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.

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*……………..

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

Signed *Date*...18/11/2013

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.

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Ganf, G.G.

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

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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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Hydrobiologia

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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 5 (submitted for publication) Pg 70

The effect of salinity on photosynthesis of *Ruppia tuberosa*

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Many thanks to Kane and Anna; you welcomed me into your family on Christmas day of my first year. I have really enjoyed being a postgraduate student, mainly due to the wonderful people who made my research achievable and also allowed me to pursue other opportunities. I would like to thank my friends, Ian, Rosalind, Trevor and Gina, and a special thanks to elders, Sung Kwan Choi, Kyung Sook Kyun and many others, for your support.

Most of all, I would like to thank my parents, family, Garry and friends back in Korea for their continued support, encouragement and love. I could not have come so far without your assistance.