Developing ecological knowledge to inform the re-establishment of *Ruppia tuberosa* in the southern Coorong.

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Executive Summary

This report is divided into two parts. In the first part, the responses of *Ruppia tuberosa* to different salinities and manipulations of salinities in both field conditions and mesocosm experiments (ponds at the University of Adelaide) are reported. These responses help to define and confirm the salinities at which the plants will perform best within the Coorong environment and hence the ideal salinities needed to facilitate successful translocation. The second part documents the ability of potential propagules (seeds, turions and sections of rhizomatous material) to disperse, to survive periods of desiccation and to be used in translocation programs.

In mesocosm experiments, *Ruppia tuberosa* performed best when placed into salinities of 30 gL^{-1}. At these salinities the plants grew rapidly and expanded into adjacent sediments and produced more above ground and below ground biomass compared to higher salinities. At the higher salinities of 60 gL^{-1} and 90 gL^{-1} the performances of the plants were reduced with very limited growth at the highest salinity. The only plants to produce floral stalks in these experiments were those exposed to the low salinity (30 gL^{-1}) treatment. Simulated grazing reduced the above ground plant biomass for plants growing in water with a salinity of 30 gL^{-1} but had little measurable effect for plants growing at the higher salinities. The provision of a sudden increase in salinity, effected by adding ocean salt to lift the salinity by 30 gL^{-1}, was not capable of eliciting a response that differed from the responses of plants already at the higher salinity. These mesocosm experiments suggest that there is a marked improvement in the ability of *Ruppia tuberosa* to establish and flourish when salinities are below 60 gL^{-1} and approach 30 gL^{-1}. They also suggest that *Ruppia tuberosa* performs poorly in the range of 60-90 gL^{-1}. This would suggest that translocation programs should be targeted to occur when salinities are below 60 gL^{-1}, if not approaching 30 gL^{-1}. However the results of mesocosm experiments bore little resemblance to the plants' performance in natural settings within the northern Coorong and Lake Cantara. As such mesocosm experiments failed to mimic the natural situation and results from them should be treated with caution.

In the northern Coorong and at Lake Cantara, *Ruppia tuberosa* plants were flowering extensively when the salinities exceeded 60 gL^{-1}. In the North Lagoon the plants were flowering profusely when the salinities were as high as 87 gL^{-1}. Thus high salinities in the range of 60-90 gL^{-1} do not prevent flowering in *Ruppia tuberosa*.

The influx of freshwater into the Coorong from October 2010 onwards freshened the northern Coorong, lowering salinities to 50 gL^{-1} or even lower at times. These lower salinities favoured filamentous green algae that blanketed the existing *Ruppia* beds during summer in the North Lagoon. Despite a further flowering burst in January 2011, the *Ruppia tuberosa* beds that had been prominent in the northern Coorong in the last few years had been all but eliminated by July 2011. Only small quantities of seeds (ca 450 seeds.m^{-2}) remained in the North Lagoon in July 2011 despite two flowering attempts in the previous season. These populations are likely to have limited capacity to recover. For comparison, seed abundances in the surface sediments at Lake Cantara were around 24,000 seeds.m^{-2} with more than 30,000 turions.m^{-2} also present.

Front page image: *Ruppia tuberosa* with flower-heads and filamentous algae at Noonameena, January 2011. Photo courtesy of Lydia Paton.

Given that salinities on or below 50 gL^{-1} favour filamentous green algae in the Coorong optimum salinities for translocations need to be set above this level. As *Ruppia tuberosa* performs best at lower salinities, an optimum salinity of 60 gL^{-1} can be set for any translocation program. The prominence of filamentous green algae at the lower salinities may actually reflect a response to increased nutrients in the incoming water rather a salinity response *per se*. Further work is required to assess the influences of salinity and nutrient loads in the incoming water on filamentous green algae. Proposals to release freshwater drained from agricultural land into the southern Coorong will need to be re-assessed in light of this.

Field observations within the Coorong found small pieces of rhizome being dispersed within the water column, but no seeds or turions. Seeds and turions sank quickly when placed on the surface of a body of water in small aquaria. In contrast, small pieces of rhizome had substantial buoyancy and remained in the water column and usually on or near the surface for at least 40 minutes. The lengths of rhizomatous material being dispersed in the North Lagoon varied but the majority of pieces were short pieces of rhizome with 1-3 leaf nodes. These dispersed readily in the water column travelling at a rate of around 3m/minute. Longer pieces tended to disperse at a slower rate than shorter pieces. Movements tended to be down-wind but there was considerable variability between individual pieces in the net directions and distances moved. Rates of movement were also greater when wind speeds were higher. Further work is still needed to determine how these fragments of rhizomes settle and whether they are capable of establishing plants once settled. This information is required before any recommendations on their use in re-establishing *Ruppia tuberosa* in the South Lagoon can be made. However, the ease with which they are dispersed, offers some promise for depositing fragments of rhizomatous material at a few selected locations to then be dispersed more broadly over suitable areas. Such a scheme may greatly reduce the need to manually plant material into sediments.

Turions were generally short-lived. In the field, turions were often abundant in or on the dry sediments in autumn, but there were negligible turions present in winter. This suggests that all of the turions sprouted shortly after water had returned to the ephemeral mudflats. Desiccation trials revealed that type II turions had the capacity to sprout 3-4 months after collection but not after 7 months. Type I turions on the other hand did not sprout at all, not even 3 months after collection. For comparison, at least some seeds germinated 3, 4, 7 and 16 months after collection but not all. Given this any turions deployed in translocation programs will need to be used more or less immediately and as all are likely to sprout at the first opportunity turions offer only a single chance to establish populations of *Ruppia*. If subsequent conditions are not suitable shortly after the turions have sprouted, then plants will not establish and all the turions will be spent. Thus translocations that only involve turions are fraught with risks of failure, particularly given the vagaries with water levels and salinities in the Coorong. Seeds on the other hand offer secondary opportunities, in that if the initial attempt fails to lead to the establishment of plants, there are likely to still be some seeds available in a subsequent year.

In previous years planting or embedding living *Ruppia* material into the sediments when these are covered with water is difficult to accomplish and such plantings are then vulnerable to being dislodged by wave action, irrespective of the size of the plugs. Until the technical difficulties of planting under water are solved, the best option for translocation involves transferring dry surface sediments containing seeds (plus or minus turions) to other exposed mudflats during autumn when water levels are lowest. While these exposed mud flats are dry the opportunity to embed any translocated material into the sediments is simplified. Previous work has shown that *Ruppia tuberosa* in the Coorong performs best on sediments that are covered by around 0.6 - 0.8 m of water during winter and spring. Initial translocation trials should now be attempted, targeting those areas where the water levels are likely to be around 0.6 - 0.8m deep during winter and spring and with salinities that approach 60 gL^{-1}.

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General Introduction

Ruppia tuberosa is a key component of the *Ruppia*-chironomid-hardyhead fish ecosystem that until recently occupied the southern half of the Coorong (all of the South Lagoon, and the southernmost 4-5 km of the North Lagoon; Paton2010). *Ruppia tuberosa* is not only an important primary producer underpinning the food chains of the southern Coorong, but is also important structurally, providing habitat for hardyhead fish and chironomid larvae. For example, hardyhead fish attach their eggs to aquatic vegetation, so without re-establishing *Ruppia tuberosa*, populations of hardyhead fish have no places for egg attachment and so may fail to flourish in the southern Coorong even once the current high salinities have been suitably reduced. Hardyhead fish are critically important for a number of piscivorous birds, notably the vulnerable Fairy Tern whose populations have plummeted from over 1500 birds in the 1980s to around 200 in 2010. *Ruppia tuberosa* may also be important for chironomid larvae, not only being a source of food (detritus) for larvae, but also dampening water movement over sediments preventing larvae from being dislodged by wave action. Dense mats of *Ruppia* may also reduce fish predation of larvae. *Ruppia tuberosa* is also an important food resource for many waterfowl, notably ducks and swans that feed on its turions and foliage. The value of the Coorong as an effective drought refuge is dependent on having suitable resources for various waterfowl, so the re-instatement of *Ruppia tuberosa* is critical for securing functional wetland refuges for water birds. Furthermore migratory waders are known to consume the seeds and turions of *Ruppia tuberosa*, and these plant products may be important foods supplementing chironomid larvae and allowing the birds to fatten prior to a migratory flight. Other migrating birds are known to add easily digested carbohydrate-rich plant material to their diets during pre-migratory fattening.

In the southern Coorong, *Ruppia tuberosa* was found primarily in water that was 30-90 cm deep, with the plants growing in the middle of this range performing best (Paton & Bailey 2010). Although *Ruppia tuberosa* can grow in water less than 30 cm deep in the southern Coorong, these plants do not survive for long because day-to-day changes in the speed and direction of winds cause day-to-day changes in water levels of the order of 30 cm, resulting in those plants being exposed to desiccation. Within the southern Coorong, *Ruppia* does not grow in water deeper than approximately 90 cm and the likely explanation for this is that the high turbidity of the water reduces the quantity of light reaching the floor of the Coorong, the reduced light levels inhibiting if not preventing plant growth (e.g. Congdon & McComb 1979).

Water levels in the southern Coorong typically change by around 1m over the course of a year, with water levels typically being highest in late winter and spring and lowest in autumn. The key drivers for changes in water levels are seasonal flows over the barrages, but seasonal changes in sea levels coupled with seasonal patterns to evaporative water loss are also important. In the absence of extraction of water for human uses, flows to the Murray Mouth would have begun to increase slowly during winter, more rapidly during spring, peaking in late spring-early summer before subsiding during summer to low flows in autumn (e.g. Paton 2010). With extraction of water for human uses, the typical seasonal pattern is truncated: not only is the seasonal increase in flow during winter delayed, but the peak flows are greatly reduced with flows subsiding during late spring and not extending through summer.

Water levels in the southern Coorong are elevated substantially when water is released over the barrages, the actual level of elevation related to the quantities of water being released (Noye 1975, Webster 2005, 2010, Paton unpubl.). This is despite over 99% of the water being released going out through the Murray Mouth. Thus flows over the Barrages to the Murray Mouth during spring and into summer maintain the water levels in the Coorong long enough to allow *Ruppia tuberosa* to flourish and set substantial quantities of seed and turions before water levels drop in autumn. However when flows are reduced and only sustained through spring, then water levels drop exposing the beds of *Ruppia tuberosa* to desiccation before they have set seeds or many turions.

During the recent 2002-2010 drought when there was negligible water being released over the barrages, water levels in the southern Coorong dropped in early spring (usually September) exposing the beds of *Ruppia tuberosa* before any *Ruppia* had reproduced (Paton 2010, pers. obs.). This drop in water levels during spring was in association with a seasonal drop in sea levels but also with the switch in frequency of strong north-westerly winds that push water southwards in the Coorong during winter. So during most of the last decade the water levels have been suitable to stimulate *Ruppia* seeds to germinate (and turions if present to sprout) during winter, but not sufficient to allow the plant to complete its reproductive cycle (Paton 2010). These conditions were re-iterated over a series of consecutive years, and have led to the virtual elimination of the seed bank (e.g. Paton 2010; Paton & Bailey 2010a, 2011a). As a consequence *Ruppia tuberosa* will not return quickly to the southern Coorong once the hydrological issues have been addressed.

The absence of flows of freshwater to the Murray Mouth during spring and early summer also account for the rise in salinity within the southern Coorong over the last decade. During summer evaporative water losses from the Coorong are substantial (>1m of water evaporates off water surfaces in this region). As water levels drop within the Coorong water is drawn in from the Murray Mouth region to offset those losses. When there are no freshwater flows to the Murray Mouth, marine water is drawn into the Coorong. This marine water carries 37,000 tonnes of salt per GL, and as much as 100GL of water may evaporate off the Coorong during summer. This salt then rapidly accumulates in the Coorong, particularly so in the southern end, lifting salinities well above any historical levels and exceeding the tolerances of key aquatic organisms, notably hardyhead fish and chironomids (Paton *et al.* 2009; Paton 2010). When there are freshwater flows to the Murray Mouth region that last through summer, then largely fresh water with little salt is drawn into the Coorong (Webster 2010). Even though some salt will enter the Coorong in those years when flows are truncated, years with more extensive and substantial flows are likely to facilitate the advective transport of some of that salt out of the southern Coorong and so keep the salinities within an acceptable range to sustain the hypersaline ecosystem (Webster 2005, 2010).

Before *Ruppia tuberosa* can be re-established across the southern Coorong, the typical water regime for the southern Coorong needs to be re-established. The two options for reducing salt loads include pumping some of the highly saline water out of the southern Coorong into the Southern Ocean or using advective removal following the return of substantial river flows to the Murray Mouth (Paton *et al.* 2009; Paton 2010, I. Webster pers. comm.). Neither offers a rapid solution to the current high salt loads. Other options such as directing water currently being drained from land south-east of the Coorong into the southern Coorong have been promoted as a solution but the volumes are insufficient to offset evaporative losses and do not address the issue of water levels for the Coorong.

Ultimately the maintenance of the ecological character of the Coorong will depend on maintaining almost continuous flows of River Murray water to the Murray Mouth.

Once the water regimes (levels and salinities) have been rectified there are still many challenges associated with re-establishing *Ruppia tuberosa* along the 50 km length of the southern Coorong, not the least being the scale of the task. The challenges include understanding the best time to translocate material into the Coorong, the nature of the material to be translocated, how to prevent this initial material from being dislodged by wave action during initial establishment, and whether there are strategic locations for the initial translocations that would optimise subsequent dispersal of propagules to other nearby areas, so facilitating more rapid and extensive re-colonisation. Whatever translocation protocols and strategies are developed, the reality of the scale of the task is that re-populating the whole southern Coorong is likely to take many years, probably at least a decade. As a consequence of this there is an urgency to develop necessary background technical knowledge that will allow translocation to commence as soon as appropriate conditions have been re-established. Failing to develop that knowledge now just delays the time to recover the Coorong, placing at risk the ability of this region to support waterbird populations during the next drought.

During the period in which *Ruppia tuberosa* was lost from the southern Coorong, *Ruppia tuberosa* expanded its distribution 20-25 km northwards and established at sites midway along the North Lagoon (e.g. Paton 2010). This colonisation did not take place until the salinities in this region were consistently hypersaline (>35g/L) and following initial establishment, the populations increased in cover slowly. These plants have established in areas that have a year-round cover of water, with some plants establishing in water deeper than 1 m. These differences reflect the reduced seasonal fluctuations of water levels in the North Lagoon, as well as reductions in turbidity that have taken place with increases in incursions of salt-bearing marine water into the Coorong. These plants have failed to produce seeds and so they remain vulnerable to changes in water levels, salinities and turbidities (Paton & Bailey 2010a, 2011a). The *Ruppia tuberosa* populations in the middle of the North Lagoon have been sustained by being permanently inundated and their expansion has been due solely to below ground vegetative growth from rhizomes. Several factors have been suggested for the failure of these populations of *Ruppia tuberosa* to set seed: a rapid increase in salinity in spring; high rates of grazing from waterfowl; and interference from filamentous green algae (Paton & Bailey 2010a).

Based on annual monitoring of *Ruppia tuberosa* in the Coorong that began in 1998 the likely mechanism by which *Ruppia tuberosa* expanded northwards into the middle of the North Lagoon was via the dispersal of small fragments of rhizomes. In the year prior to *Ruppia tuberosa* being first detected at sites in the middle of the North Lagoon, occasional small pieces of rhizomes were found washed up along the shoreline. These pieces of *Ruppia* would have been dislodged from *Ruppia tuberosa* beds further south in the North Lagoon and washed northwards by southerly winds. The small fragments may have been dislodged by wave action and or from grazing disturbance by Black Swans. In areas where *Ruppia tuberosa* was abundant large numbers of small fragments have been found washed onto the adjacent shore. Whatever the mechanism this expansion of *Ruppia tuberosa* within the North Lagoon indicates that *Ruppia* does have some ability to disperse and colonise areas. However the prevailing winds during spring and summer when abundant *Ruppia* material is available for dispersal do not favour dispersal southwards back into the South Lagoon. Furthermore the narrow channels and peninsulas that exist between the two lagoons may conspire to restrict transport of *Ruppia* material from the North Lagoon southwards into the South Lagoon. That no fragments of *Ruppia* have been detected in the South Lagoon during the last 2-3 years strengthens that argument. Thus natural re-colonisation back into the South Lagoon, and particularly southern parts, is unlikely to be substantial or quick. Even after initial colonisation at a site, *Ruppia tuberosa* may take up to five years to establish a moderate level of cover (>30%). This time line is based on the rate of establishment at Noonameena, in the middle of the North Lagoon, where even after five years the cover was less than 30% (e.g. Paton 2010; Paton & Bailey 2010a; 2011a).

Given the importance of *Ruppia tuberosa* there is a premium on quickly re-establishing populations within the southern Coorong. This re-establishment will require the translocation of *Ruppia* material (seeds, turions or fragments) back into the South Lagoon. Before this could happen an understanding of the ecological requirements and performance of the species under different ecological conditions (salinity, water level, turbidity, and in the presence and absence of competitors (e.g. filamentous algae) and grazers (e.g. waterfowl) is required, to provide guidelines around the nature of the material to be translocated, the timing of that translocation, and techniques for effective translocation.

Program of research during 2010-11

In May-June 2010, when the 2010-11 fieldwork was being planned, there was no prospect of flows returning to the Coorong. Since the research conducted in 2009-10 identified the ongoing inability of populations of *Ruppia tuberosa* to flower extensively and set seeds in the middle of the North Lagoon as a significant issue for the maintenance of that plant population, one key component of the work to be conducted in 2010-11 was to assess the factors likely to be limiting flower and seed production for *Ruppia tuberosa* in the North Lagoon. Understanding those factors also has implications for any re-established populations in the South Lagoon. From a management perspective, successful re-establishment of *Ruppia tuberosa* across the South Lagoon will require the plants to reproduce to re-establish an effective seed (or propagule) bank that will allow the plants to span annual periods of desiccation (due to anticipated regular seasonal changes in water levels that will be greater in the South Lagoon than the North Lagoon (e.g. Noye 1975: Webster 2005, 2010). Thus factors that disrupt or prevent flowering and seed production reduce the speed of re-establishment and the long-term sustainability of any translocated populations unless those factors are also addressed. Most of the assessment of factors influencing flowering in *Ruppia tuberosa* was conducted by Teagan McKillop as part of her B.Sc. Honours degree, with funds from this program used to provide substantial field and technical assistance to bolster sample sizes.

A second body of work aimed to determine whether seeds, turions or fragments of plants (rhizomes) were more likely to be dispersed, including potential for long-distance dispersal as the first step in exploring the use of natural dispersal units to facilitate widespread re-establishment of *Ruppia tuberosa* either as an additional technique or as an alternative to physically planting *Ruppia* material in the sediments.

The other work involved continuing to collect relevant information on the performances of *Ruppia tuberosa in situ* within the Coorong and Lake Cantara, and assessing the ability of different propagules to survive periods of desiccation.

The following provides a list of the tasks that were to be addressed during 2010-11.

1. Comparing the ability of seeds versus turions to germinate at different times of the year and under different salinity regimes.
2. Assessing factors that inhibit *Ruppia tuberosa* from producing flowers and seeds in the North Lagoon.
3. Assessing the ability of *Ruppia tuberosa* plants, seeds and turions to cope with different periods of desiccation.
4. Assessing the ability of seeds, turions and fragments of *Ruppia tuberosa* plants as dispersal units.
5. Investigating the size of individual plugs to influence the ability of *Ruppia tuberosa* plants to establish.
6. Continue to build a spatially-explicit demographic model for *Ruppia tuberosa*.

The intention in the 2010-11 program of research was to advance our understanding across these areas and was not aimed to complete the work required in each of these areas, since many require input over multiple years.

Importantly, no contract to fund this work was provided for consideration until late November 2010. By then much of the opportunity to advance components of this work rapidly had been compromised, because the opportunity to conduct a full set of appropriate experiments and field measurements had been lost. This is because *Ruppia tuberosa* largely grows and reproduces during winter and spring within the Coorong region. The on-going inability to provide funding in a timely fashion (from the perspective of the biology of the plant) is a serious handicap to developing the knowledge needed to re-establish *Ruppia tuberosa* in the southern Coorong.

This report will be provided in two parts. Part I will focus on factors influencing the production of flowers and hence seeds for *Ruppia tuberosa* and will include some experimental assessment of salinity thresholds for *Ruppia* and so contributes to components 1 and 2 above. Part 2 will focus on assessing the dispersal and longevity of seeds, turions and fragments of *Ruppia tuberosa* plants and initial trials investigating the influence of plug size on establishment of translocated *Ruppia tuberosa* so contributes to the next three components listed above. The sixth and final component listed above, the building of a spatially-explicit demographic model for the performance of *Ruppia tuberosa* is not covered specifically in this report. However, much of the information reported in the two parts feeds into this model. The management and translocation implications stemming from the program of research in 2010-11 are outlined at the end of the each of the two parts of this report.

Part I. Factors influencing the production of flowers and hence seeds by *Ruppia tuberosa*.

Introduction

In recent years *Ruppia tuberosa* has established in the North Lagoon but has failed to develop a seed bank, and in the absence of a seed bank these recently-established populations remain vulnerable to environmental changes such as changes in water levels and or salinity because they have no mechanism for surviving extended periods of inhospitable conditions. Similar issues may also arise during the period of re-establishment of *Ruppia tuberosa* in the South Lagoon. Three explanations were proffered to explain the lack of seed production in the North Lagoon: high salinities and particularly a rapid increase in salinity in spring; extensive grazing by waterfowl; and interference by filamentous green algae (Paton & Bailey 2010a). This component of the 2010-11 research program largely aimed to assess the influence of salinities and grazing pressure on floral production of *Ruppia tuberosa* using both *in situ* field experiments at Rob's Point and Lake Cantara as well as *ex situ* pond experiments at the University of Adelaide. Additional information on the performances of *Ruppia tuberosa* in the North Lagoon of the Coorong and Lake Cantara were gathered while establishing and monitoring the field experiments.

Methods

Timing of field and pond experiments

The field exclusion and clipping experiments were set up in late September 2010, and the field salinity experiment was set up in late October 2010 because of delays in the supply of materials. The contingency plan (pond salinity and clipping experiments) was put into place in response to high water levels interfering with the field exclusion experiment and delays in the supply of materials (stainless steel sheet metal) for the salinity experiment. The pond experiments were set up in early October 2010. The Lake Cantara field experiments were sampled at the end of the growing season (mid December 2010), and the pond experiments were concluded and sampled in late February 2011.

Salinity experiment at Lake Cantara

This experiment manipulated the rate of salinity increase over spring in plots at Lake Cantara by adding salt at regular intervals to mimic the change in salinity experienced in the North Lagoon over spring in recent years (Paton & Bailey 2010). Eight circular plots (rings) of 1.5 m diameter were set up; four were experimental plots (salt added) and four were control plots (no salt added). The plots were surrounded by a ring of stainless steel sheet metal buried into the sediment so that water movement was limited (Fig. 1). The extremely fine sediment in Lake Cantara made the rings waterproof enough for the plants contained within the experimental rings to be exposed to sustained higher salinities. Salinities were raised at a steady rate in the experimental rings by 30 gL^{-1} over three weeks during spring using Instant Ocean® synthetic sea-salt. *Ruppia tuberosa* flowers in the rings were then counted and the salinities in the rings measured once a week. Five cores from each ring were sampled upon completion of the experiment. The cores were processed and statistically compared (methodology described later) to assess whether plants experiencing relatively stable natural salinities were significantly different to plants exposed to a rapid rise in salinity over spring (hereafter referred to as a salinity burst) with respect to numbers of shoots, turions and above and below ground biomass.

It should be noted that installation of the rings was delayed by four weeks because of a delay in the supply of the metal for the rings. Consequently, *Ruppia tuberosa* plants were already flowering when the experiment was set up. This was not ideal; ultimately, the experiment should have commenced prior to plants flowering. With this in mind, the rings were positioned in an area where floral production had only just commenced. However, even this area had a mean (± s.e.) of 84 ± 18 flower heads per ring.

Fig. 1 Field salinity experiment at Lake Cantara. Rings without black stripe received no additional salt, rings with black stripe received additional salt. Photo taken from shore by Tom Bradley.

Simulated grazing experiment at Lake Cantara

This experiment simulated grazing by regularly clipping *Ruppia tuberosa* plants at Lake Cantara to mimic the grazing by waterfowl experienced in the North Lagoon. Eight artificial grazing plots of 4 m x 4 m were set up; the plants in four of the plots were clipped and the remainder were left unclipped as controls. Plants were clipped as close as possible to ground level once a week. Five cores from each plot were sampled upon completion of the experiment. The cores were processed and statistically compared (methodology described later) to assess whether plants in clipped plots were significantly different to plants in unclipped plots with respect to numbers of shoots, turions, and above and below ground biomass. Time limitations have meant that only two cores per plot have been processed and analysed to date.

Grazing exclusion experiment at Rob's Point

This experiment was designed to reduce the grazing pressure experienced by*Ruppia tuberosa* populations in the North Lagoon by excluding herbivorous waterfowl from accessing plants. The experiment was established in late September 2010 when water levels in the Coorong were low, but typical of the previous three years. Waterfowl were excluded from three 4 m x 4 m plots using bird netting strung between stakes and suspended just above the waterline to deter waterfowl from accessing plants. Three control plots of the same size had stakes but no bird netting, so allowed waterfowl to access the plants growing on the floor of the Coorong. This experimental set-up was similar to the ones established previously (see Paton & Bailey 2010a). The intention with the experiments in 2010-11 was to compare the performances of plants protected from grazing by waterfowl with those not protected, particularly their ability to set flowers and seeds. However, following major rain events across the Murray-Darling Basin, substantial volumes of water flowed to the end of the River Murray filling the Lower Lakes and allowing significant volumes of freshwater to be released over the Barrages from October onwards. With the release of this water, water levels rose by as much as a metre in the Coorong and covered the bird netting. This made it both impossible for waterfowl to access *Ruppia tuberosa* on the Coorong floor in the area of these experimental exclusion plots and impossible to relocate the marked plots as the markers and netting were now entirely under water. As a consequence, this experiment provided no results and is not discussed further. The opportunities to re-activate this experiment in 2011-12 will depend on water levels within the Coorong returning to more typical levels and on the *Ruppia tuberosa* beds remaining intact.

Ex situ salinity and clipping experiments

Salinity and clipping experiments were set up in two ponds at the University of Adelaide (Fig. 2). These experiments used a similar set-up to field trials with a 7.5 cm diameter x 4 cm deep core containing *Ruppia* material being embedded in the centre of a 2 L ice-cream container filled with sediment that lacked *Ruppia* material. These 2 L containers were then placed in large tubs and covered with water of an appropriate salinity (see below). The tubs were then placed into shallow water (~35 cm deep) within the ponds (e.g. Fig. 2). This external water around the tubs reduced extremes in water temperature. The 2 L containers with *Ruppia* material were then deployed in different salinity or clipping treatments.

Three sources of *Ruppia* material were used: cores of dry sediment collected in the previous autumn from Lake Cantara which contained around 100 seeds; cores with *Ruppia tuberosa* plants taken from Lake Cantara; and cores with *Ruppia tuberosa* plants taken from Rob's Point. In the latter two cases, *Ruppia tuberosa* plants were transported from the field to the University while covered with water. These two sites experience different conditions as Rob's Point is in the Coorong proper where salinity levels in recent years have ranged from 30 to 110 gL^{-1}, while Lake Cantara is external to the Coorong and had salinities that ranged from 17 to 88 gL^{-1} in 2009 (Paton & Bailey 2010; unpubl.).

The salinity treatments consisted of comparing the performances of plants that were being maintained in salinities that were 30, 60 or 90 gL^{-1} (this covered the typical range of field salinities experienced by *Ruppia tuberosa* in the Coorong) and then half of these were given a salinity burst by rapidly increasing the salinity a further 30 gL^{-1} and then maintaining this salinity at this higher level. The controls for this experiment were maintained at the same constant salinity. Salinities were established and manipulated using Instant Ocean® synthetic sea-salt added to seawater (and reverse osmosis water when dilution was required). Salinity levels were checked at the time of adding salt and regularly every 1-2 weeks using a portable TPS salinity metre (model WP-84).

The clipping experiment was run in conjunction with the salinity trial. Clipping involved cutting the *Ruppia tuberosa* shoots about 3mm above the sediment and was used to mimic grazing levels observed in the field (Paton & Bailey 2010a) where a very high proportion of shoots had been clipped about 1cm from the sediment. The experiment consisted of two treatments unclipped (control) and clipped (experiment). Clipping took place once a week.

Plants in experimental treatments were only subjected to a salinity burst and clipping once they were established (judged by lateral growth) and had recovered from the stress of transplantation and the initial salinities into which they were placed. A minimum of two weeks was allowed for this and by then lateral growth was visible for most of the plants (particularly those at lower salinities). Plants starting in salinities of 90 gL^{-1} never established lateral growth into the adjacent sediments so were not subjected to salinity burst, but were clipped.

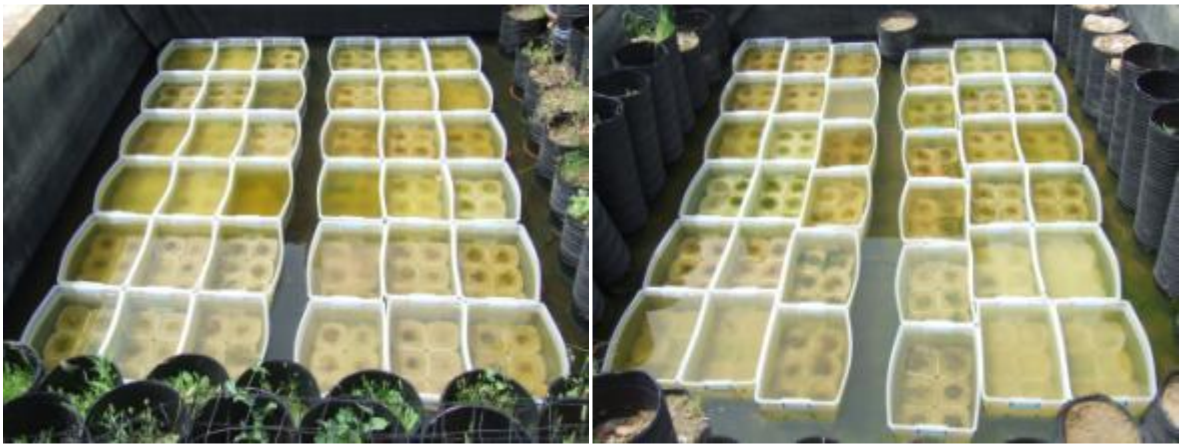


Fig. 2. Deployment of tubs in the in-ground ponds at the University of Adelaide in which the *in situ* salinity and clipping experiments were executed.

The high salinity required for the experiments meant that the ponds themselves were not able to be filled as this would cause deterioration of the ponds, so tubs were placed inside the ponds. Each tub held six 2 L ice-cream containers that had samples from one of the three sites, half of these were clipped and half were not clipped. For each source of plant material there were 24 tubs, and four tubs (= replicates) were assigned to each of the six salinity treatments (30g L^{-1}, 60 gL^{-1}, 90 gL^{-1}, and the same salinities with a subsequent 30 gL^{-1} salinity burst). With three sources of plant material (Lake Cantara plants, Rob's Point plants, and Lake Cantara seeds) this made a total of 72 tubs each with six 2 L ice-cream containers. This design with the clipping experiment undertaken within the salinity experiments allows for any interaction between the two to be assessed but this was not the focus of the study. Replicate tubs were assigned to positions within the ponds using stratified random assignment, so that only one tub replicate would be placed within each pond quadrant. This was done so that differences between positions within the ponds (e.g. shading) would not confound results. Because of time limitations and the time consuming nature of processing samples, only half of the replicates in each experiment were processed. The remainder were stored and are currently being processed.

Sampling procedure for field and pond experiments

The method of sampling used for all experiments was the same as the method used by Paton & Bailey (2010a) for the grazing exclusion experiment. In the field 7.5 cm diameter x 4 cm deep core samples were collected randomly from plots, rings or from the areas where samples were collected for the *ex situ* experiments. Each sample was passed through a 500µm Endecott sieve and the shoots, flowers, seeds and turions were counted. Maximum shoot length was also measured.

Biomass was separated into above-ground and below-ground biomass by cutting shoots and flower stems at the junction of the shoot/stem and rhizome. The plant material was then dried for 48 hours at 70° C, and weighed. Above-ground biomass included shoots, flower stems, and all turions (as most turions were type I and were found at the base of shoots; Brock 1982). Below-ground biomass included all roots and rhizomes. Seeds were not included in biomass measurements. For field experiments baseline assessments were undertaken to confirm that there was no difference between the treatments at the start of the field experiments, and no significant differences were found.

For the *ex situ* experiments, a similar process was conducted but the plant material was associated with the original 7.5 cm diameter plug deployed in the 2 L ice-cream containers was assessed separately to the additional plant material growing outside this core. Where other plant species appeared in the containers (as was the case for samples from Lake Cantara when salinities were 30 gL^{-1}) then the *Ruppia tuberosa* material was separated from the other species (*Lepilaena* sp., *Chara* sp.) and the biomass of the different species considered separately.

Field observations on *Ruppia tuberosa*

While establishing and maintaining the field experiments at Rob's Point and Lake Cantara additional information on salinities and the performances of *Ruppia tuberosa* were collected. Salinities were measured by collecting a sample of water, returning to the laboratory where the sample was diluted to bring salinities within the optimum range of the conductivity metre (labCHEM-C) before electrical conductivity (EC) was measured. The measured EC was converted to gL^{-1} using the equation of Williams (1988). Note that the ECs measured for the diluted samples were within the range of salinities used to construct the Williams' equation but the equation may slightly underestimate salinities for the Coorong (I. Webster pers. comm.). The salinity (gL^{-1}) of the original sample was then calculated by multiplying by the dilution factor. Monthly field observations were used to document flowering of *Ruppia tuberosa* at both Lake Cantara and Rob's Point during spring 2010, in addition 10-72 sediment cores (7.5 cm diameter x 4 cm deep) were taken in July, September and November. These samples were passed through a 500µm Endecott sieve and the shoots, flowers, seeds and turions counted. Overall biomass (g dry weight per core) was also determined for these samples using the same methods as described above.

Statistical analyses for field and pond experiments

The differences between experimental and control population means for each response variable (shoot, flower, seed and turion abundance, maximum shoot length and above-ground, below-ground, and total biomass) were tested for significance using Graphpad Prism©. Data were checked for normality and because of small sample sizes most of the data were not normally distributed. Data transformations did not improve normality. Therefore, (non-parametric) Mann-Whitney tests were used when comparing treatments in the field salinity and clipping experiments. However, statistical analysis of data from the pond experiments required two-way ANOVAs and as Graphpad Prism© did not offer a non-parametric equivalent, ANOVAs were conducted as these are still sufficiently robust and are able to analyse non-parametric data with confidence in the absence of non-parametric alternatives {Zar, 1984 #25}. Where significant interactions were evident ($P < 0.05$), a Bonferroni multiple comparison *post-hoc* test was carried out to establish which samples were significantly different from others.

Results

Field experiments

Salinity experiment at Lake Cantara

There were no significant differences between the responses of plants growing in the rings with a stable salinity compared to the rings with a salinity burst (Table 1). Shoot, turion, flower, and seed abundances, as well as maximum shoot length, and above-ground, below-ground and total biomasses were all similar in rings with stable salinities and salinity burst (Table 1). However, the mean abundance of flowers per ring appears to be higher (although not significantly so) in rings with a stable salinity compared to rings with a salinity burst (Table 1).

Table 1. Summary of results from the field salinity experiment. Data are means ± s.e. and *P*-values from Mann-Whitney tests comparing plant responses to stable salinity and a salinity burst. Stable salinity is Lake Cantara natural salinity (65 gL^{-1} at the start of experiment) and salinity burst raised the salinity level by 30 gL^{-1} above natural levels and maintained it at 30 gL^{-1} above natural levels. Sample size (*n*) is five for all variables except flower abundance, where *n* is four. Significant results ($P < 0.05$) are shown in bold.

Response variable	Stable salinity	Salinity burst	*P*-value
Shoot abundance	295 ± 30	315 ± 37	0.462
Max shoot length (mm)	68 ± 2	66 ± 4	0.914
Turion abundance	140 ± 14	151 ± 16	0.574
Above-ground biomass (g)	0.377 ± 0.062	0.471 ± 0.067	0.505
Below-ground biomass (g)	1.010 ± 0.175	0.861 ± 0.065	0.721
Total biomass (g)	1.390 ± 0.175	1.332 ± 0.089	1.000
Flower abundance (per ring)	117 ± 37	67 ± 32	0.686
Seed abundance	109 ± 21	128 ± 30	0.916

Clipping experiment at Lake Cantara

There were no significant differences between the responses of plants growing in clipped plots compared to unclipped plots (Table 2). Shoot, turion, and flower abundances, as well as maximum shoot length, and above-ground, below-ground and total biomasses were all similar in clipped and unclipped plots (Table 2). Although clipping did not significantly alter the response of the plants, clipping had a greater influence on above ground biomass than on any other response variable measured, and was slightly lower following clipping.

Table 2. Summary of results per core from field clipping experiment: mean ± s.e. and *P*-values from Mann-Whitney tests comparing plant responses to clipping and no clipping. Plants were clipped once a week and clipping ceased two weeks prior to sampling. Sample size (*n*) is eight. Significant results ($P < 0.05$) are shown in bold.

Response variable	Unclipped	Clipped	*P*-value
Shoot abundance	388 ± 67	291 ± 63	0.442
Max shoot length (mm)	56 ± 6	46 ± 5	0.245
Turion abundance	155 ± 25	111 ± 24	0.270
Above-ground biomass (g)	0.280 ± 0.048	0.174 ± 0.046	0.105
Below-ground biomass (g)	1.010 ± 0.127	1.030 ± 0.119	0.959
Total biomass (g)	1.290 ± 0.163	1.210 ± 0.143	0.798
Flower abundance	1.500 ± 0.500	1.880 ± 0.693	0.829

Pond experiments

Experiments starting at 30 gL^{-1} salinity

(i) Lake Cantara plants

An obvious visual difference between treatments was evident, with plants exposed to stable salinities (30 gL^{-1}) and/or no clipping appearing healthier than those exposed to a salinity burst (from 30 to 60 gL^{-1} then maintained at 60 gL^{-1}) and/or clipping (Fig. 3). Shoot abundance, maximum shoot length, and above-ground biomass were all lower when exposed to a salinity burst or clipped (Table 3 and Fig. 4). Further reductions in shoot abundance and above-ground biomass were detected in plants that were exposed to clipping and stable salinity, and further reductions in maximum shoot length were found in plants exposed to stable salinity and clipping, and a salinity burst and clipping. However, these interactions influenced plant responses less than the independent salinity and clipping treatments. The percent of total variation accounted for by salinity burst, clipping, and their interaction was 54, 13, and 9 % for shoot abundance, 33, 53, and 7 % for maximum shoot length, and 45, 23, and 15 % for above-ground biomass, respectively. Total biomass responded in the same way to the independent treatments (reducing in response to salinity burst and clipping), but did not experience an interaction effect. Below-ground biomass was reduced by a salinity burst, but was not affected by clipping. Flower and turion abundances were not affected by salinity or clipping treatments. Two flowers were produced across all containers. These flowers only occurred in tubs with stable salinities, and only in containers that were unclipped (Fig. 6). Because of the low flower abundances, the data do not provide a sound basis for assessing whether flower production was influenced by salinity and/or clipping treatments.

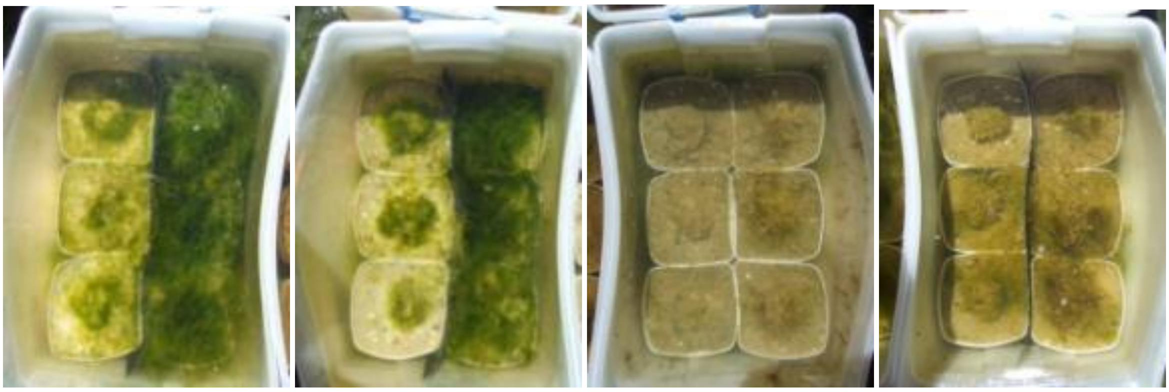


Fig. 3. Appearance upon completion of experiment of Lake Cantara plants with salinity starting at 30 gL^{-1}. Stable salinity tubs (with salinity maintained at 30 gL^{-1}) are on the left, and salinity burst tubs (with salinity raised from 30 to 60 gL^{-1} and maintained at 60 gL^{-1}) are on the right. Within each tub, the three containers on the left were clipped weekly (ceasing two weeks prior to the experiment ending) and the three on the right were unclipped.

Table 3. Summary of *P*-values from two-way ANOVAs testing significant differences in the response of Lake Cantara plants in salinity starting at 30 gL^{-1} to salinity and clipping treatments. Significant results ($P < 0.05$) are shown in bold.

Response variable	Interaction	Clipping treatment	Salinity treatment
Shoot abundance	**0.015**	**0.004**	**< 0.001**
Max shoot length (mm)	**< 0.001**	**< 0.001**	**< 0.001**
Turion abundance	0.377	0.058	0.851
Above-ground biomass (g)	**< 0.001**	**< 0.001**	**< 0.001**
Below-ground biomass (g)	0.727	0.481	**0.009**
Total biomass (g)	0.089	**0.026**	**< 0.001**
Flower abundance	0.130	0.130	0.130

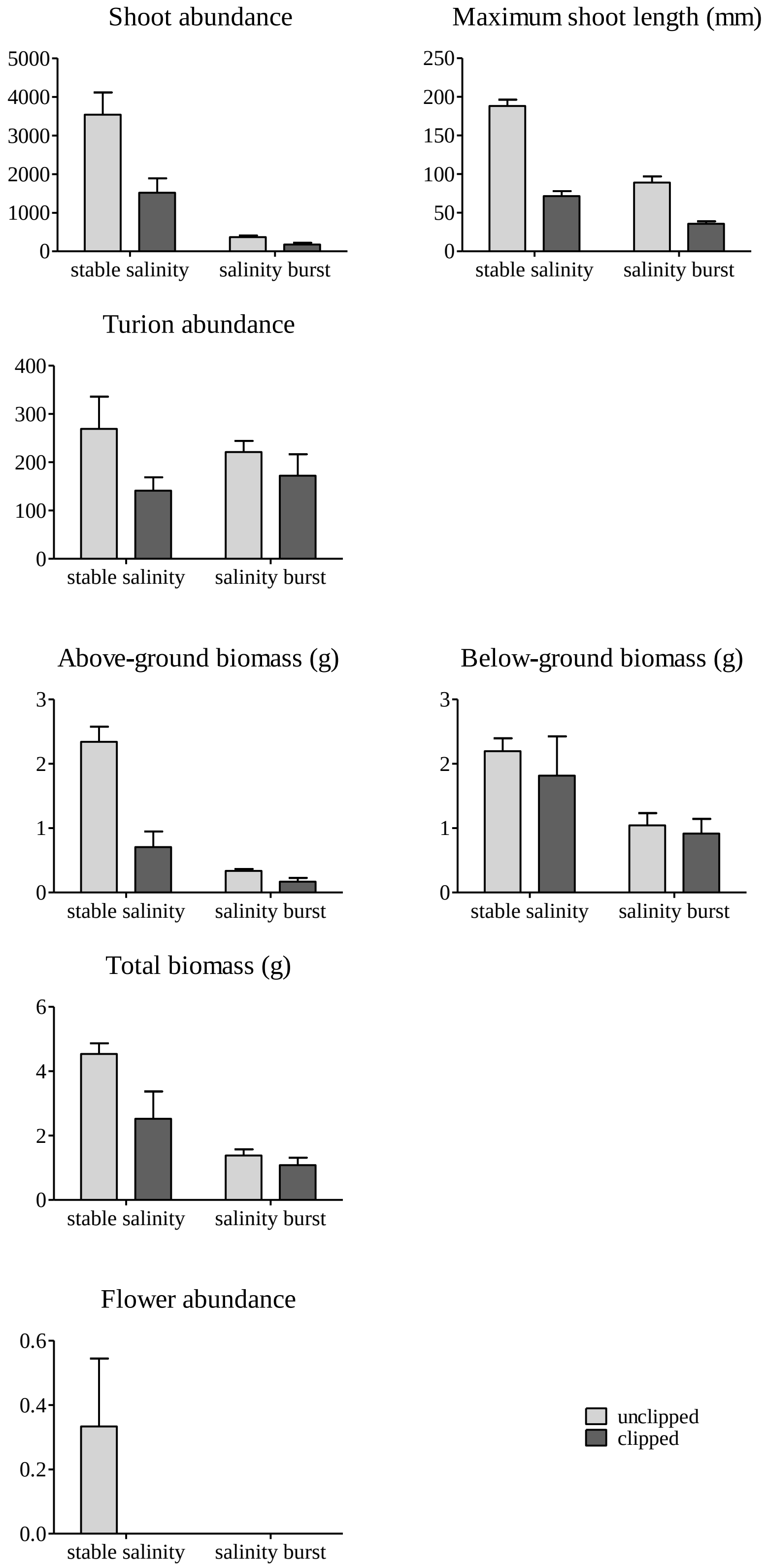


Fig. 4. Mean responses per container of samples from pond experiment using Lake Cantara plants with salinity starting at 30 gL^{-1}. Error bar represents standard error of the mean. Sample size (*n*) is six. Stable salinity is salinity maintained at 30 gL^{-1} and salinity burst is salinity raised from 30 to 60 gL^{-1} and maintained at 60 gL^{-1}. Clipping occurred weekly and ceased two weeks prior to the experiment ending. Note that y-axes differ in magnitude and units.

(ii) Rob's Point plants

The plants from Rob's Point recovered well following transplantation into 30 gL^{-1} salinity, but quickly became smothered in several types of algae. This resulted in the visual difference between treatments being not as striking as in the Lake Cantara plant experiment (Fig 5). However, there were statistical differences in the response variables. Shoot abundance and above-ground biomass responded to the salinity and clipping treatments in the same way, resulting in significant increases under salinity burst, and significant reductions when clipped (Table 4 and Fig. 6). Above-ground biomass was further reduced by the interaction of salinity burst and clipping, but this only accounted for 8 % of total variation and influenced plant responses less than the salinity burst and clipping on their own (27 % and 36 % respectively). Maximum shoot length and total biomass were not affected by salinity burst, but experienced a significant reduction when clipped. Flower abundance was significantly lower in plants exposed to salinity burst and clipping. Eight flowers were produced; all were in tubs with stable salinities, and all but one occurred in unclipped containers (Fig. 6). The interaction between salinity burst and clipping significantly reduced flower abundance and accounted for 13 % of variance, with salinity burst and clipping on their own accounting for 26 and 13 % respectively. Turion abundance was significantly increased in plants exposed to salinity burst, and just (P = 0.048) significantly decreased by clipping. Below-ground biomass did not change significantly with salinity or clipping treatments.

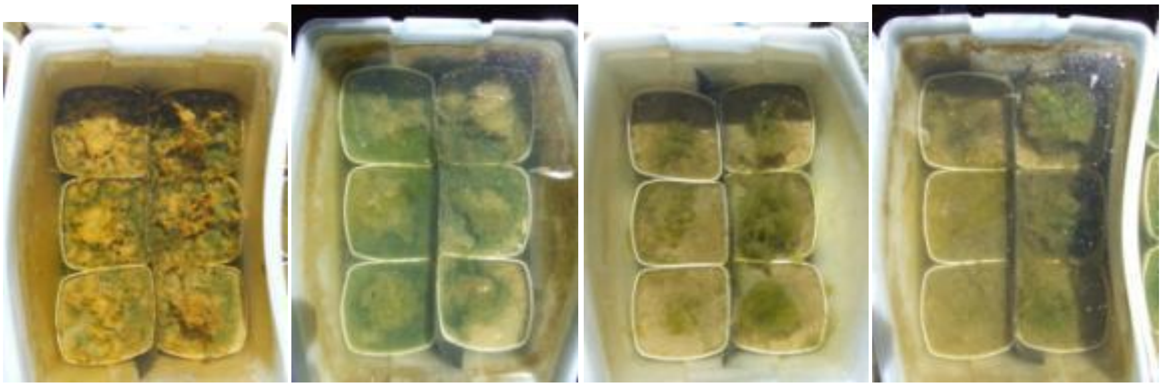


Fig. 5. Appearance upon completion of experiment of Rob’s Point plants with salinity starting at 30 gL^{-1}. Stable salinity tubs (with salinity maintained at 30 gL^{-1}) are on the left, and salinity burst tubs (with salinity raised from 30 to 60 gL^{-1} and maintained at 60 gL^{-1}) are on the right. Within each tub, the three containers on the left were clipped weekly (ceasing two weeks prior to the experiment ending) and the three on the right were unclipped.

Table 4. Summary of *P*-values from two-way ANOVAs testing significant differences in the response of Rob’s point plants in salinity starting at 30 gL^{-1} to salinity and clipping treatments. Significant results ($P < 0.05$) are shown in bold.

Response variable	Interaction	Clipping treatment	Salinity treatment
Shoot abundance	0.160	**0.019**	**0.004**
Max shoot length (mm)	0.051	**< 0.001**	0.861
Turion abundance	0.419	**0.048**	**0.005**
Above-ground biomass (g)	**0.032**	**< 0.001**	**< 0.001**
Below-ground biomass (g)	0.425	0.265	0.453
Total biomass (g)	0.186	**0.026**	0.076
Flower abundance	**0.032**	**0.032**	**0.006**

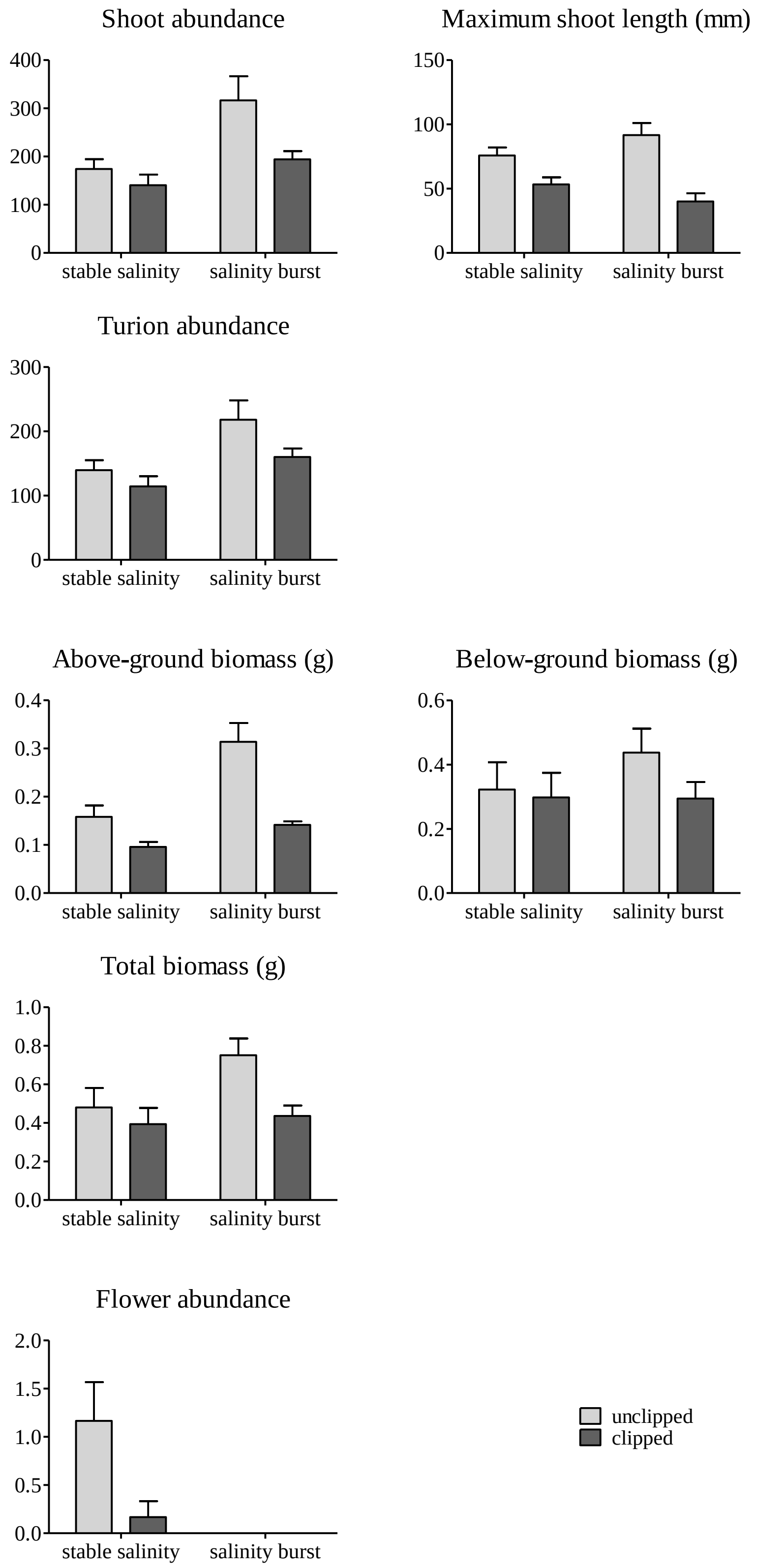


Fig. 6. Mean of samples from pond experiment using Rob's Point plants with salinity starting at 30 gL^{-1}. Error bar represents standard error of the mean. Sample size (*n*) is six. Stable salinity is salinity maintained at 30 gL^{-1} and salinity burst is salinity raised from 30 to 60 gL^{-1} and maintained at 60 gL^{-1}. Clipping occurred weekly and ceased two weeks prior to the experiment ending. Note that y-axes differ in magnitude and units.

(iii) Lake Cantara seeds

The plants that germinated from Lake Cantara seeds did not appear to be visually different between treatments by the end of the experiment (Fig. 7), but there were statistical differences in some of the response variables. Shoot abundance and total biomass significantly decreased in response to salinity burst and clipping (Table 5 and Fig. 8). A further significant reduction in total biomass was related to the interaction of stable salinity and clipping. However, this only accounted for 10 % of total variation and influenced plant responses less than the salinity and clipping treatments on their own (22 and 43 % respectively). Maximum shoot length and below-ground biomass were both significantly reduced by salinity burst and clipping. Turion abundance was only just significantly reduced ($P = 0.049$) by salinity burst, but was not affected by clipping. Conversely, above-ground biomass was significantly reduced by clipping, but was not affected by salinity burst. Seed and flower abundances were not influenced by salinity bursts or clipping. However, as with the Lake Cantara plant experiment in salinities starting at 30 gL^{-1}, the low flower abundances mean that the data does not provide a sound basis for assessing whether flower production was influenced by salinity and/or clipping treatments. Both flowers were produced in stable salinity tubs, and both were in unclipped containers.

Lepilaena and *Chara* spp also sprouted from seeds in this experiment. *Lepilaena* was more abundant than *Ruppia tuberosa*, with mean shoot abundances per container reaching 833 ± 12 compared to 263 ± 40 respectively (Fig. 8). Mirroring the response of *Ruppia tuberosa* shoot abundances, *Lepilaena* shoot numbers were significantly reduced by a salinity burst and clipping (Table 5 and Fig. 8). The species flowered prolifically in containers exposed to stable salinity and no clipping, but did not flower at all when exposed to a salinity burst or clipping. Plants in some unclipped containers in stable salinities (such as the top right container in the far left tub in Fig. 7) produced more than 25 flower stems. *Chara* occurred in low numbers in this experiment, with mean shoot abundances only reaching 4 ± 2 per container. *Chara* shoot abundances were significantly higher in stable salinities, and higher (although not significantly so) in clipped containers compared to unclipped (Table 5 and Fig. 8).

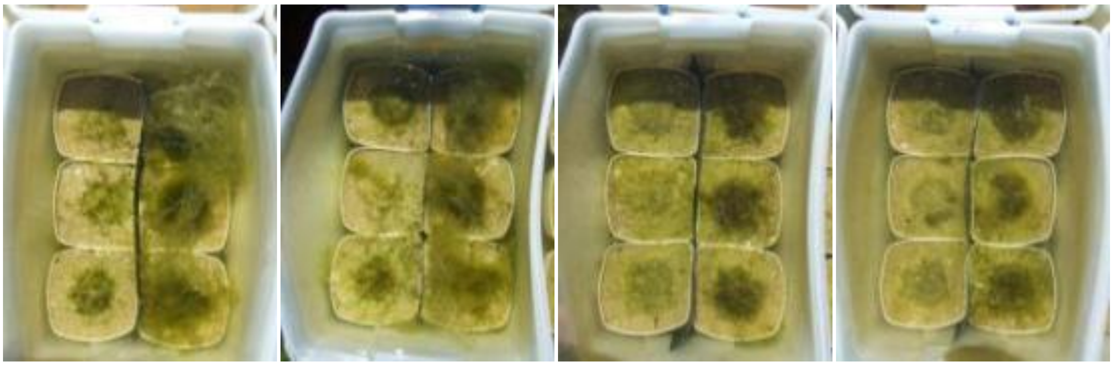
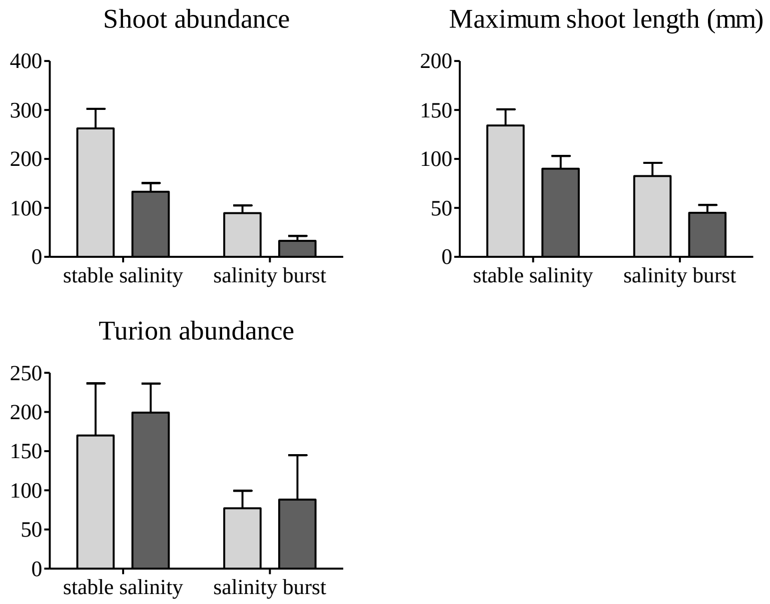
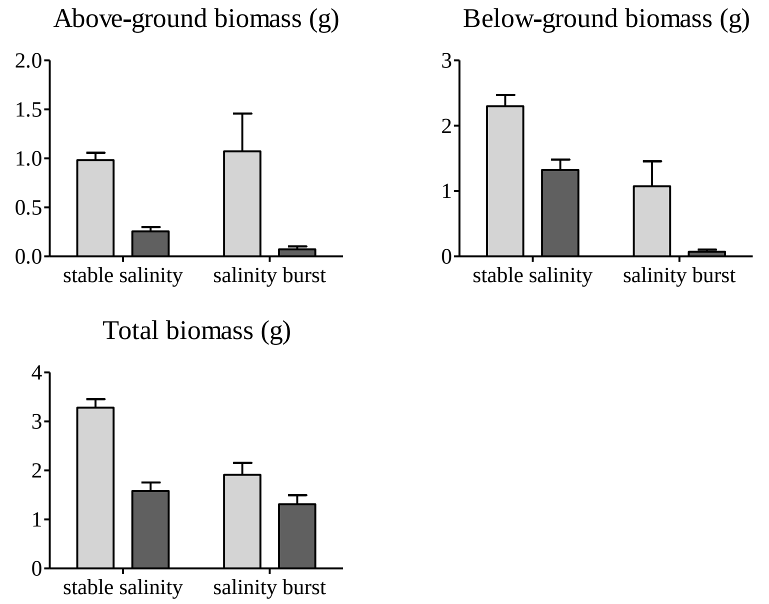


Fig. 7. Appearance upon completion of experiment of plants germinated from Lake Cantara seeds with salinity starting at 30 gL^{-1}. Stable salinity tubs (with salinity maintained at 30 gL^{-1}) are on the left, and salinity burst tubs (with salinity raised from 30 to 60 gL^{-1} and maintained at 60 gL^{-1}) are on the right. Within each tub, the three containers on the left were clipped weekly (ceasing two weeks prior to the experiment ending) and the three on the right were unclipped.

Table 5. Summary of *P*-values from two-way ANOVAs testing significant differences in the response of Lake Cantara seeds in salinity starting at 30 gL^{-1} to salinity and clipping treatments. Significant results ($P < 0.05$) are shown in bold.

Response variable	Interaction	Clipping treatment	Salinity treatment
Shoot abundance	0.142	**< 0.001**	**< 0.001**
Max shoot length (mm)	0.803	**0.006**	**0.002**
Turion abundance	0.853	0.684	**0.049**
Above-ground biomass (g)	0.496	**< 0.001**	0.814
Below-ground biomass (g)	0.956	**< 0.001**	**< 0.001**
Total biomass (g)	**0.011**	**< 0.001**	**< 0.001**
Flower abundance	0.130	0.130	0.130
Seed abundance	0.243	0.655	0.095
Lepilaena shoot abundance	0.247	**0.002**	**< 0.001**
Chara shoot abundance	0.263	0.263	**0.008**


Shoot abundance
400
300
200
100
0
stable salinity
salinity burst
Maximum shoot length (mm)
200
150
100
50
0
stable salinity
salinity burst
Turion abundance
250
200
150
100
50
0
stable salinity
salinity burst


Above-ground biomass (g)
2.0
1.5
1.0
0.5
0.0
stable salinity
salinity burst
Below-ground biomass (g)
3
2
1
0
stable salinity
salinity burst
Total biomass (g)
4
3
2
1
0
stable salinity
salinity burst

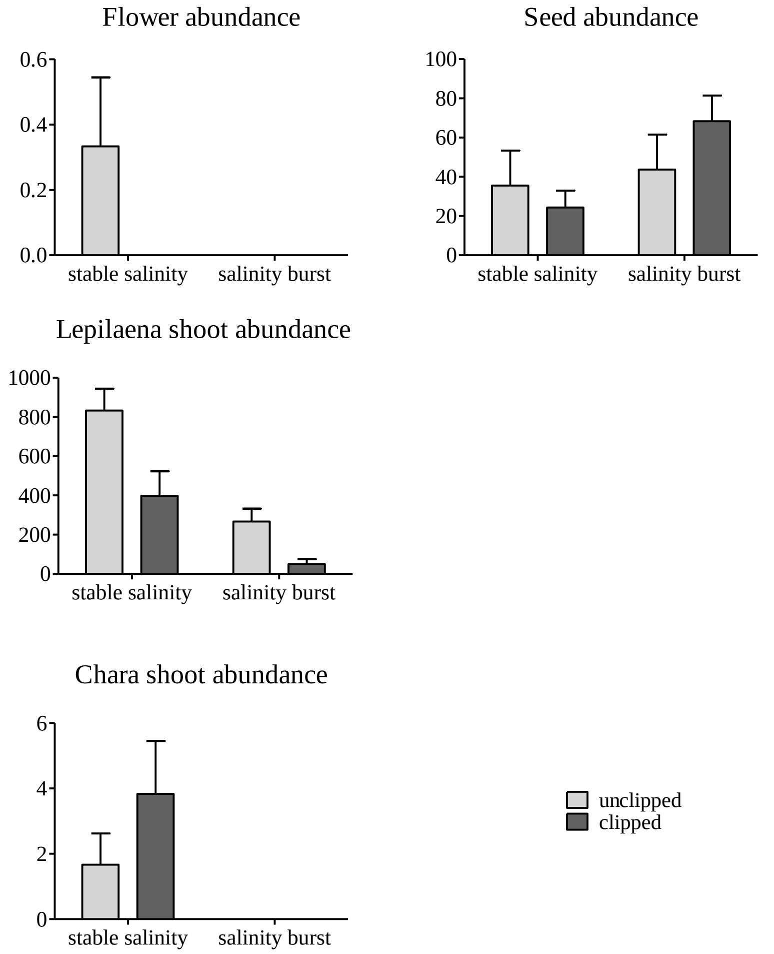


Fig. 8. Mean responses per container of samples from pond experiment using Lake Cantara seeds with salinity starting at 30 gL^{-1}. Error bar represents standard error of the mean. Sample size (*n*) is six. Stable salinity is salinity maintained at 30 gL^{-1} and salinity burst is salinity raised from 30 to 60 gL^{-1} and maintained at 60 gL^{-1}. Clipping occurred weekly and ceased two weeks prior to the experiment ending. Note that y-axes differ in magnitude and units.

Summary of results from experiments starting at 30 gL^{-1} salinity

The response of *Ruppia tuberosa* to the salinity and clipping treatments was not always the same across the Lake Cantara plant, Rob's Point plant, and Lake Cantara seed experiments. *Ruppia* shoot abundance was the only response variable affected by salinity in all experiments, albeit negatively for the Lake Cantara plants and seeds, and positively for the Rob's Point plants. In contrast, clipping reduced maximum shoot length, above-ground biomass, and total biomass in all experiments suggesting that shoots (i.e. above-ground biomass) have a disproportionate influence over total biomass in these *ex situ* experiments. Turion abundance and below-ground biomass did not respond to the salinity or clipping treatments consistently across the three experiments. Nor did flower abundance, but the low abundances mean that the data do not provide a sound basis for assessing whether flower production was influenced by the salinity and/or clipping treatments. All *Ruppia tuberosa* flowers were produced in stable salinity conditions, and 11 of the 12 flowers (92 %) were in unclipped containers.

Ruppia tuberosa shoot density was far greater in the Lake Cantara plant experiment (reaching 3539 ± 576 shoots per container) than the Rob's Point plant and Lake Cantara seed experiments (only reaching 317 ± 50 and 263 ± 40 shoots per container, respectively). Likewise, maximum shoot length was longer in the Lake Cantara plant experiment compared to the Rob's Point plant and Lake Cantara seed experiments, with maximum shoot lengths (mm) reaching 188 ± 8, 92 ± 9, 134 ± 17, respectively. Consequently, above-ground and total biomasses were also higher in the Lake Cantara plant experiment. Both the plant experiments had more than twice as much below-ground biomass per container than the seed experiment. Despite the plants from the Lake Cantara seed experiment starting from seeds, they still produced similar numbers of turions as the plants from the Lake Cantara plant experiment.

Lepilaena and *Chara* spp sprouted in the Lake Cantara seed experiment. *Chara* only occurred in stable salinities and was not abundant. *Lepilaena* was more than twice as abundant as *Ruppia tuberosa* but responded to treatments in the same way as *Ruppia tuberosa.* The Lake Cantara and Rob's Point plant experiments starting at 30 gL^{-1} salinity did not have *Lepilaena* or *Chara* in them.

Experiments starting at 60 gL^{-1} salinity

(i) Lake Cantara plants

The Lake Cantara plants were not as strikingly healthy as those that started at 30 gL^{-1} salinity, and differences between treatments were less visually obvious (Fig. 9). Maximum shoot length was significantly reduced by salinity burst and clipping (Table 6 and Fig. 10). A further significant reduction was related to the interaction between clipping and stable salinity, but this only accounted for 10 % of total variation and influenced plant responses less than individual salinity and clipping treatments (44 and 20 % respectively). Below-ground biomass and total biomass were both significantly increased by salinity burst, suggesting that below-ground biomass may disproportionately influence total biomass. Shoot and turion abundance, and above-ground biomass were not significantly affected by salinity or clipping treatments. Flowers were not produced in any treatment.

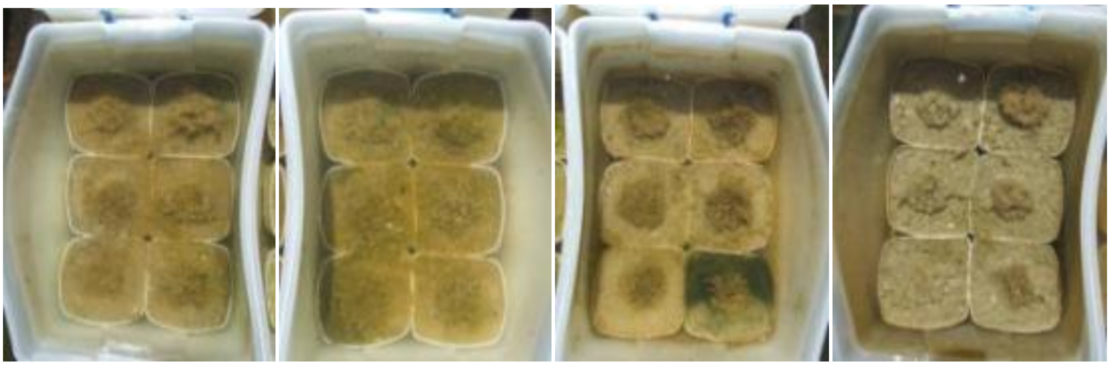


Fig. 9. Appearance upon completion of experiment of Lake Cantara plants with salinity starting at 60 gL^{-1}. Stable salinity tubs (with salinity maintained at 60 gL^{-1}) are on the left, and salinity burst tubs (with salinity raised from 60 to 90 gL^{-1} and maintained at 90 gL^{-1}) are on the right. Within each tub, the three containers on the left were clipped weekly (ceasing two weeks prior to the experiment ending) and the three on the right were unclipped.

Table 6. Summary of *P*-values from two-way ANOVAs testing significant differences in the response of Lake Cantara plants in salinity starting at 60 gL^{-1} to salinity and clipping treatments. Significant results ($P < 0.05$) are shown in bold.

Response variable	Interaction	Clipping treatment	Salinity treatment
Shoot abundance	0.370	0.435	0.143
Max shoot length (mm)	**0.011**	**< 0.001**	**< 0.001**
Turion abundance	0.718	0.822	0.314
Above-ground biomass (g)	0.185	0.707	0.738
Below-ground biomass (g)	0.295	0.172	**0.004**
Total biomass (g)	0.171	0.209	**0.005**

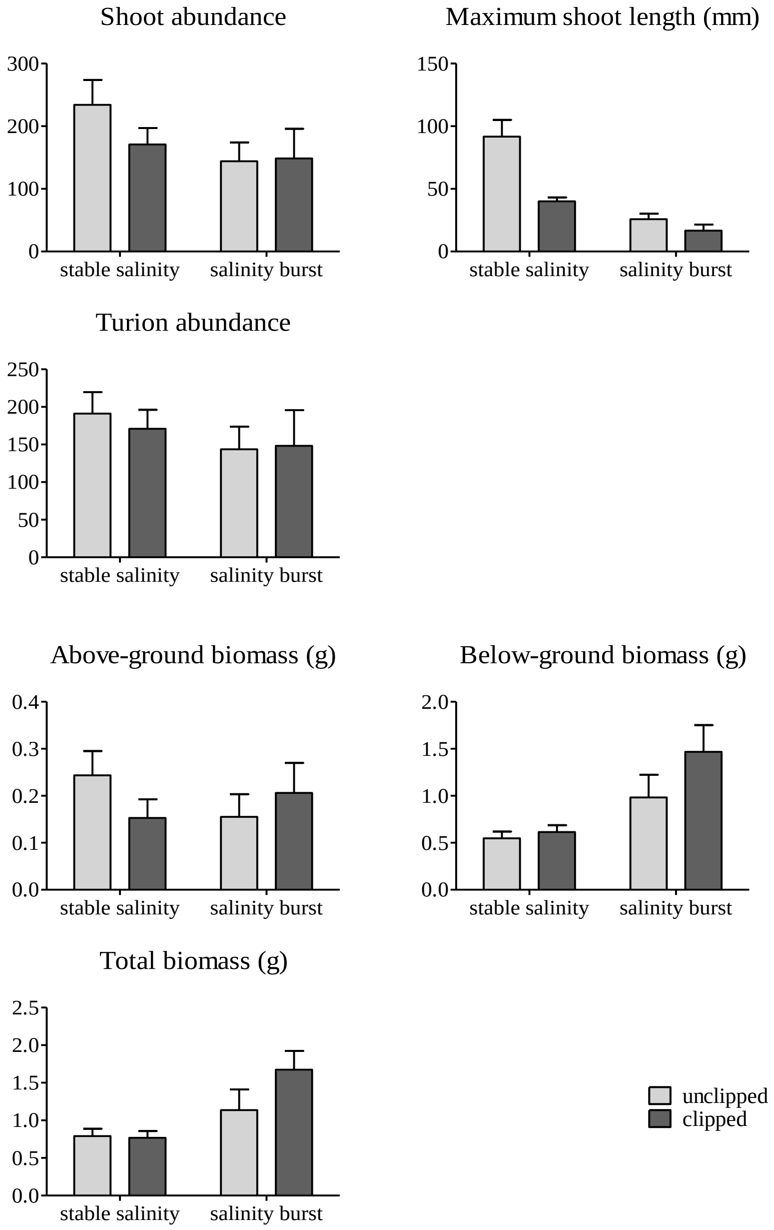


Fig. 10. Mean responses per container of samples from pond experiment using Lake Cantara plants with salinity starting at 60 gL^{-1}. Error bar represents standard error of the mean. Sample size (*n*) is six. Stable salinity is salinity maintained at 60 gL^{-1} and salinity burst is salinity raised from 60 to 90 gL^{-1} and maintained at 90 gL^{-1}. Clipping occurred weekly and ceased two weeks prior to the experiment ending. Note that y-axes differ in magnitude and units.

(ii) Rob's Point plants

The plants from Rob's Point recovered after being transplanted into 60 gL^{-1} salinity tubs, but failed to grow laterally following recovery (Fig. 11). The plants and substrate became smothered in several types of algae. Maximum shoot length was significantly reduced by salinity burst. Above-ground and total biomasses were significantly reduced by clipping (Table 7 and Fig. 12). Shoot and turion abundance, and below-ground biomass were not affected by salinity or clipping treatment. Flowers were not produced in any treatment.

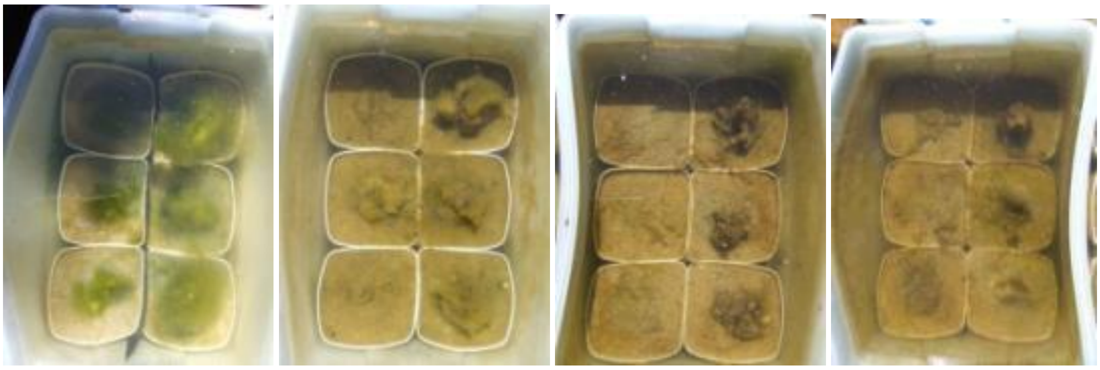


Fig. 11. Appearance upon completion of experiment of Rob's Point plants with salinity starting at 60 gL^{-1}. Stable salinity tubs (with salinity maintained at 60 gL^{-1}) are on the left, and salinity burst tubs (with salinity raised from 60 to 90 gL^{-1} and maintained at 90 gL^{-1}) are on the right. Within each tub, the three containers on the left were clipped weekly (ceasing two weeks prior to the experiment ending) and the three on the right were unclipped.

Table 7. Summary of *P*-values from two-way ANOVAs testing significant differences in the response of Rob's Point plants in salinity starting at 60 gL^{-1} to salinity and clipping treatments. Significant results ($P < 0.05$) are shown in bold.

Response variable	Interaction	Clipping treatment	Salinity treatment
Shoot abundance	0.811	0.133	0.064
Max shoot length (mm)	0.947	0.324	**< 0.001**
Turion abundance	0.991	0.099	0.338
Above-ground biomass (g)	0.134	**0.023**	0.457
Below-ground biomass (g)	0.413	0.203	0.514
Total biomass (g)	0.867	**0.016**	0.885

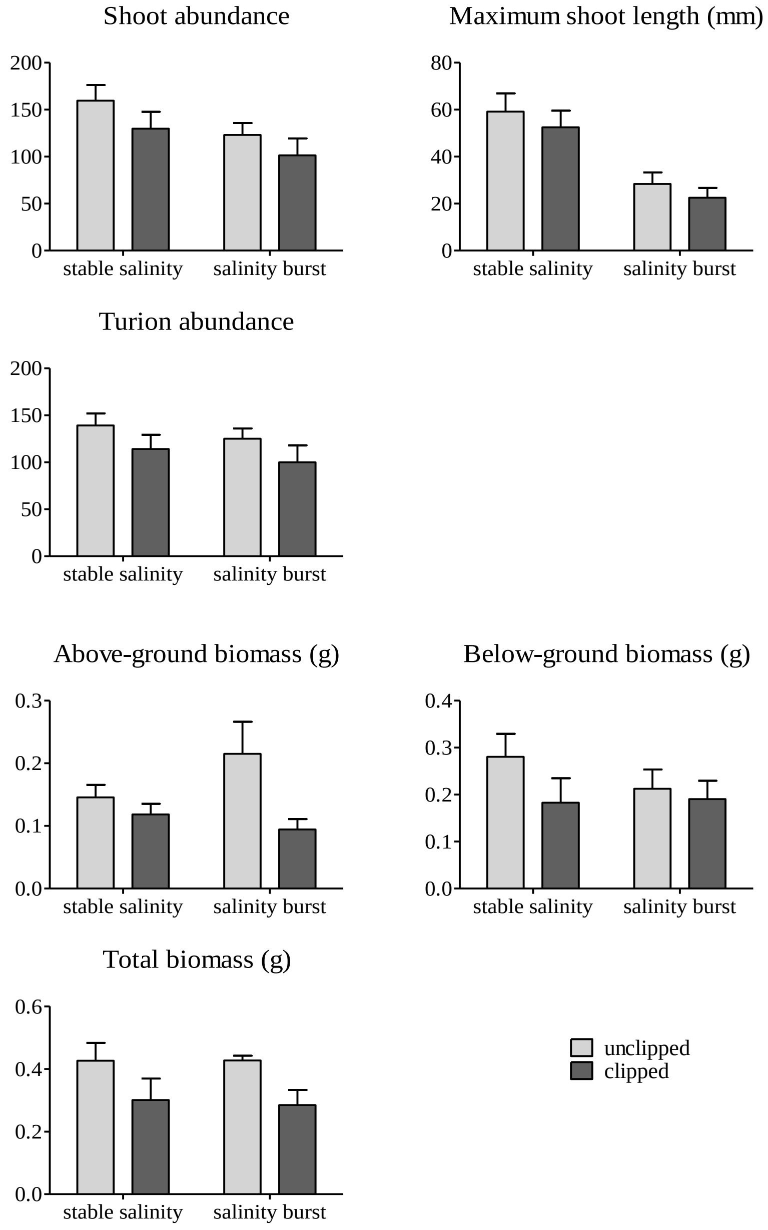


Fig. 12. Mean responses per container of samples from pond experiment using Rob's Point plants with salinity starting at 60 gL^{-1}. Error bar represents standard error of the mean. Sample size (*n*) is six. Stable salinity is salinity maintained at 60 gL^{-1} and salinity burst is salinity raised from 60 to 90 gL^{-1} and maintained at 90 gL^{-1}. Clipping occurred weekly and ceased two weeks prior to the experiment ending. Note that y-axes differ in magnitude and units.

(iii) Lake Cantara seeds

Following germination, the plants in this experiment failed to establish to a point where the experiments (raising the salinity and clipping the plants) could be carried out (Fig. 13). Therefore, all the plants in this experiment were unclipped and in a stable salinity of 60 gL^{-1}. Every seed that germinated produced one shoot and one turion (evidenced by the respective abundance per container of 32 ± 6 and 40 ± 7), but did not produce lateral growth (Table 8). Considering this, shoot abundances can be used as a crude approximation of seed germination abundance per core.

Therefore, bearing in mind there were 57 ± 8 seeds per core that did not germinate, germination abundances per core were approximately 32 ± 6, or approximately 36 % of seeds in the core. *Ruppia tuberosa* plants did not produce flowers, and *Lepilaena* and *Chara* seeds did not sprout in this experiment.

Fig. 13. Appearance upon completion of experiment of plants germinated from Lake Cantara seeds with salinity starting at 60 gL^{-1}. Note that because the plants never established after germinating, the salinity burst and clipping were not carried out, resulting in four tubs with the same salinity and clipping treatments (unclipped plants in salinity maintained at 60 gL^{-1}).

Table 8. Mean responses per container of samples from pond experiment using Lake Cantara seeds in salinity starting at 60 gL^{-1}.

Response variable	Mean	±	s.e.
Shoot abundance	32	±	6
Max shoot length (mm)	46	±	6
Turion abundance	40	±	7
Above-ground biomass (g)	0.048	±	0.007
Below-ground biomass (g)	0.754	±	0.086
Total biomass (g)	0.802	±	0.088
Seed abundance	57	±	8

Summary of results from experiments starting at 60 gL^{-1} salinity

The responses of the plants to salinity burst and clipping in the Lake Cantara plant, Rob's Point plant, and Lake Cantara seed experiments were not similar. Seeds in the Lake Cantara seed experiment germinated but failed to develop into established plants, so did not receive a salinity burst or clipping. Both the plant experiments did receive a salinity burst and clipping, but the only significant response they had in common was a shorter maximum shoot length when exposed to a salinity burst. Shoot and turion abundance did not respond to salinity burst or clipping in either plant experiment. Additionally, above-ground biomass did not respond to a salinity burst, and below-ground biomass did not respond to clipping in both plant experiments. Flowers were not produced under any treatment in any of the experiments starting at 60gL^{-1} salinity.

Ruppia tuberosa in the Lake Cantara and Rob's Point plant experiments did not respond to treatments identically. Turion abundance was higher in the Lake Cantara plant experiment, reaching

191 ± 29 compared to 139 ± 13 in the Rob's Point plant experiment. The Lake Cantara plant experiment also recorded heavier below-ground and total biomasses than the Rob's Point plant experiment. However, shoot abundance and maximum shoot length were comparable between the plant experiments.

Differences between the results from the Lake Cantara seed experiment and the results from equivalent conditions (stable 60 gL^{-1} salinity and no clipping) in the Lake Cantara plant experiment were evident. Plants transplanted into the experiment produced seven-times more shoots, longer shoots, and five-times more turions and above-ground biomass than plants germinated from seeds in the experiment. However, below-ground and total biomasses were similar in both experiments.

Experiments starting at 90 gL^{-1} salinity

(i) Lake Cantara plants

The Lake Cantara plants never recovered from being transplanted into the 90 gL^{-1} salinity tubs. The majority of plants appeared dead for the duration of the experiment (Fig. 14); the green in Fig. 14 is not *Ruppia tuberosa* leaves, but algae covering the substrate. Because of this, the plants in experimental tubs were not subjected to a salinity burst, resulting in four tubs with the same 90 gL^{-1} stable salinity treatment. However, the plants were accidently clipped twice near the start of the experiment. Clipping did not illicit significant responses from the plants (Table 9). Shoot and turion abundance, maximum shoot length and above-ground, below-ground and total biomasses did not respond to clipping, although the response of above-ground biomass ($P = 0.053$) was almost significant (Table 9). Although not significantly so, the mean values per container of all response variables were higher in the unclipped than clipped treatment. Flowers were not produced by plants in this experiment.

Fig. 14. Appearance upon completion of experiment of Lake Cantara plants with salinity starting at 90 gL^{-1}. Note that because the plants never established after being transplanted, they were not subjected to a salinity burst, resulting in four tubs with the same salinity treatment (salinity stable at 90 gL^{-1}). The plants were accidently clipped, however. Within each tub, the three containers on the left were clipped twice near the start of the experiment and the three on the right were unclipped.

Table 9. Summary of results per container from Lake Cantara plant experiment in salinity starting at 90 gL^{-1}: mean ± s.e. and *P*-values from Mann-Whitney tests comparing plant responses to clipping and no clipping. Sample size (*n*) is six. Significant results ($P < 0.05$) are shown in bold.

Response variable	Unclipped	Clipped	P-value
Shoot abundance	218 ± 25	168 ± 24	0.166
Max shoot length (mm)	29 ± 4	21 ± 2	0.145
Turion abundance	217 ± 25	168 ± 24	0.166
Above-ground biomass (g)	0.258 ± 0.032	0.184 ± 0.022	0.053
Below-ground biomass (g)	0.806 ± 0.121	0.572 ± 0.064	0.126
Total biomass (g)	1.064 ± 0.124	0.756 ± 0.065	0.073

(ii) Rob's Point plants

The Rob's Point plants responded in the same way as the Lake Cantara plants to being transplanted into the 90 gL^{-1} salinity tubs, and the majority of plants appeared dead for the duration of the experiment (Fig. 15). As with the Lake Cantara plants in 90 gL^{-1} salinity experiment, the plants in experimental tubs were therefore not subjected to a salinity burst. This resulted in four tubs with the same 90 gL^{-1} stable salinity treatment, but plants were accidently clipped twice near the start of the experiment. All response variables except maximum shoot length had a significant difference between clipped and unclipped containers (Table 10). Shoot and turion abundance, and above-ground, below-ground and total biomasses were all significantly lower in the clipping treatment (Table 10). Every shoot in unclipped and clipped treatments produced a turion; shoot and turion abundances were identical in unclipped containers (both 126 ± 13) and in clipped containers (both 68 ± 16). Flowers were not produced by plants in this experiment.

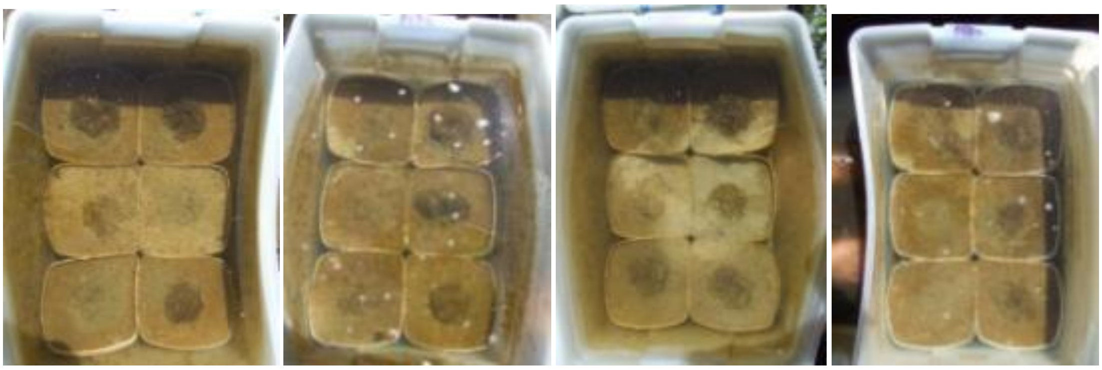


Fig. 15. Appearance upon completion of experiment of Rob's Point plants with salinity starting at 90 gL^{-1}. Note that because the plants never established after being transplanted, the salinity burst was not carried out, resulting in four tubs with the same salinity treatment (salinity stable at 90 gL^{-1}). The plants were accidently clipped, however. Within each tub, the three containers on the left were clipped twice near the start of the experiment and the three on the right were unclipped.

Table 10. Summary of results per container from Rob's Point plant experiment in salinity starting at 90 gL^{-1}: mean ± s.e. and *P*-values from Mann-Whitney tests comparing plant responses to clipping and no clipping. Sample size (*n*) is six. Significant results ($P < 0.05$) are shown in bold.

Response variable	Unclipped	Clipped	P-value
Shoot abundance	126 ± 13	68 ± 16	**0.026**
Max shoot length (mm)	11 ± 3	11 ± 2	0.951
Turion abundance	126 ± 13	68 ± 16	**0.026**
Above-ground biomass (g)	0.104 ± 0.001	0.056 ± 0.012	**0.024**
Below-ground biomass (g)	0.261 ± 0.035	0.128 ± 0.012	**0.004**
Total biomass (g)	0.364 ± 0.364	0.184 ± 0.021	< **0.001**

(iii) Lake Cantara seeds

Few seeds germinated under these high salinity conditions, and those that did germinate failed to establish into plants with lateral growth (Fig. 16). Because of this, the plants in experimental tubs and containers were not subjected to salinity burst and clipping, resulting in four tubs with the same salinity and clipping treatments (unclipped plants in 90 gL^{-1} stable salinity). As with the Lake Cantara seed experiment starting at 60 gL^{-1} salinity, every seed that germinated produced one shoot and one turion (evidenced by the respective abundance per container of 20 ± 5 and 29 ± 5), but did not produce lateral growth (Table 11). Considering this, shoot abundances can be used as a crude approximation of seed germination abundance per core. Therefore, bearing in mind there were 73 ± 7 seeds per core that did not germinate, seed germination abundance per core was approximately 20 ± 5, or 22 % of all seeds in the core. *Ruppia tuberosa* plants did not produce flowers, and *Lepilaena* and *Chara* did not sprout in this experiment.

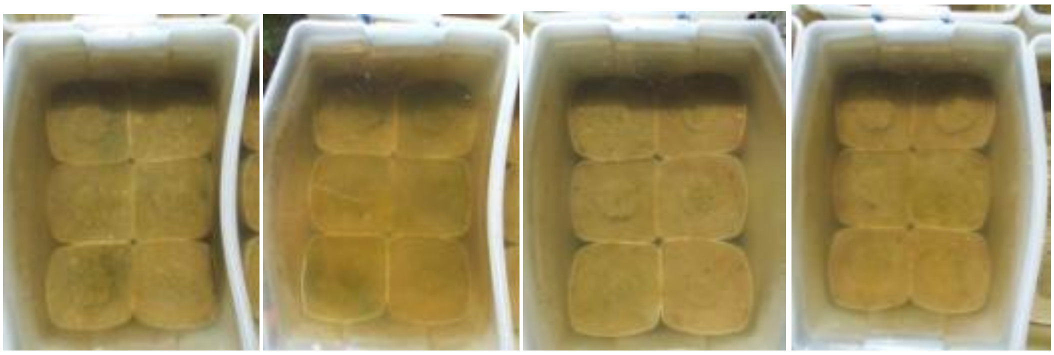


Fig. 16. Appearance upon completion of experiment of plants germinated from Lake Cantara seeds with salinity starting at 90 gL^{-1}. Note that because the plants never established after germinating, the salinity burst and clipping were not carried out, resulting in four tubs with the same salinity and clipping treatments (unclipped plants in stable 90 gL^{-1} salinity).

Table 11. Mean responses per container of samples from pond experiment using Lake Cantara seeds in salinity starting at 90 gL^{-1}.

Response variable	Mean	±	s.e.
Shoot abundance	20	±	5
Max shoot length (mm)	10	±	2
Turion abundance	29	±	5
Above-ground biomass (g)	0.029	±	0.005
Below-ground biomass (g)	1.122	±	0.120
Total biomass (g)	1.151	±	0.121
Seed abundance	73	±	7

Summary of results from experiments starting at 90 gL^{-1} salinity

The Lake Cantara and Rob's Point plants did not recover well after being transplanted into 90 gL^{-1} salinity, and spent the duration of the experiments appearing dead. Similarly, the few Lake Cantara seeds that germinated failed to grow. This failure of all plants in all 90 gL^{-1} salinity experiments to establish into plants with lateral growth meant that experimental treatments (salinity burst and clipping) could not be undertaken. However, the Lake Cantara and Rob's Point plants were accidently clipped and yielded significant responses by all variables except maximum shoot length on Rob's Point plants, but did not significantly affect any response variables in Lake Cantara plants. Flowers were not produced by plants in any of the 90 gL^{-1} salinity experiments.

Differences between the results from the Lake Cantara plant and Rob's Point plant experiments are evident. Shoot and turion abundance, maximum shoot length, and above-ground, below-ground and total biomasses were approximately two (or more) times higher in Lake Cantara plants compared to Rob's Point plants.

Differences between the results from the Lake Cantara seed experiment and the results from equivalent conditions (stable 90 gL^{-1} salinity and no clipping) in the Lake Cantara plant experiment are evident. Plants transplanted into the experiment produced ten-times more shoots and above-ground biomass, three-times longer shoots, and eight-times more turions. However, below-ground and total biomasses were similar in both experiments.

The responses of the plants in the Lake Cantara seed experiment in 90 gL^{-1} stable salinity were dampened compared to the 60 gL^{-1} stable salinity experiment. Mean values for shoot abundance (and therefore germination rate), turion abundance, maximum shoot length, and above-ground biomass were all smaller under 90 gL^{-1} stable salinities. Conversely, mean values for below-ground biomass, total biomass, and seed abundance were slightly larger under 90 gL^{-1} stable salinities.

Field observations on the performance of *Ruppia tuberosa* during 2010-11

Observations on Ruppia tuberosa *at Rob's Point and in the North Lagoon*

In late September 2010 *Ruppia tuberosa* was flowering extensively at Robs Point in the North Lagoon of the Coorong, with up to 50 flower heads per square metre. This was the first time that any substantial flowering had taken place at this site. Salinities at Robs Point in September 2010 were 87 gL^{-1} and since the salinities had increased from 62 gL^{-1} in July (Table 12), neither a high salinity nor a relatively rapid increase in salinity prevents *Ruppia tuberosa* from flowering. Despite the high salinities many of the plants and flower-heads were entangled with filamentous green algae (e.g. Fig. 17), and because the water levels had also dropped dramatically since July about half of the extensive *Ruppia tuberosa* beds including plants that had commenced flowering were now left exposed (e.g. Fig. 18). With the return of flows over the barrages water levels rose in the Coorong during October and salinities dropped at Robs Point, such that by November salinities were around 50 gL^{-1} (Table 12). Salinities dropped further and by December 2010 were around 20 gL^{-1}. This rise in water levels re-inundated the exposed mudflats. In November when samples of *Ruppia tuberosa* were collected to score abundances of seeds and turions no plants were flowering, but there were substantial numbers of type I turions present (ca 15,000 turions.m^{-2}) but few seeds. In January 2011, the plants at Robs Point were again flowering, but there were few turions or seeds present. Filamentous green algae were prominent again and largely swamped and covered all of the *Ruppia* beds (e.g. see frontispiece). In July 2011 when this site was revisited there were no plants growing and the former beds of *Ruppia tuberosa* that had established in recent years were entirely absent but there was a small number of seeds (ca 450 seeds.m^{-2}) present in the sediments.

Table 12. Changes in salinity and performance of *Ruppia tuberosa* at Robs Point during 2010-11. Values shown in brackets for some months are from nearby Noonameena as there were no quantitative data for Robs Point in the December-January period. Salinities at Noonameena were similar to those at Robs Point whenever both sites were sampled, differing by 0-9 gL^{-1} (aver 5 gL^{-1}). The diameter of the cores used to assess Ruppia tuberosa was 75 mm. To convert abundances per core to abundances per square metre multiply by 226. Note that the abundances of shoots, turions and seeds per core were based on samples of 10-200 core samples, with standard errors around 10% of the means when there were more than 5 items per core, but 30-100% of means when there were fewer than 3 items per core. Some of the fluctuations in abundances are likely to be due to small scale spatial heterogeneity, but the major changes in abundances particularly for turions and shoots reflect changes from being present in virtually all samples to being absent from all cores. (nm = present at site but not counted).

Parameter	2010								2011	
	Jan	Feb	May	Jul	Sep	Oct	Nov	Dec	Jan	Jul
salinity (gL^{-1})	(121)	146	54	62	87	81	50	(20)	(50)	36
flowering	no	no	no	no	yes	yes	no	no	yes	no
shoots/core	nm	nm		42	272		nm		(25)	0
turions/core	(6.3)	20		0	0		67.6		(0.05)	0
seeds/core	(0)	0		0.04	0.94		0.2		(0.0)	2.4

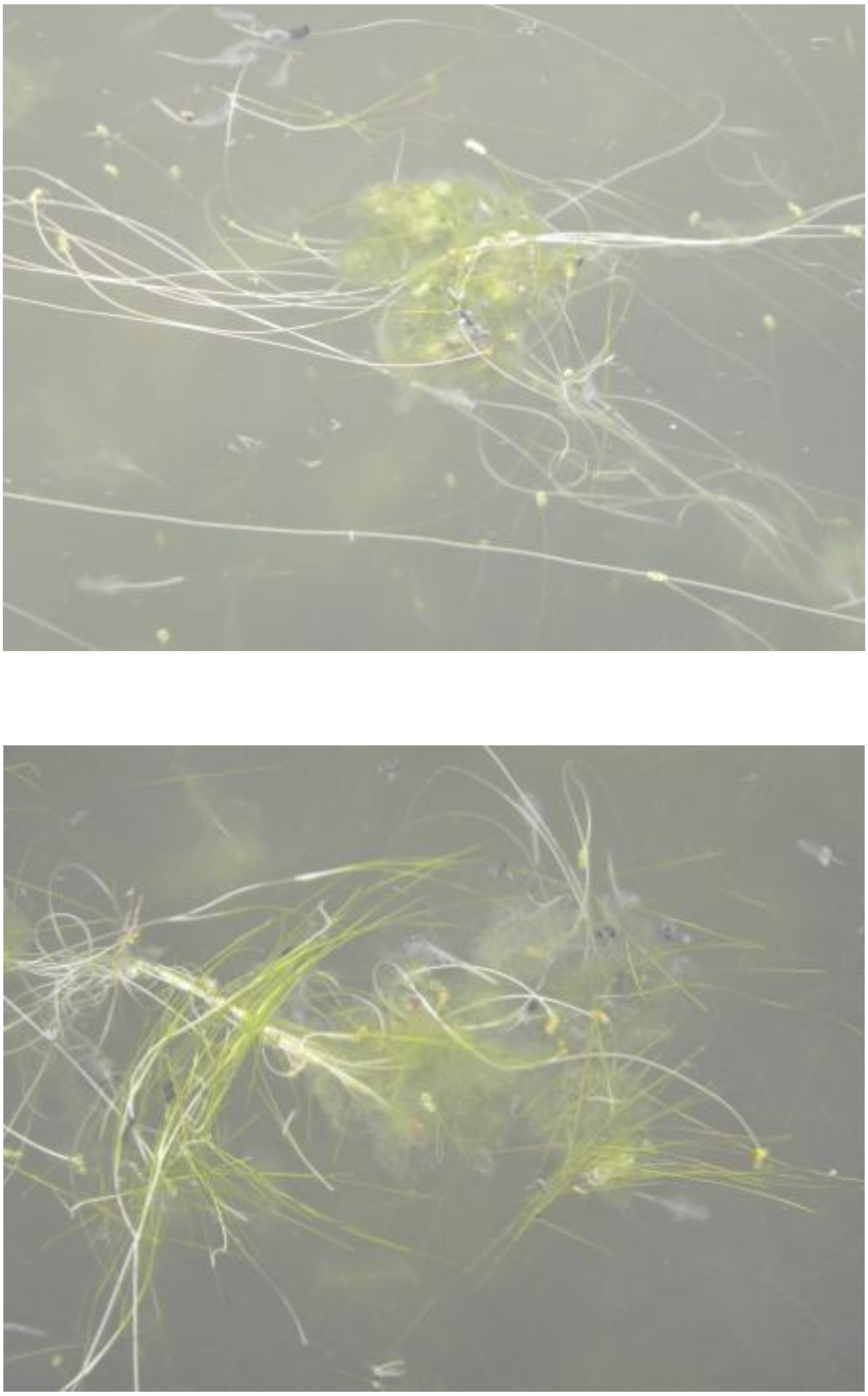


Fig. 17. Examples of filamentous algae entangled around the flower-heads of *Ruppia tuberosa* floating on the surface at Rob’s Point, September 2010. Clumps of algae are also visible below the surface.

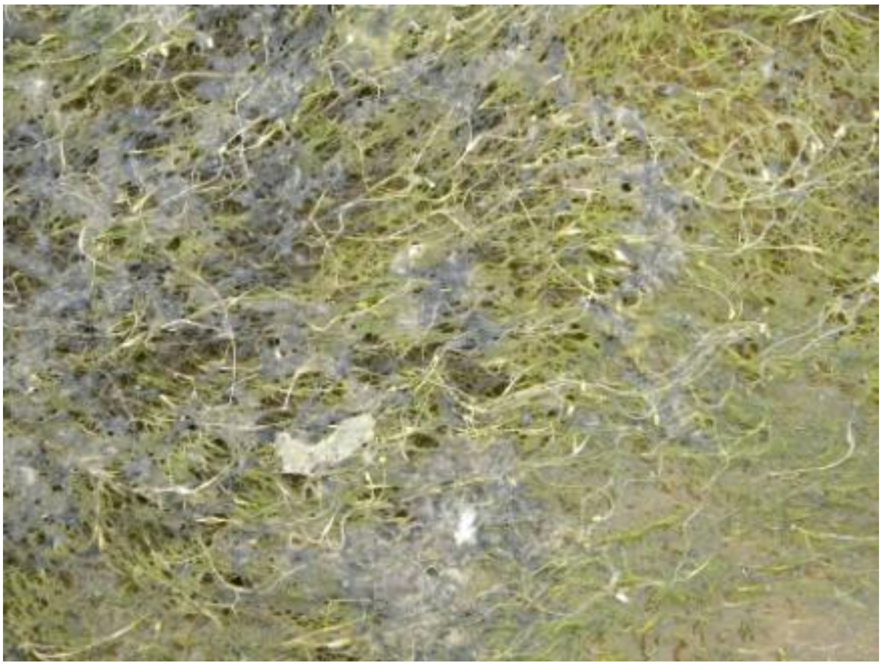


Fig. 18. Extensive mud-flats at Robs Point (top) exposed in September 2010 with *Ruppia tuberosa* and algae drying out on the exposed mudflats (bottom). Note that these exposed mudflats were covered by at least 0.5m of water by late November.

Observations on Ruppia tuberosa *at Lake Cantara*

Ruppia tuberosa plants growing in Lake Cantara were also flowering in late September 2010. Salinities at this site were 32 gL^{-1} at this time, similar to the salinities in July 2010 (Table 13). However salinities increased in October 2010 to 65 gL^{-1} as water levels dropped but the plants continued to produce floral stalks. Flowering, however, had ceased during inspections in November and December when water levels had dropped further and salinities exceeded 100 gL^{-1}. Substantial numbers of seeds and turions remained on or within the sediments in Lake Cantara just prior to the Lake drying out completely in late December 2010. The abundances of *Ruppia tuberosa* propagules at this site reached densities in excess of 30,000 turions and 24,000 seeds per square metre and were similar to the quantities present in the sediments in autumn 2010 prior to the lake filling with water. No turions remained in the sediments in July 2010 and few remained in July 2011 shortly after the mudflats had been re-inundated (Table 13), indicating that turions are relatively short-lived either sprouting when water returned to Lake Cantara in July or having decayed by then. Similarly at Robs Point, although turions were present in late summer in 2010, they had all disappeared by July 2010 (Table 12).

Table 13. Changes in salinity and performance of *Ruppia tuberosa* at Lake Cantara during 2010-11. Cores were 75mm in diameter, so to convert abundances to numbers per square metre multiply by 226. Note that the abundances of shoots, turions and seeds per core were based on samples of 10-200 core samples, with standard errors usually around 10% of the means. Some of the fluctuations in abundances from one time period to the next , however, are likely due to small scale spatial heterogeneity, but the major changes in abundances particularly for turions and shoots reflect changes from being present in virtually all core samples to being absent from nearly all cores.

Parameter	2010							2011		
	Jan	Mar	Jul	Sep	Oct	Nov	Dec	Jan	May	Jul
salinity (gL^{-1})	dry	dry	35	32	65	104	109	dry	dry	40
flowering	no	no	no	yes	yes	no	no	no	no	no
shoots/core	0	0	87		348		295	0	0	82
turions/core		104	0	0	173	76	140		58	2.1
seeds/core		123	52		91	78	109		114	53.5

Discussion

Pond Experiments

The influence of background salinities

(i) Lake Cantara plant performance

The performances of Lake Cantara plants in the pond experiments were reduced when background salinities (i.e. stable 30, 60, and 90 gL^{-1}) were higher. Performances were also dampened with an increase in salinity from 30 to 60 gL^{-1} but did not alter much from 60 to 90 gL^{-1}. This suggests the salinity threshold at which performance is affected is between 30 and 60 gL^{-1}, a conclusion supported by others {Geddes, 1987 #34; Womersley, 1975 #36}. Interestingly, turion production does not appear to be restricted by salinities in the 30 to 90 gL^{-1} range, and remains stable despite a reduction in basic plant performance. Flowering however, was stifled at the higher salinities in these pond experiments and suggests that Lake Cantara plants only flowers when salinities are below 60 gL^{-1}. These findings are supported by incidental observations reported in other studies {e.g. \Brock, 1981 #11; Brock, 1983 #14; Brock, 1983 #8; Brock, 1982b #15; Paton, 2010 #1; Geddes, 1987 #34}. However, plants growing at Lake Cantara although commencing their flowering in water with salinities around 30 gL^{-1} were still producing new flowering stalks in October when the salinities were above 60 gL^{-1}. These findings imply that the conditions in the pond experiments may have failed to mimic the conditions experienced in Lake Cantara.

(ii) Rob’s Point plant performance

An increase in background salinity did not reduce the performance of Rob’s Point plants in the same way as it did the Lake Cantara plants. Plant performances did not substantially change with background salinities of 30, 60, and 90 gL^{-1}. This suggests that Rob’s Point plants may be more tolerant of varying salinities than Lake Cantara plants. This is likely a result of the different salinities normally experienced by the source populations; salinity normally varies through the spring growing season by 30 gL^{-1} at Lake Cantara, but has varied by 30-60 gL^{-1} at Rob’s Point over this period, Tables 12,13).

Despite the apparent differences in salinity tolerances, the Rob’s Point and Lake Cantara plants were alike in their abilities to sexually and asexually reproduce. Similar to the Lake Cantara plants, the production of turions by Rob’s Point plants was not affected by salinities in the range of 30-90 gL^{-1}; turion production by Rob’s Point plants was similar for plants experiencing 30, 60 and 90 gL^{-1} stable salinities when unclipped and clipped. Additionally, just as the floral production by the plants from Lake Cantara was stifled at 60 gL^{-1}, so too was the floral production by plants from Rob’s Point. This suggests that although vegetative growth of Rob’s Point plants is tolerant of a wide range of salinities, sexual reproduction may not be. As with the Lake Cantara plants, the salinities at which flowering occurred in the field were higher than the salinities at which flowering occurred in the pond experiments. Plants were flowering at salinities of 87 gL^{-1} at Rob’s Point in September 2010, and the salinities experienced by these plants were likely to have been above 60 gL^{-1} since July (Table 12). This contrasts with the pond experiments where flowers were only produced in 30 gL^{-1} salinities. Again, this implies that the conditions in the pond experiments failed to mimic the conditions in the field.

(iii) Performance of plants germinated from seeds

The number of *Ruppia tuberosa* seeds that germinated varied depending on the salinity level. The number of shoots per container in 60 and 90 gL^{-1} salinities represent a crude germination value because germinated seeds only produced one shoot, indicating that 36 % and 22 % of seeds from respective treatments germinated. Germination rates are not as easily calculated for the 30 gL^{-1} salinity because germinated seeds produced multiple shoots. However, if it is assumed that the cores containing seeds used for the seed experiments had the same number of seeds in them because they were sourced from the same population, then a comparison can be made. This would mean that all seed cores originally had approximately 91 seeds in them. Therefore, assuming that the abundance of seeds at the end of the 30 gL^{-1} seed salinity experiment in clipped and unclipped containers was not affected by clipping, the mean (± s.e.) seed abundance per container was 30 ± 10 seeds at the end of the experiment, suggesting that around 66 % of seeds germinated. This is twice the germination rate of seeds in the 60 gL^{-1} salinity and three-times the germination rate of seeds in the 90 gL^{-1} salinity. Although some *Ruppia tuberosa* seeds from Lake Cantara are able to germinate at high salinities, they show a preference for germinating at lower (30 gL^{-1}) salinities. Paton and Bailey {, 2010 #1} had similar results. This preference seems appropriate, as plant performance is enhanced at lower salinities.

The growth of the plants that germinated from the Lake Cantara seeds also varied greatly depending on the salinity level. Plants in the 30 gL^{-1} stable salinity produced some lateral growth but had to compete for resources with *Lepilaena* and *Chara* (that also germinated in the experiment), whereas seeds that germinated in the 60 and 90 gL^{-1} stable salinities produced a shoot but failed to grow laterally. It should be noted that the 30 gL^{-1} experiment may underestimate the performance of *Ruppia tuberosa* and comparisons may therefore be conservative. This is because potential competition with *Lepilaena* and *Chara* may have reduced the performance of *Ruppia tuberosa*. *Ruppia* species have little competitive ability and their survival is often inhibited by competition in habitats that would otherwise be suitable {Verhoeven, 1979 #87}. Although some *Ruppia tuberosa* seeds are able to germinate at 60 gL^{-1} and 90 gL^{-1}, growth is limited by such salinities, so the plants immediately produce turions to ensure the seed is not wasted by a false start (germinating in unfavourable conditions). This response would allow the plant to wait-out the unfavourable conditions and resprout when conditions are more desirable. This may also be an adaptation that allows the species to continue to persist following a false start caused by summer flooding inducing seed germination. The diminished ability to grow experienced by plants originating from seeds at salinities of 60 and 90 gL^{-1} mirrors the response of the plants in the Lake Cantara plant experiments. This adds further evidence that Lake Cantara plants perform best in salinities between 30 gL^{-1} and 60 gL^{-1}.

The influence of salinity bursts

(i) Plant performance in salinities starting at 30 gL^{-1}

The performance of transplanted and germinated *Ruppia tuberosa* plants from Lake Cantara was dampened by a salinity burst from 30 to 60 gL^{-1}, whereas Rob's Point plants responded positively. The differences in response to a salinity burst may be associated with the salinities of the source sites. Lake Cantara only experiences a gradual increase in salinity during the growing season of around 30 gL^{-1}, whereas plants at Rob's Point have experienced a rapid salinity increase during the growing season of 30-60 gL^{-1} in recent years {Paton, 2010 #1}. Therefore, the responses of the transplanted and germinated *Ruppia tuberosa* plants would appear to be adapted to the conditions of their source sites. This, in addition to the fact that the plants germinated from Lake Cantara seeds had the same tolerances as the Lake Cantara plants and not the Rob's Point plants, suggests there may be genetic differences between the Lake Cantara and Rob's Point populations.

The more parsimonious explanation however, is that differences in the responses of Lake Cantara and Rob's Point plants are the product of competition (or lack thereof) with filamentous green algae. Rob's Point plants were smothered in algae, but Lake Cantara plants were not. The salinity burst negatively affected the algae because algae in the Coorong appear to prefer lower (< $60gL^{-1}$) salinities {Paton, 2010 #1}. Due to the fact that *Ruppia* species are poor competitors {Verhoeven, 1979 #1}, reducing algal competition may have improved the performance of the Rob's Point plants because this would have reduced smothering by algae and therefore allowed the *Ruppia* plants to increase their rate of photosynthesis.

Although the plants from Lake Cantara and Rob's Point responded to a salinity burst in opposing ways, it appears that their inability to produce flowers after enduring a salinity burst is universal. However, this may not be a response to the salinity burst *per se*, but rather a response to the higher salinity. As discussed earlier, floral production was limited to low (30 gL^{-1}) salinities in the pond experiments, so a salinity burst from 30 to 60 gL^{-1} may have stifled flower production because 60 gL^{-1} was too high for flowering to occur, rather than because the salinity burst itself had an effect. Note that the salinity levels discussed here relate to the pond experiments in which flowering was stifled at 60 gL^{-1}, whereas flowering occurred at salinities > 60 gL^{-1} in the field. Since the pond experiments failed to mimic field conditions, extrapolation of pond experiment results to the field is unreliable. Nevertheless, the fact remains that interpretation of plant responses to a salinity burst may be confounded by the underlying response of plants to high salinities.

(ii) Plant performance in salinities starting at 60 gL^{-1} and 90 gL^{-1}

Plants transplanted into and germinated in the experiments starting at 60 gL^{-1} did not respond to the salinity burst as drastically as the experiments starting at 30 gL^{-1}. This is likely to be because optimal performance was already diminished by the higher starting salinity (as discussed earlier, this starting salinity appears to be above the threshold at which optimal performance is achieved). Therefore, a further increase in salinity may have failed to have a substantial effect because basic performance has already been jeopardised. The minimal response to a salinity burst from 60 to 90 gL^{-1} provides further evidence that the threshold at which optimal performance is affected is between 30 and 60 gL^{-1}. Furthermore, the means of the response variables from the plants exposed to a salinity burst (from 30 to 60 gL^{-1} and from 60 to 90 gL^{-1}) in the 30 and 60 gL^{-1} starting salinity experiments were very similar to the response variable means from the plants exposed to 60 and 90 gL^{-1} stable salinities in the 60 gL^{-1} and 90 gL^{-1} starting salinity experiments, respectively. That is, Rob's Point plants exposed to a salinity burst from 30 to 60 gL^{-1} (for example) had the same performance as Rob's Point plants just exposed to 60 gL^{-1}. This suggests that the plant's response to a salinity burst may in fact be a response to high salinity. Similar comparisons cannot be made between seed experiments as the differences in germination rates at different salinities would confound the comparisons.

The influence of simulated grazing by waterfowl

The response to clipping of all transplanted and germinated plants was more significant at stable salinities of 30 gL^{-1} than at 60 or 90 gL^{-1}. Under salinities of 30 gL^{-1} almost all response variables in all experiments were significantly reduced by clipping, whereas at higher salinities this was not the case. This diminished effect of clipping at higher salinities parallels the diminished effect of salinity burst discussed in the previous section. This adds yet more weight to the argument that *Ruppia tuberosa* performs best when salinities are between 30 and 60 gL^{-1} above which plant performance is reduced.

The lack of flowers in the clipping treatments at 30 gL^{-1} stable salinity suggests that *Ruppia tuberosa* flower production is reduced by waterfowl grazing the plant. These findings are not conclusive however, as the low flower abundances in the experiments mean that the data do not provide a sound basis for assessing the influence of clipping on flower production. Nevertheless, the findings are similar to those of other studies on submerged macrophytes. For example, O'Hare *et al.* {, 2007 #37} found that the flowering of *Ranunculus pseudofluitans* was significantly reduced in areas grazed by mute swans, and Rodriguez-Villafane *et al*. {, 2007 #38} reported an 85% increase in the abundance of *Myriophyllum alterniflorum* flowers when waterfowl were excluded.

Field observations

The field observations at both Lake Cantara and Rob's Point during the 2010-11 growing season provide a stark contrast to the performances of plants taken from these two sites and used in the pond experiments. In the field, plants from both populations produced floral stalks and flowered extensively when salinities were in the range 60-90 gL^{-1}. In both populations salinities had increased by around 30 gL^{-1} over a period of one or more months prior to or during flowering and those increases did not curtail the production of flowers. These observations eliminate high salinities and salinity bursts as explanations for the lack of flowering by populations in the middle of the North Lagoon in previous years. Intensive grazing by waterfowl particularly by Black Swans and potential interference competition from filamentous green algae, however, cannot be eliminated as factors causing lack of flowering in previous years. Grazing pressure in July 2009 and July 2010 was high at Rob's Point with 76% and 96% of 200 cores showing evidence of grazing respectively (Paton *et al.* unpubl. data) but there was no quantification of the intensity of that grazing pressure (i.e. per cent of shoots grazed). In September 2010 when the plants were flowering, all of 72 cores showed evidence of past grazing pressure but only about 5% of shoots within the cores had been grazed. In 2009 the intensity of grazing at Rob's Point was much higher with over 80% of shoots grazed in February 2009 and over 70% grazed in March 2009 (Paton & Bailey 2010a). Populations of *Ruppia tuberosa* despite this grazing pressure nevertheless increased their area of occupation between July 2008 and July 2010 (Paton & Bailey 2011a). During this period there was negligible change and certainly no increase in the numbers of waterfowl in the North Lagoon (e.g. Paton & Bailey 2010b, 2011b). With no increase in the numbers of grazing waterfowl and with more plants present, the overall grazing intensity on *Ruppia tuberosa* during 2010 should be reduced. A reduction in grazing pressure particularly between July and September 2010, therefore, may have allowed *Ruppia tuberosa* to flower in the North Lagoon in spring 2010. The low number of *Ruppia tuberosa* shoots showing evidence of grazing in September is indicative of negligible grazing in recent months.

The other important field observation was the prominence of filamentous green algae at Rob's Point, not just when the salinities were on or below 50 gL^{-1} in summer, but also in September 2010 when salinities at Rob's Point were close to 90 gL^{-1}. This suggests that high salinity *per se* does not prevent filamentous green algae from interfering with *Ruppia tuberosa*. However, filamentous green algae were far more prominent in January 2011 than September 2010, suggesting high salinities at least dampen the extent of the interference. Two types of interference exist. First, the algae easily become entangled around the foliage of the plants potentially blocking light and or facilitating plant material being dislodged by waves because of increased drag. Second, the algae also become easily entangled in the flower-heads of *Ruppia* plants and this in turn facilitates dislodgement of flowering stalks, affecting reproductive outputs for *Ruppia tuberosa*. Relatively few seeds were produced at Rob's Point despite two flowering events during 2010-11, with densities of seeds post-flowering being at best around 450 $seeds.m^{-2}$ in July 2011. This compares with seed banks that exceeded 20,000 $seeds.m^{-2}$ for *Ruppia tuberosa* at Lake Cantara, where there was no interference from filamentous algae and no serious wave action.

Despite flowering extensively in 2010-11 and producing reasonable numbers of turions during spring (ca 15,000 $turions.m^{-2}$ in November 2011), *Ruppia tuberosa* was largely eliminated from Rob's Point (and other monitoring sites within the middle sections of the North Lagoon) by July 2011. The large numbers of turions present in November had disappeared by January 2011 when the plants were flowering. This suggests that the resources held in turions have been used for the production of flowers as well as rhizomes and leaves. Turions, therefore, may not just be asexual perenniating organs but act as important reserves of resources that are subsequently used during periods of growth and sexual reproduction.

Ruppia tuberosa in the North Lagoon, however, did not produce additional turions post the January flowering period, despite being known to produce turions in previous years during autumn (Paton & Bailey 2010a). Two factors could account for the failure of plants to produce additional turions over autumn in 2011: interference from the extensive mats of filamentous green algae; or lower salinities inhibiting turion production. The latter explanation can be quickly eliminated because extensive turion production took place in the pond experiments at salinities as low as 30 gL^{-1}. In fact rates of turion production were higher at lower salinities. Thus the demise of *Ruppia tuberosa* from the middle sections of the North Lagoon (Long Point, Noonameena, Rob's Pt) by July 2011 was likely linked to the influx of freshwater during spring that stimulated another round of flowering, exhausting turions, and to the freshening benefiting filamentous green algae that then essentially smothered the plants. Higher levels of dissolved nutrients accompanying the freshwater flows may have also contributed. Increased concentrations of nutrients in the water column generally favour free-floating algae that take their nutrients from the water column rather than submerged macrophytes, like *Ruppia tuberosa,* that take their nutrients from the sediments (e.g. Linden *et al.* 2004; Ibelings *et al.* 2007). Whatever the mechanisms involved the return of freshwater to the Coorong in late October 2010 is likely to have triggered the responses of plants and contributed to the demise of *Ruppia tuberosa* from the middle sections of the North Lagoon. Ongoing freshening of the Coorong further south may cause similar issues for any remaining populations of *Ruppia tuberosa* that still exist within the Coorong. The demise also highlights how vulnerable *Ruppia tuberosa* is to single perturbations of water regimes (salinities and levels) particularly when it lacks an adequate seed bank.

These observations are extremely relevant to re-establishing *Ruppia tuberosa* in the South Lagoon of the Coorong, in two senses. First, successful re-establishment of *Ruppia tuberosa* in the South Lagoon of the Coorong is only attained when the translocated plants have been able to reproduce sexually and accumulate and maintain an adequate seed bank. Until then any populations that are established will remain vulnerable to once off perturbations to water levels and salinities.

Implications for management and future research

The marked differences in the performances of plants in the pond experiments compared to their performances *in situ* within the Coorong and Lake Cantara suggest that mesocosm experiments, like those in the ponds at the University of Adelaide, do not mimic the natural environments adequately to be surrogates for field conditions. Given this, information on the biology and responses of *Ruppia tuberosa* to different treatments collected from mesocosm experiments should be treated cautiously. Some key environmental features that are likely to be important particularly in the Coorong, like wave action, are extremely difficult to replicate in mesocosm experiments. Given this, various translocation methods need to be tested in the Coorong and not in experimental mesocosms.

The mesocosm experiments that have been conducted however have shown that, all other things being equal, the performances of *Ruppia tuberosa* plants (germination, growth, reproduction) were higher at lower salinities. Thus the lower the salinity at the time of translocation the more likely the translocated material is to establish plants. There is a catch, however, in that at the lower salinities interference from other aquatic macrophytes (e.g. *Lepilaena*) and filamentous green algae are increased. Thus there will be a limit to how low the salinities should be before attempting widespread translocation. The optimum salinity is probably around 60 gL^{-1}. However further work is still needed on the performances of filamentous green algae at different salinities and whether concentrations of nutrients in the water column also influence the performance of the algae. This has relevance for assessing proposals to release increased amounts of freshwater into the southern Coorong, because of potentially reduced salinities near the outlet and because of the potential for this water to carry increased loads of nutrients.

At present there is little extant *Ruppia tuberosa* left within the Coorong that could be used for translocation into the South Lagoon. External sources of material to translocate are likely to be needed, and the logical source material will be the nearby ephemeral lakes, like Lake Cantara. Sediments from these ephemeral lakes are likely to contain seeds of other plant species (e.g. *Lepilaena*) and oogonia of various algae (e.g. *Lamprothamnium, Chara*). Although these aquatic plants have not been recorded in the southern Coorong for more than a decade, there are historical data suggesting *Lepilaena* and *Lamprothamnium* were once relatively widespread in different parts of the Coorong (e.g. Womersley 1975). These species generally have low tolerances of higher salinities and so are more likely to have been eliminated before *Ruppia tuberosa*. Translocating sediments that might contain the seeds or oogonia of these other species is not considered inappropriate for the Coorong. Whether these other plants establish will depend on the salinities that eventual establish in the Coorong.

During the period when *Ruppia tuberosa* was establishing in the North Lagoon grazing pressure was high, and potentially high enough to prevent widespread flowering for several years. Grazing by waterfowl is known to influence performances and flowering in other aquatic plants (e.g. O'Hare *et* *al.* 2007; Rodriguez-Villafane *et al.* 2007). Given this there may be a requirement to protect some of the initial plantings from grazing to improve initial rates of establishment and to allow the plants that do establish opportunities to flower and set seeds as soon as possible. The need to do this is likely to diminish as populations expand and as more populations are established.

Finally given the sensitivity of *Ruppia tuberosa* to water level fluctuations and indirectly to low salinities within the Coorong, greater consideration needs to be given to how water levels will be maintained during spring and to limiting the extent to which low salinities as well as excessively high salinities might establish across the Coorong in the future.

Part II. Understanding the ecology of *Ruppia tuberosa* to guide the development of an effective translocation program.

Introduction

Ruppia tuberosa has disappeared from much of the South Lagoon over the last decade and has a greatly diminished if not non-existent seed bank (e.g. Paton 2010). Given this, the ability of *Ruppia tuberosa* to recover quickly once the water regime (salinity, water level) has been rectified is likely to be very limited. Thus the re-establishment of *Ruppia tuberosa* throughout the South Lagoon in a timely fashion (a few years) is likely to require *Ruppia* material to be translocated from other areas back into the South Lagoon. This translocation is likely to be needed to a large number of sites on both sides of the South Lagoon and over most of its 40-50km length.

There are a suite of knowledge gaps around developing an effective translocation proposal to re-establish *Ruppia tuberosa* in the southern Coorong. These include assessing the type of material (seeds, turions, plant fragments) that should be translocated, the timing of that translocation, and whether the translocated material needs to be embedded into the sediment and if so the planting densities and procedures that should be used. The potential to use strategic deposition of *Ruppia* material in the water column to take advantage of passive (wind-induced) dispersal to spread the plant as opposed to physically planting material in the sediments also needs to be assessed. Filling these knowledge gaps requires information on natural dispersal, the longevity of potential propagules and the ability of these to tolerate periods of desiccation. Field observations and small experimental manipulations can be used to advance our knowledge in these areas but need to be placed in the context of the biology of the plant within the Coorong.

Seeds, turions and small fragments of *Ruppia* plant material may all contribute to dispersal. In the Coorong small fragments of rhizomes, consisting of 1 or more leaf nodes and up to 10cm in length, are often washed onto the shoreline, or observed floating in the water column. This suggests *Ruppia tuberosa* is mainly dispersed by fragments of rhizomes rather than by seeds or turions. An assessment of the dispersal abilities of seeds, turions and fragments of rhizomes should consist of assessing the relative abundances of each of these within the water column, documenting their buoyancy, and tracking their movements. However this is not just about the availability of different dispersing units and how readily they can be dispersed, there is also a need for any potential dispersing unit to settle in areas where the plant can establish.

Ruppia tuberosa primarily exploits the ephemeral mud flats that fringe the Coorong lagoons. These mud flats are typically covered with water during winter, spring and early summer but become exposed to varying degrees during autumn. The timing and extent of exposure depends on the timing and extent of reductions and or the cessation of flows to the Murray Mouth. Over a year water levels can vary by as much as a metre in the South Lagoon (e.g. Paton 2010). Within this system *Ruppia tuberosa* grows during winter and spring on the ephemeral mudflats when these are covered by water that is typically 0.3-0.9 m deep (Paton & Bailey 2010a). The plant, however, will grow in water less than 0.3 m but within the Coorong such plants rarely establish because the day-to-day fluctuations in water levels due to changes in the direction and intensity of winds causes water level fluctuations of up to 0.3 m, leaving these plants out of the water on occasions.

The normal annual cycle for *Ruppia tuberosa* exploiting the ephemeral mudflats of the Coorong is for the seeds and turions to germinate and sprout when water returns to the exposed mudflats during late autumn and winter. The plants then grow over winter and spring, flower during spring and continue to grow and produce turions post-flowering until water levels retreat. This annual cycle largely dictates the opportunities and timing for translocations. Two opportunities exist, one is the translocation of dry sediment containing seeds and turions during autumn when water levels are lowest and depositing these in areas that are likely to be covered by 0.3-0.9 m of water during winter and spring. The other would involve translocating actively growing plant material and or additional seeds and turions in winter or early spring. Translocating material later than this would increase the risks that the translocated material would have insufficient time to establish and produce seeds and turions before being exposed in late summer or autumn. The timing and methods of translocation requires knowledge of the ability and extent to which translocated material survives the translocation process and is able to germinate, sprout and establish. Not only do the plants need to establish quickly but they need to grow into the adjacent sediment and successfully reproduce before water levels retreat. This is likely to be determined by the speed with which translocated seeds and turions can germinate or sprout when covered again with water and by the proportion that do so, and whether different- sized fragments of plant material differ in their ability to establish. An assessment of the risks of failure to initially establish is also important. For example, if all the seeds or turions germinate or sprout when first re-inundated with water, then the risks of the translocation failing are high because subsequent inappropriate conditions in spring would result in eliminating all of the translocated material. If, however, not all of the seeds germinated or all of the turions sprouted and these survived to germinate in a subsequent year then there is a second opportunity for establishment. An important research question is the extent to which seeds and turions can remain dormant and then subsequently germinate.

There are also several technical issues around the translocation process itself in terms of the amount of material that is translocated. In the previous year, field experiments where plugs of *Ruppia tuberosa* that were 7.5 cm in diameter were translocated to other sites and planted into the sediments while covered with water were vulnerable to being dislodged by wave action, as were 2 litre ice-cream containers (15cm x 15cm) that were used to house some of the translocated material. Given that wave action was capable of dislodging transplanted material, translocations that involve planting material in the sediment while covered with water may be regularly disrupted. Smaller plugs, however, may be less prone to being dislodged but before smaller plugs could be used some assessment of whether plants could establish and grow into adjacent un-occupied sediments is required. If small plugs (ca 2cm in diameter) were still capable of establishing *Ruppia tuberosa* plants and growing into the adjacent sediments, then the use of smaller plugs may help to alleviate the potential disruption of translocated material by wave action. The first step in assessing this is to determine if plants can establish from smaller plugs of plant material.

Methods

Identifying potential long-distance dispersal units of *Ruppia tuberosa*

Field observations and sampling to identify potential dispersal units for *Ruppia tuberosa* were conducted in September 2010 and January 2011. This consisted of three activities: (1) recording identifiable material that was dispersing in the water column in and amongst existing plants at both Lake Cantara (September only) and Rob's Point; (2) recording *Ruppia tuberosa* material that had been washed onto the shoreline at Lake Cantara and Rob's Point ; and (3) towing a 125 µm plankton net behind a small dinghy immediately beyond existing *Ruppia* beds in the North Lagoon in January 2011 in water that was 1 to 1.5m deep. Twelve replicate trawls were run each approximately 50m in length. In addition we recorded the nature of any *Ruppia* material that had been washed ashore (seeds, turions, plant fragment) and in the case of plant fragments scored the length of the fragments (measured as the number of leaf nodes present along the length of the rhizome) and also whether the fragment included a flowering stalk (with flower-head) and had any attached filamentous green algae.

Assessing the potential for effective long-distance dispersal

(i) Buoyancy of seeds, turions and plant fragments

An initial assessment of buoyancy consisted of placing individual seeds, individual turions (type II) and individual fragments of plants in an aquarium and recording if and when the material sank to the bottom. 50 individual seeds, 50 type II turions, and 50 fragments of *Ruppia* plant (2-3 leaf nodes) were tested. Each seed (approximately 1mm in size), turion (2-3 mm in diameter) and plant fragment (2-5 cm in length) was placed on the surface of the water in a separate white plastic container, and observed for up to 40 minutes or until they had sunk, with the time recorded.

(ii) Rates and patterns of dispersal of fragments of Ruppia tuberosa

Patterns of dispersal for fragments of *Ruppia tuberosa* plants were assessed in September 2010 and January 2011. This involved collecting fragments of *Ruppia tuberosa* plants from the water column or shoreline at Rob's Point. These fragments were then cleared of any filamentous green algae if present before being deployed in dispersal trials. This involved releasing individual fragments at a particular point (marked with a stake) and following that fragment for up to 40 minutes, with the position of the fragment marked at regular intervals (1, 5 or 10 minutes) with further wooden stakes, depending on the length of the observation time. To map the movements of the fragments, a second baseline post 10 or 20m away from the release post was established and used to allow each of the marked points along a fragment's trajectory to be determined by triangulation. This simply involved measuring the distance of each position from the release post and the second baseline post, and then plotting the points on a map. These data were then used to determine the distances, directions and rates of movement for each fragment. Our observations were conducted during periods of light onshore winds and negligible wave action (easier to keep track of the fragments). As the only material that was observed in the water column and washed on to the shore were fragments of *Ruppia tuberosa* plants, similar trials were not considered for other potential dispersal units (seeds, turions).

Longevity of seeds and turions

Multiple samples of surface sediment containing seeds and turions of *Ruppia tuberosa* were taken from the dry surface of Lake Cantara in March 2010 and stored. At the same time an equivalent set of samples was collected from Rob's Point in the North Lagoon of the Coorong, from an area where there were still *Ruppia tuberosa* plants growing and also stored dry (ambient temperature, with natural light). Inspection of a subset of the samples taken from Rob's Point showed that these only contained type I turions and no seeds, while those from Lake Cantara contained seeds and turions (both type I and type II) at the time of collection. The intention with these samples was to check on the viability of the propagules (particularly turions) at regular intervals (3, 6 or more months) by covering some of the containers with water and measuring subsequent sprouting of turions or germination of seeds.

Impact of exposure to differing periods of desiccation

An experiment to assess the ability of *Ruppia tuberosa* to cope with short periods of exposure was established at Rob's Point during September 2010. This involved removing large plugs (15cm x 15cm) of dense *Ruppia tuberosa* from extensive and well-established beds of *Ruppia tuberosa* (e.g. Fig. 19). These plugs were then placed in 2 litre ice-cream containers, flush with the lip of the containers. These were then left out of the water for varying lengths of time (6h, 24h, 3 days, and 12 days) before being re-deposited back in and amongst the existing *Ruppia* beds in water that was 0.35m deep in late September 2010. Ten replicates of each treatment were established. However the return of water to the area and extremely high water levels prevented these pots from being relocated (marker posts were all under water). This experiment will be repeated at Lake Cantara during September-October 2011 where excessive water levels will not be an issue.

Assessing optimum plug sizes for translocation of *Ruppia tuberosa*

Both field and pond experiments were used to assess the potential of different-sized plugs for establishing *Ruppia tuberosa*. The experiments involved two treatments. One treatment consisted of placing a single plug (7.5 cm in diameter) of dry sediment taken from Lake Cantara that contained seeds in the centre of a 2 L ice-cream container that had been filled with compacted sediment devoid of *Ruppia* material. To do this a 7.5 cm diameter plug to ca 4 cm deep was removed from the centre of the container and replaced with a 7.5 cm x 4 cm deep plug that contained the seeds. The other treatment consisted of removing a series of 14 plugs that were 2 cm in diameter x 4 cm deep from the sediment contained within a 2 L ice-cream container and replacing these with an equivalent number of plugs 2 cm in diameter and 4 cm deep that did contain seeds. These 14 plugs provided the same surface area of translocated material to the pots receiving a single larger diameter plug. Replicate series of 12 ice-cream containers that were then established in the field by digging each container into the sediment such that the lip of the container was flush with the natural sediment surface. The containers were positioned in water that was 0.4- 0.5 m deep at Noonameena and in water 0.2 m deep at Lake Cantara in November. These pots were then collected four months later (in March) and the biomass of plant material produced and the extent to which the interstitial sediment outside the original plugs had been colonised were assessed. To determine the extent of cover a grid divided into 36 squares was placed over the pot and the presence of *Ruppia* stolons present in the sediments scored for each. Determining the biomass of *Ruppia* material involved carefully separating out the above and below ground biomass for the cores and the interstitial sediment outside the original cores, and then drying this material at 70°C for 48 hours before weighing the samples to determine dry-weight. In addition to these field experiments, a similar experiment with 16 replicate pots with either one 7.5 cm core or fourteen 2 cm cores was conducted in a pond at the University of Adelaide over the same period.

Figure 19. Assessing the impact of short-periods of desiccation on *Ruppia tuberosa* at Rob's Point, September 2010: collecting *Ruppia* material for deployment in trials (top left); re-burying plugs of *Ruppia tuberosa* after different periods of exposure (top right), posts marking re-buried ice-cream containers containing *Ruppia* exposed for differing lengths of time (bottom left), and measuring dispersal of *Ruppia* plant fragments (bottom right).

Results and preliminary discussion

Potential dispersal units of *Ruppia tuberosa*

The only *Ruppia tuberosa* material detected in the water column at Rob's Point were fragments of plant material. These fragments differed in length and contained from 1 to 10 leaf nodes (e.g. Fig. 20), but smaller fragments (1-3 leaf nodes) were more common. Of the 200 fragments scored in January 2011, 27.5% of them had at least a floral stem often with a flower-head attached. Overall 36.5% of the plant fragments had filamentous green algae still attached. The rate of attachment of algae to these floating pieces of *Ruppia* was similar for fragments with and without floral stalks. These rates of entanglement, however, may not be representative of the extent to which filamentous green algae interfere with *Ruppia tuberosa*, because the algae is loosely attached and easily removed. Strands of algae that are entangled amongst the individual flowers within a flower-head and or leaves may easily dislodge from small pieces of *Ruppia* once the pieces are detached from the embedded *Ruppia tuberosa*. Small detached pieces of *Ruppia* plant move within the water column, both horizontally and vertically and, as they move, the pieces twist and tumble and change direction. This may facilitate the disentanglement of the algae from the *Ruppia* plant and allow the two to separate. Inspections of over 200 flower-heads that were still attached to embedded plants in September 2010 and January 2011 revealed that they all had filamentous green algae entangled amongst the flowers within an individual flower-head. However the quantity of algae entangled amongst the flowers varied from few short (<1cm long) strands that would appear to have little impact on the flowers to substantial floating masses of filamentous algae up to 10 cm across. Often several flower-heads were buried within the same mat of algae.

At Lake Cantara, there was no *Ruppia tuberosa* washed on to the shorelines, nor any naturally occurring pieces of *Ruppia tuberosa* floating or moving in the water column. However, fragments of *Ruppia* material were dislodged when researchers waded through the existing beds of *Ruppia tuberosa*. The marked differences between the presence and absence of *Ruppia tuberosa* material within the water column near beds, and washed onto shorelines, between Rob's Point and Lake Cantara reflect substantial differences in the extent of wave action between the two sites. Lake Cantara is a shallow lake with maximum water depths that approach 30 cm. Any waves that develop are of low amplitude and low energy. In the Coorong lagoons, however, the waves have much greater amplitude and energy because of the much greater fetch and deeper water.

In previous years observations on the material washed ashore along the Coorong often included pieces of *Ruppia tuberosa plants* with type I turions still attached. Type I turions are those that develop as swellings at the base of leaves (leaf axils) on or just below the surface of the sediment. They differ from type II turions that form at the end of the rhizomes (Brock 1982). The relationship, however, between type I and type II turions is not clear and whether the two types of turions serve different functions or are just at different stages of development is not known. Type II turions are larger and buried a little deeper in the sediments and are less likely to be easily dislodged.

The absence of type I turions amongst the material washed on to shorelines in the Coorong during our inspections in September 2010 and January 2011, was not unexpected, since there were few if any turions present on plants at this times (e.g. Table 12).

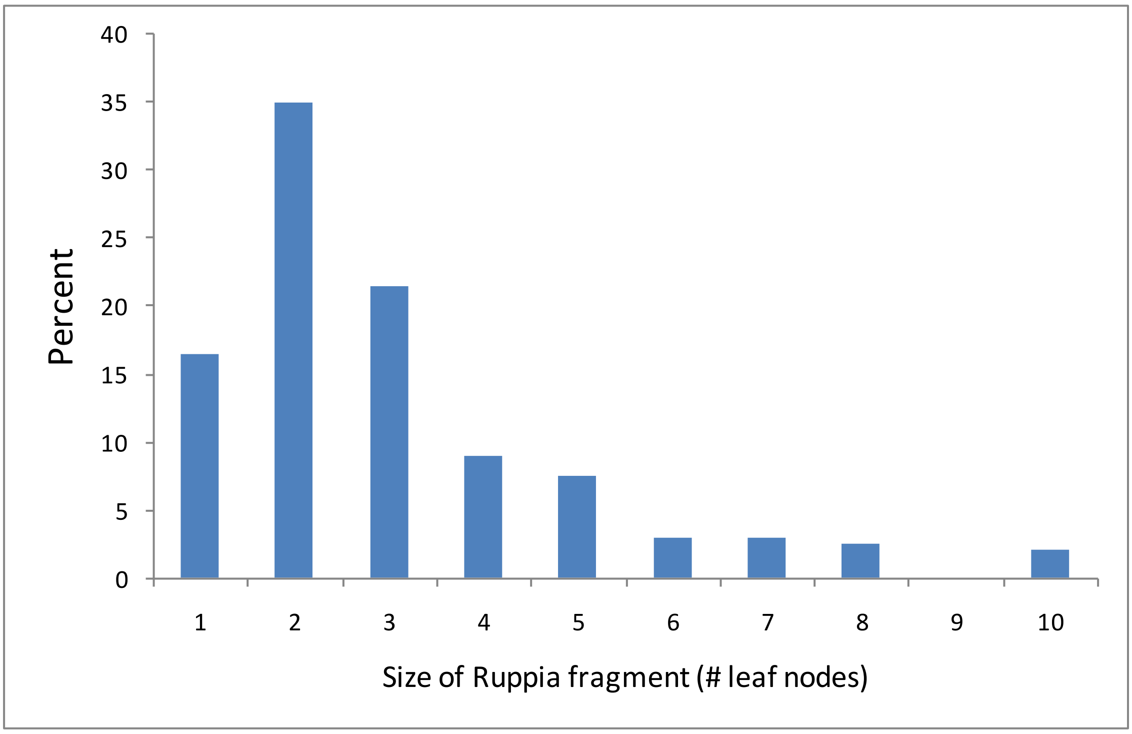


Fig. 20. Percent of different-sized pieces of *Ruppia tuberosa* plant found being dispersed in the Coorong. Data based on a sample of 200 fragments collected near Rob's Point in January 2011.

The twelve plankton trawls taken immediately beyond the major *Ruppia tuberosa* beds present in the North Lagoon in January 2011 contained up to 40 leaf blades or fragments of leaf blades of *Ruppia tuberosa*. No seeds, turions or pieces of rhizome were detected.

Seed, turion and plant fragment buoyancy

Of the 50 seeds placed on the surface of still water, all 50 had sunk within 2 minutes, with most sinking within a few seconds. Similarly all 50 type II turions had sunk within 5 minutes. However the 50 pieces of *Ruppia* plant material were all still suspended in the water after 40 minutes. This suggests that seeds and type II turions were unlikely to be readily dispersed within the water column, although wave action may pick up both and wash them towards the shore just above the surface of the sediments. No type I turions were tested as these were not freely available in January 2011. However type I turions which are swellings at leaf bases are likely to be dispersed with fragments of plant material which have good buoyancy and so are more likely to be dispersed within the water column.

Rates of dispersal of fragments of *Ruppia tuberosa*

Fragments of *Ruppia tuberosa* plants when tracked at Lake Cantara were highly variable in the directions in which they moved. When the pieces were close to the surface they moved in the general direction of the wind-induced water movements near the surface, but as the pieces shifted their vertical position within the water column, the direction of movement was often reversed. In this shallow lagoon water on the surface was pushed in the direction of the wind, but below the surface was a counter current moving in the opposite direction. The movements of pieces of *Ruppia* were very variable with some pieces moving in the same direction as the wind, some moving in the opposite direction to the wind, and others moving in both directions for shorter periods of time. The net distance moved was often small. The variability in net movement depended on the orientation of the fragments of *Ruppia* material relative to the different currents, and the length of the fragment. The orientation relative to the currents influenced the speed and direction of movements. Orientations could change from the long axis of the plant material being in line with one current or across it, or if the piece aligned vertically in the water column potentially influenced by two currents depending on the depth of the fragment and their overall length. Essentially the pieces of *Ruppia* tracked at Lake Cantara would twist and move within the water column allowing the different currents to act on them. Some pieces would twist and sink a little deeper allowing the counter-current to influence their movements others would rise closer to the surface where the surface current would have a greater influence. The outcome of this was that the net distances moved by the fragments even after long periods (20 minutes or longer) were often short (< 5m).

Within the Coorong, the presence of counter currents close to the surface was not prominent, consistent with this system being a much larger and open lagoonal system. Within the Coorong, fragments of *Ruppia tuberosa* tended to move in the same general direction as the wind and any associated wave action but there was a wide range of actual directions moved during any one 10 minute observation period (Tables 14, 15). The average distances moved by short fragments (2 leaf nodes) were generally greater than long fragments (5 leaf nodes; Table 14) in September 2010, with an overall rate of movement of 3.75 ± 0.24 m/min (n = 29) for short fragments compared with 3.0 ± 0.22 m/min (n = 29) for long fragments. Rates of movement were generally higher when wind speeds were higher, with rates of net movement being around 2-3 m/min when wind speeds were around 7 knots, to 4-5m/min when average wind speeds were 14 knots depending on the size of the fragment (Table 14).

In January 2011 when wind speeds were comparable to the lower speeds experienced in September, different-sized fragments dispersed at similar rates. Small fragments that consisted of just 2 leaf nodes dispersed at a rate of 2.87 ± 0.26 (se) m/minute, while medium sized fragments (fragments of rhizome with 4-6 leaf nodes) dispersed at a rate of 2.43 ± 0.15 m/min and long fragments (fragments of rhizome with 8-10 leaf nodes) dispersed at a rate of 2.63 ± 0.17 m/min (Table 15). The overall rate of dispersal was 2.64 ± 0.12 (se) m/min. The actual net distance moved from the point of release was nearly always less than the total distance travelled, averaging 2.05 ± 0.14 (se) m/min. The trajectories of fragments of rhizomes were again not constant, with the directions (and speeds) of movement for individual fragments changing over the 10 minute observation period (Table 15). In general fragments generally travelled towards the shore, particularly small fragments or parallel to the shore when in shallow water (<10cm deep) and close to shore, but there were also times when fragments moved away from the shore during these observations (Table 15).

Table 14. Distances and directions of movement for short (2 leaf nodes) and long (5 leaf nodes) fragments of *Ruppia tuberosa* in September 2010. Wind speeds and direction based on 9am and 3pm measurements by the Bureau of Meterology.

VARIABLE	23 Sept 2010	25 Sept 2010	26 Sept 2010
WIND SPEED & DIRECTION			
Average wind speed (knots)	6.5	14.5	7.0
Average wind direction*	23	79	101
Range in directions	0-45	45-113	45-158
DIRECTION OF MOVEMENT			
Average	64	147	110
Range	1-135	120-166	73-138
RATE OF MOVEMENT (m/min)			
Short fragments (mean ± s.e.(n))	2.86 ± 0.16 (10)	5.24 ± 0.47 (8)	3.47 ± 0.19 (11)
Long fragments (mean ± s.e.)	2.26 ± 0.32 (10)	3.73 ± 0.59 (8)	3.14 ± 0.22 (11)

*measured as the direction that the wind was travelling, wind direction is 180° more than this.

Table 15. Distances travelled by fragments of *Ruppia tuberosa* deployed in the Coorong in January 2011. Individual fragments were released approximately 30 m out from the shoreline in water that was approximately 30 cm deep. Fragment sizes were determined by the number of leaf nodes along the piece of stolon and by total length, as the density of leaf nodes differed between samples of plants. These data were collected with a mild onshore afternoon sea breeze (approx 5-8 knots).

Bout	Obs	Fragment size		Time Followed (min)	Total distance travelled (m)	Linear distance from start (m)	Comments	Direction travelled
		# leaf nodes	Length (cm)					
1	1	2	5	10	35.1	32.5		Parallel to shore
	2	2	2.5	10	19.5	10.6		Shoreward
	3	4	33	10	23.8	17.8		Parallel to shore
	4	6	35	10	34.8	32.5		Parallel to shore
	5	8	35	10	31.1	11.1		Parallel to shore
	6	8	40	10	34.7	12.6		Parallel to shore
2	1	2	3	10	29.4	21.5		Parallel to shore
	2	2	3.5	10	27.3	19.6		Parallel to shore
	3	5	33	10	29.7	27.9		Parallel to shore
	4	5	18	10	28.3	27.5		Parallel to shore
	5	10	31	10	28.9	27.9		Parallel to shore
	6	8	18	10	34.5	29.8		Parallel to shore
3	1	2	5	6 m 50 s	29.6	29.4	hit shore	Shoreward
	2	2	5.5	10	18.2	8.3		Shoreward
	3	5	21	10	24.2	20.4		Parallel to shore
	4	6	27	10	21.2	18.6		Parallel to shore
	5	10	31	10	36	28.5		Parallel to shore
	6	10	55	10	19.4	14.9		Parallel to shore
4	1	2	3	10	22.6	11.9		away from shore
	2	2	6	10	25.6	24.7		away from shore
	3	5	13.5	10	30.1	14.3		away from shore
	4	5	8	10	25.2	24.5		away from shore
	5	9	44	10	25.9	25.7		away from shore
	6	10	47	10	24.7	24.7		away from shore
5	1	2	3	5 m 40 s	31.8	31	hit shore	Shoreward
	2	2	3	10	9.2	1		Shoreward
	3	5	26	10	15.7	13		Parallel to shore
	4	5	28	10	18.3	8.4		Parallel to shore
	5	8	42	10	21.6	8.9		Parallel to shore
	6	10	45	10	25.7	25		away from shore
6	1	2	4	9 m 48 s	32.3	26	hit shore	Shoreward
	2	2	4.6	10	25.8	22		Shoreward
	3	5	4.5	10	21.6	8.9		away from shore
	4	5	15	10	11.3	5.4		Shoreward
	5	8	22	10	12.7	18.9		away from shore
	6	10	46	10	20.7	16.3		away from shore

7	1	2	4	10	26.8	21.8	Shoreward
	2	2	5	10	30.2	23.8	Shoreward
	3	5	28	10	24.8	21.3	away from shore
	4	5	17	10	24.3	14.5	Shoreward
	5	8	26	10	24.2	19.6	away from shore
	6	8	24	10	32.2	21.5	Parallel to shore
8	1	2	2	10	32.4	22.3	Parallel to shore
	2	2	3	10	24.7	22.3	Shoreward
	3	5	18	10	28.9	21.9	Parallel to shore
	4	5	13	10	26.7	18.3	Shoreward
	5	8	28	10	16.9	9.6	Parallel to shore
	6	8	4	10	31.8	26.8	Parallel to shore

Longevity of seeds and turions

The seasonal patterns to the availability of turions at both Rob’s Point and Lake Cantara suggest that all of the turions that were present sprouted when the ephemeral mudflats were re-inundated during late autumn or early winter. In both July 2009 and July 2010 there were virtually no turions remaining in the sediments at both sites (see Tables 12 & 13). Many of the seeds also appeared to have germinated at this time judging by the reductions in numbers of seeds at this time. However, not all seeds germinated. At Lake Cantara up to half of the seeds had not germinated and these non-germinated seeds are potentially available to germinate on a subsequent occasion.

Exposure of samples of seeds and turions that had been collected from Lake Cantara in March 2010 and May 2011, and from Rob’s Point in March 2010 were consistent with these field observations (Table 16). The data suggest that type I turions may have limited ability to survive even the typical lengths of time (3- 6 months) that ephemeral mud flats may be exposed to desiccation over autumn, as no type I turions sprouted in samples that were re-inundated with water 3 months, 4 months, 7 months or 16 months after collection. Type II, turions, however, sprouted 3 and 4 months after being collected (or 6-7 months after being initially exposed), but not at 7 or 16 month intervals. Some seeds however germinated at each of these time intervals. Those turions that were still present in samples 7 and 16 months after collection, however often turned dark particularly on their exterior when re-inundated and appeared to begin to rot. These data indicate that the viability of turions is short-lived (ca 6 months) and that they have a limited capacity to tolerate periods of desiccation.

This particular experiment may however over-emphasise the speed with which turions deteriorate because samples were kept dry. When lakes dry some moisture may remain within the sediments and this may dampen the speed of deterioration. A second series of experiments that measures the survival of turions *in situ* is now required to determine the ability of turions to survive in the absence of a covering of water. This is particularly true for type I turions which failed to sprout at all, even after 3 months. The time lines for these experiments are probably of the order of weeks rather than months (as was originally proposed). This *in situ* assessment can probably be conducted at Lake Cantara once the Lake dries in late summer 2011-12 and would involve assessing abundances and sizes of turions remaining in the sediments at weekly intervals and their ability to sprout on re-inundation.

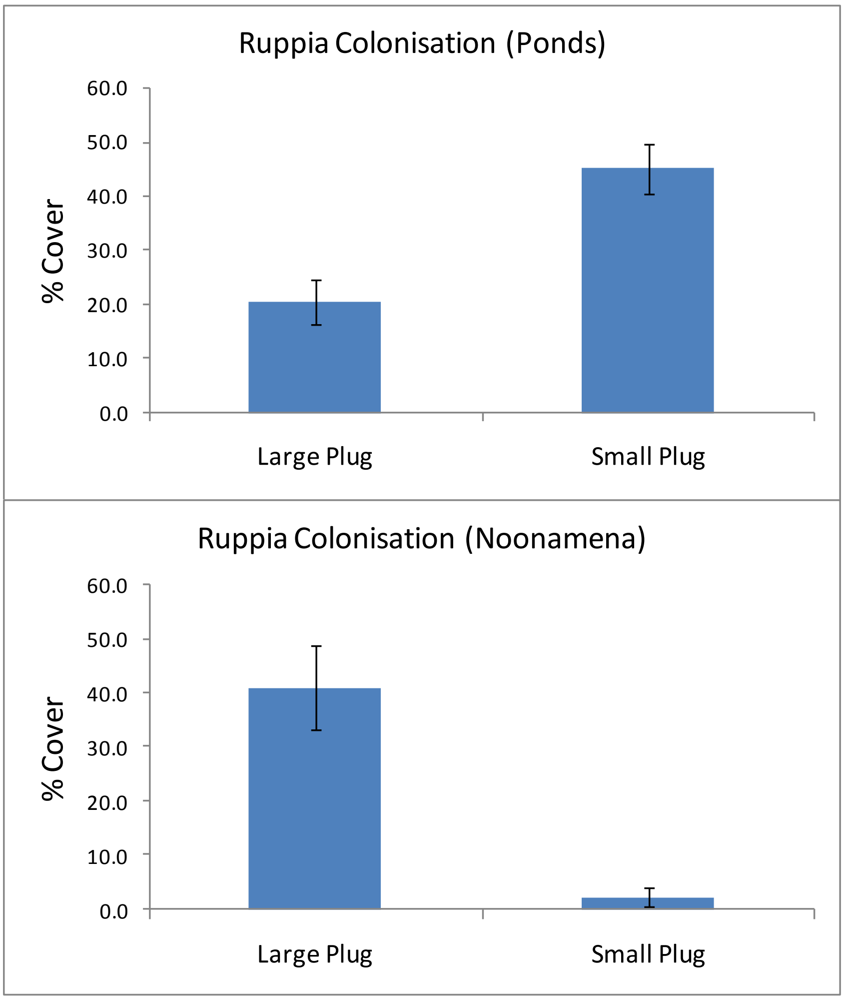
Table 16. Ability of seeds and turions of *Ruppia tuberosa* to withstand differing lengths of dessication. The samples from Rob's Point were covered by water in March 2010, while the samples collected from Lake Cantara were collected from the dry mudflats that had become exposed in late December or early January.

Source material	Collection date	Types of propagules	Time after collection			
			3 months	4 months	7 months	16 months
Rob's Point	March 2010	Type I turions		no	no	no
Lake Cantara	March 2010	Type I turions		no	no	no
		Type II turions		yes	no	no
		Seeds		yes	yes	yes
Lake Cantara	May 2011	Type I turions	no			
		Type II turions	yes			
		Seeds	yes			

From the evidence to date, turions may only allow *Ruppia tuberosa* to re-establish after a short period of desiccation. They do not provide any potential for re-establishment of *Ruppia tuberosa* after a more extended period of desiccation. Given this, populations of *Ruppia tuberosa* in the North Lagoon which are only setting significant numbers of turions and few if any seeds (e.g. Table 12) are vulnerable to periods of low water levels, and will remain so until a seed bank is established. This is doubly so as most of the turions being set by these populations of *Ruppia tuberosa* are also type I turions with an even lower capacity to sprout following a period of desiccation.

Experiments to assess the size of translocated plugs

The pond experiments involving two different-sized plugs containing seeds revealed that multiple small plugs provided a greater cover of *Ruppia tuberosa* material in pots than a single large plug (Fig. 21). However the biomass produced outside the original cores was similar (Fig. 22). Overall the single large plug produced more overall biomass (Fig. 22). The *ex situ* data contrasted with the field data from Noonameena where very little *Ruppia tuberosa* material penetrated into the sediments beyond the original 2cm diameter cores for the small plugs, while there was substantial growth into the interstitial sediments for the single large plug (Figs 21, 22). Although the experiment was repeated at a second field site (Lake Cantara), those samples had dried out before the samples could be collected.


Ruppia Colonisation (Ponds)

Ruppia Colonisation (Noonamena)

Figure 21. Growth of *Ruppia* into sediments outside cores for two different sized cores (large 1 x 7.5cm diam core; small 14 x 2cm diam cores) *ex situ* (pond) and *in situ* (Noonameena) after 4 months. Data are percent of 36 cells that contained *Ruppia tuberosa* plant material in the sediment.

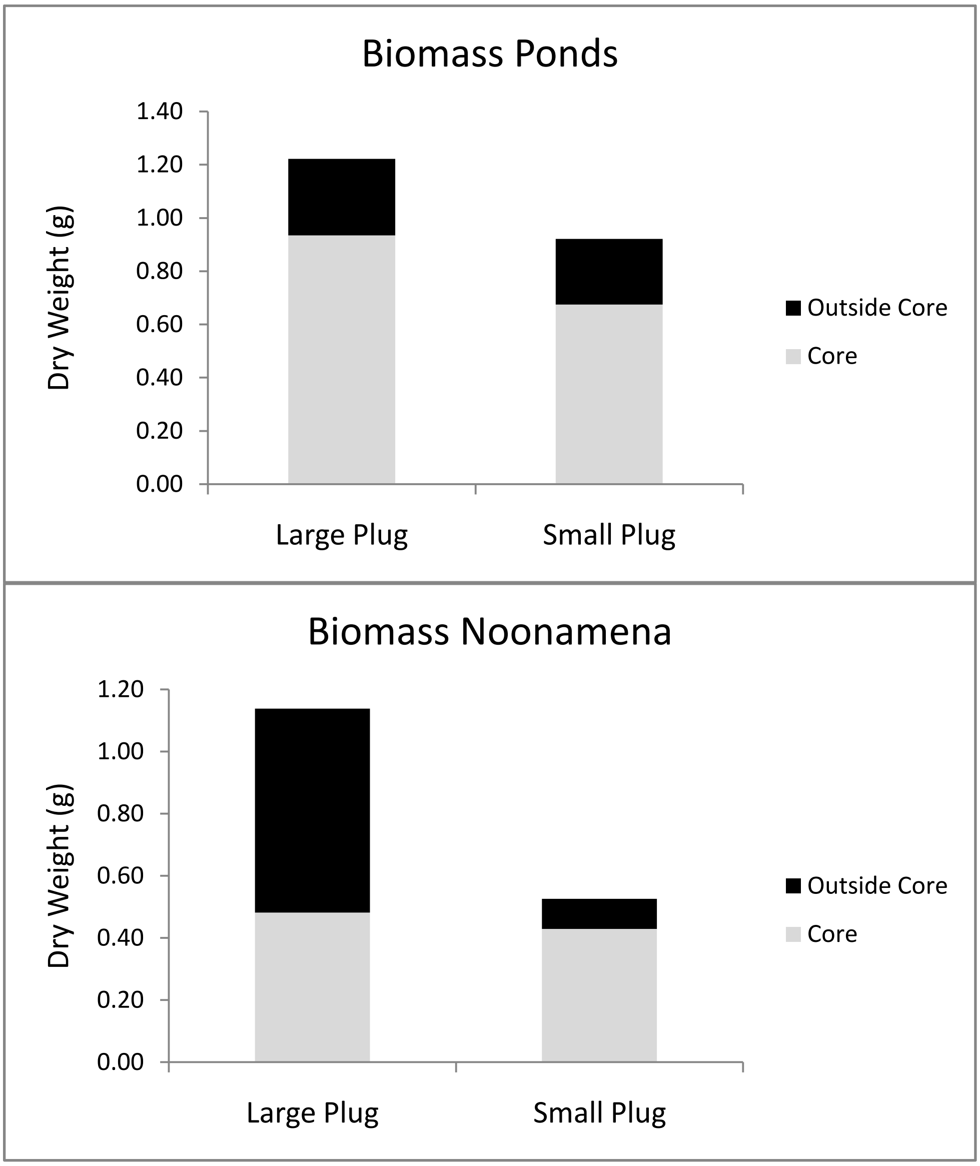


Figure 22. Mean biomass (g dry weight) of *Ruppia tuberosa* produced within and beyond the original cores from two different sized cores (large 1 x 7.5cm diam core; small 14 x 2cm diam cores) *ex situ* (pond) and *in situ* (Noonameena) after 4 months.

General discussion

Based on field observations over the last few years and the performances of plants when challenged with high salinity *ex situ*, the functions of seeds and the two types of turions for *Ruppia tuberosa* are likely to be different. Turions are short-lived and allow the plants to sequester resources even when the conditions for growth are not ideal. If the ecological conditions improve then those stored resources may allow the plants to grow quickly to immediately exploit the favourable conditions. This would account for the rapid disappearance of turions in winter following the return of a cover of water over ephemeral mudflats. Seeds on the other hand must first germinate and then the seedlings need to establish, so the ability to exploit favourable conditions is delayed. However, seeds have the ability to remain viable when water is absent for extended periods, unlike turions. Thus seeds allow the plant to exist in an area through extended periods (> a few months) when the environmental conditions prevent growth (e.g. the lake is dry) and turions deteriorate.

The two types of turions may also serve different functions. Type I turions may provide an immediate reserve of resources for a period of rapid growth of rhizomes, floral stalks and or foliage rather than acting as perenniating organs. That none sprouted after a period of time out of the water suggests their capacity to act as perenniating organs is limited at least. Type II turions on the other hand had capacity to survive and sprout following short periods of desiccation. Type II turions also tended to be produced and be more prominent at the end of the growing season consistent with a perenniating function. Further work is required to unravel the primary functions of these two types of turions. The first step in doing this is to determine how their availability varies across the growing season. If type I turions function primarily as reserves of resources to allow periods of rapid growth their abundances should fluctuate seasonally, by being abundant prior to a period of growth or flowering but then scarce during the times of rapid growth. The general absence of turions during flowering at Rob's Point in September 2010 and January 2011, despite being abundant between these two periods (Table 12), is consistent with this function.

To a large extent areas which permitted growth of *Ruppia* in the past are likely to be the places where growth is most likely possible in the future and so the majority of the seeds and turions produced are likely to be primarily aimed at maintaining the presence of the plant at that site. That intact seeds and type II turions readily sink and are not buoyant is consistent with this. In Lake Cantara, where there was minimal disturbance to plants, negligible *Ruppia tuberosa* material was being dispersed within the water column. At the end of flowering, flower-heads with developing seeds retreated from the surface of the water by sinking in amongst the remaining plant material, largely trapping the seeds at the site. If local dispersal within a wetland system was important then some of the developing seeds would be expected to remain on the surface until fully mature to maximise any wind-induced dispersal. Similarly, because the viable type II turions are produced within the sediments they are unlikely to be dispersed unless disturbed. In this population long-distance dispersal of propagules is perhaps unlikely. However because of the sticky nature of the sediments, waterfowl that visit this wetland may inadvertently transfer seeds that are held within any sediment that sticks to their feet.

The situation is very different in the Coorong, where regular wave action as well as disturbance from grazing waterfowl, notably Black Swans, can result in fragments of a plant's above ground or near surface material being dislodged into the water column. Other disturbances such as interference from filamentous green algae may also facilitate dispersal.

In the Coorong where there are many substantial wind-generated waves, seeds and turions could potentially be dispersed by wave action along the floor of the Coorong, These movements will be almost invariably towards the shore. Few (if any) seeds or turions are likely to be dispersed to deeper water in the Coorong because offshore winds do not produce waves that travel away from the shore at least not close to the shoreline where *Ruppia* grows. This is in part because the shoreline and adjacent coastal dunes with their terrestrial vegetation and rocky headlands shield the near coastline from any offshore wind.

In the Coorong buoyant fragments of *Ruppia tuberosa* are transported within the water column. These fragments usually consist of pieces of rhizome with 1-3 leaf nodes and travel at rates of around 2-3m per minute depending on wind speeds. The pieces tend to be broadly washed towards the shore but they do not all follow the exact same trajectory with pieces spreading out broadly from a release point. These pieces may remain buoyant for extended periods of time (at least 40 minutes and probably much longer) and so can potentially travel hundreds of metres. The initial expansion of *Ruppia tuberosa* into the middle sections of the North Lagoon was probably aided by successful dispersal of pieces of *Ruppia tuberosa*, as small fragments of *Ruppia tuberosa* appeared at sites in the North Lagoon in the year prior to *Ruppia* establishing at those sites in the North Lagoon. Since seeds and turions were not detected until after the plants had established (in the case of seeds some years after), reinforces the notion that seeds and turions are not long-distance units of dispersal for *Ruppia tuberosa*. Thus a potential mechanism by which *Ruppia tuberosa* might colonize the southern Coorong is through the dispersal of small pieces of rhizomatous plant material transported in the water column. This method of dispersal could be used strategically to increase the rate of re-establishment across the southern Coorong.

At present there is no indication of how pieces of rhizomatous material settle on sediments and become embedded in the sediment and this needs to be understood before promoting the deployment of fragments of *Ruppia tuberosa* plants for re-establishing populations in the South Lagoon. A substantial number of fragments may not settle in appropriate places and not establish, so this could be very wasteful of plant material. An understanding of any relationship between fragment size and ability to establish once settled is also required.

The trials using different sized plugs to re-establish *Ruppia tuberosa* suggest that the larger plugs of sediment containing *Ruppia tuberosa* plants performed best, and this is certainly the case in field conditions. Several factors may account for this. First, some of the smaller plugs may not contain viable pieces of plant material close because of their size, and so plants do not develop from those cores. Second, removing a core of material and replacing it disturbs the sediment and with more small plugs there may be an increased chance of losing some of those to wave action. The impact of wave action was reduced in the field by placing the experimental pots in water 0.4-0.5m deep but it was not eliminated. In the pond trial wave action was eliminated and the small cores performed comparably. This difficulty in translocating living plant material reinforces the findings from the previous year. Translocating a plug of *Ruppia tuberosa* and embedding it into sediments covered by water is not only difficult to do but is fraught with risks of the plug being dislodged by wave action, at least initially. Further field tests are required to determine if plug sizes and planting densities could alleviate the frequency of this. However advancing some of these technical issues is likely to be best accomplished by incorporating some different plug sizes into the design of any initial translocation trial rather than waiting for additional findings before proceeding. Otherwise the recovery of *Ruppia tuberosa* in the southern Coorong is further delayed. An adaptive management approach could be used with some of the initial trials until the most efficient method is found. There is certainly little benefit in continuing to use mesocosms to test different methods of translocating *Ruppia tuberosa* material because they do not adequately mimic conditions in the Coorong.

Implications for the management and translocation of *Ruppia tuberosa* in the southern Coorong

The re-establishment of the once extensive beds of *Ruppia tuberosa* within the southern Coorong and the subsequent management of the re-established populations will ultimately depend on the timing and quantities of water that are released over the barrages, as these will largely determine the water levels and salinities that will be experienced at different points along the Coorong. Based on field observations on *Ruppia tuberosa* within the Coorong over the last decade, years in which there are no flows over the barrages in spring are likely to prevent *Ruppia tuberosa* from setting seeds in the southern Coorong in that year, primarily because of low water levels rather than high salinities. Similarly, periods when there are substantial flows over the barrages, resulting in higher water levels, lower salinities and potentially higher concentrations of dissolved nutrients, may also disadvantage *Ruppia tuberosa* favouring competitive interference from filamentous green algae. Provided an adequate seed bank has been established in intervening years, populations of *Ruppia tuberosa* should have some resilience to these events. This is because not all seeds germinate in any one year. Thus, if the populations fail to set seeds in one year, there are still viable seeds remaining within the seed bank that can germinate in the following year and allow *Ruppia* to recover. Thus to be successful translocation programs not only need to re-establish plants but the re-established plants must also reproduce and quickly accumulate an effective seed bank. Once an adequate seed bank is in place, the newly established populations will have some resilience to extremes in river flows to the Murray Mouth. Assuming that the new Murray Darling Basin Plan delivers at least a small increase in environmental flows, the risks of having years with negligible flows in spring, however, should be reduced compared to recent times.

These arguments suggest that any translocation proposal should be timed to occur when the conditions are likely to lead to good germination rates and rapid growth coupled with opportunities to flower shortly after translocation.

The germination of seeds and the ability of *Ruppia tuberosa* plants to establish and grow rapidly into unoccupied adjacent sediments are dampened by high salinities. *Ex situ* trials suggested that *Ruppia* plants performed best at salinities around 30 gL^{-1} (within the 30-90 gL^{-1} range tested) but the field observations showed that established populations of *Ruppia tuberosa* were nevertheless capable of producing turions and even flowering when salinities approached 90 gL^{-1}. More importantly the extent to which filamentous green algae interfered with the plants and their flowering was greater at lower salinities. In 2010-11, filamentous green algae were more prominent when salinities were around 50 gL^{-1} or lower. Based on this if seeds were to be translocated then the ideal conditions within the Coorong to do this would be when the salinities in winter and spring were expected to be around 60 gL^{-1}, balancing the benefits of increased germination rates at lower salinities with reducing the potential competitive interference of filamentous green algae at the lower salinities. Historically the South Lagoon had winter salinities (when most seeds germinate) that ranged from 30-50 gL^{-1} (Paton 2010, unpubl.).

At present, translocations will need to be limited to translocating seeds. This is largely due to the physical difficulty of embedding plugs of living plant material into sediments under water that is around 0.4 m deep or deeper. A depth of at least 0.3 m is needed to avoid short-term day-to-day fluctuations in water levels of 0.3 m, which might otherwise leave plugs exposed. A further factor limiting these translocations is that they remain vulnerable to being dislodged by wave action. The translocation of seeds, on the other hand, could take place during autumn and involve embedding plugs of sediments containing seeds into the exposed mudflats when water levels are at their lowest. This is technically much easier to do, and has the potential to be more automated allowing substantial areas to be re-seeded reasonably quickly, assuming that there is sufficient source material. For example, small plugs of surface sediment from Lake Cantara could be scattered over the exposed mud flats in the Coorong that would normally be covered by water 0.6 - 0.8 m deep during winter and spring (see Paton & Bailey 2010a). These are the depths at which *Ruppia tuberosa* has done best in the Coorong in previous years (Paton & Bailey 2010a). Because the mudflats are firm it may be possible to use a roller to quickly press the samples into the sediment. There is merit in embedding the material that contains seeds into the surface sediment of the Coorong since this should reduce risks that waves shift the seeds towards the shore and away from the ideal growing locations (linked to water depth). Note that for surface sediments collected from Lake Cantara, there are also likely to be reasonably large numbers of turions (both types) as well as seeds. Having two types of propagules present also increases the chances of successful establishment. The turions although short-lived may provide a more rapid rate of establishment than seeds.

Seeds may also provide additional advantages over other plant material (plants, turions) in a translocation program in that not all seeds are likely to germinate in the first year, and so there is the potential for subsequent opportunities for additional seeds to germinate, spreading the risk of failure over several years. In the case of translocating turions and living plant material, if *Ruppia* does not establish in the first year then there is no second opportunity because plants and turions are short-lived on ephemeral mudflats.

An alternative to physically translocating large numbers of seeds to extensive areas of sediment is to explore the possibilities of using wind-induced water movement to disperse small fragments of rhizomes within the Coorong. However, at present there is no understanding of how or where these short fragments of rhizomatous material settle on sediments and whether they are capable of establishing. Until this is known it is not possible to assess this as an alternative or supplementary method for re-establishing populations of *Ruppia tuberosa* in the Coorong, other than to note that these small fragments of *Ruppia tuberosa* have the potential to be dispersed quickly over large distances.

The loss of the recently established beds of *Ruppia tuberosa* in the North Lagoon following freshening of the Coorong has largely eliminated the option for using the existing *Ruppia tuberosa* within the Coorong as the source material for translocation, at least for the time being. Thus, the Lake Cantara population and populations of *Ruppia tuberosa* on other nearby ephemeral wetlands are now increasingly the key source populations, at least until *Ruppia tuberosa* can re-establish somewhere within the Coorong.

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References

Brock MA (1981) The ecology of halophytes in the Southeast of South-Australia. *Hydrobiologia* **81-2**: 23-32.

Brock MA (1982) Biology of the salinity tolerant genus *Ruppia* in saline lakes in South-Australia .2. Population ecology and reproductive biology. *Aquatic Botany* **13**: 249-268.

Brock MA, Lane JAK (1983) The aquatic macrophyte flora of saline wetlands in Western-Australia in relation to salinity and permanence. *Hydrobiologia* **105:** 63-76.

Congdon RA, McComb AJ (1979) Productivity of *Ruppia*: seasonal changes and dependence on light in an Australian estuary. *Aquatic Botany* **6**: 121-132.

Geddes MC (1987) Changes in salinity and in the distribution of macrophytes, macrobenthos and fish in the Coorong Lagoons, South Australia, following a period of River Murray flow. *Transactions of the Royal Society of South Australia* **111**: 173-181.

Ibelings BW, Portielje R, Lammens ERRH, Noordhuis R, van den Berg MS ,Joosse W, Meijer, ML (2007) Resilience of alternative stable states during the recovery of shallow lakes from eutrophication: ake Veluwe as a case study. *Ecosystems* **10**: 4–16

Linden LG, Lewis DM, Birch M , Brookes JD (2004) Interannual variability in rainfall and its impact on nutrient load and phytoplankton in Myponga Reservoir, South Australia. *Intl. J. River Basin Management* **2**: 1–11

Nicol JM, Ganf GG (2000) Water regimes, seedling recruitment and establishment in three wetland plant species. *Marine and Freshwater Research* **51**: 305-309.

Noye BJ (1975) Waters of the Coorong Lagoons. pp 59-79 *In* Noye J. (ed.) T*he Coorong.* (Dept Adult Educ., Univ of Adelaide, Adelaide)

O'Hare MT, Stillman RA, McDonnell J, Wood LR (2007) Effects of mute swan grazing on a keystone macrophyte. *Freshwater Biology* 52, 2463–2475.

Paton DC (2010) *At the End of the River: The Coorong and Lower Lakes*. (ATF Press: Adelaide)

Paton DC, Bailey CP (2010a) Restoring *Ruppia tuberosa* to the southern Coorong. (Report for the SA Dept of Environment and Natural Resources. University of Adelaide, Adelaide).

Paton DC, Bailey CP (2010b) *Condition monitoring of the Lower Lakes, Coorong and Murray Mouth: waterbirds using the Coorong and Murray Estuary 2010.* (Report to the SA MDB NRM Board & MDBA, University of Adelaide, Adelaide)

Paton DC, Bailey CP (2011a) Annual monitoring of Ruppia tuberosa in the Coorong region of South Australia. July 2010. (Report for the SA Dept of Environment and Natural Resources. University of Adelaide, Adelaide)

Paton DC, Bailey CP (2011b) *Condition monitoring of the Lower Lakes, Coorong and Murray Mouth: waterbirds using the Coorong and Murray Estuary 2010.* (Report to the SA Dept for Water & MDBA, University of Adelaide, Adelaide)

Paton DC, Rogers DJ, Aldridge K, Deegan B, Brookes J (2009) A future for the Coorong and Lower Lakes. *Pacific Conservation Biology* **15**:7-10

Rodriguez-Villafane C, Becares E, Fernandez-Alaez M (2007) Waterfowl grazing effects on submerged macrophytes in a shallow mediterranean lake. *Aquatic Botany* **86***:* 25–29

Verhoeven JTA (1979) The ecology of *Ruppia* dominated communities in Western Europe. 1. Distribution of *Ruppia* representatives in relation to their autecology. *Aquatic Botany* : 197-268.

Webster IT (2005) *An overview of the hydrodynamics of the Coorong and Murray Mouth*. (CSIRO Water for a Healthy Environment Research Flagship, Canberra)

Webster IT (2010) The hydrodynamics and salinity regime of a coastal lagoon - the Coorong, Australia- Seasonal and multi-decadal timescales. *Estuarine, Coastal and Shelf Science* 90: 264-274

Womersley HBS (1975) Plant life in the Coorong lagoons. pp 81-88. *In* Noye BJ (ed.) *The Coorong*. (The University of Adelaide: Adelaide)