The Influence of Salinity on Various Life Stages of *Ruppia tuberosa* and implications for its distribution in the Coorong, South Australia

by

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Abstract

Despite the obvious decline in the distribution of *Ruppia* in the Coorong, there have been few extensive studies investigating the factors that control the distribution of this genus. In particular, few studies have focused on how salinity causes changes in the distribution and abundance of various life stages of *Ruppia tuberosa* J. Davis and Tomlinson. To enable the appropriate and restorative management of *R. tuberosa* in the Coorong, it is necessary to improve our understanding of the environmental factors that impact this species. The primary aim of this study was to determine the principle factors in controlling the germination, growth and reproduction of *R. tuberosa* in the Coorong, which ultimately controls its distribution. This was examined through a range of techniques, including field distribution surveys, germination experiments, pond experiments and transplantation experiments to enable for the management of this species in the Coorong.

The first objective (Chapter 2) was to determine the effect of physico-chemical conditions on the distribution and abundance of various life stages of *R. tuberosa* in the Coorong region. In investigating this aim, salinity thresholds for various *R. tuberosa* life stages were assessed. The distribution and abundance of shoots, flowers, seeds and turions of *R. tuberosa* were monitored in the Coorong and neighbouring lakes, Lake Pipe Clay and Lake Cantara to assess their response to 10 physico-chemical factors. The second objective (Chapter 3) was to determine the optimal salinity levels for the germination stage of *R. tuberosa* and *Ruppia megacarpa* R. Mason. It was unknown whether the form of the relationship between salinity and germination is a simple linear response or whether there is a critical threshold salinity above which neither seeds and/or turions will not germinate. Furthermore, it was unknown whether exposure to high salinities is irreversible or whether seeds retain their ability to germinate when transferred to lower salinity. The third objective (Chapter 4) was to assess whether salinity altered the concentrations of ions in plant tissues and whether this was related to growth and reproductive responses of *R. tuberosa*. These responses were investigated by growing plants in ponds, and comparing pond plant responses to *R. tuberosa* grown in the field. The last objective (Chapter 5) was to investigate whether the concentrations of ions in plant tissue was related to photosynthesis of *R. tuberosa*, and to determine the extent to which growth responses could be explained by the function of PSII,

photosynthesis (Y_{II}), photochemical (Y_{NO}) and non-photochemical quenching (Y_{NPQ}). The study also aimed to evaluate the plant's capacity for photo-protection against photo-damage, and to examine possible roles of ion concentrations accumulated in response to increasing salinity in explaining effects on growth.

Salinity and water depth were identified as the principal physico-chemical conditions driving changes in the distribution and abundance of various life stages of *R. tuberosa*. There were strong spatial differences in shoot abundance of *R. tuberosa* among sites. The highest *R. tuberosa* shoot abundance was at salinities of 30-100 mS cm^{-1}. However, shoot abundance declined between salinities of 100 and 150 mS cm^{-1}, but increased again in salinities of 160-170 mS cm^{-1}. *R. tuberosa* shoots were present consistently in water depths of 0.2 to 0.6 m. Flower abundance was influenced by salinity and water depth. The highest flower abundance was observed at salinities of 70 to 90 mS cm^{-1} in water depths of 0.1 to 0.4 m. The highest seed density was maintained at salinities of 40 to 90 mS cm^{-1} in water depths of 0.1 to 0.4 m. In comparison to seeds, *R. tuberosa* turions had a positive relationship with increasing salinity, with turions most likely to occur in areas with salinity above 100 mS cm^{-1}, and the highest turion density observed when salinity was approximately 160 mS cm^{-1} in water depths of 0.1 to 0.4 m. This study has shown that extremely high salinity levels led to reduced flower and seed production and seed and turion germination and thus reduced shoot abundance in the Coorong. The reduced abundances of *R. tuberosa* at water depths >0.6 m suggest light limitation and that *R. tuberosa* is a high light adapted species. Salinity levels in Lake Pipe Clay were favourable for germination and results suggested that this was also the case for shoot growth during winter and spring. No flowering and relatively low seed densities indicate the high salinities during spring may affect the flowering of *R. tuberosa*. Unlike other sites, however, physico-chemical conditions in Lake Cantara were not only favourable for the establishment and development of *R. tuberosa* shoots, but also for flower production and seed production and germination. In ephemeral systems provided adequate salinities for germination (seed and turion) and growth during autumn and winter *R. tuberosa* is able to withstand elevated spring salinities through the either sexual production or the production of turions, depending on salinity levels.

Increased salinity led to a decrease in germination rates for *R. tuberosa* and *R. megacarpa*. The salinity thresholds for germination were 120-125 mS cm^{-1} for *R.*

tuberosa seeds, 165 mS cm^{-1} for *R. tuberosa* turions and 45 mS cm^{-1} for *R. megacarpa* seeds. An increase in salinity also led to an increase in mean time to germination of 15 days for *R. tuberosa* seeds (at 120 mS cm^{-1}), 24 days for *R. tuberosa* turions (at 165 mS cm^{-1}) and 10 days for *R. megacarpa* seeds (at 45 mS cm^{-1}). Seeds of *R. tuberosa* that failed to germinate at elevated salinities germinated on transfer to low salinities at an enhanced rate compared with the initial rates. Turions of *R. tuberosa* did not respond in a similar manner because of ion toxicity after exposure to high salinities. This study suggests that salinity affects imbibition, inducing ion toxicities in seeds and turions, consequently influencing the germination process. Using recent elevated salinity levels of 60 to over 165 mS cm^{-1} in the Coorong, modelling suggests that seed germination of *R. tuberosa* is likely to be restricted to the less saline northern half of the Coorong, but *R. megacarpa* germination is unlikely to occur. The modelling also suggests that riverine inputs that reduce salinity levels would restore *R. tuberosa* and *R. megacarpa* communities in the water-bodies where they were once abundant, but have declined in response to human induced salinisation.

As salinity increased from 45 to 165 mS cm^{-1} in the pond experiment, shoot concentrations of Na^+ increased, and K^+ concentrations decreased, and Na^+/K^+ ratios increased. These ions were significantly correlated with the inhibition of biomass growth and measures of sexual and asexual reproduction. A similar response was found in the field situation, where shoot concentrations of Na^+ and Cl^- increased as salinity increased in *R. tuberosa*. The total biomass of *R. tuberosa* was affected by the ions of Na^+ and K^+ and Na^+/K^+ ratios. The inhibition of flowering in these field plants was correlated with low B and high Na^+/K^+ ratios, whereas turion density was correlated with low K^+ and high Na^+/K^+ ratios. Longer shoot lengths of field *R. tuberosa* were correlated with high Ca^{2+} concentrations. It is concluded that increased Na^+ and Cl^- induced by elevated salinity would inhibit plant growth, and low B and high Na^+/K^+ ratios would inhibit sexual reproduction, while low K^+ and high Na^+/K^+ ratios would induce asexual reproduction. The study indicates that reproduction failure induced by low B and high Na^+/K^+ ratios may be an important factor causing the observed reduction in the distribution and abundance of *R. tuberosa* in the Coorong.

The results of photosynthetic responses to salinity showed that Na^+ concentrations and Na^+/K^+ ratios were positively related to increased salinity in the tissues of *R. tuberosa*, but there was no relationship between salinity and K^+ concentrations. There was a rapid decline in Y_{II} from pre-dawn to midday, with a concomitant increase in Y_{NPQ} in all salinities of 60, 75 and 125 mS cm^{-1}, but subsequently Y_{II} increased with a decrease in Y_{NPQ} during the afternoon, whereas Y_{NO} remained relatively stable throughout the day. The Fv/Fm ratio was negatively related to increased Na^+ concentrations and Na^+/K^+ ratios, but not to K^+ concentrations, which affected positively the Fv/Fm ratio of *R. tuberosa*. The concentrations of B in *R. tuberosa* tissues were higher at the lower Na^+/K^+ ratios, but a significant decrease in B was observed as Na^+/K^+ ratios increased. The low B concentration in plant cells may cause physiological changes, resulting in inhibited leaf expansion and consequently causing a loss of photosynthetic capacity. This can be associated with reductions in growth and development of *R. tuberosa*.

Overall, the results indicate that elevated salinities decreased the opportunity of *R. tuberosa* to complete its life cycle. If salinity levels in the Coorong continue to increase *R. tuberosa* may disappear completely, resulting in an ecosystem depauperate of submerged macrophytes. It is concluded that *R. tuberosa* has a number of mechanisms that allows it to survive in a range of conditions. However, the findings of this study suggest that the provision of appropriate riverine inputs to the Coorong to maintain salinities below 100 mS cm^{-1} in the southern end of the Coorong are required to restore the *R. tuberosa* community. The habitat restoration requiring reduced salinity levels can only be achieved by providing adequate flow of fresh water from the River Murray to the Coorong to ensure successful sexual reproduction of *R. tuberosa*. This study has provided a greater understanding of the influences of different environmental parameters on *R. tuberosa*, which in turn would allow for more effective biological and ecological management tools and methods to be developed.

Declaration of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Dae Heui Kim 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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Dae Heui Kim

November 2013

Foreword

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 2: How *Ruppia tuberosa*'s Life cycle adapted to contrasting habitats, Chapter 3: The effect of salinity on the germination of *Ruppia tuberosa* and *Ruppia megacarpa* and implications for the Coorong: A coastal lagoon of southern Australia, Chapter 4: The effect of salinity on growth and reproduction of *Ruppia tuberosa* in the Coorong and Chapter 5: The effect of salinity on photosynthesis of *Ruppia tuberosa*, have been submitted (Chapter 2, Chapter 4 and Chapter 5) and published (Chapter 3) in the interest of continuity of the thesis, this chapter has been included as part of the word document. In the publications, salinity was reported in g/L but these have been converted to mS cm^{-1} for inclusion in the main body of the thesis. Copy of this publication has been added as Appendix I respectively.

Publications Associated with this thesis

Chapter 2 (submitted for publication) Pg 15

How *Ruppia tuberosa*'s life cycle adapts to contrasting habitats

D.H. Kim, K.T. Aldridge, J.D. Brookes, G.G. Ganf

School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

Inland Waters

Kim, D.H. (Candidate)

Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate

Signed…… …….*Date*……..

Aldridge, K.T.

Supervised development of work, helped in data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis

Signed *Date*15/11/2013

Brookes, J.D.

Supervised development of work, helped in data interpretation and manuscript evaluation.

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Ganf, G.G.

Supervised development of work, helped in data interpretation and manuscript evaluation.

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Chapter 3 (published) Pg 33

The effect of salinity on the germination of *Ruppia tuberosa* and *Ruppia megacarpa* and implications for the Coorong: A coastal lagoon of southern Australia

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Aquatic Botany (2013) 111:81-88

Kim, D.H. (Candidate)

Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate

Signed…… …….*Date*……..

Aldridge, K.T.

Supervised development of work, helped in data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis

Signed *Date*15/11/2013

Brookes, J.D.

Supervised development of work, helped in data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis

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Ganf, G.G.

Supervised development of work, helped in data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis

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Chapter 4 (submitted for publication) Pg 53

The effect of salinity on growth and reproduction of *Ruppia tuberosa* in the Coorong, a coastal lagoon of southern Australia

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Chapter 5 (submitted for publication) Pg 70

The effect of salinity on photosynthesis of *Ruppia tuberosa*

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Chapter 1 General Introduction

Estuaries, coastal lagoons and wetlands worldwide are regions of high productivity and diversity (Lirman et al., 2008), providing important habitats for invertebrates, fishes and waterbirds (Congdon and McComb, 1979; Lirman et al., 2008). However, the functions of these ecosystems have deteriorated as a consequence of chemical pollution, eutrophication, physical impacts and upstream water extraction for an irrigated agriculture (Orth et al., 2006; Lirman et al., 2008). In particular, salinisation has emerged as a major threat to these unique ecosystems (Keighery et al., 2000). A large proportion of Australia's inland waters have become saline, with salinity levels continuing to increase through evaporation and agricultural irrigation/depletion (Keighery et al., 2000; Nielsen et al., 2003b; Lotze et al., 2006). This has resulted in the loss and decline of many aquatic macrophyte communities, which are key components of these ecosystems (Orth et al., 2006; Lirman et al., 2008). Salinisation is not only a problem within Australia, but is recognised as an issue in Argentina, China, India, South Africa and the United States of America (Lazar and Dawes, 1991; Santamaría and Hootsmans, 1996; Nielsen et al., 2003b; Parida and Das, 2005; Ye et al., 2005; Taylor et al., 2006; Nejrup and Pedersen, 2008; Song et al., 2008).

1.1 The salinisation of aquatic ecosystems

Secondary salininisation is recognised as a major environmental problem in arid and semi-arid regions of the world (Redondo-Gómez et al., 2009), including many riverine and wetland ecosystems in Australia (Hart et al., 1990; Williams, 1999; Hart et al., 2003; James et al., 2003). Salinisation is caused by a number of factors including: increasing irrigated agriculture (Cramer and Hobbs, 2002) and anthropogenic modification to water regimes (Nielsen et al., 2003a; Nielsen et al., 2003b; Brock et al., 2005). For example, in Australia, it is estimated that 17 million ha of land will be at high risk of elevated salt concentrations within the next 40 years (NationalLandandWaterResourcesAudit, 2001). One of the main reasons for the rise in salinity in aquatic ecosystems in Australia is the extractions of water from the aquatic ecosystems, which results in the accumulation of salts. These extractions are for agricultural, industrial and domestic uses (Cramer and Hobbs, 2002; Nielsen et al., 2003b). With decreased inflows from snow and rainfall, lake and wetland volumes decrease with consequent rises in salinity, leading to salinity problems in both

permanent and temporary aquatic ecosystems (Hart et al., 1990; Hart et al., 2003; James et al., 2003). Furthermore, salinisation of aquatic ecosystems can be caused increased groundwater levels and increased saline groundwater inputs into aquatic ecosystems that result from replacing deep-rooted perennial vegetation with shallow-rooted annual crops (Clarke et al., 2002).

1.2 Impacts of salinisation on aquatic macrophyte communities

There is a recognition that increased salinity can result in changes to river, stream and wetland ecosystems. At the global scale, reductions in abundance and diversity of aquatic plants have been largely attributed to increases in salinity (Cramer and Hobbs, 2002; Nielsen et al., 2003b). However, it is recognised that while salinity is a crucial factor controlling diversity and abundance, it is not be the only factor causing of macrophyte decline. At the local scale, salinisation has also resulted in a reduction in the distribution and abundance of individual aquatic macrophyte species (Nielsen et al., 2003a; Sim et al., 2006; Song et al., 2008) and can lead to localised extinctions of macrophyte communities (Keighery et al., 2000). Since macrophytes play an integral part in the functioning of ecosystems, this has had major impacts for the ecosystems more broadly (Khan and Ungar, 1996; Katembe et al., 1998; Tessier et al., 2000; Jarvis and Moore, 2008; Lirman et al., 2008; Elsey-Quirk et al., 2009). Salinity can affect aquatic plants by interfering with the physiology of various life stages, thereby affecting their distribution and abundance (Brock et al., 2005; Sim et al., 2006). This is the case for both halophytes (Khan and Ungar, 1996) and glycophytes (Brock et al., 2005), as discussed below.

1.2.1 Germination response to salinisation

Germination is a pivotal stage of a plant life-cycle and salinity is known to influence germination success. In fact, the success of halophyte populations is largely dependent on their germination salinity tolerance (Pujol et al., 2000; Ye et al., 2005). Whilst the salinity thresholds for glycophytes vary considerably, most halophytes are able to germinate at salinities greater than seawater (Vollebergh and Congdon, 1986; Sim et al., 2006).

High salt concentration in soil prevents germination and seeds only germinate when conditions become favourable (i.e. inundation and appropriate salinity levels). The initial germination process can be delayed by salinity stress (Brock et al., 2005; Kim et al., 2013), due to ion toxicity and/or osmotic effects (Katembe et al., 1998). Water movement across cell membranes into seed cells is determined by the difference between the osmotic potential of the seed and that of the external medium (Katembe et al., 1998). High ion concentrations in seed cells may induce changes in protein activity because ions affect the structure of hydration water, causing an inhibition of enzyme activity (Pujol et al., 2000), in turn inhibiting germination (Kim et al., 2013). However, salinity has been often attributed to osmotic effects (Keiffer and Ungar, 1997; Pujol et al., 2000), as it is thought that the movement of water across cell membranes into the embryo of seed leads to breakage of seed and increased enzyme activity, enhancing metabolic rates (Katembe et al., 1998).

1.2.2 Physiological and growth responses to salinisation

Internal excesses of sodium (Na^+) and chloride (Cl^-) ions cause membrane damage or shifts in nutrient concentrations and interfere with ion balances, thus affecting physiology and morphology, and consequently causing a reduction in growth and reproduction of aquatic plants (Jampeetong and Brix, 2009). Salinity stress at the early growth stage is potentially fatal for aquatic macrophytes (Khan and Ungar, 1996; Katembe et al., 1998; Tessier et al., 2000; Jarvis and Moore, 2008; Lirman et al., 2008; Elsey-Quirk et al., 2009). Causative processes are excessive ion concentrations within the plants (resulting in a low external water potential), ion toxicity, osmotic stress and nutrient imbalance due to high specific ions flowing through plant tissues (Pujol et al., 2000).

It is well known that Na^+ is preferentially delivered to older leaves, thereby protecting developing leaves. However, excessive amounts of salt entering the plant will increase salt concentrations to toxic levels in the older transpiring leaves, causing premature senescence (Munns, 2002). Salinity stress is generally thought to have a depressive effect on photosynthesis, and inhibits photosynthesis mostly through photosystem II activity (Naumann et al., 2007). Reduction in photosynthesis under saline conditions has been reported for glycophyte and halophytic species (Ball and Farquhar, 1984; Seemann and Critchley, 1985). Salinity can predispose plants to photo-inhibition and

photo-damage of PSII (Mishra et al., 1991). However, halophytes are able to maintain photosynthetic performance over a wide range of light and salinity conditions (Ralph and Gademann, 2005). This is due to photo-protective mechanisms to dissipate excess excitation energy, which protect the photosynthetic apparatus (Demmig-Adams and Adams III, 1992).

There are also indirect physiological effects of salinity on macrophyte growth resulting from ion imbalances. For example, plants growing in salinity water may not be able to obtain enough potassium (K^+). This problem exists because Na^+ competes with the uptake of K^+ throughout the elongation zone (Naidoo, 1994), which subsequently results in high Na^+/K^+ ratios (Maas and Grieve, 1987; Reid and Smith, 2000). The inhibition of K^+ accumulation due to salinity, increases with distance from the leaf base, inducing growth inhibition (Bernstein et al., 1995).

Ion toxicity also inhibits the uptake of calcium (Ca^{2+}, (Reid and Smith, 2000), leading to Ca^{2+} deficiency (Cramer et al., 1989) and consequently causing high Na^+/Ca^{2+} ratios (Maas and Grieve, 1987; Cramer et al., 1989; Cramer et al., 1990). This can result in inhibition of leaf growth (Bernstein et al., 1995). The presence of Ca^{2+} is known to be crucial for growth and if sufficient Ca^{2+} is present, a high-affinity uptake system having a preference for transport of K^+ can function, and the plants can then obtain sufficient K^+ and restrict Na^+ uptake (Davenport et al., 1997).

High Na^+ and Cl^- concentrations in plant tissues can also inhibit the uptake of Boron (B, (El-Motaium et al., 1994; Goldbach et al., 2007), resulting in B deficiency (López-Gómez et al., 2007). A reduce leaf expansion caused through limiting cell enlargement and cell division by B deficiency, in turn resulting in a loss of photosynthetic capacity (Dell and Huang, 1997).

1.3 The importance of water regime on aquatic macrophytes

Water regime is a complex variable, as described by water depth, duration, frequency, drawdown, the rate of change in water level and seasonal variability, has a significant influence on the distribution of aquatic regions (Duarte et al., 1986; Grace, 1989; Rea and Ganf, 1994; Blanch et al., 1999; Blanch et al., 2000). Water regime is the primary factor determining pattern and process in wetland macrophytes (Brock, 1994; Rea and

Ganf, 1994; Blanch et al., 1999). In systems where water levels fluctuate, pattern and process change is common, with biomass, production, recruitment and photosynthesis in aquatic macrophytes (Brock, 1988; Froend and McComb, 1994).

1.4 Ecological importance of halophyte of *Ruppia*

Ruppia spp. are an important contributor to the primary productivity of aquatic ecosystems and are considered to be one of the most important carbon sources to estuarine and coastal lagoon food-webs (Brock, 1981b, 1982b). *Ruppia* forms an important component in the diets of waterfowl and fish species; forms a major source of detritus of detritivores; provides important habitats for fish, invertebrates and waterbirds (Congdon and McComb, 1979; Nicol, 2005); and plays a critical role in the overall ecology of these ecosystems (Congdon and McComb, 1979; Nicol, 2005; Lirman et al., 2008). However, despite its ecological importance, little is known about the ecology of *Ruppia* and the environmental requirements of this genus. In particular, there is limited information regarding the environmental requirements of *R. tuberosa* in comparison to that on other *Ruppia* species.

1.4.1 Description of *Ruppia*

Ruppia is a genus of cosmopolitan submerged angiosperms with worldwide distribution, recorded in varying salinities of 4.7 to 360 mS cm^{-1} (Brock, 1979, 1982a; Nicol, 2005) in both permanent and temporary water bodies. *Ruppia* consists of annual and perennial species that complete their life cycles within a few months from germination to reproduction (Brock, 1982b). Brock (1982b) found that the annual species are considerably less productive than the perennial species because the former occur in wetlands subject unfavourable environmental conditions such as high salinity levels and desiccation.

1.4.2 Classification of *Ruppia*

The classification of *Ruppia* at both the genus and family level has been debated between taxonomists (Jacobs and Brock, 1982), with a distinction recognised between the Ruppiaceae and the Potamogetonaceae (cited in (Jacobs and Brock, 1982). Hutchinson (1959, cited in (Jacobs and Brock, 1982) classified *Ruppia* in the family Ruppiaceae as separate from that of Potamogetonaceae. Kartesz and Kartesz (1980,

cited in (Jacobs and Brock, 1982) classified *Ruppia* in the family Zosteraceae. They recognised that North America has three species of *Ruppia*: *Ruppia anomala*, *Ruppia cirrhosa* (Petagna) Grande and *Ruppia maritima* L. (Kantrud, 1991). In Europe, *Ruppia* is considered to be in the family Potamogetonaceae and two species, *R. cirrhosa* and *R. maritima* are recognised. Jacobs and Brock (1982) investigated further to clarify the species of *Ruppia* and they recognised four species of *Ruppia* in Australia: *R. maritima*, *R. megacarpa*, *R. polycarpa* and *R. tuberosa*. They placed the genus in the family Potamogetonaceae.

1.4.3 Distribution of *Ruppia*

Ruppia is represented on all continents of the world and on many islands (Kantrud, 1991). The northern and southern limits are approximately 69ºN and 55ºS respectively, and the altitudinal limit is at least 3,800m above sea level (Verhoeven, 1979). In North America, *R. maritima* occurs in inland saline habitats along the east coast from Newfoundland to Florida (Phillips, 1960). In South America, *R. cirrhosa* occurs in mountain lakes in the Andes and in Argentina (Verhoeven, 1979). In Africa, *Ruppia* occurs all along the Mediterranean coast (Verhoeven, 1979). In Asia, *Ruppia spiralis* is reported from the Black Sea and *R. maritima* in India and Iran (Verhoeven, 1979). In Europe, *R. cirrhosa* and *R. maritima* occur along all coasts of Denmark in belt waters and in brackish and coastal pools (Verhoeven, 1979). In Britain and France, *Ruppia* spp. occurs in blocked brackish water habitats, in ponds and lagoons along the Atlantic, as well as the Mediterranean coast (Verhoeven, 1979). In Australia and New Zealand, *Ruppia,* including *R. maritima, R. megcarpa*, *R. polycarpa* and *R. tuberosa*, has been found in coastal and brackish areas (Brock, 1979; Verhoeven, 1979). Within Australia, *R. megacarpa, R. polycarpa* and *R. tuberosa* are restricted to New South Wales, Victoria, South Australia, Tasmania and Western Australia (Brock, 1982b). *R. megacarpa* and *R. tuberosa* were once common in saline lakes and wetlands in the Coorong, South Australia. *R. polycarpa* is present in the saline lakes in the southeast of South Australia, while *R. maritima* is not common in South Australia (Nicol, 2005).

1.4.4 Life-cycle of *Ruppia*

Ruppia megacarpa R. Mason, a perennial species, has peak above-ground biomass in midsummer, then flowers and fruits, and produces seed during late summer and autumn.

It resumes growth through the growth of rhizomes after winter (Brock, 1981b, 1982a). *R. megacarpa* leaves are 5-25 cm long with 1-2 mm wide. The inflorescence is presented at the water surface for pollination on a peduncle up to 1 m or more long, which coils after pollination.

In contrast, the annual species, *Ruppia tuberosa* J. Davis and Tomlinson and *Ruppia polycarpa* R. Mason grow in mid to late autumn or early winter and produce seeds and turions during spring and early summer, (from September to December in Australia) (Brock, 1981b, 1982a). *R. tuberosa* leaves are 5-30 cm long with 1 to 2 mm wide. The inflorescence is presented at the water surface for pollination on a peduncle up to 1 m, which coils after pollination (Figure 1.1). *R. polycarpa* stems are not always obvious, up to 20-40 cm long, with leaves 5-15 cm long and usually less than 1 mm wide. The inflorescence is presented at the surface for pollination on a peduncle less than 1 m long, which coils after pollination. *R. polycarpa* and *R. tuberosa*, reproduce asexually by rhizomes and stem fragments and produce large numbers of seeds (Brock, 1982a). Lateral spread of these annual species by rhizomes and the development of asexual reproductive organs (turions) are important in aquatic ecosystems short growing seasons (Brock, 1982b). In South Australian ecosystems, two types of turions are recorded on *R. tuberosa*: type I turions (Figure 1.2), which are a swelling at the leaf base, and type II turions (Figure 1.3), which are a swelling at the rhizome tip (Brock, 1982a). However, only type I turions are recorded in *R. polycarpa*. Both turion types occur in ephemeral habitats and serve as perennating organs when conditions are favourable. Brock (1982a) has shown that turions of type I and II are both structures containing dormant meristematic areas which can be stimulated to resume growth by favourable environmental conditions. Turions formed by *R. polycarpa* and *R. tuberosa* are readily detached from the parent plants and dispersed (Brock, 1982b). Both seeds and turions of these two species survive drying periods in the sediment and germinate in the subsequent wet phase. Turions have not been recorded in the perennial *R. megacarpa*.

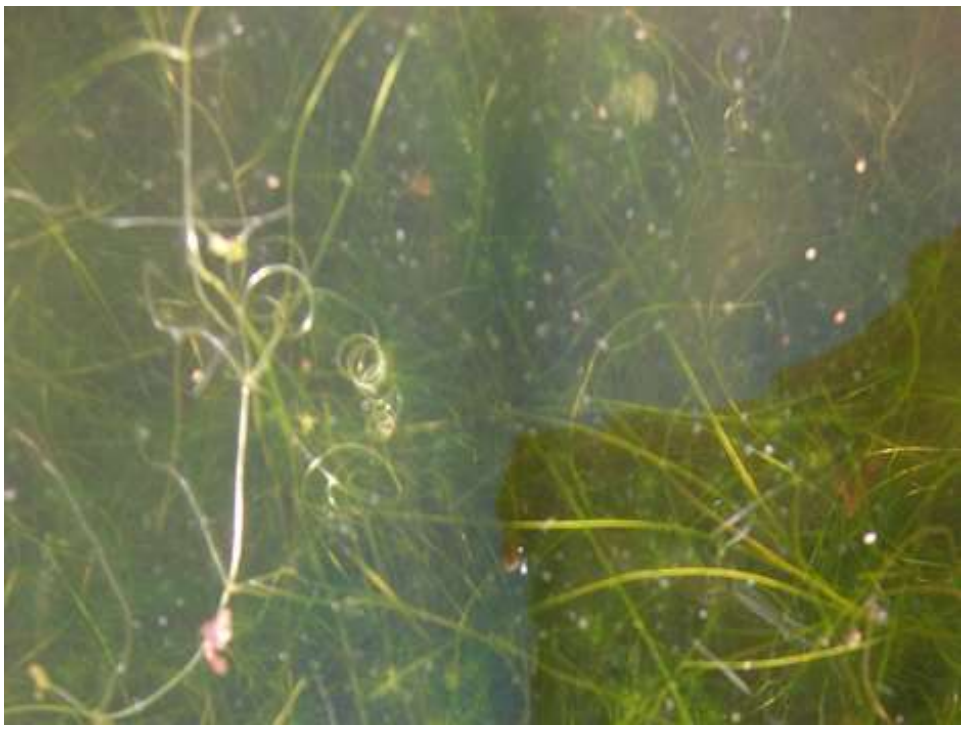

Figure 1.1: Image of *Ruppia tuberosa*.

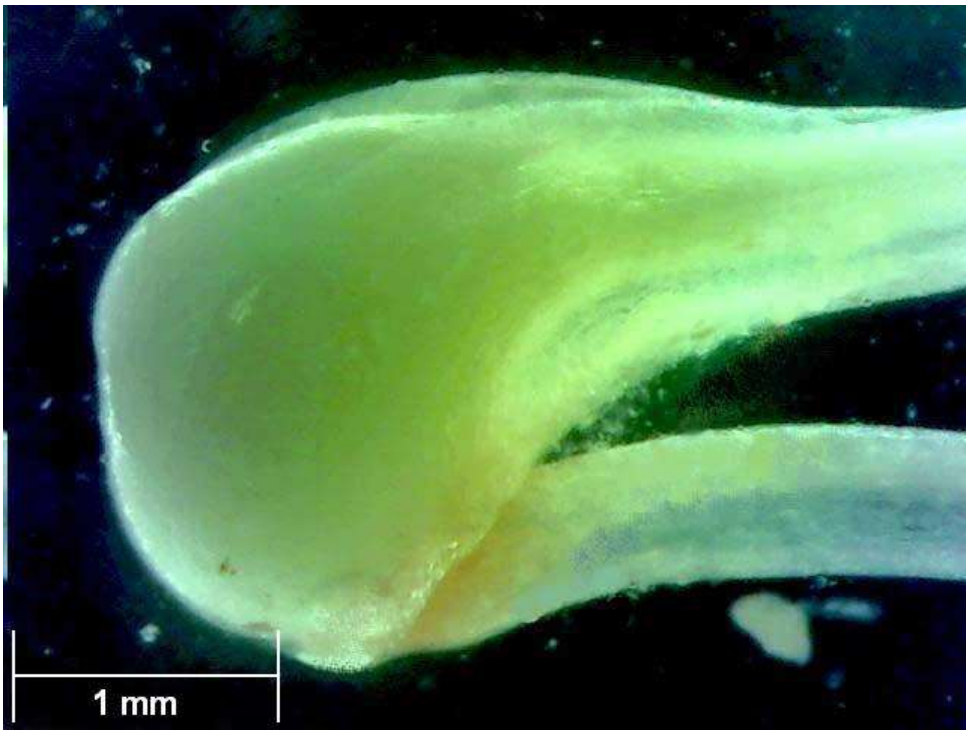

Figure 1.2: Image of the turion type I of *Ruppia tuberosa*.

Figure 1.3: Image of the turion type II of *Ruppia tuberosa*.

1.5 *Ruppia* populations in the Coorong region

1.5.1 Study area: The Coorong region of southern Australia

The Coorong, located in South Australia (35°59′S, 139°02′E) is a coastal lagoon confined by a coastal barrier dune extending southeast from the Mouth of the River Murray for approximately 140 km^2 (Figure 1.4). The Coorong is part of one of the most important wetland systems in Australia (Ramsar Convention on Wetlands: http://www.ramsar.org/), covering 467 km^2 and composed of ocean beach, the mouth of the River Murray, the freshwater Lower Lakes (Lake Alexandrina and Lake Albert) and the estuary. The Coorong is connected to the ocean at its northern end via the River Murray Mouth, and is separated into a North and South Lagoon about halfway at Parnka Point (PA).

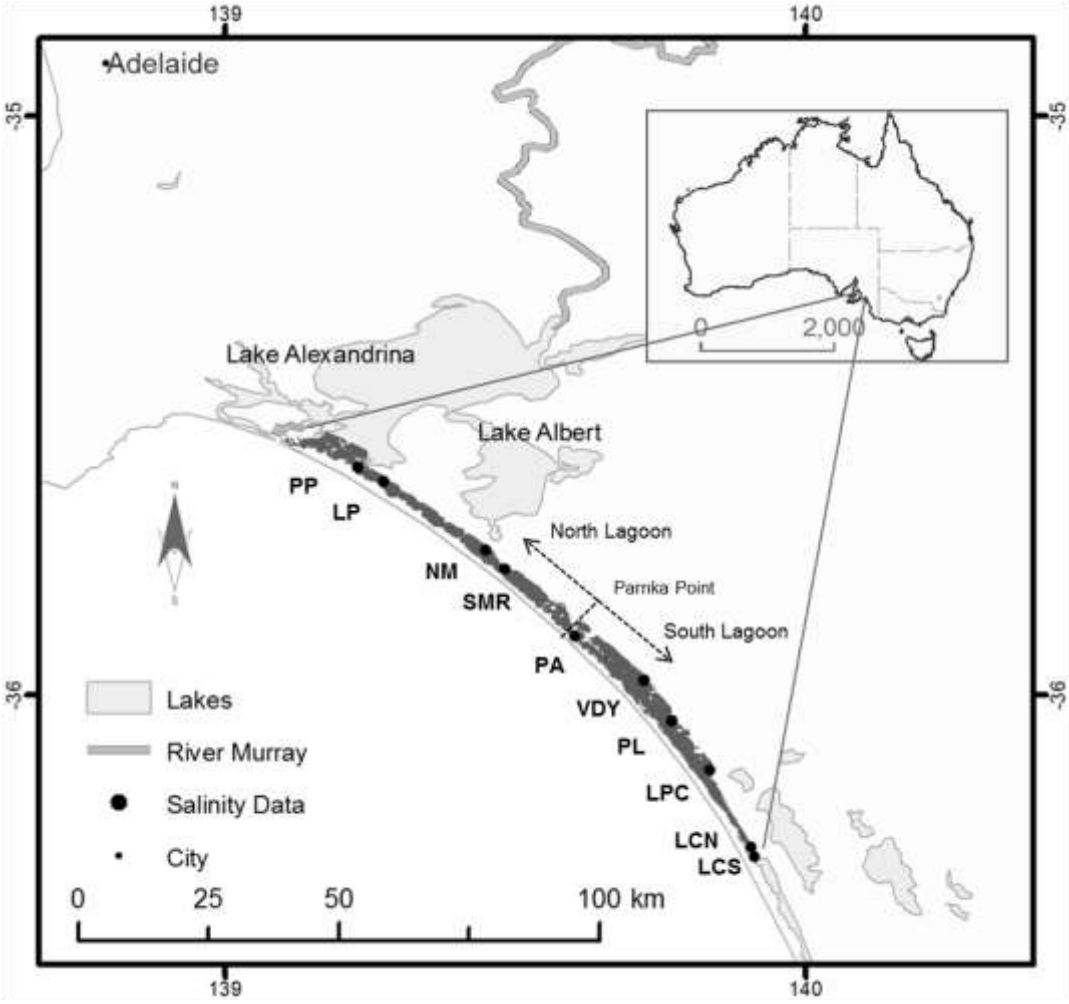

Figure 1.4: The location of study sites in the Coorong and neighbouring Lake Pipe Clay and Lake Cantara (North and South), South Australia (PP = Pelican Point; LP = Long Point; NM = Noonameena; SMR = Seven Mile Road; PA =Parnka Point; VDY = Villa dei Yumpa; PL = Policeman's Point; LPC = Lake Pipe Clay; LCN = Lake Cantara North and LCS = Lake Cantara South).

The Murray Mouth and Coorong region, experience a Mediterranean climate where evaporation exceeds precipitation over summer, causing water levels within many wetlands to drawdown. The more southerly sections of the Coorong are micro-tidal and water levels are determined predominantly by wind, evaporation and inflows. In summer, wide areas of mudflat are exposed due to reduced freshwater inflows and lower water levels within the Southern Ocean.

Typically, the Coorong has a strong salinity gradient from north to south, ranging from fresh to brackish in the North Lagoon depending upon inflows from Lake Alexandrina to hyper saline in the South Lagoon. Lake Alexandrina is separated from the Coorong

by a series of barrages constructed in the late 1930s to minimise tidal incursions into the lakes and thus ensure fresh-water supplies for both metropolitan consumption and agricultural uses. Flows to the Coorong can be controlled by opening gates in the barrages. Reduced freshwater inflows resulting from water resource development have resulted in increased salinity levels (Brookes et al., 2009). Historically, the Coorong had high biological productivity and diversity, especially with regard to migratory waterbirds. However, like many other coastal systems across the world, the Coorong has experienced degradation over the last 100 years. More recently, major significant ecological changes have been observed, including the rapid decline of *R. tuberosa*, and the complete loss of *R. megacarpa* (Paton and Rogers, 2007). Water resource development combined with a regional drought meant that no inflows to the Coorong from Lake Alexandrina between 2006 and 2010. This resulted in increased salinity levels and extreme hyper-salinisation of the South Lagoon (Figure 1.5).

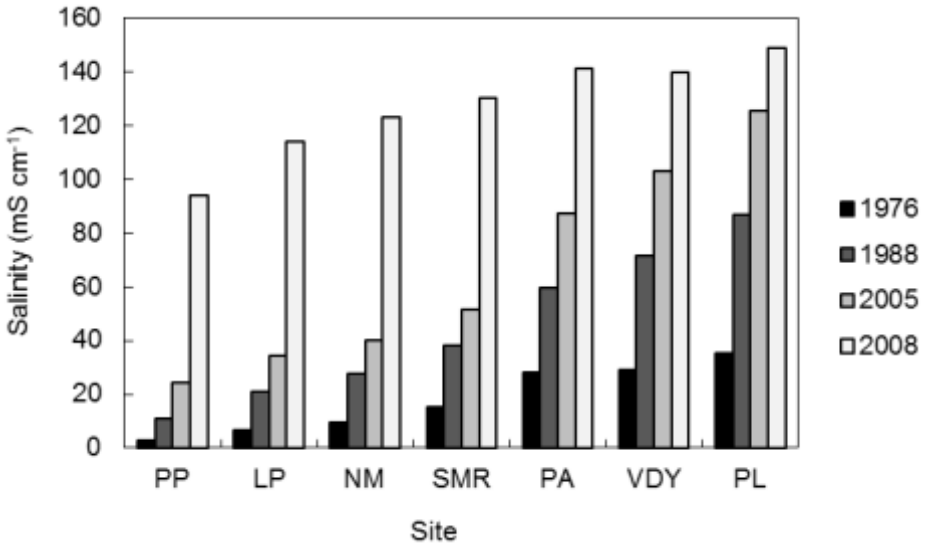

Figure 1.5: Salinity at seven sites in the Coorong sampled in July 1976, 1988, 2005 and 2008 (PP = Pelican Point; LP = Long Point; NM = Noonameena; SMP = Seven Mile Road; PA =Parnka Point; VDY = Villa dei Yumpa; PL = Policeman's Point).

Lake Pipe Clay is an ephemeral lake located in south of the Coorong (36°32′S, 139°75′E). The lake is shallow (approximate water depth of ~0.5m in winter) and is dry during summer (December-February). Salinities within the lake range from 40 to 160 mS cm^{-1} from August to November, and *R. tuberosa* is highly abundant. The lake currently has no direct connection to the Coorong.

Lake Cantara is an ephemeral lake located adjacent to the Coorong, about 250 km from Adelaide, South Australia (35°59′S, 139°02′E). The lake is small (144 ha or ~ 1.5 km^2), shallow (approximate water depth of ~0.5m in winter) and is dry during summer (December-February). Salinity at Lake Cantara ranges from 27 to 115 mS cm^{-1} from August to November, and *R. tuberosa* is highly abundant. The lake is presently athalassic with no direct connection to either the Coorong or the sea. The lake is separated into two water bodies (Lake Cantara North and South).

However, the Coorong is currently under threat as a result of ongoing changes in the hydrological regime (e.g. reduced fresh water inflow) of the River Murray (Brookes et al., 2009). While a number of projects, including protecting and enhancing the ecological values of this region, are underway to reverse the environmental decline, rehabilitation/restoration efforts are hampered by the lack of the field of knowledge about the links between flows and ecological responses in the system. This involves research to understand the links between the key ecosystem drivers for the region and key ecological processes (e.g. macrophyte communities). Thus, this study attempts to advance the understanding of the effects of environmental factors on submerged macrophyte species, using a combination of field surveys in the Coorong and neighbouring Lake Pipe Clay and Lake Cantara, pond and field experiments.

1.5.2 Distribution of *Ruppia tuberosa* in response to salinity and water depth in the Coorong region

Despite the obvious decline in the distribution of *Ruppia* in the Coorong, there have been few extensive studies investigating the factors that control the distribution of this genus (Brock, 1981b). In particular, few studies have focused on how salinity causes changes in the distribution and abundance of various life stages of *R. tuberosa*. To enable restoration of *R. tuberosa* in the Coorong, it is necessary to improve our understanding of the environmental factors that impact this species. This study utilises habitat differences within the Coorong region to determine the habitat requirements for the life cycle of *R. tuberosa*. The environmental factors that control the distribution of *R. tuberosa* in the Coorong are as yet unknown but salinity and water level have been implicated as key drivers. Because *R. megacarpa* disappeared from the Coorong and *R. tuberosa* disappeared from the southern basin it is hypothesised that the salinity levels found in the Coorong reflect the salt tolerance of the two *Ruppia* species. The first aim

of this study (Chapter 2) was to determine how the distribution and abundance of different life-stages of *R. tuberosa* were related to physico-chemical conditions in the Coorong and neighbouring Lake Pipe Clay and Lake Cantara. This aim was investigated through field surveys, with the distribution and abundance, was assessed by measuring shoot, flower, seed and turion abundance.

1.5.3 Germination of *Ruppia tuberosa* and *Ruppia megacarpa* response to salinity

The success of halophyte populations is dependent on their germination salinity tolerance (Pujol et al., 2000; Ye et al., 2005). Since, the distribution and abundance of *R. tuberosa* and *R. megacarpa* have declined in the Coorong region during a period of salinisation, it is important to document the salinity tolerance of these species. While it is understood that salinity affects seed germination of *R. tuberosa* and *R. megacarpa*, the aims of this study (Chapter 3) were to: provide detailed information on the response of both species to salinisation, including a response to a dynamic salinity regime; compare the response of seed and turion germination of *R. tuberosa* to salinisation; explore the mechanisms of the response; and use this information to examine the relationship between riverine inputs and *Ruppia* germination in the Coorong.

1.5.4 The effect of salinity on growth and reproduction of *Ruppia tuberosa*

Salt stress at the growth stage is critical for aquatic macrophyte communities (Khan and Ungar, 1996; Katembe et al., 1998; Tessier et al., 2000; Jarvis and Moore, 2008; Elsey-Quirk et al., 2009). The growth and flowering responses of various species to salinity stress varies. Salinity affects plant growth in a variety of ways; decreasing water uptake, causing ion toxicity and reducing nutrient availability. Flowering is a particularly sensitive phase in a plant's ontogenetic development. However, very little is known about factors controlling flowering of halophytes. It is hypothesised that the reduced growth and reproduction of *R. tuberosa* under high salinity concentrations includes inhibition of ion uptake and shoot ion (K^+, Ca^{2+} or B) deficiency. This study aimed to test this hypothesis. This will allow for an assessment of the duration and timing of favourable salinities that are required to permit the growth and reproduction. The purpose of this study (Chapter 4) was to investigate the effects of salinity on growth and reproduction of *R. tuberosa*. These responses were investigated by growing plants in ponds at varying salinities which were compared with *R. tuberosa* grown *in situ*.

1.5.5 The effect of salinity on photosynthesis of *Ruppia tuberosa*

It is well known that internal excesses of Na^+ and Cl^- ions can cause membrane damage, interfering with ion balances, and cause shifts in nutrient concentrations, thus affecting morphological and physiological characteristics of macrophytes (Jampeetong and Brix, 2009). This study was designed to elucidate growth and photosynthetic responses of *R. tuberosa* to salinity and light. It is hypothesised that a possible cause of reduced growth of *R. tuberosa* under high salinity concentrations includes inhibition of photosynthesis due to ion toxicity. The aims of this study (Chapter 5) were to investigate the effects of salinity on photosynthesis of *R. tuberosa*, and to determine the extent to which growth responses could be explained by the function of PSII, photosynthesis (Y_{II}), photochemical (Y_{NO}) and non-photochemical quenching (Y_{NPQ}). The study also aimed to evaluate the plant's capacity for photo-protection against photo-damage, and to examine possible roles of ion concentrations accumulated in response to increasing salinity in explaining effects on growth.

Chapter 2 How *Ruppia tuberosa*'s Life cycle is adapted to contrasting habitats

2.1 Introduction

Secondary salinisation is recognised as a major environmental problem in arid and semi-arid regions of the world (Redondo-Gómez et al., 2009), with many riverine and wetland ecosystems impacted in Australia (Hart et al., 1990; Williams, 1999; Hart et al., 2003; James et al., 2003). Secondary salinisation is caused by a number of factors including increasing irrigated agriculture (Cramer and Hobbs, 2002) and anthropogenic modification to water regimes (Nielsen et al., 2003a; Nielsen et al., 2003b; Brock et al., 2005).

Salinisation of inland water ecosystems has resulted in a reduction in the abundance and distribution of submerged aquatic macrophytes (Sim et al., 2006; Song et al., 2008), which play an integral part in the functioning of these ecosystems (Khan and Ungar, 1996; Katembe et al., 1998; Tessier et al., 2000; Jarvis and Moore, 2008; Lirman et al., 2008; Elsey-Quirk et al., 2009). In some cases, salinisation has led to localised extinctions of macrophytes species (Keighery et al., 2000). Previous studies have revealed that salinity stress influences the establishment, growth and survival of glycophytes and halophytes (Vicente et al., 2004; Brock et al., 2005; Song et al., 2008). Increased salinity can have detrimental effects not only on the condition of mature plants, but also on their reproductive capacity, thereby influencing propagule availability (Baldwin and Mendelssohn, 1998; Parida and Das, 2005). Often the impacts of salinisation upon macrophytes coincide with other co-related impacts, such as altered water depth and temperature (Carruthers et al., 1999). For example, reduced riverine inputs to estuaries can not only result in elevated salinities but also in lower water levels that are unsuitable for *Zostera marina* L. to complete its life-cycle (Orth and Moore, 1988).

Significant losses of glycophytes from river and stream ecosystems have been relatively well studied (Williams, 1999; James et al., 2003; Nielsen et al., 2003b), but impacts on halophytes have received less attention (Vicente et al., 2004; Song et al., 2008) despite their importance to saline inland water ecosystems (Sim et al., 2006; Song et al., 2008). Species of *Ruppia* (*Ruppia megacarpa* R. Mason, *Ruppia polycarpa* R. Mason and

Ruppia tuberosa J. Davis and Tomlinson) are halophytes that provide crucial habitats for fish and invertebrates and food for waterfowl (Brock, 1979; Congdon and McComb, 1979). They grow in a wide range of salinities from 20 - 300 mS cm^{-1}, but display large differences in their salinity preferences and tolerances for different life stages (Brock, 1982b; Kantrud, 1991; Carruthers et al., 1999).

R. tuberosa and *R. megacarpa* were once widespread in the Coorong, a Ramsar wetland in South Australia, recognised for its diverse and abundant waterbird community (Nicol, 2005; Brookes et al., 2009). Although once abundant in the Coorong (Brock, 1982b), *R. megacarpa* has now disappeared, coinciding with a period of reduced riverine inputs associated with water extractions for human purposes (Brookes et al., 2009). *R. tuberosa* is now the most widely distributed angiosperm in both the Coorong and a series of ephemeral lakes to the south of the Coorong, including Lake Pipe Clay and Lakes Cantara North and South. It is an annual angiosperm that is capable of both sexual (seeds) and vegetative (turions) reproduction (Brock, 1982b). However, the abundance and distribution of *R. tuberosa* in the Coorong has also reduced significantly (Brookes et al., 2009).

The environmental factors that control the distribution of *R. tuberosa* in the Coorong are as yet unknown. Because *R. megacarpa* disappeared from the Coorong and *R. tuberosa* disappeared from the southern basin it is hypothesised that the salinity levels found in the Cooong reflect the salt tolerance of the two *Ruppia* species. The aims of this study were to determine the effect of physico-chemical conditions on the distribution and abundance of various life stages of *R. tuberosa* in the Coorong region. In investigating this aim, salinity thresholds for various *R. tuberosa* life stages were assessed. The distribution and abundance of shoots, flowers, seeds and turions of *R. tuberosa* were monitored in the Coorong, and neighbouring lakes and compared to a range of physico-chemical conditions.

2.2 Meterial and methods

2.2.1 Site description

The Coorong is a permanent coastal lagoon located in the south-east of South Australia in the Murray-Darling Basin (Figure 2.1). The Coorong is one of Australia's largest wetland systems and along with the Lower Lakes (Lake Alexandrina and Lake Albert) is listed as a wetland of international importance under the Ramsar convention (Ramsar

Convention on Wetlands: http://www.ramsar.org/), largely due to the abundance and diversity of the Coorong's waterbird community.

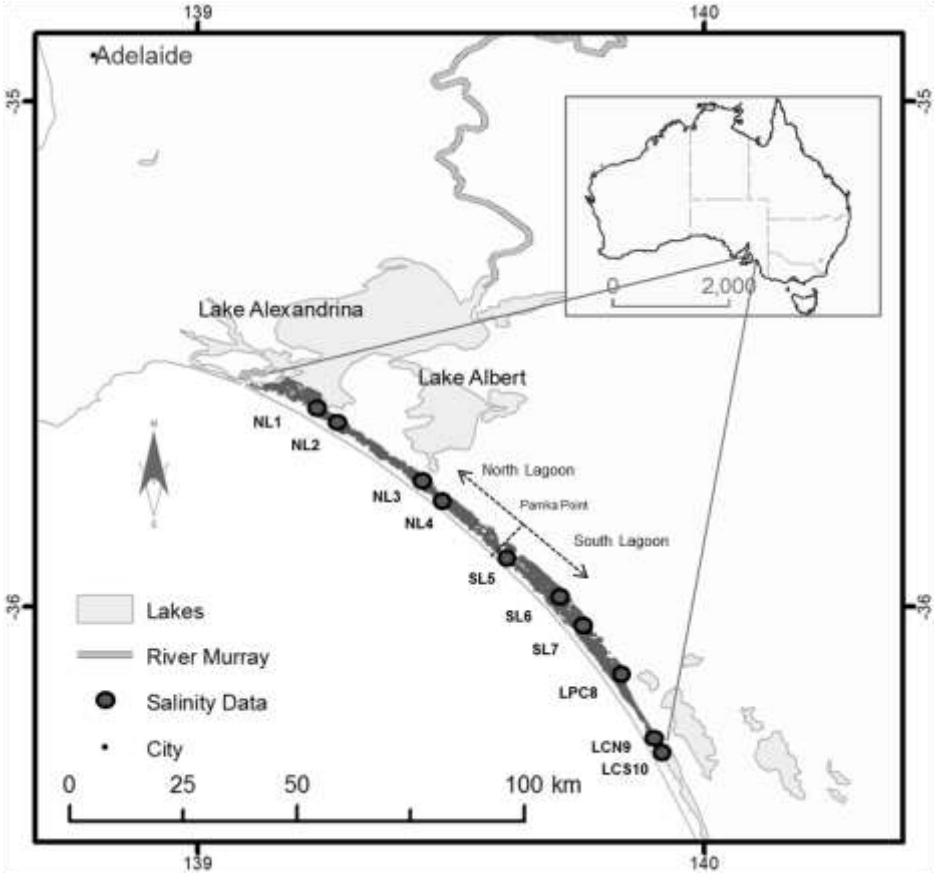

Figure 2.1: The location of study sites in the Coorong and neighbouring Lake Pipe Clay and Lake Cantara, South Australia (NL1 = Pelican Point; NL2 = Long Point; NL3 = Noonameena; NL4 = Seven mile Point; SL5 = Parnka Point; SL6 = Villa dei Yumpa; SL7 = Policeman Point; LPC8 = Lake Pipe Clay; LCN9 = Lake Cantara North and LCS10 = Lake Cantara South).

The climate of the region is Mediterranean with hot and dry summers (December-February) and cool and wet winters (June-August). The Coorong has an area of 467 km^2 and is comprised of two main lagoons (North and South) separated by a narrow channel at Parnka Point. Flow to the Coorong is controlled by five barrages that separate the North Lagoon of the Coorong from Lake Alexandrina, a large freshwater lake. At the northern end of the North Lagoon is the Murray Mouth. Due to evapoconcentration, salinity within the Coorong increases with distance from the Murray Mouth with fresh to saline conditions in the North Lagoon and saline to hyper-saline conditions in the South Lagoon. Due to the development of an extensive irrigation industry within the Murray-Darling Basin, inflows to the Coorong are now 40% of natural inflows and salinities within the Coorong have been increasing steadily (Brookes et al., 2009). At the time of this study salinities varied from 55 to 160 mS cm^{-1} from north to south and

typically the southern end of South Lagoon of the Coorong in summer was hyper-saline (over 220 mS cm^{-1}). Water levels are typically highest in July to September and fall to a minimum in February. South of the Coorong are Lake Pipe Clay (LPC8), Lake Cantara North (LCN9) and Lake Cantara South (LCS10, Figure 2.1), which have abundant *R. tuberosa* populations. The lakes are shallow (approximate water depth of ~0.5m in winter) and dry completely during summer. Salinity at Lake Pipe Clay ranges from 40 to 170 mS cm^{-1} intra-annually and salinities at lakes Cantara North and South range from 30 to 90 mS cm^{-1} intra-annually.

2.2.2 Field surveys

Field surveys were carried out in winter (August 2008), spring (October 2008), summer (February 2009) and autumn (May 2009). Surveys were conducted at seven sites spanning the salinity gradient that exists in the Coorong, as well as three ephemeral lakes at the southern end of the Coorong (Table 2.1). At each site, five 100 m transects were established 50 m apart, form the shoreline towards the centre of the waterbody (T1-T5 at NL1, T6-T10 at NL2, T11-T15 at NL3, T16-T20 at NL4, T21-T25 at SL5, T26-T30 at SL6, T31-T35 at SL7, T36-T40 at LPC8, T41-T45 at LCN9 and T46-T50 at LCS 10). At each end and in the middle of each transect, 3m x 1m quadrats were located which were further divided into three 1m x 1m cells, and visual assessments were taken. 45 replicates from each site were used. Within each cell measurements of water quality, sediment characteristics and the presence of various life stages of *R. tuberosa* were taken on each sampling occasion.

Table 2.1: Summary of co-ordinates, location and habitats of the field survey sites.

Site label	Co-ordinates	Location	Habitat description
NL1	S 35.59922 E 139.02795	Pelican Point	Permanent hypersaline
NL2	S 35.69341 E 139.16479	Long Point	Permanent hypersaline
NL3	S 36.77335 E 139.27347	Noonameena	Permanent hypersaline
NL4	S 35.79682 E 139.31752	Seven mile Point	Permanent hypersaline
SL5	S 35.90227 E 139.39922	Parnka Point	Permanent hypersaline
SL6	S 35.89423 E 139.43538	Villa dei Yumpa	Permanent hypersaline
SL7	S 36.05955 E 139.58617	Policeman Point	Permanent hypersaline
LPC8	S 36.13424 E 139.64636	Lake Pipe Clay	Ephemeral saline lake
LCN9	S 36.32808 E 139.75046	Lake Cantara North	Ephemeral saline lake
LCS10	S 36.32888 E 139.74950	Lake Cantara South	Ephemeral saline lake

Measurement of physico-chemical conditions

Water depth, specific electrical conductivity, turbidity, temperature, dissolved oxygen (DO) and pH were recorded using a calibrated TPS 90-FL series probe (TPS Pty. Ltd., Brisbane, Australia). Specific electrical conductivity was used as a measure of salinity. Due to insufficient water, water quality measurements could not be taken during summer and autumn at sites LPC8, LCN9 and LCS10.

Sediment samples were collected from each cell using a sampler corer with an internal diameter of 40 mm for the determination of sediment pH, sediment redox potential (SRP) and sediment organic content (SOC). A sediment corer (5cm ø) were pushed approximately 4 cm into the sediment and the sediment was extracted. Soil pH was measured immediately upon collection using a pH test-kit. Similarly, redox potential was measured immediately using a Hanna combination platinum oxidation-reduction potential electrode. Remaining sediments were stored in plastic bags and returned to the laboratory, where sediment organic content was measured as volatile solids using method 2540E described by Eaton et al. (2005). For these measurements, 25-50 g (fresh-weight) of sediment from the three subsamples from each core was analysed. The sediment organic content was calculated as the difference between combusted (550°C for 1 hour) and dry (103 to 105°C overnight prior to combustion) weights, expressed as a percentage of the dry-weight.

Presence of various life stages of R. tuberosa

In each cell the cover/abundance of shoots and flowers of *R. tuberosa* were assessed using the classification of Braun-Blanquet (Brock, 1979). Percent cover was expressed in six and five categories for shoots and flowering, respectively (Tables 2.2 and 2.3). Within each cell, additional sediment samples (5cm ø x 4cm depth) were collected for the determination of seed and turion density. Sediment (150 g) was sieved through a 600µm sieve and all the seeds and turions were counted.

Table 2.2: Shoot cover-abundance scale by the Braun-Blanquet method.

Braun-Blanquet scale	Range of cover (%)
5	76-100
4	51-75
3	26-50
2	6-25
1	≤ 5
0	absent

Table 2.3: Percentages of flowering by the Braun-Blanquet method.

Braun-Blanquet scale	Range of cover (%)
4	51-100
3	26-50
2	11-25
1	≤ 10
0	absent

2.2.3 Statistical analyses

To assess temporal and spatial differences in *R. tuberosa* abundance (shoot, flower, seed and turion) non-parametric two-way analysis of variances were conducted on non-normal data using GraphPad (version 5, GraphPad Software, San Diego, United States of America). For this analysis the effects of season, site and the interaction between season and site were assessed. Nonparametric Multiplicative Regressions (NPMR, (McCune and Mefford, 2004) were used to derive optimal fit models describing the response of *R. tuberosa* (shoots, flowers, seeds and turions) to the physico-chemical conditions (McCune and Mefford, 2004). For this analysis only data from winter and spring were used as physico-chemical conditions could not be measured during summer and autumn at LPC8, LCN9 and LCS10. This was conducted using Hyperniche Software (version 1.12, MjM Software, Oregon, United States of America), with a local mean multiplicative smoothing function with Gaussian weighting, with a standard deviation of a Gaussian weighting function (McCune and Mefford, 2004). Forty five replicates from each site on each sampling occasion were used. The relationships between physico-chemical conditions and *R. tuberosa* were initially assessed individually to determine the independent variable with the strongest relationship with *R. tuberosa* abundance (McCune and Mefford, 2004). To evaluate the importance of independent variables in the NPMR models, sensitivity analyses were conducted using Monte Carlo tests; with sensitivity value of 0.0 indicating that an independent variable has no detectable effect on the dependent variable, and 1.0 indicating that a change in the independent variable has an equal effect on the dependent variable.

2.3 Results

2.3.1 Salinity changes

There was a longitudinal salinity gradient in the Coorong (Figure 2.2). The lowest salinity occurred at the northern end, NL1 and increased towards the southern end, SL7, with salinity ranging from 55 mS cm^{-1} to over 160 mS cm^{-1} in autumn, winter and

spring. Salinities in the South Lagoon of the Coorong (SL5, SL6 and SL7) were approximately 220 mS cm^{-1} in summer, almost four times higher than seawater. Salinities at LCN9 and LCS10 were between approximately 30 and 90 mS cm^{-1} in autumn and spring, respectively. Salinity at LPC8 varied from 40 mS cm^{-1} in autumn to 170 mS cm^{-1} in spring (Figure 2.2).

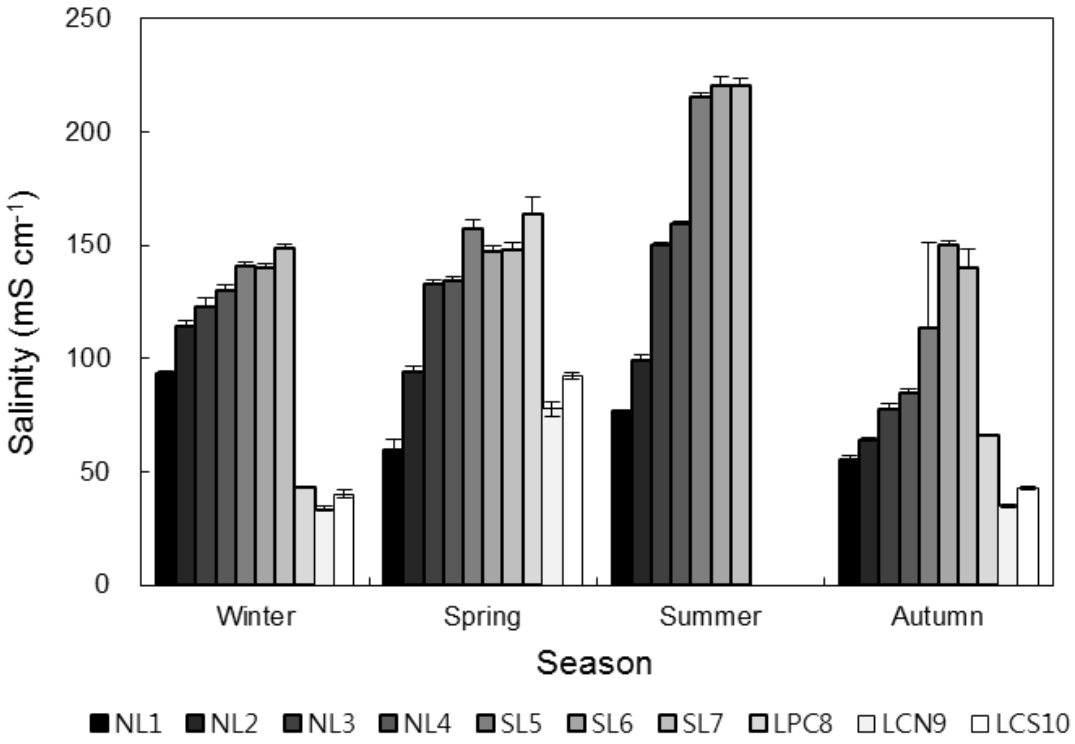

Figure 2.2: Salinities of the seven study sites in the Coorong (NL1-NL4 and SL5-SL7) and neighbouring Lake Pipe Clay (LPC8) and lakes Cantara North (LCN9) and South (LCS10). Mean (± S.D). n = 450.

2.3.2 Shoot abundance

There were strong spatial differences in shoot abundance of *R. tuberosa*. There were no shoots at NL1 and SL7 in any season and low abundances at NL2, SL5 and SL6 (Table 2.4). All other sites had relatively high abundances and showed seasonal differences with shoot abundance generally highest in winter and spring and lowest in summer. The contrasting seasonal responses between sites meant there was an interaction effect between site and season (Table 2.5).

Table 2.4: Average abundance of shoots, flowers, seeds and turions of *R. tuberosa* in the Coorong and the three associated lakes. Values are mean (± S.D).

Abundance	Site	Season			
		Winter	Spring	Summer	Autumn
Shoots	NL1	0	0	0	0
	NL2	1 (1)	1 (1)	1 (1)	1 (1)
	NL3	5 (1)	5 (0)	1 (1)	5 (0)
	NL4	3 (2)	3 (1)	1 (2)	2 (1)
	SL5	2 (2)	1 (1)	0	0
	SL6	0	2 (1)	0	0
	SL7	0	0	0	0
	LPC8	5 (0)	5 (0)	0	5 (0)
	LCN9	5 (0)	5 (0)	0	5 (0)
	LCS10	4 (0)	5 (0)	0	5 (0)
Flowers	NL1	0	0	0	0
	NL2	0	0	0	0
	NL3	0	1 (1)	0	0
	NL4	0	1 (1)	0	0
	SL5	0	0	0	0
	SL6	0	0	0	0
	SL7	0	0	0	0
	LPC8	0	0	0	0
	LCN9	0	3 (1)	0	0
	LCS10	0	2 (1)	0	0
Seeds (No. m^{-2})	NL1	0	0	0	0
	NL2	0	0	0	0
	NL3	375 (559)	625 (625)	0	125 (279)
	NL4	0	0	0	0
	SL5	3625 (2774)	3125 (2688)	2500 (1531)	2625 (3074)
	SL6	7000 (368)	750 (1118)	2250 (2364)	875 (1369)
	SL7	3875 (436)	4000 (7560)	0	1875 (1531)
	LPC8	375 (342)	375 (559)	500 (815)	1250 (884)
	LCN9	22250 (7888)	24250 (1172)	20500 (5667)	15500 (5735)
	LCS10	4125 (2236)	17125 (1144)	8875 (5047)	6625 (2781)
Turions (No. m^{-2})	NL1	0	0	0	0
	NL2	0	0	0	0
	NL3	0	3750 (4351)	4500 (6224)	0
	NL4	0	3125 (6988)	6500 (13511)	0
	SL5	0	0	0	0
	SL6	0	0	0	0
	SL7	0	0	0	0
	LPC8	0	12625 (2539)	6375 (3106)	0
	LCN9	0	0	0	0
	LCS10	0	4000 (2404)	0	0

R. tuberosa shoot and flower abundance were estimated as % cover. Shoot abundance categories were: 0, absent; 1, ≤ 5% cover; 2, 6-25% cover; 3, 26-50% cover; 4, 51-75% cover; 5, 76-100% cover. Flower abundance categories were: 0, absent; 1, ≤ 10% cover; 2, 11-25% cover; 3, 26-50% cover; 4: 51-100% cover.

Table 2.5: Results of two-way ANOVA analysis on abundance of shoots, flowers, seeds and turions of *R. tuberosa*.

Variable	Source	DF	F	*P* value
Shoots	Season	3	128.39	< 0.0001
	Site	9	116.43	< 0.0001
	Season*site	27	17.14	< 0.0001
Flowers	Season	3	87.50	< 0.0001
	Site	9	21.15	< 0.0001
	Season*site	27	21.15	< 0.0001
Seeds	Season	3	3.41	0.0198
	Site	9	44.61	< 0.0001
	Season*site	27	2.23	0.0017
Turions	Season	3	5.12	0.0023
	Site	9	6.19	< 0.0001
	Season*site	27	2.65	0.0002

The model relating *R. tuberosa* shoot abundance to physico-chemical conditions included salinity, water depth, turbidity, DO, water pH and sediment redox potential explained 73% of variation in *R. tuberosa* shoot abundance, with salinity having the highest sensitivity value (Table 2.6). The highest *R. tuberosa* shoot abundance was at salinities of 30-100 mS cm^{-1} with a maximum score of 5 (Figure 2.3). However, shoot abundance declined between salinities of 100 and 150 mS cm^{-1}, but increased again to salinity of 170 mS cm^{-1}. The influence of water depth had the second highest sensitivity value (Table 2.6). *R. tuberosa* shoots were present consistently in water depths of 0.2 to 0.6 m.

Table 2.6: Summary of nonparametric multiplicative regression models describing the relationships between physico-chemical conditions and the abundance of *R. tuberosa* life stages. DO, dissolved oxygen; SRP, sediment redox potential; SOC, sediment organic content; Tol, tolerance and Sen, sensitivity.

Physico-chemical parameter	Shoots x R^2 = 0.73		Flowers x R^2 = 0.76		Seeds x R^2 = 0.27		Turions x R^2 = 0.43	
	Tol.	Sen.	Tol.	Sen.	Tol.	Sen.	Tol.	Sen.
Salinity	7.135	0.523	7.135	0.415	14.27	0.398	7.135	0.429
Depth	8.600	0.224	8.600	0.174	0.825	0.536	82.95	0.090
Turbidity	190.4	0.031	95.20	0.079	190.4	0.081	142.8	0.055
Temperature								
DO	3.359	0.105						
Water pH	0.682	0.114	0.512	0.111			1.875	0.014
SRP	110.6	0.146						
SOC							12.63	0.027

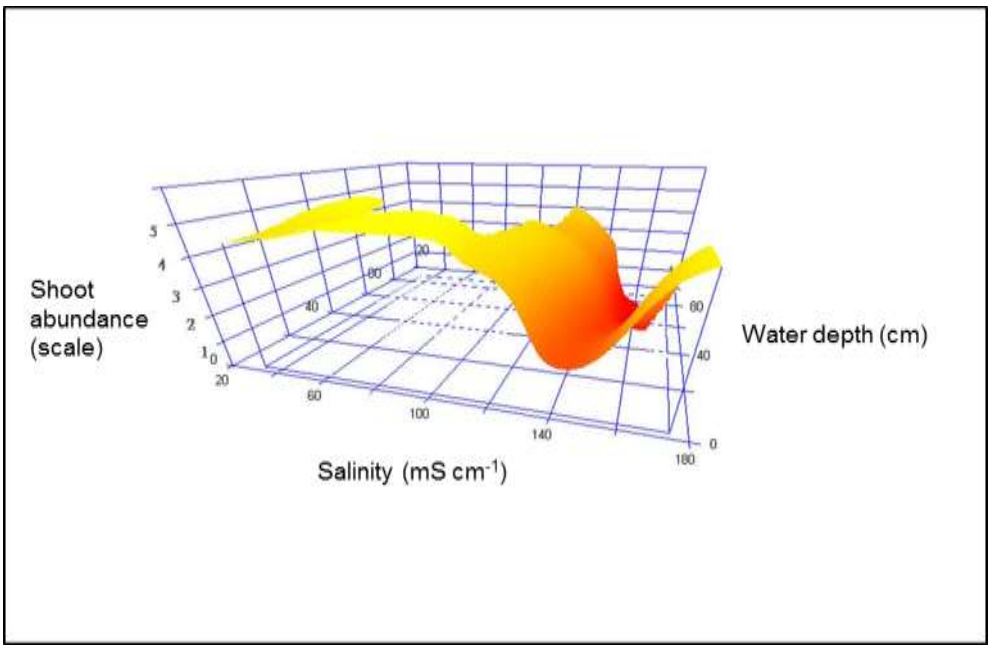

Figure 2.3: The relationship of shoot abundance of *R. tuberosa* to salinity and water depth. Abundance of shoots scales are 0, absent; 1, ≤ 5% cover; 2, 6-25% cover; 3, 26-50% cover; 4, 51-75% cover; 5, 76-100% cover.

2.3.3 Flower abundance

Flowering occurred in spring at NL3, NL4, LCN9 and LCS10 but did not occur at all at other sites (Table 2.4) and as such there was significant interaction effect of site and season (Table 2.5). Highest flowering occurred at LCN9, followed by LCS10, NL3 and NL4.

Seventy-six percent of the variation in flower abundance was explained by salinity, water depth, turbidity and water pH (Table 2.6). Flower abundance was most strongly influenced by salinity, followed by water depth (Figure 2.4 and Table 2.6). The highest flower abundance was observed when salinity was between approximately 70 and 90 mS cm^{-1} in water depths of 0.1 to 0.4 m. Little flowering was detected at salinities above 100 mS cm^{-1} and below 70 mS cm^{-1}.

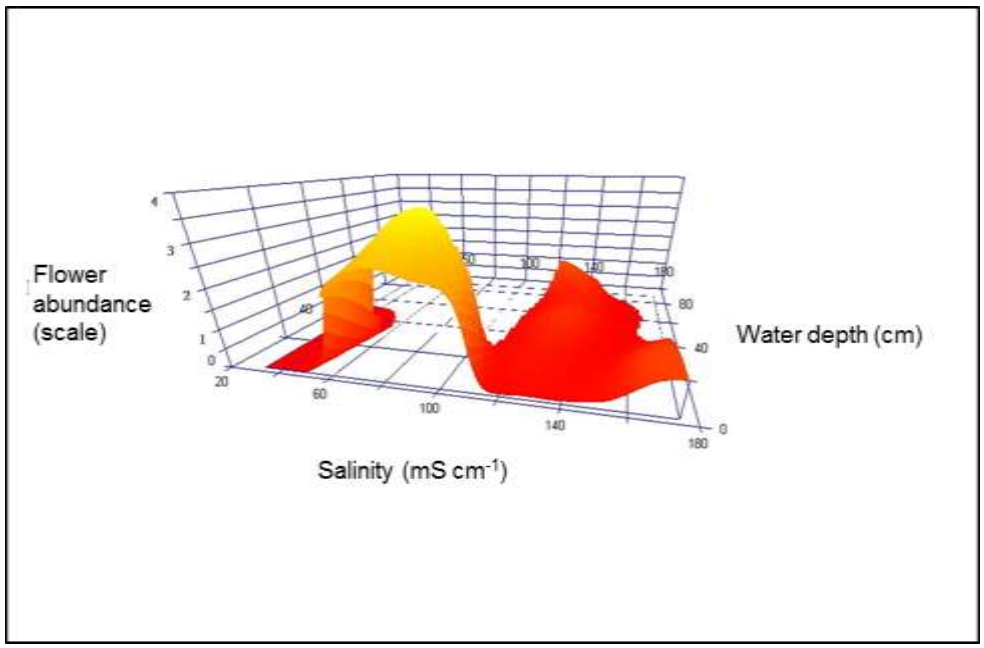

Figure 2.4: The relationship of flower abundance of *R. tuberosa* to salinity and water depth. Abundance of flowers scales are 0, absent; 1, ≤ 10% cover; 2, 11-25% cover; 3, 26-50% cover; 4: 51-100% cover.

2.3.4 Seed abundance

Seeds of *R. tuberosa* were consistently more abundant at sites LCN9 and LCS10 (Table 2.4) in all seasons. There were relatively small numbers of seeds present in sediments at sites NL3, SL5, SL6, SL7 and LPC8. Where seed were present, seed abundance tended to be highest in Spring, but no seeds were detected at sites NL1, NL2 and NL4 in all seasons and as such there was an interaction effect of site and season (Table 2.5).

The model relating seed density to physico-chemical conditions explained 27% of the variation in seed abundance (Table 2.6). Seed abundance had strongest (inverse) relationships with salinity and water depth (Table 2.6 and Figure 2.5). The highest seed density was maintained at salinities of 40 to 90 mS cm^{-1} in water depths of 0.1 to 0.4 m.

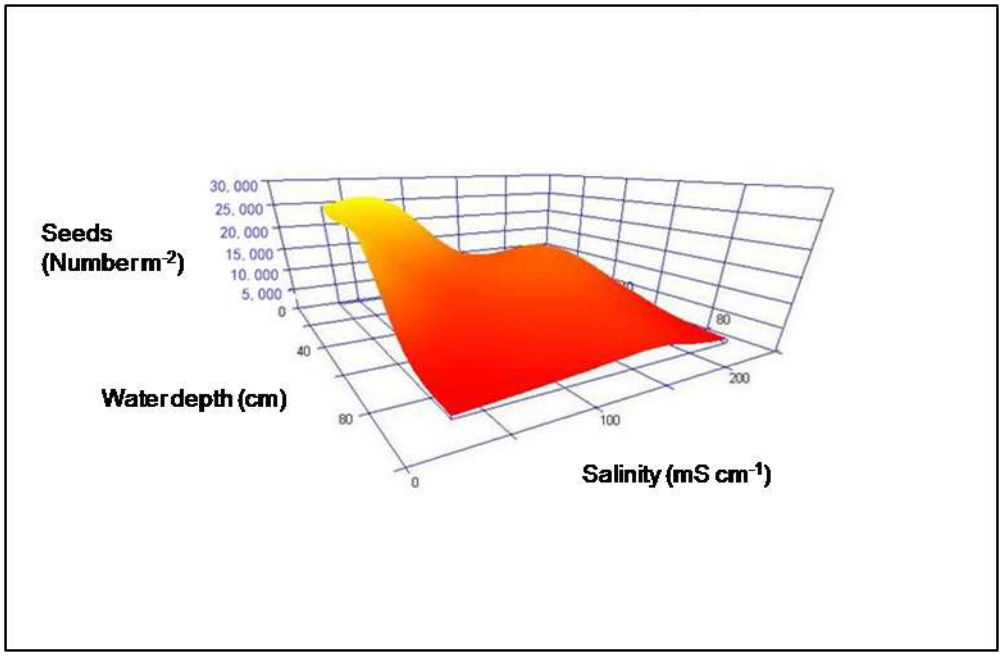

Figure 2.5: The relationship of *R. tuberosa* seed density to salinity and water depth. Mean number of seeds per m^2.

2.3.5 Turion abundance

Turions were not present in all seasons at numerous sites but were present in spring and summer at NL3, NL4, LPC8 and LCS10 (Table 2.4) and as such there was a significant interaction effect of site and season (Table 2.5). The highest numbers of *R. tuberosa* turions were detected at LPC8 in spring, followed by NL4 during summer.

The model relating turion density to physico-chemical conditions explained 43% of variation in turion density, with salinity and water depth having the greatest influence (Table 2.6). In comparison to seeds, *R. tuberosa* turions had a positive relationship with increasing salinity (Figure 2.6), with turions most likely to occur in areas with salinity above 100 mS cm^{-1} (Figure 2.6), and the highest turion density observed when salinity was approximately 170 mS cm^{-1}. Water depth had detectable effect on turion density, with highest densities in water depths of 0.1 to 0.4 m.

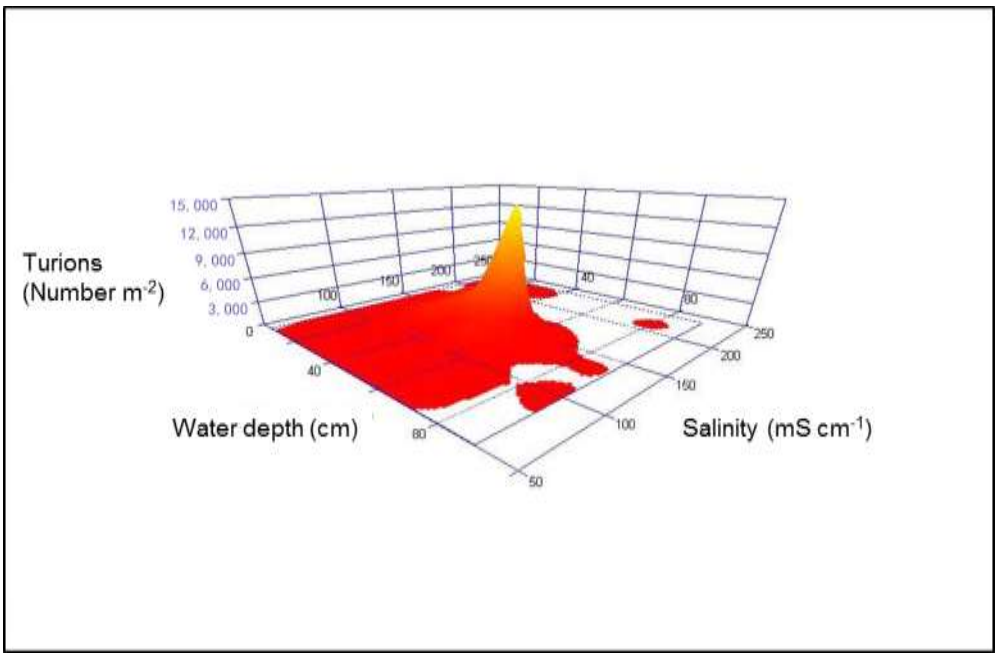

Figure 2.6: The relationship of *R. tuberosa* turion density to salinity and water depth. Mean number of turions per m^2.

2.4 Discussion

Macrophytes have adapted to a wide range of aquatic environments and have developed different physiological mechanisms to varying environmental habitats (Hart et al., 1991). For example, environmental factors, including salinity, turbidity and water depth drove changes in the abundance of *R. megacarpa* (Carruthers et al., 1999). Santamaría et al. (1995) reported temperature as a major role influencing flowering of *R. drepanensis* Tineo. Nejrup and Pedersen (2008) also reported water temperature that exceeded 25°C for long periods caused the extensive disappearance of eelgrass. Numerous studies have identified the importance of salinity in determining the distribution of aquatic macrophytes (Brock et al., 2005; Sim et al., 2006; Song et al., 2009; Kim et al., 2012), although the mechanisms are not always simple. For example, Song et al. (2009) showed that under high salinities the osmotic role of NO_3^- was more important than Cl^- for a euhalophyte, *Suaeda physophora* compared to a xerophyte, *Haloxylon persicum*.

This study has shown that *R. tuberosa* was able to survive in a broad range of physico-chemical conditions, thought to be result of its adaptive life cycle. In particular, *R. tuberosa* is able to reproduce both sexually and vegetatively. Salinity and water depth were identified as the principal physico-chemical conditions driving changes in the distribution and abundance of various life stages of *R. tuberosa* in the Coorong region. Salinity appeared to be particularly important with the highest abundance of *R. tuberosa*

found at salinities of 30-100 mS cm^{-1} and declined sharply in response to increasing salinity from 100 to 150 mS cm^{-1}. These results correspond with the germination thresholds for *R. tuberosa* (Kim et al., 2013). However, the modeled response of *R. tuberosa* shoot abundance was not a simple unimodal response, but instead bimodal. The shoot abundance increased again to salinity of 170 mS cm^{-1}. As explained below, it is believed that this response was a result of differing life-cycle responses of *R. tuberosa* in a permanent water body with a broad salinity gradient (the Coorong, salinities of 55 to 160 mS cm^{-1}), an ephemeral water body with a moderate spring salinity (Lake Cantara, salinities of 30 to 90 mS cm^{-1}) and an ephemeral water body with a high spring salinity (Lake Pipe Clay, salinities of 40 to 170 mS cm^{-1}).

2.4.1 How *Ruppia tuberosa*'s life cycle is adapted to different habitats

A permanent water body (the Coorong)

R. tuberosa was absent in both the Northern and Southern extremes of Coorong (55 mS cm^{-1} to 160 mS cm^{-1}), with the distribution thought to be a result of two separate phenomenon: sub-optimal physico-chemical conditions in the south and the ongoing but incomplete colonisation in the north. There were no *R. tuberosa* shoots at the northern end of the North Lagoon (NL1, approximately 55 mS cm^{-1}) despite apparently ideal salinity conditions, as indicated by the modeled response and observations within the ephemeral lakes, which experienced comparable salinities at times when *R. tuberosa* was present. Relatively low abundance of *R. tuberosa* shoots at NL2 is most likely result of recent colonisation, not a reflection of sub-optimal physico-chemical conditions. The colonisation may be limited by propagule availability, as propagule dispersal is an important determinant of macrophyte abundance (Tilman, 1993). *R. tuberosa* has only recently started to colonise the northern area of the North Lagoon as previously this was dominated by *R. megacarpa*, while *R. tuberosa* dominated in the South Lagoon (Brock, 1979). *R. tuberosa* restriction to the South Lagoon may have been a result of being outcompeted by *R. megacarpa* in the North Lagoon as salt-tolerant species are often out competed by salt-sensitive species in less stressful environments (Katembe et al., 1998). In fact, since the distribution of *R. megacarpa* has disappeared in the North Lagoon, the distribution of *R. tuberosa* has drifted northwards (Paton and Rogers, 2007).

In contrast to NL1, the absence of shoots in the southern end (SL7) is most likely due to ion toxicity. Similarly, both Carruthers et al. (1999) and Sim et al. (2006) reported that

the abundance of *R. megacarpa* and *R. polycarpa* was negatively affected by salinity. In general, plant's distribution is associated with success of all stages of its life cycle (Carruthers et al., 1999): germination, establishment, growth and reproduction Here, highly saline conditions were experienced throughout the year and so it appears that salinities were always too high for *R. tuberosa* to establish and complete its life cycle (Santamaría and Hootsmans, 1996; Carruthers et al., 1999). Indeed, salinities at SL7 exceeded salinity thresholds for seed and turion germination for *R. tuberosa* (Kim et al., 2013). It was also apparent that the salinity levels in parts of the South Lagoon exceeded thresholds for flower production. The negative influence of salinity on flower abundance of *R. tuberosa* is in agreement with Sim et al. (2006) for *R. polycarpa*, *Lamprothamnium macropogon* (A. Braun) Ophel and *Lamprothamnium succinctum* (A. Braun in Ascherson) Wood. The observed effect of salinity on flowering resulted in an apparent decrease in sexual reproduction (seeds) and an increase in asexual reproduction (turions) in the Coorong. Over time, this would result in the depletion of the seedbank in areas that have suitable salinity levels for a portion of the year to allow germination, but unsuitable salinity levels for the whole year to allow flowering and sexual reproduction. The small numbers of seed present in the South Lagoon of the Coorong suggest that seed production was minimal due to elevated salinities, with *R. tuberosa* instead relying on turion production for reproduction. However, turion cell death by ion toxicity occurs after immersion in high salinity and turions are more vulnerable to extreme salinities than seeds (Kim et al., 2013). This means that turion viability will be lost on exposure to elevated salinities during summer.

Water depth was shown to be the second most important factor in the abundance of *R. tuberosa* in the Coorong. These findings contrast with those of Carruthers et al. (1999) who found that water depth was a primary factor controlling the distribution of *R. megacarpa* in Wilson Inlet, Western Australia. However, in that case salinities were much lower than for the Coorong. For *R. tuberosa* in the Coorong shoots were in highest abundances between 0.2 and 0.6 m and flowers, seeds and turions between 0.1 and 0.4 m. Wind driven turbulence at the sediment surface and dislodgement of shoots, as well as periodic desiccation associated with wind seiching may limit the abundance of macrophytes in shallow areas (Keddy, 1983). However, given these processes are likely to have been relatively consistent between sites of the Coorong, it is unlikely that they contributed to the observed distribution and abundance along the length of the Coorong, but instead to the distribution and abundance along transects. The reduced

abundances of *R. tuberosa* at depths >0.6 m suggest light limitation and that *R. tuberosa* is a high light adapted species although it may be associated with the relatively high turbidity of the water. Carruthers et al. (1999) found that the abundance of *R. megacarpa* was inversely related turbidity and turbidity has previously been considered important in limiting depth distribution of seagrasses (Verhoeven, 1980). Similarly, *Ruppia cirrhosa* (Petagna) Grande grew to only 34% the biomass of an unshaded control when subjected to 75% shading for 3 months (Verhoeven, 1980). Orth and Moore (1988) found that decreased abundance of *R. maritima* L. as water depth increased to 1 m, suggesting reduced light availability may be the principal causative factor affecting the distribution in the Chesapeake Bay, USA. They considered *R. maritima* as a high-light adapted species and *Z. marina* as a low-light adapted species, suggesting these two varied photosynthetic capacities allow *R. maritima* to dominate in the shallow (water depth of ~0.3 m) high-light intensity, while *Z. marina* dominate in the deep (water depths of 0.8 m to 1.2 m) low-light intensity.

An ephemeral water body with a high spring salinity (Lake Pipe Clay)

Salinity did not affect the distribution and abundance of shoots of *R. tuberosa* in Lake Pipe Clay (LPC8), an ephemeral water body with salinities ranging from 40 to 160 mS cm^{-1} at the time of this study. Although the highest abundance of *R. tuberosa* were observed at 40 mS cm^{-1}, the high abundance at salinities of 160 to 170 mS cm^{-1} were a result of the abundances observed at Lake Pipe Clay during spring. The elevated salinities at this time were a result of evapoconcentration as water levels fell during Spring in Lake Pipe Clay appear to be favourable for germination in autumn (Kim, 2013) and results suggested that this was also the case for shoot growth during winter and spring. However, no flowering and relatively low seed densities were detected in this site. The high salinities during spring may affect the flowering of *R. tuberosa*. Sim et al. (2006) also reported limited flower production as salinity increased and Santamaría et al. (1995) also found tissue ion concentration limited flower production of *R. drepanensis*. The findings of this study indicate a decrease in sexual reproduction capacity of *R. tuberosa* at high salinities. In contrast, the highest turion density was maintained in LPC8 at salinities of 150-170 mS cm^{-1}. This suggests the high shoot abundance of Lake Pipe Clay is maintained through the production and germination of turions and that shoot growth and turion production have extremely wide tolerance to salinity fluctuations (Vollebergh and Congdon, 1986; Sim et al., 2006). Brock & Lane (1983) found *R. tuberosa* occurs at salinities of 26-215 mS cm^{-1}. Brock & Lane (1983)

also recorded that *R. polycarpa* and *R. megacarpa* occurred in seasonally ephemeral ecosystems at salinities of 2.2-170 mS cm^{-1} and of 17-200 mS cm^{-1}. In contrast to shoot and turion production, flowering, seed production and germination and turion germination are the most sensitive (salinity) components of the life cycle of *R. tuberosa*.

An ephemeral water body with a moderate spring salinity (Lake Cantara)

Like Lake Pipe Clay, salinity did not affect the distribution and abundance of *R. tuberosa* shoots in Lake Cantara, an ephemeral water body with moderate spring salinities ranging from 30 to 90 mS cm^{-1} at the time of this study. However, unlike other sites, physico-chemical conditions in Lake Cantara were not only favourable for the establishment and development of *R. tuberosa* shoots, but also for flower production and seed production and germination. The highest flowering of *R. tuberosa* was observed in Lake Cantara at salinities of 70-90 mS cm^{-1}. This agrees with the findings of Sim et al. (2006), who reported *R. polycarpa* flowering at salinities of 60-74 mS cm^{-1}. Water depth appeared to be an important factor affecting flowering of *R. tuberosa*, but this is not clear due to restricted limits of dataset. The highest flowering density observed at shallow depths of 0.1-0.2 m was likely associated with highest flowering in Lake Cantara (suitable salinity) in spring, when water levels are lower due to evaporation. In contrast to the Coorong and Lake Pipe Clay, the *R. tuberosa* population of Lake Cantara was associated with seed production and germination. The availability of seed bank is well known as the important factor for distribution and abundance of halophytes (Santamaría and Hootsmans, 1996). Seeds provide important functions for submerged macrophytes to continue persisting populations, colonizing populations into new environments (Jarvis and Moore, 2008). Santamaría and Hootsmans (1996) reported seed bank density is the main controlling factor in the development of *R. drepanensis*, and they also found a positive relationship between biomass and seed yield, which agrees with the findings of Santamaría et al. (1995), who found flower production is positively correlated with biomass. The importance of seed production for *R. tuberosa* populations is supported by the fact that the highest shoot abundances of *R. tuberosa* were observed at salinities of 30-90 mS cm^{-1} with highest germination rates at salinities of 0-90 mS cm^{-1} (Kim et al., 2013). Overall, it appears that the intra-annual salinities of 30 to 90 mS cm^{-1} in Lake Cantara may be optimal salinities for all life stages of *R. tuberosa*.

2.4.2 Management implication for the restoration of *Ruppia*

From this study it is concluded that while optimal conditions of *R. tuberosa* are shallow, moderate-high salinity levels, *R. tuberosa* has a number of mechanisms that allows it to survive in a range of conditions. Despite its high salinity tolerance it is apparent that extremely high and constant salinity levels, lead to reduced flower and seed production and seed and turion germination and thus reduced shoot abundance. In ephemeral systems provided adequate salinities for germination (seed and turion) and growth during autumn and winter *R. tuberosa* is able to withstand elevated spring salinities through the either sexual production or the production of turions, depending on salinity levels. Any increases in salinities during autumn and winter are likely to have negative impacts on *R. tuberosa*. In particular, *R. tuberosa* populations that rely heavily on turion production and germination are likely to be particularly vulnerable because of the low salinity tolerance of turion germination to salinity in comparison to seeds (Kim et al., 2013). The loss of *R. tuberosa* from parts of the Southern Lagoon of the Coorong and the general northward distribution shift is of concern. This shift is associated with reduced riverine inflows and increasing salinity concentrations, which is also a likely explanation for the complete absence of *R. megacarpa* in the North Lagoon. It is clear that if unsuitable salinity levels continue then habitat for *R. tuberosa* may be restricted or may disappear completely, resulting in the complete loss macrophytes in the Coorong ecosystem. This would have negative implications for the abundance of certain migratory shorebirds (Paton and Rogers, 2007). This study suggests that salinity, which has been found to be the primary driving environmental factor controlling the distribution of *R. tuberosa*, should be reduced to below 100 mS cm^{-1} in the southern end of the Coorong, to ensure flower production and successful *R. tuberosa* life cycle. This may be achieved by adequate inflows of fresh water to the Coorong, promoting germination and establishment of the species.

Chapter 3 The effect of salinity on the germination of *Ruppia tuberosa* and *Ruppia megacarpa* and implications for the Coorong

3.1 Introduction

Salinisation is a major problem, affecting over 800 million hectares of agricultural land throughout the world (Song et al., 2008). It is also a major threat to aquatic biota, including aquatic plants, interfering with the physiology of various life stages and affecting their distribution (Brock et al., 2005; Sim et al., 2006). It is widely recognised that salt stress at the germination stage is critical for the abundance and distribution aquatic macrophyte communities (Khan and Ungar, 1996; Katembe et al., 1998; Tessier et al., 2000; Jarvis and Moore, 2008; Elsey-Quirk et al., 2009). Salinity reduces germination of both halophytes (Khan and Ungar, 1996) and glycophytes (Brock et al., 2005). Increased salinity inhibits water uptake by seeds (Katembe et al., 1998). This leads to a high salinity-induced dormancy (Boorman, 1968; Woodell, 1985). Water movement across cell membranes into seed cells is determined by the difference between the osmotic potential of the seed and that of the external medium (Katembe et al., 1998). High sodium concentrations in seed cells may induce changes in protein activity because ions affect the hydration of nuclear contents, causing an inhibition of enzyme activity (Katembe et al., 1998), leading to a high salinity-induced dormancy (Woodell, 1985).

In contrast Keiffer and Ungar (1997) reported a positive germination effect due to the relaxation of osmotic stress following salt stress. Halophytes, including *Arthrocnemum macrostachyum* (Moric) Moris and *Halocnemum strobilaceum* (Pallas) M. Bieb., have enhanced germination rates when salinity and osmotic stress are reduced after exposure to hypersaline conditions (Pujol et al., 2000). They suggest that the movement of water across cell membranes into the embryo leads to breakage of the seed coat and enhances metabolic rates and germination (Pujol et al., 2000).

Ruppia spp. are submerged halophytes that are an important components of many saline aquatic ecosystems throughout the world, including Australian estuarine and coastal lagoon food-webs (Brock, 1979, 1981b; Nicol, 2005; Paton, 2005). They are found in

brackish to hypersaline habitats in many continents (Verhoeven, 1979). *Ruppia cirrhosa* (Petagna) Grande occurs at salinities of 3-55 mS cm^{-1}, and *Ruppia maritima* L. occur in salinities of 0.8-12.5 mS cm^{-1} (Verhoeven, 1979). In Australia, *Ruppia tuberosa* J. Davis and Tomlinson occurs over a wide range of salinities of 20-359 mS cm^{-1}, and reproduces both sexually and vegetatively via seeds and turions. *Ruppia megacarpa* R. Mason occurs in salinities of 8-72 mS cm^{-1} (Brock, 1982b) and reproduces sexually. However, the distribution of *Ruppia* species has declined considerably throughout the world due to human impacts, such as water extractions (Brookes et al., 2009). For example, *R. tuberosa* and *R. megacarpa* were once a keystone species in the Coorong (Brock, 1981b), but elevated salinities resulting from reduce riverine inputs have led to a rapid decline of *R. tuberosa* and complete loss of *R. megacarpa* (Brookes et al., 2009). Brock (1982b) reported negative effect of increasing salinity on the seed germination of *R. megacarpa* and *R. tuberosa*. Sim et al. (2006) also demonstrated that an increase in salinity led to a decrease in the number, and an increase in lag time, of germination of *Ruppia polycarpa* R. Mason.

While it is known that salinity affects germination of *R. tuberosa* and *R. megacarpa* (Brock, 1979), it is unknown whether the form of the relationship between salinity and germination is a simple linear response or whether there is a critical threshold salinity above which neither seeds and/or turions will not germinate. Furthermore, it is unknown whether exposure to high salinities is irreversible or whether seeds retain their ability to germinate when transferred to lower salinity. If seed viability is lost on exposure to critical salinities exposure to freshwater inputs will not enhance germination of an existing seed bank. Here we investigated the ability of seeds and turions of *R. tuberosa* and seeds of *R. megacarpa* to germinate in responses to salinity and apply the knowledge to the Coorong to examine the relationship between riverine inputs and *Ruppia* germination.

3.2 Material and methods

3.2.1 Site description

The Coorong, located in South Australia, is a coastal lagoon confined by a coastal barrier dune extending southeast from the mouth of the River Murray for approximately 140 km (Figure 3.1). It has an area of 467 km^2 and is comprised of two main lagoons (North and South) separated by a narrow channel at Parnka Point (PA). The Coorong is part of one of the most important wetland systems in Australia (Ramsar Convention on

Wetlands: http://www.ramsar.org/), consisting of ocean beach, the mouth of the River Murray, the estuary and two freshwater lakes, Albert and Alexandrina. Its listing as a Ramsar wetland was largely due to its high biological productivity and diverse waterbird community, including migratory birds.

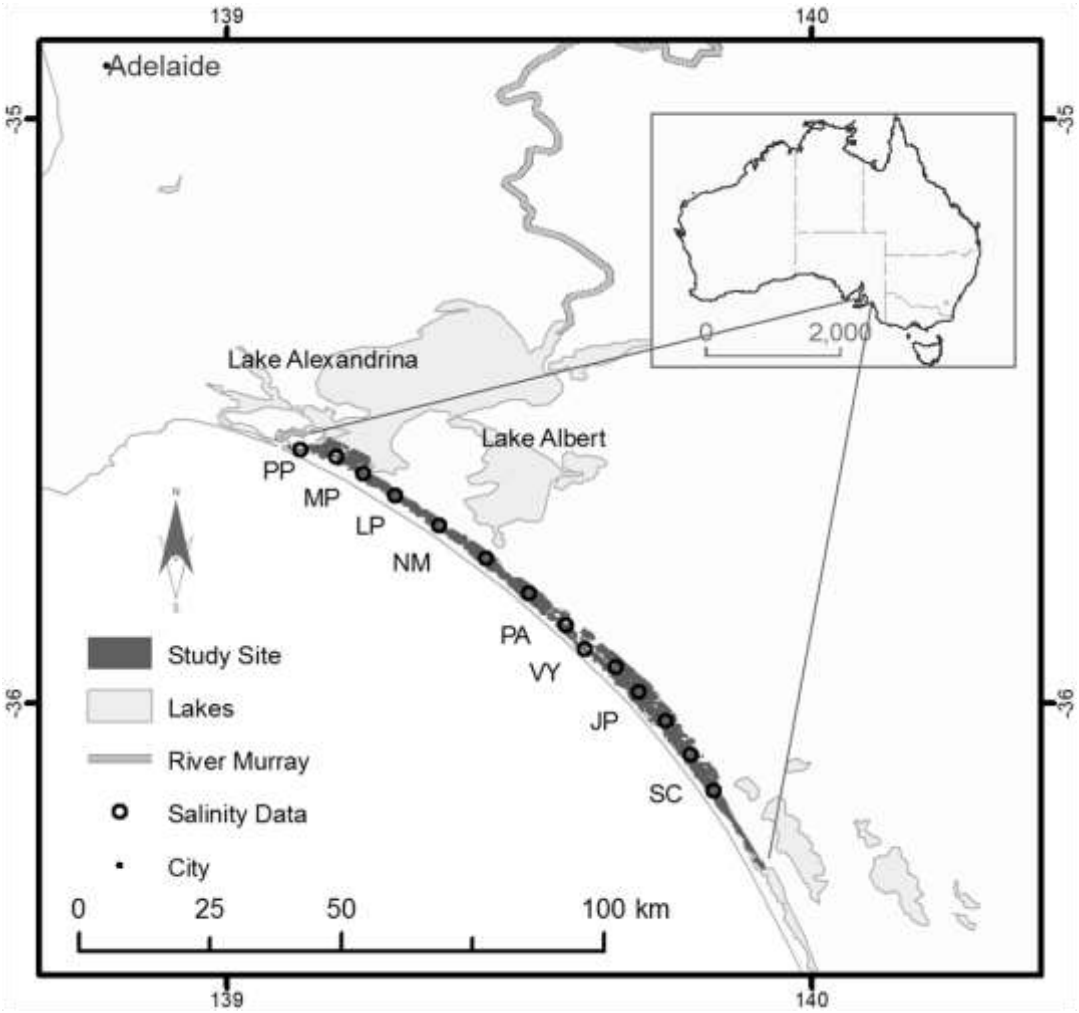

Figure 3.1: Location of the Coorong, South Australia (PP = Pelican Point; MP = Mark Point; LP = Long Point; NM = Noonameena; PA = Parnka Point; VY = Villa dei Yumpa; JP = Jack Point and SC = Salt Creek).

Typically, the Coorong has a strong salinity gradient, ranging from fresh to brackish in the North Lagoon depending upon inflows from Lake Alexandrina, to hypersaline in the South Lagoon due to evaporation and poor exchange with freshwater from Lake Alexandrina that has been separated by a series of barrages, constructed in the late 1930s. Flows to the Coorong can be controlled by opening gates in the barrages. Reduced freshwater inflows resulting from water resource development have increased salinity levels (Brookes et al., 2009). Water resource development combined with a regional drought resulted in no inflows from Lake Alexandrina between 2006 and 2010,

which consequently increased salinity to extreme hyper-salinisation (over 230 mS cm^{-1}) throughout the South Lagoon.

3.2.2 Seed and turion collection and storage

During May 2008, *R. tuberosa* seeds and turions were collected from the Coorong (35°77′S, 139°27′E) and the neighbouring Lake Cantara (36°32′S, 139°74′E). *R. megacarpa* seeds were collected from a small wetland near the township of Keith (36°11′S, 140°37′E), South Australia in February 2010. The seeds and turions were sorted using a 600 µm sieve, cleaned and stored in a plastic bag.

3.2.3 Experimental design and procedure

Effects of salinity and sediment on germination

Germination experiments were conducted in a culture room from October 2008 to March 2009 for *R. tuberosa* and from May to July 2010 for *R. megacarpa*. Germination experiments without sediment were carried out in 90 mm diameter plastic petri dishes on Whatman's grade No. 1 filter paper. This paper was treated with 10 mL of solutions of various salinities made from Instant Ocean sea salt (Aquarium systems Inc., France): 0, 25, 45, 65, 85, 105, 125, 145, 165, 185, 200, 220 and 240 mS cm^{-1}. These values are beyond the range normally associated with *R. tuberosa* and *R. megacarpa*. Ten replicates of twenty seeds for both species and four replicates of ten turions for *R. tuberosa* were used for each salinity treatment. Seeds and turions were placed on the filter paper and dishes were covered to prevent evaporation. Dishes were randomly placed on four shelves, in the culture room. The dishes were exposed to alternating 12 hour light periods (white fluorescent lamps, 60 µmol photons m^{-2} s^{-1}) at 18°C and 12 hour dark periods at 10°C. The number of seeds germinated on each dish was recorded daily to obtain the final percentage germination (G_F) and mean time to germination (T_G) over a period of 60 days. Seeds and turions were considered to have germinated when the emerging radicle length was at least 1 mm (Song et al., 2008). The viabilities of seed and turion were taken into account with the final percentage germination.

Since *R. megacarpa* did not germinate in the saline solutions, a similar experiment was conducted but with the presence of sediment. Sediment (sandy loam) was collected from the Coorong (35°46′S, 139°16′E) in June 2011, sieved to remove seeds and stored

at 4°C. The experiment was run in the culture room from September 2011 to October 2011. Germination experiments with sediment were carried out using plastic trays (15 x 20 x 7 cm), containing 2 cm of sediment (~20% organic content), overlain with 500 mL of saline solution. Experimental salinities were 0, 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 mS cm^{-1}. Three replicates of twenty seeds were used for each salinity treatment. Trays were randomly placed in the culture room, with a 12 hr/12hr light-dark cycle, a light intensity of 60 µmol photons m^{-2} s^{-1} and temperature cycle of 18°C/ 10°C The number of seeds germinated on each dish was recorded daily to obtain G_F and T_G over the 60 day period.

Effect of transfer from high to low salinity

After 60 days, un-germinated *R. tuberosa* seeds and turions from the salinity treatments greater than 125 and 165 mS cm^{-1} were placed in lower salinity treatments to test the effect of the exposure to elevated salinities on the subsequent capacity for germination at lower salinities. These experiments were carried out in 90 mm diameter plastic petri dishes on Whatman's grade No. 1 filter paper. The paper was treated with 10 mL of saline solution. For seeds, five replicates of ten seeds from each of the original treatments (145, 165, 185, 200, 220 and 240 mS cm^{-1}; hence forth referred to as pre-treatments) were exposed to post-treatment salinities of 0, 45, 85 and 125 mS cm^{-1}. For *R. tuberosa* turions, four replicates of ten turions from each pre-treatment (185, 200, 220 and 240 mS cm^{-1}) were used for post-treatment salinities of 0, 45, 85, 125 and 165 mS cm^{-1}. These dishes were placed in the culture room, with a 12 hr/12hr light-dark cycle, a light intensity of 60 µmol photons m^{-2} s^{-1} and temperature cycle of of 18°C/ 10°C. The number of seeds germinated on each dish was recorded daily to obtain G_F and T_G over a period of 60 days.

Effects of salinity on imbibition and Na concentrations of seeds

Salinity effects on imbibition and Na concentrations of *R. tuberosa* seeds were examined by comparing three constant (0, 60 and 180 mS cm^{-1}) salinities to one fluctuating salinity regime (initial exposure to 180 mS cm^{-1} followed by exposure to 60 mS cm^{-1}). The values of 60 mS cm^{-1} and 180 mS cm^{-1} were selected as intermediate values where germination occurred previously or did not previously occur (i.e. at 180 mS cm^{-1}). The experiment was carried out in 90 mm diameter plastic petri dishes on

Whatman's grade No. 1 filter paper. Dishes were treated with 30 mL of salinity treatments of 0, 60, 180 and 180-60 mS cm^{-1}. Three replicates of one hundreds seeds were used for each salinity treatment. Dishes were randomly placed in the culture room, with a 12 hr/12hr light-dark cycle, a light intensity of 60 µmol photons m^{-2} s^{-1} and temperature cycle of 18°C/ 10°C. The experiment ran for four weeks with seeds of fluctuating treatment exposed to 180 mS cm^{-1} for two weeks and then 60 mS cm^{-1} for two weeks. The number of seeds germinated on each dish was recorded daily for four weeks. Every week, including at the start of the experiment, twenty seeds from each treatment were randomly selected, weighed (fresh-weight) and placed back into each treatment. After two and four weeks of incubation, ten seeds were randomly selected from each replicate and the sodium (Na^+) concentration of seeds and seed embryos were determined. For this, five seeds from each replicate had their embryos removed from their seed coat. The samples were dried at 60°C for 2 days and Na^+ concentrations were determined by flame photometry following extraction in 8 mL of 0.1 mM nitric acid in a boiling water bath for 30 min.

3.2.4 propagule (seed and turion) viability test

Twenty *R. tuberosa* seeds and turions and *R. megacarpa* seeds were tested for viability using the tetrazolium staining method (Hendry and Grime, 1993). Seed embryos were removed from their seed coats and soaked in a 1% tetrazolium chloride solution for 2 hours in the dark before examination with a dissecting scope. Positive tests occurred when the embryo stained red (Hendry and Grime, 1993). *R. tuberosa* and *R. megacarpa* seeds had 70 ± 3% (Figure 3.2) and 92 ± 2% (Figure 3.3) viabilities, respectively, while turions of *R. tuberosa* had 100 % viability.

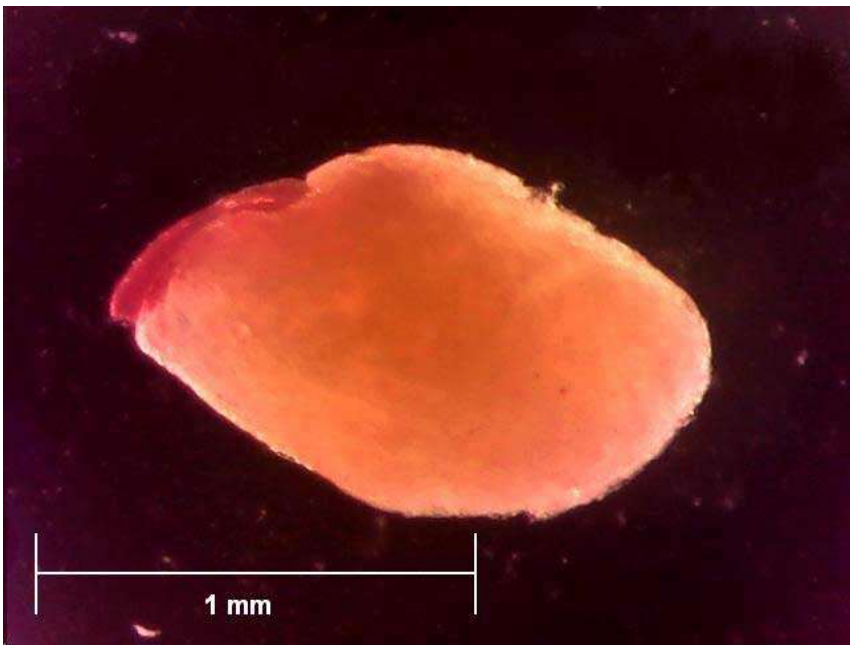

Figure 3.2: Image of *Ruppia tuberosa* seed without seed coat after viability test.

Figure 3.3: Image of of *Ruppia megacarpa* seed without seed coat after viability test.

3.2.5 Statistical analyses

Data were analysed using generalized lineal models (GLMs) and one-way analysis of covariance (ANCOVA) with PASW statistics and JMP IN version 404 (SAS Institute Inc., 1989-2001). All data were tested for normality and homogeneity with the Shapiro-Wilk and Bartlett's tests, respectively. On occasions $\log_{10}$ or Arcsine square-root transforms were required. The effect of salinity as a continuous independent variable on

G_F and T_G was performed using GLMs (Wald test). A poisson distribution with log-link was applied. ANCOVA was used to determine whether the presence/absence of sediment influenced G_F and T_G of *R. tuberosa* seeds under the salinity regime. To test the effect of the exposure to elevated salinities on the subsequent capacity for germination at lower salinities, a two-factor model was performed using GLMs. The model included two fixed independent variables (pre-treatment and post-treatment) and the interaction between them. A two-way ANOVA was used to test the effects of salinity and duration of salinity exposure on fresh-weight of *R. tuberosa* seeds. To test the effects of seed weight on the germination of *R. tuberosa* seeds, general log-linear regression was used. A three-way ANOVA was used to determine if there were differences in Na^+ concentrations of seeds in response to the salinity regime (constant, fluctuating), the presence/absence of seed coats and duration of salinity exposure.

3.2.6 Mapping the probability of germination in the Coorong

To map the probability of germination of seeds and turions of *R. tuberosa* and seeds of *R. megacarpa* in the Coorong, the historic salinity data of July 1976, 1988, 2005, August 2008 and 2011 were imported into a GIS using ArcGIS 9 (ArcMap Version 9.3). Salinity data for each year were interpolated using Inverse Distance Weighting (IDW). These interpolated salinities were overlain with an additional layer of the probability of germination for *R. tuberosa* seeds and turions and *R. megacarpa* seeds, based on the germination threshold identified by this study, with the final percentage germination of *R. tuberosa* and *R. megacarpa* seeds with sediment and *R. tuberosa* turion without sediment. The model demonstrates the probability of germination for *R. tuberosa* seeds present in the Coorong and assumes experimental conditions (temperature and light cycles, constant salinity) in the Coorong.

3.3 Results

3.3.1 Effects of salinity and sediment on germination

Ruppia tuberosa seeds without sediment germinated in salinities of 0 to 125 mS cm^{-1} with 21-42% germination, but no germination occurred above 125 mS cm^{-1} (Figure 3.4a), and *R. tuberosa* seeds with sediment germinated in salinities of 0 to 120 mS cm^{-1} with 14-64% germination, but no germination occurred above 120 mS cm^{-1} (Figure 3.4a).

Salinity influenced the germination of *R. tuberosa* seeds without and with sediment at salinities of 0-125 mS cm^{-1} and of 0-120 mS cm^{-1} (Wald = 77.32, $P < 0.0001$; Wald = 17.47, $P < 0.0001$; Table 3.1). Differences between the presence and absence of sediment, however, became apparent in germination rates and mean time to germination. Mean time to germination increased significantly with increasing salinity, and extended to 37 days at the salinity of 125 mS cm^{-1} for *R. tuberosa* seeds without sediment (Wald = 162.14, $P < 0.0001$; Figure 3.4b) and to 15 days at the salinity of 120 mS cm^{-1} for *R. tuberosa* seeds with sediment (Wald = 33.91, $p < 0.0001$; Figure 3.4b and Table 3.1).

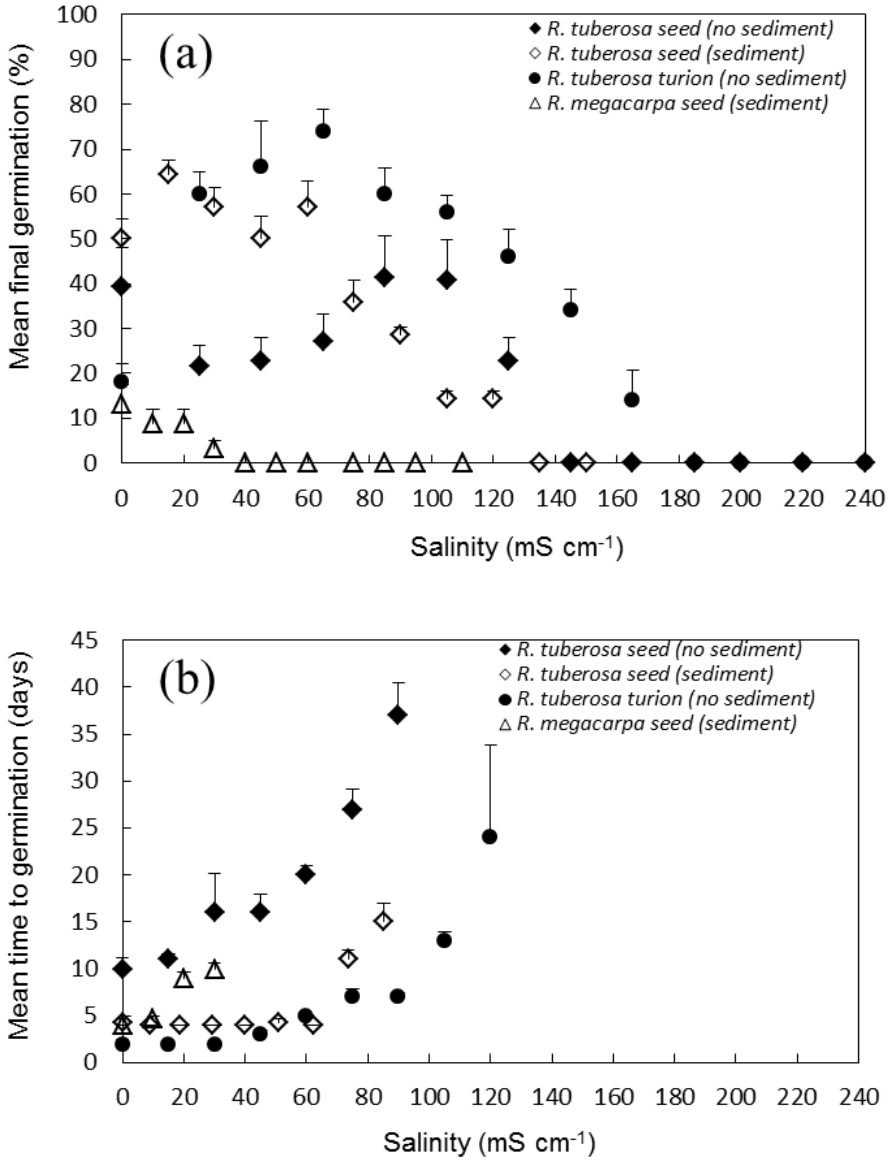

Figure 3.4: Effects of salinity on mean final germination percentage of *R. tuberosa* seeds and turions without sediment and of *R. tuberosa* and *R. megacarpa* seeds with sediment (a) and on mean final percentage germination of *R. tuberosa* seeds and turions without sediment and of *R. tuberosa* and *R. megacarpa* seeds with sediment (b) after 60 days.

Table 3.1: Results of log-linear regression model applied to the germination parameters, G_F (final percentage germination**)** and T_G (time to germination), for seeds and turions of *R. tuberosa* and seeds of *R. megacarpa* with and without sediment, as a function of the salinity.

					95% confidence interval		
		Estimate	SE	Wald	Lower	Upper	*P*
R. tuberosa seed	G_F	0.984	0.002	77.32	0.980	0.987	0.0001
(without sediment)	T_G	1.012	0.001	162.14	1.010	1.013	0.0001
R. tuberosa turion	G_F	0.988	0.002	42.53	0.984	0.991	0.0001
(without sediment)	T_G	1.024	0.002	171.34	1.020	1.028	0.0001
R. tuberosa seed	G_F	0.984	0.004	17.47	0.976	0.991	0.0001
(with sediment)	T_G	0.017	0.003	33.91	1.012	1.023	0.0001
R. megacarpa seed	G_F	0.943	0.018	10.08	0.910	0.978	0.001
(with sediment)	T_G	1.029	0.010	8.45	1.009	1.049	0.004

Analysis of covariance of successful germinations of *R. tuberosa* seeds across the salinity range 0 to 125 mS cm^{-1} with and without sediment indicated that the presence / absence of sediment (p = 0.002) and salinity (p = 0.001; Table 3.2) influenced germination rates. Mean time to germination was significantly longer when sediment was absent ($p < 0.0001$), coupled with a significant delay in germination when salinity increased ($p < 0.0001$).

Table 3.2: Results of one-way analysis of covariance performed on the effect of salinity on the GF (final percentage germination) and TG (time to germination) for *R. tuberosa* seeds across the salinity ranges from 0 to 120-125 mS cm^{-1} with and without sediment.

	Source	SS	DF	F	*P* value
G_T	Salinity	58.23	1	11.32	0.001
	Sediment	51.12	1	9.94	0.002
T_G	Salinity	49.76	1	66.41	< 0.0001
	Sediment	75.40	1	100.63	< 0.0001

Turions of *R. tuberosa* germinated at salinities of 0 to 165 mS cm^{-1}, with 14-74 % germination, but no germination occurred above 165 mS cm^{-1} (Figure 3.4a). Salinity had effect on germination of *R. tuberosa* turions (Wald = 42.53, $p < 0.0001$; Table 3.2). Mean time to germination increased significantly with increasing salinity (Wald = 171.34, $p < 0.0001$; Figure 3.4b and Table 3.1), and it extended to 24 days at the salinity of 165 mS cm^{-1} for *R. tuberosa* turions. Germination of *R. megacarpa* seeds was significantly influenced by salinity (Wald = 10.08, p = 0.001; Table 3.1). *R. megacarpa*

germinated at salinities between 0 and 45 mS cm^{-1} with 3-13 % germination, but, no germination occurred above 45 mS cm^{-1} (Figure 3.4a). Mean time to germination increased significantly with increasing salinity (Wald = 8.45, p = 0.004; Figure 3.4b and Table 3.1), and it extended to 10 days at the salinity of 45 mS cm^{-1} for *R. megacarpa* seeds.

3.3.2 Effect of transfer from high to low salinity

Un-germinated seeds of *R. tuberosa* showed high recovery germination rates (Figure 3.5a). *R. tuberosa* that failed to germinate initially at salinities of 145 to 240 mS cm^{-1}, did so at lower post-treatment salinities (Wald = 9.76, p = 0.002; Table 3.3). The pre-treatment salinity, however, had no effect (Wald = 0.08, p = 0.776), but interactive effect with post-treatment salinity (Wald = 37.87, p = 0.0001). The pre-treatment of seeds at high salinities enhanced the germination rates, compared with seeds that had no pre-treatment. Seeds initially incubated at salinities of 145 to 240 mS cm^{-1} had 94% germination rates once placed in salinity of 45 mS cm^{-1}. This compares to 22% germination rates for seeds exposed to 45 mS cm^{-1} with no pre-treatment at elevated salinities.

In comparison to seeds, turions showed relatively lower recovery germination rates. Turions that failed to germinate at salinities of 185 to 240 mS cm^{-1} germinated when transferred to 0, 45, 85, and 125 mS cm^{-1} treatments, with 5-10% germination, but no germination occurred at 165 mS cm^{-1} (Figure 3.5b).

Table 3.3: Results of log-linear regression model applied to the germination parameter G_F (final germination percentage) for *R. tuberosa* seeds, as function of the pre-treatment (145, 165, 185, 200, 220 and 240 mS cm^{-1}) and post-treatment (0, 30, 60 and 90 mS cm^{-1}) salinities and the interaction between both factors.

				95% confidence interval		
Effect	Estimate	SE	Wald	Lower	Upper	*p*
Pre-treatment	1.000	0.001	0.08	0.998	1.001	0.776
Post-treatment	1.007	0.002	9.76	1.002	1.011	0.002
Pre-treatment x post-treatment	1.000	0.000	37.87	1.000	1.000	0.0001

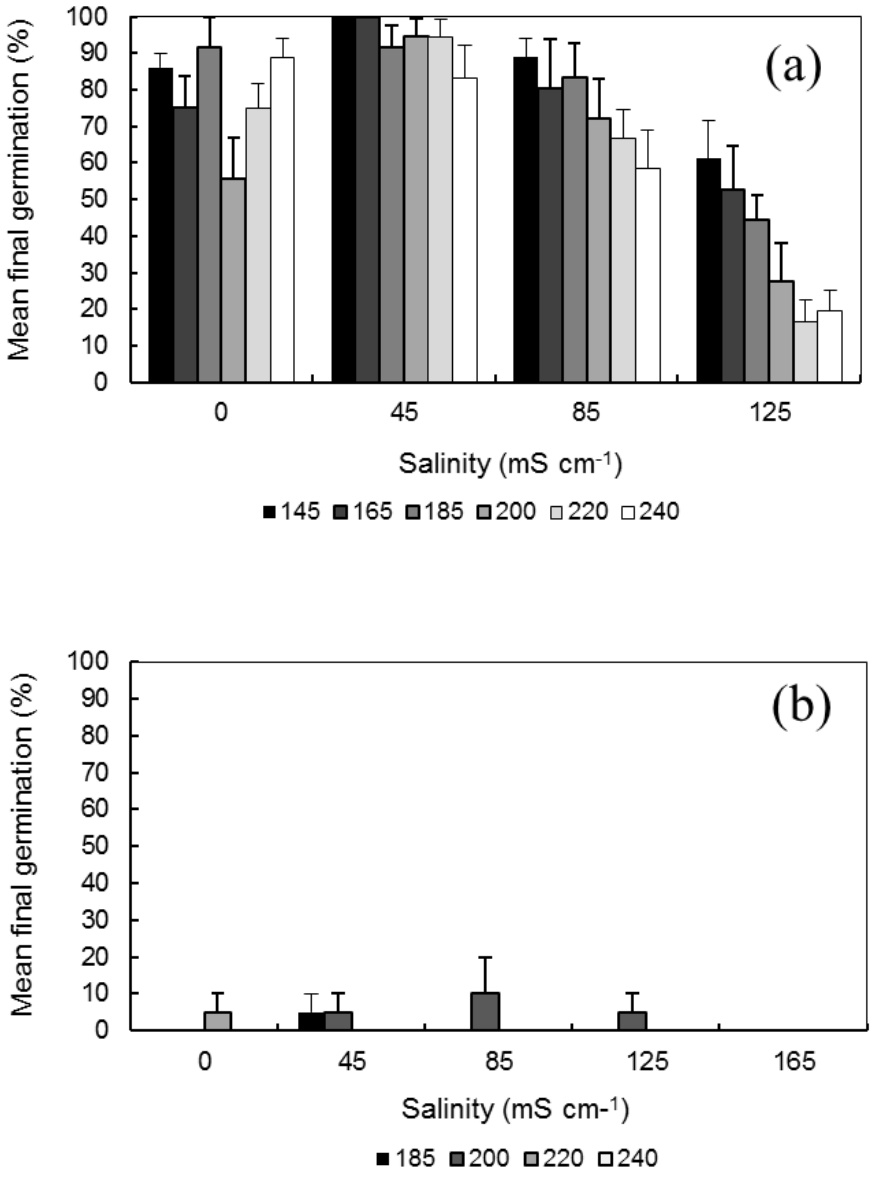

Figure 3.5: Effects of salinity on mean final germination percentage for *R. tuberosa* seeds (a) and *R. tuberosa* turions (b), following incubation in initial salinity treatments. Pre-treatment were 145, 165, 185, 200, 220 and 240 mS cm^{-1} for seeds and 185, 200, 220 and 240 mS cm^{-1} for turions.

3.3.3 Effects of salinity on imbibition and Na concentrations of seeds

The fresh-weight of *R. tuberosa* seeds was influenced by salinity (p = 0.0061) and duration of incubation (p < 0.0001), but it was not influenced by their interaction (Table 3.4). The weight of *R. tuberosa* seeds was highest at 0 and 60 mS cm^{-1} treatments by week 4. However, when seeds were transferred to 60 mS cm^{-1} after exposure to 180 mS cm^{-1} for two weeks, the fresh-weight of *R. tuberosa* increased rapidly by week 4, but the weight of seeds in the 180 mS cm^{-1} treatment were significantly lower than for other

treatments (Figure 3.6a). The fresh-weight of seeds affected the final percentage germination of *R. tuberosa* (y = 6.73 + 69.84 In(x), $r^2 = 0.46$, $F_{1, 46} = 38.83$, $p < 0.0001$; Figures 3.6b and 3.7). Na^+ concentrations in the seeds were influenced by an interaction between salinity, the presence/absence of seed coat and duration of incubation ($p < 0.0001$; Table 3.5). There was a significant effect on Na^+ concentrations in seeds with seed coats, with higher Na^+ concentrations at the higher salinity treatment of 180 mS cm^{-1} by week 4. These effects were highly dependent upon interactions with each other and with duration. The Na^+ concentration of seeds with seed coats was highest in the treatment of 180 mS cm^{-1} by week 4, but the Na^+ concentration of the 180-60 mS cm^{-1} treatment fell to that of the treatment of 60 mS cm^{-1} (Table 3.6).

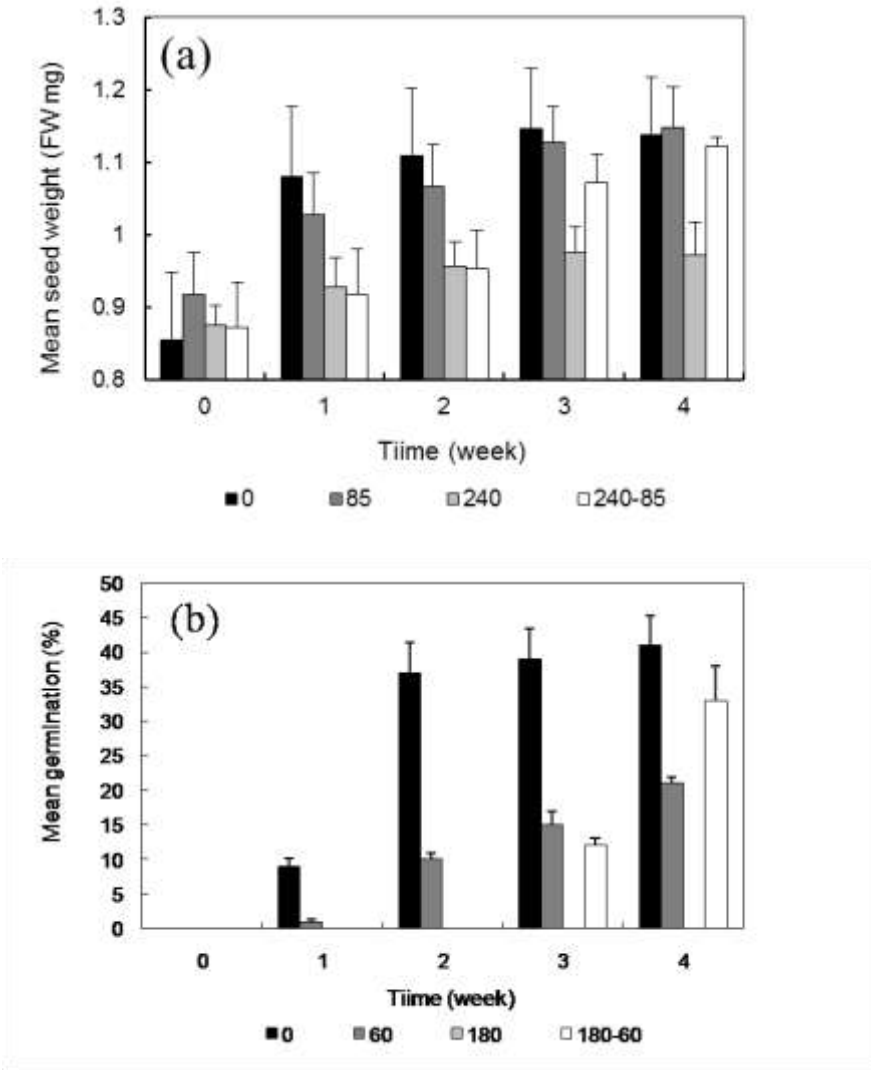

Figure 3.6: The effect of salinity on the change in seed weights of *R. tuberosa* (a) and on germination percentage for *R. tuberosa* seeds (b) within 28 days.

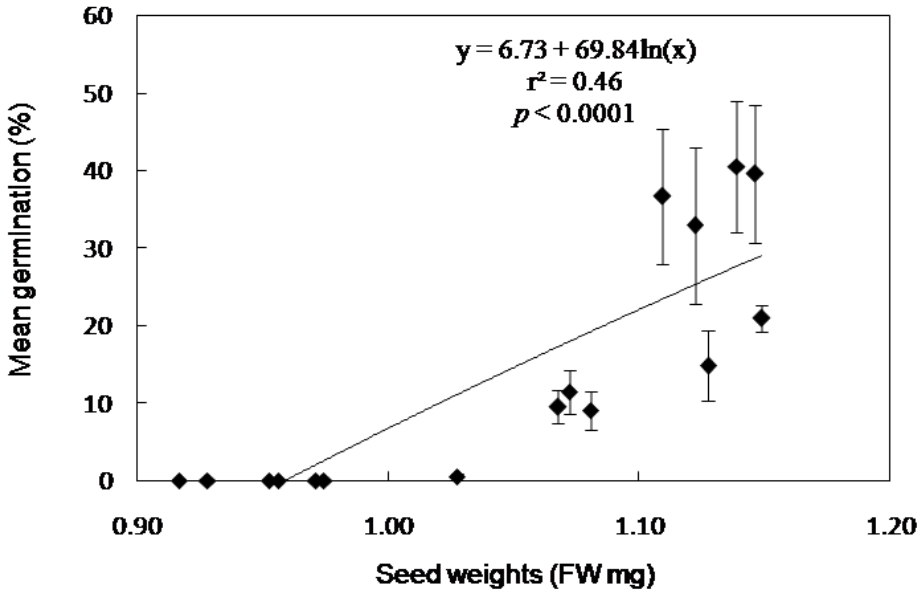

Figure 3.7: The effect of seed weights on the germination percentages of *R. tuberosa*. Log-linear regression lines included.

Table 3.4: Results of a two-way ANOVA performed on the effect of salinity on the fresh-weights of *R tuberosa* seeds at three constant (0, 60 and 180 mS cm^{-1}) and one fluctuating salinity regime (180-60 mS cm^{-1}) during the duration of 4 weeks (0, 1, 2, 3 and 4).

Source	% SS	DF	F	*P* value
Salinity	16.05	3	4.79	0.0061
Duration	36.67	4	7.97	< 0.0001
Salinity x Duration	7.50	12	0.56	< 0.8614

Table 3.5: Results of a three-way ANOVA performed on the effect of salinity on sodium (Na^+) concentrations of *R tuberosa* seeds at three constant (0, 60 and 180 mS cm^{-1}) and one fluctuating salinity regime (180-60 mS cm^{-1}), with and without seed coats after 2 and 4 weeks.

Source	SS	DF	F	*P* value
Salinity	43.86	3	133.18	< 0.0001
Seed coat	33.11	1	308.42	< 0.0001
Duration	0.44	1	4.10	0.0513
Salinity x Seed coat	22.30	3	69.19	< 0.0001
Salinity x Duration	11.76	3	36.52	< 0.0001
Seed coat x Duration	2.56	1	23.85	< 0.0001
Salinity x Seed coat x Duration	4.09	3	12.70	< 0.0001

Table 3.6: The effect of salinity on sodium (Na^+) concentrations of *R tuberosa* seeds with and without seed coats. Shown are Na^+ concentrations (mean (s.e.) after 2 and 4 weeks of incubation at three constant (0, 60 and 180 mS cm^{-1}) and one fluctuating salinity regime (180-60 mS cm^{-1}, which had 2 weeks incubation at 180 mS cm^{-1} followed by 2 an additional 2 weeks incubation at 60 mS cm^{-1}).

Duration	Seed coat	Na^+ concentrations (mg/g)			
		0 mS cm^{-1}	40 mS cm^{-1}	130 mS cm^{-1}	130–40 mS cm^{-1}
2 Wk	Present	0.58 (0.14)	3.05 (0.19)	4.33 (0.21)	4.64 (0.20)
	Absent	0.95 (0.06)	1.03 (0.08)	1.05 (0.04)	1.07 (0.03)
4 Wk	Present	0.47 (0.13)	1.88 (0.43)	5.76 (0.27)	1.87 (0.22)
	Absent	0.86 (0.07)	0.99 (0.23)	2.28 (0.08)	1.06 (0.12)

3.3.4 The probability of germination of *Ruppia* in the Coorong

Construction of GIS habitat maps showing the probability of germination for *R. tuberosa* seeds and turions and *R. megacarpa* seeds were based on their salinity responses coupled with the different annual salinity gradients observed along the Coorong (Figure 3.8). In salinity below 60 mS cm^{-1} a germination probability of > 50% would be expected for seeds of *R. tuberosa*, but at salinities between 60 mS cm^{-1} and 125 mS cm^{-1} germination would be < 50%, while the germination of turions of *R. tuberosa* would have been expected to occur in salinities between 30 and 125 mS cm^{-1} (> 50% germination). Decline in germination of *R. tuberosa* was most pronounced in the South Lagoon (Figure 3.8) as salinity increased. Germination of seeds of *R. megacarpa*, however, occurred between 0 and 30 mS cm^{-1} (> 10% germination success).

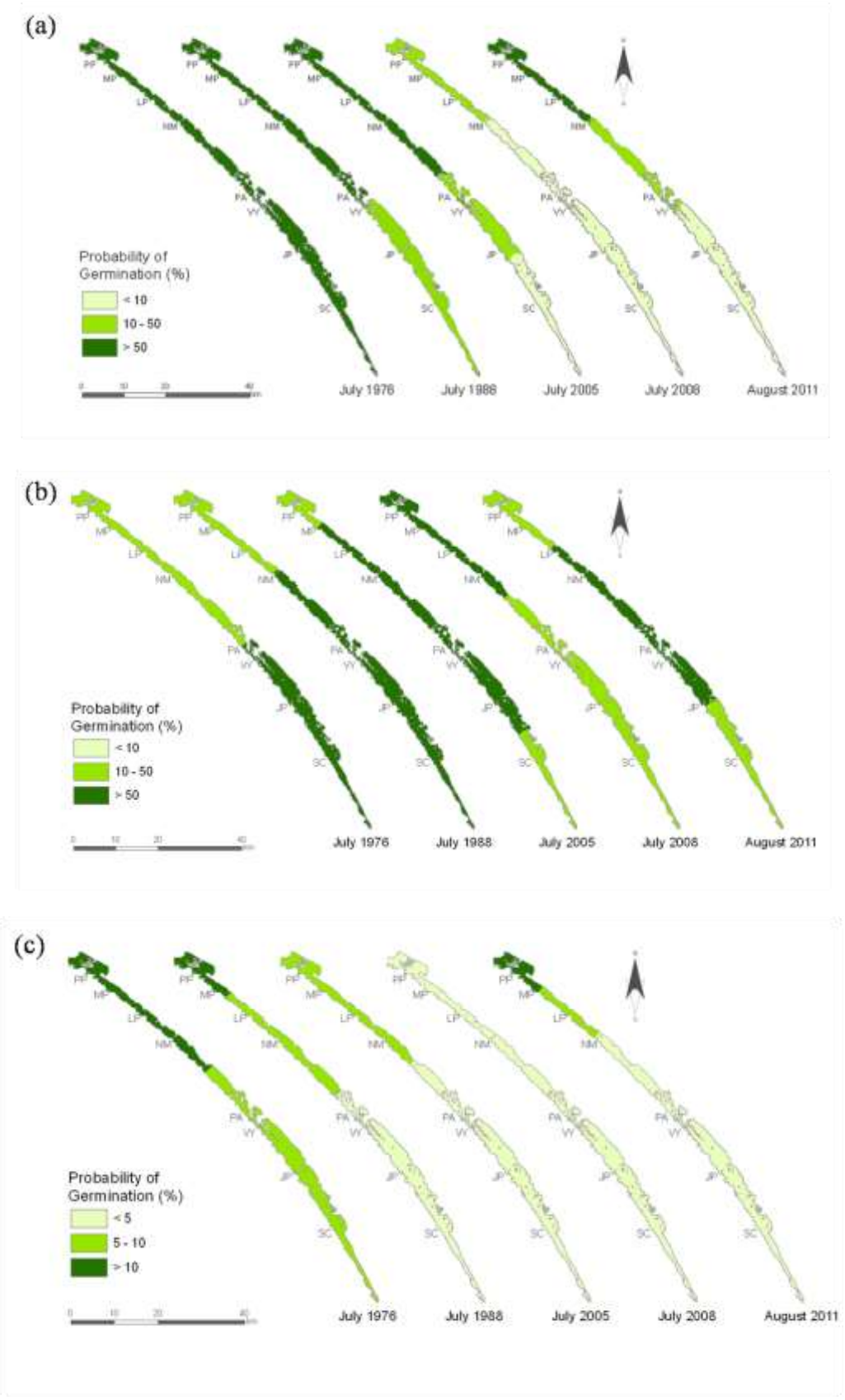

Figure 3.8: The models of probability of *R. tuberosa* seed (a) and turion (b) and *R. megacarpa* seed (c) germination in the Coorong in July 1976, July 1988, July 2005, July 2008 and August 2011. PP = Pelican Point; MP = Mark Point; LP = Long Point; NM = Noonameena; PA = Parnka Point; VY = Villa dei Yumpa; JP = Jack Point and SC = Salt Creek.

3.4 Discussion

3.4.1 Effects of salinity and sediment on germination

The success of halophyte populations is dependent on their germination salinity tolerance (Pujol et al., 2000; Ye et al., 2005). Most halophytes are able to germinate at salinities greater than seawater (Vollebergh and Congdon, 1986; Sim et al., 2006). However, salt stress responses and tolerances to germination vary between species, and also within species with different survival strategies for seeds and turions, that may have different capacities of coping with exposure to high salinity. *R. tuberosa* and *R. megacarpa* seed germination at salinities of 0-125 mS cm^{-1} and of 0-45 mS cm^{-1}, and turion germination of *R. tuberosa* at salinities between 0 and 165 mS cm^{-1} suggest that these are the salinity thresholds for these species. The observed germination thresholds for *R. tuberosa* and *R. megacarpa* correspond with their distribution in the Coorong (Chapter 2). In 2008 *R. tuberosa* was present in the North Lagoon where salinities were 60 to 120 mS cm^{-1}, but absent from the South Lagoon where salinity exceeded 125 mS cm^{-1}. The disappearance of *R. megacarpa* correlates with the observed salinities exceeding the germination threshold identified by this study.

A similar germination response to salinity was obtained by Sim et al. (2006), who found limited *R. megacarpa* germination at salinities of 30 and 45 S cm^{-1}. Brock (1979) also found *R. megacarpa* germinated at salinities of 0 to 30 mS cm^{-1} with 2-28% of seeds germinating, while *R. tuberosa* germinated at salinities of 0 to 65 mS cm^{-1} with 1-24% germination. *R. megacarpa* appears unable to germinate at salinities higher than seawater and has a lower salinity tolerance than other *Ruppia* species (Sim et al., 2006). Vollebergh and Congdon (1986) and Sim et al. (2006) found that germination of *R. polycarpa* R. Mason occurred between freshwater and 60 mS cm^{-1} and 0 and 60-78 mS cm^{-1}, respectively. In comparison to seeds, turions of *R. tuberosa* seem to occur over a wide range of salinities, and exhibit greater tolerance to salinity than seeds. Vollebergh and Congdon (1986) found that germination of *R. polycapa* turions was optimum between 28 and 56 mS cm^{-1} with 60% germination, and it was higher in salinity of 127 mS cm^{-1} than in fresh-water after 30 days, resulting in 40% germination.

An increase in salinity led to an increase in mean time to germination for seeds of both species and turions of *R. tuberosa*, as observed in other studies of *Halosylon recurvum* Bunge ex. Boiss and *Vallisneria americana* Michx (Khan and Ungar, 1996; Jarvis and Moore, 2008) and *R. polycarpa*, *Lamprothamnium macropogon* (A. Braun) Ophel and

Lamprothamnium succinctum (A. Braun in Ascherson) Wood (Sim et al., 2006). Why the presence of sediment should influence germination and mean time to germination of *R. tuberosa* and *R.megacarpa* requires further investigation.

3.4.2 Effect of transfer form high to low salinity

Un-germinated seeds of *R. tuberosa* recovered germination when transferred to lower salinities after 60 day exposure to high salinities indicating that viable seeds could remain in the sediment when salinity levels exceed their tolerance limits, and germinate when salinity levels are reduced. Unlike seeds, turions that failed to germinate at initial salinities of 185 to 240 mS cm^{-1} showed lower germination when transferred to lower salinities. This may be caused by specific ion toxicities after immersion in salinities above 165 mS cm^{-1}. Increased salinity can result in toxic ion accumulation in the cell membrane (Katembe et al., 1998). Specific ion toxicity of Na^+ and Cl^- ions lead to changes in protein hydration, affecting the hydration structure surrounding the protein molecule, thereby inhibiting the activities of some enzymes or causing cell death (Katembe et al., 1998). This indicates that turions may be more vulnerable to extreme salinities than seeds despite the findings under static salinities supporting the opposite.

3.4.3 Effects of salinity on imbibition and Na concentrations of seeds

High salinity-induced dormancy is well known for most halophyte seeds (Khan and Ungar, 1996; Katembe et al., 1998). Pujol et al. (2000) found enforced dormancy for seeds of *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa* (L) AJ Scott as the osmotic stress increased, which is in agreement with Katembe et al. (1998) for *Atriplex patula* L. Our study indicates that increased salinity reduced imbibition of water by seeds of *R. tuberosa* since imbibition is determined by the Na^+ concentration difference between the seeds and the external medium (Katembe et al., 1998). Reduced imbibition resulted in high Na^+ concentrations in seeds, thereby inducing dormancy to protect enzymes from the elevated salinity (Katembe et al., 1998). In this study, the seed coat appeared to protect the embryo of *R. tuberosa* from elevated salinities. Indeed, the increase in Na^+ concentration in seeds was dependent upon the presence/absence of a seed coat. Furthermore, salinity pre-treatment had no negative effect on germination success, indicating that intact seeds are not affected or damaged in highly saline conditions. In fact, when seeds were transferred to lower salinities germination success was enhanced. It appeared that this was associated with reduced water uptake by the seeds when exposed to high salinities. The movement of water across cell membranes

into the embryo leads to a decrease in Na^+ concentrations, which enhances germination. Others have reported germination of halophyte seeds after the seeds experienced high osmotic stress (Keiffer and Ungar, 1997; Pujol et al., 2000). In contrast, germination success was not enhanced under the fluctuating salinity of 180-60 mS cm^{-1} in the imbibition experiment (Table 3.6). This may be because seeds were pre-treated at high salinity for only 2 weeks. Indeed, there was no significant difference in the Na concentration of seeds between salinities of 60, 180 and 180-60 mS cm^{-1} for a two week period. Vollebergh and Congdon (1986) also found that pre-treatment at high salinities elevated germination success. These findings indicate that the germination success is likely to be related to the degree of osmotic stress.

3.4.4 Summary of *Ruppia tuberosa* and *Ruppia megacarpa* germination

R. tuberosa seed germination appears to correspond to type 3 of Woodell (1985) for halophytes. Type 3 plants are those that are stimulated by immersion in high salinity. Salt-stimulated germination may be an advantage to *R. tuberosa* which often lives in highly variable environments. On the other hand, this may be a disadvantage to *R. tuberosa*, because if all seeds germinate at one time after subjected to high salinity, the population is vulnerable to any future environmental change. However, under a static salinity regime the germination success of *R. tuberosa* and *R. megacarpa* were highly variable at given salinities. This variability may be associated with: the previous history of the parent plant; the age of seed; and morphological differences. This may also indicate that the species bet hedge, which is an important ecological strategy for plant communities in temporary or unpredictable habitats.

3.4.5 Implications for the Coorong

During large freshwater flows in 1976 salinities in the North Lagoon of the Coorong were less than 12.5 mS cm^{-1} and approximately 84 mS cm^{-1} in the South Lagoon. Under these conditions *R. tuberosa* would be likely to germinate throughout the Coorong, as would *R. megacarpa* in the North Lagoon (Figure 3.6). However, the distribution of *R. megacarpa* was limited to the North Lagoon and *R. tuberosa* to the South Lagoon, perhaps due to competition as influenced by salinity (Sim et al., 2006). Salt-tolerant species are out competed by salt-sensitive species in less stressful environments (Katembe et al., 1998). However, in 2008 during a period of extremely low freshwater inflows and elevated salinities, germination of *R. tuberosa* was likely to have been restricted to the northern half of the Coorong and *R. megacarpa* germination unlikely.

Indeed, *R megacarpa* has not been observed in the system and *R. tuberosa* is shifting northwards with complete loss from the South Lagoon as salinities increase. The study suggests that the demise of both *Ruppia* species would denude the seed bank, particularly if the seeds are short lived.

Similar changes in the distribution of submerged macrophytes resulting from elevated salinity have been observed in other regions of the world, including South America, South Africa and Australia. For example, there are significant ecological changes associated with increased salinisation during a drought in the St Lucia Estuary in South Africa. The system became hyper-saline associated with closed-mouth conditions, resulting in die-off of submerged macrophytes such as *Potamogeton pectinatus* L., *Ruppia cirrhosa* (Petagna) Grande and *Zostera capensis* Setch (Taylor et al., 2006). In addition, it is estimated that 450 aquatic plant species are threatened with extinction due to increased salinity (Keighery et al., 2000). As aquatic plants play an integral role in the functioning of aquatic ecosystems their loss has major ramifications for wetland ecosystems (Smith et al., 2009). In Western Australia there has been a 50% reduction in the numbers of waterbirds using wetlands as a consequence of the loss of aquatic plants due to increasing salinisation (Keighery et al., 2000). The partial loss of *Ruppia* from the Coorong has resulted in an increasingly simplified habitat and food-web and will reduce ecosystem function and resilience. *Ruppia* habitat restoration may be achieved by reducing salinity through adequate inflows of fresh water to the Coorong. The restoration of macrophyte communities has been shown to be a slow process if a seed bank no longer exists (Orth et al., 2006b), but it has been shown to be a rapid regrowth of *Ruppia cirrhosa* from the available seed bank (Riddin and Adams, 2009), and consequently recovery of the community may require assistance through transplantation of seeds or adult shoots (Meehan and West, 2002).

Chapter 4 The effect of salinity on the growth and reproduction of *Ruppia tuberosa*

4.1 Introduction

Despite macrophyte communities in aquatic ecosystems constituting a significant biomass and playing an important role in the ecology of estuaries and coastal lagoons (Sim et al., 2006; Song et al., 2008), the communities continue to be threatened by secondary salinisation. Secondary salinisation, along with extraction and flow reduction, is regarded as the greatest threat facing the ecology of a wide range of aquatic ecosystems throughout the world (Smith et al., 2009), including Australia (Keighery et al., 2000). In Australia, extensive areas are affected by rising salinities, and these areas have continuously increased as a consequence of natural and anthropogenic impacts (Kingwell and John, 2007). For instance, extensive regulation, diversions of water across the Murray-Darling Basin, regional droughts, high evaporation rates, and inflows of saline groundwater have contributed to high salinity in the South Australian reaches of the Murray River (Brookes et al., 2009). Significant changes have occurred with ecological effects, particularly changes in the natural character of aquatic ecosystems, decreased biodiversity and lower productivity (Brock et al., 2005).

Salinity strongly influences the survival, growth and reproduction of aquatic plants (Vollebergh and Congdon, 1986; Bernstein et al., 1995; Vicente et al., 2004; Song et al., 2008), and therefore affects its overall population dynamics and distribution (Brock et al., 2005). Santamaría et al. (1995) report significant reductions in aquatic macrophyte abundance due to increased salinity. A reduction in growth and reproduction as a result of increased salinity may be caused by ion toxicity, ion imbalance or physiological processes (Seemann and Critchley, 1985). Jampeetong and Brix (2009) demonstrated that high ion concentrations impose both ionic toxicity and osmotic stress on plants, thereby inducing several morphological and physiological changes, leading to reduction of the growth and development of plants. This may be caused by high sodium (Na^+) and chloride (Cl^-) concentrations in the plant cells that are inhibitory to many enzyme processes (Jampeetong and Brix, 2009). In addition, ion toxicity resulting from elevated Na^+ and Cl^- concentrations in plant tissues may inhibit the uptake of other ions, including potassium (K^+, (Naidoo, 1994), calcium (Ca^{2+}, (Reid and Smith, 2000) and Boron (B, (El-Motaium et al., 1994; Goldbach et al., 2007), leading to K^+ (Davenport et

al., 1997), Ca^{2+} (Maas and Grieve, 1987) and B deficiencies (López-Gómez et al., 2007), and subsequently this results in high Na^+/K^+ and Na^+/Ca^{2+} ratios (Maas and Grieve, 1987; Reid and Smith, 2000). Moreover, it is well known that sexual reproduction is often more sensitive to B than vegetative growth (Dell and Huang, 1997). Reproductive growth among species, and even within the same species can be affected by low B in plant tissues, impairing microsporogenesis and pollen tube growth, thereby reducing male fertility (Dell and Huang, 1997).

Ruppia species, submerged halophytes, are important primary producers in brackish to hyper-saline habitats in many continents (Verhoeven, 1979). In Australia, *Ruppia tuberosa* J. Davis and Tomlinson occurs over a wide range of salinities of 22-300 mS cm^{-1}, and reproduces both sexually and vegetatively via seeds and turions. *Ruppia megacarpa* R. Mason occurs in salinities of 9-67 mS cm^{-1} (Brock, 1982b) and reproduces sexually. *R. megacarpa* and *R. tuberosa* were the dominant aquatic plants of the Coorong (Brock, 1979, 1981a), which is part of one of Australia's most important wetland systems, covering 467 km^2 and consisting of ocean beach, the mouth of the River Murray, the estuary, and Lakes Albert and Alexandrina (Figure 4.1). However, the Coorong has experienced degradation over the last decades by changes in water quality, including increased turbidity and salinity and reduced fresh water inflows from the River Murray. Furthermore, considerable biological changes have accompanied these biophysical changes to the Coorong, particularly the rapid decline of *R. tuberosa* and complete loss of *R. megacarpa* in the system (Paton and Rogers, 2007).

Despite the obvious decline in the distribution of *Ruppia* species in the Coorong, the effect of salinity on the growth and reproduction of *R. tuberosa* is not well understood. It is hypothesised that reduced growth and reproduction of *R. tuberosa* under high salinity concentrations includes inhibition of ion uptake and shoot ion (K^+, Ca^{2+} or B) deficiency. This study aimed to test this hypothesis. The aim of this study was to investigate whether the concentrations of ions in plant tissue was related to growth and reproductive responses of *R. tuberosa*. These responses were investigated by growing *R. tuberosa* plants in ponds at salinities of 45, 75, 105, 135 and 165 mS cm^{-1}, and comparing pond plant responses to *R. tuberosa* grown in the field. This enabled an assessment of favourable salinities that would permit the growth and reproduction of *R. tuberosa*.

4.2 Material and methods

4.2.1 Site description

The Coorong is a coastal lagoon confined by a coastal barrier dune (the Younghusband Peninsula), extending southeast from the mouth of the River Murray for approximately 140 km, in South Australia (Figure 4.1). The Coorong is comprised of two main lagoons, (North and South), separated by a narrow channel at Parnka Point. The Coorong is recognised as a wetland of international importance under the Ramsar convention (Ramsar Convention on Wetlands: http://www.ramsar.org/), and supports high biological productivity and diversity, with regard to macro-invertebrates, fish and migratory waterbirds. Historically, the Coorong has a strong salinity gradient from north to south, ranging from fresh to marine in the North Lagoon depending upon freshwater inflows from Lake Alexandrina. The South Lagoon historically maintained salinity of approximately twice that of sea water. Reduced freshwater inflows resulting from water resource development resulted in increases in salinity levels throughout the Coorong (Brookes et al., 2009). Continual evaporation and replenishment of water from marine ingress resulted in extreme salinity levels, with hyper-salinity in the South Lagoon.

Lake Pipe Clay and Lake Cantara, are ephemeral salt lakes located about 220 and 250 km south of Adelaide, South Australia (Figure 4.1). The lakes are shallow (approximate water depth of ~0.5m in winter) and are dry during summer (December-February). Salinities range from 40 to 160 mS cm^{-1} (Lake Pipe Clay) and 35 to 90 mS cm^{-1} (Lake Cantara) during Autumn to Spring (May-November). The lakes presently have no direct connection to the Coorong. The lakes lie above the Pleistocene calcrete and between Quaternary dunes running parallel to the coast. The region is characterized by slow tectonic uplift, low coastal plain gradients, and eustatic sea level oscillations.

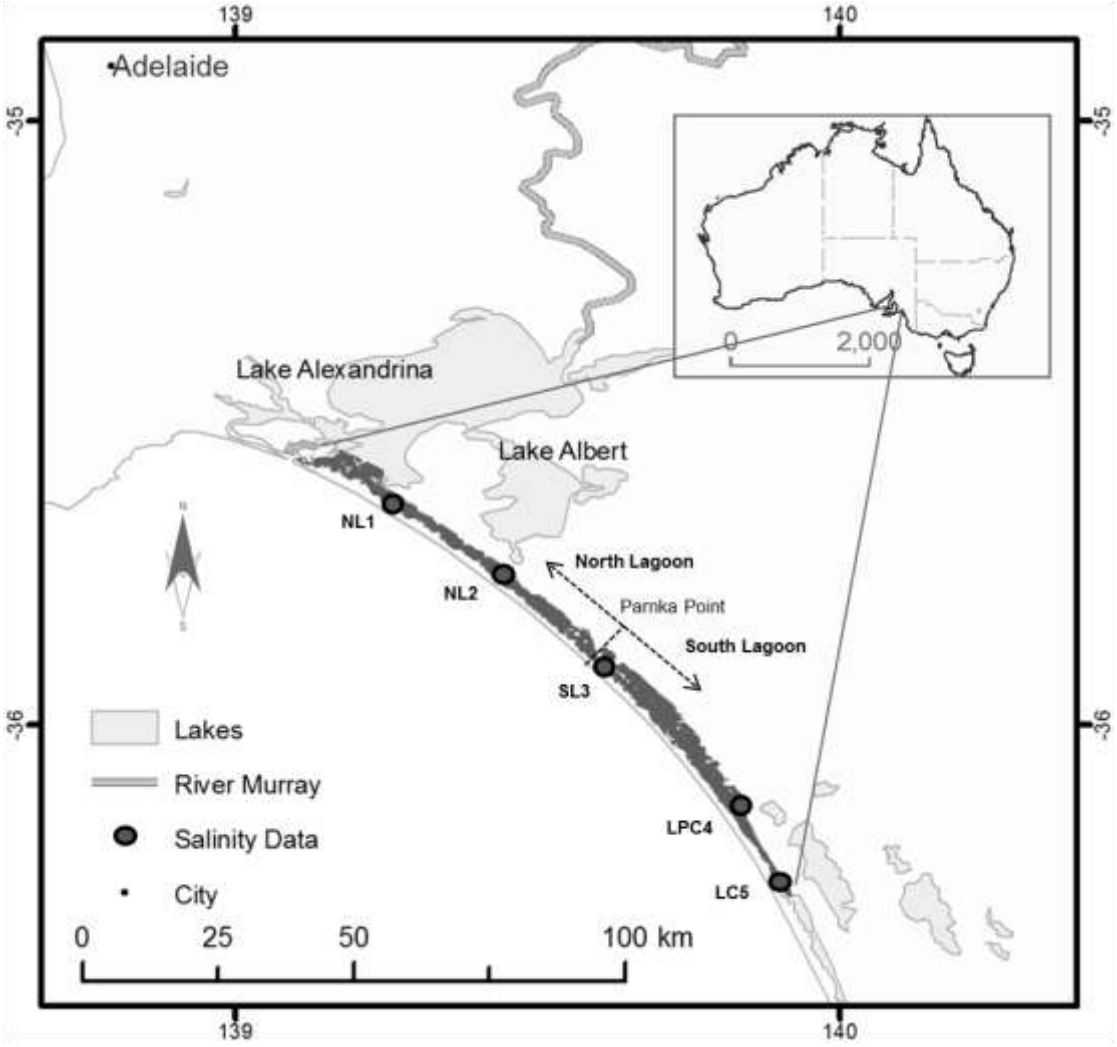

Figure 4.1: Location of the Coorong and neighbouring Lakes Pipe Clay and Cantara (NL1 = Mulbin Yerrok Point; NL2 = Noonameena; SL3 = Parnka Point; LPC4 = Lake Pipe Clay and LC5 = Lake Cantara), South Australia.

4.2.2 Experimental design and procedure

Experimental salinity regimes

This study consisted of a pond and a field experiment. For the pond experiment, five salinities chosen were: 45, 75, 105, 135 and 165 mS cm^{-1}. This salinity range extended beyond the field salinity range that *R. tuberosa* has been experienced at 85-135 mS cm^{-1} (Kim unpublished data). For the field experiment, five sites were chosen based on the salinity gradient from North to South in the Coorong: North Lagoon 1 (NL1), North Lagoon 2 (NL2), South Lagoon 3 (SL3), Lake Pipe Clay (LPC4) and Lake Cantara (LC5, Table 4.1). However, no data was obtained from the sites of NL 1 and 2, due to the disturbance caused by strong winds and storms during the period of the *in situ* experiment. In addition, no data obtained from the salinities of 105, 135 and 165 mS cm^{-1} in the pond experiment due to shoot mortality during November 2009. Thus, only

data for three months (from August to October) was analysed.

Table 4.1: Summary of GIS co-ordinates and conductivities of the five study sites in the Coorong and neighbouring Lake Pipe Clay and Lake Cantara.

Site	GIS co-ordinates	Location	Salinity (mS cm^{-1})	
			June	October
N1	S 35.66960 E 139.13887	Mulbin Yerrok Point	45	50
NL2	S 35.77409 E 139.27287	Noonameena	60	69
SL3	S 36.90286 E 139.39903	Parnka Point	118	123
LPC4	S 36.13626 E 139.64706	Lake Pipe Clay	42	81
LC5	S 36.32827 E 139.74910	Lake Cantara	35	63

Pond experiment design

The pond experiment was conducted in 4 outdoor ponds (4 x 4 x 1.6m) at the University of Adelaide for four months from August to November 2009. Seedlings (approximately 1200) of *R. tuberosa* of similar size and biomass were collected from Lake Cantara (36°33′S, 139°75′E) in July 2009. Twenty young *R. tuberosa* plants were potted in plastic pots (0.15 x 0.15 x 0.1m) filled to a depth of 0.05 m with sandy clay collected from Lake Cantara (36°33′S, 139°75′E). Three replicates were used for each salinity. The total numbers of sixty pots, twelve pots per treatment, were exposed to salinities of 45, 75, 105, 135 and 165 mS cm^{-1} and inundated to a water depth of 0.3m above the sediment surface. The design enabled monthly harvesting from each of five salinity treatments, allowing measurements of growth and reproduction for each of the four months from August to November; thus every month one pot from each replicate was selected and harvested to determine ion concentrations in plant tissues. To avoid salinity shock, salinity levels were increased sequentially from 45 mS cm^{-1} to the desired level, with 2 days at each salinity step. The water levels were monitored and topped up with tap water to the marked level as necessary to maintain constant salt concentration.

The following data were collected monthly: the number of flowers, seeds and turions per pot and the shoot lengths. Every 30 days, including at the start of the experiment until the end of the experiment, plants were harvested, cleaned and their fresh-weight measured. Plants were then dried at 60°C for three days and dry-weight measured. Relative growth rate (RGR) was calculated using the formula $(\ln (W_1) - \ln (W_0))/\Delta t$ (Harper, 1977), where W_0 and W_1 are weights at the initial and final stages of the sampling period, respectively, and t is the length of the sampling period in days.

Determination of Na^+ and K^+ concentrations in the tissue of *R. tuberosa* occurred every 30 days, commencing at the start of the experiment. Plants were randomly selected from each replicate and the Na^+ and K^+ concentrations of shoots were determined. For this, the samples were dried at 60°C for three days and Na^+ and K^+ concentrations were determined by flame photometry following extraction in 8 mL of 0.1 mM nitric acid in a boiling water bath for 30 min.

However, there was no data obtained from the salinities of 105, 135 and 165 mS cm^{-1} treatment, due to shoot mortality during the period of the pond experiment in November 2009. Thus the data of three months (from August to October) only was provided and analysed in this study.

Field experiment design

A similar experiment was conducted in 2011 but *in situ*. Seedlings of *R. tuberosa* were grown in the Coorong: NL1, NL2 and SL3; and the neighbouring lakes: LPC4 and LC5, from July 2011 to October 2011 for four months (Table 4.1). Seedlings of *R. tuberosa*, of similar size and biomass were collected from Lake Cantara (36°33′S, 139°75′E) in June 2011. Three replicates were used for each site, with twenty young *R. tuberosa* planted in each plastic pot (0.15 x 0.15 x 0.1 m) filled to a depth of 0.05 m with sandy clay collected from Lake Cantara (36°33′S, 139°75′E). The total number of fifteen pots, three pots per site, were distributed at three sites in the Coorong and the two in the neighbouring lakes, Lake Pipe Clay and Lake Cantara. These pots were exposed to the natural water level fluctuations, where water depth ranged from 0.2 to 0.5m for 120 days.

At the end of the experiment the following data were collected: the number of flowers and turions per pot and the shoot lengths. The plants were then harvested, cleaned and their fresh-weight measured. Seeds were harvested as they matured. The plants were separated into the different tissue type and dried at 60°C for three days and dry-weight measured. RGR was calculated as detailed previously for the pond samples.

For the determination of Na^+ and K^+ concentrations in *R. tuberosa* tissues, Na^+ and K^+ concentrations of shoots were determined as described previously for the pond samples. To determine tissue ion concentrations of Ca^{2+}, Cl^- and B, plants were randomly selected from each replicate and the shoot concentrations determined on oven dried

material using inductively coupled plasma optical emission spectrometry. The samples for Ca^{2+} and B were digested, using nitric acid and hydrogen peroxide, in 50 mL polypropylene centrifuge tubes, with lids to prevent contamination (Wheal et al., 2011). For the determination of Cl^- concentration, additional samples were extracted using warm 4% nitric acid in 50ml polypropylene tubes, with lids to prevent contamination (Wheal and Palmer, 2010).

4.2.3 Statistical analyses

All analyses were carried out using the software JMP IN version 404 (SAS Institute Inc., 1989-2001). All data were tested for normal distribution and homogeneity of variance, using the Shapiro–Wilk and Bartlett's tests, respectively. Log-transforms were required to ensure homogeneity of variance. A two-way ANOVA test was used to investigate significant differences in abundance of shoots, flowers, seeds, turions and RGRs between months, and salinity for the pond samples. For this, the effects of month, salinity and the interaction between them were assessed. However, one-way analysis of variances (ANOVA) was conducted for the field samples to assess spatial differences in *R. tuberosa* abundance (shoots, flowers and turions) and RGRs. In addition, one-way ANOVA was conducted to test the effects of salinity on ion concentrations and Na^+/K^+, Na^+/Ca^{2+} ratios in the tissue of *R. tuberosa* under different salinity/site regimes for both the pond and the field experiments. To determine the relationship between salinity and ion concentrations in the plant tissue, a linear regression analysis was conducted. Additionally, a linear regression analysis was used to determine the relationship of ion concentrations of Na^+, K^+, Ca^{2+}, B and Cl^-, and Na^+/K^+ and Na^+/Ca^{2+} ratios and total biomass, RGRs, flowers, seeds, turions and shoot lengths of *R. tuberosa*.

4.3 Results

4.3.1 Salinity and ion concentrations in *Ruppia tuberosa* tissues under pond conditions

There was a significant effect of salinity on Na^+ and K^+ concentrations and Na^+/K^+ ratios in the tissues of *R. tuberosa* with higher Na^+ concentrations, lower K^+ concentrations and higher Na^+/K^+ ratios at the higher salinity of 165 mS cm^{-1} (Figure 4.2).

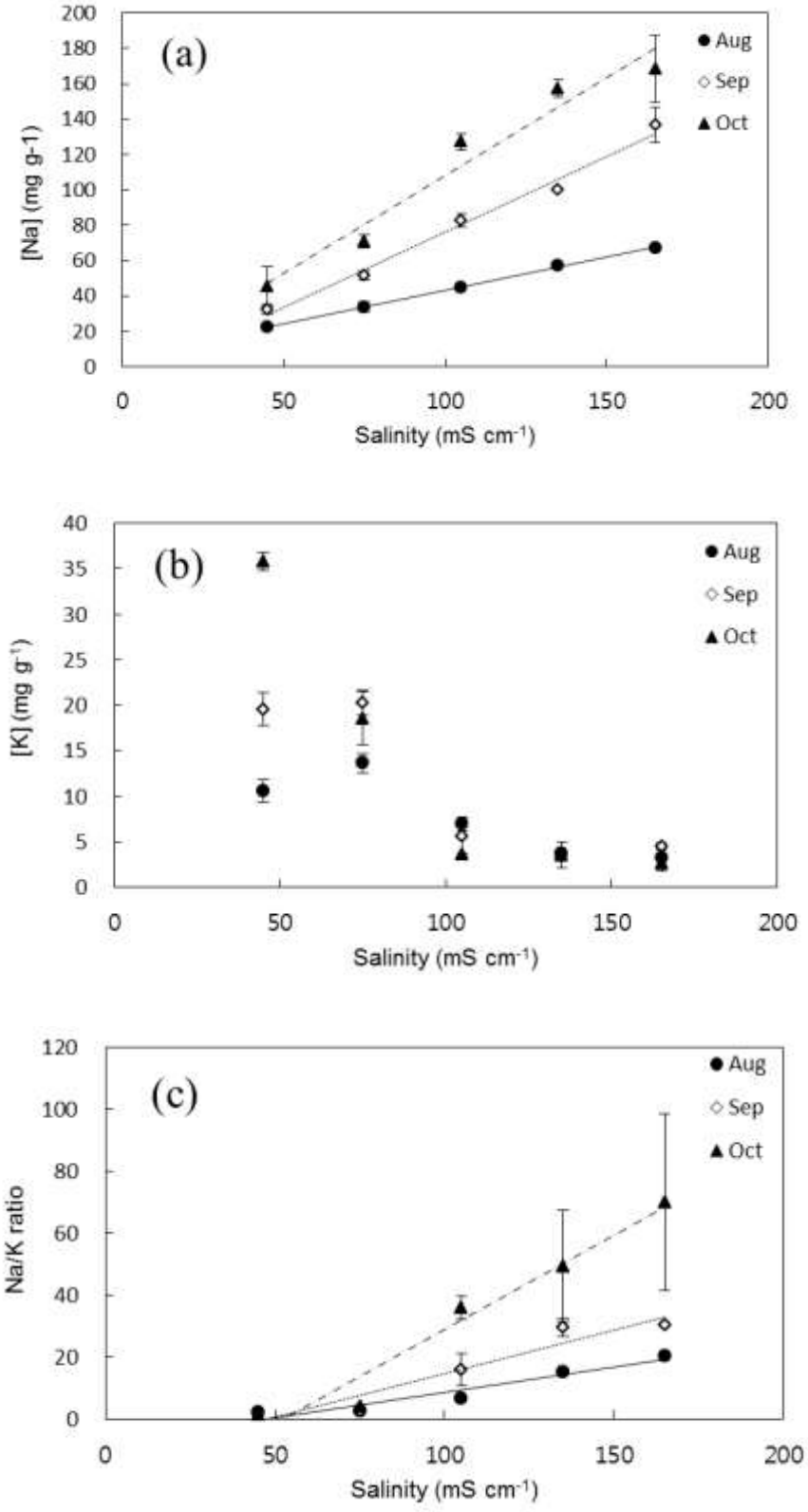

Figure 4.2: Effects of salinity on ion concentrations of Na^+ (a) and K^+ (b) and Na^+/K^+ ratios (c) in the tissue of *R. tuberosa* grown in the pond in 2009. Mean (± S.D). n = 15.

The Na^+ concentrations in *R. tuberosa* tissues were all positively related to increased salinity ($p < 0.0001$, $y = 0.38x + 5.11$, $r^2 \geq 0.96$), but the Na^+ concentration per unit

salinity increased month by month (Figure 4.2a). At the lower salinities K^+ concentrations in August increased as salinity rose, remained the same on September and were high in October (Figure 4.2b). The overall response was a decline in the K^+ as salinity increased ($p < 0.0001$, $r^2 \geq 0.86$). The Na^+/K^+ ratios were all positively related to salinity ($p < 0.0001$, $y = 0.17x - 7.99$, $r^2 \geq 0.92$), and increased over time as the ratio per unit salinity increased from month to month (Figure 4.2c).

4.3.2 Growth of *Ruppia tuberosa* under pond conditions

The total biomass, relative growth rate, flowering, seeds, turions and shoot lengths were influenced by an interaction between month and salinity (Table 4.2). The total biomass of *R. tuberosa* showed significant monthly changes under the different salinity treatments ($F = 4.06$, $p = 0.0023$, Table 4.2 and Figure 4.3a).

Table 4.2: Results of two-way ANOVA analysis on the total biomass, relative growth rate (RGR), flowering, seed and turion densities and shoot length of *R. tuberosa* from the pond experiment conducted in 2009.

Variable	Source	DF	SS	F	P
Total biomass	Month	2	0.07	12.76	< **0.0001**
(DW g)	Salinity	4	0.16	15.13	< **0.0001**
	Month*Salinity	8	0.08	4.06	**0.0023**
RGR	Month	2	0.00	46.42	< **0.0001**
(mg $g^{-1}d^{-1}$)	Salinity	4	0.01	34.67	< **0.0001**
	Month*Salinity	8	0.00	11.47	< **0.0001**
Flowering	Month	2	2.18	7.00	**0.0032**
(flowers pot^{-1})	Salinity	4	1.69	2.71	**0.0485**
	Month*Salinity	8	3.38	2.71	**0.0223**
Seed density	Month	2	34.84	6.47	**0.0046**
(seeds pot^{-1})	Salinity	4	29.69	2.76	**0.0458**
	Month*Salinity	8	59.37	2.76	**0.0206**
Turion density	Month	2	1472.18	39.96	< **0.0001**
(turions pot^{-1})	Salinity	4	189.69	2.57	0.0578
	Month*Salinity	8	379.37	2.57	**0.0287**
Shoot length	Month	2	1.23	30.70	< **0.0001**
(cm)	Salinity	4	1.15	14.41	< **0.0001**
	Month*Salinity	8	0.43	2.67	**0.0241**

The month that the total biomass changes occurred differed significantly across the salinity treatments. Biomass increase was greatest at the salinity of 45 mS cm^{-1}, followed by 75 mS cm^{-1} during September, but it decreased rapidly with further increase in salinity over time under the pond condition. *R. tuberosa* conditions seemed to be depressed when plants were grown in salinity above 105 mS cm^{-1}, and generally

performed poorly. The total biomass observed at the salinity of 165 mS cm^{-1} had relatively lower density in all months.

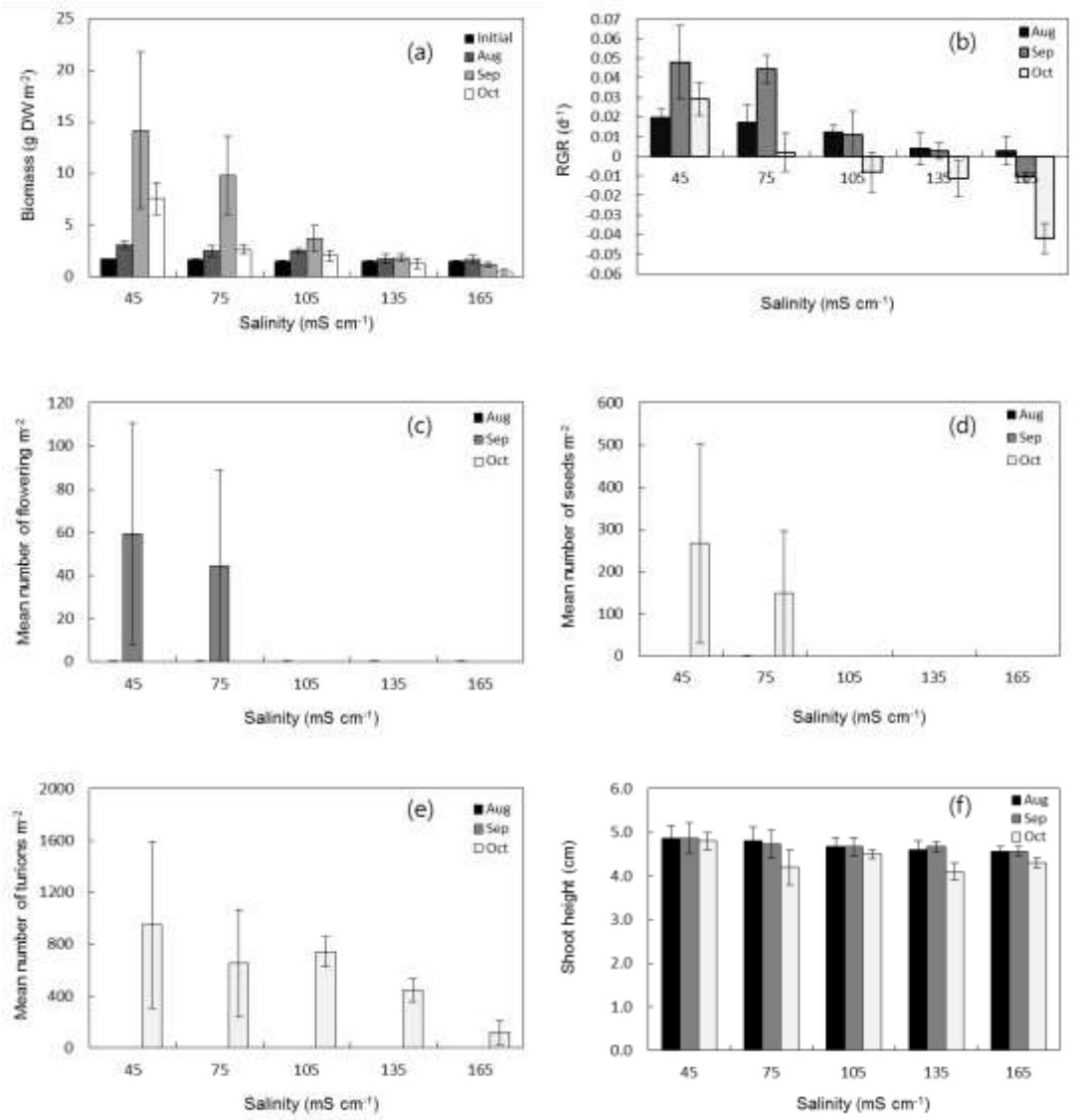

Figure 4.3: Effects of salinity on the total biomass (g DW m^{-2}, (a)), relative growth rate (RGR (d^{-1}), (b)), flowering (flowers pot^{-1}, (c)), seed (seeds pot^{-1}, (d)) and turion densities (turions pot^{-1}, (e)) and shoot length (cm, (f)) of *R. tuberosa* grown in the pond in 2009. Mean (± S.D). n = 15.

The growth rates generally reflected the patterns for the total biomass, having the maximum growth rates at the salinity of 45 mS cm^{-1}, followed by 75 mS cm^{-1}, with the RGRs of 0.047 and 0.044 (d^{-1}) (F = 11.47, p < 0.0001, Table 4.2 and Figure 4.3b). However, the growth rates were all negative in salinities above 75 mS cm^{-1} during October, when shoots and leaves died. Flowering was highest at conductivity of 45, followed by 75 mS cm^{-1} during September but no flowering was observed above the salinity of 105 mS cm^{-1} (F = 2.71, p = 0.0223, Table 4.2 and Figure 4.3c).

Seed production differed between salinities, having the maximum density during October at the salinity of 45 mS cm^{-1}, followed by 75 mS cm^{-1}, but no seeds were detected at the salinities of 105, 135 and 165 mS cm^{-1} (F = 2.76, p = 0.0206, Table 4.2 and Figure 4.3d). Turion density at the salinity of 45 mS cm^{-1} was significantly greater than other salinity treatments during October (F = 2.57, p = 0.0287, Table 4.2 and Figure 4.3e). There were differences in shoot lengths of *R. tuberosa*, but differences between salinity treatments were not consistent in the different months resulting in an interactions between month and conductivity (F = 2.67, p = 0.0241, Table 4.2). Longer shoot lengths were found at the salinity of 45 mS cm^{-1} during August and September, and relatively shorter shoot lengths with further increase in salinity during October, across all salinity treatments (Figure 5.3f).

4.3.3 Effects of ion concentrations on the performance of pond grown *Ruppia tuberosa*

The total biomass, RGRs, flowering, seeds and turions of *R. tuberosa* were significantly affected by Na^+, K^+ and Na^+/K^+ ratios but shoot lengths were only influenced by Na^+ concentrations (Table 4.3). The total biomass, RGRs, flowering, seeds, turions and shoot lengths of *R. tuberosa* were all negatively correlated with increasing salinity.

Table 4.3: Effects of Na^+ and K^+ concentrations and Na^+/K^+ ratios on total biomass (g DW m^{-2}), relative growth rate (RGR (d^{-1})), flowering (flowers pot^{-1}), seed (seeds pot^{-1}) and turion densities (turions pot^{-1}) and shoot length (cm) of *R. tuberosa* under the pond experiment in 2009, when flowering commenced.

Variable		Na^+	K^+	Na^+/K^+ ratio
Total biomass (g DW)	Equation	y = 1.3 – 0.3 In(x)	y = -0.5 + 0.3 In(x)	y = 0.4 – 0.1 In (x)
	r^2	= 0.66	= 0.60	= 0.65
	P	**= 0.0005**	**= 0.0012**	**0.00005**
RGR ($mg\ g^{-1}d^{-1}$)	Equation	y = 0.2 – 0.1 In(x)	y = -0.1+0.1 In (x)	y = 0.1 – 0.1(x)
	r^2	= 0.81	= 0.82	= 0.86
	P	**< 0.0001**	**< 0.0001**	**< 0.0001**
Flowering (flowers pot^{-1})	Equation	y = 6.6 – 1.4 In(x)	y = -2.4 +1.2 In (x)	y = 1.9 – 0.7 In(x)
	r^2	= 0.54	= 0.34	=m0.47
	p	**= 0.0029**	**= 0.0301**	**= 0.0072**
Seed density (seeds pot^{-1})	Equation	y = 27.9 – 5.9 In(x)	y = -1.1 + 5.1 In(x)	y = 7.8 – 2.9 In(x)
	r^2	= 0.54	= 0.32	= 0.46
	p	**= 0.0018**	**= 0.0282**	**= 0.0057**
Turion density (turions pot^{-1})	Equation	y = 93.8 – 18 In(x)	y = 34 +20 In(x)	y = 28 + 42.3(x)
	r^2	= 0.48	= 0.47	= 0.48
	p	**= 0.0042**	**= 0.0051**	**= 0.0042**
Shoot length (cm)	Equation	y = 6.2 – 0.4 In(x)	y = 3.5 + 0.4 In(x)	y = 4.8 – 0.2 In(x)
	r^2	0.32	= 0.18	= 0.26
	p	**= 0.0276**	= 0.1102	=0.0539

4.3.4 Effects of ion concentrations in *Ruppia tuberosa* tissue *in situ*

In the field situation, similar trends to those described above were found for higher Na^+ concentrations with higher salinity (Table 4.4). Lake Cantara (LC5), where the lowest salinity occurred, showed the lowest Na^+ concentrations, but differed with Lake Pipe Clay (LPC4) and Parnka Point (SL3). There were no differences in K^+ concentrations and Na^+/K^+ ratios between LC5 and SL3, but the concentrations and ratios differed at LPC4 where the lowest K^+ concentrations and highest Na^+/K^+ ratios were found. B concentrations were highest at LC5, while highest Ca^{2+} concentrations were found at SL3 (Table 4.4). There was no difference in ion concentrations of Cl^- and Na^+/Ca^{2+} ratios of the plant tissues between the sites.

Table 4.4: Effects of salinity on ion concentrations of Na^+, K^+, Ca^{2+}, B and Cl^- and Na^+/K^+ and Na^+/Ca^{2+} ratios in the tissue of *R. tuberosa* for the field experiment during October 2011 when flowering commenced. Mean (± S.D.). Different letter superscripts indicate significant differences between sites.

	Site		
	SL3	LPC4	LC5
Salinity (mS cm^{-1})	122.97 (0.84) ^{c}	80.67 (1.53) ^{b}	62.57 (2.71) ^{a}
Na^+ (mg DW g^{-1})	56.33 (12.01) ^{b}	48.67 (7.51) ^{b}	38.33 (11.02) ^{a}
K^+ (mg DW g^{-1})	25.77 (7.60) ^{a}	16.77 (2.40) ^{b}	31.33 (3.51) ^{a}
Ca^{2+} (mg DW g^{-1})	38.33 (8.50) ^{a}	17.87 (10.24) ^{b}	24.67 (0.58) ^{b}
B (mg DW g^{-1})	0.13 (0.01) ^{b}	0.13 (0.02) ^{b}	0.27 (0.03) ^{a}
Cl^- (mg DW g^{-1})	115.00 (27.84) ^{a}	87.00 (9.85) ^{a}	72.67 (21.94) ^{a}
Na^+/K^+	2.19 (0.27) ^{a}	2.90 (0.03) ^{b}	1.22 (0.43) ^{a}
Na^+/Ca^{2+}	1.47 (0.52) ^{a}	2.72 (0.50) ^{a}	1.55 (0.43) ^{a}

4.3.5 Species performance of *Ruppia tuberosa in situ*

Flowering of *R. tuberosa* only occurred in LC5, where the lowest salinity of 63 mS cm^{-1} was found during October (Table 4.5). LC5 had the lowest turion density relatively while LPC4 had the highest. Longer shoot lengths were detected at SL3, where the highest salinity occurred, but no difference in shoot lengths occurred between sites of LPC4 and LC5.

Table 4.5: Species performance of flowering (flowers pot^{-1}) and turion densities (turions pot^{-1}) and shoot length (cm) of *R. tuberosa* grown *in situ* in 2011. Mean (± S.D).

Variables	Site		
	SL 3	LPC 4	LC 5
Flowering (flowers pot^{-1})	0	0	237 (93)
Turions (turions pot^{-1})	326 (282)	1807 (372)	89 (44)
Shoot length (cm)	11.4 (1.04)	5.1 (0.21)	5.1 (0.15)

4.4 Discussion

4.4.1 The total biomass and relative growth rate in response to ion concentrations

Many aquatic macrophytes are undergoing dramatic declines in their population and distribution due to increasing salinisation, influenced by global climate change, changing land-use and flow regulation, all a consequence of natural and anthropogenic impacts. *R. tuberosa* in the Coorong region, South Australia, is significantly influenced

by increased salinity. Reduction in the total biomass as a result of increased salinity is well known for most halophyte species (Santamaría et al., 1995; Carruthers et al., 1999; Sim et al., 2006). Sim et al. (2006) found a significant reduction for *R. polycarpa*, *Lamprothamnium macropogon* (A. Braun) Ophel and *Lamprothamnium succinctum* (A. Braun in Ascherson) Wood as salinity increased, which is in agreement with Jampeetong and Brix (2009) for other macrophytes such as *Salvinia natans* L. when exposed to high salinity.

RGRs generally reflected the patterns for the total biomass. The decline in RGRs was reflected in a lower number of leaves and shorter shoots when exposed to high salinity, and manifested in reduction in RGRs and subsequent reduction in total biomass. The reduction in biomass was correlated with increased ions of Na^+ and K^+ and Na^+/K^+ ratios in the tissue of *R. tuberosa* with exposure to salinity stress. It has generally been assumed that Na^+ and Cl^- ions are toxic to plants when present in high concentrations in plant cells, leading to several morphological and physiological changes (Cramer et al., 1990; Jampeetong and Brix, 2009). This study showed that a positive correlation exists between Na^+ and Cl^- concentrations in plant tissues and the inhibition of growth. It was apparent that Na^+ concentration increased continuously in the tissue of *R. tuberosa* when exposed to high salinity. Yeo et al (1991) demonstrated that Na^+ levels increased in expanding leaf tissue by exposure to salinity, and the increases were accompanied by reduced leaf elongation, inducing the growth reduction by cytoplasmic Na^+ toxicity or turgor reduction in elevated salinity (Bernstein et al., 1995). This is because ion toxicity in plant cells correlating with high Na^+ concentrations and high Na^+/K^+ ratios due to Na^+ interfering with K^+ uptake and concentrations in plant cells (Davenport et al., 1997), and includes membrane dysfunction and generation of reactive oxygen species in cells (Cramer et al., 1990). Indeed, K^+ concentrations in the tissue of pond grown *R. tuberosa* were decreased dramatically when salinity increased to 105 mS cm^{-1}, and further decreased with further increase in salinity, resulting in very high Na^+/K^+ ratios which are a crucial factor in salt injury, as has been shown in other studies (Davenport et al., 1997; Jampeetong and Brix, 2009).

4.4.2 Sexual reproduction in response to ion concentrations

Flowering of *R. tuberosa* was strongly affected by salinity, which is similar to *R. polycarpa* (Sim et al., 2006). The difference, however, between the pond and the field plants became apparent in the timing of flower production. Flowering of *R. tuberosa*

was detected in September in the pond, whereas flowering was detected in October in the field. This difference may be related to the warming trend of the pond tending to promote flowering (McMillan, 1976), and may suggest that high temperature may restrict flower production, or an interaction between salinity and temperature may be contributing to ion accumulations in plant tissues, resulting in high ion concentrations in plant cells that may restrict flowering. In fact, even though ion concentrations were determined in September for pond plants when flowering was observed, this ion concentration for pond plants at 45 mS cm^{-1} was much higher than for field plants, determined in October, at 63 mS cm^{-1}. Pond plants showed lower numbers of flower and lower numbers of seed at only 45 and 75 mS cm^{-1} treatments. This is likely to be related to high Na^+/K^+ ratios and B deficiency. In fact, as mentioned earlier, high Na^+ concentrations was shown to reduce K^+ concentrations, resulting in very high Na^+/K^+ ratios. In this study, B concentrations decreased dramatically with increasing Na^+/K^+ ratios. Therefore, the inhibition of flowering may have resulted from the low B concentration in plant tissues. Dell and Huang (1997) demonstrated that B concentrations in plant cells is more sensitive to sexual reproduction than vegetative growth. Furthermore, Blevins and Lukaszewski (1998) reported that B deficiency causes flower malformations and sterility. B deficiency inhibits the growth and reproduction of plants through limiting cell enlargement and cell division.

4.4.3 Asexual reproduction in response to ion concentrations

Turion density was significantly greater at 81 mS cm^{-1} in Lake Pipe Clay than at salinities of 63 mS cm^{-1} in Lake Cantara, or at 123 mS cm^{-1} in Parnka Point. In the pond, the turion density appeared to be positively correlated to total biomass and plant performance. In the pond experiment, the plants seemed to be affected by environmental conditions, such as salinity and high water temperature, that consequently influenced the total biomass and plant performance, and salinity stress tended to develop over time in parallel with water temperature. However, in general, turion density was positively correlated with increasing salinity. In contrast to flowering, the production of turions appeared to be related to low K^+ and high Na^+/K^+ ratios. The interactive effects of Na^+ with other ions such as K^+ in *R. tuberosa* contributed to producing turions at high salinity.

4.4.4 Shoot length in response to ion concentrations

Ion toxicity induced by high salinity results in inhibition of Ca^{2+} uptake and concentration (Reid and Smith, 2000), and this results in high Na^{+}/Ca^{2+} ratios that cause nutritional imbalance, thereby inducing Ca^{2+} deficiency (Maas and Grieve, 1987). Previous studies demonstrated Ca^{2+} deficiency symptoms in several plant species under salinity stress (Davenport et al., 1997), leading to reduction of the growth of shoots and shorter leaves of plants (Maas and Grieve, 1987). This present study showed a positive correlation between Ca^{2+} concentrations and shoot length under salinity stress. This may indicate that plants may have a greater requirement for calcium under saline conditions.

4.4.5 Summary of growth and reproduction

The effect of salinity on plant development is reflected in altered patterns of plant growth and development. Continuous exposure to elevated salinity progressively decreases leaf size over time. This may be a direct effect of salinity on rate of cell division, to a slower rate of expansion, or a decrease in the duration of expansion. If cell division was affected, even if cell growth potential was not affected, final leaf size would be limited due to reduced cell numbers. This is because internal excesses of particular ions may cause membrane damage, interfere with solute balances, or cause shifts in nutrient concentrations. Na^{+} accumulation in *R. tuberosa* tissues is the primary factor depressing growth and reproduction, and Na^{+} is specifically toxic to *R. tuberosa* interfering with metabolism by competing with K^{+} at high salinity. High Na^{+} concentrations in the cells inhibit growth by affecting uptake of K^{+} and Ca^{2+}, or by direct toxicity in the cytoplasm (Reid and Smith, 2000).

Ion toxicity resulting from elevated Na^{+} and Cl^{-} concentrations in the tissue of plants may inhibit the uptake of other ions such as Ca^{2+} (Cramer et al., 1990). Subsequently, this can result in high Na^{+}/Ca^{2+} ratios, leading to Ca^{2+} deficiency (Reid and Smith, 2000). Maas and Grieve (1987) reported that the high Na^{+}/K^{+} ratios impair the selective permeability of membranes, and cause nutritional imbalance, thereby inducing serious Ca^{2+} deficiency. Reid and Smith (2000) reported that Ca^{2+} deficiency in barley induced by salinity is due to inhibition of Ca^{2+} transport. Therefore, the reduction of growth by salinity is likely to be related to these factors. However, supplemental Ca^{2+} may protect the plants from ion toxicity by reducing Na^{+} uptake, thereby reducing the toxic effect of Na^{+} in plants (Reid and Smith, 2000). This may be because Ca^{2+} plays a role in the reduction of Na uptake by inhibiting the passive influx of Na^{+} across the plasmalemma

of root cells (Davenport et al., 1997). Ca^{2+} is also likely to improve K^+ uptake under high salinity, consequently reducing the Na^+/K^+ ratios in the tissues of plants (Davenport et al., 1997), thereby reducing K^+ deficiency and also B deficiency. Increasing Ca^{2+} concentration is thought to be of importance in salt tolerance, and is hence suspected to be of importance to growth and reproduction of *R. tuberosa*.

4.4.6 Management implications

The study of the growth and reproduction of *R. tuberosa* in the Coorong suggests that the inhibition of growth and reproduction of *R. tuberosa* is due to the alteration in magnitude of Na^+, K^+, and Ca^{2+} concentrations in plant cells. The study indicates that the failure of sexual reproduction induced by high Na^+/K^+ ratios or low B may be an important factor causing the observed reduction in the distribution and abundance of *R. tuberosa* in the Coorong. Thus, to ensure successful reproduction of *R. tuberosa* in the southern end of the Coorong salinity levels should be maintained below 105 mS cm^{-1}. *Ruppia* habitat restoration via reduced salinity levels may be assisted by providing adequate inflows of fresh water to the Coorong to decrease Na^+ concentrations and Na^+/K^+ ratios in the plant cells, and by supplying Ca^{2+}, which is likely to improve K^+ uptake under high salinity. In general, additional Ca^{2+} restores K^+ uptake, and will overcome the problem of Ca^{2+} deficiency, because Ca^{2+} is needed for many important processes in plants under high salinity stress (Reid and Smith, 2000).

Chapter 5 The effect of salinity on photosynthesis of *Ruppia tuberosa*

5.1 Introduction

In many regions, the combination of inappropriate irrigation and evapotranspiration exceeding precipitation is responsible for extending soil and water salinisation (Cramer and Hobbs, 2002). Salinity is one of the major environmental constraints influencing aquatic plants by interfering with the physiology of various life stages, thereby affecting distribution and abundance in semi-arid and arid regions of the world (Sudhir and Murthy, 2004; Munns and Tester, 2008; Parida and Jha, 2010). It is widely recognised that unfavourable salinities restrict the growth and reproduction of aquatic macrophyte communities (Khan and Ungar, 1996; Tessier et al., 2000; Elsey-Quirk et al., 2009). Salinity stress result in osmotic and ionic stress at both whole plant and cellular levels (Pujol et al., 2000). High sodium (Na^+) and chloride (Cl^-) concentrations are known to induce changes in protein activity, because ions affect the hydration of water, causing an inhibition of enzyme activity (Volkmar et al., 1997). Internal excesses of Na^+ and Cl^- may cause membrane damage, interfering with ion balances by reducing uptake of other ions including potassium (K^+) and calcium (Ca^{2+}), or cause shifts in nutrient concentrations, thus affecting the plant growth, physiology and survival (Jampeetong and Brix, 2009).

It is known that salinity stress has detrimental effects on plant growth, via the impairment of diverse physiological processes through photosynthesis (Munns, 2002; Naumann et al., 2007). Salinity stress can inhibit photosynthetic capacity mostly through photosystem II activity (Naumann et al., 2007) by factors affecting stomatal closure, leading to a reduction intracellular CO_2 partial pressure, inhibition of biochemical reactions and feedback carbon metabolism (Seemann and Critchley, 1985), and through non-stomatal factors (Bethke and Drew, 1992). The reduction in photosynthesis induced by ion toxicity stimulates excess excitation energy, causing photo-damage to photosystem II, thereby resulting in limited energy dissipation (Gilmore, 2001).

Halophytes are known for their ability to tolerate/maintain photosynthetic performance over a wide range of light and salinity conditions (Ralph and Gademann, 2005). This

may be due to photo-adaptive and photo-protective mechanisms at levels ranging from the whole plant, the leaf or cell, to the photosynthetic membranes (Masojídek et al., 2000), via biochemical changes in the content of cell pigments (Chlorophylls and carotenoids). One of the photo-protective mechanisms is dissipation of excess excitation energy by the xanthophyll cycle (Demmig-Adams and Adams III, 1992; Gilmore, 1997). The xanthophyll cycle represents one of the most important systems to prevent photo-damage, because it allows the dissipation of the enhanced excitation energy as heat, which can be assessed by changes in the non-photochemical quenching (Y_{NPQ}) at high light (Müller et al., 2001). Y_{NPQ} is indicative of ability to cope with oxidative stress following over-reduction of reaction centres (Redondo-Gómez et al., 2006). In addition, the maximum quantum yield (Fv/Fm) is a robust indicator of photosynthetic stress used to diagnose thermal stress, salinity and other environmental stresses (Campbell et al., 2006; Biber et al., 2009).

It is reported that salinity induced accumulations of organic compounds, such as proline and glycine betaine, are compatible solutes that accumulate in response to osmotic stress, and the accumulation of these osmolytes represents an important adaptive response to salinity stress (Moghaieb et al., 2004). Halophytes are also known to retain high levels of salt without salt injury, preferentially accumulating Na^+ into cell vacuoles, where it is used to balance the osmotic potential of the salts outside the plants (Flowers et al., 1977). Previous studies suggest that Na^+ is the main contributor to osmotic adjustment under salinity stress (Brock, 1981a; Moghaieb et al., 2004; Parida and Jha, 2010). Osmotic adjustment is one of the major cellular protection mechanisms against the negative effects of salinity, drought and temperature stress (Moghaieb et al., 2004).

Ruppia species, submerged halophytes, are important primary producers in brackish to hyper-saline ephemeral and permanent habitats throughout the world (Verhoeven, 1979). In Australia, *Ruppia tuberosa* J. Davis and Tomlinson and *Ruppia megacarpa* R. Mason occur in salinities of 22-300 mS cm^{-1} and 9-67 mS cm^{-1} respectively (Brock, 1982b). *R. tuberosa* is distributed at water depth of ~0.6 m throughout the Coorong, South Australia (Brock, 1979, 1981b; Paton, 2005), a coastal lagoon that is of international importance under the Ramsar convention. *R. tuberosa* in the Coorong is normally subject to high salinity and high light under field conditions, and is subject to seasonal hyper-salinity. Although reduction in growth of *R. tuberosa* caused by ion toxicity was demonstrated in an earlier chapter, (Chapter 4), growth responses need further

investigation to understand mechanisms of adaptions to high salinity concentrations and high light conditions. This study was designed to elucidate growth and photosynthetic responses of *R. tuberosa* to salinity and light. It is hypothesised that a possible cause of reduced growth of *R. tuberosa* under high salinity concentrations includes inhibition of photosynthesis due to ion toxicity. The aims of this study were to investigate whether the observed growth reduction was related to photosynthesis as tissue ion concentrations changed in response to rising salinities and whether *R. tuberosa* possessed a mechanism for photo-protection. This was examined through measurements of PSII, photosynthesis (Y_{II}), photochemical (Y_{NO}) and non-photochemical quenching (Y_{NPQ}).

5.2 Material and methods

5.2.1 Site description

The Coorong, a Ramsar wetland located in South Australia (Ramsar Convention on Wetlands: http://www.ramsar.org/), is an inter-dune coastal lagoon that extends southeast from the mouth of the River Murray for approximately 140 km (Figure 5.1). It is comprised of two main lagoons (North and South) separated by a narrow channel at Parnka Point. There is a longitudinal salinity gradient in the Coorong. The lowest salinity was observed at the northern end of the system, and salinity increased towards the southern end with salinity ranging from 55 mS cm^{-1} to over 160 mS cm^{-1} in autumn, winter and spring. Salinities in the south lagoon of the Coorong were typically hyper-saline, around 220 mS cm^{-1} in summer due to evaporation, almost four times higher than seawater.

Lake Cantara is an ephemeral lake located adjacent to the Coorong, about 250 km from Adelaide, South Australia (Figure 5.1). The lake is small (144 ha or ~ 1.5 km^2), shallow, (approximate water depth of ~ 0.5m in winter), and is dry during summer, (December-February). Salinity at Lake Cantara ranges from 27 to 115 mS cm^{-1} from August to November, and there is a large abundance of *R. tuberosa*. The lake is presently athalassic with no direct connection to either the Coorong or the sea.

5.2.2 Field sampling

R. tuberosa samples were collected in August, September and October in 2009 at four sites (water depths of 0.2 to 0.5m) where *R. tuberosa* was present: North Lagoon 1 (NL1), North Lagoon 2 (NL2), North Lagoon 3 (NL3) and South Lagoon (SL4),

spanning the salinity gradient that exists in the Coorong, as well as at one ephemeral lake, Lake Cantara (LC5, Figure 5.1; Table 5.1). Chlorophyll a fluorescence of *R. tuberosa* was measured on each sampling occasion at each site *in situ*. After these measurements samples were stored in plastic bags for return to the laboratory for measurement of ion concentrations in the plant tissue.

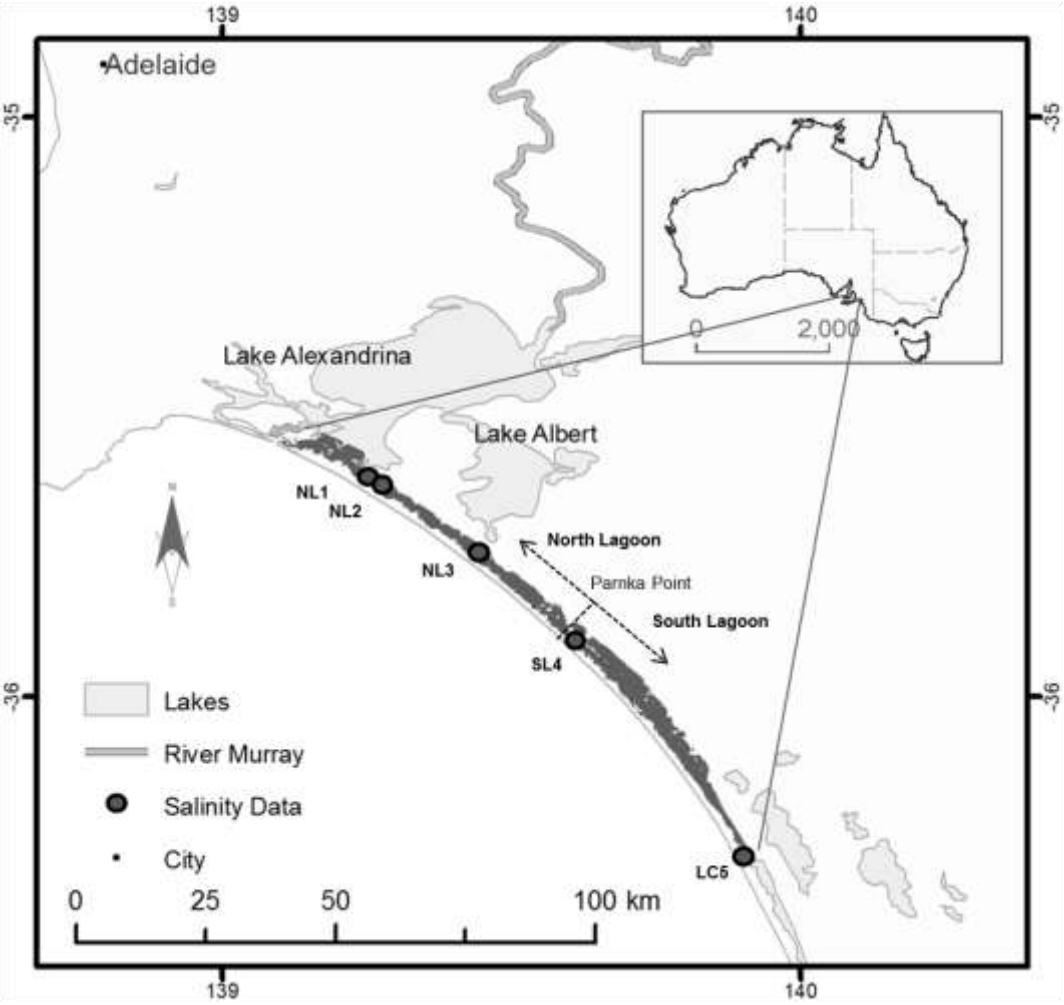

Figure 5.1: Location of the Coorong, South Australia (NL1 = Mulbin Yerrok Point; NL2 = Long Point; NL3 = Noonameena; SL4 = Parnka Point and LC5 = Lake Cantara).

Table 5.1: Summary of GIS co-ordinates, location and habitat description of the field survey sites.

Site	GIS	Location	Habitats
NL1	S 35.67004 E 139.13876	Mulbin Yerrok Point	Permanent saline to hyper-saline
NL2	S 35.69321 E 139.16457	Long Point	Permanent saline to hyper-saline
NL3	S 35.77388 E 139.27284	Noonameena	Permanent saline to hyper-saline
SL4	S 35.90279 E 139.39842	Parnka Point	Permanent hyper-saline
LC5	S 36.33338 E 139.74283	Lake Cantara	Ephemeral saline lake

5.2.3 Data collection

Measurement of chlorophyll fluorescence

Chlorophyll a fluorescence was measured using a pulse amplitude modulated fluorimeter (PAM-2003, Walz GmbH, Effeltrich, Germany). It enables quantification of the three possible outcomes of light absorbed by chlorophyll a in PSII (Klughammer and Schreiber, 2008). The energy absorbed from the light can either drive photosynthesis Y_{II}, be passively dissipated as heat and fluorescence (Y_{NO}) or be transferred to the xanthophyll cycle and harmlessly dissipated as heat (Y_{NPQ}). Samples were randomly collected from NL1, NL2, NL3, SL4 and LC5, at water depths of ~ 0.3 m, on 30th August, 30th September and 30th October 2009. At this time salinities at the Coorong from NL1 to SL4 varied from 40 to 105 g/L, and salinity at LC5 ranged from 15 to 29 g/L. Three replicates of leaves were used for each site, and stored for the measurement of ion concentrations. The leaves were dark-adapted for 30 minutes before measurements were taken in order to allow complete oxidation of the PSII reaction centers, followed by a saturating pulse, and then maximum photochemical efficiencies were estimated by measuring Fv/Fm ratios (Ralph and Burchett, 1998).

To assess the effect of ion concentrations and Na^+/K^+ ratios at low and high irradiances on Y_{II}, Y_{NO} and Y_{NPQ}, fluorescence measurements were made early in the morning (32 µmol m^{-2} s^{-1}) and in the afternoon (1464 µmol m^{-2} s^{-1}) on 30th September 2009. Samples of *R. tuberosa* shoots were randomly collected from the Coorong: NL1 (35°67′ S, 139°14′E), NL2 (35°69′S, 139°16′E), NL3 (35°77′S, 139°27′E) (35°77′S, 139°27′E), SL4 (35°90′S, 139°39′E); and the neighbouring lake: LC5 (36°33′S, 139°74′E), at water depths of ~ 0.3 m.

To assess the effect of salinity on diurnal changes of Y_{II}, Y_{NO} and Y_{NPQ}, fluorescence measurements were conducted on samples collected from pre-dawn to sunset at two to three hours intervals on 3rd October 2009. Samples were randomly collected from the Coorong: NL1 (60 mS cm^{-1}), NL3 (75 mS cm^{-1}) and SL4 (125 mS cm^{-1}), at water depths of ~ 0.3 m. Chlorophyll a fluorescence measurements of maximum quantum yield (Fv/Fm) were made for each sample at each site. Irradiance (µmol photons m^{-2} s^{-1}) measured on 3rd October 2009 at Mt Bold Reservoir were used.

Measurement of ion concentrations

Monthly measurements of Na^+ and K^+ concentrations in the tissue of *R. tuberosa* were performed on the same plant leaves used for the measurements of Chlorophyll fluorescence. Samples (leaves) were dried at 60°C to constant weight, and Na^+ and K^+ concentrations were determined by flame photometry following extraction in 8 mL of 0.1 mM nitric acid in a boiling water bath for 30 min.

To determine Boron (B) tissue concentrations in *R. tuberosa*, samples of three shoots were randomly selected from each replicate when plants commenced flowering in September 2009. B concentrations of shoots were determined on oven dried materials using inductively coupled plasma optical emission spectrometry. The samples were digested using nitric acid and hydrogen peroxide in 50 mL polypropylene centrifuge tubes with lids in place to prevent contamination (Wheal et al., 2011).

5.2.4 Statistical analyses

All statistics were carried out using the software JMP IN version 404 (SAS Institute Inc., 1989-2001). All data were tested for normal distribution and variance homogeneity using the Shapiro–Wilk and Bartlett's tests, respectively. To determine the effect of salinity on Na^+ and K^+ concentrations and Na^+/K^+ ratios in the tissue of *R. tuberosa*, log-linear regression analyses were conducted. Log-linear regression analyses were conducted to determine the relationship between Fv/Fm and ion concentrations (Na^+ and K^+) and Na^+/K^+ ratios, and between B and Na^+/K^+ ratios for *R. tuberosa* tissues. Absorbed excitation energy in PS II between three fundamental pathways, expressed in terms of the complementary quantum yields of photochemical conversion (Y_{II}), regulated thermal energy dissipation related to NPQ (Y_{NPQ}) and non-regulated heat dissipation and fluorescence emission (Y_{NO}). The sum of these parameters is always unity: $Y_{II} + Y_{NO} + Y_{NPQ} = 1$. The three parameters were calculated using the formula as $Y_{II} = (Fm'-F)/ Fm' = \Delta F/ Fm'$, $Y_{NPQ} = F/ Fm'-F/ Fm$ and $Y_{NO} = F/Fm$, (Klughammer and Schreiber, 2008), where F is fluorescence yield measured briefly before application of a saturation pulse, Fm and Fm' are maximal fluorescence yields of dark-adapted and illuminated samples with all PS II centers closed, and ΔF is the increase of fluorescence yield, Fm'-F, induced by a saturation pulse.

5.3 Results

5.3.1 Effects of salinity on ions (Na^+ and K^+) and Na^+/K^+ ratios in *Ruppia tuberosa*

Na^+ (y = 43.50 In(x) – 119.73, r^2 = 0.97, $p < 0.0001$) concentrations and Na^+/K^+ ratios (y = 1.23 In(x) – 3.40, r^2 = 0.89, $p < 0.0001$) showed a significant increase in the tissues of *R. tuberosa* as salinity rose, ranging from 37.57 ± 0.21 to 88.50 ± 15.26 (mg g^{-1} DW) for Na^+ concentrations and from 0.94 ± 0.01 to 2.38 ± 0.05 for Na^+/K^+ ratios (Figure 5.2).

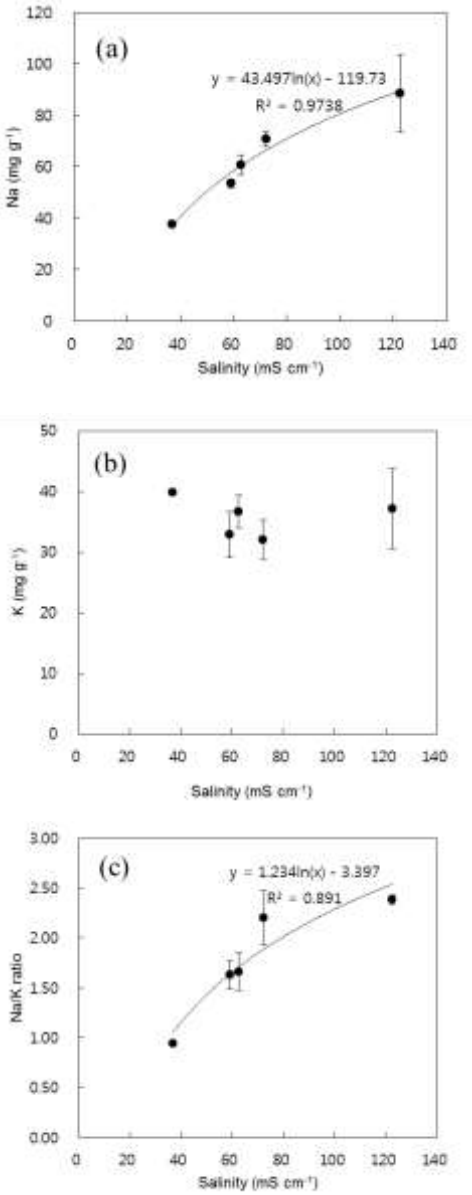

Figure 5.2: The effect of salinity on ion concentrations of Na^+ (a) and K^+ (b) and Na^+/K^+ ratios (c) in the tissue of *R. tuberosa* during September. Mean (± S.D). Mean (± S.D). n = 15.

5.3.2 Effects of salinity on the chlorophyll fluorescence parameter of Fv/Fm

Spatial and temporal changes on Na^+ and K^+ concentrations and Na^+/K^+ ratios under different salinity regimes

There were spatial and temporal differences in salinity levels, and a longitudinal salinity gradient in the Coorong (Figure 5.3a). The lowest salinity occurred at the northern end, NL1, and salinity increased towards the southern end, SL4, with salinity ranging from 59 mS cm^{-1} to over 145 mS cm^{-1} from August to October. Lake Cantara, LC5, had the lowest salinity and showed monthly variations ranging from 27 to 43 mS cm^{-1} (Figure 5.3a).

Salinity varied over time between sites affecting ionic uptake and concentrations of Na^+ and K^+ and Na^+/K^+ ratios in plant tissue (Figure 5.3). Increased salinity led to a significant increase in Na concentrations over time at all sites, ranging from 21.98 ± 1.55 to 45.82 ± 0.55 mg g^{-1} DW (Figure 5.3b). The concentrations of K^+ in the tissue of plants varied considerably over time, being generally highest in September at all sites, ranging from 15.13 ± 0.66 to 39.86 ± 0.42 mg g^{-1} DW (Figure 5.3c). A significant increase in Na^+/K^+ ratios with an increase in salinity was observed over time, ranging from 0.94 ± 0.0 to 4.87 ± 1.84 (Figure 5.3d).

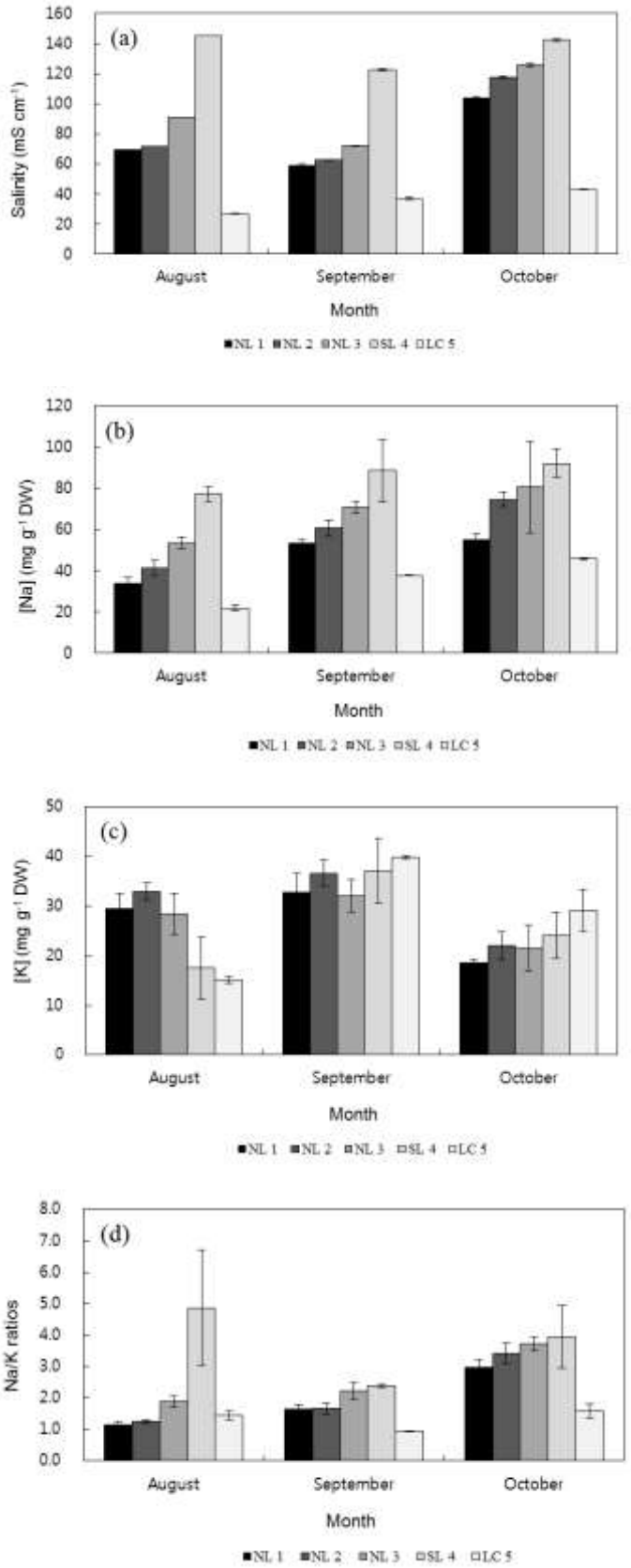

Figure 5.3: Salinity changes (a) and ion concentrations of Na^+ (b) and K^+ (c), and Na^+/K^+ ratios (d), in the tissue of *R. tuberosa* for the three months of August to October 2009 at the five study sites: NL1 (black column); NL2 (dark-grey column); NL3 (grey column); SL4 (light-grey column); and LC (white column). Mean (± S.D). n = 15.

Effects of Na^+ and K^+ concentrations and Na^+/K^+ ratios on the chlorophyll fluorescence parameter of Fv/Fm

The Fv/Fm ratios of *R. tuberosa* were significantly affected by Na^+ concentrations and Na^+/K^+ ratios in the tissues of plant, but not by K^+ concentrations (Figure 5.4). The Fv/Fm ratio was negatively related to increased Na^+ concentrations and Na^+/K^+ ratios (Table 5.2). Generally the highest Fv/Fm ratio was observed at the lowest Na^+ concentrations and Na^+/K^+ ratios in the tissues of *R. tuberosa* during September, but it decreased considerably over time with increasing Na^+ and Na^+/K^+ ratios, ranging from 0.67 ± 0.05 to 0.39 ± 0.07.

Table 5.2: Effects of Na^+ and K^+ concentrations and Na^+/K^+ ratios in the tissues on Fv/Fm ratios of *R. tuberosa*.

Month		Na^+	K^+	Na^+/K^+ ratio
August	Equation	y = 1.30 – 0.20 In(x)	-	y = 0.63 – 0.15 In(x)
	r^2	0.66		= 0.57
	P	**0.0002**		**0.0011**
September	Equation	y = 1.15 – 0.13 In(x)	-	y = 0.67 – 0.12 In(x)
	r^2	0.48		= 0.52
	p	**0.0043**		**= 0.0026**
October	Equation	y = 1.15 – 0.23 In(x)	-	y = 0.72 – 0.20 In(x)
	r^2	0.60		0.75
	p	**0.0007**		**< 0.0001**

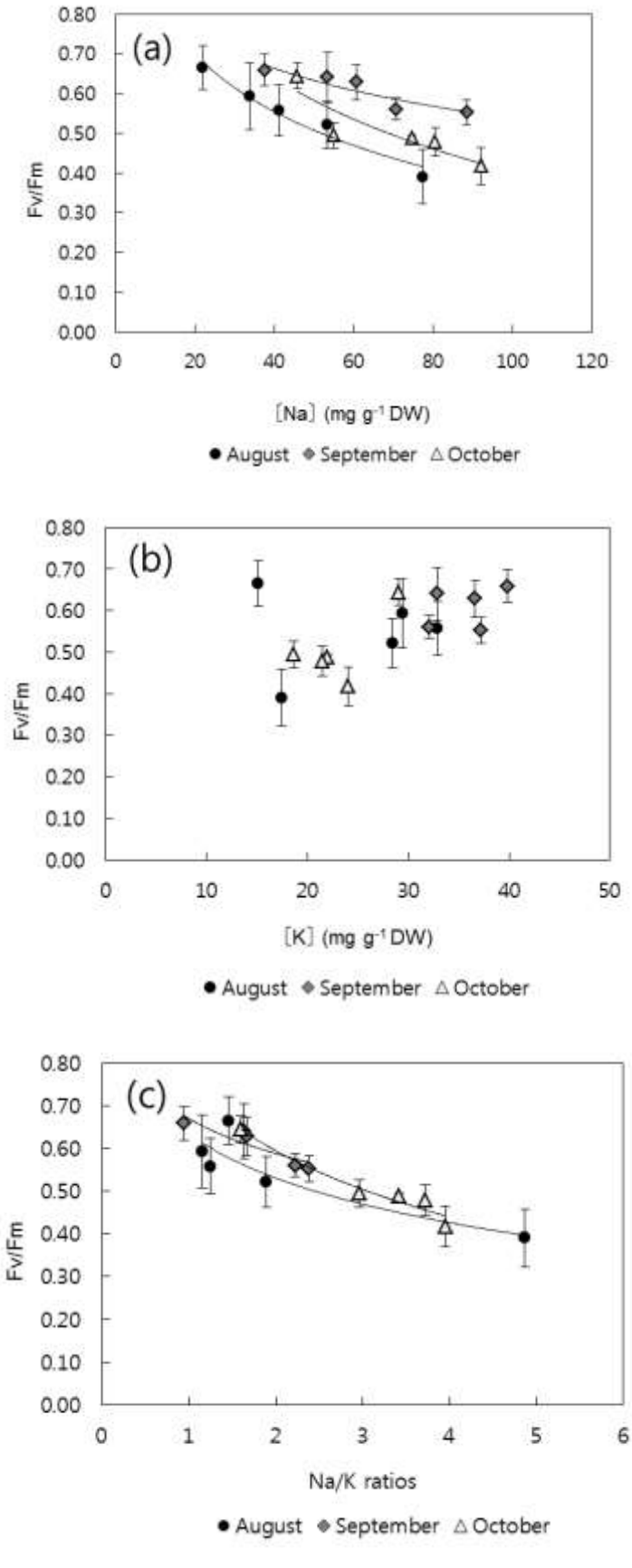

Figure 5.4: The effect of ion concentrations of Na^+ (a), K^+ (b) and Na^+/K^+ ratios (c) on Fv/Fm under field conditions for the three months of August to October 2009. Mean (± S.D). n = 15.

The relationship of Na^+/K^+ ratios and B concentrations

The concentrations of B in *R. tuberosa* tissues were higher at the lower Na^+/K^+ ratios, but a significant decrease in B was observed as Na^+/K^+ ratios increased, ranging from 0.32 ± 0.02 to 0.10 ± 0.001. The levels of B were negatively influenced by increasing Na^+/K^+ ratios, y = 0.28 – 0.19 In(x), $r^2 = 0.86$, $p < 0.0001$ (Figure 5.5).

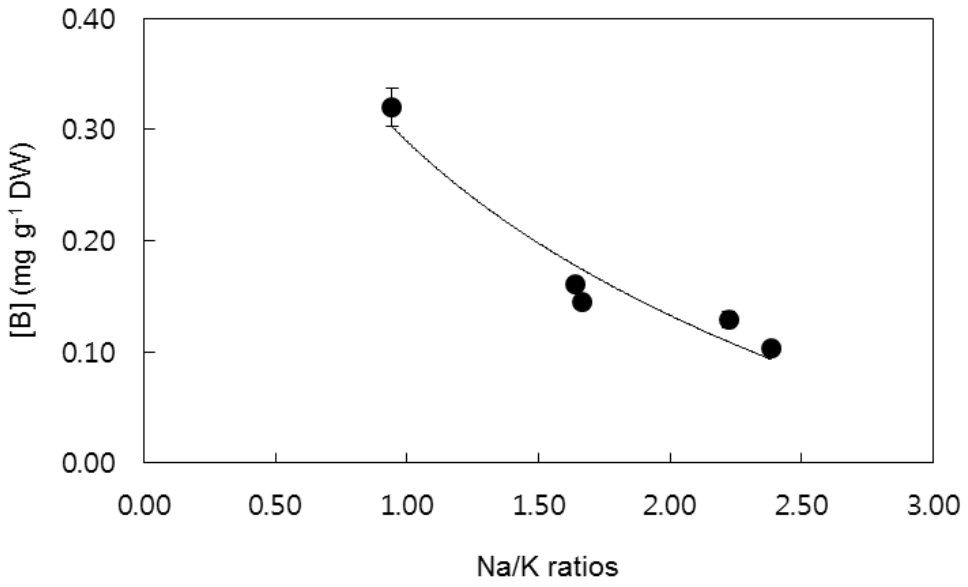

Figure 5.5: The relationship of Na^+/K^+ ratios and B concentrations in the tissue of *R. tuberosa*. Mean (± S.D). n = 15.

5.3.3 Effects of Na^+, Na^+/K^+ ratios at high and low light on Y_{II}, Y_{NO} and Y_{NPQ}

Y_{II} varied significantly within high and low light intensities, and within different salinity regimes. Y_{II} was highest at the lower Na^+ concentrations and Na^+/K^+ ratios in low light intensity, but subsequently Y_{II} decreased with increases in Na^+ concentrations and Na^+/K^+ ratios, while Y_{NO} remained relatively stable (Figure 5.6). Y_{NPQ} increased markedly at high light intensity, but it remained relatively stable at low light intensity, except for the highest values of Na^+ and Na^+/K^+ ratios.

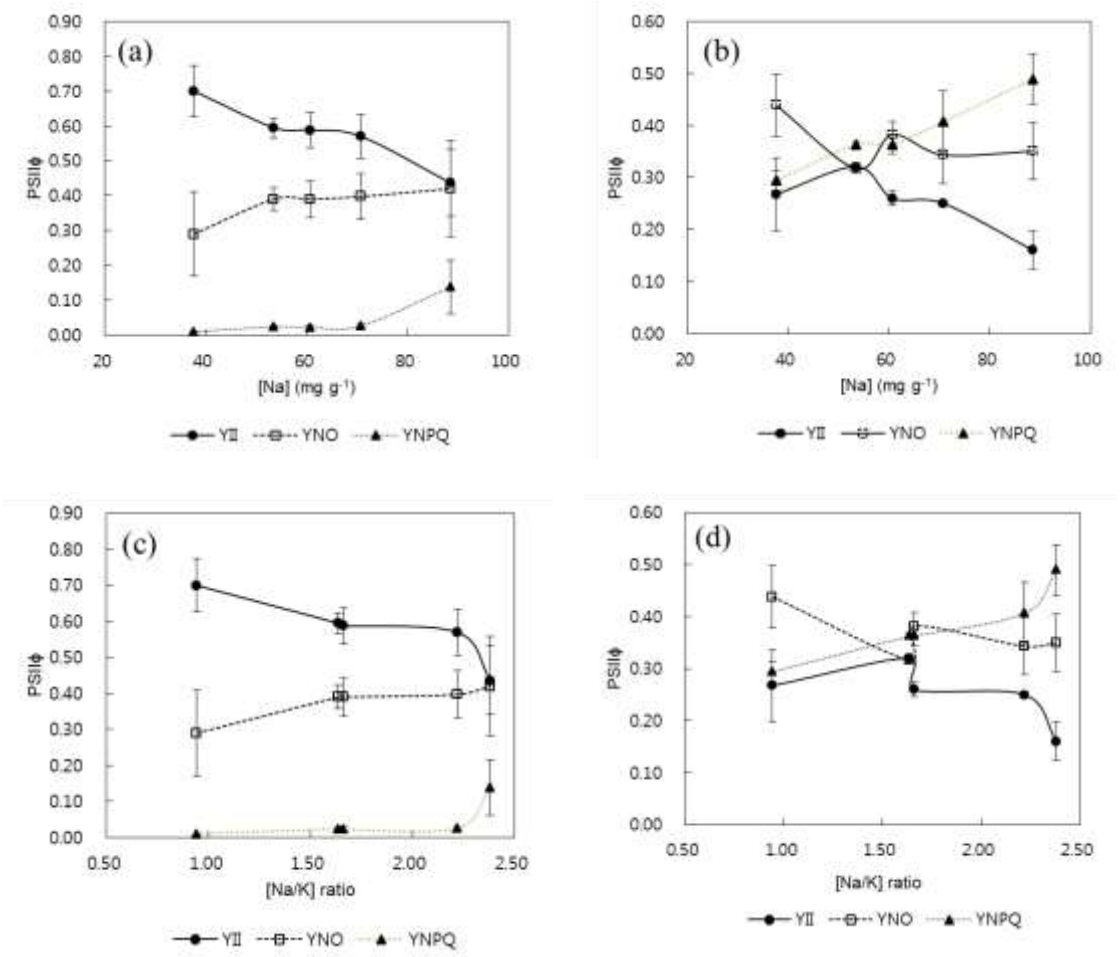

Figure 5.6: The effect of ion concentrations of Na^+ at low (32 µmol quantum m^{-2} s^{-1}, (a)) and high (1464 µmol quantum m^{-2} s^{-1}, (b)) light irradiances, and Na^+/K^+ ratios at low (32 µmol quantum m^{-2} s^{-1}, (c)) and high (1464 µmol quantum m^{-2} s^{-1},(d)) light irradiances on the complementary yields of YII, YNO and YNPQ. Mean (± S.D). n = 15.

5.3.4 Effects of salinity on diurnal changes in Y_{II}, Y_{NO} and Y_{NPQ}

Diurnal changes in Y_{II}, Y_{NO} and Y_{NPQ} occurred under different salinities of 60, 75 and 125 mS cm^{-1} in *R. tuberosa* as irradiance and decreased (Figure 5.7). There was a rapid decline in Y_{II} from pre-dawn to midday with a concomitant increase in Y_{NPQ} in all salinities of 60, 75 and 125 mS cm^{-1}, but subsequently Y_{II} increased with a decrease in Y_{NPQ} during the afternoon, whereas Y_{NO} remained relatively stable throughout the day. However, the values of Y_{NO} increased as salinity increased from 60 to 125 mS cm^{-1}. It is worth noting that Y_{II} was inhibited with an increase in salinity, but the decrease in Y_{II} was quantitatively compensated by a corresponding increase of Y_{NPQ}.

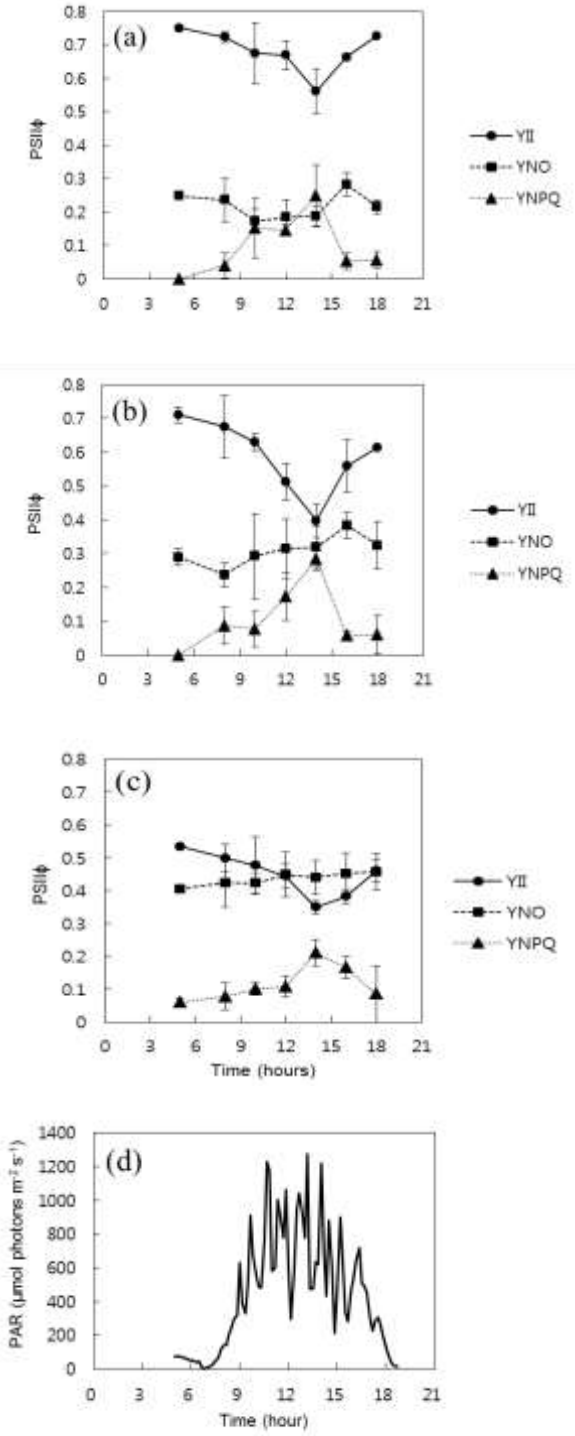

Figure 5. 7: The effect of salinity on diurnal changes of Y_{II}, Y_{NO} and Y_{NPQ} under different salinity regimes of (a) 60 mS cm^{-1}, (b)75 mS cm^{-1} and (c) 125 mS cm^{-1} in *R. tuberosa*, and (d) daily incident irradiance at Mt Bold Reservoir on 3rd October 2009. Mean (± S.D). n = 15.

5.4 Discussion

5.4.1 Effects of ion concentrations and Na^+/K^+ ratios on plant growth

Ion toxicity generated by high salinity represents a common environmental stress limiting plant growth and reproduction due to the impairment of vital enzymes and/or loss of osmotic balance flowing ion accumulation, membrane instability resulting from Ca^{2+} displacement by Na^+ and decreased photosynthetic efficiencies (Maas and Grieve, 1987; Naidoo, 1994; Bernstein et al., 1995; Naidoo et al., 2002). The difference, however, between the pond (Chapter 4) and the field plants became apparent in ion concentrations (Na^+ and K^+) and Na^+/K^+ ratios. The field Na^+ tissue concentrations and Na^+/K^+ ratios data in this study showed a logarithmic increase, while the identical pond data showed a linear increase (Chapter 4). This may have been caused by K^+ and Ca^{2+} deficiencies. Ion toxicity induced by excessive accumulations of Na^+ and Cl^- in plant tissues can inhibit the uptake of other ions such as K^+ (Naidoo, 1994) and Ca^{2+} (Maas and Grieve, 1987; Cramer et al., 1989; Cramer et al., 1990). Subsequently, this can result in high Na^+/K^+ and Na^+/Ca^{2+} ratios (Maas and Grieve, 1987; Reid and Smith, 2000), leading to K^+ (Davenport et al., 1997) and Ca^{2+} (Cramer et al., 1989) deficiencies as mentioned earlier (Chapter 4). Maas and Grieve (1987) reported that the high Na^+/Ca^{2+} ratios impair the selective permeability of membranes, and cause nutritional imbalance, thereby inducing serious Ca^{2+} deficiency. It is also well known that K^+ concentrations decrease as salinity increases, as shown in the pond study (Chapter 4) and other studies (Jampeetong and Brix, 2009). In fact, K^+ concentrations in *R. tuberosa* tissue under field conditions were much higher than under pond conditions. This is likely to be related to the presence of Ca^{2+} (Chapter 4). If sufficient Ca^{2+} is present, a high-affinity uptake system having preference for transport of K^+ can function, and the plants can then obtain sufficient K^+ and restrict Na^+ (Davenport et al., 1997).

5.4.2 Fv/Fm response to salinity

Reductions in photosynthesis resulting from elevated Na^+ and high Na^+/K^+ ratios under high salinity conditions have also been reported for glycophyte and halophyteic species in other regions of the world, including Spain, China and South Africa (Naidoo et al., 2002; Qiu et al., 2003; Redondo-Gómez et al., 2006). In this study, increasing ion concentrations in *R. tuberosa* tissues with an increase in salinity influenced Fv/Fm ratios. This may be due to increasing Na^+/K^+ ratios or the decrease in water potential at high salinity, which is in agreement with the findings of Qiu et al. (2003) for *Suaeda salsa*

and Jampeetong and Brix (2009) for *Salvinia natans* L.. Ralph (1998) also reported that *Halophila ovalis* responded significantly to salinity stress, with a progressive decline in photosynthetic efficiency as stress continued, suggesting the decline in photosynthetic efficiency is attributed to ion imbalances, including Na^+ influx, intracellular ionic competition, membrane rupture and permeability. In fact, the Fv/Fm ratio was negatively related to increased Na^+ concentrations and Na^+/K^+ ratios, but not by increased K^+ concentrations which positively affected the Fv/Fm ratio of *R. tuberosa*. In fact, it is noteworthy that the Fv/Fm ratio increased as K^+ concentrations increased during the period of September in this study. This may indicate the capacity of plants to tolerate salinity may depend on the status of their K^+ availability. Similar findings were obtained by Naidoo et al. (2002), who reported limited photosynthesis correlated with K^+ deficiency for *Avicennia marina*, *Bruguiera gymnorrhiza* and *Hibiscus tiliaceus*. Furthermore, in this study, high Na^+/K^+ ratios generated by elevated Na^+ appeared to contribute to displacement of B (Goldbach et al., 2007), and a consequent B deficiency (López-Gómez et al., 2007). Dell and Huang (1997) demonstrated that inhibition of vegetative growth caused through limiting cell enlargement and cell division by B deficiency, results in a loss of photosynthetic capacity. Thus, the low B concentration in *R. tuberosa* tissues may cause physiological changes, in turn resulting in inhibited leaf expansion and consequently a loss of photosynthetic capacity. This may be associated with reductions in growth and reproduction of *R. tuberosa*. A previous study of physiological parameters showed that changes in ion concentrations correlate with reductions in growth of *Salicornia europaea* and *Suaeda maritima* (Moghaieb et al., 2004).

5.4.3 Y_{II}, Y_{NO} and Y_{NPQ} in responses to salinity

Plants are known to more be resistant to salinity at the initial stages of imposed salinity but more sensitive with increasing duration of salinity. The physiological condition of plants is indicative of plant growth and reproduction, adaptability to stress and is also a general indication of the environment in which they grow. Plant growth depends on photosynthesis, which is particularly sensitive to environmental conditions, including salinity (Naumann et al., 2007). The progressive Na^+ accumulation and continued Na^+/K^+ ratios increases seen with increasing salinity in *R. tuberosa* tissues are consistent with the findings of Jampeetong and Brix (2009), who found that salinity led to an increase in Na^+ concentrations and Na^+/K^+ ratio in *Salvinia natans* L. tissues. In this study, there were strong correlations between increases in ion concentrations and

reductions in photosynthesis, which were in agreement with those presented for many other halophytes, including *Ruppia drepanensis* Tineo, *Ruppia maritima* L., *Sarcocornia fruticosa* (L) AJ Scott and *Thalassia testudinum* (Garcia et al., 1991; Murphy et al., 2003; Redondo-Gómez et al., 2006), thereby reducing plant growth (Jampeetong and Brix, 2009). Plants may experience ionic stress during exposure to high salinity, leading to premature senescence of adult leaves, and thus a reduction in photosynthesis (Munns, 2002), leading to limited plant growth (Sudhir and Murthy, 2004). In general, reduction in photosynthesis is attributed to ion toxicity, which may trigger photo-inhibition or photo-damage (Foyer and Noctor, 2005).

In this study, Y_{II} showed a significant reduction at mid-day, but recovered completely by the following dawn, indicating photo-inhibition associated with an over-reduction of PSII, and the photo-inhibition observed in *R. tuberosa* may be due to photo-protective processes, but not to photo-damage. PSII was affected by light, which led to a significant reduction of Y_{II}, accompanied by an increase of Y_{NPQ}, suggesting the dissipation of excess excitation energy as heat, and indicating that carotenoids, which play a potent role in photo-inhibitory protection of plants as PSII heat dissipation (Gilmore, 2001), was accumulated under high light intensity (Ralph and Burchett, 1998). This was consistent with previous studies for halophytes, including *Atriplex centralasiatica* (Qiu et al., 2003) and *Sarcocornia fruticosa* (Redondo-Gómez et al., 2006). Furthermore, the result of decreased Y_{II}, concomitant with an increase of Y_{NPQ} during periods of high-light, indicates that *R. tuberosa* may possess mechanisms to prevent PSII photo-inhibitory damage. Increased Y_{NPQ}, representing excess light energy released as heat, suggests *R. tuberosa* has a major photo-protective mechanism of the photosynthetic apparatus, and the increase of Y_{NPQ} at high salinity is indicative of ability to cope with oxidative stress following over-reduction of reaction centres. This is in agreement with the findings of Redondo-Gómez et al. (2006), who reported efficient photo-protective mechanisms to preserve the PSII integrity, hence sustaining photosynthetic activity and thereby growth for halophytes of Chenopodiaceae, including *Atriplex genera* and *S. fruticosa*. In addition, increased Y_{NPQ} as salinity increased indicates that salinity may affect photosynthetic components such as carotenoids, which contribute to non-photochemical quenching of chlorophyll fluorescence (Gilmore, 2001). Qiu et al. (2003) reported that the xanthophyll cycle may protect photosynthetic apparatus in the halophyte *Atriplex centralasiatica*, which is well adapted to increased salinity by a high tolerance of photosystem II to salinity and photo-inhibition. In the

xanthophyll cycle, photo-pretective dissipation of excess excitation energy as heat occurs through the formation of zeaxanthin by de-epoxidation of viloaxanthin via the intermediate antheraxanthin, so trapping surplus excitation energy in the PSII antennae (Qiu et al., 2003). Qiu et al. (2003) suggested that violaxanthin/zeaxanthin, which plays an important role in the photo-protective dissipation of surplus excitation energy in the antennae complexes of PSII as heat (Demming & Adam 1992, Gilmore 1997), is able to dissipate excess excitation as heat in the PSII antennae.

In this study, however, non-photochemical quenching (Y_{NPQ}) increased as salinity rose to 125 mS cm^{-1} at the low-light condition. This may indicate that ion concentrations exceed the critical thresholds for *R. tubersoa*, suggesting there is critical threshold salinity between 75 and 125 mS cm^{-1}, affecting growth and reproduction of *R. tuberosa*. Previous studies suggested that stresses such as temperature, salinity, desiccation and mechanical stresses can cause massive accumulation of secondary carotenoids such as astaxanthin in algal cells of *Haematococcus pluvialis* (Chaumont and Thépenier, 1995). Ralph (1998) demonstrated that non-photochemical quenching processes dominate under high-light conditions, which means physiological changes are caused by salinity stress induced at salinities between 75 and 125 mS cm^{-1}, affecting reproduction of *R. tuberosa*. Indeed, flowering of *R. tuberosa* was affected by salinity above 105 mS cm^{-1} in the pond experiment study (Chapter 4), and this corresponds with the findings of low flower abundance at the Coorong where salinity was above 100 mS cm^{-1} (Chapter 2). This may also explain that inhibition of germination at salinity of 125 mS cm^{-1} (Chapter 3). High sodium concentrations may induce changes in protein activity because ions affect the hydration of nuclear contents, causing an inhibition of enzyme activity (Katembe et al., 1998).

5.4.4 Physiological responses to salinity

Na^+, K^+ and Cl^- are known as the predominant solutes in the vacuole for charophyte *Lamprothamnium* (Wichmann and Kirst, 1989) and contribute to turgor pressure (Bisson and Kirst, 1980), defined as the alteration of the internal osmotic pressure following changes in external osmotic pressure (Wichmann and Kirst, 1989). Accumulation of Na^+ provides an osmotic driving force for the uptake of water in highly saline environments (Moghaieb et al., 2004). Parida and Jha (2010) suggested that Na^+ is the main contributor to osmotic adjustment under salt stress for *Salicornia brachiata* Roxb. Salt induced accumulations of organic compounds, such as proline and glycine betaine, are

compatible solutes that accumulate in response to osmotic stress, and the accumulation of these osmolytes represents an important adaptive response to salt stress (Moghaieb et al., 2004). Moghaieb et al. (2004) demonstrated that *Suaeda maritima* accumulate higher levels of proline in the leaves under salt conditions than *Salicornia europaea*, but *S. europaea* exhibit higher glycine betaine content in the leaves than *S. maritima*, suggesting different halophytes deal with osmotic stress by accumulating different types of osmolytes. Brock (1981a) reported that proline concentration increased with increasing salinity in *Ruppia* species, *R. tuberosa*, *R. polycarpa* and *R. megacarpa*. Osmotic adjustment is a mechanism used to maintain turgor and to reduce the deleterious effects of water stress on vegetative and reproductive tissue. Although Na^+ accumulation increased as salinity stress increased in *R. tuberosa*, Na^+ may have been compartmentalized into vacuoles, thereby contributing to osmotic adjustment (Moghaieb et al., 2004). Furthermore, Papageorgiou & Murata (1995) suggested that compatible osmolytes can stabilise and protect the PSII. Parida and Jha (2010) also suggested that salt induced accumulation of organic compounds including proline and glycine betaine in salt treated *Salicornia brachiata* may have contributed to maintaining PSII functioning. Furthermore, Ca^{2+} as a main second messenger acting in concert with ethylene, abscisic acid, and salicylic acid protects plant tissues against oxidative damage (Clarke et al 2004). It is concluded that the results of this study indicate that *R. tuberosa* may have a complex survival strategy at high salinity, involving photo-protective mechanisms to prevent photo-damage, and growth of *R. tuberosa* under high salinity could be partly explained by efficient control of salt, in conjunction with improved photosynthetic capacity.

Chapter 6 General Discussion

Differences in species tolerances and responses to environmental factors (e.g. salinity tolerance) are important in determining the distribution and abundance of individual species and thus, community responses to changing conditions (Brock, 1988; Froend and McComb, 1994). The tolerances and responses of individual plants and populations may be a result of various adaptations. Anthropogenic impacts, such as increasing irrigated agriculture (Cramer and Hobbs, 2002); modification to water regimes (Nielsen et al., 2003a; Nielsen et al., 2003b; Brock et al., 2005), will decrease the availability of habitats and so species well adapted to particular physico-chemical conditions may become threatened. The nature of relationships determining the distribution of species may not be simply understood by spatial and temporal distribution of individuals. It is also important to understand the environmental factors controlling the distribution and abundance (Chapter 2); adaptive plant features to different habitats (Chapter 2); and physiological adaptations to survive a certain environmental condition (Chapter 3, Chapter 4 and Chapter 5).

6.1 Distribution of *Ruppia tuberosa*

The components of a plant's environment that impact on various plant life-cycle stage will modify the initial pattern of propagule supply, hence influencing the distribution and abundance of adult plants (Harper, 1977). A successful plant's recruitment and life-cycle depend upon both the availability of propagules and the suitability of the post-dispersal environment, including abiotic and biotic factors for seedling emergence and growth (Harper, 1977). The availability of propagules can not only influence plant distribution patterns (Aguiar and Sala, 1997), but also explain spatial variation in plant density across habitats (Reader and Buck, 1986). The availability of propagules was demonstrated to limit the extent of plant populations (Primack and Miao, 1992), and propagule dispersal can be an important determinant of species composition, abundance and richness in plant communities (Tilman, 1993). Patterns of recruitment and dispersal can influence local populations and community dynamics, surpassing the influences of post-dispersal factors, such as abiotic stress, which have been focused upon in community ecology (Tilman, 1993).

It is well known that the combination of abiotic and biotic factors are required for successful plant germination, establishment, growth and reproduction (Froend and McComb, 1994). This study (Chapter 2) supports the finding of Carruthers et al. (1999), who demonstrated that abiotic environmental factors, including salinity and water depth, influence both the distribution and abundance of submerged macrophytes. From this study, it appears that the distribution of *R. tuberosa* in the Coorong is limited by propagule availability, primarily due to salinity (Chapter 2). There was a positive association of plant numbers and propagule density in the Coorong. As discussed in Chapter 2, there appears to be two separate phenomenon controlling the distribution of *R. tuberosa* in the Coorong at the time of this study: the ongoing but incomplete colonisation in the North Lagoon, and sub-optimal physico-chemical conditions in the South Lagoon (Chapter 2, Table 2.4). The low abundance of *R. tuberosa* shoots in areas of the North lagoon with relatively low salinity, NL1, Pelican Point and NL2, Long Point, is likely to be a result of limited propagules availability, not a reflection of post dispersal factors, such as salinity. In fact, the North Lagoon of the Coorong was previously a stronghold for *R. megacarpa,* while the South Lagoon was occupied by *R. tuberosa* during a period of relatively high inflows to the Coorong in the 1970s and 1980s (Brookes et al., 2009). *R. tuberosa* restriction to the South Lagoon at that time may have been caused by biotic factors, such as competition with *R. megacarpa* (Katembe et al., 1998).

In contrast to the North Lagoon, the absence of shoots in the southern end of the South Lagoon is associated with an unsuitable post-dispersal environment. In particular, salinity exceeds the germination threshold of *R. tuberosa* (Kim et al., 2013). Previous studies have revealed that elevated salinity leads to a reduction in the abundance and distribution of submerged aquatic macrophytes in inland saline water ecosystems, including *R. megacarpa* and *R. polycarpa* (Sim et al., 2006). This can ultimately lead to localised extinctions of macrophyte species (Keighery et al., 2000). Moreover, other studies demonstrate that the impacts of salinity on macrophytes coincide with co-related physical impacts such as altered water depth (Carruthers et al., 1999). Indeed, water depth appeared to be an important factor controlling the abundance of *R. tuberosa* in the Coorong. It is well known that water depth influences biomass, recruitment and photosynthesis in both emergent and submersed macrophytes (Brock, 1988; Froend and McComb, 1994). Previous studies have demonstrated that light availability is the best descriptor of the cover and biomass of submerged plants (Duarte et al., 1986). As

demonstrated in this study, the reduced abundances of *R. tuberosa* at water depths >0.6 m suggest light limitation and that *R. tuberosa* is a high light adapted species.

6.1.1 The influence of salinity on various life stages of *Ruppia tuberosa*

Salinity is well recognised as a major factor controlling the changes in species composition, distribution, abundance and performance of macrophyte populations (Thorne-Miller et al., 1983). Salinity can affect germination and establishment of glycophytes and halophytes (Vicente et al., 2004; Brock et al., 2005; Song et al., 2008). Salinity tolerance of germination varies between species. Furthermore, seeds and turions have different capacities for coping with exposure to high salinity, meaning that having both seeds and turions provides different survival strategies (Chapter 3; (Kim et al., 2013). Several patterns of germination have been characterised for halophytes (Boorman, 1968; Woodell, 1985; Keiffer and Ungar, 1997; Pujol et al., 2000), including different mechanisms of dormancy to delay germination until the optimal conditions in the environment are met (Chapter 3). Indeed, high salinity-induced dormancy of seeds may be considered a selective advantage (Chapter 3). On the other hand, however, this may be a disadvantage to *R. tuberosa* in habitats with highly variable salinity conditions, such as the Coorong, because if all seeds germinate at one time, the population is vulnerable to any future environmental change.

The physiological condition of a plant is indicative of its capacity to grow and reproduce and its capacity to adapt to stress, and is a general indication of the environment in which it grows. Thus, understanding the physiological mechanisms of environmental stress is critical in predicting how environmental stressors influence plant community dynamics and distribution. The results of this study's field and pond experiments found a reduction in growth as a result of increased salinity (Chapter 4 and Chapter 5). This supports the findings of other studies (Seemann and Critchley, 1985; Santamaría and Hootsmans, 1996). It is well known that plant growth depends on photosynthesis, which is affected by environmental factors such as light, salinity, temperature and water availability (Naumann et al., 2007). In particular, salinity stress enhances photo-damage of photosystem II by inhibiting the repair of photosystem II through the suppression of transcription and translation of light-dependent genes (Allakhverdiev et al., 2002). Chapter 5 demonstrated an inverse relationship between ion concentrations and photosynthesis (Figure 5.6), supporting those presented for many other halophytes, including *Ruppia drepanensis*, *Ruppia maritima*, *Sarcocornia*

fruticosa and *Thalassia testudinum* (Garcia et al., 1991; Murphy et al., 2003). Damage from high salinity at the cellular level is associated with different mechanisms. High cellular Na^+ and Cl^- concentrations cause an increased formation of active oxygen species, with possible oxidative damage of cellular constituents. Ion toxicity is caused by excessive accumulation of Na^+ and Cl^- ions in the cytoplasm leading to ionic imbalance (Chapter 4). Impacts of salinity on the photosynthesis of *R. tuberosa* can be attributed mostly to Na^+/K^+ ratios (Chapter 5). High Na^+/K^+ ratios generated by elevated Na^+ concentrations contribute to displacement of Boron (B), and a consequent B deficiency in *R. tuberosa* tissue (Chapter 5, Figure 5.7). This may cause physiological changes, in turn resulting in inhibited leaf expansion and consequently causing a loss of photosynthetic capacity (Dell and Huang, 1997). This is in agreement with the findings of Yeo et al. (1991), who demonstrated that Na^+ levels increased in expanding leaf tissue. Leading to cytoplasmic Na^+ toxicity and reduced leaf elongation, inducing growth reduction (Bernstein et al., 1995). In addition, ion stress induced by high salinity results in inhibition of Ca^{2+} uptake (Reid and Smith, 2000), causing a nutritional imbalance and a reduction in leaf and shoot growth (Maas and Grieve, 1987). A positive correlation between Ca^{2+} concentrations and shoot length across of range of salinities observed in this study indicates *R. tuberosa* may have a greater requirement for calcium under saline conditions (Chapter 4). The results of this study (Chapter 4), support the finding of Ca^{2+} deficiency symptoms associated with high salinity that have been observed for several plant species (Maas and Grieve, 1987; Davenport et al., 1997).

In general, reductions in photosynthesis is attributed to either stomatal closure or non-stomatal factors, including ion stress (Bethke and Drew, 1992), which is related to a decline in the photosynthetic activity that may trigger photo-inhibition or photo-damage (Foyer and Noctor, 2005). A reduction in photosynthesis induced by ion stress stimulates excess excitation energy, causing photo-damage to photosystem II in the case of limited energy dissipation (Gilmore, 2001). Protection of the photosynthetic machinery is crucial in maintaining plants fitness in sub-optimal conditions (Gilmore, 2001). Studies on the photo-chemical aspects of photosystem II in halophytes have shown that salt-tolerant plants maintain high CO_2 assimilation rate even at extreme temperatures above 40°C, suggesting enhanced thermo-tolerance of halophytes (Wen et al., 2005). Increased activity of enzymatic antioxidants is proposed as a mechanism for the increased protection of photosystem II of salt-tolerant species (Meloni et al., 2003). One of strategies allowing halophytes to grow in high saline environments is increased

tolerance of photosystem II against photo-damage that is caused by a combination of high salinity and high light. Y_{NPQ} is indicative of ability to cope with oxidative stress following over-reduction of reaction centres (Redondo-Gómez et al., 2006). In this study (Chapter 5), decreased Y_{II}, and increased Y_{NPQ} during periods of high light, indicates that *R. tuberosa* may possess mechanisms to prevent PSII photo-inhibitory damage and a photo-protective mechanism for the photosynthetic apparatus (Chapter 5, Figure 5.3 and 5.4). This finding is consistent with previous studies for halophytes, including *Atriplex centralasiatica* (Qiu et al., 2003) and *Sarcocornia fruticosa* (Redondo-Gómez et al., 2006). In addition, the xanthophyll cycle may protect the photosynthetic apparatus in the halophyte *Atriplex centralasiatica*, which is well adapted to increased salinity by a high tolerance of photosystem II to salinity and photo-inhibition (Qiu et al., 2003). Moreover, increased Y_{NPQ} as salinity increased indicates that salinity may affect photosynthetic components such as carotenoids, which contribute to non-photochemical quenching of chlorophyll fluorescence (Gilmore, 2001). Carotenoids play an important role in the photo-inhibitory protection of plants as agents for PSII heat dissipation (Gilmore, 2001).

However, increased Y_{NPQ} as salinity rose to 125 mS cm^{-1} at low-light conditions (Chapter 4, Figure 4.3) indicates that ion concentrations exceed the critical thresholds of *R. tuberosa*, suggesting there is critical threshold salinity below 125 mS cm^{-1}, affecting reduced growth and reproduction of *R. tuberosa* (Chapter 4). In fact, flowering of *R. tuberosa* was affected by salinity above 105 mS cm^{-1} in the pond experiment study (Chapter 4). This corresponds with the findings of low flower abundance at the Coorong where salinity was above 105 mS cm^{-1} (Chapter 2) and inhibition of germination at salinity of 125 mS cm^{-1} (Chapter 3). Internal excesses of Na^+ and Cl^- concentrations may induce changes in protein activity, causing an inhibition of enzyme activity (Volkmar et al., 1997). Indeed, the maintenance of high salinities led to a decline in flower production, resulting in a decrease in sexual reproduction (seeds) and an increase in asexual reproduction (turions), in the Coorong (Chapter 2 and Chapter 4). This may explain why only turions are now present throughout the Coorong (Chapter 2). As mentioned earlier, however, turions are more vulnerable than seeds to extreme salinities when salinity levels exceed their tolerance limits (Kim et al., 2013), as turion cell death by ion toxicity occurs after immersion in high salinity water (Chapter 3). This means that if turion viability is lost on exposure to elevated salinities during summer, exposure to subsequent freshwater or saline-water inputs will not enhance germination of turions.

Osmotic adjustment is one of the major cellular protection mechanisms against salinity, drought and temperature stress (Nuccio et al., 1999). The capacity to adjust osmotic potential may reflect a plant's ability to withstand a variety of abiotic stressors. Different species accumulate various types of chemical substances as osmolytes, such as polyols and sugars (e.g mannitol, trehalose), amino acids (proline) and to ammonium compounds (glycine betaine) (Nuccio et al., 1999). In *Ruppia* species, proline significantly increased under high salinity (Brock, 1981a), suggesting that Na^+ is the main contributor to the osmotic adjustment of *Ruppia* under high salinity. Parida and Jha (2010) suggested that Na^+ was also the main contributor to osmotic adjustment under salt stress for *Salicornia brachiata*. In these plants it is likely that Na^+ would be diverted to the production of proline as an osmolyte. Accumulation of high levels of proline in the leaves of *Suaeda maritima* under salt condition has been reported by Moghaieb et al. (2004). The use of organic compounds for osmotic adjustment is an energy consuming process at the cellular level and synthesis of proline requires more energy compared to that required in the uptake of Na^+. Although Na accumulation increased as salinity stress increased in *R. tuberosa*, Na^+ may have also been compartmentalised into vacuoles, thereby contributing to osmotic adjustment (Moghaieb et al., 2004).

6.1.2 The influence of water depth on the distribution of *Ruppia tuberosa*

Water regime is the primary factor determining distribution patterns and processes in wetland macrophytes (Rea and Ganf, 1994; Blanch et al., 1999; Blanch et al., 2000). Water regime, as described by the changes in water depth, drawdown and wave action, has a significant influence on the distribution of *R. tuberosa* in the Coorong. As demonstrated in this study, water depth was the second important factor in the abundance of *R. tuberosa* in the Coorong (Chapter 2). *R. tuberosa* is a high light adapted species, allowing it to survive in shallow water (Chapter 2). It has a major photo-protective mechanism of the photosynthetic apparatus to prevent photo-damage (Chapter 5). However, it is difficult to conclude whether the low abundance of *R. tuberosa* in deep water of the Coorong is due to decreased photosynthetic capacity, or wave action. The distribution of adults and propagules are intrinsically dependent upon each other with propagule distribution matching that of adult plant population, as shown for mangrove communities (Rabinowitz, 1979). A high abundance of *Ruppia* propagules, especially turions, in the shallow water may be due to loss of propagules from the deep

water and deposition on the substrate surface. Similar results have been reported in other coastal habitats (Ehrenfeld, 1990). Seasonal water level drawdown associated with reduced inflows in the Coorong is so large today that many of the mudflats that were previously considered to be good *Ruppia* habitat are now dry before the plants have had a chance to germinate seeds or turions.

6.2 Knowledge Gaps

Many studies have focused on the effects of elevated salinity on the distribution of submerged macrophytes and the importance of patterns in distribution and water regime in determining opportunity for recruitment (Brock, 1988; Brock et al., 2005). This study has also focused on the effect of salinity on the distribution, germination, growth, reproduction and photosynthesis of *R. tuberosa* (Figure 6.1). However, a knowledge of the response to fluctuations of water regime (or wave action) of plant population characteristics such as distribution, seed production, and seedling recruitment is critical in determining population water regime requirements need investigation, especially for *R. megacarpa*. Conservation of the ecological values of wetlands can require management to meet the water requirements of wetland systems. To determine these requirements, quantitative information is needed about the interactions between plants and water regime. Further the degree to which plant distribution varies between wetlands relative to water regime, and the importance of other environmental parameters such as nutrient uptake mechanisms in altering plant response to water regime are also not well understood. Moreover, the influence of sediment characteristics (nutrients, texture, types) on the distribution (especially the germination and mean time to germination of *R.megacarpa*) requires further investigation (Figure 6.2). Furthermore it is known that B deficiency inhibits the growth and reproduction of plants through limiting cell enlargement and cell division (Dell and Huang, 1997). However, although Blevins and Lukaszewski (1998) reported that B deficiency causes flower malformations and sterility, and B deficiency inhibits the growth and reproduction of plants through limiting cell enlargement and cell division, complete physiological process for plant reproduction is not fully understand (Chapter 5).

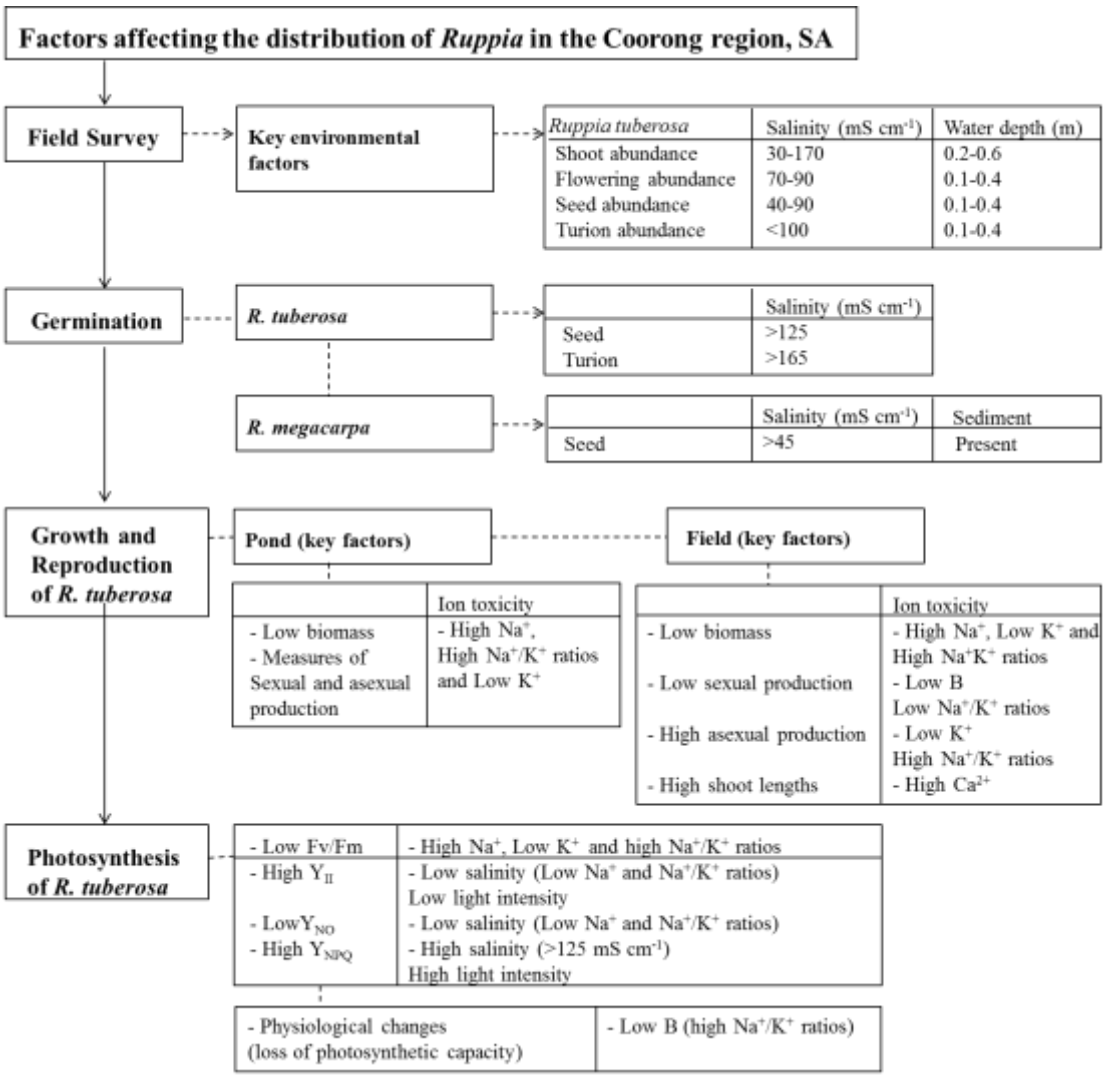

Figure 6.1: Conceptual diagram for the major findings linking the effects of salinity on the distribution of *Ruppia* in the Coorong, Southern Australia.

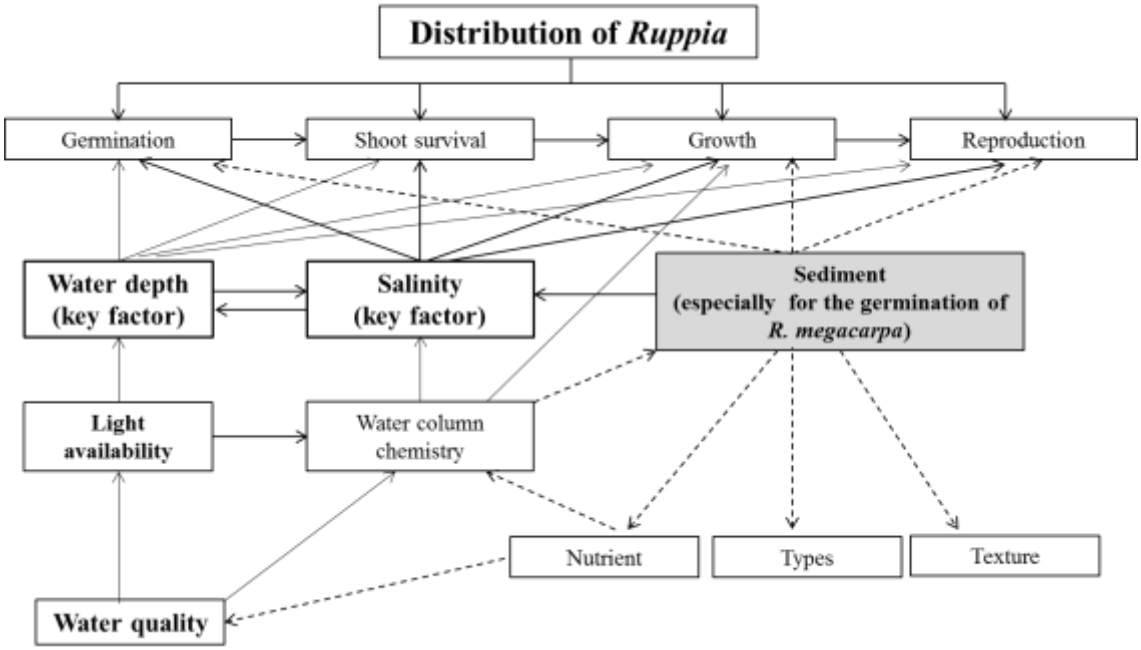

Figure 6.2: Conceptual model of the interactions of various environmental parameters on *Ruppia* species (line, research completed in this study; broken line, for future work).

6.3 Conclusions

The effect of salinity and water depth on plant development is reflected in altered distribution patterns. There is a recognition that increased salinity can result in changes to aquatic ecosystems and reductions in the abundance and diversity of aquatic plants at a global level have been largely attributed to increases in salinity (Cramer and Hobbs, 2002; Nielsen et al., 2003b). Changes of distribution will result from the effects of salinity on propagule germination and formation, photosynthesis, growth and reproduction. As salinity of the world's waterways continue to increase salt-sensitive and even salt-tolerant plant communities will continue to decline and may become extinct. Locally, if salinities increase, all stages of the life cycles will be impacted and the plants will either tolerate, adapt or the distribution will shift if suitable habitat exists. As demonstrated in this study, the partial loss of *Ruppia* from the Coorong has resulted in an increasingly simplified habitat and food-web and will reduce ecosystem function and resilience (Brookes et al., 2009). Detailed knowledge of a plant life-cycle to abiotic parameters is valuable in understanding and predicting the response of aquatic macrophytes to environmental management practices. This study suggests that the restoration of both *R. tuberosa* and *R. megacarpa* in the Coorong may be achieved by providing suitable habitat. In particular, the provision of appropriate riverine inputs aimed at meeting salinity targets and necessary water levels would be an appropriate restoration tool. The restoration of macrophyte communities has been shown to be a

slow process and consequently recovery of the community may require assistance through transplantation of seeds or adult shoots.

Appendix

Appendix I. Copy of publication from Chapter 3

Kim, D.H., Aldridge, K.T., Brookes, J.D. & Ganf, G.G. (2013) The effect of salinity on the germination of *Ruppia tuberosa* and *Ruppia megacarpa* and implications for the Coorong: A coastal lagoon of southern Australia.
Aquatic Botany, 111, pp. 81-88

Appendix II. A physico-chemical condition data (winter) of the seven study sites in the Coorong (NL1 – SL7) and neighbouring Lake Pipe Clay (LPC8) and lakes Cantara North (LCN9) and South (LCS10).

Site	Turbidity (NTU)	Tempera-ture (°C)	DO (ppm)	Water pH	Sediment pH	Soil Redox Potential (mV)	Soil Organic Content (%)
NL1	8.7 (5.1)	15.8 (1.4)	10.4 (6.4)	8.1 (0.4)	5.3 (0.3)	-149 (13)	2.7 (0.2)
NL2	1.6 (0.6)	15.4 (1.6)	10.6 (0.5)	8.6 (0.2)	5.3 (0.3)	-121 (88)	1.2 (0.3)
NL3	6.5 (3.6)	12.5 (2.1)	9.2 (1.5)	9.0 (0.3)	5.3 (0.3)	-106 (24)	2.1 (2.1)
NL4	4.5 (0.7)	16.5 (0.6)	13.7 (1.0)	9.1 (0.3)	5.3 (0.3)	-183 (70)	1.7 (0.2)
SL5	20.3 (0.8)	12.8 (0.6)	11.4 (2.4)	8.1 (0.1)	5.3 (0.3)	-225 (29)	5.6 (5.0)
SL6	8.1 (0.4)	16.6 (0.8)	1.9 (2.2)	8.1 (0.2)	5.5 (0.0)	-297 (11)	5.6 (1.1)
SL7	8.6 (1.1)	21.2 (4.5)	13.5 (1.4)	8.4 (0.6)	5.2 (0.3)	-160 (7)	1.4 (0.4)
LPC8	11.4 (1.4)	18.8 (2.7)	9.6 (0.8)	9.3 (0.2)	5.5 (0.0)	-307 (70)	26.7 (4.1)
LCN9	6.8 (4.4)	13.8 (0.2)	10.2 (0.3)	8.8 (0.0)	6 (0.0)	-272 (86)	18.5 (2.7)
LCS10	2.2 (2.7)	10.8 (0.8)	9.5 (0.8)	8.2 (0.1)	5.8 (0.3)	-262 (36)	35.0 (2.7)

Appendix III. A physico-chemical condition data (spring) of the seven study sites in the Coorong (NL1 – SL7) and neighbouring Lake Pipe Clay (LPC8) and lakes Cantara North (LCN9) and South (LCS10).

Site	Turbidity (NTU)	Tempera-ture (°C)	DO (ppm)	Water pH	Sediment pH	Soil Redox Potential (mV)	Soil Organic Content (%)
NL1	1.4 (2.5)	18.0 (0.5)	9.9 (0.7)	8.2 (0.5)	5.5 (0)	-122 (69)	1.7 (0.1)
NL2	0.6 (0.9)	20.2 (0.9)	11.0 (0.3)	8.3 (0.1)	5.5 (0)	-232 (22)	1.0 (0.3)
NL3	6.0 (4.4)	14.9 (0.8)	9.3 (1.3)	8.0 (0.1)	5.5 (0)	-196 (52)	1.6 (0.2)
NL4	5.6 (4.6)	16.6 (0.5)	9.7 (0.4)	8.0 (0.3)	5.5 (0)	-228 (22)	1.6 (0.2)
SL5	20.9 (20)	20.8 (2.2)	8.8 (0.5)	7.6 (0.2)	5.3 (0.3)	-288 (19)	9.9 (6.7)
SL6	16.9 (3.5)	18.8 (0.8)	10.8 (3.2)	8.6 (0.1)	5.5 (0)	-256 (13)	6.2 (2.8)
SL7	15.1 (2.2)	16.6 (0.9)	8.6 (0.9)	8.1 (0.5)	5.3 (0.3)	-209 (99)	2.7 (1.5)
LPC8	5.3 (0.9)	23.4 (2.0)	9.7 (1.9)	8.9 (0.2)	5.3 (0.3)	-318 (64)	30.9 (5.9)
LCN9	2.3 (2.2)	18.3 (1.7)	9.3 (1.7)	8.5 (0.1)	5.5 (0)	-299 (16)	14.8 (1.2)
LCS10	8.1 (7.1)	14.1 (0.9)	9.1 (2.7)	7.5 (0.2)	5.5 (0)	-180 (47)	16.0 (4.7)

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