CHAPTER 2:

EFFECTS OF SALINITY AND TEMPERATURE ON THE GERMINATION OF *PHRAGMITES AUSTRALIS, JUNCUS ACUTUS*, AND *JUNCUS KRAUSSII*

2.1   Summary

This chapter describes the effects of salinity and temperature on germination characteristics of *P. australis*, *J. acutus* and *J. kraussii*. In particular, it was determined if timing restoration projects to coincide with natural germination cycles or seasonal conditions of high salinity would be disadvantageous to *P. australis* or *J. acutus* germination. Germination trials lasted 25 days, under two temperature range treatments (l0-25 and 15-30°C) and a salinity gradient (0-30 ppt). Many *P. australis* seeds commenced decomposition after three days (up to 58%). Increased salinity lowered germination in all species; however, only *P. australis* was influenced by temperature. *Phragmites australis* germinated in all conditions, although germination rate was low (2 % ± 1.7) in the highest salinity treatment, at high temperature regime. Both *Juncus* species reached 100% germination in freshwater, failed to germinate in the highest salinity and seed viability was not affected by 25 days immersion in high salinity. It is suggested that tidal reinstatement should be initiated in late autumn when *P. australis* seed banks are low. Additionally, periods of heavy rainfall, which reduce soil salinity, may help other species colonize the area. Further studies are required to determine characteristics of *J. acutus*, which can be used to repress the species spread along Australia's eastern coast. Currently, active measures involving chemical and physical weed suppression, litter removal and mass planting of native species are likely to be required to achieve management goals.

A modified version of this chapter has been published (Greenwood and MacFarlane 2006), see Appendix P15.

2.2   Introduction

Wetland rehabilitation projects are either presently being undertaken ((Kooragang Island (Svoboda 2004)) or are proposed to commence ((Hexham Swamp, 2008 (HCRCMA (2007)) Tomago Wetlands (unknown). The proposal is to restore natural tidal conditions, with the expectation that increasing soil salinity levels will promote the natural regeneration of original salt marsh communities (Svoboda 2004). *Phragmites australis*, *J. acutus* and *J. kraussii* occur in the sites planned for tidal reinstatement and occupy areas with overlapping soil and hydrologic characteristics.

2.2.1 Salinity affects on seed germination

Germination of many wetland species is influenced by a combination of salinity, temperature and light, enabling plants to respond to seasonal variations in surface conditions (Ekstam et al. 1999; Khan et al. 2000a; Kellogg et al. 2003). High, low, or fluctuating temperatures may signify the commencement of a particular season that favors seedling establishment (Ekstam et al. 1999; Khan et al. 2000a). Salinity affects germination by delaying or preventing germination altogether, with or without losing viability (Bewely and Black 1994; Khan et al. 2000b). The ability of seeds to recover and germinate after saline immersion (recovery rate) is frequently observed in salt marsh species (Baskin and Baskin 1998). A light requirement is often observed in small seeds, which aids in maintaining seed-banks and ensures that seedlings do not germinate deep underground (Thompson and Grime 1983; Redondo et al. 2004).

There appears to be some disagreement as to the viability of fresh *P. australis* seeds. Meyerson et al. (2000) maintain that, although variable, germination rate is low. Conversely, 100% germination has been reported in other studies (Ekstam and Forseby 1999; Ekstam et al. 1999; Mauchamp and Mesleard 2001). *Phragmites australis* is reported as being able to germinate in salinity ≤ 25 ppt but with high variability (Lissner and Schierup 1997; Mauchamp and Mesleard 2001). Few studies have evaluated the germination characteristics of either *Juncus* species. Clark and Hannon (1970) and Zedler et al. (1990) documented *J. kraussii* germination success in NaCl concentrations of ≤ 10 ppt but not ≥ 20 ppt. Jones and Richards (1954) reported 90, 65 and 0% germination in 5,

9 and 17.5 ppt salinity respectively for *J. acutus*. It is not known if salinity prevents or merely retards the germination process in *Juncus* species. There is also some debate as to whether *Juncus* species possess a light requirement. Jones and Richards (1954) state that *J. acutus* will not germinate without light; however, in freshwater at 15-25°C, Martinez-Sanchez, et al. (2006) reported <75% germination under dark conditions. Determining optimal germination conditions for each species may facilitate the goal of management in enhancing native salt marsh (*J. kraussii*) habitat over the introduced estuarine species *J. acutus* and the predominantly freshwater macrophyte *P. australis*, by prescribing seasons for restoration work (Svoboda 2004).

2.2.2   Aims

Objectives of the study were 1) to establish germination ability, rate, and viability of the three species across an environmentally relevant salinity gradient, 2) to determine if a difference in temperature range influences salinity tolerance or mitigates the light response of *Juncus* spp. and 3) to provide recommendations for estuarine restoration initiatives.

2.3   Material and Methods

2.3.1   Study Species

All three species are anemophilous (wind pollinated) and produce large quantities of small seeds. Anemochorous (wind dependent) dispersion takes place (Isacch et al.2003); additionally, *Juncus* are hydrochorous (water dispersed) (Jones and Richards 1954, Ekstam and Forseby 1999). Both *J. kraussii* and *J. acutus* are tussock-forming species, and expansion is mainly driven by seed germination (Harden 1993). *Phragmites australis* seedlings account for initial colonization of an area, whereas expansion is dominated by rhizome development (Ailstock et al. 2001; Bart and Hartman 2003).

2.3.2 Seeds

Seeds of *J. acutus* and *J. kraussii* were local genotypes obtained from Kooragang Island (32°52′S, 151°43′E) on 2 July 2003. Although fully developed panicles of *P. australis* were harvested three times over a six-week period, few fully developed seeds were found. An expert in grass seed collection agreed that most of the seed was not, and would not become, viable. Within the Hunter, August is the month for *Phragmites* seed to disperse and by September stems are bare. Therefore, *P. australis* seeds were collected from a coastal wetland at Cleveland Qld (27°30′S, 153°21′E) on 3 August 2003 (AustraHort Pty. Limited). Seeds were sorted and firm seeds with unbroken seed coat selected. Seeds were similar in size within each species. All seeds were then stored, dark and dry (20 ±3°C) until germination trial commencement.

2.3.3 Experimental Design

Throughout the germination experiment, warm fluorescent light (12 μmol m^{-2} s^{-1} 400-700 nm) was maintained for 12 h (0700-1900) photoperiods within an environmental control room (Thermolight, Australia). The temperature cycled diurnally throughout the experiment, with two temperature regimes maintained. Temperatures simulated local bare earth conditions during spring (10-25°C) or summer (15-30°C), coinciding with the same 12 h periods as the light dark regime. Replication with regard to temperature was not carried out due to a limitation of control room facilities.

.

For each treatment, four replicates of twenty-five seeds were placed on filter paper in 90 mm petri dishes. Artificial seawater (Aquasonic, NSW, Aus.) was used to obtain salinity concentrations of 0, 5, 10, 15, 20, 25 and 30 ppt (four replicates x three species x two temperatures x seven salinity treatments = 4200 seeds). Ten milliliters of solution treatment was added to each replicate and seeds allowed to sink at their own rate. Petri dishes were sealed with para-film and placed in the control environment room in a randomized fashion. Percent germination was recorded daily. A seed was considered germinated when the seed coat ruptured (Baskin and Baskin 1998). The initial trial lasted 25 days; subsequently, decayed (soft) seeds were removed and all remaining ungerminated seeds were transferred to new containers containing 10 ml of fresh water.

After five days in fresh water, any additional germination was recorded. To establish if any species had a light requirement, an additional four freshwater (control) replicates were wrapped in double-layered aluminum foil and placed with the 10-25°C treatment, as the lower treatment was known to be desirable for *P. australis* and represented spring field conditions. These control replicates were left undisturbed until day 25, at which time foil was removed and germination recorded.

Many *P. australis* seeds failed to germinate in the 0 ppt salinity (control) treatment. To determine if *P. australis* seeds were viable at the commencement of the trial, 50 seeds with cut embryos were placed in 5.0% 2, 3, 5-triphenyl-2H-tetrazolium-chloride solution for 24 hrs and tested for color change. Sixty four percent of *P. australis* seeds were found to be viable.

2.3.4 Statistical Analyses

Due to the low germination recorded, recovery rates of *P. australis* could not be computed, as it was not able to replicate germination requirements. Therefore, all subsequent analyses were based on percentage germinants relative to the control average equaling 100% (maximum germination potential). Analyses were preformed using Statistica release 6.0 (StatSoft Inc.). Percentage data were arcsine-transformed prior to statistical analysis to achieve normality (Anderson-Darling Normality Test; $P = 0.12$, *P. australis*; $= 0.74$, *J. acutus*; $= 0.92$, *J. kraussii*). Speed of germination was determined using a speed of germination index (Chiapusio et al. 1997).

$$S = ((N_1 * 1) + (N_2 - N_1) * \tfrac{1}{2} + (N_3 - N_2) * \tfrac{1}{3} + \ldots + (Nn - Nn - 1) * {}^1/^n) * 100$$

where N was the proportion of germinated seeds obtained the first (1), (2), (3), (n) days.

Final germination percentages and speed of germination index rates were examined ($\alpha = 0.05$) by species, using a two-way Analysis of Variance (ANOVA) model, for independent variables of salinity and temperature. Recovery potential was evaluated through a three-way ANOVA (salinity, temperature and with or without recovery period). Where main effects or interactions were detected, Tukey's HSD test was used to separate factors within these effects ($\alpha = 0.05$) (Fowler et al. 1998). Student *t*-tests were used to

compare germination differences between dark and light conditions within and between species.

2.4   Results

2.4.1 Effect of Salinity

Germination decreased for all species ($F_{6, 21}$ = 9.66, p = <0.001, *P. australis*; = 154.4, p = <0.001, *J. acutus*; = 113.8, p = <0.001, *J. kraussii*) in response to increasing salinity (Figure 2-1; Appendix A). *Phragmites australis* recorded 2% (± 1.7 SE) and 43% (± 8.9 SE) germination in 30 ppt salinity at high and low temperatures respectively, but both *Juncus* species failed to germinate at the highest salt concentration. Percent germination of *J. kraussii* decreased rapidly at >15 ppt in high and >20 ppt salinity at low temperatures. *Juncus acutus* displayed the same trend, decreasing rapidly >10 ppt for high and >15 ppt salinity at low temperature treatments. However, percent germination of *J. acutus* and *J. kraussii* were similar at both temperature regimes (t_{68} = 0.05, p = 1.0, high; = 0.04, p = 0.9, low). *Juncus* species recorded superior germination in treatments ≤10 ppt salinity; however, at 15 ppt salinity, germination was greater in *P. australis*. Germination speed decreased as salinity increased ($F_{6, 21}$ = 33.07, p = <0.001, *P. australis*; = 277.52, p = <0.001, *J. acutus*; = 265.36, p = <0.001, *J. kraussii*) (Figure 2). *Phragmites australis* germinated faster than *J. acutus* at all salinities (t_{68} = 2.96, p = 0.004). Although not statistically significant (t_{68} = 0.88, p = 0.4), *J. kraussii* germinated fastest in high temperature amplitudes (temperature fluctuation range) ≤10 ppt salinity. Above 10 ppt salinity, in low temperature treatments, *P. australis* germinated more rapidly than either *Juncus* spp.

2.4.2 Effect of Temperature

Temperature influenced both the final percentage ($F_{1, 68}$ = 30.86, p = <0.001) and rate ($F_{1, 68}$ = 31.12, p = <0.001) of germination for *P. australis*, being greater and faster at lower temperature. Lower temperature amplitudes increased the salinity tolerance of *P. australis* ($F_{12, 63}$ = 2.47, p = 0.04) at mid-salinity treatments to above that recorded in freshwater treatments. Temperature alone did not affect final germination values of *J.*

acutus; however, an interaction between salinity and temperature occurred ($F_{12, 63} = 3.15$, $p = 0.01$), salinity tolerance increasing under lower temperature treatment. Additionally, temperature and salinity combined to affect the speed of *J. acutus* germination ($F_{12, 63} = 3.59$, $p = 0.006$); germination was faster at 15 ppt in lower temperatures but slower at all other salinity concentrations. Lower temperatures stimulated rate of germination ($F_{1, 68} = 5.65$, $p = 0.02$) but not the percent germination of *J. kraussii* ($F_{1, 68} = 3.72$, $p = 0.6$); no interaction between salinity and temperature was found ($F_{12, 63} = 1.16$, $p = 0.35$).

2.4.3 Recovery

At completion of the recovery period, germination of *P. australis* (relative to control) was consistently lower than either *Juncus* species; *P. australis* had 26 and 71%, *J. acutus* 83 and 98% and *J. kraussii* 70 and 99% germination at 30 ppt salinity, in high and low temperature amplitudes, respectively (Figure 3). The five-day recovery period did not stimulate additional germination in *P. australis*. ($F_{1, 68} = 2.02$, $p = 0.16$). However, both *Juncus* species responded to being placed in fresh water for five days ($F_{1, 68} = 344.15$, $p = <0.001$, *J. acutus*; $= 257.1$, $p = <0.001$, *J. kraussii*). The combination of salinity and temperature affected recovery ($F_{12, 63} = 3.83$, $p = 0.006$, *J. acutus*; $= 3.65$, $p = 0.003$, *J. kraussii*) with lower recovery values recorded in higher salinity at higher temperatures.

2.4.4 Light Requirement

No difference in the germination of *P. australis* under dark or light conditions was discerned ($t_{68} = 0.27$, $p = 0.8$). Relative to light controls, *P. australis* achieved 96% ($\pm$ 11 SE) germination in dark conditions, as compared to 75% ($\pm$ 7.3 SE) in high and 88% ($\pm$ 12 SE) in low temperature treatments. *Juncus* species achieved $\leq$100% germination in light at both temperature regimes, and a decrease was recorded under dark conditions ($t_{68} = 4.18$, $p = 0.006$ *J. acutus*; $= 10.83$, $p = <0.001$ *J. kraussii*). However, although both species were able to germinate in the dark, *J. kraussii* achieved greater germination at 71% than *J. acutus* 43% ($t_{68} = 2.05$; $p = 0.047$).
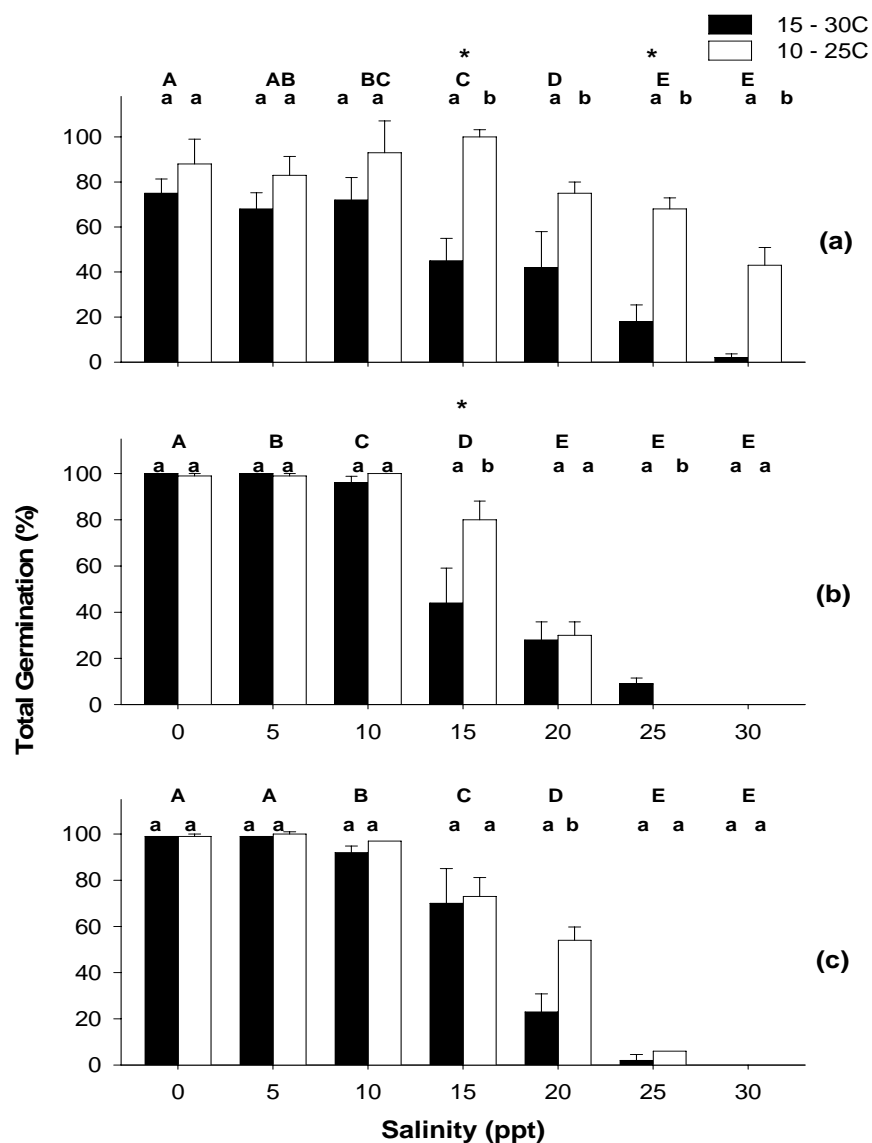
Figure 2-1 Final mean ± SE (N=4) germination percentages, relative to control achieving 100%, of (a) *Phragmites australis*, (b) *Juncus acutus,* and (c) *Juncus kraussii* seeds. Seeds subjected to seven salinity treatments and two temperature regimes. Trial conducted over 25 days. Trial performed with 12h temperature and photoperiods. All seeds stored in dark conditions at 20 ± 2°C prior to trial. Different letters indicate differences in germination between different salinity treatments (uppercase) or different temperature treatments at a particular salinity level (lowercase). An asterisk indicates interaction between salinity and temperature occurred at a particular salinity treatment, ANOVA with post-hoc Tukey's HSD tests (α = 0.05).

Figure 2-2 Germination speed index of (a) *Phragmites australis*, (b) *Juncus acutus*, and (c) *Juncus kraussii* seeds (mean ± SE (N=4)). Seeds subjected to seven salinity treatments and two temperature regimes. Trial performed over 25 days, with 12h temperature and photoperiods. Different letters indicate differences in germination between different salinity treatments (uppercase) or different temperature treatments at a particular salinity level (lowercase). An asterisk indicates interaction between salinity and temperature occurred at a particular salinity treatment, ANOVA with post-hoc Tukey's HSD tests (α = 0.05).
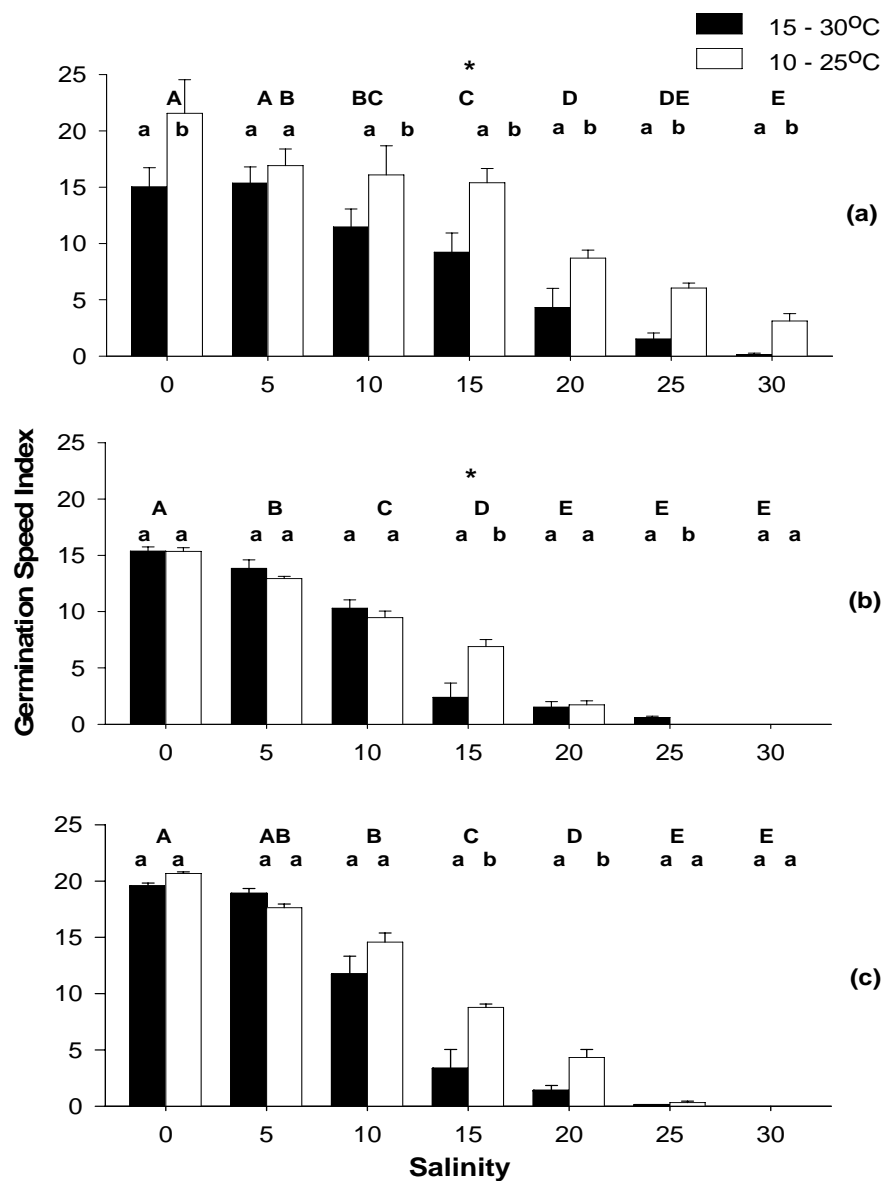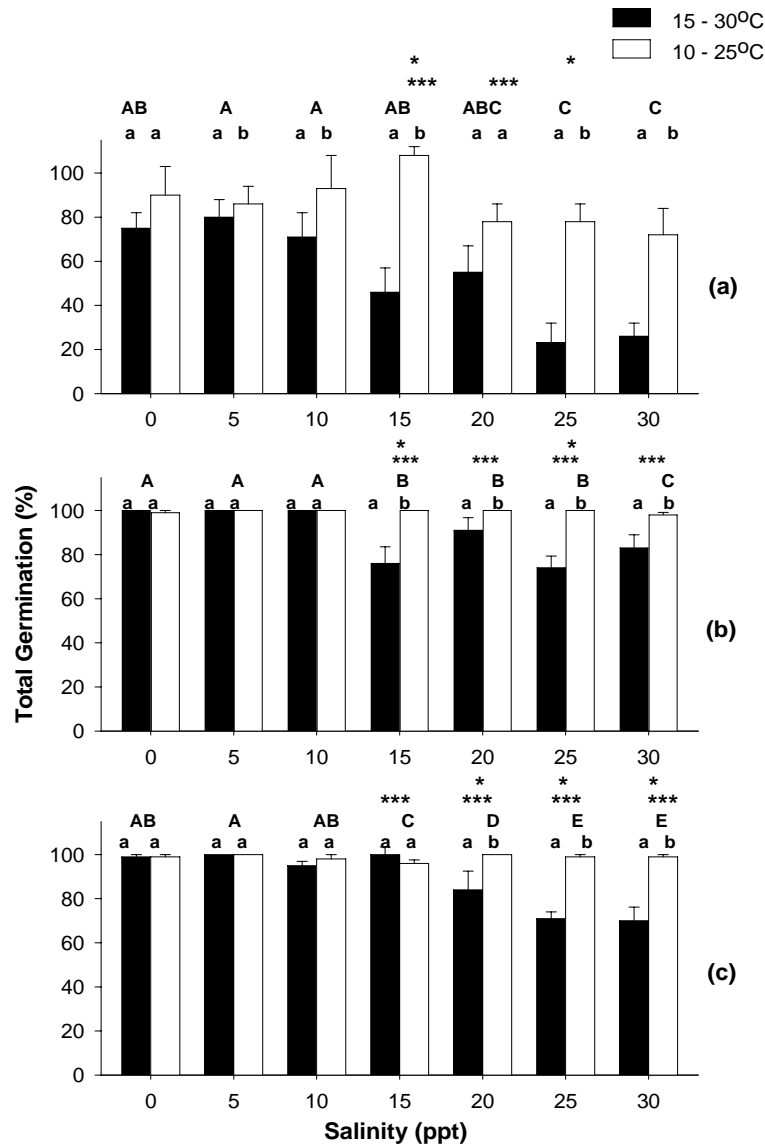
Figure 2-3 Final mean ± SE (N=4) germination percentages after recovery period (a) *Phragmites australis***, (b)** *Juncus acutus,* **and (c)** *Juncus kraussii* **seeds. Seeds subjected to seven salinity treatments and two temperature regimes, followed by a 5-day recovery period in fresh water. Different letters indicate significant differences in germination between, different salinity treatments (uppercase) or, different temperature treatments at a particular salinity level (lowercase). An asterisk indicates interaction between salinity and temperature occurred at a particular salinity treatment. Three asterisks indicate interaction between salinity, temperature, and recovery period, ANOVA with post-hoc Tukey's HSD tests (α = 0.05).**

2.5   Discussion

All study species were able to germinate in salinities up to or in excess of 20 ppt and spring was the optimum germination season. Many salt marsh species germinate in spring after rains, coinciding with mild temperatures and reduced soil salinity (Allison 1996). As salinity within the Hunter estuary marsh system varies considerably, ground-water salinity ranges between 0 and 25 ppt (Hughes 1998), the range of possible habitat would appear large. After being subjected to 25 days of high salinity treatments *Juncus* species were able to germinate fully in fresh water at spring temperatures, implying that high summer temperatures are less favorable for germination. However, prolonged saline contact apparently affected *P. australis* seed viability, and recovery was minimal. Overall, *Juncus* species possessed an advantage in germination capabilities over *P. australis* when salinities were below 10 ppt. However, contrary to expectations, this advantage was reversed when salinity was maintained above 10 ppt.

It is not known why a high percentage of *P. australis* seeds failed to germinate. Percent germination recorded in earlier studies ranged from 0 (Harris and Marshall 1960) to 100% (Ekstam et al. 1999). Rapid seed decomposition, coupled with the loss of viability due to salinity immersion and lack of light dependency, could be expected to contribute to the previously reported transient seed-bank of the species (Koppitz et al. 1997; Keller 2000). This transient seed-bank, where large quantities of seeds are produced but do not persist, is thought to be indicative of plants with a great dependency on seasonal cues (Baskin and Baskin 1998). Conversely, as so many seeds are produced, the loss of 50% would still allow ample opportunity for colonization and, therefore, may not be ecologically significant.

Where light helps control germination timing, large numbers of seeds may accumulate in the seed-bank and therefore be available for future colonization under favorable conditions (Grillas et al 1993). Although Martinez-Sanchez, et al. (2006) determined *J. acutus* was able to germinate under dark conditions, it is generally accepted that *Juncus* species possess some sort of light requirement (Jones and Richards 1954). Thompson and Grime (1983) observed germination of *J. effusus* L. to be fully inhibited by darkness and

enhanced under small (2°C) fluctuating temperature in light regimes. A small percentage of *Juncus* species seeds were germinated in dark conditions under fluctuating temperatures; however, both species maintained a light dependency. The capacity to respond to fluctuating temperatures in light, but not darkness, is thought to be a mechanism whereby spring germination is initiated by increasing irradiance and darkness used as a method of depth-sensing, thus overriding any temperature response (Thompson and Grime 1983).

In general, germination characteristics of *J. kraussii* and *J. acutus* were similar. Both species required light to achieve maximum germination, displayed rapid and high final percentage germination in salinities ≤15 ppt, and seed viability was not compromised by salinity. The major difference in germination traits between *Juncus* species was that germination decreased above 10 ppt salinity in *J. acutus*, while *J. kraussii* maintained a high final germination percentage until salinity exceeded 15 ppt, indicating that *J. kraussii* possesses a slightly higher degree of salinity tolerance. These salinity thresholds can be modified, with lower temperature amplitudes enhancing germination capability. In this trial, germination values recorded for *Juncus* species under saline conditions were higher than those previously documented (Jones and Richards 1954; Clark and Hannon 1970; Zedler et al. 1990). Artificial seawater which possesses a high percentage of $MgCl_2$ was used throughout the present trial, whereas previous studies were performed with NaCl and/or under static temperatures. NaCl solutions represent a highly unnatural situation and results should be treated with caution. Additionally, many plants react differently to different types of salt (Wright and Wellbourn 2002) and temperature fluctuation is a known germination inducer in many marsh species (Ekstam et al. 1999) and probably accounted for these differences.

Germination differences observed for *P. australis* have led some (Lenssen et al. 1998; Koppitz 1999; Mauchamp and Mesleard 2001) to suggest that the species shows great genotypic variation among locations, is adapted to local conditions, and possesses phenotypic plasticity in its ability to modify its germination strategy in response to variable environments at a local scale. To my knowledge, there have been no studies on

the heritage of *P. australis* ecotypes in Australia. It may therefore be informative that *P. australis* was found to have lower germination rates than those recorded in most European studies (Ekstam et al. 1999; Mauchamp and Mesleard 2001) but similar to those documented in North American literature (Harris and Marshall 1960; Galinato and van der Valk 1986; Wijte and Gallagher 1996). Although germination of *P. australis* was affected by salinity, a combination of low mean temperature and mid-range salinity increased germination. The ability of mild salinity regimes (5-10 ppt) to stimulate *P. australis* germination has been reported and is thought to give *P. australis* a competitive advantage in saltmarsh conditions that receive freshwater inputs (Wijte and Gallagher 1996).

2.5.1 Management Implications

These results, together with the phenology of each species help illustrate the threat *P. australis* and *J. acutus* may present to *J. kraussii*. *Phragmites australis* flowers in late summer, and non-dormant seeds persist on the plant until late winter (Auld and Medd 1987). Spring winds disperse seeds and if germination does not take place immediately many seeds could be expected to disintegrate. Viable seeds prevented from germinating through adverse conditions, such as high salinity, may enter the seed-bank (Bewely and Black 1994). As the seed-bank is only replenished in spring, seed numbers gradually decrease due to numerous factors such as ephemeral persistence and predation. Therefore, by late autumn or early winter the amount of viable *P. australis* seed available for colonization is low. Conversely, *Juncus* species flower in early spring and fruit ripen by mid-summer (Jones and Richards 1954). Fruit may disperse immediately or remain with the parent plant for up to six months. Mature seeds germinate quickly in freshwater and light. However, if buried in sediment or exposed to high salinity, seeds could be expected to remain viable but not undergo germination. The ability to produce seedlings throughout the year indicates that soil disturbances, particularly during early winter, should benefit establishment of either or both *Juncus* species over *P. australis*.

Under natural conditions germination of all three species is probably initiated on bare soil. Dead standing crops of freshwater vegetation are likely to occur following tidal

reinstatement, influencing community succession (Xiong et al. 2003). Excess litter may prevent desirable seeds from germinating due to inadequate light availability. Having no light requirement *P. australis* seeds would be able to germinate under heavy leaf litter, while *Juncus* seeds would enter the seed bank. Additionally, vegetative components, such as ramets and rhizomes, might be mixed through the dead standing crops and these often display higher salinity tolerance than seedlings (Zedler et al. 1990; Lissner and Schierup 1997).

Initiating restoration programs in autumn or winter, after rain lowers salinity levels, will coincide with *P. australis'*s low seasonal germination capabilities. This will assist in restricting seedling growth and allow alternative species to become established. Although germination of *J. acutus* is adversely affected by a combination of increased salinity and high temperatures, timing events and manipulating salinity regimes will do little to favour *J. kraussii* over *J. acutus*. It seems that expectations of native salt marsh reestablishment through natural processes are ill-founded. Active measures, involving chemical and physical weed suppression, litter removal and mass planting of native species are likely to be required to achieve management goals.

CHAPTER 3:

RELATIVE SALINITY TOLERANCE OF *PHRAGMITES AUSTRALIS*, *JUNCUS ACUTUS* AND *J. KRAUSSII:* ACUTE AND CHRONIC SALINITY EFFECTS DURING SEEDLING GROWTH AND DEVELOPMENT

3.1   Summary

I compared the effect of a salinity gradient (0 - 40 ppt) on *Phragmites australis* and *Juncus* spp. (*acutus* and *kraussii*). Factors studied were photosynthetic capability, Na^+ accumulation and distribution, photosynthetic pigments, biomass accumulation and morphology *(*height, and density) of the three species. *Phragmites australis* is able to exclude Na^+ from entering root systems for over one month, after which time Na^+ accumulated in all tissue, toxic effects and mortality occurring in treatments of 30 ppt and above. Conversely, although both *Juncus* species recorded decreased growth rates with increased salinity, plants withstood four months exposure at 40 ppt salinity without visible signs of necrosis. These results indicate that *P. australis* will decrease in vigour in the field where salinity is maintained ≤ 20 ppt. Both *Juncus* species accumulated Na^+ and are highly salt tolerant. The key difference in salinity adaptation is that *J. acutus* placed above-ground reserves into short, thick culms, whereas *J. kraussii* maintained height at the expense of culm numbers. Additionally, *J. acutus* maintained a high level of control over accumulation and regulated to reduce Na^+ in shoot tissue after one month; whereas over time, *J. kraussii* increased Na^+ concentrations in roots at low salinity while maintaining shoot concentrations around 10 ppt. Overall, *J. acutus* will not be overly disadvantaged by increased salinity levels. There is a need to investigate water depth, the other major factor associated with increased tidal conditions, to find possible ecological differences between the two species.

3.2   Introduction

3.2.1   The affect of salinity on plants

Salinity is considered the main chemical stressor of coastal marsh systems (Critchley 1982; Wilson and Keddy 1986; Ungar 1998; Allakhverdiev et al. 2000). Salinity affects plants through osmotic effects, decreasing water availability, or by toxicity of excess ions present in the plant (Ralph 1998; Alarcon et al. 1999). The potentially toxic effects of salinity depend not only on the concentration, but also on temporal exposure and is associated with a species' ion accumulation and tolerance mechanisms (Greenway and Munns 1980; Munns 2002). Sodium-specific damage results in leaf necrosis, growth reductions and shorter lifespan (Tester and Davenport 2003). When excessive salt enters a plant concentrations may rise to toxic levels, leading to changes in nutrient uptake, disruption of metabolic function, premature senescence, reduced growth and eventual mortality (Kalaji and Pietkiewicz 1993; Serrano et al. 1999; Zhao et al. 2003). The major salts causing plant stress in coastal environments are Na^+ and Cl^-, as NaCl is the primary salt encountered in sea water (Munns 2002).

3.2.1.2 Glycophytes

Plants utilise a variety of different mechanisms to avoid or alleviate salt induced toxicity. The most common method recorded in glycophytic plants is avoidance or exclusion. Sensitive leaf tissue is protected through excess salts being excluded from, or trapped in, root systems. The mechanism for this may be 1) a selectivity of ion uptake at the root epidermis, exodermis or, if the flow is apoplastic across the root cortex, the endodermis, 2) a preferential loading of K^+ rather than Na^+ into the xylem or 3) removal of salts from the xylem and retaining Na^+ in older tap roots and stem bases, thereby reducing plant water potential (Munns 2002).

3.2.1.2 Halophytes

Halophytic plants tolerate salt by alleviating the effects of salinity, typically translocating accumulated salts from roots to above-ground tissue without altering internal resource allocation ratios (Waisel 1972; Jordan et al. 2002). To maintain osmotic balance Na^+ is

often stored within vacuoles, with a corresponding increase in cytoplasmic K^+ and organic compatible solutes, many of which contain nitrogen (i.e. *Suaeda* spp.) (Hogarth 1999; Zhao et al. 1999). Excess Na^+ may also be excreted from aboveground tissue. For example the mangrove species *Avicennia marina* (Forsk) Vierh accumulates and excretes excess salts through glandular trichomes in its leaves to protect against salt damage (Ye et al. 2005).

Species referred to as facultative halophytes (often brackish species), are plants possessing some degree of salinity regulation. These species may tolerate, albeit at lower growth rates, mild increases in soil salinity without experiencing mortality. Differences in salinity tolerance can be observed between closely related species, or even among ecotypes. For example, *Limonium latifolium* Sm was found to be more salt resistant, due to efficient exclusion of both Na^+ and Cl^- ions, than the closely related species *L. caspia x L. latifolium cv.*, (Alarcon et al. 1999). Lissner et al. (1999a) found Spanish populations of *P. australis* possessed higher Na^+ accumulation and tolerance than populations in Denmark.

3.2.2 Evaluating relative salinity tolerance

A common way of evaluating salinity tolerance is through quantifying the salinity level resulting in a 50% reduction in yield; or alternatively, the salinity level where a significant decline in yield occurs (Marcum 2006). Change in stomatal conductance is the first manifestation of water stress, followed by changes in respiratory and photosynthetic function, as dehydration constricts guard cells, reduces intercellular spaces and lowers CO_2 uptake (Ralph 1998; Taiz and Zeiger 2000; Gandul-Rojas et al. 2004; Li et al. 2004). Increased respiration occurs due to an energy demand for maintaining normal functions (growth, ion regulation, osmotic adjustment and membrane integrity) (Keiper et al. 1998). Although excess salts generate no effect on PSII photochemistry (Belkhodja et al. 1999; Lu et al. 2003) it inhibits the repair of photodamaged PSII (Allakhverdiev et al. 2002). Many photosynthetic enzymes are activated by K^+; therefore high Na^+/ K^+ ratios can disrupt enzymatic function, as Na^+ successfully competes with K^+ at binding sites (Tester and Davenport 2003).

Chlorophyll pigment analysis is frequently used as a surrogate for photosynthetic potential (Ralph 1998). Salinity has been shown to impact upon production and retention of pigments by 1) inactivation, or over stimulation, of particular enzymes thereby increasing reactive oxygen species production, 2) increasing irradiance stress through diminished, or enhanced, photon availability or 3) disruption of proteins, such as the chroplast membrane protein FLU (Sudhir and Murthy 2004; Tanaka and Tanaka 2006).

Changes may also occur in chloroplast structure and function via disruption of water splitting reactions and photosynthetic electron transport (Critchley 1982; Lechno et al. 1997; Keiper et al. 1998; Parida and Das 2005; Navarroc et al. 2007). Salinity causes reductions in photosynthetic pigments such as chlorophylls *a* and *b* and carotenoids in glycophytes (Garcia et al. 1997; Belkhodja et al. 1999; Husain et al. 2003; Sayed 2003). For halophytic species, photosynthetic pigment values may decrease (Ervin and Wetzel 2000; Colom and Vazzana 2002; Farnsworth and Meyerson 2003) increase (Lu et al. 2003; Redondo-Gòmez et al. 2006) or not change with increasing salinity (Ashraf and Harris 2004; Garcia-Valenzuela et al. 2005). Ultimately, changes in photosynthetic pigment response affects carbon assimilation, growth, fecundity and longevity (Munns and Termatt 1986; Ashraf 2004; Parida and Das 2005).

Salinity has been reported to affect membrane permeability through various mechanisms; including, increases in planar free steroids, increases in saturation of membrane fatty acids and changes to membrane transport proteins (Mansour and Salam 2004). This combination of lower CO_2 uptake, photosynthetic pigment degradation and disruption of cell membrane permeability may result in reduced carbon fixation (total biomass), smaller leaves, decreased height and reduced stem thickness and density (Taylor 1939; Waisel 1972; Turner et al. 2004). Additionally, shoot/root biomass ratios may change as energy resources are reallocated to actively transport Na^+ back into the soil medium (Maggio et al. 2001).

3.2.3 Effect of temporal exposure on salinity tolerance

Quantifying differences in salt tolerance between species, especially closely related species, is confounded by the fact that potentially toxic effects of salinity depend not only on the concentration, but also the temporal exposure (Greenway and Munns 1980; Munns 2002). Table 1-3 summarises the sequence of response that take place in plants subjected to an increase in salinity. The initial phase, minutes to days, is due to an osmotic stress, caused by the difference in water potential within and outside plant roots (Munns 2002). Over time, some amount of recovery may take place as plants adjust by lowering internal water pressure. The second phase in salinity stress results from internal injury and, being due to an accumulation of salts in plant tissue, takes time to develop. Accumulation of excess amounts of salts in transpiring leaves may exceed the capacity of the plant to sequester salts in the vacuole. This will reduce supply of carbohydrates to growing cells, affecting growth of young leaves (Bajji et al. 2002; Munns 2002). Over weeks and months older foliage may die, reducing the amount of photosynthetic material. With the decline of healthy green leaves flowering and seed set can be affected.

Table 3-3 Plant responses to increased salinity at different temporal scales

Time	Water stress	Salt-specific stress
	Effect on salt tolerant plant	Additional effect on salt-sensitive plant
Minutes	An instant reduction in leaf and root elongation occurs.	
Hours	Reduced, but steady, rate of leaf and root elongation	
Days	Affects on leaf growth are higher than on root growth. Reduction of new leaves	Visible injury in old leaves
Weeks	Reduction in leaf size Reduction of lateral shoots	Death of older leaves
Months	Change in time of flowering, reduced seed set, reduced seed viability	Death of young leaves, plant may die before seed maturity

From (Munns 2002)

3.2.4 Salinity tolerance of target species

3.2.4.1 Phragmites australis

Outside Australia, the salinity tolerance of *P. australis* has been well studied (Hanganu et al. 1999; Lissner et al. 1999a; Lissner et al. 1999b; Meyerson et al. 2000; Mauchamp and Mesleard 2001). The species has been documented as growing most successfully in freshwater or salinity < 10 ppt, above this level vigour decreases. However, salinity tolerances of > 45 ppt have been reported in mature stands, especially when supplied with occasional freshwater inputs (Hellings and Gallagher 1992; Lissner and Schierup 1997; Chambers et al. 1998; Burdick et al. 2001; Hartzendorf and Rolletschek 2001; Mauchamp and Mesleard 2001; Fogli et al. 2002). In a search of the records relating to Australian populations, no published research on salinity tolerance or toxicity effects caused by salinity could be sourced. It is possible that tolerances of local (Australian) ecotypes may be notably different to those previously reported.

3.2.4.2 Juncus species

Little research has been conducted on either *Juncus* species. Zonation patterns of *J. kraussii*, with regard to salinity and inundation, have been documented in South Africa, New Zealand and Australia (Congdon and McComb 1980; Russell 2003; Thomsen et al. 2005; Naidoo and Kift 2006). Findings indicate *J. kraussii* is tolerant of 70% seawater, although maximum growth was recorded < 10%, under flooded conditions (Naidoo and Kift 2006). Zedler et al. (1990) found the species to be tolerant of salinity as high as 40 ppt, when mature. Both *Juncus* species are able to achieve limited germination in 25 ppt salinity (Chapter 2 p. 45). Limited habitat information is available for *J. acutus*, the most informative source being Jones and Richards (1954), who state that the species is tolerant of mildly saline habitats. Introduced to Australia, the species is documented as growing on Garden Island (WA) and mainland estuarine environments along Australia's coast (Burkett 2000; Williams and Meehan 2004; Paul et al. 2007).

3.2.5 Aims

In order to predict potential zonation trajectories of the three species, detailed information on salinity tolerances are required prior to reinstatement of more natural tidal flows. Equally important is understanding relative sub-lethal toxic responses of the aforementioned species growing in various salinity regimes. The aim of the study was to 1) assess the effect of increasing salinity on early (acute) physiological responses (Na^+ accumulation, respiration and photosynthetic capability) of the three species; 2) assess the effect of increasing salinity on long-term (chronic) production of photosynthetic pigments, biomass and morphological responses (height and density) of the three species; 3) determine relative sub-lethal toxic effects of salinity and EC_{50} values for each species and finally 4) to provide recommendations for estuarine restoration initiatives.

3.3 Materials and Methods

3.3.1 Plant material

Seedlings of both *Juncus* species were local genotypes, seed being obtained from Kooragang Island (32°52′S, 151°43′E) on July 2nd 2003. *P. australis* plants were obtained as seedlings (ABULK Pty Ltd, NSW), seed material being collected from sites within the Sydney region of NSW, Australia. It is a concern that the genetic source of *P. australis* is not fully known, introducing a source of variation that is not controlled for.

3.3.2 Experimental Design

Trials were conducted under glasshouse conditions, maintained at 26 ± 4ºC day and 14 ± 2ºC night temperatures, with an 11.03 ± 0.0004 hr light period. Humidity was not controlled for; however, all pants were located in one glasshouse and therefore under similar conditions. To characterise a typical saltmarsh sediment, seven sediment samples were taken from three marsh locations within the Hunter River estuary (N = 7 per location). Samples (20-30 cm depth) were collected within and around areas that contained communities with combined *P. australis*, *J. acutus* and *J. kraussii* stands, using a 90mm round cylinder. Samples were combined and analysed for particle size

(mechanical fraction analysis) and percent organic matter (LOI at 400ºC) (Allen JRC (2000). Results indicated a typical marsh soil consisted of sand = 47 ± 2.1; silt/clay = 27 ± 6.1 particle matter and organic matter = 26 ± 1.3 percent. Experiments were conducted in soil prepared to resemble a typical saltmarsh sediment (i.e. 50% washed river sand, 25% loam soil and 25% organic material (coconut fibre).

Where potentially toxic levels of elements, such as boron, are present in the soil medium, raising salinity levels may release the element and affect plant growth (Mitsch and Gosselink 2000; Tester and Davenport 2003). To determine if any element was present at elevated levels, potting mix samples (N = 3) were taken and levels of 23 elements determined through Inductively Coupled Plasma Mass Spectroscopy (Advanced Mass Spectrometry Spectrometer Unit, University of Newcastle). All elements analysed were found to be below minimum concentrations required to exert adverse biological effects (ANZECC/NHMR 1992) (Appendix B).

Each pot (10 litres) was supplied with 10 g (N 14%, P 9%, K 15%) of Nutricote® slow (6 month) release fertiliser (Chisso Asahi Fertilisers, Tokyo) and received 40 ml Wuxal® liquid fertiliser (Ag Nova Technologies, Australia) at monthly intervals. A single seeding was placed in the centre of each pot. Pots stood in individual holding trays. Plants were watered (tap-water) to saturation point, with an additional 1 litre added. Throughout the trial, water, at appropriate treatment level, was added twice-weekly to maintain water levels. After a one-month equilibration period, *P. australis* plants were thinned to 4-5 young shoots. The study commenced three weeks later, at which time all seedlings were 18-20 weeks of age (Plate 3-1).

The experimental design for each species consisted of five replicates and seven salinity treatments (0, 5, 10, 15, 20, 30, 40 ppt). Throughout the duration of the study, treatment values remained constant. A number of temporal monitoring events were included. Non destructive, net photosynthesis and respiration were assessed at 24, 48, 96 and 168hrs (1 week). Photosynthetic pigments, Na^+ concentrations in roots and shoots, and growth parameters were assessed at three harvest periods (1 week, 1 month and four months). A

total of 315 pots were thus used in the experiment (3 species. x 5 replicates x 7 treatments x 3 harvests). Pots were randomly distributed within each harvest period. As each harvest reduced pot numbers, pots were condensed to maintain spatial constancy.

At trial commencement, plants were removed from containers, left to drain for 1-2 hours, watered with appropriate salinity treatment and returned to containers with 1 litre of appropriate treatment water. A mark was placed on the holding tray and subsequent watering was made up to this mark. Salinity concentrations were produced from artificial seawater (Ocean Nature, Aquasonic, Australia), salinity composition being 85% NaCl, 7% SO_4^2, 3% Mg^{2+}, 1% each Ca^{2+} and K^+, plus trace amounts of unspecified salts. Soil-water salinity was monitored daily for the first week and then at two-week intervals, by a hand-held salinity meter (Conductivity meter YSI, Ohio), and adjusted if necessary ($\pm$ 5%).

3.3.2.1 Determination of photosynthetic capability

Respiration and gross photosynthetic rates, taken as CO_2 net exchange (μmol m^{-2} s^{-1}) between leaf and atmosphere under dark and light conditions, were recorded at 24, 48, 96 and 168 hrs (1 week), using a LiCor Inc™ portable photosynthesis system (model number LI-6200) coupled with a carbon dioxide analyser (model number LI 6250) (Plate 4-2). Readings were normalised for relative leaf area. Readings were taken at approximately ambient CO_2 (350-370 ppm), temperature (26-28° C) and relative humidity (> 52%). Data used was an average of three consecutive recordings. Net photosynthetic rate was calculated as

$$PN \quad = PG - R$$

where, PN = net photosynthesis, PG = gross photosynthesis and R = respiration occurring under dark conditions.

3.3.2.2 Photosynthetic pigment analysis

Photosynthetic pigments were determined using the *N,N-dimethyl-formamide* (DMF) method of Inskeep and Bloom (1985). For *P. australis*, the three uppermost, fully extended, leaves from the tallest stem of each plant were removed. For *Juncus* species,

basal leaves were uses where possible, otherwise uppermost section of three green stems was evaluated. Leaves were washed and dried with absorbent paper. Approximately 30 mg (fresh weight) of tissue was weighed, cut into fine slivers and placed in brown glass bottles with 10 ml DMF. Samples were refrigerated (4°C) for seven days before spectrophotometric determination (1 cm path length, UV/VIS model Biomate3, Thermo Spectronic Pty Ltd). Absorbance was measured at 647, 664 and 480 nm, for Chl *a*, *b* and carotenoids. Results were calculated as mg/g dry weight, applying the absorption coefficient equations described by Wellburn (1984).

3.3.2.3 Growth evaluation

Prior to study commencement, numbers of live shoots and the height of the second tallest shoot were recorded. The second tallest stem was used to compensate for outliners and to allow a degree of protection from stem brakeage. The selected shoot was then tagged and used in subsequent measurements. At each harvest period shoot numbers and shoot height were established. Plants were then harvested, washed and separated into above-below-ground biomass. Plant tissue was oven-dried for at least 96 hrs at 60°C, or until further drying did not reduce mass. Plant tissue was dry weighed (DW) to three decimal places. Total biomass (shoot + root) data were analysed based on total grams harvested. Due to the large difference in height and growth patterns between the two genera, numbers of live shoots and maximum shoot heights were recorded as percentage change between trial commencement and harvest.

3.3.2.4 Analysis of Na⁺ accumulation and distribution

Following harvest procedures, shoot (leaf, *P. australis*; uppermost stem section, *Juncus* spp) and fine-feeding root tissue were ground and 250 mg (DW) digested in a nitric acid, hydrogen peroxide mixture, made to 25 ml volume (Krishnamurty et al. 1976; Mudroch et al. 1997) Sodium analysis was performed on resulting digest, using air/acetylene atomic absorption spectroscopy (AAS; Varian AA-1275, Australia). Standards used contained 2% HNO_3 matrix (ACR Elemental Standard).Results in ppm were converted to mg/g dry weight of tissue.

Plate 3-1 Recording height and density of *Phragmites australis* plants prior to trial commencement.

Plate 3-2 Recording respiration and gross photosynthetic rates, using a LiCor Inc™ portable photosynthesis system (model number LI-6200) coupled with a carbon dioxide analyser.

3.3.3 Statistical Analysis

Analyses were preformed using Statistica 7.1 release (Stat-Soft 2005). Data that did not pass Kolmogorov-Simirnov normality test were transformed (Table 3-1); subsequently, data were normally distributed. Levenes test for homogeneity was still significant for some factors. However, as ANOVA is generally robust in respect to heterogeneous variances (Underwood 1997), transformed data were analysed using two-way ANOVAs for interactions between time and salinity. It is known that different species can possess different optimal salinity requirements (Hootsmans and Wiegman 1998; Van Zandt et al. 2003). However, for this study freshwater treatments consistently produced the optimum responses in dependant variables measured and were therefore used to determine the effective concentration that produced a significant change from optimum.

Table 3-2. Data transformations and results of Kolmogorov-Simirnov normality and Levenes homogeneity tests of *P. australis* (Pa), *Juncus acutus* (Ja) and *J. kraussii* (Jk) data sets.

Factor	Transformation			K-S test			Levenes test		
	Pa	Ja	Jk	Pa	Ja	Jk	Pa	Ja	Jk
Root Na$^+$ accumulation	Log	Raw	Log	Pass	Pass	Pass	Pass	**Fail**	Pass
Shoot Na$^+$ accumulation	Log	Raw	Raw	Pass	Pass	Pass	Pass	Pass	Pass
Respiration	Log	Raw	Raw	Pass	Pass	Pass	Pass	Pass	**Fail**
Net photosynthesis	Raw	Raw	Raw	Pass	Pass	Pass	Pass	Pass	**Fail**
Chlorophyll *a*	Log	Log	Raw	Pass	Pass	Pass	**Fail**	Pass	**Fail**
Chlorophyll *b*	Log	Log	Raw	Pass	Pass	Pass	Pass	Pass	**Fail**
Carotenoids	Log	Log	Raw	Pass	Pass	Pass	Pass	Pass	**Fail**
Total biomass	Log	Raw	Raw	Pass	Pass	Pass	**Fail**	**Fail**	**Fail**
Density change	Sqrt	Sqrt	Sqrt	Pass	Pass	Pass	Pass	Pass	Pass
Height change	Sqrt	Sqrt	Sqrt	Pass	Pass	Pass	Pass	Pass	Pass

A series of non-linear regression analyses were performed in SigmaPlot (9.01) (Systat 2004) to best describe trend lines of Na$^+$ accumulation in root and shoot tissue. Effective concentrations values, resulting in a 50% decrease from optimum values, (EC$_{50}$) were determined using the sigmoidal method advocated by Morelock et al. (2005) and the associated software, BioFit 1.02 (Chang Bioscience).

3.4   *Results*

3.4.1   *Sodium accumulation and distribution*

Sodium in *P. australis* root tissue showed a positive relationship over the salinity gradient at all time periods (R^2 = 0.44, 1 week; 0.72, 1 month; 0.74, 4month; *p* < 0.001). Initially, a small linear increase in Na$^+$ concentration occurred over the gradient studied. However, by four months a dramatic increase was observed in higher treatments, salinity of 40 ppt inducing a seven fold increase in Na$^+$ concentration and an exponential regression model best explaining Na$^+$ accumulation to roots (F$_{12, 84}$ = 7.53, *p* < 0.001; Table 3-2; Figure 3-1a). In *P. australis* leaf tissue, a positive relationship between salinity treatment and leaf Na$^+$ concentration was initially discerned (R^2 = 0.48, 1 week; 0.49, 1 month; *p* < 0.001) and exponentially later (R^2 = 0.75, 4 months; *p* < 0.001). As exposure time increased,

accumulation increased and the treatment level required to result in a significant increase in Na^+ accumulation to shoots decreased ($F_{12, 84} = 5.97$, $p < 0.001$). At four months, a six fold increase was apparent in the two highest treatments (Table 3-2; Figure 3-2a).

For *J. acutus* root tissue, there was a positive linear relationship between soil salinity and Na^+ in root tissue across the salinity gradient at all time-periods ($R^2 = 0.55$, 1 week; 0.55, 1 month; 0.40, 4 month; $p < 0.001$). However, the pattern varied with time ($F_{12, 84} = 2.31$, $p < 0.05$), initial accumulation increases occurring at lower salinities as exposure time increased (Table 3-2, Figure 3-1b). A positive association, which plateaued above 15 ppt salinity treatment, occurred between salinity and shoot Na^+ concentration at all time periods ($R^2 = 0.49$, 1 week; 0.53, 1 month; 0.44, 4 month; $p < 0.001$). The pattern of Na^+ increase in shoot tissue varied with time $F_{12, 84} = 3.78$, $p < 0.001$). Higher concentrations and sharper Na^+ accumulation responses became apparent after one month exposure, with the lowest and more moderate occurring after four months (Table 3-2; Figure 3-2b).

In *J. kraussii* root tissue, Na^+ concentration rose in a polynomial relationship over the salinity gradient ($R^2 = 0.56$, 1 week; 0.55, 1 month, $p < 0.001$; $R^2 = 0.4$, 4 month, $P < 0.05$) An interaction between salinity and time occurred ($F_{12, 84} = 1.76$, $p < 0.05$). Higher Na^+ concentrations in roots at lower salinities were observed at one and four months compared to one week. A greater relative increase (slope) in root Na^+ concentration versus salinity treatment occurred at one week, with slopes decreasing over time due to increased Na^+ concentrations only at low and moderate salinity application (Table 3-2; Figure 3-1c). For shoot tissue, accumulation relationships were quadratic; increased exposure time increasing amount of curvature. ($R^2 = 0.55$, 1 week; 0.55, 1 month; 0.44, 4 month; $p < 0.001$). Responses were strongest after one month and the lowest after four months (Table 3-2; Figure 3-2c). After four months exposure Na^+ levels were found to plateau at 10 ppt salinity ($F_{12, 84} = 2.8$, $p < 0.05$).

Sodium concentration (mg/g DW) was higher in *P. australis* roots, but lower in shoot tissue than either *Juncus* species. Throughout the trial, the character of Na^+ increase was consistent. All species recorded significant positive relationships over the salinity

gradient with moderate to low variance. However, the pattern of the relationship differed among species. In *P. australis,* increasing salinity treatment did not unduly increase Na^+ within root tissue until after one month, and low translocation to leaf tissue was maintained throughout the trial (less than 0.6 at any time/concentration). Although Na^+ was restricted in leaf tissue ≤ 20 ppt salinity, concentration doubled in the two highest salinity treatments with increasing exposure time, with an exponential increase in Na^+ to both root and shoot. Conversely, both *Juncus* species experienced an increase in Na^+ earlier and at lower salinity treatments, without further increases at treatment concentrations of ≥ 20 ppt in shoot tissue, suggesting regulation of Na^+ transport to leaf tissue. Sodium increased in low and intermediate salinities, higher treatments causing no additional increase. Sodium accumulation in *Juncus* leaf tissue fell between one and four months. However, in *J. acutus* the fall was approximately uniform over the salinity gradient; whereas, in *J. kraussii* the fall occurred only at the highest treatments. At one month *J. acutus* placed Na^+ in root and shoot tissue equally, while increasing time lowered translocation to shoots by half. A translocation factor of 2.1 ($\pm$ 0.37) was observed at 10ppt salinity in *J. kraussii* after one week. Although reduced at each time period, over the salinity gradient, translocation factor was consistently higher in *J. kraussii* than *J. acutus* throughout the study period.

Table 3-3 Summary of ANOVA results into the effects of salinity and time on Na^+ concentration in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

		P. australis		*J. acutus*		*J. kraussii*	
	df	*F*	*p*	*F*	*p*	*F*	*p*
		Root Na $^+$					
Time	2,84	388.81	**< 0.001**	4.79	**0.011**	42.28	**< 0.001**
Salinity	6,84	73.79	**< 0.001**	29.73	**< 0.001**	22.14	**< 0.001**
Interaction	12,84	7.53	**< 0.001**	2.31	**0.013**	1.76	**0.049**
		Shoot Na $^+$					
Time	2,84	115.14	**< 0.001**	136.42	**< 0.001**	63.84	**< 0.001**
Salinity	6,84	34.59	**< 0.001**	45.91	**< 0.001**	80.47	**< 0.001**
Interaction	12,84	5.97	**< 0.001**	3.78	**< 0.001**	2.8	**0.003**
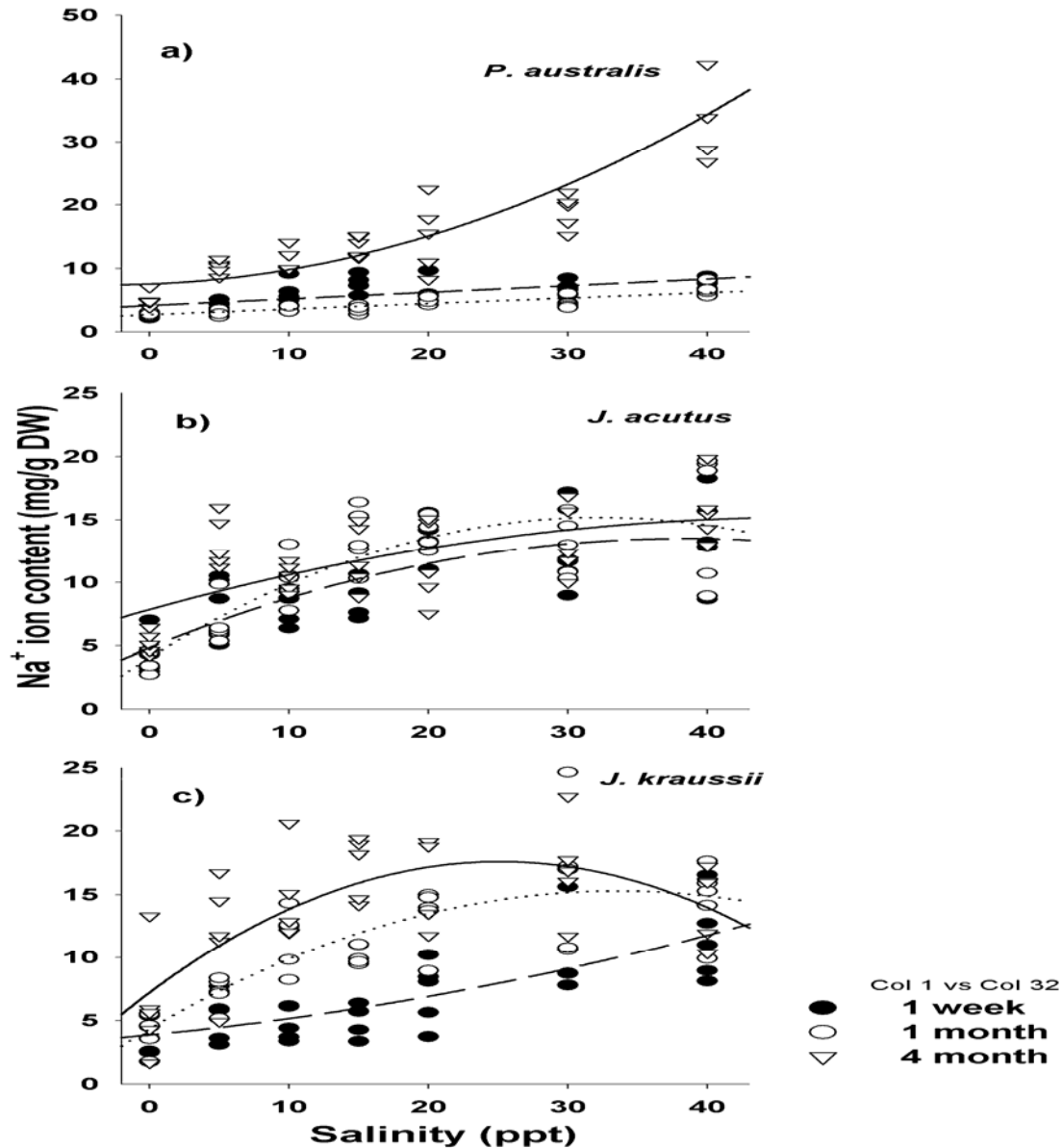
Figure 3-1 Effect of salinity over time on root Na$^+$ ion content in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

Note scale change between *Phragmites* and *Juncus*.

Phragmites australis, **curve equation: y = y0+ax.**

y0 = 4.443, a = 1.05^{-1}, R^2 = 0.442, 1 week; y0 = 2.696, a = 8.738^{-2}, R^2 = 0.715, 1 month.

Curve equation: y = y0+ax+bx^2. y0 = 5.019, a = 1.259, b = -6.799^{-2}, R^2 = 0.79, 4 month.

Juncus **curve equation: y = y0+ax+bx^2**

Juncus acutus, **y0 = 4.837, a = 4.518^{-1}, b = -5.888^{-3}, R^2 = 0.614,1 week; y0 = 4.05, a = 6.89^{-1}, b = -1.066^{-2}, R^2 = 0.683, 1 month; y0 =7.855, a = 3.078^{-1}, b = -3.216^{-3}, R^2 = 0.412, 4 month.**

Juncus kraussii, **y0= 3.862, a = 1.069^{-1}, b = -2.271^{-3}, R^2 = 0.598,1 week; y0 = 4.332, a = 6.526^{-1}, b = -9.715^{-3}, R^2 = 0.545,1 month; y0 = 7.218, a = 18.280^{-1}, b = -1.647^{-3}, R^2 = 0.401, 4 month.**
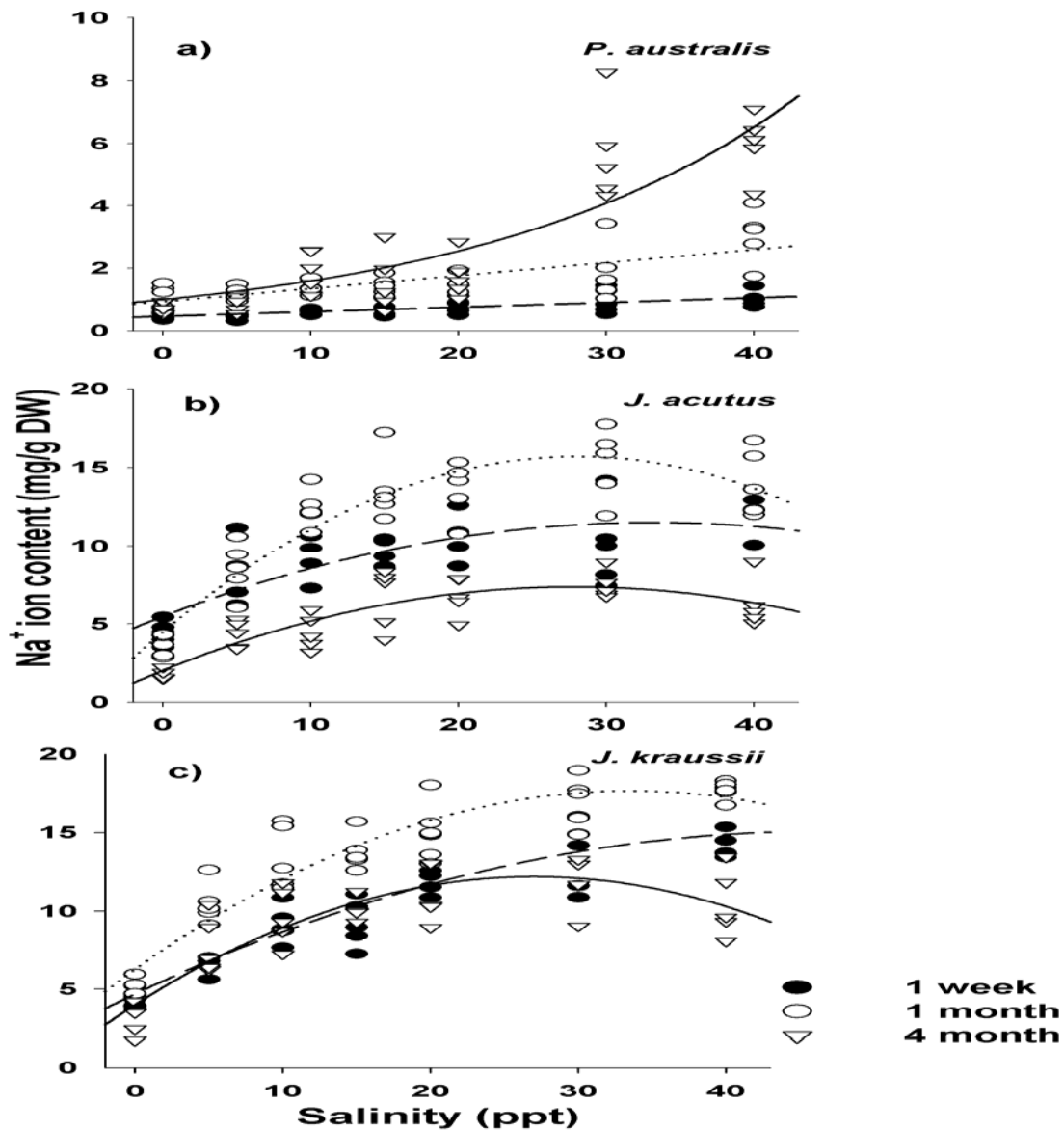
Figure 3-2 **Effect of salinity over time on shoot Na$^+$ ion content in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.**

Note scale change between *Phragmites* and *Juncus*.

Phragmites australis, **curve equation: y = y0+ax.; y0; = 4.443, a = 0.105, R^2 = 0.442, 1 week; y0 = 2.696, a = 8.738^{-2}, R^2 = 0.715, 1 month.curve equation: y = e^{ax}. a = 4.688^{-2}, R^2 = 0.753, 4 month**

Juncus, **curve equation: y = y0+ax+bx^2**

Juncus acutus, **y0 = 5.48, a = 3.63^{-1}, b = -5.5.48^{-3}, R^2 = 0.486,1 week; y0 = 4.49, a = 8.0^{-1}, b = -1.47^{-2}, R^2 = 0.525, 1 month; y0 = 2.04, a = 3.8308^{-1}, b = -6.89^{-3}, R^2 = 0.441, 4 month.**

Juncus kraussii, **y0= 3.86, a = 1.07^{-1}, b = 2.27^{-3}, R^2 = 0.795, 1 week; y0 = 3.92, a = 7.39^{-1}, b = -1.1^{-2}, R^2 = 0.721, 1 month; y0 = 7.29, a = 8.28^{-1}, b = -1.48^{-2}, R^2 = 0.426, 4 month.**

3.4.2 Determination of photosynthetic capability

Salinity caused reductions in respiration in *P. australis,* which varied with duration of exposure ($F_{18,\ 112}$ = 2.93, *p* < 0.001) (Table 3-3; Figure 3-3a). Respiration fell at 15 ppt after 24 hr and 40 ppt salinity after 96 hr exposure, but did not vary significantly across the salinity gradient at other time periods. For *J. acutus,* no difference was discerned between freshwater control and any salinity treatment. Interactions between salinity and time suggested salinity induce changes in respiration, which varied with exposure duration ($F_{18,\ 112}$ = 1.88, *p* <0.05). Exposure time did not alter respiration ≤10 ppt salinity (Table 3-3; Figure 3-3b); although, at higher salinities respiration was greater in early (24 -48 hr) than later periods. Likewise, for *J. kraussii,* respiration rate was influenced by the combined effects of salinity and exposure duration ($F_{18,\ 112}$ = 1.85, *p* < 0.05). Although no difference was discerned from the control treatment, the increase in rate of respiration recorded at 40 ppt salinity up to 48 hr was not observed with increased time of exposure (Table 3-3; Figure 3-3c).

Overall, respiration was consistently lower in *P. australis* than either *Juncus* species (Figure 3-3). During the initial two days, increasing salinity caused respiration to fall in *P. australis*, but rise in both *Juncus* spp. However, by one week salinity had no affect on any species. After one week exposure, no species reached an EC_{50} for respiration over the experimental salinity gradient.

For *P. australis,* increased salinity caused progressive declines in net photosynthesis at 5, 15 and 40 ppt ($F_{6,\ 112}$ = 63.75, *p* < 0.001). Photosynthetic activity increased as exposure time increased ($F_{3,\ 112}$ = 5.4, *p* < 0.05) (Table 3-3; Figure 3-4a). Similarly, in *J. acutus*, increasing salinity caused a decrease in net photosynthesis at 10, 20 and again at 30 ppt salinity ($F_{6,\ 112}$ = 52.47, p < 0.001). Net photosynthetic activity significantly increased at 48 hr and decreased at 1 week (Table 3-3; Figure 3-4b) ($F_{3,\ 112}$ = 24.02, *p* < 0.001). Effects of salinity concentration on net photosynthesis varied with exposure time for *J. kraussii* ($F_{18,\ 112}$ = 2.24, *p* < 0.05). Net photosynthesis declined at 20 ppt salinity after 24 hrs, 15 ppt after 48 hrs, did not differ at 96 hrs and fell at 40 ppt after one week (Table 3-3; Figure 3-4c).

Under ambient conditions, *P. australis* recorded higher net photosynthesis than either *Juncus* species. Net photosynthesis of all species was initially affected by rising salinity concentrations; however, at 40 ppt net photosynthesis of *P. australis* was reduced to extremely low levels up to one week of exposure. *Juncus acutu*s was affected by salinity with an initial decline at 10 ppt salinity. At first, increasing time of exposure caused an increase in photosynthesis; however, continued exposure lowered net photosynthesis. *Juncus kraussii* was the least affected, showing increasing tolerance as the trial progressed. A 50% decline in net photosynthesis was apparent for all species over the salinity gradient (19.68 ppt (SE ± 3.95), *P. australis*; 18.80 ppt (± 3.29), *J. acutus*; 21.71 ppt (± 4.86) *J. kraussii*).

Table 3-4   Summary of ANOVA results into the effects of salinity and time on respiration and net photosynthesis in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

		P. australis		*J. acutus*		*J. kraussii*	
	df	*F*	*p*	*F*	*p*	*F*	*p*
Respiration							
Salinity	6,112	6.57	**< 0.001**	2.84	**0.013**	2.82	**0.014**
Time	3,112	5.29	**0.002**	42.04	**< 0.001**	7.58	**< 0.001**
Interaction	18.112	2.93	**< 0.001**	1.88	**0.024**	1.85	**0.027**
Net Photosynthesis							
Salinity	6,112	63.75	**< 0.001**	52.47	**< 0.001**	24.23	**0.016**
Time	3,112	5.4	**0.002**	24.02	**< 0.001**	5.44	**< 0.001**
Interaction	18,112	0.816	0.789	1.51	0.101	2.24	**0.006**

Figure 3-3 **Effect of salinity over time on respiration in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.**

Note scale change between *Phragmites* and *Juncus*.

Means (± SE) (N = 5).

***** indicates initial significant change from control at each time period.**

Significant changes between individual treatments not shown.

Figure 3-4 **Effect of salinity over time on net photosynthesis in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.**

Note scale change between *Phragmites* and *Juncus*.

Means (± SE) (N = 5).* indicates initial significant change from control at each time period.

**** indicates change over salinity gradient, where effect is not affected by exposure duration.

Significant changes between individual treatments not shown.

3.4.3 Photosynthetic pigments

Chlorophyll *a* concentration in *P. australis* was similar over the salinity gradient at one week, but decreased at 40 ppt salinity at one month and beyond, (Table 3-4; Figure 3-5a) ($F_{12,84}$ = 2.79, p = < 0.05). Salinity concentrations caused significant decreases in chlorophyll *a* levels, which varied with exposure duration in *J. acutus* ($F_{12,84}$ = 2.85, p < 0.05). Chlorophyll *a* decreased at 20 ppt salinity after one week and four month exposures, but at one month no difference across the salinity gradient was discerned (Table 3-4; Figure 3-5b). At no time period were chlorophyll *a* concentrations in *J. kraussii* affected by salinity (Table 3-4; Figure 3-5c), primarily due to the large variation observed at 20 ppt salinity; however, concentrations were higher after four months than other time periods ($F_{2,84}$ = 42.58, p = <0.001).

Salinity did not affect Chl *b* concentration of *P. australis* at one week (Table 3-4; Figure 3-6a). A decrease was detected after one month at 40 ppt salinity and at 30 ppt after four months ($F_{12,84}$ = 2.93, p = < 0.05). In *J. acutus* Chl *b* decreased at 20 ppt salinity at one week and four month exposure but at one month, due to low values recorded in freshwater treatment, an increase was discerned at 20 ppt ($F_{12,84}$ = 7.04, p < 0.001) (Table 3-4; Figure 3-6b). Chlorophyll *b* values in *J. kraussii* did not vary significantly over the salinity gradient or through time (Figure 3-6c).

Salinity did not affect carotenoid concentration in *P. australis* at one week (Table 3-4; Figure3-7a), but decreases were observed at 40 and 30 ppt salinity after one and four months respectively ($F_{12,84}$ = 2.64, p = < 0.05). For *J. acutus*, carotenoid results were similar to those of other pigments (Table 3-4; Figure 3-7b). An interaction between salinity and time caused a significant decrease at 20 ppt salinity after one week and four months, but not at one month ($F_{12,84}$ = 3.74, p < 0.001). *Juncus kraussii* recorded high variation in mid range salinities. Values were similar at each time period and no difference between freshwater and any salinity treatment was detected. However, salinity did affect carotenoid readings ($F_{6,84}$ = 3.41, p = <0.05) (Table 3-4; Figure 3-7c), at 10 ppt salinity carotenoid concentrations were lower than at 20 ppt and both 5 and 10 ppt were lower than at 30 ppt salinity.

Salinity affected *P. australis* only at treatment levels $\geq$ 30 ppt; however, when reductions occurred the decline was rapid. Conversely, Chl *a* declined in *J. acutus* at 20 ppt and the reduction was gradual. All photosynthetic pigment concentrations measured were lower in *J. kraussii.* Pigment concentrations in *J. kraussii* showed great variation in mid range treatments; however, over the four month trial period, salinity had little consistent negative effect on pigments.

Table 3-5   Summary of ANOVA results into the effects of salinity and time on photosynthetic pigment in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

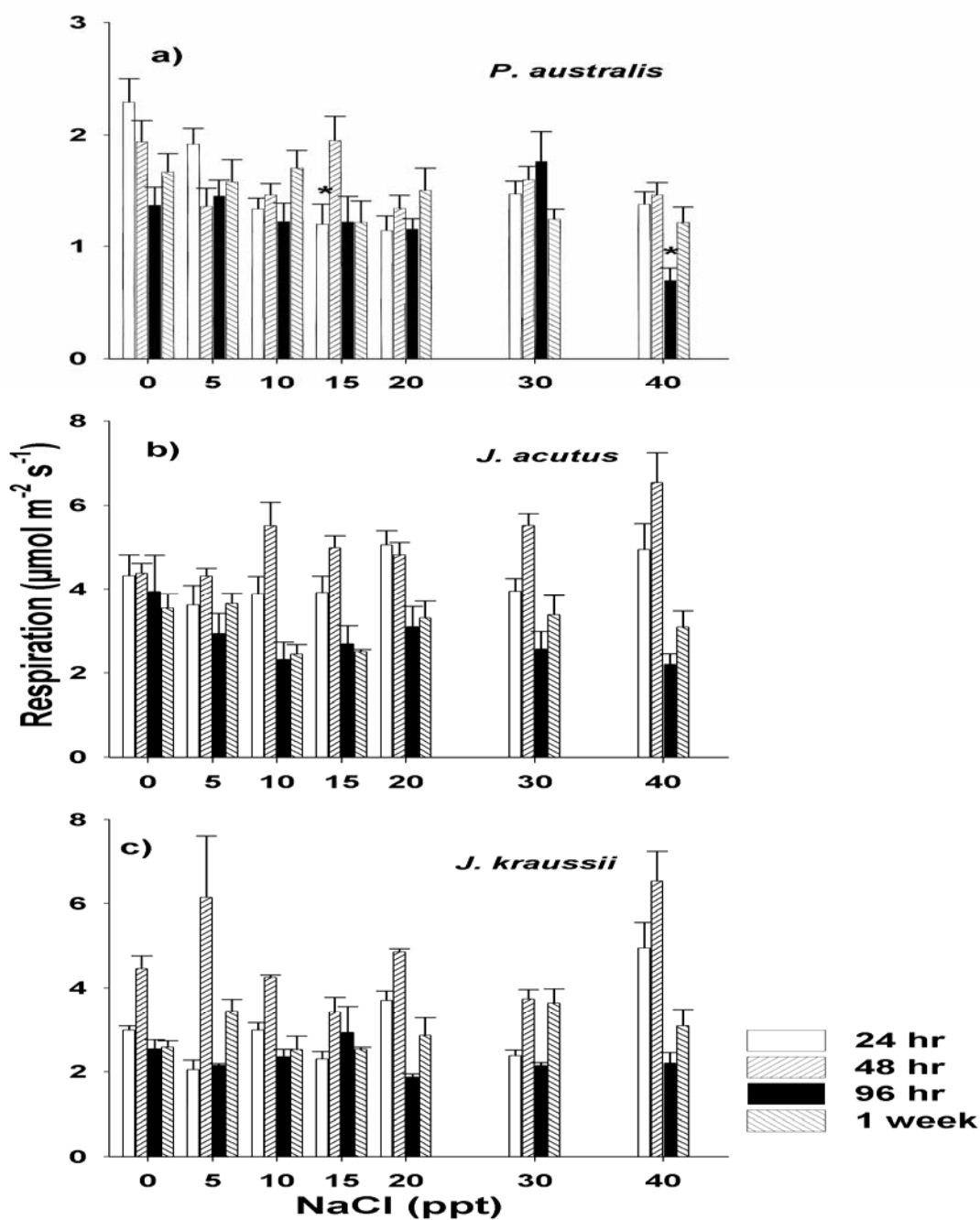|		*P. australis*		*J. acutus*		*J. kraussii*	
	df	*F*	*p*	*F*	*p*	*F*	*p*
		Chl *a* mg/l					
Salinity	6,84	10.23	**< 0.001**	8,15	**< 0.001**	0.51	0.802
Time	2,84	6.84	**< 0.001**	1.2	0.305	42.58	**< 0.001**
Interaction	12,84	2.79	**0.003**	2.85	**0.003**	0.732	0.717
		Chl *b* mg/l					
Salinity	6,84	10.54	**< 0.001**	1.622	0.152	1.47	0.199
Time	2,84	11.2	**< 0.001**	6.07	**0.003**	2.01	0.141
Interaction	12,84	2.93	**0.002**	7.04	**< 0.001**	1.093	0.377
		Carotenoids mg/l					
Salinity	6,84	10.3	**< 0.001**	4.79	**< 0.001**	3.41	**0.046**
Time	2,84	13.39	**< 0.001**	2.6	0.081	2.73	0.071
Interaction	12,84	2.64	**0.005**	3.74	**< 0.001**	1.72	0.077

Figure 3-5   Effect of salinity over time on leaf Chl *a* content in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

Note scale change in *J. kraussii*.

Means (± SE) (N = 5).

* indicates initial significant change from control at each time period.
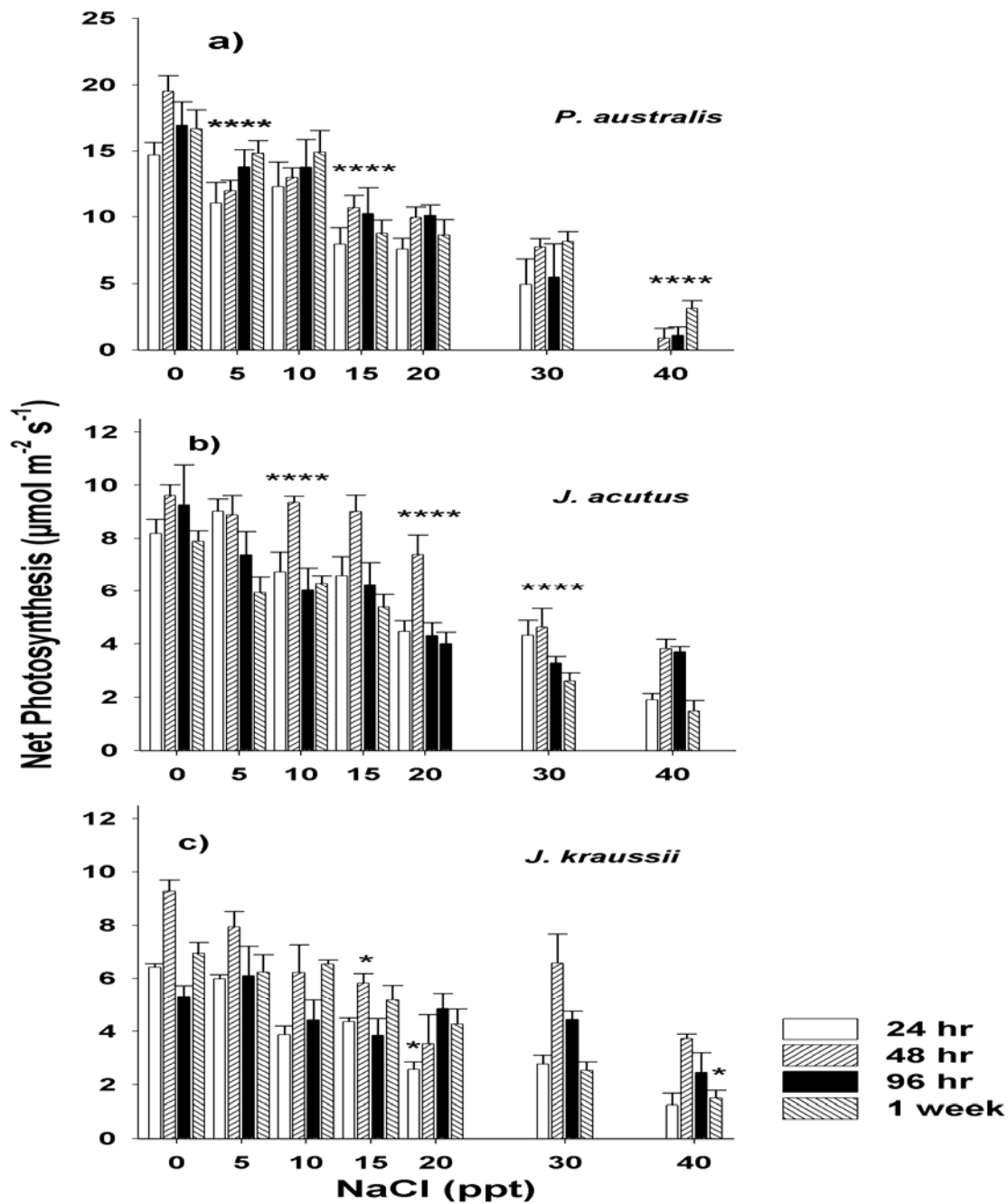
Significant changes between individual treatments not shown.
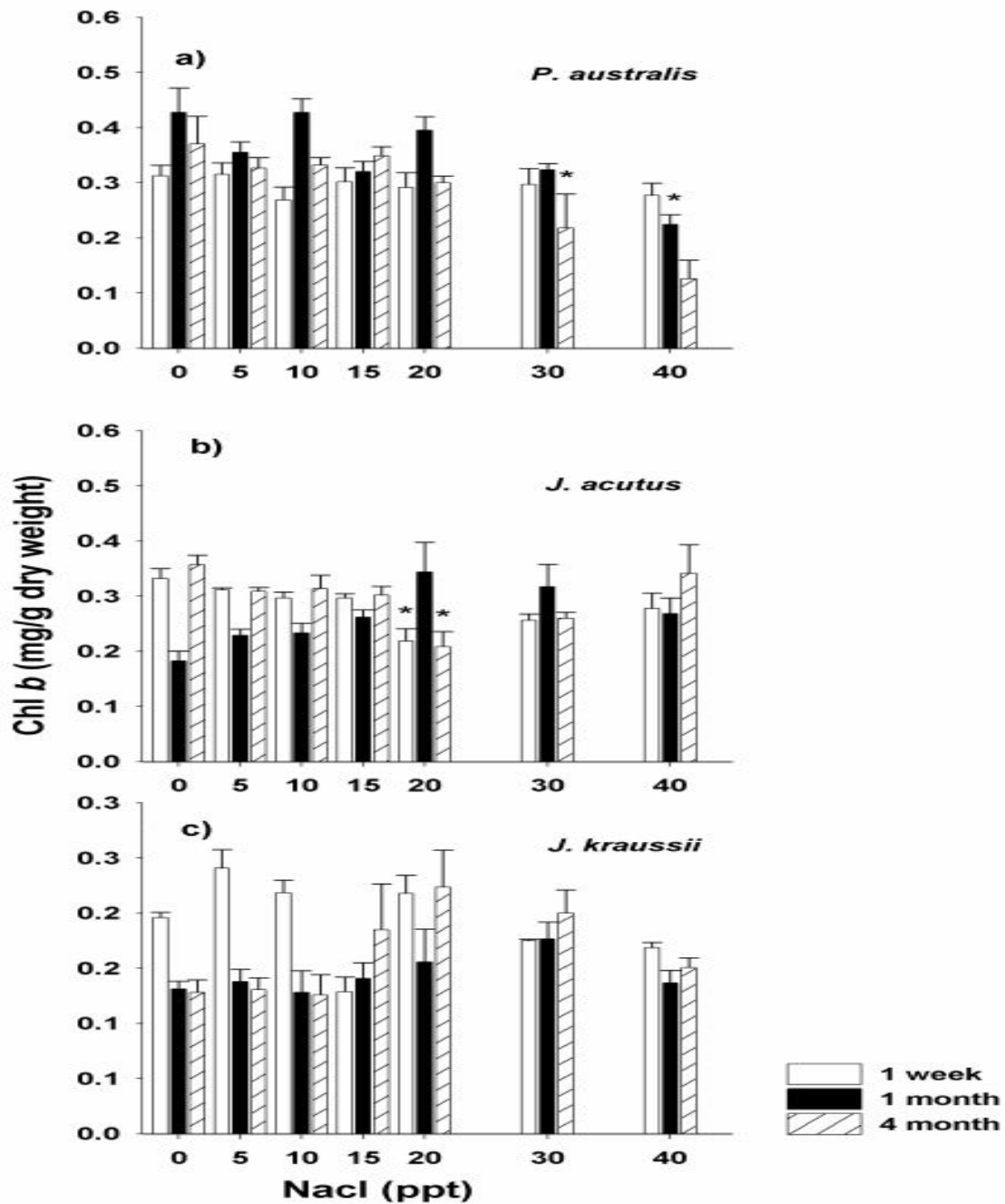
Figure 3-6 Effect of salinity over time on leaf Chl *b* content in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

Note scale change in *J. kraussii*.

Means (± SE) (N= 5). * indicates initial significant change from control at each time period.

Significant changes between individual treatments not shown.
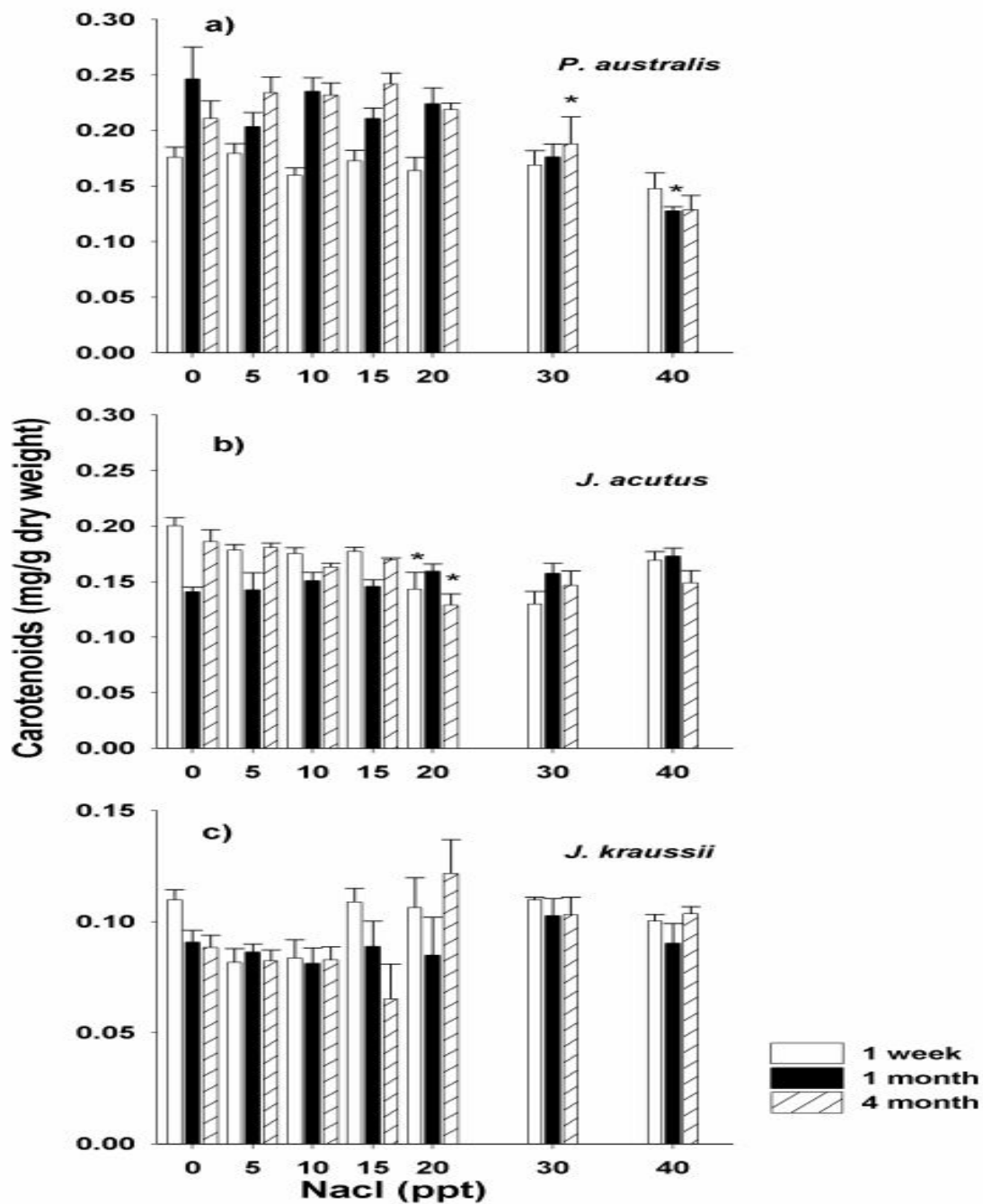
Figure 3-7 Effect of salinity over time on carotenoids content in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

Note scale change in *J. kraussii*.

Means (± SE) (N = 5).

* indicates initial significant change from control at each time period.

Significant changes between individual treatments not shown.

3.4.4 Biomass evaluation

For *P. australis,* exposure duration facilitated the negative effect of salinity at lower treatment concentrations ($F_{12,84} = 26.1$, $p < 0.001$). Increasing salinity caused no difference in total biomass of *P. australis* after one week; by one month exposure a decrease occurred at 40 ppt. At four months, *P. australis* biomass was similar in freshwater and 5 ppt salinity, after which decreases occurred at 10, 15, 20 and 30 ppt (Table 3-5; Figure 3-8a). *Juncus acutus* was able to substantially increase biomass in all treatments after 4 months (Figure 3-8b). However, an interaction with time showed salinity beyond one month caused less biomass increase at 30 ppt salinity than lower treatments (Table 3-5) ($F_{12,84} = 2.85$, $p < 0.05$). Biomass of *J. kraussii* was also affected by salinity and time of exposure ($F_{12,84} = 8.16$, $p <0.001$). Biomass increased in all salinity treatments after 4 months exposure, relative to prior exposure durations. Salinity did not affect total biomass until four months, at which time biomass declined at 10 ppt (Table 3-5; Figure 3-8c).

All species were able to increase biomass ≤20 ppt salinity over time. *P. australis* was affected by salinity at an earlier time period than either *Juncus* species and by four months the rate of biomass decline was sharper over the salinity gradient for *P. australis*. Over the salinity gradient, biomass gain was evidenced for both *Juncus* species over time. Although at 4 months, the increase observed was significantly reduced at 10 ppt for *J. kraussii* and 30 ppt salinity for *J. acutus*. Salinity of 15.3 ppt caused an EC_{50} value in *P. australis* biomass, but a 50% reduction was not detected in either *Juncus* species during the study period.

Table 3-6   Summary of ANOVA results into the effects of salinity and time on biomass in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.

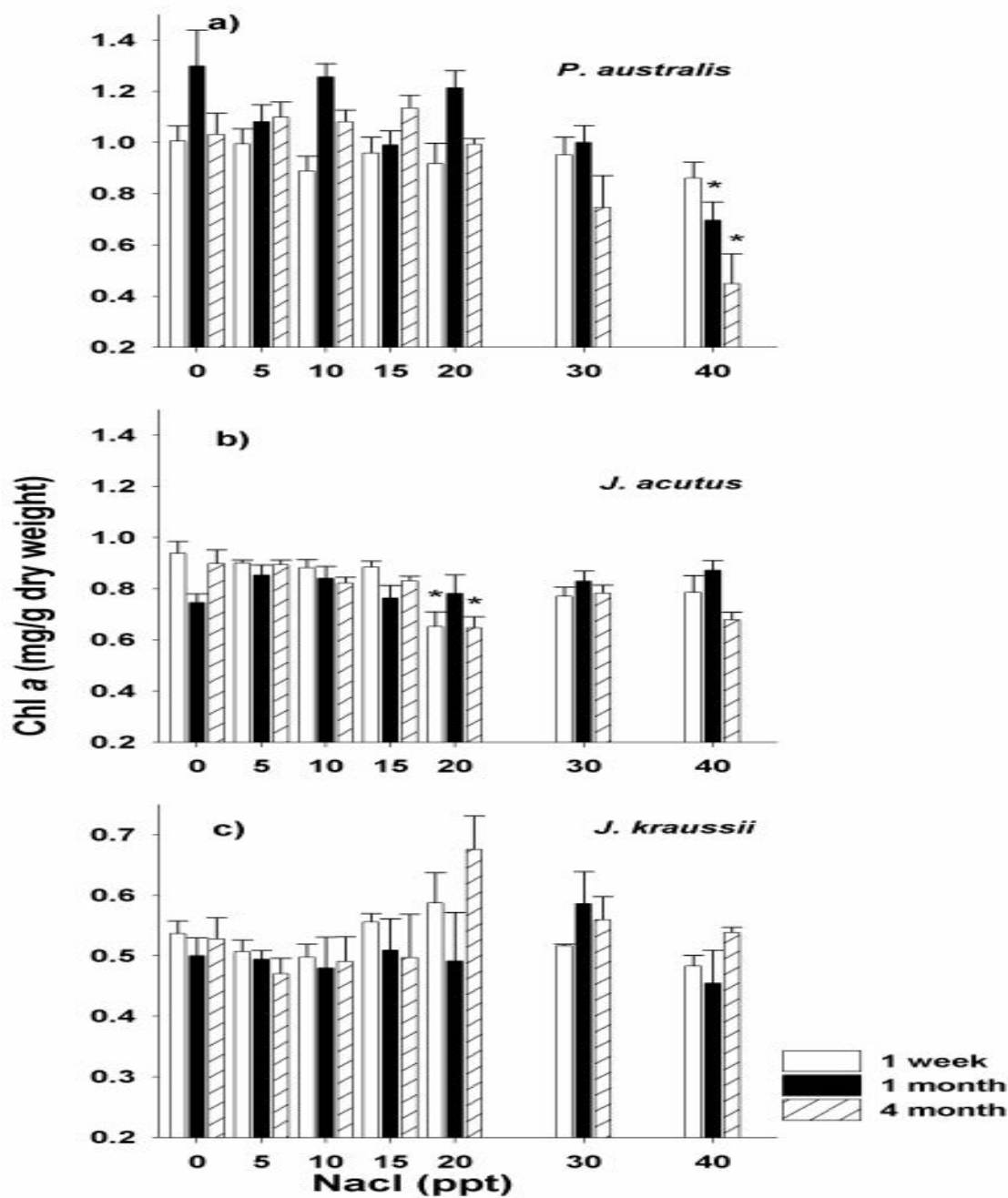|		*P. australis*		*J. acutus*		*J. kraussii*	
	df	F	p	F	P	F	p
				Total Biomass			
Time	2,84	77.7	**< 0.001**	693.52	**< 0.001**	2951.29	**< 0.001**
Salinity	6,84	36.5	**< 0.001**	3.62	**0.003**	9.52	**< 0.001**
Interaction	12,84	26.1	**< 0.001**	2.85	**0.002**	8.16	**< 0.001**
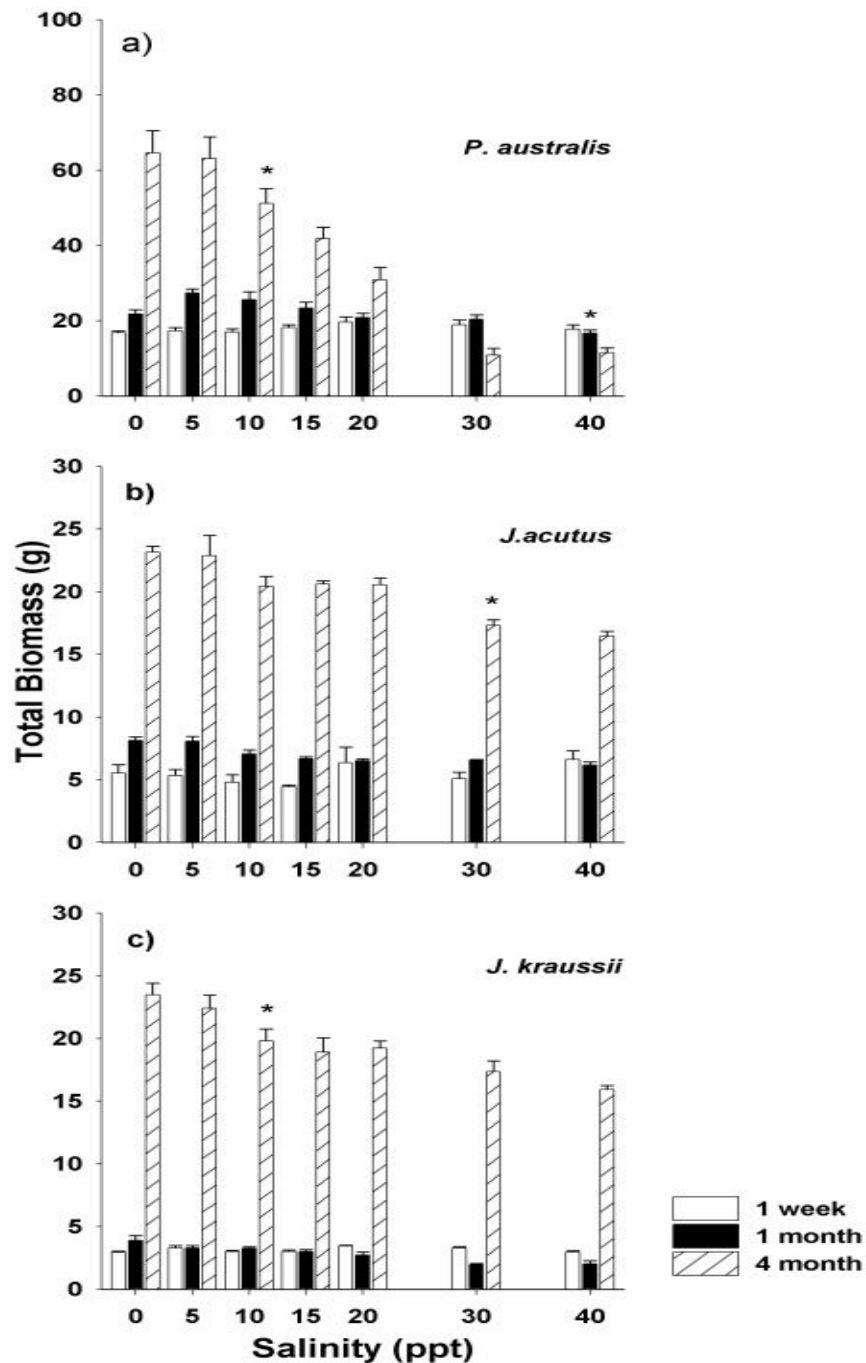
Figure 3-8 **Effect of salinity over time on total biomass in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.**

Note scale change between *Phragmites* and *Juncus*.

Means (± SE) (N = 5). * indicates initial significant change from control at each time period.

**Significant changes between individual treatments not shown.**

3.4.5 Morphology

For *P. australis,* the manner in which density increased (% change from t_0) was dependant on duration of exposure ($F_{12.84}$ = 5.64, *p* < 0.001). Salinity did not affect density at one week. However, the ability to increase stem numbers fell at 40 ppt at one month exposure and 30 ppt at four months (Table 3-6; Figure 3-9a). By four months many shoots had died in the two highest treatments, some plants recording a complete loss of above-ground vegetation (negative change). For *J. acutus*, density increase was not affected across the salinity gradient until four months exposure, when a fall in the ability to increase density occurred at 40 ppt salinity ($F_{12.84}$ = 2.95, p < 0.001) (Table 3-6; Figure 3-9b). In *J. kraussii,* no shoot augmentation occurred until four months exposure (Table 3-6; Figure 3-9c). An interaction with time caused a large increase in shoot number in freshwater after four months, which was not apparent at 10 ppt salinity ($F_{12.84}$ = 5.37, *p* < 0.001).

At one month salinity affected the ability to increase density in *P. australis* but not *Juncus* species. By four months, a reduced capacity to increase density was observed in all species. During the trial period, at salinity treatments ≤ 20 ppt, *P. australis* was capable of increasing shoot numbers four times more than either *Juncus* species. After four months, salinity concentrations affected density of *J. kraussii* (10 ppt) before either *J. acutus* (40 ppt) or *P. australis* (30 ppt). Recorded EC_{50} values of 18.9; 21 ppt and 9.2 ppt salinity were detected in *P. australis J. acutus* and *J. kraussii* respectively.

For *P. australi*s, height increased quickly at low salinity, relative increases in shoot height being affected at 40 ppt after one month exposure, and 30 ppt after four months exposure ($F_{12.84}$ = 7.97, < 0.001) (Table 3-6; Figure 3-10a). In *J. acutus*, the capacity to increase height was not apparent until four months exposure (Table 3-6; Figure 3-10b). At four months, 5 ppt salinity and greater caused a reduction in height change ($F_{12.84}$ = 5.45, *p* < 0.001). For *J. kraussii*, height only increased over time ≤ 20 ppt salinity. Within exposure intervals, a decrease in percentage height change was detected in *J. kraussii* at 10 ppt salinity after one month ($F_{12.84}$ = 4.59, *p* <0.001) and a significant reduction in height occurred at 20 ppt salinity after four months (Figure 3-10c).

The ability of all species to increase height was affected by salinity; salinity ≤20 ppt salinity causing no increase in height to take place beyond one week exposure. Height augmentation in *P. australis* and *J. kraussii* was both initiated and affected by salinity after one month exposure. Whereas similar effects occurred in *J. acutus* after four months exposure, due to *J. acutus* being slower to initiate shoot growth. By four months, height increase in *P. australis* fell rapidly in higher treatments. Height increase in *J. acutus* dropped sharply at 5 ppt salinity and again at 30 ppt. The effect on *J. kraussii* height was more gradual, being significant at 20 ppt salinity, higher salinity producing no additional effect. After four months exposure *P. australis* recorded an EC_{50} value of 21.0 ppt, *J. acutus* 6.7ppt and 14.1 ppt salinity for *J. kraussii*.

Table 3-7 **Summary of ANOVA results into the effects of salinity and time on height and density in** *Phragmites australis*, *Juncus acutus* **and** *Juncus kraussii*.

		P. australis		*J. acutus*		*J. kraussii*	
	df	*F*	*p*	*F*	*p*	*F*	*p*
Density							
Time	2,84	119.13	**< 0.001**	39.24	**< 0.001**	14.83	**< 0.001**
Salinity	6,84	16.24	**< 0.001**	1.64	0.147	4.27	**< 0.001**
Interaction	12,84	5.64	**< 0.001**	2.95	**0.002**	5.37	**< 0.001**
Height							
Time	2,84	81.26	**< 0.001**	28.29	**< 0.001**	16.31	**< 0.001**
Salinity	6,84	11.48	**< 0.001**	5.92	**< 0.001**	17.37	**< 0.001**
Interaction	12,84	7.97	**< 0.001**	5.45	**< 0.001**	4.59	**< 0.001**
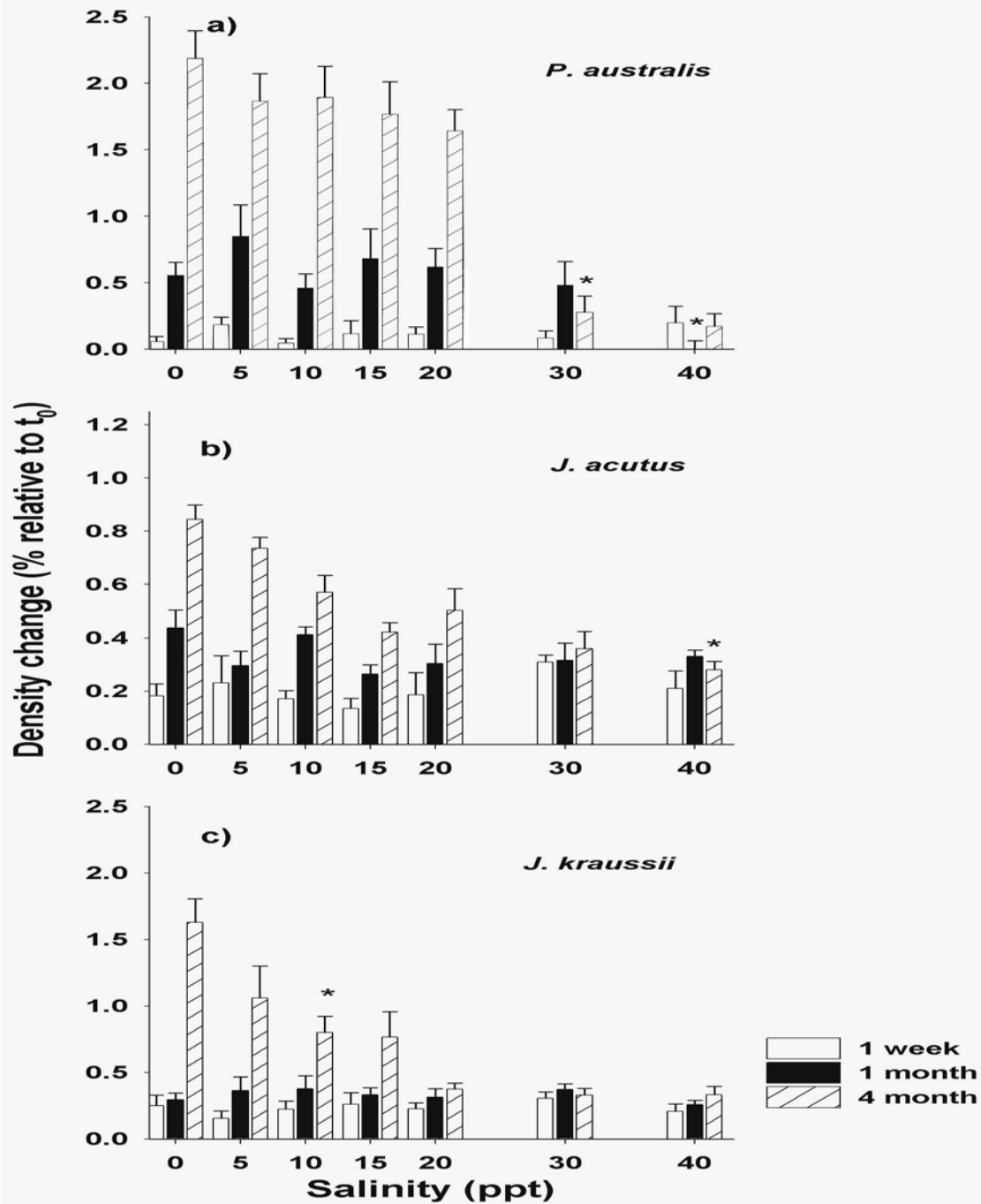
Figure 3-9   **Effect of salinity, over time, on stem density increase in *Phragmites australis*, *Juncus acutus* and *Juncus kraussii*.**

Percentage change is relative to zero time.

Means (± SE) (N = 5). * indicates initial significant change from control at each time period.

Significant changes between individual treatments not shown.
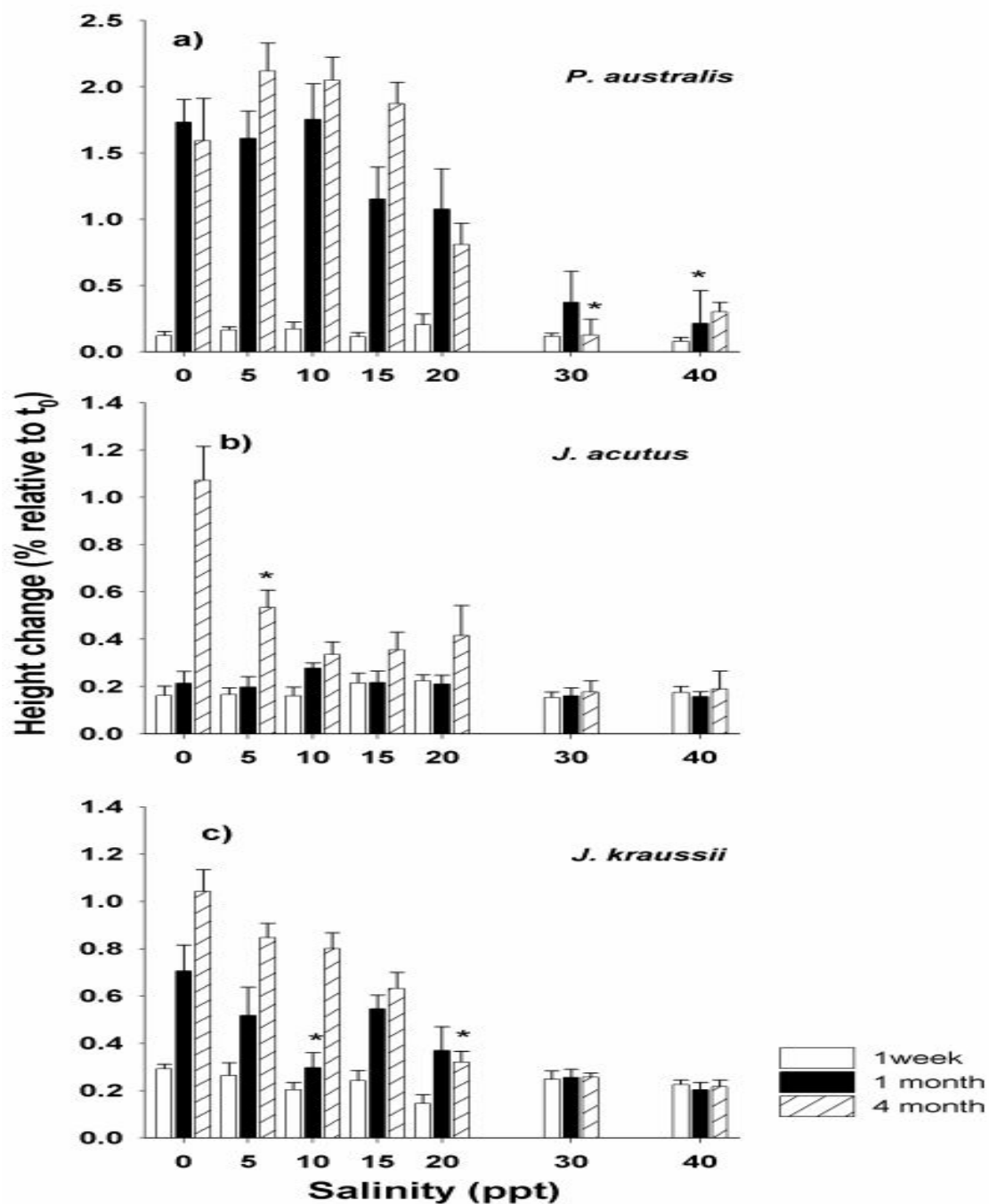
Figure 3-10 **Effect of salinity, over time, on stem height in *Phragmites australis*, *Juncus acutus* and**

***Juncus kraussii*.**

P. australis, *J. acutus* and *J. kraussii*

Percentage change is relative to zero time.

Note scale change between *Phragmites* and *Juncus*.

Means (± SE) (N = 5). * indicates initial significant change from control at each time period.

Significant changes between individual treatments not shown.

3.5   Discussion

3.5.1   Sodium accumulation and distribution

Overall, all species showed high salinity tolerance. However, differences, relating to timing of effect and degree of salinity tolerance occurred among species. It would appear these differences are due to particular Na^+ accumulation strategies of individual species. In general, glycophytes minimise Na^+ entry into the plant and maintain low translocation to shoots. Conversely, halophytes rapidly absorb Na^+ and possess high root to shoot translocation (Greenway and Munns 1980; Munns 2002; Parida and Das 2005). Excess Na^+ accumulation for both glycophytes and halophytes may result in physiological stress, leading to possible plant mortality (Kalaji and Pietkiewicz 1993; Ashraf 2004; Parida and Das 2005). As the Na^+ recorded in plant tissue was higher than that required to elicit cellular damage it is logical that, if not restricted to vacuoles, toxicity effects will occur.

It would seem the mechanism employed by *P. australis* to protect tissue from Na^+ uptake is one of exclusion. Sodium in the cytoplasm becomes progressively toxic above 6 mg/g (100 mM) (Munns 2002). Irrespective of salinity treatment, *P. australis* maintained low Na^+ concentration in root tissue up to one month. This suggests Na^+ regulation takes place at the root surface, the initial barrier being the epidermal cell plasma membrane (Apse and Blumwald 2007). Similar to Matoh et al. (1988), this study found exceedingly low concentrations of Na^+ in leaf tissue up to 20 ppt salinity treatment, again suggesting an efficient mechanism for Na^+ exclusion. However, with time, higher ($\geq$ 30 ppt) soil salinity caused Na^+ to enter the root at equivalent rates (up to 40 mg/g). This implies complete and passive uploading of Na^+ into the root cortex was taking place. At the same time increases in leaf Na^+ content were observed; inferring potential barriers to Na^+ at the root endodermis were compromised. The low translocation factor suggests some regulation was maintained, either by Na^+ being passively uploaded and then unloaded into basal portions of roots and shoots or selectively uploaded via weakly charged transporters within the membrane (Levitt 1980; Munns 2002; Apse and Blumwald 2007). These results are in line with those of Zhao et al. (1999); who found that Na^+ content was higher in roots than in leaves of brackish *P. australis* stands along the Yellow River

Delta. *Phragmites australis* does not possess salt glands that excrete excess Na^+ (Lissner and Schierup 1997). Although redistribution was reported by Matoh et al. (1988), without an increase in shoot growth there appears limited opportunity for lowering Na^+ concentration in leaf tissue. Unless compartmentalised, high accumulation of Na^+ is likely to cause Na^+ toxicity, disrupting K^+/Na^+ ratios, increasing cell permeability, interrupting metabolic function and, ultimately, causing cell death (Levitt 1980; Lissner et al. 1999b; Munns 2002).

Both *Juncus* species behaved like typical halophytes, showing high levels of Na^+ accumulation at low salinities and saturation and/or regulation of Na^+ to leaf tissue at higher salinities (Hwang and Chen 1995). It is generally thought ion accumulation is a superior mechanism for growth under saline conditions. Sequestration of Na^+ and other ions in the vacuole act as osmotic adjustment to decrease cellular water potential and organic solutes, such as proline, accumulate in the cytoplasm and organelles to balance the then low osmotic potential in the vacuole (Hootsmans and Wiegman1998; Greenway and Munns 1980). However, Levitt (1980) argues avoidance in the form of exclusion is more energy economic, as energy is not diverted for the internal control of saline effects.

In *J. acutus*, Na^+ rose linearly to almost 15mg/g (DW) in root tissue at all time periods, suggesting Na^+ was accumulated, albeit at reduced rates to the surrounding media. As the level of Na^+ remained constant in roots over time, but fell by half in shoot tissue, this indicates that regulatory mechanisms controlling the flow of Na^+ to the xylem are in force. It is unlikely active salt excretion occurs in the stem tissue; therefore, the fall in shoot Na^+ levels at four months would be a result of Na^+ redistribution into basal or actively growing shoot tissue, or dilution of Na^+ through additional shoot growth.

Sodium accumulation was apparent in *J. kraussii*. Initially Na^+ was sequestered in shoots at higher concentrations than roots, which may indicate a preference for Na^+ over other ions as an osmotic regulator (Kalaji and Pietkiewicz 1993; Hootsmans and Wiegman 1998; Munns 2002). Similar results have been reported in many monocotyledonous halophytes, whereby culms serve as a sink for ions (Munns and Termatt 1986; Tremblin

and Ferard 1994; Donovan et al. 1996; Khan et al. 2000a; Naidoo and Kift 2006). As exposure time increased, Na^+ concentrations in roots of *J. kraussii* increased, in low, but not high, salinity treatments, with concentration limited to 15 mg/g (DW) irrespective of soil salinity. Sodium accumulation in shoot tissue decreased between one and four months and accumulation arrested at 10 mg/g (DW). It appears Na^+ is accumulated in *J. kraussii* until a threshold value is reached; after which time, exclusion takes place possibly at both the root boundary and Casparian-strip. These results are similar to those reported for other halophytic species, such as *Buchlon dactyloides* Nutt. and S*porobolus cryptandrus* Torr., whereby Na^+ accumulates at high levels in low salinity but is maintained at plant specific concentrations irrespective of salinity increase in the media (Marcum 2006).

3.5.2   Acute effects of salinity

Acute reactions to salinity stress are normally attributed to an osmotic response, as cells endeavour to lower water potential and maintain turgor (Bohnert et al. 1995; Munns 2002). Generally, respiration increases with mild and decreases with severe stress (Long and Baker 1986; Parida and Das 2005). Conversely, osmotic stress may be expected to decrease photosynthesis via decreased diffusion and metabolic disturbance caused by loss of intercellular space and increased mesophyll resistance. A salinity concentration of approximately 20 ppt produced a 50 % reduction in net photosynthesis in all species in the current experiments; implying effects on carbon fixation and that growth reductions may be expected at later time intervals.

3.5.2.1 Phragmites australis

Salinity did not overtly affect respiration of *P. australis* over a one-week period, suggesting *P. australis* is not unduly affected by drought stress. These results are in line with those of Pagter et al. (2005) who attributed no effect on the dark respiration rate of *P. australis* due to water deficit, on high intrinsic water-use efficiency. The structure of young *P. australis* leaves have been found to be C_4, with older leaves anatomically characteristic of C_3 plants (Antonielli et al. 2002). Although Antonielli et al. (2002) considered it unlikely C_3-C_4 metabolism occurs in *P. australis*, the high respiration rates

observed in this study are in line with those found in the C_3-C_4 plant *Flaveria* spp (Holaday and Chollet 1984). It may be *P. australis* is a facultative CAM plant, switching from C_3 photosynthetic carbon metabolism to C_4 in response to changes in salinity, as reported in the grass *Aeluropus litoralis* (Gouan) Parl. (Long and Baker 1986).

In *P. australis,* a large fall in net photosynthesis occurred with increased salinity, which showed no change after one week exposure. *Phragmites australis* has a particularly high density of leaf stomata on upper and lower leaf surfaces (ave. 406, upper; 622 lower mm^2) (Ailstock et al. 2001). Stomatal closure, with consequential decrease in stomatal conductance due to initial osmotic effects of saline exposure, would be expected to account for some portion of the reduction in CO_2 assimilation (Long and Baker 1986), as the correlation between leaf water deficit and rate of photosynthesis has been previously explained by the complete, or partial, closure of stomata (Levitt 1980). However, the exceptionally low reading at 40 ppt salinity (relative to control) suggests a decreased ability to assimilate CO_2. Although photosynthesis and respiration are coupled as metabolic functions, the rate of photosynthesis is far higher than that of respiration. Therefore, although the quantity of O_2 provided by photosynthesis is limited under osmotic stress conditions, it would be still high enough for respiration to take place.

3.5.2.2 Juncus species

There appeared to be no difference between *Juncus* species in the respiratory response to increased salinity. During the first two days of exposure, respiration rose with increasing salinity. Beyond 96 hrs, both *Juncus* species were able to acclimatise and normal respiratory functions were maintained over the salinity gradient. Salinity caused a decrease in net photosynthesis of both *Juncus* species over the gradient. However, at 48 hr, an increase in net photosynthesis occurred, observations returning to predicted levels (reduced over salinity gradient) with increasing time of exposure. It appears optimum net photosynthesis rates of *J. acutus* were impaired $\geq$ 20 ppt salinity. Conversely, in *J. kraussii* adaptation to salinity appeared to improve as exposure time increased. Naidoo and Kift (2006) state CO_2 exchange in *J. kraussii* is unaffected at salinity $\leq$ 11ppt, but decreases at higher treatments. However measurements taken by Naidoo and Kift (2006)

were after an equilibrium period occurred and are perhaps not comparable to the initial acute effects observed in *J. kraussii* in the current experiment.

3.5.3 Chronic effects of salinity

Over a period of weeks and months, where osmotic pressures are other than optimum, plants need to adapt or face mortality. Plants with the ability to compartmentalise excess Na^+ show development similar to that seen under water stress; for example, reduced cell expansion and division, reduced leaf area and number and reduced growth. Where Na^+ is unable to be adequately compartmentalized, additional injury occurs in the form of enzyme disruption and decreased photosynthetic pigment production. Decreased area available for carbon sequestration occurs, due to death of older and reduced size of new leaves, nutrient uptake is disrupted and leaf, shoot and root death occurs (Bohnert et al. 1995; Munns 2002; Parida and Das 2005; Ashraf and Orooj 2006).

3.5.3.1 Phragmites australis

In *P. australis*, all photosynthetic pigment concentrations decreased at the highest salinity treatment level. Decreases in chlorophyll *a* and *b* production may suggest an over-regulation of chlorophyllase, the major enzyme involved in chlorophyll degradation (Sultana et al. 1999; Santos 2004). However Santos (2004) has shown chlorophyllase in sunflower plants is reduced above 50 mM salinity. Therefore, it is more likely salinity decreased chlorophyll synthesis in *P. australis*. Inhibition of 5-aminolaevulinic acid, the precursor of protochlorophyllide, due to salinity stress has previously been reported and would directly impact on chlorophyll synthesis (Breierova et al. 1997; Santos 2004).

As exposure time increased, Chl *a* was less affected by salinity than Chl *b*; Chl *a* decreasing at 30 and Chl *b* at 40 ppt salinity after four months exposure. Mechanisms of chlorophyll synthesis and degradation are complex (Garcia et al. 1997; Belkhodja et al. 1999; Husain et al. 2003; Gandul-Rojas et al. 2004; Garcý-Valenzuelaa et al. 2005). Increased salinity induces water stress, enhancing production of reactive oxygen species, which affects plants at the physiological, biochemical and molecular level (Srivastava et al. 2005). Recent research suggests Chl *b* may be oxidized independently of Chl *a*

(Eggink et al. 2001; Gandul-Rojas et al. 2004). As decreases in leaf water potential have been shown to increase chlorophyll a/b ratio (Long and Baker 1986; Sultana et al. 1999), it may be that under saturated conditions employed in this trial, metabolic pathways producing Chl *a* may be less affected by high salinity than Chl *b.*

At the whole plant level, height and density declined and mortality was observed ≥ 30 ppt salinity. However, the decline in biomass was more pronounced after four months, decreasing at 10 ppt salinity. This implies that, compared to shoot growth, root growth declined rapidly between one and four months. At this time, Na^+ concentrations in both root and shoot tissue increased exponentially in higher salinity treatments, indicating Na^+ accumulation is taking place. The implication is that significant toxic effects occurred at the root level, resulting in a reduction in ability to exclude Na^+ and preventing further growth. Normal and even additional root growth has been reported in *P. australis* under saline conditions, being attributed to the species endeavouring to locate fresh water (Adams and Bate 1999), which was unavailable under trial conditions. It would seem that similar to saltmarsh species results recorded by Turner et al. (2004) root production is decreased, producing an obvious effect on above-ground biomass.

3.5.3.2 *Juncus acutus*

With the exception of values at one month photosynthetic pigments were depressed in *J. acutus* at 20 ppt salinity, additional salinity causing no further decrease. However, over all salinity treatments pigment concentrations were highly variable and no effect between control and 40 ppt salinity was discerned. Previous studies have found no effect (Lu et al. 2003), increases (Husain et al. 2003) and decreases (Belkhodja et al. 1994; Garcia et al. 1997; Gandul-Rojas et al. 2004) in chlorophyll and carotenoid content due to salinity addition. It may be photosynthetic pigments in *J. acutus* are adversely affected by salinity but protective mechanisms, such as an increase in antioxidants, are initiated to counter effects above a particular threshold.

Increased salinity decreased total biomass of *J. acutus*; however, plants continued to grow and no mortality was observed. The loss in biomass with increased salinity

corresponded with a loss in shoot production, root biomass being maintained at 90% of control (Appendix C). Density decreased gradually while shoot height fell rapidly with the addition of even low (5 ppt) salinity, higher treatments causing little or no further effect. Many halophytes react this way; whereby fresh water initiates higher than normal growth rates, or, conversely, mild salinity increases initiate a morphological response (Waisel 1972; Zhao et al. 2003; Parida and Das 2005). As Na^+ levels in shoot tissue fell at four months it poses the question as to why culm height was affected at mild salinity treatments. Personal observations indicate that as stems shortened they became thicker and this thickening contributed to the continued growth. An increase in succulence is a common adaptation in halophytes. Cells enlarge, due to an increase in water content and corresponding salt concentration dilution (Donovan et al. 1996; Khan et al. 2001). It would appear *J. acutus* is displaying signs of salinity stress, albeit small, and the salinity treatments used in this trial may be approaching the species upper limit. It would be interesting to investigate the plants reaction to longer exposure periods.

3.5.3.3 *Juncus kraussii*

Juncus kraussii recorded a slight increase in photosynthetic pigmentations over time and salinity gradient. Research into increased chlorophyll accumulation in agricultural crops due to drought and or salinity stress has shown total chlorophyll (*a* and *b*) are often enhanced at low levels of NaCl (Garcia et al. 1997; Matsumura et al. 1998), with some halophytes requiring high Cl- concentrations to maintain optimum PS11 activity (Garcia et al. 1997; Garcia-Valenzuela et al. 2005). It is worth noting that *J. kraussii* failed Levene's test for biochemical variables. Additionally, results might be attributed to a seasonal effect, rather than being related to the experimental treatment. However, as both *Juncus* species were of similar age and at similar growth stages, similarity in distribution and seasonal variations should be apparent in both species after 4 months. Although high variability in the salinity tolerance of individual *J. kraussii* plants may occur, maintaining normal pigment levels suggests *J. kraussii* is able to preserve light capturing ability and carbon acquisition.

Morphological results recorded for *J. kraussii* were similar to those of *J. acutus*. Salinity caused a reduction in total biomass, but plants continued to increase carbon sequestration at all treatment levels. As with *J. acutus*, both density and height decreased and culms remained green and appeared healthy. Unlike *J. acutus*, however, culms of *J. kraussii* did not thicken with increased salinity. Biomass was allotted to shoot height, with culm density decreasing at before height. Overall, results from this study agree with the findings of Naidoo and Kift (2006) with respect to ion accumulation and morphological growth and the conclusion that *J. kraussii* is a highly salt tolerant species.

3.5.4 Relative sub-lethal toxic effects of salinity

P. australis has the ability to withstand 40 ppt salinity for a limited period (1-5 weeks), or moderate salinity ($\leq$ 20 ppt) for at least 4 months. Under controlled conditions, salinity concentrations maintained $\geq$ 30 ppt for a period of four months can cause irreversible metabolic damage of *P. australis*. Under the same conditions, 20ppt salinity is likely to cause a 50% decrease in photosynthesis, biomass and morphological characteristics. Conversely, although *Juncus* species were affected by salinity; effects were, in general, observed at a later stage and not lethal. It appears *Juncus* species grow best under freshwater conditions. Initial salinity response occurred at low salinity; additional salinity application, up to 40 ppt, lowering growth rate, but not resulting in 50% biomass reductions. Both *Juncus* species displayed high salinity tolerance, through accumulation and regulation of Na^+, while *P. australis* attempted to exclude Na^+. The exclusion mechanism employed by *P. australis* was disrupted with time at higher salinities. Differences between *Juncus* species include reduced photosynthetic pigmentation in *J. acutus,* but not *J. kraussii,* at brackish salinity values and biomass reduction induced in *J. kraussii* at lower salinity. Additionally, in *J. acutus* aboveground biomass was allocated to maintaining culm numbers, which became shorter and thicker, whereas *J. kraussii* retained culm height at the cost of culm numbers. Morris and Ganf (2001) state that retaining number, rather than culm height, maintains space occupation and may infer competitive advantages; although culm height may be advantageous under flooded conditions maximising culm height may also aid light capturing capabilities.

3.5.5 Management Implications

Direct comparison between this and most European/USA studies is problematic, due to controlled vs field evaluations. However, in this study salinity had little effect on *P. australis* until 20 ppt, whereas it is frequently reported that an effect occurs at 10-15 ppt (Hanganu et al. 1999; Lissner et al. 1999a; Lissner et al. 1999b; Meyerson et al. 2000; Mauchamp and Mesleard 2001). Restoration projects requiring a decline in *P. australis* populations would need to implement a continuous salinity regime for a minimum duration of four months. This is important ecologically, as it has been shown that the species is able to regain normal growth if supplied with fresh water periodically (Chambers 1997; Lissner and Schierup 1997; Burdick et al. 2001; Mauchamp and Mesleard 2001; Bart and Hartman 2003). Maintaining soil salinity conditions is not easy in a large marsh. Additionally, salt tolerance increases in many species with plant age (Zedler et al. 1990; Munns 2002). Mature *P. australis* stands can be expected to display higher tolerance levels and field data is required to validate tolerance levels and the relationships observed here under controlled conditions.

This study indicates *J. acutus* will not be overtly affected by the increased salinity anticipated. Although biomass of *J. kraussii* was affected at lower salinities than *J. acutus*, variability was high and the pattern of effect appears parallel. Similarly, the decrease in photosynthetic pigment levels in *J. acutus,* but not *J. kraussii,* may possibly be explained as a variation in data, although the fall was seen in all pigments. *Juncus acutus* places aboveground reserves into short, thick culms, whereas *J. kraussii* maintains height at the expense of culm numbers. Therefore, the major difference in salinity adaptation is how individual species manage Na^+ accumulation. *Juncus acutus* maintains tight control over accumulation and regulates to reduce Na^+ in shoot tissue after one month, whereas *J. kraussii* increases Na^+ concentrations in roots at low salinity and only regulates shoot concentrations when salinity reaches 10 ppt.

Only Na^+ was investigated in this study. Further research is required into the types of ions and organic solutes used for osmotic regulation present at various salinities, as well as increasing salinity and or treatment duration to levels that induce a visible response. In

conclusion, the combined data suggests *J. kraussii* may possess a slight advantage over *J. acutus*; in that, less energy would be required by *J. kraussii* to actively alleviate effects of high Na^+ concentrations. With regard to restoration practices, both species appear to occupy similar ecological niches. Other parameters, such as hydrology or sulphur levels may yield an ecological answer for reducing vigour in *J. acutus*, but not *J. kraussii*. It would also be interesting to study the architecture of each *Juncus* species and the way in which carbon sequestration changes over a salinity gradient with duration of exposure.

Restoration of any ecosystem is a complex process, made even more difficult where there is a potential for exotic species to invade the newly disturbed system, such as *J. acutus* encroachment due to demise of *P. australis*. Improved understanding of salinity tolerance levels for these three dominant species will help shape restoration programs, determine vegetation trajectories and contribute to ecosystem management decisions along the Australian coastline as a whole.

CHAPTER 4:

SALINITY STRESS INDICATORS IN *PHRAGMITES AUSTRALIS;* EVALUATION OF TEMPORAL INFLUENCES ON THE AFFECT RESPONSE, WITH COMPARISON TO CONTROLLED CONDITIONS

4.1 Summary

Determining outcomes of wetland rehabilitation projects is often achieved through monitoring a particular indicator, or suite, of indicators. A specific objective for the restoration of the Hunter Estuary Wetlands is to reduce the dominance of *P. australis* and increase saltmarsh area through increasing tidal flushing and thereby soil salinity. The response of *P. australis* and effectiveness of a number of stress indicators, found to be reliable under controlled conditions, were tested at four time-periods in a field situation. Mature *P. australis* stands, growing within project area (32º 50' - 57'S, 151º 36' - 47'E), were studied between June 2003 and April 2004. Sites ranged from relatively undisturbed salt marsh to created freshwater wetlands, having a soil salinity range of 0.03 - 26.8 ppt. Stress indicators tested were photosynthetic pigment concentrations, leaf Na^+ concentration, percentage foliage cover, stem height and stem density. No measured biochemical parameter was found to be a reliable indicator of salinity stress, over time and under controlled conditions. Height of *P. australis* was also not a dependable factor, as freshwater inputs alleviated effects. However, density and PFC results were consistent predictors of salinity stress. Most importantly, the relationship between soil salinity and density/PFC displayed no seasonal variation and was similar to previous data obtained from glasshouse studies. Stem density has all the trademarks of an ideal indicator (large and easy to count, available anytime, little expertise required to sample and indicative of a complex situation). The implication is that stem density may be a useful indicator in evaluating the projects' performance, enabling achievable goals to be set. Conversely, monitoring stem counts, at any point in time, could be useful as an indicator of rising or falling soil salinity concentrations and therefore hydrological changes.

4.2 Introduction

Restoration of the Hunter's wetlands is a major project of the Hunter-Central Rivers Catchment Management Authority (HCRCMA)(HCRCMA 2007) Figure 4-1). Increasing tidal flushing is expected to protect remaining and augment saltmarsh (a threatened community), enhance estuarine habitat, manage weed species, and transform existing freshwater plant communities back to saltmarsh, through increasing soil salinity.

Determining the functional outcomes of wetland restoration projects is most often achieved through monitoring a particular biological indicator, or a suite of bioindicators (Ozdemir and Sagiroglu 2000; Short et al. 2000; Ashraf and Harris 2004; HCRCMA 2007). However, monitoring the trajectory of restoration initiatives requires the selection of reliable bioindicators that provide an accurate assessment of both restoration progression and desired endpoints.

4.2.1 Assessment of indicators

The word indicator is, at times, misleading. For practical purposes, within a management context, an indicator should possess certain attributes. Foremost, an indicator ought to exhibit a direct linear relationship with the environmental variable of interest (impact) which is expected to change (Ewing et al. 1995), i.e. salinity. A robust indicator not only exhibits an association with the variable(s) of environmental change, but also provides information as to the ecological effects of the variable(s). An indicator needs to relate directly to a larger or more complex situation. For example, measuring vegetation transplant survival and biomass production, under various saltwater flooding regimes, may predict potential plant community responses in a saltmarsh undergoing tidal hydrology alterations (Konisky and Burdick 2004). Indicators also need to be robust in character. As such, temporal or spatial scales should have no effect on the relationship between the indicator and environmental variable(s). The ideal indicator is simple to count and understand, inexpensive, and able to be accurately and repeatedly recordable by persons with little experience. Finally, the ability to set targets and endpoints are desirable in an indicator; so that when targets/endpoints are met individual project

objectives may be considered achieved (Wackernagel and Rees 1996; Pastorok et al. 1997; Short et al. 2000; Kurtz et al. 2001; Walmsley et al. 2001). For example, measurements of plant biomass, density, leaf length, leaf width, canopy height, leaf area and percent cover (indicators) were taken over a four year period and used to evaluate restoration of natural primary production values of eelgrass (*Zostera* spp.) habitat (Short et al. 2000). Of these, the indicators most cost effective and representative of change were density and biomass. These factors indicated the time period required for restoration of eelgrass structure within the estuary to be three years (Short et al. 2000).

In a review of 69 Australian wetland rehabilitation projects habitat improvement was reported to be the main objective, with 52 projects implementing monitoring programs (Streever 1997). Indicators are useful to management in a variety of different applications, indicating both positive and negative effects of environmental change. Most often they are utilised as early warning systems, whereby particular traits of a species are linked to possible unwanted changes in abiotic factors. In a study of 30 wetlands in the Midwestern United States, stem height and aboveground biomass of *Typha* spp. and *Phalaris arundinacea* L. were found to indicate nutrient enrichment and eutrophication (Craft et al. 2007). Burdick et al. (2001), linked increases in biomass, canopy height and foliage cover of *P. australis* to freshwater inputs and variable groundwater salinity, concluding that the spread of *P. australis* is a useful indicator of salt marsh degradation.

The use of indicators, which measure negative impacts on existing plant communities, to determine whether restoration of degraded ecosystems is progressing along planned trajectories is rarely reported. However, Buchsbaum et al. (2006) used decreased *P. australis* and increased *Spartina alterniflora* Loisel. vigour as indicators of saltmarsh restoration, due to increased tidal flushing, in a New England (Massachusetts) marsh. Cover of *S. alterniflora,* used as an indicator of long-term establishment and health of desired plant communities, rose exponentially after an initial lag period. Although overall *P. australis* declined, the change was highly variable. Some areas recorded complete elimination, others remained unchanged and one increased by about 50 %. Buchsbaum et

al. (2006) determined the marsh was still adjusting to the hydrological changes after four years.

Determining appropriate and reliable restoration bioindicators can only be achieved by first establishing what major environmental changes will arise and then systematically validating that the bioindicator(s) of interest respond sensitively to the proposed changes (Thom et al. 2005; Laegdsgaard 2006). In restoring degraded estuarine wetland areas, through the reintroduction of tidal inundation, salinity change is perhaps the major expected environmental change (Winning 2006), as soils are mostly already waterlogged and anoxic. Negative sub-lethal and/or lethal effects on freshwater vegetation currently dominating these degraded areas are perhaps the most appropriate and rapid indicators to determine if restoration initiatives are following planned trajectories in the short-term.

4.2.2 Plant response to salinity

Environmental stress, be it anoxia, drought, nutrient levels, sulphide or salinity contribute to the species ability to grow, compete and reproduce in wetlands (Shumway and Bertness 1992; Emery et al. 2001; Pezeshki 2001; Crain et al. 2004; Konisky and Burdick 2004). Within a saltmarsh, salinity is thought to be the major stressor (Shumway and Bertness 1992; Ungar 1998; Emery et al. 2001; Pennings et al. 2005). Plant responses to salinity are well documented, indicators of salt stress having been studied at the biochemical (Thomas and Bohnert 1993; Ashraf and Harris 2004), physiological (Ewing et al. 1997; Naidoo and Kift 2006) and whole plant level (Hootsmans and Wiegman 1998; Hester et al. 2001; Mauchamp and Mesleard 2001).

Upon the reintroduction of natural or regulated, tidal flow to degraded saltmarsh, true glycophytic species would be expected to experience an immediate and complete negative effect. However, degraded saltmarsh often supports an array of salt-tolerant glycophytes, or brackish (5-15 ppt salinity) species. It is these salt-tolerant glycophytes species, such as *P. australis*, that are of particular interest to restoration managers, as they are often dominant and their responses to the new regime tend to be less certain (Short et al. 2000; Svoboda 2004). The ability to measure the physiological and morphological

state of a validated salinity bioindicator may allow the observer to gain an insight into the initial progression of negative impacts from salinity in a time and concentration dependant manner.

4.2.3 Controlled conditions vs. field responses

When developing and validating a robust indicator, experimental laboratory trials are required to demonstrate a direct cause-effect relationship between the measurable property (indicator) and system change (e.g. salinity) (Fairweather 1999) as field based correlation does not imply causation. The complex and dynamic nature of natural environments has led to criticism that controlled experiments are artificial, recreating processes in artificially simplified environments (Heugens et al. 2001). The implication is that findings of such research may not be applicable to real and complex environmental situations (Schwenk 1982; Heugens et al. 2001). Few studies have compared results of plant stress under both field and controlled scenarios. However, Ewing et al. (1997) compared indicators (leaf spectral reflectance, net CO_2 exchange rate, leaf expansion and leaf proline concentration) of sub-lethal salinity stress in *Spartina patens* (Ait.) Muhl under controlled and field conditions. Ewing et al. (1997) found the ability of indicators to detect plant responses changed during the growing season and that reflectance indicator responses were less responsive under field conditions than in a glasshouse situation.

4.2.4 Choice of sub-lethal stress indicators

Chapter 3 details the results of a four month glasshouse experiment conducted on *P. australis*. Factors studied were photosynthetic capability, Na^+ accumulation and distribution, photosynthetic pigments, biomass accumulation and morphology *(*height, and density).

Net CO_2 exchange, as an indicator of respiration activity, and photosynthetic activity possess the ability to detect early stress responses from salinity under controlled conditions (Ewing et al. 1995; Hester et al. 2001). However, the process of collecting data in the field (equipment, time of day, light and temperature intensity) was felt to be

above that required of an indicator useful to management. Indicators evaluated were photosynthetic pigments, Na^+ accumulation in leaf tissue, percentage foliage cover (PFC) and plant height and density, as these were found to respond to salinity in *P. australis* in laboratory trials and are amenable to field-based assessment.

Chlorophyll concentrations are a common indicator of general photosynthetic ability and extensively used for assessing plant responses to environmental stress (Eggink et al. 2001; Parida et al. 2002), as high salinity decreases chlorophylls (Ashraf and Harris 2004; Parida and Das 2005). After four months in glasshouse conditions, photosynthetic pigments in *P. australis* decreased at 30 ppt salinity (Chapter 3, p. 71).

Excess Na^+ can accumulate in leaf tissue inducing cytoplasmic toxicity, dehydrating cells, reducing growth and ultimately causing mortality (Greenway and Munns 1980; Khan et al. 2000a; Munns 2002). Under glasshouse conditions, *P. australis* is able to exclude Na^+ from entering root systems for over one month (Chapter 3, p.62). However, with time Na^+ accumulates in all tissue, with toxic effects and mortality occurring in treatments of 30 ppt salinity and above.

Both height and density have been found reliable indicators of stress in many species (Munns and Termatt 1986; Sanchez et al. 1998; Parida and Das 2005; Villagra and Cavagnaro 2005; Ye et al. 2005). Under glasshouse conditions, height and density of *P. australis* decreased at 30 ppt under glasshouse conditions, while biomass was affected at 10 ppt salinity (Chapter 3, p.76-78).

Although PFC has been criticized as an indicator (Cole 2002) and is not readily adapted to glasshouse conditions it may be a useful tool under field conditions, as it is easy to measure and has been shown, in certain species, to indicate response to stressors and competitive ability (Aan et al. 2006).

4.2.5 Aims

The aim of this study was to evaluate the effectiveness of a number of known stress indicators in detecting a negative response in *P. australis,* subjected to increasing soil salinity, under natural conditions. Objectives were to 1) verify if indicators exhibit linear exposure-response relationships under field situations and 2) determine if indicator responses are subject to temporal variability. Additionally, the relevance of data obtained from controlled studies compared to field situations was evaluated.

4.3   Materials and Methods

An initial five mature *P. australis* stands growing within Kooragang Wetland Rehabilitation Project area (32º50' - 52'S, 151º41' - 47'E) were selected for evaluation. Criteria for site selection were based on existing salinities, the history, topography and vegetation associations of each area. Stands needed to be large enough to accommodate independent sampling (N = 5) at four sampling times, three months apart. An additional 11 sites, within and outside the rehabilitation project area, were sampled to enable evaluation of a more complete range (gradient) of soil salinities (Figure 4-1). Sites ranged from relatively undisturbed salt marsh to created freshwater wetlands. Table 4-1 displays site characteristics, vegetation associations, soil salinity and sampling times. A subset of the dataset from the glasshouse trials analysed in Chapter 3, consisting of treatments 0 – 30 ppt salinity, at the four month exposure period (N = 5), was used to compare field and controlled results. This subset was deemed most appropriate as, at four months, control plants were considered relatively mature, height being similar to that recorded under field conditions. Additionally, removal of the highest treatment (40 ppt salinity) allowed for evaluation over a similar environmental gradient, as soil salinity in the field was always less than 30 ppt.
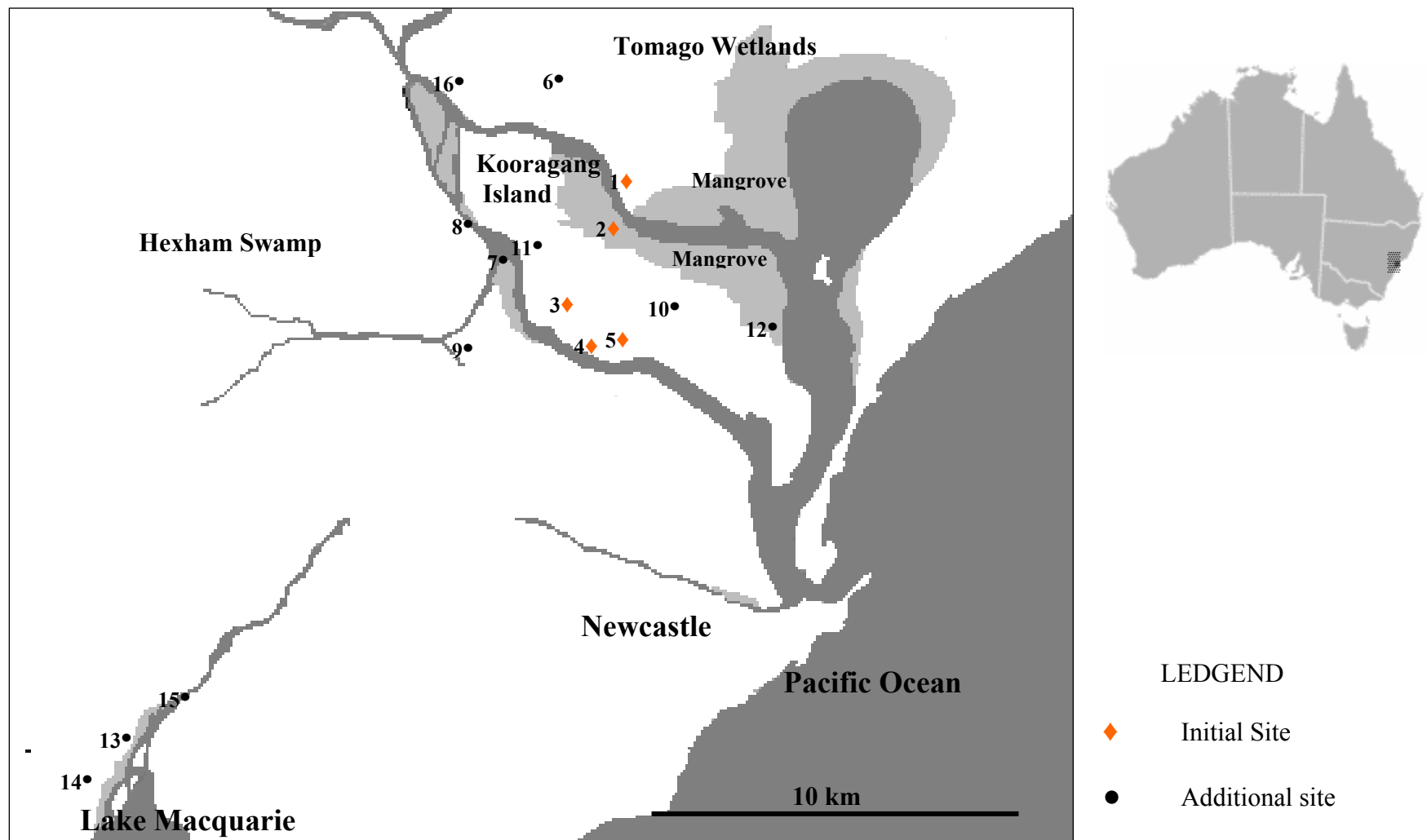
Figure 4-1 Map depicting the major wetland areas within the Lower Hunter NSW, Australia (32º50' - 52'S, 151º41' - 47'E), Initial and augmented *P. australis* sampling site locations shown. Field sampling undertaken June, 2003 - April, 2004

4.3.1 Field Sampling

At each site, and at each time, five $1m^2$ quadrats were randomly selected for evaluation. To minimise edge effects, all quadrats were a minimum of 6 m into the stand, with individual quadrats being at least 6 m apart. A template for the collection of vegetation data is contained in Appendix D. Within each quadrat Plant Foliage Cover (PFC) was estimated as a percentage for each species present (Daubenmire cover scale). Height of the tallest five stems of *P. australis* was recorded in cm. Density was determined by counting the total number of stems, taken as any green shoot above ground level, within the quadrat. Three leaves, being the third leaf from the growing apex of three non-flowering stems, were removed, placed in sealed containers and transported on ice to the laboratory for photosynthetic pigment determination and leaf Na^+ content.

4.3.2 Soil salinity analysis

Three sediment samples (7 x 5 cm) were taken within each quadrat from opposite diagonal corners and centre. Samples taken were from soil 20-30 cm deep. The majority of *P. australis* root zone is included at this depth, although it is expected some roots and rhizomes would penetrate further. Sediment cores within each quadrat were pooled, stored in air-tight plastic bags and returned to the laboratory for analysis. A standard technique throughout the life of the field work allowed comparison between soils with varying moisture levels (Burdick et al. 2001).Soil salinity was determined using a ratio of 1:5, aired dried, homogenized sediment and distilled water. The mixture was shaken overnight (SWB20 Ratek Shaking Waterbath), filtered through a 0.45 μm filter and analysed for salinity with a conductivity meter (YIS, Model 85).

4.3.3 Photosynthetic pigment analysis

Photosynthetic pigments were determined using the N,N-dimethyl-formamide (DMF) method of Inskeep and Bloom (1985) and absorption coefficient equations detailed by Wellburn (1984). For details refer to Chapter 3 (p. 59).

4.3.4 Leaf sodium concentration

Leaf Na+ concentrations were obtained by digestion analysis reported in Chapter 3 (p.60) (Krishnamurty et al. 1976; Mudroch et al. 1997).

4.3.5 Statistical analysis

Statistical analysis utilised Statistica 7 software package (Stat-Soft 2005). Due to the standard deviation of *y* increasing as salinity increased, hetroscedasticity was apparent in PFC and density datasets; therefore data were transformed to log (*y* +1) prior to analysis (Zar 1999). For other parameters, statistical analysis was employed on raw data. Analysis consisted of a three-tiered approach. Firstly, bivariate linear regression analysis between soil salinity and measured plant parameters was undertaken on individual datasets of each sampling date and the subset of data obtained from the glasshouse study. Secondly, in order to assess temporal relationship maintenance, where significant linear relationships existed at two or more time-periods, a students t tests (two time-periods) (Fowler et al. 1998) or ANCOVA analysis (Homogeneity of regression model, for more than two time-periods) (Zar 1999) determined if there was a significant difference between the slopes of regression lines. Where ANCOVA indicated significant differences, subsequent analysis (students t test) determined which sampling periods were the same and which different (Underwood 1997).

Where the slope was not significantly different between two or more time-periods, datasets were merged to construct a common regression line. Finally, the common regression slope obtained from the field data was compared to the regression slope acquired from the four-month glasshouse study subset of data, via a students t test to determine if the nature of relationship was similar.

Table 4-1 Site descriptions, *Phragmites australis* plant associations, GPS coordinates, salinity and sampling times. Sampling undertaken June, 2003 - April, 2004

Bolboschoenus caldwellii **(B.c);** *Hydrocotyle bonariensis* **(H.b);** *Juncus acutus* **(J.a)** *J. kraussii* **(J.k);** *J. usitatus* **(J.u);** *Paspalum distichum* **(P.d);** *Sarcocornia quinqueflora* **Sq);** *Sporobolus virginicus* **(S.v);** *Typha orientalis* **(T.o). Soil salinity, based on mean site observations (n=5)**

| Site No. | Site Location | Site Description | GPS 32S-151E | Land Tenure | Species Associations | Salinity (ppt) | | Oct. | Jan. | Apr. | June |
						All Seasons X (+/- SE)	Range Min-Max				
1	Tomago east	Between mangroves and levee bank	50 19 06S 44 56 01E	Protected	S.p.	16.21 (0.86)	21.04 – 12.4	✓	✓	✓	✓
2	Ash Island, Creek 6	Salt marsh reserve	51 18 70 44 08 12	Protected	J.k.; S.p.	16.31 (0.58)	18.76 – 13.72	✓	✓	✓	✓
3	K. Island, BHP pond	Edge of large ephemeral deep freshwater pond	52 16 00 43 56 00	Abandoned Industrial	T.o.; B.c.	6.10 (0.55)	11.1 – 3.32	✓	✓	✓	✓
4	K. Island, Tafe pond	Shallow pond. Reclaimed area Highly variable water table,	52 45 16 44 57 48	Crown land	J.a.; B.c.	6.9 (0.44)	10.28 – 3.52	✓	✓	✓	✓
5	K. Island, south-arm	Impounded marsh, originally salt marsh	52 53 80 45 06 50	Crown land	J.a.; J.k.; H.b.	11.14 (0.33)	12.22 – 10.60	✓	✓	✓	✓
6	Tomago West	Freshwater marsh/pasture	50 34 42 43 26 05	Agricultural	J.u.; P.d.	1.12 (0.19)	1.14 – 1.1	✓			✓
7	Hexham, floodgates	Impounded marsh, originally salt marsh	52 23 60 42 34 62	Crown land	Pure stand	2.06 (.06)	2.22 – 1.90	✓			✓
8	Pacific Hwy, Hexham	Freshwater inputs from rail line	51 18 75 41 49 57	Crown land	H.b.; J.a.	1.66 (0.28)	2.18 – 1.14	✓			✓
9	Newcastle Wetlands	Created freshwater wetland	53 07 89 43 38 99	Urban	T.o.	1.86 (0.16)	2.3 – 1.42	✓			✓
10	K. Island, Cormorant Rd	Impounded marsh, reclaimed area.	52 50 55 45 18 10	Crown Land	B.c.;.T.o.	1.1	1.1 – 1.1				✓
11	Ash Island, Creek 3	Degraded area. Fresh pasture	51 33 90 43 06 80	Protected	P.c.	1.3	11.3 – 1.13	✓			
12	K. Island, Weighbridge	Between mangroves and industrial land	52 29 80 46 15 20	Industrial	H.b.; P.d.	2.2	2.2 – 2.2			✓	
13	Speers Pt., Five Island Bridge	Close to banks of salt lake	57 42 46 36 46 71	Crown land	J.k.; S.v;	12.82	12.82 – 12.82			✓	
14	Teralba, Five Islands	Caravan Park	57 53 45 36 29 25	Caravan Park	S.v.	13.1	13.1 – 13.1			✓	
15	Cockle Creek	Close to disused Sulphide Works	56 50 90 37 20 85	Abandoned site	J.k.; P.d.	16.5 (0.71)	16.5 – 16.5			✓	
16	Tomago West levee	Degraded salt marsh	51 05 78 42 15 30	Future Industrial	S.v.; S.q.	8.94	8.94 – 8.94			✓	

4.4 Results

Soil salinity range was 0.03 - 26.8 ppt, being highest at site 2 during January and lowest at sites 3 and 9 during April and October (Table 4-1; Appendix E). October sampling occurred after heavy rain. Previous studies have indicated a 20 cm depth is adequate for determining ground water salinity levels (Lissner and Schierup 1997; Hughes 1998) and readings, taken from same stands, were similar to June but lower than those from the January and April period. Vegetation associations were compatible with the literature on the range of soil salinity levels tested (Clarke and Hannon 1970; MacDonald 2001; Svoboda 2004). In freshwater sites *P. australis* was associated with *B. caldwellii* and *T. orientalis* in locations with standing water, being replaced with *Hydrocotyle bonariensis* Lam., *Juncus usitatus* L. and *P. distichum* where drainage increased. *S. quinqueflora, S. virginicus* and *J. kraussii* were the main species associations recorded in mid and high salinity sites. *J. acutus* appeared to dominate sites with mid level salinity values and/or a history of disturbance.

4.4.1 Response of indicators

4.4.1.1 Photosynthetic pigments

A positive linear relationship between soil salinity and Chl *a* was apparent at the January sampling period ($F_{1,\ 48}$ = 4.27, *p* <0.05), although variability was high (R^2 = 0.081) (Figure 4-2). For Chl *b*, a positive linear association occurred in October ($F_{1,\ 48}$ = 9.9, *p* <0.05, R^2 = 0.17) (Figure 4-2). No relationship was apparent between soil salinity and either chlorophyll at any other time-period. During the October sampling period a negative relationship was detected between soil salinity and Chl *a/b* ratio ($F_{1,\ 48}$ = 6.69, *p* <0.05, R^2 = 0.12). Conversely, the ratio of Chl *a* to Chl *b* increased with increasing soil salinity during June and January ($F_{1,\ 48}$ = 4.65, *p* <0.05, R^2 = 0.09, June; = 6.28, *p* <0.05, R^2 = 0.12, January) (Figure 4-3). Salinity affected carotenoid concentrations during the April period ($F_{1,\ 23}$ = 5.5, *p* <0.05, R^2 = 0.19), carotenoids increasing with soil salinity, but not at other times (Figure 4-3).

4.4.1.2 Leaf sodium concentration

Leaf Na^+ content increased with increasing salinity treatments during June and April sampling periods ($F_{1, 48}$ = 4.61, p <0.05, R^2 = 0.087 June; $F_{1, 23}$ = 8.42, p <0.001, R^2 = 0.64, April), but the relationship was not significant in October or January (Figure 4-4).

4.4.1.3 Percentage Foliage Cover and Density

Increasing soil salinity caused a negative effect upon PFC at all sampling periods ($F_{1, 48}$ = 5.21, p <0.05, R^2 = 0.08, June; = 8.42, p <0.05, R^2 = 0.149, October; = 17.2, p <0.001, R^2 = 0.24, January; $F_{1, 23}$ = 4.7, p <0.05, R^2 = 0.205, April (Figure 4-4). Increasing soil salinity also produced a negative relationship in the density of *P. australis* at all time periods ($F_{1, 48}$ = 24.96, p <0.001, R^2 = 0.351, June; = 13.38, p <0.001, R^2 = 0.434, October; = 13.77, p = <0.001, R^2 = 0.423, January; $F_{1, 23}$ = 4.518, p <0.05, R^2 = 0.702, April (Figure 4-5).

4.4.1.4 Height

No relationship between soil salinity and height was recorded at the June or January sampling period. A negative relationship was detected at October and April ($F_{1, 48}$ = 31.85, p <0.001, R^2 = 0.399, October; $F_{1.23}$ = 9.52, p<0.05, R^2 = 0.293, April) (Figure 4-6).
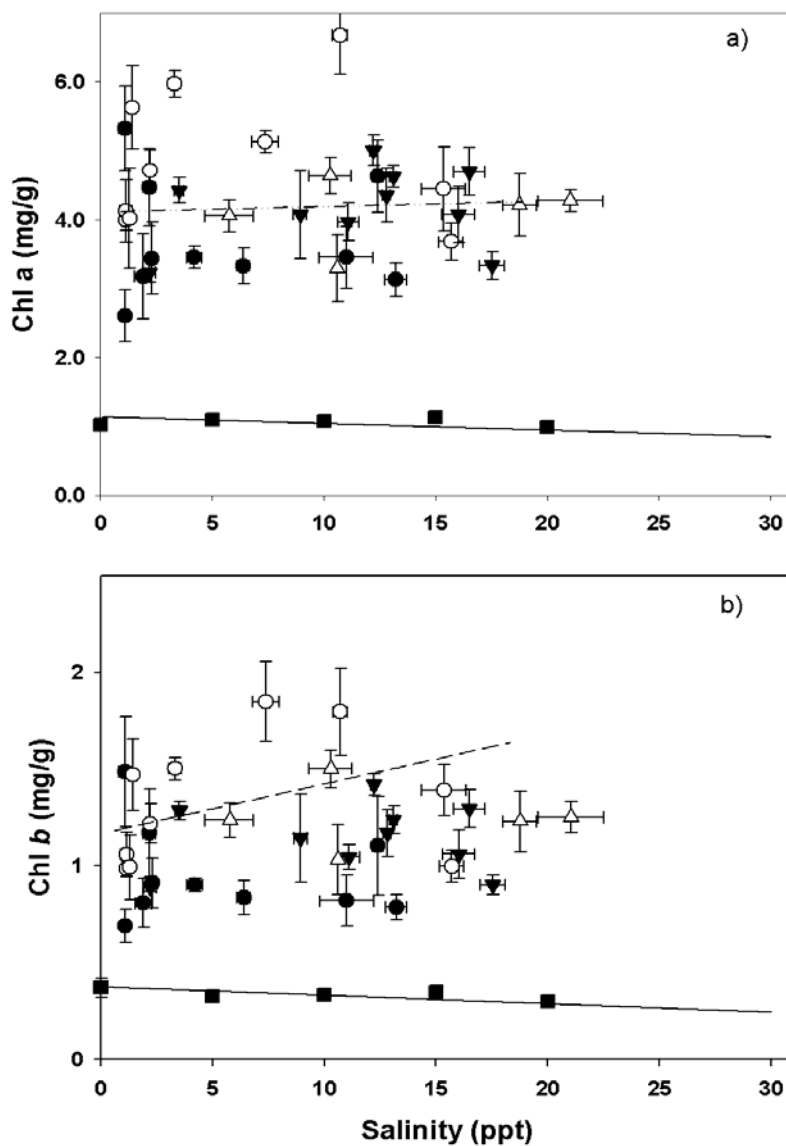
Figure 4-2 Changes in photosynthetic pigmentation mg/g, dry weight leaf tissue of *Phragmites australis* under different soil salinity concentrations. Chlorophyll *a* (a); Chlorophyll *b* (b)

June ● − − − − − ;   October ○ - - - - - - - ;   January ▼ − ·· − ·· − ;   April △ ·······························;

Glasshouse ■ ——————— trial at four months exposure

Curve equation: y = ax + b.

Chlorophyll *a*; June and October non-significant; January, a = 0.567^{-3}, b = 3.67, R^2 = 0.081; April non significant; Glasshouse trial, a = -0.951^{-3}, b = 1.142, R^2 = 0.156.

Chlorophyll *b*; June, non-significant; October, a = 2.571^{-2}, b =1.167, R^2 = 0.173. January and April non-significant;   Glasshouse,   a   = -0.437^{-3},   b   =   0.376, R^2 =   0.260.
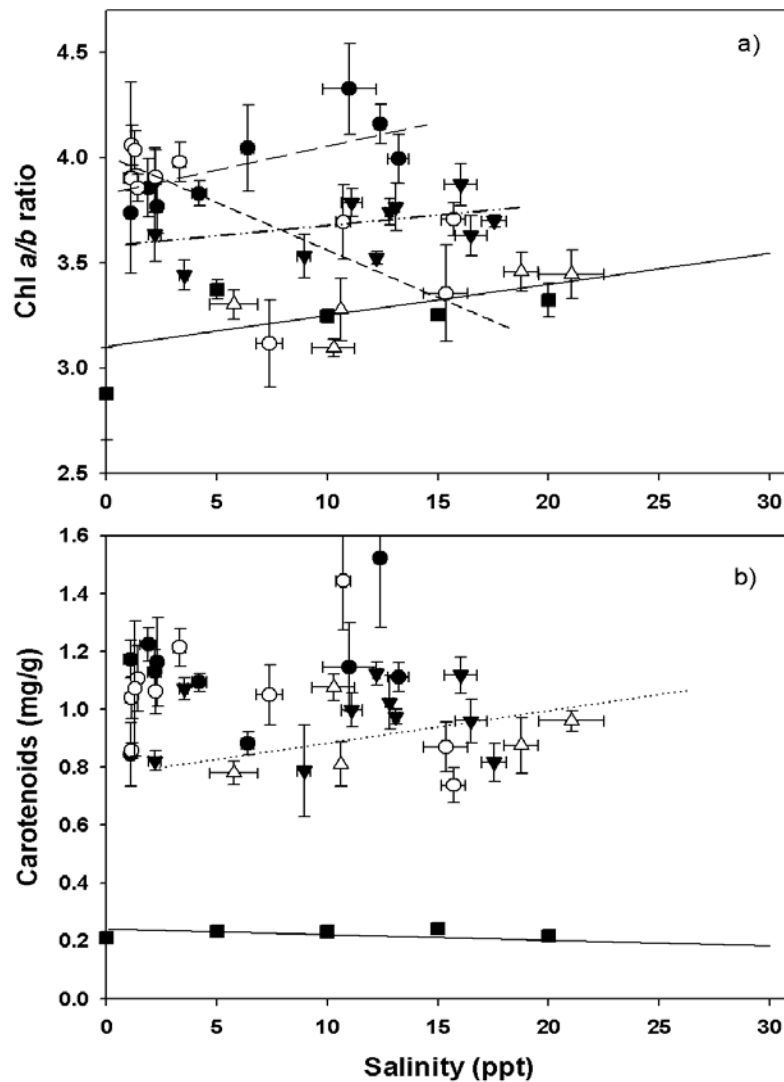
Figure 4-3 Photosynthetic pigmentation of *Phragmites australis* under different soil salinity concentrations. Leaf Chl *a/b* ratio (a), Carotenoids mg/g, dry weight leaf tissue (b)

June ● − − − − − ;   October ○ - - - - - - ;   January ▼ − ·· − ·· − ;   April △ ···························;

Glasshouse ■ ———— trial at four months exposure   Curve equation: $y = ax + b$.

Curve equation: $y = ax + b$

Chlorophyll *a/b* ratio; June, a = 2.89^{-2}, b = 3.77, R^2 = 0.089; October, a = -4.504^{-2}, b = 4.011, R^2 = 0.121; January, a = 1.901^{-2}, b = 3.290, R^2 = 0.116; April, non-significant; Glasshouse trial, a = 1.463^{-2}, b = 3.102, R^2 = 0.268.

Carotenoids; June, October and January non-significant; April, a = 7.64^{-2}, b = 0.796, R^2 = 0.19; Glasshouse trial, a = -1.93^{-2}, b = 0.241, R^2 = 0.151.
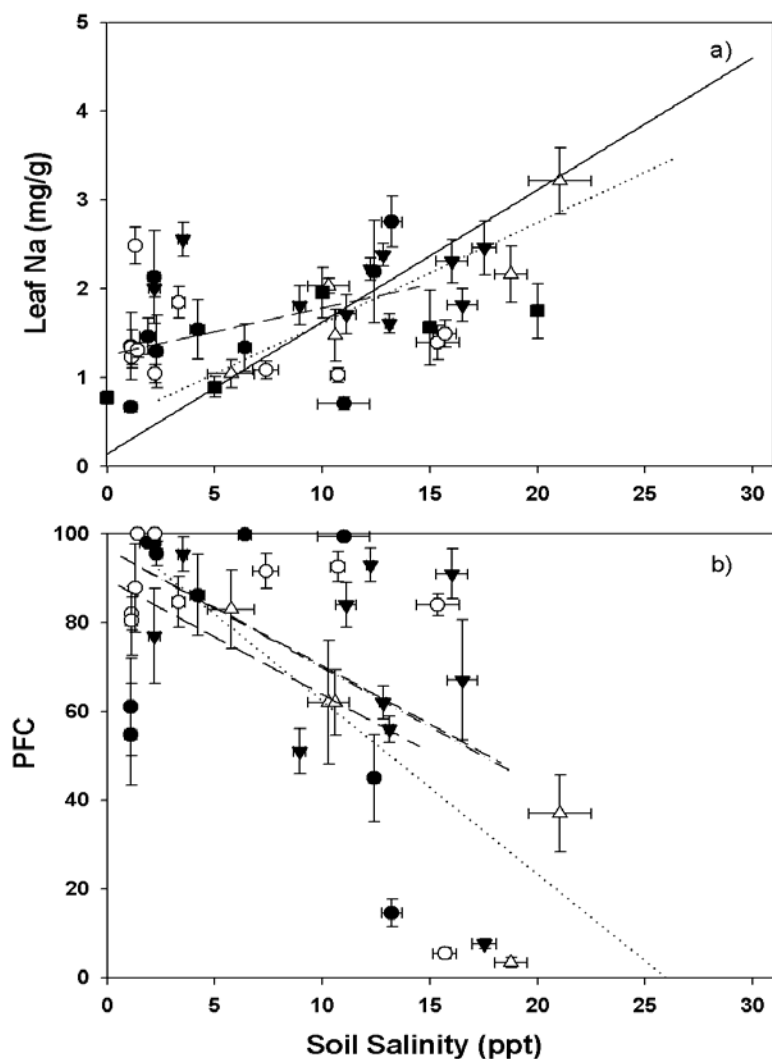
Figure 4-4 Changes in *Phragmites australis* under different soil salinity concentrations. Leaf Na$^+$ content (a), Percentage Foliage Cover (b)

June ● − − − − − **; October ○** - - - - - - **; January ▼** − · · − · · − **; April △** ·················;

Glasshouse ■ ⎯⎯⎯⎯ **trial at four months exposure**

Curve equation: y = b - ax

Leaf Na$^+$ content; June, a = 0.305 b = 1.22, R^2 = 0.072; October and January not significant; April, a = -1.02^{-2}, b = 1.106, R^2 = 0.641; Glasshouse trial, a = 5.75^{-2}, b = 0.923, R^2 = 0.296.

Percentage Foliage Cover; June, a = -9.17, b = 105.6, R^2 = 0.079; October, a = -1.38^{-2}, b = 91.7, R^2 = 0.149; January, a = -1.175, b = 76.4, R^2 = 0.24; April, a = -3.05, b = 92.5, R^2 = 0.205.

4.4.2 Indicator response to temporal variability

Individual photosynthetic pigment concentrations were affected by soil salinity at one time-period only, time-periods being linked to a particular pigment (Figures 4-3 - 4). Due to the obvious difference in Chl a/b ratio between the October sampling period and other time-periods; October data was not tested for similarity of regression slope. June and January sampling data displayed a difference in the slope of relationship (T_{96} = 3.356, p<0.001) and, therefore, data could not be combined. Likewise, for leaf Na^+ concentration (Figure 4-4) seasonal data could not be combined as the nature of the relationship between soil salinity and leaf Na^+ was significantly different between sampling periods (June and April) (T_{71} = 8.76, p<0.001).

Percentage Foliage Cover data displayed similar regression slopes at all time-periods (Figure 4-4). Similarly, the manner in which salinity affected density did not differ between sampling periods (Table 4-2; Figure 4-5). Comparable regression slopes were also observed for soil salinity and height between the October and April sampling times (T_{71} = 2.57, p > 0.05) (Figure 4-5).

Table 4-2 Summary of ANCOVA (Homogeneity of slope) results for percentage foliage cover and density of *Phragmites australis*: Effects of temporal variation on the slope of regression over a salinity gradient. Slopes with significant relationships were tested (N = 5). Sampling times, June, October, January and April 2003-4.

		PCF		Density	
	df	*F*	*P*	*F*	*P*
Intercept	1	518.42	**<0.001**	350.6	**<0.001**
Time	3	0.236	0.734	0.95	0.416
Soil NaCl	1	64.07	**<0.001**	42.18	**<0.001**
Interaction	3	0.632	0.595	0.15	0.925
Error	167				
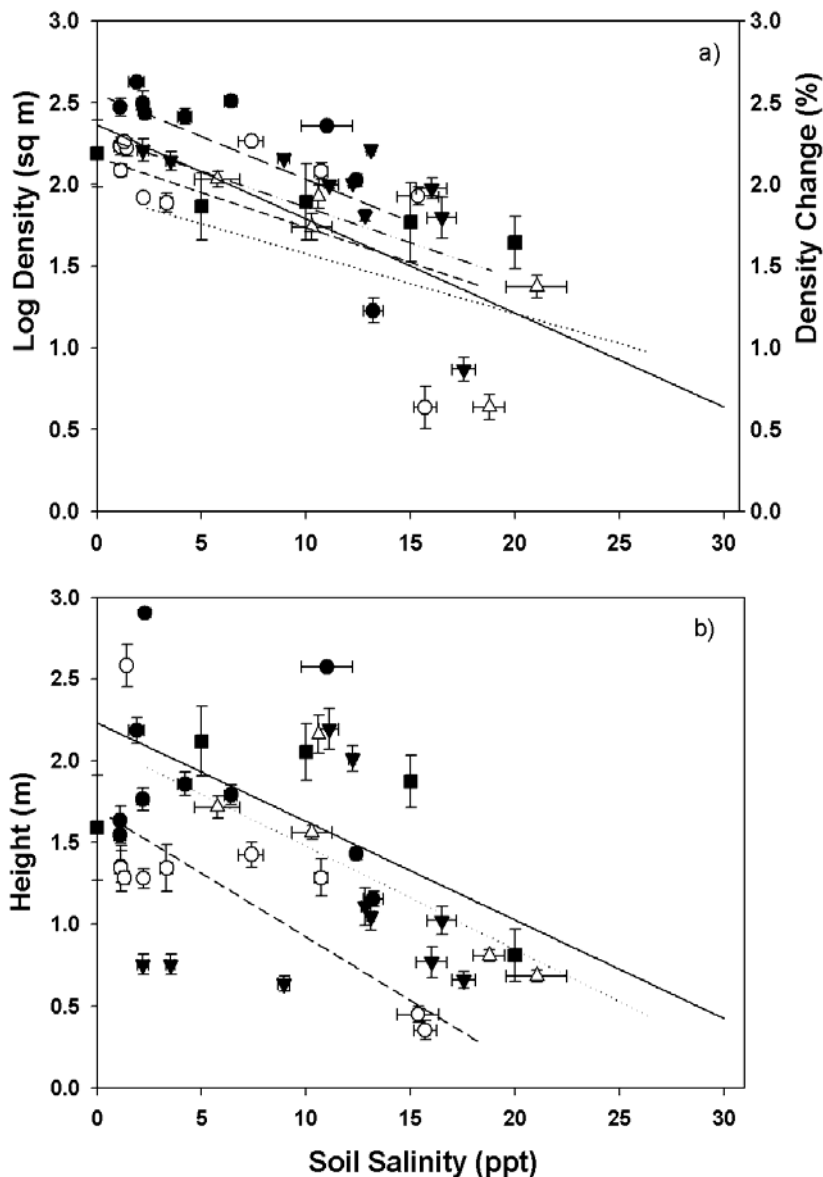
Figure 4-5 Changes in *Phragmites australis* under different soil salinity concentrations. Height (a), Density (b)

June ● – – – – – ;   October ○ - - - - - - - ;   January ▼ – ·· – ·· – ;   April △ ·························· ;

Glasshouse ■ ———— trial at four months exposure   Curve equation: y = ax + b.

Curve equation: y = ax + b.

Density; June, a = -5.213^{-2}, b = 2.552, R = 0.351; October, a = -4.29^{-2}, b = 2.164, R^2 = 0.434; January, a = -4.37^{-2}, b = 2.298, R^2 = 0.423; April, a = -3.661^{-2}, b = 1.942, R^2 = 0.702; Glasshouse trial, a = -5.755^{-2}, b = 2.365, R^2 = 0.588.

Height; June, non-significant; October, a = -7.76^{-2}, b = 1.7, R^2 = 0.399; January, non-significant; April, a = -3.6^{-2}, b = 1.77^2, R^2 = 0.293; Glasshouse trial, a = -6.02^{-2}, b = 2.231, R^2 = 0.510.

4.4.3 Glasshouse trial results revisited

Under controlled conditions, a decrease in Chl a was recorded at 20 ppt salinity after four months exposure ($F_{12, 84} = 2.79$, $p = < 0.05$). Both Chl b and carotenoid values declined at 30 ppt salinity ($F_{12, 84} = 2.93$, Chl b; = 2.64, carotenoids; $p = < 0.05$;Figure 3-5 – 7; pp 73-75). All photosynthetic pigments displayed a negative linear relationship to salinity (30 ppt) after four months exposure ($F_{1, 23} = 5.17$, $R^2 = 0.156$, Chl a; = 9.80, $R^2 = 0.260$, Chl b; = 4.98, $R^2 = 0.151$, carotenoids; $p = <0.05$). A positive relationship between Chl a/b ratio and salinity (to 30 ppt) was observed under controlled conditions ($F_{1, 23} = 10.28$, $p = <0.05$, $R^2 = 0.268$) (Figures 4-2 – 4-3). Ratio of Chl a to b increased at 30 ppt after four months exposure ($F_{2, 96} = 2.28$, $p = <0.05$) (Figure omitted, see Appendix E), most probably as a result of greater osmotic stress in higher treatments. Sodium in leaf tissue dramatically increased in higher treatments (30-40ppt) under controlled conditions, suggesting an exclusion threshold was reached around 20 ppt, Na^+ accumulation taking place above 20 ppt ($F_{12,84} = 5.97$, $p< 0.001$;Figure 3-2; p 66). Soil salinity, up to 30 ppt, caused a significantly positive linear relationship to occur in leaf Na^+ concentration during the glasshouse trial ($F_{1.23} = 44.76$, $p<0.001$, $R^2 = 0.615$) (Figure 4-4).

There was no measurement of PFC under controlled conditions. In the glasshouse, density fell linearly as salinity treatments increased ($F_{1, 23} = 4.477$, $p <0.001$, $R^2 = 0. 588$) (Figure 4-5). At 20 ppt salinity, density was similar to control values but fell significantly beyond 20 ppt ($F_{12, 84} = 5.64$, $p < 0.001$; 3-9; p80). Likewise, the height of $P.$ $australis$ stems were not affected until 30 ppt salinity ($F_{12, .84} = 7.97$, $p < 0.001$; Figure 3-10; p 81). A significant negative relationship was observed between height and soil salinity (30 ppt) during the controlled glasshouse trial ($F_{1, 23} = 4.93$, $p = <0.001$, $R^2 = 0.510$) (Figure 4-5).

4.4.4 Relevance of controlled studies, compared to field situations

Compared to field data, concentrations of Chl $a,$ b and carotenoids were all highly depressed under glasshouse conditions c (Figures 4-2 -3). For Chl a, the nature of the relationship was similar between the January sampling period and the controlled study

(T_{71} = 1.66, p >0.5). For Chl b and carotenoids, the relationship was significantly different between field and controlled datasets (T_{71} = 3.35, Chl b / *October*; T_{46} = 2.45, carotenoids / April; p >0.5). Although the nature of the relationship between soil salinity and Chl a/b ratio was different among individual time-periods, the relationship (slope) was similar between the glasshouse trial and individual (June or January) sampling times (T_{71} = 1.616, June; = 0.26, January; p >0.05).

The relationship between soil salinity leaf Na^+ concentration under controlled conditions is depicted as an exponential relationship in Chapter 3 (p. 66); however, the response was linear up to 20ppt. There was no similarity between the nature of the relationship under controlled conditions and either sampling period (T_{71} = 9.26, June; T_{46} = 8.76, April, p <0.001) (Figure 4-4).

No measurement of PFC in the controlled trial was recorded. Density fell as salinity treatments increased under glasshouse conditions and the slope of regression obtained from the combined seasonal data was similar to the slope obtained from four months exposure in the controlled study (T_{196} = 0.263, p <0.05). There was no similarity between the nature of regression slopes for the glasshouse and combined (October/April) field data for height (T_{96} = 3.57, p= >0.001).

4.5   *Discussion*

4.5.1   Indicator of salinity stress in the field, over time

Ecologically, the sites examined extend from pure *P. australis* stands, to brackish saltmarsh habitat dominated by *J. kraussii.* The vegetation communities were consistent with the literature relating to species-mix over a salinity gradient of fresh- (*T. orientalis* and *P. australis*) to salt-marsh (*J. kraussii*) habitat in New South Wales estuaries (Clarke and Hannon 1967; Coutts-Smith and Downey 2006; Laegdsgaard 2006; Ling et al. 2006), with a range in hydrology from permanently moist, to standing water. Due to the

disturbed history of the study area environmental conditions are complex. Unexpected freshwater inputs were present at some sites and the water table is highly variable at others. Additionally, initial measurements were made in June, at the beginning of a new growing season, and ended in April when biomass was high but senescence had begun.
It would be interesting to sample all locations at all times, as individual sites may exhibit confounding attributes, such as inundation and soil characteristics or high heavy metal loads, which may maintain variation between sites.

No measured biochemical parameter was found to be a reliable indicator of salinity stress over time. The response of individual photosynthetic pigments varied with season. Additionally, the relationship between soil salinity and leaf Na^+ concentration response altered as soil salinity increased. Height of *P. australis* was also not a dependable factor, probably responding to variability in rainfall patterns. However, density and PFC results were constant predictors of the negative effect of salinity stress. Most importantly, the relationship between soil salinity and density/PFC did not show any seasonal variation and, for density, the nature of the relationship was consistent with data obtained from the controlled glasshouse study.

4.5.1.1 Photosynthetic pigments

Photosynthetic pigments of *P. australis* do not appear to be useful indicators of salinity stress. In a controlled environment with standardised light availability, controlled temperatures and regulated nutrient and water levels, it is clear that photosynthetic pigments are adversely affected by salinity. Interpretation of photosynthetic pigment content under field conditions is less clear. Sampling at one time period, (Farnsworth and Meyerson 2003) found chlorophyll content in *P. australis* leaves similar in fresh and brackish (≤ 17 ppt) marsh habitats. In this study, concentrations of individual pigments increased at different times/seasons. Halophytes, and some salt tolerant glycophytes, can display elevated photosynthetic electron transport and increased pigments in high salt concentrations (Critchley 1982; Garcia-Valenzuela et al. 2005). Other common environmental parameters can impact upon pigment type and quantity. Stress induced

changes in pigment fluorescence have been reported in many species, including beech (*Fagus spp.*) seedlings due to drought (Galle and Feller 2007), mangroves (*Avicennia spp.*) due to heavy metals (MacFarlane and Burchett 2001) and lettuce (*Lactuca*) due to atmospheric pollution (ozone and acid rain) (Catlatayud 2007). The negative effect of herbicides on photosynthetic pigments in duckweed (*Lemma*) and milfoil (*Microphyllum*) (Marwood et al. 2001) has been reported, along with temperature and light effects of on seagrasses (Ralph et al. 2007) and yams (Liao et al. 2004). Although flooding is known to decrease chlorophyll content in wheat (*Triticum*) (Collaku and Harrison 2002), it increases content in cattails (*Typha*) (Li et al. 2004). Additionally, phenology of the species will influence pigment combinations (Lichtenthaler 1987), short day-lengths in autumn signifying a reduction in chlorophylls and corresponding increase in carotenoids and xanthophylls (Starr and Taggert 1995). Therefore, photosynthetic pigments may not be particularly appropriate for reliably detecting toxic responses to salinity over time.

The ratio of Chl *a* to Chl *b* increased as salinity increased in the laboratory and during January, April and June; however, this trend was reversed during the spring (October) sampling period, and coincided with the increase in Chl *b*. Immediately prior to October freshwater inputs were apparent. Decreases in leaf water potential have been shown to increase chlorophyll *a/b* ratio (Long and Baker 1986; Sultana et al. 1999) and enhanced chlorophyll production (*a* + *b*) has been reported under flooded conditions. Eggink et al. (2001) and Pagter et al. (2005) found photosynthetic parameters in *P. australis* relatively unaffected until very low water availability occurred. Although previous studies had indicated that a 20 cm depth is adequate for determining ground water salinity levels (Lissner and Schierup 1997; Hughes 1998), soil salinity values for the October time period were constantly lower than those recorded during other seasons. It is possible the increase in Chl *b* was due to the influx of fresh water, with corresponding drop in salinity. Additionally, an increase in a particular pigment may be a factor of a particular growth phase, such as carotenoid increase in autumn when senescence is taking place (Taiz and Zeiger 2000).

4.5.1.2 Sodium content

Leaf Na^+ content increased and all morphological parameters decreased with increasing soil salinity under controlled conditions and during the winter (June) period; when accumulation in mature leaves would be highest (Munns and Termatt 1986; Munns 2002). These results appear to contradict a previous study, where no physical effect and limited (3mg/g dry weight) Na^+ uptake at 15ppt soil salinity was reported (Matoh et al. 1988). Matoh et al. (1988) attributed their findings to efficient Na^+ exclusion by *P. australis*. However, soil salinity recorded during this study reached 26ppt (June, field) and 30ppt (controlled), with leaf Na^+ increasing significantly after 20ppt. These results are in line with the findings by Lissner and Schierup (1997) and Adams and Bate (1999) that *P. australis* is severely affected above 20 ppt salinity and Na^+ accumulation takes place above 10 ppt. Considered a fresh or brackish water species (Fogli et al. 2002), *P. australis* may regulate Na^+ translocation to leaf tissue, or exclude Na^+ uptake into root tissue (Lissner et al. 1999a). It is more probable that, based on glasshouse findings, *P. australis* excludes Na^+ at the root level until a threshold is reached, additional Na^+ accumulating to toxic levels.

Seasonal variation was detected in interactions between salinity and leaf Na^+. The species is known to alleviate salt stress, and presumably lower cellular Na^+ concentration, when periodically supplied with fresh water (Hootsmans and Wiegman 1998; Adams and Bate 1999; Burdick et al. 2001). Weather patterns within the Hunter generally consist of dry winters and wet summers, with high variability. For example, April mean rainfall is 116 mm (N = 137); however, zero monthly rainfall has been recorded in some years and the highest daily recording is 231 mm (Bureau-Meteorology 2008). Under such conditions the marsh may experience excessively high or low soil salinity during any month, with leaf Na^+ values expected to follow similar fluctuations.

4.5.1.3 Stem height

Only two sampling periods produced a relationship between height and soil salinity. Contrary to expectations, it therefore appears height is not a good indicator of long-term

salinity stress under field conditions. Decreased height is a known indicator of environmental stress (Lissner and Schierup 1997; Adams and Bate 1999; Howard and Mendelssohn 1999; Ashraf and Harris 2004; Buchsbaum et al. 2006). During a controlled study into salinity effects on *P. australis*, the major negative morphological response recorded was height change (Howard and Rafferty 2006). However, in a comparison of North American freshwater and coastal tidal marshes no salinity effect on height was discerned (Meyerson et al. 2000). Additionally, following a controlled (25-day) trial subjected to 25 ppt salinity treatment, Mauchamp and Mesleard (2001) documented similar heights after a 25-day freshwater recovery period. The ability to recover quickly from stress effects supports the hypothesis that periodical freshwater influxes, such as precipitation, make conclusions based on height data collected from controlled studies ambiguous. As such, at sites with salinities around 10-15 ppt, results from the January period were higher than might be expected, due to the influence of freshwater in October and November.

4.5.1.4 Stem density and PFC

Stem density and PFC are often used as an indicator of one-another in herbaceous species, with the assumption that as stem number increases PFC increases (Beard 1975). In the field both these parameters consistently decreased with rising soil salinity. Importantly, no difference in the nature of decline at different time periods was apparent. This suggests that, in *P. australis,* the response of both parameters to salinity is predictable at the temporal scale and not masked by other confounding environmental factors. Additionally, the pattern of effect obtained under controlled conditions was similar to those obtained in the field.

4.5.2 Management Implications

Management resources are often limited. If density and PFC are considered to correlate with each other it would seem appropriate to include a single indicator, rather than measure both. Although quantitative, PFC is open to interpretation by different observers, whereas simple density counts are less likely to suffer from observer bias and are thus

more likely to be comparable. Cole (2002) states that the use of herbaceous plant PFC as an indicator of functional outcome in created wetlands in the USA is "inadequate at best and misleading at worst". Cole (2002) found little or no relationship existed between herbaceous PFC, commonly used to assess performance of created wetland projects, and the wetland functions it was reported to indicate (short- and long-term surface water storage; maintenance of high water table, element cycling; retention/removal of dissolved elements; accumulation of inorganic sediments). Additionally, as the phenology of herbaceous and grass-like plants is cyclic, when used as a function of primary productivity or fauna habitat, PFC may lead to different conclusions, depending on season. However, in this study the primary objective was to determine the effectiveness of individual indicators in detecting a response in *P. australis,* subjected to increasing soil salinity. To this end, PFC was shown to be an excellent indicator and if carried out in a uniform manner may be useful in assessing not only the vigour of *P. australi*s but, possibly, also the variation in salinity conditions within a marsh system.

However, it is suggested that the more appropriate indicator is density as 1) density is directly comparable between time periods and controlled studies, 2) density is a simple count and not as susceptible to observer bias based on the proficiency of the observer and 3) density is more likely to relate to total biomass and primary production (Cole 2002). Although other factors in the field environment would have contributed to the results, similar to PFC, density was found to be a highly reliable indicator of salinity stress in *P. australis*. Additionally, as the effect was similar between controlled and field situations it is reasonable to presume data derived under glasshouse conditions are useful in predicting in-situ observations.

4.5.2.1 Conclusion

Eliminating indicators that appear to have potential, but are unreliable at spatial or temporal scales, is as vital as locating those robust enough to be transferred from controlled to field conditions (Ewing et al. 1997). This study highlights the danger of simply extrapolating data collected under controlled conditions to field situations. The

introduction of natural tidal regimes to the marsh is expected to bring about increased soil salinity. Salinity effects on *P. australis* are visible as lower shoot numbers and it appears the effect is sturdy enough to produce a consistently reliable reaction in stem density, independent of growing season. Future manipulative field testing is required to verify these findings.

Although considered a glycophyte or brackish species, *P. australis* appears to possess numerous halophytic characteristics. Elucidating the complexities of indicators for marsh restoration purposes is therefore confounded by the physiology of *P. australis*. Stem density is one indicator that shows considerable promise having many of the trademarks of an ideal indicator (large and easy to count, available anytime, little expertise required to sample).

The next step in establishing the reliability of density as a robust and practical indicator of negative salinity effect on *P. australis,* is to monitor the species under a BACI (before-after control-impact) design once restoration processes begin; or alternatively, conducting a reciprocal translocation study through transplanting *P. australis* to areas of higher salinity and vice versa. This would confirm or refute the ability to detect sub-lethal effects through measurement of stem numbers. Monitoring *P. australis* stem counts might also provide a useful indicator of rising or falling soil salinity concentrations and associated core hydrological changes. Ideally, trajectories would be established at higher salinities than those observed in the current field study and density changes linked to eventual mortality of the species. Eventually the aim is to construct predictive models for the management of *P. australis*. Such models would enable the construction of time-lines for decreasing vigour/mortality in *P. australis* at various salinity regimes. Practical and achievable goals might then be set for performance standards of initial stages of coastal wetland restoration projects.

Globally, wetland rehabilitation, along with creation of new wetlands for mitigation purposes are increasingly used to compensate past and predicted future wetland losses (Streever 1997). There is a large body of work showing that individual wetlands develop differently, and at different time scales, and that no one attribute captures the full character of a restoration project (Keddy 1999; Zedler and Callaway 1999; Konisky and Burdick 2004). However, for more than two decades scientists have been arguing for clear objectives and performance standards (Streever 1997). Recommendations for performance standards, along with associated indicators evaluating progress of desired trajectories are many (Boesch et al. 1994; Brix 1994; Streever 1997; Mayer and Galatowitsch 2001; Harty 2004; Laegdsgaard 2006). Less frequently encountered are performance standards and indicators evaluating initial change, such as the demise of problematic species (Chapman 1998). It appears that degradation of existing habitat and creation of an alternative state are irrefutably linked. It is equally as important to track early-phase progression as it is to monitor desired restoration endpoints for the project over five or more years. The concept of impact assessment on existing habitat is a useful initial approach to assess ecosystem response and change to an altered salinity regime, but under-utilised. Including assessment of impact indicators in designing restoration projects not only enhances understanding of temporal scales but also permits restoration timeframes of individual wetlands to take place.

CHAPTER 5:

EFFECTS OF SALINITY ON THE COMPETITIVE INTERACTIONS BETWEEN TWO *JUNCUS* SPECIES

5.1 Summary

A glasshouse study investigated the effect of salinity on growth and competitive interactions between two closely related rush species, an Australian native (*Juncus kraussii*) and an exotic (*J. acutus)* species. Overall, both species exhibited decreases in height and total biomass with increasing salinity, although tolerance of *J. acutus* was marginally lower. Asymmetric responses were observed at each salinity level, due to the presence of the other species. In fresh-water, co-presence of *J. kraussii* facilitated the growth (increases in height and total biomass) of *J. acutus.* However, at 10 ppt salinity direct interspecific competition with *J. kraussii* adversely affected total biomass of *J. acutus.* When grown with *J. acutus,* at 5 but not at 10 ppt, salinity reduced total biomass of *J. kraussii*. Interspecific interactions vary, dependant on relative salinity tolerance of each species. It would appear that in areas receiving regular fresh-water inputs, which reduce salinity stress, *J. acutus* has the potential to displace *J. kraussii.*

A modified version of this chapter has been published (Greenwood and MacFarlane 2008), see Appendix P16.

5.2 Introduction

The distribution and abundance of species within estuarine plant assemblages are both an indication of an individual species relative physiological tolerance to abiotic conditions and the outcome of competitive interactions among species (Grime 1979; Davis et al. 2000; Pennings et al. 2005). Salinity is perhaps the dominant physicochemical gradient in estuarine settings, and thus the most important variable modifying competitive

interactions among plant species. Ungar, (1998) reviewed the influence of abiotic and biotic factors in determining plant zonation patterns of saline habitats. Ungar, (1998) suggested that lower limits of halophyte distribution are governed by physicochemical factors (predominantly salinity), while upper limits (areas of periodic freshwater inputs and thus lower salinity) are largely the outcome of competitive interactions among species. Much of the literature to date suggests that obligate halophytes find refuge from facultative halophytes and glycophytes by being relegated to marginal habitats of high salinity (Grime 1973; Grime 1979; Bertness et al. 1992; Emery et al. 2001; Crain et al. 2004; MacDougall et al. 2006). Conversely, lower performance is observed by obligate halophytes when present with facultative halophytes and/or glycophytes at lower salinities (Wilson and Keddy 1986; Ungar 1998; Emery et al. 2001; MacDonald 2001; Crain et al. 2004; Konisky and Burdick 2004).

Bertness (1991) used transplant studies to investigate competitive interactions between three marsh species. Grown alone, all species obtained maximum growth in low salinity habitat. However, when grown together at low salinity, *Juncus gerardi* Loisel. displaced *Spartina patens* (Aiton) Muhl. and both *J. gerardi* and *S. patens* displaced *Distichlis spicata* (L.) Greene. Conversely, in areas of high evaporation and soil salinity, where the other species grew poorly, *D. spicata* (the most halo-tolerant species) became dominant. In a fourteen month glasshouse study on three marsh plants conducted by Greiner La Peyre et al. (2001) over a salinity gradient (0 – 8 ppt), (*S. patens*, *Sagittaria lancifolia* L. and *Panicum hemitomon* J. A. Schultes), competitive interactions differed depending on the relative salinity tolerance of the three species. *Panicum hemitomon* (glycophyte) affected other species in fresh-water but growth declined with increasing salinity, 100% mortality occurring in the highest treatment. Effects of competition on the brackish species (*S. lancifolia*) were highest in fresh-water and decreased as growth (and dominance) of *P. hemitomon* decreased. At the highest salinity *S. patens* (halophyte) growth was unaffected.

Thus, resource competition is considered highest under ambient growing conditions, with increasing abiotic stress (i.e. salinity) modifying competitive interactions among species (Bertness and Callaway 1994; Konisky and Burdick 2004; Craine 2005; Lortie and Callaway 2006). Despite progress in the assessment of competitive interactions of species with differing salinity tolerances across salinity gradients, little is known concerning competitive interactions of closely related species with similar physiology (salinity tolerance) and autecology over salinity gradients. As such, general models of competitiveness over a stress gradient may not be applicable in habitats where changes in salinity regimes are coupled with the introduction of new species with similar tolerances to salinity and analogous life histories.

5.2.1 Relevance to saltmarsh restoration

Saltmarsh in NSW is vulnerable to invasive species, notably the introduced species, *J. acutus* (Williams and Meehan 2004). Within south-eastern Australian estuaries *J. acutus* and the Australian native, *J. kraussii* occupy similar mid-marsh habitat (Flanagan 1997; Burkett 2000; Paul et al. 2007). Field based observations suggest *J. acutus* is able to displace *J. kraussii* at lower salinities i.e. areas of the marsh that are elevated or receive regular fresh-water input (Flanagan, 1997). Elevated salinity levels may thus favour *J. kraussii* and therefore confer a competitive advantage over *J. acutus*. Consequently, interactions between individuals of these two species are expected to differ across a salinity gradient.

5.2.2 Aims

In a preliminary experiment, an appropriate density for each species at which resources become limiting and competition initiated was first established. This enabled testing of two hypotheses relating to the susceptibility of *J. kraussii* to invasion in an increasingly stressful saline environment. It was predicted that 1) increased salinity would affect *J. acutus* more than *J. kraussii* and 2) increasing salinity would increase the competitive ability of *J. kraussii*.

5.3   Materials and Methods

Seeds of both species were collected, at homogenous habitat locations within the Hunter River estuary marsh system, NSW, Australia (32°52´S, 151°43´E), (February 2004). Seeds were cleaned and stored in paper bags at room temperature (20ºC ±3) until trials commenced (June 2004 and February 2005).

Trials were conducted under glasshouse conditions (see Chapter 3, p.57 for details). Each pot (150 mm diameter) stood in its own holding tray (165 mm diameter) and was supplied with 5g Nutricote® slow (6 month) release fertiliser (N 14%, P 9%, K 15%; Chisso Asahi Fertilisers, Tokyo) (Plate 5-1). Wet-marsh conditions, favoured by *J. kraussii,* were maintained by flushing the appropriate salinity treatment water through pots three times per week. Saturation was achieved by placing additional treatment water into each holding tray, to a 2 cm standing-water mark. Pots received 40ml Wuxal® liquid fertiliser (Ag Nova Technologies, Australia) at monthly intervals.

Within the glasshouse, pots were randomly distributed and seeds germinated in-situ under appropriate treatment conditions. Seeds were spaced equally apart. Seed size (1 - 1.3 mm) and weight (0.03 - 0.09 mg) ranges were similar for both species. Germination is initiated between two and five days after planting (Greenwood and MacFarlane, 2006). Germination rate was over 98 percent. Additional plants, expressly grown in appropriate treatments for use as replacements, replaced plants that failed to germinate. The two experiments lasted eight months, and were carried out successively. Prior to harvesting, the maximum height of each plant was recorded. Soil was washed from plants. Roots and shoot tissue were separated and placed in paper bags. After drying at 60˚C for a minimum of three days, the weight of shoot and root tissue was recorded.

5.3.1 Experiment 1: Density at which resources are limiting: intraspecific competition

5.3.1.1   *Experimental design*

To examine competitive interactions it was first necessary to establish the density at which resources are limiting for individuals, i.e. negative effects on height and/or biomass as evidence of intraspecific competition. Non-saline soil was used. For each species, four density treatments were evaluated (1, 2, 3, and 6 plants). Five replicates for each treatment gave a total number of 40 pots. Data, height and shoot-to-root biomass ratios was analysed using the mean weight of individual plants per pot.

5.3.1.2 Data analysis

ANOVA is generally robust to minor heterogeneity of variance (Underwood, 1997). To improve homogeneity of variance, total biomass data of both species were transformed (Log +1) prior to analysis. For each species, the density at which significant growth reductions (per pot average, N = 5) occurred was determined through one-way ANOVA, followed by Tukey's HSD. A more conservative p value ($p < 0.025$) was employed via Bonferroni correction to reduce family-wise Type 1 error rate when directly comparing two contrasts simultaneously (two one-way ANOVAs) (Zar, 1999).

Plate 5-1 Glasshouse study on interspecific competition of *Juncus acutus* and *Juncus kraussii* over a salinity gradient

Plate 5-2 *Juncus kraussii* (six plant density), grown under 0 and 10 ppt salinity treatments

Biomass variation among individual plants, at the six plant density and under fresh-water conditions, was used to discern a possible self-thinning effect. Data passed the Kolmogorov - Smirrnov normality test. Levene's Test was employed to identify potential differences in asymmetry and variance ($n = 30$) for each of the two species (Zar, 1999).

5.3.2 Experiment 2: Tolerance and interspecific competition across a salinity gradient

5.3.2.1 Experimental design

A subsequent experiment examined the relative salinity tolerance and interspecific competition dynamics across an environmentally relevant salinity gradient (Plate 5-2). The experiment consisted of four salinity treatments, three species treatments (*J. acutus* alone, *J. kraussii* alone, mixed species) and five replicates per treatment for a total of 60 pots. Because the species are broadly similar in size and morphology a replacement series design, whereby the number of individuals per species changes, so that the number of individuals per replicate (density) remains static (Snaydon, 1991) could be applied. Each pot contained six plants of one species (monoculture), or three plants of each species (mixed culture). Previous germination trials established that both species were able to germinate in NaCl > 20 ppt, but not 30 ppt (Chapter 2, p. 45). Prior to trial commencement salinity treatments of 0, 5, 10 and 20 ppt were applied to each pot (Ocean Nature, Aquasonic, Australia). Water salinity was monitored weekly, using a hand-held salinity meter (Conductivity meter YSI, Ohio), and adjusted if necessary (± 5%). Although both species germinated, over 70% mortality occurred within the first three weeks at 20 ppt; therefore this treatment was not included in the final analysis.

5.3.2.2 Data analysis

5.3.2.2.1   Tolerance and interspecific competition across a salinity gradient

Height and weight of individual plants (average per pot, per species) were used as response variables. Above- and below-ground biomass data were transformed (log+1)

prior to analysis, to allow comparison between species, and improve homogeneity of variance.

5.3.2.2.2 Salinity tolerance of individual species

For each species, effects of salinity were determined through one-way ANOVA, followed by Tukey's HSD, preformed on monoculture treatments. Although species are similar in size and morphology at maturity, inherent differences in growth patterns are expected to exist, making direct comparison between species an invalid approach. Likewise, percentage change from control/optimum could not be employed, as the species examined (estuarine halophytes) may differ in their optimal growth response across the salinity gradient tested. To directly compare the two tests simultaneously a Bonferroni correction ($p < 0.025$) was employed to reduce family-wise Type 1 error rate (Zar, 1999).

5.3.2.2.3 Interspecific competition across a salinity gradient

A two-way ANOVA with salinity and species-mix as factors tested interspecific competition effects, allowing assessment of possible competitive or facilitatory interactions due to the presence of another species at each salinity treatment level. Data for each species mixed-treatment analysis was an average of the three plants *(J. acutus/J. kraussii*) obtained from the same pots. Tukey's HSD tests followed ANOVAs where significant main effects and/or interactions were detected (Stat-Soft, 2005).

5.4   Results

5.4.1 Experiment 1: Density at which resources are limiting: intraspecific competition

Plant height declined with density in both species ($F_{3, 16} = 6.2$, *J. acutus*; $= 17.2$, *J. kraussii*, $p < 0.005$), but the pattern was different between the two. Density decreased the average height of individual *J. acutus* plants only when six plants were grown together; however, *J. kraussii* height decreased significantly at three individuals per pot (Figure 5-1). For *J. acutus,* a significant biomass decrease occurred at a plant density of three ($F_{3, 16}$

= 14.6, $p < 0.001$). Biomass of *J. kraussii* was not affected (Figure 5-1, Plate 5-3). There were no differences in the s/r ratio with density increases for either species (data not shown, see Appendix F). The experimental density that consistently exhibited significantly lower average height and biomass per individual for both species was six plants per pot. Subsequent mixed species experiments contained six plants per pot.

At a density of six plants per pot, the biomass variance among individuals was significantly greater for *J. acutus* compared to *J. kraussii* (F_{58} = 10.3, $p = < 0.002$). For both species, size distribution was negatively skewed, with *J. acutus* showing a greater negative response (Sk = -0.871, *J. acutus; = -0.267 J. kraussii*). Range of total biomass of individual plants was 0.93 – 8.71 g, *J. acutus*; 2.3 – 6.69, *J. kraussii*.
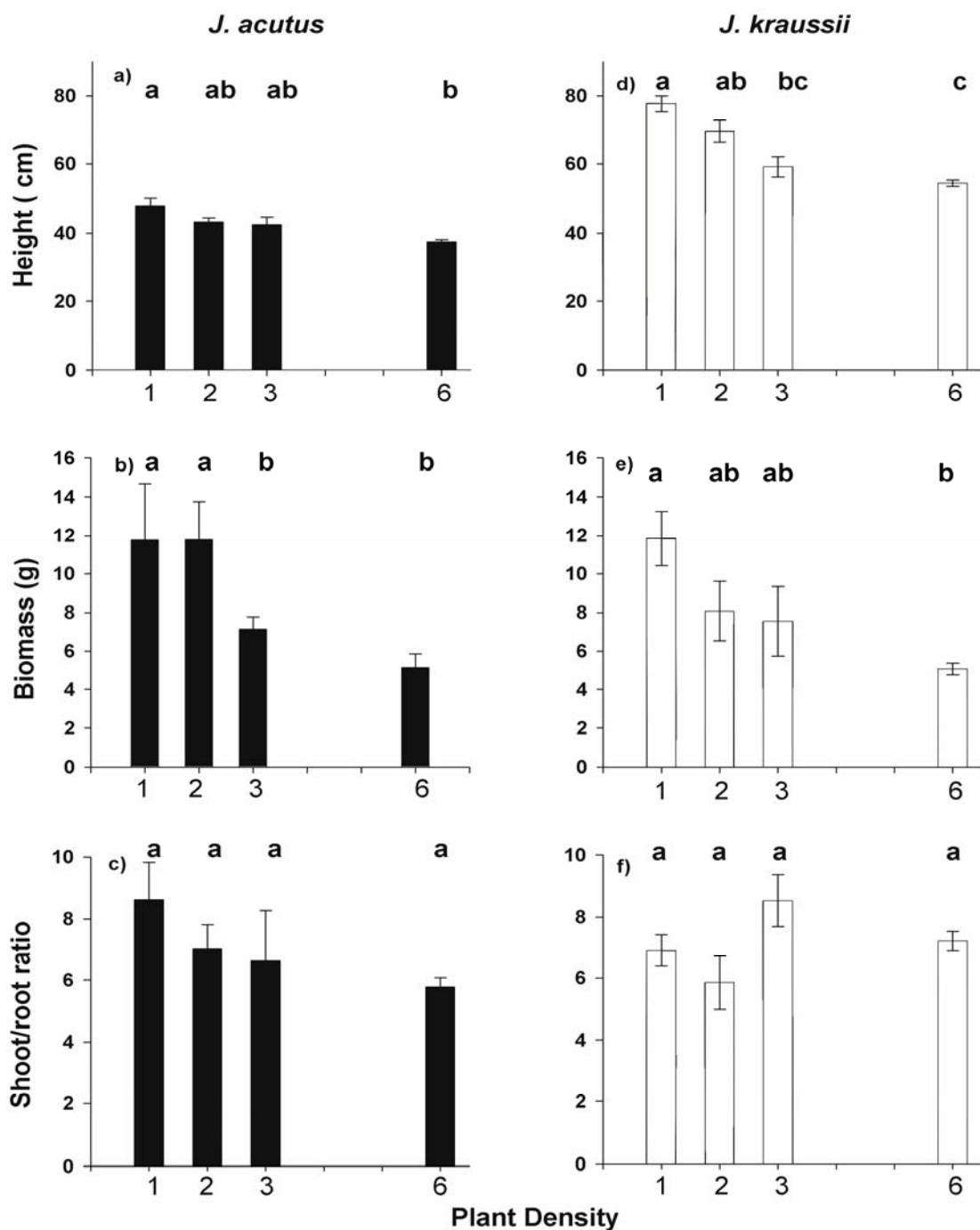
Figure 5-1. Height and biomass values of *Juncus acutus* and *Juncus kraussii*. Mean values of individuals ± SE (N = 5) at four density levels of plant growth. Letters indicate significant differences among treatments (α = 0.025).

Plate 5-3 *Juncus kraussii*, **grown at 1, 2, 3 and 6 plant density**

5.4.2 Experiment 2: Tolerance and interspecific competition across a salinity gradient

5.4.2.1 Salinity tolerance of individual species

Under monoculture conditions, salinity caused a decrease in height ($F_{2, 12}$ = 20.1, *J. acutus*; = 33.4, *J. kraussi*i, $\underline{p}$ < 0.001) and total biomass ($F_{2, 12}$ = 16.1, *J. acutus*; = 85.6, *J. kraussii*, *p* < 0.001) of both *J. acutus* and *J. kraussii* at 10 ppt salinity (Figure 5-2i – ii, v – vi). Shoot biomass did not differ among treatments for *J. acutus* (Figure 5-2iii); whereas decreases in shoot biomass were recorded for *J. kraussii* at 10 ppt salinity ($F_{2, 12}$, 35.9, $\underline{p}$ < 0.001) (Figure 5-2vii). Decreases in root biomass were observed at each treatment level for *J. acutus* ($F_{2, 12,}$ =34.4, p = < 0.001) (Figure 5-2iv), while decreases in root biomass were observed for *J. kraussii* only at 10ppt salinity ($F_{2, 12,}$ =99.4, *p* < 0.001) (Figure 5-2viii).
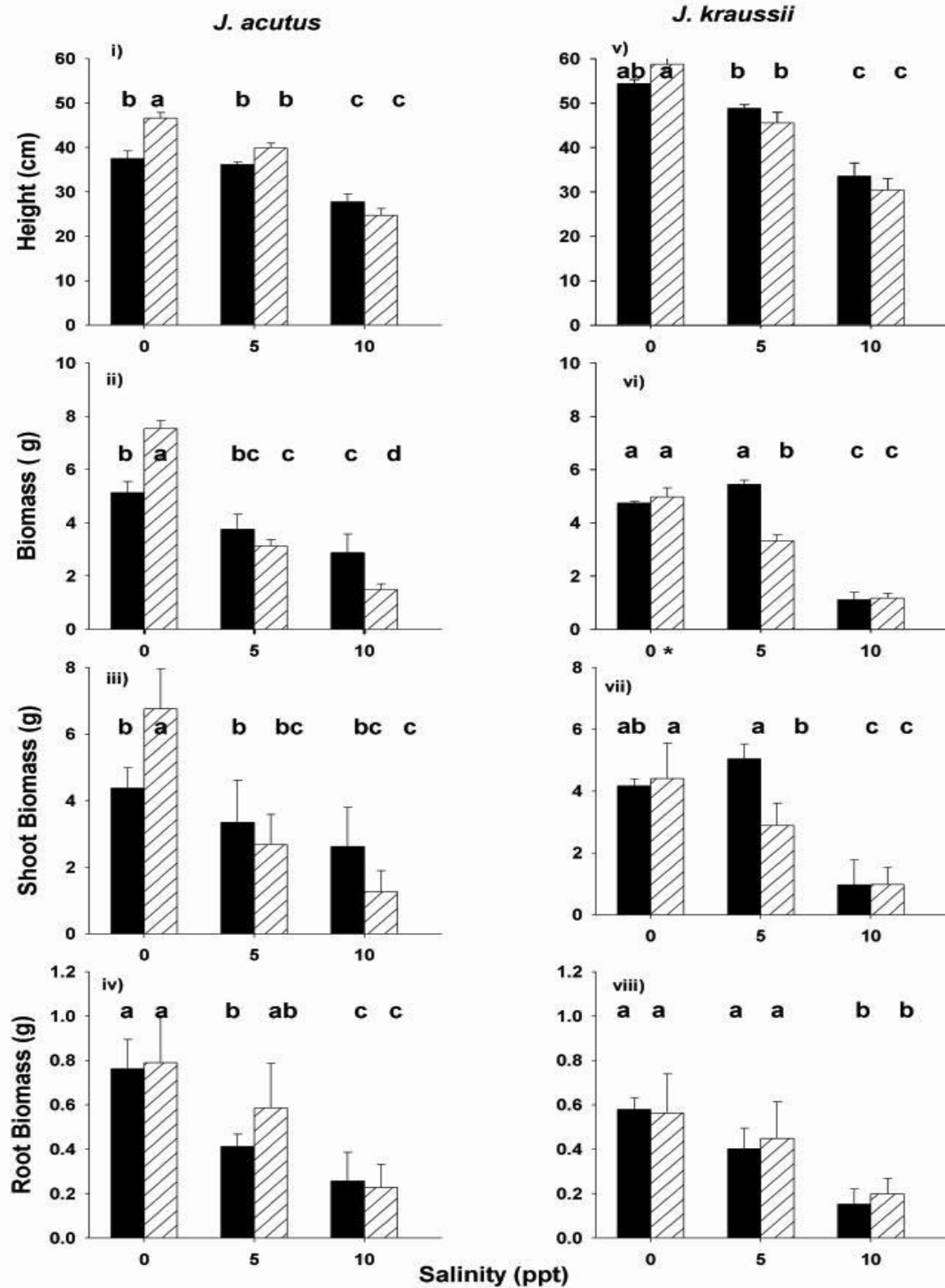
Figure 5-2 Height and biomass values of *Juncus acutus* (i-iv) and *Juncus kraussii* (v-viii) in response to salinity in mono or mixed culture. Mean values of individuals ± SE (*n* = 5). Plants grown alone (monoculture) plain bars, or together (mixed culture) hatched bars. Letters (a = highest, d = lowest, value) indicate significant differences among treatments (Tukey's HSD test, α = 0.05) between mono/mixed cultures at each salinity.

5.4.2.2 *Interspecific competition across a salinity gradient*

5.4.2.2.1 *Juncus acutus*

Significant interactions between salinity and species mixture were apparent for height, total biomass and shoot biomass ($p < 0.001$); indicating response to salinity differed depending on whether individuals were grown in mono or mixed culture (Table 5-1; Figure 5-2i–iii). Under fresh-water conditions, average height of *J. acutus* was greater when grown with *J. kraussii* culture than when grown alone. Total biomass was also greater under fresh-water mixed-species treatment than monoculture conditions. Salinity reduced the average height of *J. acutus*, in both mono and mixed culture, with an overall significant decline recorded at 10 ppt salinity. At 5 ppt salinity, total biomass was similar between species treatments; but at 10 ppt salinity *J. acutus* recorded a reduction in total biomass when grown with *J. kraussii,* compared to growing alone. Shoot biomass was lower under fresh-water monoculture treatment than in mixed species conditions. At other salinities, there was no difference in response between mono and mixed treatments (Table 5-1; Figure 5-2iii). Root biomass for *J. acutus* decreased with increasing salinity ($p < 0.001$), whether grown alone or with *J. kraussii* (Table 5-1; Figure 5-2iv),

5.4.2.2.2 *Juncus kraussii*

Higher salinity caused significant decreases in height ($p < 0.001$) of *J. kraussii* whether grown alone or with *J. acutus*, but no competition was discerned (Table 5-1; Figure 5-2v). For total and shoot biomass, however, similar interactions were observed between salinity and species mixture ($p < 0.05$). At 5 ppt salinity, the presence of *J. acutus* caused a decrease in total and shoot biomass of *J. kraussii* that was not evident under the monoculture treatment (Table 5-1; Figure 5-2vi –vii). For root biomass, significant reductions occurred at 10 ppt salinity ($p < 0.001$), but no competition was observed (Table 5-1; Figure 5-2viii).

Table 5-1 Summary of two-way ANOVA results, effects of salinity, species combination (monoculture vs. mixed culture) and interaction on *Juncus acutus* and *Juncus kraussii*

		J. acutus		*J. kraussii*	
Source	df	F	p	F	p
Height (cm)					
Salinity	2, 24	85.96	**< 0.001**	69.28	**< 0.001**
Mono-Mixed	1, 24	10.09	**0.004**	0.61	0.692
Interaction	2, 24	12.00	**< 0.001**	2.14	0.141
Biomass (g)					
Salinity	2, 24	108.79	**< 0.001**	13.98	**< 0.001**
Mono-Mixed	1, 24	0.25	0.621	5.935	**0.023**
Interaction	2, 24	23.64	**< 0.001**	5.499	**0.011**
Shoot Biomass (g)					
Salinity	2, 24	35.90	**< 0.001**	49.71	**< 0.001**
Mono-Mixed	1, 24	0.06	0.80	2.13	0.212
Interaction	2, 24	10.75	**< 0.001**	6.91	**0.004**
Root Biomass (g)					
Salinity	2, 24	42.34	**< 0.001**	58.5	**< 0.001**
Mono-Mixed	1, 24	0.01	0.804	1.66	0.210
Interaction	2, 24	0.16	0.854	3.135	0.083

5.5   *Discussion*

5.5.1   Density at which resources are limiting: intraspecific competition

Overall, when grown at a density of six plants per pot, both species exhibited a density effect in the volume of soil employed throughout the trial. Therefore, it was determined that competitive interactions, due to resource limitation, would be likely to occur in the subsequent salinity/mixed cultures.

Intraspecific competition (total biomass) affected *J. acutus* at a lower plant density (3 plants) than *J. kraussii* (6 plants). This may imply that either *J. acutus* possesses greater resource requirements than *J. kraussii*, or *J. kraussii* is more efficient in acquiring and/or utilising decreasing resources available.

In a species with high resource requirement, grown at higher densities, individual plants may be expected to achieve similar, abet lower, growth rates. However, it has been suggested that a characteristic of self-thinning is where some successful

individuals acquire most of the available resources as density increases at the expense of others, and is often characteristic of highly resource competitive species (MacDougall and Turkington 2004; Stoll and Bergius 2005). The higher variability in final biomass among individual *J. acutus* plants together with the truncated upper limit (apparent as greater, albeit non significant, negative skew) suggests many *J. acutus* plants obtained maximum growth within the limited resources, the remaining being progressively disadvantaged.

It would appear *J. kraussii* experiences height reductions at lower and biomass reductions at higher densities than *J. acutus*, suggesting a more equitable photosynthetic resources sharing response. Resource sharing among individuals has been shown to assist in maintaining monospecies stands in stressful habitats (Bertness and Callaway 1994; Harley and Bertness 1996; Hacker and Bertness 1999; Bruno et al. 2003). As such, *J. kraussii* may be exhibiting a similar resource sharing response as those reported for the halophyte *Atriplex prostrate* (Boucher) when grown at 16 plant density; whereby, all individuals survived yet reductions in height, biomass production and leaf area, along with a 50% reduction in net photosynthetic rate were recorded (Wang et al. 2005).

5.5.2 Salinity tolerance and interspecific competition across a salinity gradient

5.5.2.1 *Salinity tolerance of individual species*

In general, the pattern of growth decline was similar in both species, with declines evidenced at 10 ppt salinity. These results are in keeping with other studies, as it is well established excessive cellular salt concentrations impose an osmotic limitation to water uptake, thereby reducing cell expansion and shoot growth at elevated salinities (Evans and Etherington, 1991; Alarcon et al., 1999; Munns, 2002). (Evans and Etherington (1991) found lowering soil water potential from -0.05 to -1.5 MPa produced significant decreases in both leaf and root length of *J. acutus*. Increasing salinity could reasonably be expected to induce lower water potential and account for the reductions observed. For *J acutus,* root biomass decreased at each salinity treatment, while shoot biomass fell at 10 ppt salinity. For *J. kraussii,* salinity impacted equally on both root and shoot biomass, with reductions recorded at 10 ppt. This

indicates that the salinity response (in the range observed) differed between species, via differences in resource allocation.

In order to maintain root and rhizome survival under increasing salinity stress, halophytes divert energy from aboveground to belowground production (Konisky and Burdick, 2004; Craine, 2005). Shoot biomass was maintained in *J. acutus,* while root biomass decreased, suggesting the species was under duress. Conversely, although *J. kraussii* decreased with increasing salinity it maintained similar biomass partitioning within the salinity range examined. Maintaining root architecture and production is common in halophytes, additional root production increasing water accessibility/availability by maintaining osmotic homeostasis (Munns and Termatt, 1986; Ungar, 1998; Maggio et al., 2001; Bart and Hartman, 2003). These results suggest *J. kraussii* root growth exhibits greater tolerance to salinity during early establishment, supporting the hypothesis that salinity stress affects *J. acutus* more than *J. kraussii.*

5.5.2.2 *Interspecific competition across a salinity gradient*

Asymmetric responses were observed at each salinity treatment. For *J. acutus* facilitation occurred in fresh-water, while *J. acutus* was disadvantaged when grown with *J. kraussii* at 10 ppt salinity. Conversely, the presence of *J. acutus* did not affect *J, kraussii* in fresh-water or at 10 ppt salinity. However, at 5 ppt salinity competition from *J. acutus* caused biomass to decrease.

5.5.2.2.1 *Juncus acutus*

Enhanced total biomass values recorded under non-saline mixed-culture conditions implies a facilitative effect took place. It may be the co-occurrence of *J. kraussii* partially releases *J. acutus* from its own self-thinning response, or that *J. acutus* is better able to access available resources than *J. kraussii* under fresh-water conditions. Species can exhibit preferential uptake of different chemical forms of soil nitrogen (i.e. ammonium, nitrate or organic nitrogen as various free amino acids), with less competitive species being limited to utilising the least available form of nitrogen (nitrate) in mixed treatments (McKane et al., 2002; Van Ruijven and Berendse, 2005; Ba et al., 2006). It is possible *J. acutus* is better able to utilise preferred forms of nitrogen under non-saline conditions.

At the highest salinity, direct interspecific competition from *J. kraussii* reduced *J. acutus* biomass, supporting our second hypothesis. The effect occurred primarily on *J. acutus* shoot biomass. Results agree with current hypotheses that, when grown with a species possessing greater physiological tolerance, energy is diverted from resource exploitation (shoot growth) to survival as stress increases (Grime, 1979; Craine, 2005). Importantly, a reduction in *J. acutus* shoot growth may allow *J. kraussii* to locate leaves and stems above *J. acutus* and secure greater access to radiation and photosynthetic resources.

5.5.2.2.2   *Juncus kraussii*

Although under non-saline conditions *J. acutus* accesses additional resources, due to a lower resource requirement *J. kraussii* may be still able to maintain normal growth rates in fresh water. At 5 ppt salinity <u>J</u>. *acutus* is perhaps still within its salinity tolerance and able to aggressively compete for resources. The observed reduction in shoot biomass, but not height, suggests individual shoots were thinner and/or culm numbers lower when grown with *J. acutus*. However, *J. acutus* appears to possess a relatively lower salinity tolerance than *J. kraussii* and at 10 ppt salinity *J. kraussii* is better able to access resources and maintain growth. This suggests that the nature of interspecific interactions is largely dependant on subtle differences in the relative salinity tolerance of each species.

The original experiment included an additional concentration of 20 ppt salinity. Although germination was seen to take place at 20 ppt salinity plants did not grow. Lack of soil moisture is a major source of seedling mortality, when seedlings switch from internal to external reserves, suggesting that salinity levels may play a more important role in seedling establishment than initial germination. The use of a wider salinity range, applied to established seedlings, is perhaps required to further support our preliminary findings.

5.5.3 Management Implications

Increasing species globalisation requires a better understanding of closely related plant/plant relationships if vulnerable habitats are to be protected. Overall, our results concur with current hypotheses on the role of competition across abiotic gradients,

which predict that under low stress conditions, invasive species are often highly competitive, but that this competitiveness is reduced as physiological stress increases (Grime, 1979; Craine, 2005). Increasing and maintaining higher levels of salinity (i.e. 10ppt and potentially higher) may impart some competitive benefit to *J. kraussii* over *J. acutus.* higher)

Niche differentiation and facilitatory interactions may enable *J. acutus* to invade brackish marsh areas that experience fresh water influxes. Once established, *J. acutus* is likely to benefit from the presence of *J. kraussii*. Therefore, a better understanding of the facilitative relationship between these two species at low (1-7 ppt) salinity needs exploring. Increasing tidal flow increases depth and duration of flooding, as well as changing sedimentation and, at least short-term, nutrient cycling (Long and Mason, 1983; Chambers et al., 1998; Mitsch and Gosselink, 2000). As *J. kraussii* is typical of saline, waterlogged and potentially acid sulphate soils, an understanding of the combined effects of co-occurring stressors on competitive interactions between the two species also requires investigation.

CHAPTER 6:

CONCLUSIONS AND RECOMMENDATIONS

6.1 Overview

The influence of topography and hydrology, including the chemistry of hydrological flows, namely salinity, broadly determines wetland type. By definition, estuarine wetlands rely on tidal flows to maintain typical vegetation patterns, such as mangrove and saltmarsh communities. Anthropogenic structures that modify normal tidal flows are implicated in saltmarsh decline. The cumulative loss of coastal saltmarsh in NSW has led to the listing of the community on the Threatened Species Conservation Act 1995 (amended 2002) as an endangered ecological community, which, in turn, has generated numerous initiatives to protect wetlands in general and saltmarsh in particular.

Mitigation structures, such as floodgates and culverts, modify saline flow regimes, often changing the nature of the marsh upstream of the structures. Over time, the system may change from salt to freshwater communities. Within the complex of Lower Hunter estuary marshes, tidal flow is severely restricted by flood mitigation structures. Reintroduction of a more natural tidal regime is proposed through the modification or removal of hydrological barriers, with major changes in both hydrology and salinity anticipated. Although changing the level and duration of flooding will affect vegetation boundaries, much of the marsh is already anoxic. Therefore, changes in soil and water salinity are considered the most important factor in predicting the demise of freshwater and creation of saltmarsh communities.

An understanding of initial tidal reinstatement impacts on existing vegetative communities is required along with assessment of the long-term trajectories of restoration (i.e. the establishment and development of functional saltmarsh communities). Presently, the wetlands are dominated by the freshwater and salt-tolerant glycophyte, *P. australis*. The extent to which the reintroduction of saline waters will affect this species is not known.

As the most saline dominant native rush, managerially, the most desirable species as a replacement for *P. australis* is *J. kraussii*. However, a closely related exotic *Juncus* species is present within the region. There is a possibility that the demise of freshwater vegetation may allow *J. acutus* to increase in dominance at the expense of *J. kraussii*. Effects of salinity on these three dominant species (*P. australis, J. acutus* and *J. kraussii*) were examined at a number of key life stages and asked the following questions:

❖ Will target species be able to germinate under the new environmental conditions?
❖ What are the sub-lethal physiological and/or morphological responses of the target species to an environmentally relevant salinity gradient, and does the response vary with development stage and/or impact of exposure time?
❖ Are physiological and/or morphological indicators in *Phragmites australis* reliable predictors of salinity stress under both laboratory and field situations, and thus amenable to monitor the early stages of progression of tidal reinstatement initiatives?
❖ How competitive are the two closely related saltmarsh species (*J. kraussii* and *J. acutus*) with each other at various salinity levels?

The methodology used to explore these relative effects included controlled experimental studies into the effects of salinity on the germination capabilities, establishment and longer-term growth of the three species. A number of established salinity stress indicators were assessed, to determine responses to an environmentally relevant salinity gradient over both time/dose dependant scales. This also provided an insight into the salinity tolerance mechanisms employed by each species to survive under increased salinity concentrations. The reliability of stress indicators in *P. australis,* as potential measures of comprised growth and function, were tested by comparing the nature of relationships between salinity and stress indicators established under laboratory conditions against those collected in-situ. This determined indicator potential as a future tool to monitor the early stages of restoration progression. Finally, competitive/facilitative interactions between the two closely related *Juncus* species were studied under controlled conditions, in order to

comment on potential restoration outcomes under modified salinity regimes between the exotic *J. acutus* and native saltmarsh species, *J. kraussii*.

This chapter presents a summary of the findings, together with potential consequences for saltmarsh restoration projects. Management implications are discussed and gaps in the knowledge identified.

6.2   Will target species be able to germinate under modified saline conditions?

It appears that seed set and viability in local stands of *P. australis* are low. During the 2003 winter period, *P. australis* was harvested three times. Few seeds were located within the awns. The majority of seeds were not fully mature and appeared to be undergoing decay (Chapter 2, p. 42). This phenomena has been reported elsewhere (Harris and Marshall 1960). Various causes have been suggested, such as fungal or insect attack, unfavourable environmental conditions and pollen incompatibility caused by the low genetic variation within clonal stands (Ishii and Kadono 2002).

As seed was not available from local genotypes, results should be viewed with a modicum of caution. However, results from east-coast populations are relevant, given the local traffic of genetic material along this section of Australia. Introducing new genotypes into local populations is considered problematic. Plants sourced from Sydney are readily available for purchase and are probably already contributing to the overall genetic compositions in the Hunter. All seeds (Qld) used in the germination trial were destroyed. The majority of plants sourced from Sydney (100-200 km from the Hunter) were used in trials. However, around 50 were provided to local farmers for bank stabilisation projects. The seed viability of these plants is not known.

Using commercially purchased *P. australis* seed, it was found that even low salinity affected viability. Germination can take place under full-dark conditions, which suggests viable seeds can germinate on bare earth, within dense vegetation, or when covered by soil or wrack. This, in conjunction with low viability results, implies *P.*

australis does not possess a large persistent seed-bank, as seeds either germinate immediately in freshwater or become unviable under saline conditions.

For *P. australis,* around 80 percent germination was obtained under freshwater conditions. Salinity caused percent germination of *P. australis* to progressively decrease, with significant decreases occurring at 10, 20 and 25 ppt salinity. Temperature played an important role in the germination ability of *P. australis* seeds. The combination of high salinity and high temperatures reduced seed viability, germination percentage and speed of germination. Germination ability was highest under low (fluctuating 10-25 °C) temperatures. These temperatures are consistent with spring bare earth temperatures.

In general, germination characteristics of *J. kraussii* and *J. acutus* were similar. Both species germinated fully in 10 ppt salinity, germination being higher and faster under spring conditions. However, *J. acutus* appeared slightly less tolerant to salinity than *J. kraussii*. Viability and germination of *J. acutus* was affected above 10 ppt while *J. kraussii* maintained high germination percentages until salinity exceeded 15 ppt salinity.

In comparison to *P. australis*, *Juncus* species achieved 100 % germination in freshwater and almost total germination until salinity exceeded 10 ppt. Even after being subjected to 25 days of high salinity ($\leq$ 30 ppt) both *Juncus* species were able to achieve full germination when returned to fresh water under a spring temperature regime. *Juncus* species were found to possess a light requirement. Therefore, germination of *Juncus* species is retarded under high salinity and or/dark conditions, but remain viable. Under these conditions, large quantities of seed would accumulate in soil seed-banks. Periodically throughout the year freshwater inputs, through storm and urban runoff, would release the pressure of high soil salinity and initiate germination. In- and -around areas once dominated by either *Juncus* species it is logical to speculate regeneration will occur under suitable conditions.

Overall, *Juncus* species possessed an advantage in germination capabilities over *P. australis* when salinities are below 10 ppt. However, contrary to expectations *P.*

australis was the only species capable of germinating in 30 ppt salinity. Although *P. australis* seed used in this study were not local genotypes, these findings shed light on the possible cause of *P. australis* invasion into mangrove and saltmarsh communities along the eastern coast. Previous work has suggested invasion of highly saline habitats occurs only through asexual propagation (Amsberry et al. 2000; Bart and Hartman 2003); or that the ability of *P. australis* to spread into mangroves and areas with high soil salinity is linked with periodic freshwater inputs (Adams and Bate 1999; Burdick et al. 2001). However, as germination can take place under tidal salinity conditions there is an opportunity for germination and potential seedling establishment in these highly saline areas.

6.3 What are the sub-lethal physiological and/or morphological responses of the target species to an environmentally relevant salinity gradient, and does the response vary with developmental stage and/or exposure duration?

Chapter 3 reported the results of a four-month trial on seedlings of the three species of interest. At the commencement of the study all seedlings were in active juvenile growth phase, being 4/5 months old. Nutrient deficiencies, dehydration, and excess ions of many elements retard growth and in extreme cases cause mortality. Increasing salinity affects plants through decreasing hydration and increasing ion concentrations, especially in rapidly growing tissue (Munns 2002; Parida and Das 2005).

Both acute and chronic stress under controlled conditions is directly attributable to salinity. Increasing exposure duration can generate an accumulation effect, whereby sub-lethal effects are recorded at lower salinity concentrations as time increases. However, with continued expose plants may be able to adapt and maintain, or even minimise, stress responses. A limited number of highly adapted plant species show no reaction to salinity stress and in extreme cases salinity is required for optimum growth (Erdei et al. 1998; Ungar 1998; Munns 2002; Ashraf 2004). A common way of evaluating sub-lethal effects is through determining where a 50% reduction in yield occurs (EC_{50}); or alternatively, the level where a significant decline in yield occurs (LOEC).

6.3.1 Differences in Na+ accumulation uptake mechanism of individual species

It was demonstrated that *P. australis* is able to almost totally exclude Na^+ at the root tissue for up to one month, thereby protecting the more sensitive leaf tissue. During this time Na^+ levels in all tissue were maintained below the critical threshold of 6 mg/g (100 mM) (Munns 2002). However, as exposure duration increased the ability to exclude Na^+ was impeded and Na^+ accumulated. In order to protect photosynthetic tissue Na^+ was sequestered predominantly in roots and possibly rhizomes. Nonetheless, Na^+ ions ultimately reached shoot tissue, causing necrosis and eventually cell mortality. This system of exclusion is considered typical of glycophytes, although *P. australis* possess a salinity tolerance above most freshwater species. By four months exposure growth was suspended in plants subjected to continuous salinity concentrations of 30 ppt and above. Many individuals suffered irreversible damage, with shoot and root mortality.

Conversely, even at low salinities both *Juncus* species rapidly accumulated Na^+ in both root and shoot tissue, sequestering Na^+ ions (possibly in cell vacuoles and utilising them as osmoregulants) and thereby maintaining normal water balance. As exposure duration increased it became obvious each species possesses a salinity threshold, over which accumulation ceases and ion regulation mechanisms are initiated to maintain Na^+ ion concentrations at acceptable levels. High accumulation and translocation ratios, together with selective exclusion of particular ions, are indicative of halophytes species. Although growth was retarded and a redistribution of resources occurred, plants remained green and no loss of function was obvious.

6.3.2 Physiological responses to a salinity gradient

It has been reported *P. australis* possesses high water use efficiency and is able to continue photosynthesis under severe water stress (Pagter et al. 2005). Therefore *P. australis* may not be unduly affected by decreases in water availability associated with increased salinity. Results from Chapter 3 give some support to this statement as, over a one-week period, it was found that salinity did not overly affect respiration of four-month old *P. australis* seedlings. However, there was a general trend for increased salinity to decrease respiration. This indicates an interruption in cellular

respiration occurred, probably due to Na$^+$ toxicity rather than water deficiency. Logically, this would eventually result in decreased function at all organizational levels. In contrast, respiration of both *Juncus* species rose initially as salinity increased and plants struggled to maintain normal function. By one week, plants had adapted to the new salinity regime and respiratory functions returned to normal levels.

Salinity concentrations of approximately 20 ppt caused a 50 % reduction in net photosynthesis after one week exposure, irrespective of species. However, in *P. australis* and *J. acutus* the nature of the effect was not time dependent. Whereas the effect was less dramatic in *J. kraussii* and the concentration required to elicit a response was reduced as time increased. It is suggested that with time *J. kraussii* is probably able to regain normal photosynthetic production in salinities up to 40 ppt.

Decreases in photosynthetic pigment concentrations occurred in *P. australis* at 40 ppt salinity. This was not surprising as, at this salinity level, plants showed signs of physical distress in the form of leaf necrosis. However, salinity did not immediately affect photosynthetic pigment values. Continued exposure reduced pigments, suggesting the ability of *P. australis* to maintain typical values is increasingly compromised in high salinity. Over time, Chl *a* of *P. australis* was less affected by salinity than Chl *b*. Chlorophyll *a* contributes most to the photosynthetic pigment pool and is a higher energy provider than Chl *b* (Gandul-Rojas et al. 2004). In drier sites, or during drought and natural drawdown periods, salinity may be more effective in reducing total chlorophyll concentrations in *P. australis* plants than under moist conditions.

Photosynthetic pigments of neither *Juncus* species were affected by 40 ppt salinity. However, salinity did affect *J. acutus*, whereby photosynthetic pigments were reduced at 20 ppt salinity. It appears that above this value *J. acutus* may be able to initiate a protective response, which stimulates pigment production. However, in order to maintain normal photosynthetic pigment production a redistribution of energy resources would be required. Over a four month period *J. kraussii* was able to maintain similar photosynthetic pigmentation concentrations under all salinity treatments. As photosynthetic pigment production in *J. kraussii* was not affected

under any salinity regime, *J. kraussii* may be better adapted to higher salinity than *J. acutus*.

6.3.3 Morphological responses to a salinity gradient

At the whole plant level biomass, height and density of *P. australis* declined as salinity rose. Although compromised at 10 ppt, biomass continued to increase in *P. australis* at salinities of 20 ppt and below. At 30 ppt salinity and above a percentage of plants died after four months exposure. Additionally, the decline in root growth was more pronounced than that of shoot growth, suggesting high ion intake was causing irreversible damage. A 50% decline in *P. australis* biomass occurred at 15.3 ppt salinity. With increased exposure duration it is quite feasible that above 20 ppt most, if not all, *P. australis* would eventually die.

Salinity up to 40 ppt retarded, but did not halt, growth of both *Juncus* species. Both density and height decreased with increasing salinity. For *J. acutus* density was reduced gradually, while shoot height fell at low salinity concentrations. It appears *J. acutus* either positively responds to freshwater, through increasing height of stems, or is detrimentally affected at even low salinity. Conversely, in *J. kraussii* stem height was retained at the expense of increasing culm numbers.

6.3.4 Comparison of salinity effects among species

Overall, all species show high salinity tolerance. However, differences among species, relating to timing of effect and degree of salinity tolerance, occur. In the short term *P. australis* was able to exclude Na^+ (up to 40 ppt) from entering root tissue. This implies *P. australis* is able to withstand salinity values higher than seawater for a limited period of time. The inability of *P. australis* to continue to exclude Na^+ at high salinity, together with either mortality or large biomass decreases, confirms toxicity is occurring. On the other hand, *Juncus* accumulated Na^+ in both root and shoot tissue without noticeable damage. Over time, as salinity increases above a physiological threshold, mechanisms for regulating excess ions are initiated. There is little difference in the salinity tolerance of the two *Juncus* species. However, *J. acutus* appears to initiate salinity regulation at lower concentrations than *J. kraussii*. This tighter control of ion transport and uptake would require additional energy, again at the expense of overall vigour.

Salinity affects *P. australis* biomass at 10 ppt and beyond. By four months exposure, both height and density are affected at 30 ppt salinity. Although salinity limits biomass production of *J. acutus* at 30 ppt and *J. kraussii* at 10 ppt, the overall nature of decrease was very similar and differences in tolerance at any one treatment level was not apparent. The difference in statistical data results would seem to stem from the lower variation observed in *J. kraussii* data. Both species were able to accumulate biomass where salinity exceeds 30 ppt without nutrient deficiencies or necrosis being apparent. Physical characteristics of both species alter with increased salinity. *Juncus acutus* decreased height at 5 and 30 ppt, while maintaining density until 40 ppt. Although no measurements where taken, it appeared an increase in the girth of individual *J. acutus* culms takes place as salinity increases. In contrast, the opposite growth pattern was observed in *J. kraussii*. *Juncus kraussii* maintained height increases until 20 ppt, but decreased density at 10 ppt salinity.

6.4   Are physiological and/or morphological indicators in Phragmites australis reliable predictors of salinity stress under both laboratory and field situations, and thus amenable to monitor the early stages of progression of tidal reinstatement initiatives?

Restoration projects are intended to either return an area to some predetermined desired state or enhance structure and function of a system. However, the act of restoration affects existing species. Prior to the reinstatement of favourable species it is important to understand these initial effects. *Phragmites australis* is known to be an aggressive coloniser of impounded and disturbed estuarine marsh areas (Chambers et al. 1999). *Phragmites australis* is the dominant species within the wetlands. Restoration projects, which increase the amount of tidal exchange between the Hunter River and surrounding marsh areas, are intended to eradicate or weaken *P. australis* communities.

Using *P. australis* as an indicator for evaluating a change in the environment is commonplace. However, using reduced vigour of *P. australis*, or other undesirable dominant species, as an indicator of tracking initial desired restoration outcomes is

novel. Used in this way, the indicator species can assist management in setting early performance targets and monitoring early progress of restoration projects. In Chapter 3 a number of indicators of sub-lethal/lethal salinity effects in *P. australis* were identified, each possessing a linear relationship with salinity. Field evaluations (Chapter 4) determined if these indicators remained reliable across an environmentally relevant salinity gradient under field conditions and if seasonal variation was evident.

Results indicated that although biochemical parameters, such as photosynthetic pigments, are affected by increased salinity under controlled conditions, relationships are not reliably transferable to field situations. This is most probably due to the phenology of the species, whereby seasonal variability in microclimatic variance and growth patterns overwhelm any change solely attributed to an increase in salinity.

Sodium uptake into leaf tissue was also not a reliable indicator under natural conditions. It was apparent that hydrology affected Na^+ uptake, with periods of high rainfall changing the chemistry of available Na^+, soil water and increasing water potential in the surrounding media. For the same reason, no relationship between the height of *P. australis* stems and soil salinity was detected during summer and winter monitoring periods; as, soil salinity data collected during these seasons did not reflect previous freshwater inputs, which had initiated height increases.

On the other hand, density and PFC were reliable predictors of salinity stress. The nature of the negative linear relationship that existed between soil salinity and density/PFC is similar at various time periods throughout the year. Importantly, the relationship between density and soil salinity was similar to that recorded under controlled conditions.

As density data collected under controlled conditions was transferable to real-life scenarios, it has the potential to be used to evaluate the initial demise of *P. australis* after the recommencement of tidal flushing. Potentially, this may provide a valuable tool for managers, facilitating an adaptive management approach during the early phases of marsh restoration.

6.5   How competitive are the two closely related saltmarsh species (*J. kraussii* and *J. acutus*) with each other at various salinity levels?

Competition for resources is assumed to increase in less stressful environments, such as where tidal restrictions allow brackish species to invade native habitat. Conversely increasing a stress gradient should offer an advantage to niche saltmarsh species. Predicting outcomes of competitive interactions at higher salinities between two halophytes with similar autecologies however is more problematic. Both *J. acutus* and *J. kraussii* were detrimentally affected by increasing salinity. Both *J. acutus* and *J. kraussii* were also affected by being grown with the other species. However, the nature of interactions changed as salinity increased. Under freshwater conditions *J. acutus* was facilitated by the presence of *J. kraussii*. Conversely, at 10 ppt salinity *J. acutus* suffered a competitive inhibition when grown in the presence of *J. kraussii*. In contrast, *J. kraussii* was unaffected by *J. acutus* in freshwater and 10 ppt salinity but experienced growth reductions at 5 ppt salinity.

Facilitation has been previously identified in marsh species communities. In particular, *Juncus gerardii* has been shown to benefit non *Juncus* species, such as *Spartina alterniflora* and *S. patens* (Bertness and Hacker 1994; Bertness and Yeh 1994; Konisky and Burdick 2004). This facilitation effect has been attributed to oxygenating soils and reducing salinity through shading soils. There is a possibility that *J. acutus* may have physically benefited from the presence of *J. kraussii*; however, it is more likely *J. kraussii* possesses lower resource requirements than *J. acutus*, thereby enabling individual *J. acutus* plants access to resources under freshwater conditions. In the same conditions, although *J. acutus* consumed a greater proportion of available resources, enough remained for *J. kraussii* to be relatively unaffected.

Results suggest *J. acutus* is a superior competitor under ambient conditions. As salinity increases *J. acutus* was required to trade competitive capability for general growth and survival ability. At 5 ppt salinity growth of *J. kraussii* was stimulated when grown alone, probably due to increased water uptake via accumulated Na^+

acting as an osmoregulant. However, a salinity concentration of 5 ppt was not high enough to counterbalance the competitive ability of *J. acutus* and therefore *J. kraussii* suffered competitive effects. At 10 ppt salinity *J. acutus* was beginning to suffer physiological effects and was less able to successfully compete against *J. kraussii*. At this concentration, perhaps due to its marginally higher salinity tolerance, *J. kraussii* became the superior competitor. Salinity affected the resource allocation of each species differently. Salinity did not affect shoot biomass of *J. acutus* under mono-conditions; whereas, root biomass fell at each salinity treatment. Conversely, both shoot and root biomass of *J. kraussii* was maintained until 10 ppt salinity, thereby maintaining normal biomass allocation ratios.

It appears competitive interactions between the two species is strongly linked to differences in salinity tolerance. Appreciating how these two closely related salt-tolerant species interact with each other increases our understanding of plant invasions and succession in saltmarsh.

6.6   Implications for management

Barriers that restrict tides often result in the replacement of saltmarsh species with invasive (native and or exotic) brackish plants. Resource managers seek to reduce weed species and re-establish native halophytes, through altering hydrological flows and increase tidal flushing. However, many projects report less than optimal results in attempts to reverse vegetation trajectories (Streever 1997; Zedler and Callaway 1999; Callaway 2005). It may be that existing brackish species continue to persist in sites marked for restoration efforts. Therefore the new conditions may not be conducive to initial colonisation by desired saltmarsh species. Where colonisation does take place, the slow growth and small stature of saltmarsh species may allow existing or new weed species to remain/become dominant.

Within the Hunter estuary wetlands, *P. australis* has been identified as the dominant fresh/brackish species that will be affected by increased tidal exchange between the river and marsh areas. Management expects that the area presently occupied by *P. australis* will decrease and that saltmarsh species will eventually establish successfully at the expense of less halotolerant gylcophytic reed species. This is not

an unrealistic assumption, as increases in both flooding and salinity will influence vegetation survival.

Open water may replace *P. australis* stands in the lowest elevated areas of the marsh. Being unable to photosynthesise underwater, prolonged submergence greatly reduces the growth of *P. australis* (Mauchamp et al. 2001; Mauchamp and Methy 2004). Over-time density of *P. australis* is reduced due to affects on young emerging shoots (Hayball and Pearce 2004). Consequently, flooding will cause decomposition of much of the present *P. australis* standing crop, along with associated problems such as an increase of soluble nitrogen and phosphorus into surrounding waters (Gessner 2000; Mitsch and Gosselink 2000). Similarly, decomposition of *P. australis* will occur where soil salinity increases above the species tolerance limits. Therefore, the length of time required for mortality of *P. australis* to take place is an important consideration for managers wishing to implement restoration programs in sites vacated by *P. australis*.

Phragmites dominated communities are known to accumulate high levels of organic matter, through the yearly decomposition of old reeds. Reducing *P. australis* distribution will lessen these accumulation rates and may impact on the marshes ability to withstand possible rises in sea level (Rooth et al. 2003). Where *P. australis* does suffer a decline in vigour, decomposition of the standing crop will occur. Asaeda et al. (2002) estimated that after one year 52% of standing dead shoots of *P. australis* would remain undecomposed. Measuring density, or PFC as a cost alternative substitute, can give insights into the initial demise and long-term health of *P. australis*. The ability to monitor the collapse of *P. australis* would also enable managers to better understand whether anticipated soil salinity increases occur and are maintained.

It has been previously established that when periodically relieved of salinity stress, *P. australis* possess that ability to fully recover from high salinity regimes (Mauchamp and Mesleard 2001). Therefore, under restoration scenarios the importance of maintaining a particular salinity regime cannot be over emphasised.

This study has shown *P. australis* is tolerant of salinity values up to and including 20 ppt for at least four months. This figure is high for a species that relies on exclusion mechanisms to maintain ions at acceptable levels. Increasing the duration of exposure reduces the salinity concentration required to produce a detrimental affect. It is quite possible that as the duration of exposure increases beyond four months salinity tolerance will continue to decrease. At the very least, this may allow other species to compete with *P. australis*, promoting higher diversity of both vegetation and associated fauna within the marsh.

Problems with this scenario occur where freshwater inputs are frequent. Over the past decade Australia has experienced harsh drought conditions. During the first half of 2007 96.3% of NSW was classified as being in drought, including the Hunter/Newcastle area (DPI, 2008) In June 2007 the area received rainfall five times the monthly average, and above average values have been reported for five of the previous eight months (Bureau-Meteorology 2008). Presently (April 2008), 42.9% of the state is drought affected and the Hunter/Newcastle area is considered satisfactory (DPI, 2008). As Australia enters the *La-Nina* period of its climatic cycle wetter conditions and more erratic storm patterns are expected to occur. Additionally, changes in global climate conditions are forecast to increase the intensity of extreme climatic events, such as floods and storms. Therefore, it is entirely likely that *P. australis* will retain its dominant hold on the marsh where agricultural and urban runoff occurs.

Both *Juncus* species exhibit halophytic characteristics. Although increasing salinity will reduce the ability to increase biomass, the new regime will not exclude either species from the area. It would seem that *J. kraussii* is slightly better adapted to higher salinity than *J. acutus*, but only relatively so. Maintaining the narrow band of salinity concentrations (15-20 ppt) that favour *J. kraussii* over *J. acutus* is not realistic within a large marsh. Therefore, it is reasonable to expect that *J. acutus* will continue to compete with *J. kraussii* after tidal reinstalment occurs.

During the early stages of restoration, openings for germination and seedling establishment are likely to occur. Species with existing seed-banks, greater niche

breaths or possessing rapid germination and growth are likely to contribute most to the new community. *Phragmites australis* germinates in salinity reaching that of seawater. Conversely, germination of *Juncus* species is limited to 20 ppt salinity. The germination characteristics of both *Juncus* species are similar. Subtle differences do occur, such as high temperatures affecting germination of *J. acutus* at lower salinity concentrations than *J. kraussii*; however, variations in summer temperatures would allow conditions suitable to both species. Additionally, *P. australis* seed does not require light in order to germinate, whereas *Juncus* does. This is important in a management context as germination of *P. australis*, but not *Juncus*, could take place where decaying vegetation exists. As *P. australis* does not possess a large or permanent seed-bank, timing restoration programs to coincide with autumn, when seed banks are at their lowest, could reduce the risk of *P. australis* recolonising the area. However, in *P. australis* vegetative rhizome growth is the prime form of regeneration. Vegetation originating from this type of growth is thought to out-compete seedlings, which require a longer establishment period. Therefore, where suitable vegetative material exists, *P. australis* will out-perform species that rely mainly on seed production.

Juncus kraussii possess limited advantages over *J. acutus*; therefore, where conditions are inducive to germination and growth, establishment of both species is likely to take place simultaneously. Management hope that competition between the two species will favour *J. kraussii* in areas of high salinity. This may be the case, as *J. acutus* becomes progressively less competitive as salinity rises. However, wherever periodic freshwater inputs or lower salinity conditions exist, *J. acutus* will be facilitated, or *J. kraussii* disadvantaged when both species are present. This process can be expected to take place where the marsh is elevated or excess runoff occurs. Many areas of the marsh are fragmented by creek banks, road and rail verges, infrastructure installations and nominal walking tracks. Plate 1-5 highlights the outcome that can be expected under such circumstances. Unless high soil salinity can be maintained, which is managerially difficult, *J. acutus* is likely to become a major problem species within newly created marshes.

6.7   Recommendations for the management of saltmarsh restoration programs on the east coast of NSW Australia

Resource managers need to create conditions conducive to the recolonisation of saltmarsh species if biodiversity of habitat is to be preserved. The preferred approach to long-term restoration is the removal of hydrological barriers. Limiting freshwater input into the marsh will aid the restoration process. Timing programs to coincide with desirable seasonal or environmental conditions will enhance desired outcomes. Monitoring the initial demise of unwanted species, at specific sections of the marsh, will allow tracking of immediate goals and set the scene for the establishment of species considered necessary for sustainable saltmarsh restoration. Finally, to maintain and increase native biodiversity, there is a need to understand the relationship between additional environmental factors and plant-plant interactions of endemic and exotic species.

Within any saline wetland restoration program, ecological monitoring is required. Standard protocols for monitoring restoration of salt marshes, adapted from Chapman (1998) and Neckles et al.(2002) include the following approaches:

- Evaluate restoration sites against multiple control and reference marshes.

- Ensure restoration, control and reference sites are similar in characteristics, such as size, potential tidal range, water quality and adjacent land use.

- Ensure control sites remain unrestored for the life of the project.

- Conduct monitoring for a minimum of 1 year before restoration and annually for 2 or 3 years after initial restoration.

- Conduct long-term monitoring at five year intervals, or until all project criteria are met.

- Monitor variables to include hydrology (tidal signal and elevation), soils and sediments, pore–water salinity, nekton, avian and vegetation.

- Monitor composition, abundance and height of vegetation through permanent plots along transects

- Monitor density through permanent plots established within stands of species of concern

The following specific recommendations are based on species responses to the major chemical stressor of tidal regimes, salinity. Other environmental parameters are expected to modify the significance of salinity. Further, although adaptable to other coastal areas, these recommendations are specific to wetland restoration within the Hunter and include both passive and active restoration proposals.

6.7.1 Managerial preferred, passive restoration initiatives

1)   Restoration programs need to be carefully timed and adaptive to changes caused by environmental factors. October and November are considered the most appropriate months for tidal reinstatement in SE Australian estuaries; however, spontaneous opportunities should not be ruled out.

Rationale: The Hunter experiences low rainfall between August-December, which aids in maintaining high soil salinity levels. *Phragmites australis* will be most disadvantaged where tidal flushing is reintroduced during low rainfall. Additionally, low numbers of *P. australis* seed would be available for colonisation during this time. Soil temperatures are increasing and this may assist germination of *J. kraussii* over other species.

2)   Extensive monitoring programs on the density of *P. australis* are required immediately before and at staged intervals after tidal reinstatement. Suggested timings for monitoring events are 1, 2, 4, 8, 12, 18 and 24 months, with subsequent yearly monitoring.

Rationale: Density of *P. australis* is strongly coupled with soil salinity. Monitoring density will assist management in tracking the effect of increased tidal flushing and determine if expected responses are met. The suggested sampling timeframe would reflect when initial effects, related to the stressor, are most likely to be detected. In turn, this would provide clarification as to the validity of *P. australis* density as an indicator of successful restoration of impounded marshes. Thereafter, annual sampling is recognised as adequate for monitoring indicators of biotic integrity (Karr and Chu 1999).

6.7.2 Active restoration initiatives required

3)   Prior to the reintroduction of tidal hydrology, creation of freshwater retention areas within the marsh needs to take place.

Rationale: Artificial wetlands can accommodate urban and agricultural inflows. During normal weather conditions, these areas would act as storage and retention basins for freshwater and excess nutrients. However, freshwater overflow from these areas would arise during periods of abnormally high rainfall, such as experienced in June 2007. At such times, overflow would be diverted into areas not intended for saltmarsh establishment. In turn, this would preserve soil salinity and lower nutrient levels throughout the remaining marsh. Over time, the formation of fresh, brackish and saltmarsh areas within the marsh would take place, thereby recreating historical mosaic patterns.

4)   Upstream, in areas where water but not soil salinity levels are expected to increase (thereby not compromising monitoring activities); harvesting of *P. australis* stands is desirable prior to removal of hydrological barriers.

Rationale: Harvesting will lessen the environmental and social impact of large areas of decomposing vegetation. Additionally, an economic benefit may occur. The food and economic value of *P. australis* is low (Jiang et al. 2007), but it has previously been used, mixed with more palatable grasses, as animal feed. In Sweden, large-scale summer harvesting of *P. australis* is performed using machinery, with the resulting product processed and used as organic fertiliser (Hansson and Fredriksson 2004). *Phragmites* has also been used for thatching and insulation of roofs (Boar et al. 1999; Ksenofontova 1989). Removal of vegetation will also reduce nutrient flow into the marsh, favouring saltmarsh and disadvantaging nutrient dependant weed species. Additionally, the removal of decaying vegetation may be more acceptable to the general public in terms of aesthetics.

Phragmites australis is highly flammable, especially when previous years canes are present. Harvesting will decrease the fire potential of *P. australis* dominated areas. Although controlled burning has been used to decrease vigour of *Phragmites* (Thompson and Shay 1989), fire is considered detrimental to the marsh restoration

(Kong et al, 2007), as many other species including *Juncus* do not recover from fire as rapidly as *P. australis* (Thompson and Shay 1989).

Post-tidal reintroduction, removal of dead and decaying *P. australis* and other freshwater species may create areas of bare-earth, promoting germination of saltmarsh species. Both *Juncus* species accumulate large numbers of seed in the soil. Disturbance of the soil surface associated with mechanical removal of decaying standing crop will facilitate transportation of seed to the soil surface.

5) Chemical control and or physical removal of *J. acutus* is required.

Rationale: Salinity alone is unlikely to halt the advancement of *J. acutus* into Australian coastal marsh systems. Chemical control, physical removal or the annual harvesting of stems will be required to reduce the spread of *J. acutus*. Chemical control is useful in monospecies stands, where native *Juncus* spp are not present and employing low toxicity non-persistent herbicides applied to minimise entry to waterways. Physical removal has been shown to be practical in areas of high infestation (Streever 1997); however, high seedling reinfestation is likely to occur. Harvesting of stems (prior to seed-set) and/or seed removal is resource intensive; but will eventually reduce seed availability and increase the competitiveness of *J. kraussii*, due to relative seedling numbers.

6.8 Recommendations for further research identified from this study

During this study, a number of unanswered research questions were identified. An understanding of the following issues will support the assessment of affects of salinity on the macrophytes studied. In turn, this will assist saltmarsh restoration programs within NSW and enhance the overall understanding of restoration ecology.

1) Production of an initial model determining the time taken for *P. australis* to decrease/experience mortality at various salinity levels will assist predictions of final vegetative community compositions.

2) Implementation of translocation studies will confirm, or contest, the ability of density to detect sub-lethal affects of salinity in *P. australis*. Inclusion of salinity and hydrology regimes, along with other species of interest, would improve predictions of changes in vegetation community trajectories due to restoration disturbances.

3) A better understanding of the salinity tolerance of *P. australis* plants grown from propagules, rather than seed, will allow management to target areas that receive high quantities of wrack.

4) Additional competition studies between the two *Juncus* species, which extend the salinity gradient between species, would confirm if *J. acutus* is progressively disadvantaged with rising salinity.

5) Both laboratory and field-based assessment of how interactions between salinity and inundation (frequency and depth) affect survival of current and establishment of new species is required to better understand individual species responses to tidal reinstatement. In the field, this would require the amalgamation of detailed topographic and LiDAR/bathymetric maps, presently available for the Hunter area, with site-specific annual salinity charts.

6) Analysis of decomposition rates of *P. australis* under local conditions, to determine if rates reported in Northern hemisphere marshes are similar under Australian conditions.

7) Appraisal of available seed-banks, at the individual site and marsh level, will help determine if seed of desirable target species still exist in historically impounded sites.

8) Post-restoration, there will be opportunities to study plant colonisation patterns and the development of successional plant communities.

9) A comprehensive study of the autecology of *J. acutus* is required to better understand the species' environmental niche, quantify the rate of expansion / invasiveness and identify possible avenues of control.

10) A better understanding of differences in form, functionality and quality of *J. kraussii* vs. *J. acutus* habitat is essential, in order to establish the level of threat *J. acutus* possess to native and migratory fauna.

11) Manipulative field experiments, which create meso-communities, focusing on the influence of flooding and salinity may enable management to produce a passive restoration scenario for the control of *J. acutus.*

12) Determining how proposed increased port activities, river dredging and climate change will affect tidal levels, and therefore salinity regimes, will clarify the need to retain or modify elevation patterns within the marsh.

6.9   Application to Ecological Restoration

Restoration ecology aims to understand how species react to disturbances, both detrimental and beneficial. Processes related to tolerances of physical disturbance, such as saltwater flooding, and interspecific competition are thought to be key elements in determining temporal and spatial vegetation patterns within a saltmarsh. The aim of this thesis was to clarify the effect of increasing salinity, due to reinstatement of natural tidal regimes, might have upon three dominant species in a degraded saltmarsh system.

As ecological systems are dynamic, the assumption that the target goal of the restoration project is the only logical outcome is incorrect. Conditions that existed 50+ years ago, given extrinsic factors such as pollution or extinction, are not recreatable. As saltmarsh are constrained by tidal range and sea level, plants dependent on the intertidal zone are expected to modify distribution with changing sea level. Climate change may mean that species end up outside their optimal environmental gradient. As more frequent and intensive storm events occur,

disturbance increases the opportunity for colonisation by new species, many of which may be weed species.

Elevation is the key requisite for obtaining desired vegetation communities within estuarine sites, as it controls duration, timing and depth of flooding salt water. Knowledge of relative salinity tolerances and plant-plant interactions can help explain and predict the processes and outcomes of establishment and persistence of plant communities.

Resource managers could use the information provided in this thesis to help assess the likelihood of certain species establishing and persisting at specific locations within the marsh. Within the Hunter, post-restoration models of tidal flood elevations are available that allow estimations of the area to be covered by new flood/salinity regimes. Restoration scenarios based on soil salinity levels can be used to predict in which areas *P. australis* will remain dominant, become sub dominant or disappear. Eventually the aim is to construct predictive models for the management of *P. australis*. Such models would enable the construction of time-lines for decreasing vigour/mortality in *P. australis* at various salinity and hydrological regimes.

The three species studied possess overlapping environmental niches. Creating environmental conditions specifically targeted to promote the growth of one species at the expense of another is highly problematic and probably not practicable, given the limited resources of many projects. *Phragmites australis* has the ability to continue to increase biomass at salinities ≤ 20 ppt for at least 4 months. Given the inevitable periodic input of freshwater, although the species may not grow at optimum rates it can survive and possibly increase distribution at salinity concentrations around 20 ppt. Although higher salinity will advantage *Juncus* species, conditions that maintain salinity above seawater concentrations also place *Juncus* under stress. However, it is only under these conditions, where *P. australis* and other invasive species are outside of their salinity tolerance that species such as *J. kraussii* can remain, or become dominant.

Even less predictable is whether the native saltmarsh reed (*J. kraussii*) or the exotic weed species (*J. acutus*) will become dominant in areas of high salinity. Specific flooding regimes may yield an environmental advantage to *J. kraussii,* as the species can withstand periods of inundation. However, if only salinity is evaluated. *J. acutus* possess all the attributes to become dominant in large areas of the marsh. Additional information on the autecology of *J. acutus* is urgently required prior to implementation of large-scale passive restoration projects. Without adequate knowledge restoration programs could facilitate the spread of *J. acutus* throughout coastal wetlands. Ultimately, *J. acutus* has the potential to become the most significant weed species of restored and unrestored wetlands in Australia.

The most plausible scenario is that *P. australis* will continue to dominate the freshwater and brackish marsh. However, with time, where soil salinity rises above 30 ppt *P. australis* distribution will decrease, bring replaced by *Juncus* and dry-saltmarsh species. When environmental variables are considered, *Juncus acutu*s and *J. kraussii* are too closely correlated to determine the eventual outcome with current knowledge available. *J. kraussii* will most probably remain relegated to areas of high stress, being out competed by *J. acutus* under moderate conditions.

LITERATURE CITED

Aan A, Hallik L, Kull O (2006) Photon flux partitioning among species along a productivity gradient of an herbaceous plant community. Journal of Ecology 94: 1143-1155

Adam P (1990) Saltmarsh Ecology. Cambridge University Press, Cambridge, UK

Adam P (1995). In: Zann, LP (ed) State of the Marine Environment Report for Australia: The Marine Environment - Technical Annex: 1. Department of the Environment, Sport and Territories Canberra, ACT, Australia

Adam P (1997) Absence of creeks and pans in temperate Australian salt marshes. Mangroves and Salt Marshes 1: 239-241

Adam P (2002) Saltmarshes in a time of change. Environmental Conservation 29: 39-61

Adams JB, Bate GC (1999) Growth and photosynthetic performance of *Phragmites australis* in estuarine waters: a field and experimental evaluation. Aquatic Botany 64: 359-367

Ailstock MS, Norman CM, Bushmann PJ (2001) Common reed *Phragmites australis*: Control and effects upon biodiversity in freshwater nontidal wetlands. Restoration Ecology 9: 49-59

Alarcon JJ, Morales MA, Torrecillas A, Sanchez-Blanco MJ (1999) Growth, water relations and accumulation of organic and inorganic solutes in the halophyte *Limonium latifolium* cv. Avignon and its interspecific hybrid *Limonium caspia* x *Limonium latifolium* cv. Beltlaard during salt stress. Journal of Plant Physiology 154: 795-801

Allakhverdiev SI, Nishiyama Y, Miyairi S, Yamamoto H, Inagaki N, Kanesaki Y, Murata N (2002) Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in *Synechocystis*. Plant Physiology 130: 1443–1453

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiology 123: 1047-1056

Allen JRC (2000) Morphodynamics of Holocene salt marshes: a review sketch from the Atlantic and Southern North Sea coast of Europe. Quaternary Science Reviews 19: 1155-1231

Allison S, K. (1996) Recruitment and establishment of salt marsh plants following disturbance by flooding. American Midland Naturalist 136: 232-247

Amsberry L, Baker MA, Ewanchuk PJ, Bertness MD (2000) Clonal integration and the expansion of *Phragmites australis*. Ecological Applications 10: 1110-1118

Antonielli M, Pasqualini S, Batini P, Ederli L, Massacci A, Loreto F (2002) Physiological and anatomical characterisation of *Phragmites australis* leaves. Aquatic Botany 72: 55-66

ANZECC/NHMR (1992) Australian and New Zealand guidelines for the assessment and management of contaminated sites. Schedule B(1)

Apse MP, Blumwald E (2007) Na^+ transport in plants. FEBS Letters 581: 2247-2254

Armstrong J, Afreen-Zobayed F, Blyth S, Armstrong W (1999) *Phragmites australis*: effects of shoot submergence on seedling growth and survival and radial oxygen loss from roots. Aquatic Botany 64: 275-289

Armstrong W, Wright EJ, Lythe S, Gaynard TJ (1985) Plant zonation and the effects of the spring-neap tidal cycle on soil aeration in a Humberr salt marsh. Journal of Ecology 73: 3232-3393

Asaeda T, Nam LH, Hietz P, Tanaka N, Karunaratne S (2002) Seasonal fluctuations in live and dead biomass of *Phragmites australis* as described by a growth and decomposition model: implications of duration of aerobic conditions for litter mineralization and sedimentation. Aquatic Botany 73: 223-239

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. Flora 199: 361-376

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Science 166: 3-16

Ashraf M, Orooj A (2006) Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant Ajwain (*Trachyspermum ammi* [L.] Sprague). Journal of Arid Environments 64: 209-220

Auld BA, Medd RW (1987) Weeds: An Illustrated Botanical Guide to the Weeds of Australia. Inkata Press, Melbourne, Australia

Austin MP, Fresco LFM, Nicholls AO, Groves RH, Kaye PE (1988) Competition and relative yield: Estimation and interpretation at different densities and under various nutrient concentrations using *Silybum marianum* and *Cirsium vulgare*. Journal of Ecology 76: 157-171

Ba L, Wang D, Hodgkinson KC, Xiao N (2006) Competitive relationships between two contrasting but coexisting grasses. Plant Ecology 183: 19-26

Bajji M, Kinet JM, Lutts S (2002) Osmotic and ionic effects of NaCl on germination, early seedling growth, and ion content of *Atriplex halimus* (Chenopodiaceae). Canadian Journal of Botany-Revue Canadienne De Botanique 80: 297-304

Bart D, Burdick DM, Chambers RM, Hartman JM (2006) Human facilitation of *Phragmites australis* invasions in tidal marshes: A review and synthesis. Wetlands Ecology and Management 14: 53-65

Bart D, Hartman JM (2000) Environmental determinants of *Phragmites australis* expansion in a New Jersey salt marsh: an experimental approach. Oikos 89: 59-69

Bart D, Hartman JM (2003) The role of large rhizome dispersal and low salinity windows in the establishment of common reed *Phragmites australis* in salt marshes: New links to human activities. Estuaries 26: 436-443

Baskin CC, Baskin JM (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, CA, USA

Baskin JM, Tyndall RW, Carol C, Baskin CC (1998) Effect of salinity on germination and viability of nondormant seeds of federal-threatened species *Aeschynomene virginica* (Fabaceae). Journal of the Torrey Botanical Society 125: 246-248

Beard JS (1975) The vegetation survey of Western Australia. Plant Ecology 30: 179-189

Belkhodja R, Morales F, Abadia A, Medrano H, Abadia J (1994) Chlorophyll fluorescence as a possible tool for salinity tolerance screening in Barley (*Hordeum vulgare* 1.). Plant Physiology 104: 667-673

Belkhodja R, Morales F, Abadia A, Medrano H, Abadia J (1999) Effects of salinity on chlorophyll fluorescence and photosynthesis of Barley (*Hordeum Vulgare* L.) Grown under a Triple-Line-Source sprinkler system in the field. Photosynthetica 36: 375-387

Beritognolo I, Piazzai M, Benucci S, Kumzminsky E, Sabatti M, Mugnozza GS, Muleo R (2007) Functional characteristics of three Italian *Populus alba* L. genotypes under salinity stress. Trees 21: 465-477

Bertness MD (1991) Interspecific interactions among high marsh perennials in a New-England salt-marsh. Ecology 72: 125-137

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends in ecology & evolution 9: 191-193

Bertness MD, Ewanchuk PJ, Silliman BR (2002) Anthropogenic modification of New England salt marsh landscapes. Proceedings of the National Academy of Sciences of the United States of America 99: 1395-1398

Bertness MD, Gough L, Shumway SW (1992) Salt tolerances and the distribution of fugitive salt-marsh plants. Ecology 73: 1842-1851

Bertness MD, Hacker SD (1994) Physical stress and positive associations among marsh plants. American Naturalist 144: 363-372

Bertness MD, Shumway SW (1993) Competition and facilitation in marsh plants. American Naturalist 142: 718-724

Bertness MD, Yeh SM (1994) Cooperative and competitive interactions in the recruitment of marsh elders. Ecology 75: 2416-2429

Bewely JD, Black M (1994) Seeds: Physiology of Development and Germination. Plenum Press, New York, NY, USA

Blanch SJ, Ganf GG, Walker KF (1999) Tolerance of riverine plants to flooding and exposure indicated by water regime. Regulated rivers research & management 15: 43-62

Boesch DF, M.J. Josselyn, A.J. Mehta, J.T. Morris, W.K. Nuttle, C.A. Simenstad, Swift DJP (1994) Scientific assessment of coastal wetland loss, restoration and management in Louisiana. Journal of Coastal Research 103: 1-103

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. Plant Cell 7: 1099-1111

Boar RR, Kirby JJH, Leeming DJ (1999) Variations in the quality of the thatching reed Phragmites australis from wetlands in East Anglia, England Geological Society Special Publication 163: 145-151

Bradshaw AD (1996) Underlying principles of restoration. Canadian Journal of Fisheries and Aquatic Sciences 53: 3-9

Breierova E, Stratilova E, Sajbidor J (1997) Salinity affects fatty acid and extracellular glycoprotein composition of *Dipodascus australiensis*. Food Technology and Biotechnology 35: 237-241

Brewer JS, Rand T, Levine JM, Bertness MD (1998) Biomass allocation, clonal dispersal, and competitive success in three salt marsh plants. Oikos 82: 347-353

Bridgewater PB, Cresswell ID (1999) Biogeography of mangrove and saltmarsh vegetation: conservation and management in Australia. Mangroves and Salt Marshes 3: 117–125

Briggs SV (1978) Hexham Swamp - vegetation and waterbird habitats. NPWS, NSW, unpublished report

Brix H (1994) Functions of macrophytes in constructed wetlands. Water Science & Technology 29: 71-78

Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18: 119-125

Buchsbaum RN, Catena J, Hutchins E, James-Pirri M-J (2006) Changes in salt marsh vegetation, *Phragmites australis*, and nekton in response to increased tidal flushing in a New England salt marsh. Wetlands 26: 544–557

Burchett MD, Allen C, Pulkownik A, MacFarlane G (1998) Rehabilitation of saline wetland, Olympics 2000 site, Sydney (Australia) - II: Saltmarsh transplantation trials and application. Marine Pollution Bulletin 37: 526-534

Burdick DM, Buchsbaum R, Holt E (2001) Variation in soil salinity associated with expansion of *Phragmites australis* in salt marshes. Environmental and Experimental Botany 46: 247-261

Bureau-Meteorology (2008) Climate Averages for Australian Sites - Averages for Newcastle. Commonwealth of Australia 2006, Bureau of Meteorology. //www.bom.gov.au/climate/tables/ca_nsw_names.shtml. Assessed April 21 2008.

Burkett K (2000) Spiny Rush (*Juncus acutus*) Garden Island, Western Australia. (unpublished report) Defence Forces, Western Australia

Callaway JC (2005) The challenge of restoring functioning salt marsh ecosystems. Journal of Coastal Research 21: 24-36

Callaway JC, Zedler JB, Ross DL (1997) Using tidal salt marsh mesocosms to aid wetland restoration. Restoration Ecology 5: 135-146

Catlatayud A (2007) Chlorophyll a fluorescence as an indicator of atmospheric pollutant effects. Toxicology and Environmental Chemistry 2007: 89: 627–639.

Chambers RM (1997) Porewater chemistry associated with *Phragmites* and *Spartina* in a Connecticut tidal marsh. Wetlands 17: 360-367

Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. Aquatic Botany 64: 261-273

Chambers RM, Mozdzer TJ, Ambrose JC (1998) Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal saltmarsh. Aquatic Botany 62: 161-169

Chapman MG (1998) Improving sampling designs for measuring restoration in aquatic habitats. Journal of Aquatic Ecosystem Stress and Recovery 6: 235-251

Clarke LD, Hannon NJ (1967) The mangrove swamp and salt marsh communities of the Sydney district: I. Vegetation, soils and climate. The Journal of Ecology 55: 753-771

Clarke LD, Hannon NJ (1970) The mangrove swamp and salt marsh communities of the Sydney district: III. Plant growth in relation to salinity and waterlogging. The Journal of Ecology 58: 351-369

Clarke PJ, Jacoby CA (1994) Biomass and above-ground productivity of salt-marsh plants in south-eastern Australia. Australian Journal of Marine and Freshwater Research 45: 1521-1528

Chiapusio G, Sanchez AM, Reigosa MJ, Gonzalez L, Pellissier F (1997) Do germination indices adequately reflect allelochemical effects on the germination process? Journal of Chemical Ecology 23: 2445–2453

Cole CA (2002) The assessment of herbaceous plant cover in wetlands as an indicator of function. Ecological Indicators 2: 287-293

Collaku A, Harrison SA (2002) Losses in wheat due to waterlogging. Crop Science 42: 444-450

Colom MR, Vazzana C (2002) Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. Environmental & Experimental Botany 49: 135-144

Commonwealth Government (2004) Turning back the tide: The invasive species challenge. Report on the regulation, control and management of invasive species and the Environment Protection and Biodiversity Conservation Amendment (Invasive Species) Bill 2002. Environment Communications Information Technology and the Arts References Committee, Canberra, ACT, Australia

Congdon RA, McComb (1980) Productivity and nutrient content of *Juncus kraussii* in an estuarine marsh in south-western Australia. Australian Journal of Ecology 5: 221-234

Connolly RM (2004) Differences in trophodynamics of commercially important fish between artificial waterways and natural coastal wetlands. Estuarine Coastal & Shelf Science 58: 929-936

Conroy B, Lake P (1992) A vegetation analysis of Hexham Swamp. Department of Biological Sciences, Newcastle University, Newcastle, Australia

Coutts-Smith A, Downey P (2006) Impact of weeds on threatened biodiversity in New South Wales. CRC for Australian Weed Management, Adelaide, Australia

Craft C, Krull K, Graham S (2007) Ecological indicators of nutrient enrichment, freshwater wetlands, Midwestern United States (U.S.). Ecological Indicators 7: 733-750

Crain CM, Silliman BR, Bertness SL, Bertness MD (2004) Physical and biotic drivers of plant distribution across estuarine salinity gradients. Ecology 85: 2539-2549

Craine JM (2005) Reconciling plant strategy theories of Grime and Tilman. Journal of Ecology 93: 1041-1052

Critchley C (1982) Stimulation of photosynthetic electron transport in a salt-tolerant plant by high chloride concentrations. Nature 298: 483-485

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invisibility. Journal of Ecology 88: 528-534

Davis MA, Slobodkin LB (2004) The science and values of restoration ecology. Restoration Ecology 12: 1-3

DEC (2004) Note101 Threatened Species Conservation Act 1995. Department of Environment and Conservation, ACT, Australia

Deegan B, Harrington TJ, Dundon P (2005) Effects of salinity and inundation regime on growth and distribution of *Schoenoplectus triqueter*. Aquatic Botany 81: 199-211

Deng Y, Ogden J, Horrocks M, Anderson SH, Nichol SL (2004) The vegetation sequence at Whangapoua estuary, Great Barrier Island, New Zealand. New Zealand Journal of Botany 42: 565-588

Dickinson KJM, Mark AF (1999) Interpreting ecological patterns in an intact estuary, south-west New Zealand World Heritage Area. Journal of Biogeography 26: 913-932

Donnelly JP, Bertness MD (2001) Rapid shoreward encroachment of salt marsh cordgrass in response to accelerated sea-level rise. Proceedings of the National Academy of Sciences of the United States of America 98: 14218-14223

Donovan LA, Richards JH, Muller MW (1996) Water relations and leaf chemistry of *Chrysothamnus nauseosus* ssp consimilis (Asteraceae) and *Sarcobatus vermiculatus* (Chenopodiaceae). American Journal of Botany 83: 1637-1646

DPI (2008) Drought maps and drought assistance eligibility status. Department of Primary Industries. Assessed April 21 2008. .www/dpi.nsw.gov.au/agriculture/emergency/drought/situation/drought-maps.

Dubbert M, Tscharntke T, Vidal S (1998) Stem-boring insects of fragmented *Calamagrostis* habitats: herbivore-parasitoid community structure and the unpredictability of grass shoot abundance. Ecological Entomology 23: 271-280

Dybas CL (2004) Invasive species: The search for solutions. Bioscience 54: p.617

Eggink LL, Park H, Hoober JK (2001) The role of chlorophyll *b* in photosynthesis: Hypothesis. BMC Plant Biology 1:2: 1-7

El-Demerdash MA, Zahran MA, Serag MS (1990) On the ecology of the deltaic Mediterranean coastal land, Egypt. III. The habitat of salt marshes of Damietta, Port Said coastal region. Arab Gulf Journal of Scientific Research 8: 103-119

Emery NC, Ewanchuk PJ, Bertness MD (2001) Competition and salt-marsh plant zonation: Stress tolerators may be dominant competitors. Ecology 82: 2471-2485

Erdei L, Szegletes Z, Barabas KN, Pestenacz A, Fulop K, Kalmar L, Kovacs A, Toth B, Der A (1998) Environmental stress and the biological clock in plants: Changes of rhythmic behavior of carbohydrates, antioxidant enzymes and stomatal resistance by salinity. Journal of Plant Physiology 152: 265-271

Ekstam B, Forseby A (1999) Germination response of *Phragmites australis* and *Typha latifolia* to diurnal fluctuations in temperature. Seed Science Research 9: 157-163

Ekstam B, Johannesson R, Milberg P (1999) The effect of light and number of diurnal temperature fluctuations on germination of *Phragmites australis*. Seed Science Research 9: 165-170

Ervin GN, Wetzel RG (2000) Allelochemical autotoxicity in the emergent wetland macrophyte *Juncus effusus* (Juncaceae). American Journal of Botany 87: 853-860

Evans CE, Etherington JR (1991) The effect of soil water potential on seedling growth of Some British plants. New Phytologist 118: 571-579

Ewanchuk PJ, Bertness MD (2004) Structure and organization of a northern New England salt marsh plant community. Journal of Ecology 92: 72-85

Ewing K, McKee K, Mendelssohn I (1997) A field comparison of indicators of sublethal stress in the salt-marsh grass Spartina patens. Estuaries 20: 48-65

Ewing K, McKee K, Mendelssohn I, Hester M (1995) A comparison of indicators of sublethal salinity stress in the salt marsh grass, *Spartina patens* (Ait.) Muhl. Aquatic Botany 52: 59-74

Fagan RH, Webber M (1996) Global Restructuring: The Australian Experience. Oxford University Press, Melbourne, Australia

Fairweather PG (1999) Determining the "health" of estuaries: Priorities for ecological research. Austral Ecology 24: 441-451

Farnsworth EJ, Meyerson LA (2003) Comparative ecophysiology of four wetland plant species along continuum of invasiveness. Wetlands 23: 750-762

Flanagan N (1997) Competition between *Juncus kraussii* and *Juncus acutus* on Kooragang Island. Honours (unpublished). Biological Sciences, Newcastle, Australia

Florentine SK, Westbrooke ME (2005) Invasion of the noxious weed *Nicotiana glauca* R. Graham after an episodic flooding event in the arid zone of Australia. Journal of Arid Environments 60: 531-545

Fogli S, Marchesini R, Gerdol R (2002) Reed (*Phragmites australis*) decline in a brackish wetland in Italy. Marine Environmental Research 53: 465-479

Fowler J, Cohen L, Jarvis P (1998) Practical Statistics for Field Biology. John Wiley and Sons, Chichester, UK

Fynn RWS, Morris CD, KIirkman KP (2005) Plant strategies and trait trade-offs influence trends in competitive ability along gradients of soil fertility and disturbance. Journal of Ecology 93: 384-394

Galatowitsch SM, Anderson NO, Ascher PD (1999) Invasiveness in wetland plants in temperate North America. Wetlands 19: 733-755

Galinato MI, van der Valk AG (1986) Seed germination traits of annuals and emergents recruited during drawdowns in the delta Marsh, Manitoba, Canada. Aquatic Botany: 26 89-102

Galle A, Feller U (2007) Changes in photosynthetic traits in beech saplings (*Fagus sylvatica*) under severe drought stress and during recovery. Physiologia Plantarum 131: 412-421

Gandul-Rojas B, Roca M, Mínguez-Mosquera MI (2004) Chlorophyll and carotenoid degradation mediated by thylakoid associated peroxidative activity in olives (*Olea europaea)* cv. Hojiblanca. Journal of Plant Physiology: 499 - 507

Garcia AB, Engler JD, Iyer S, Gerats T, Vanmontagu M, Caplan AB (1997) Effects of osmoprotectants upon NaCl stress in rice. Plant Physiology 115: 159-169

Garcia-Valenzuela X, Garcia-Moya E, Rasco-Cruz Q, Herrera-Estrella L, Aguado-Santacruz GA (2005) Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. Journal of Plant Physiology 162: 650-661

Gessel FV (1983) Waders, sub order: Charadrii. In: Briggs SV (ed) Wetlands in New South Wales. NSW Parks and Wildlife Service, Sydney, NSW

Gessner MO (2000) Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Botany 66: 9-20

Grillas P, Garcia-Murillo P, Geertz-Hansen O, Marba N, Montes C, Duarte CM, Tan Ham L, Grossmann A (1993) Submerged macrophyte seed-bank in a Mediterranean temporary marsh: abundance and relationship with established vegetation. Oecologia 94: 1-6

Greenway H, Munns R (1980) Mechanisms of salt tolerance in non halophytes. Ann Review of Pant Physiology 31: 149-190

Greenwood ME, MacFarlane GR (2006) Effects of salinity and temperature on the germination of *Phragmites australis*, *Juncus kraussii*, and *Juncus acutus*: implications for estuarine restoration initiatives. Wetlands 26: 854-861

Greiner La Peyre MK, Grace JB, Hahn E, Mendelssohn IA (2001) The importance of competition in regulating plant species abundance along a salinity gradient. Ecology 82: 62-69

Grime JP (1973) Competition exclusion in herbaceous vegetation. Nature 242: 344-347

Grime JP (1979) Plant strategies and vegetation processes. John Whiley and Sons, London UK

Groves RH (1998) Recent incursions of weeds to Australia 1971 - 1995. CRC for Weed Management Systems, Technical Series No. 3, Adelaide

Gurnell J, Wauters LA, Lurz PWW, Tosi G (2004) Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. Journal of Animal Ecology 73: 26-35

Hacker SD, Bertness MD (1999) Experimental evidence for factors maintaining plant species diversity in a New England salt marsh. Ecology 80: 2064-2073

Hamer AJ, Lane SJ, Mahony MJ (2002) Management of freshwater wetlands for the endangered green and golden bell frog (*Litoria aurea*): Roles of habitat determinants and space. Biological Conservation 106: 413-424

Hanganu J, Mihail G, Coops H (1999) Responses of ecotypes of *Phragmites australis* to increased seawater influence: a field study in the Danube Delta, Romania. Aquatic Botany 64: 351-358

Hansson PA, Fredriksson H (2004) Use of summer harvested common reed (*Phragmites australis*) as nutrient source for organic crop production in Sweden. Agriculture, Ecosystems & Environment 102: 365-375

Harden GJ (1993) Flora of New South Wales. University of NSW Press, Sydney, Australia

Harley CDG, Bertness MD (1996) Structural interdependence: An ecological consequence of morphological responses to crowding in marsh plants. Functional Ecology 10: 654-661

Harris SW, Marshall WH (1960) Experimental germination of seed and establishment of seedlings of *Phragmites communis*. Ecology 41: 395

Hartig EK, Grozev O, Rosenzweig C (1997) Climate change, agriculture and wetlands in Eastern Europe: Vulnerability, adaptation and policy. Climatic Change 36: 107-121

Harty C (2004) Planning strategies for mangrove and saltmarsh changes in southeast Australia. Coastal Management 32: 405-415

Hartzendorf T, Rolletschek H (2001) Effects of NaCl-salinity on amino acid and carbohydrate contents of *Phragmites australis*. Aquatic Botany 69: 195-208

Hattori A, Mae S (2001) Habitat use and diversity of waterbirds in a coastal lagoon around Lake Biwa, Japan. Ecological Research 16: 543-553

Hayball N, Pearce M (2004) Influences of simulated grazing and water-depth on the growth of juvenile *Bolboschoenus caldwellii*, *Phragmites australis* and *Schoenoplectus validus* plants. Aquatic Botany 78: 233–242

HCRCMA (2007) Hunter-Central Rivers Catchment Action Plan. Hunter-Central Rivers Catchment Management Authority, Paterson, NSWHellings SE, Gallagher JL (1992) The effects of salinity and flooding on *Phragmites australis*. Journal of Applied Ecology 29: 41-49

Hester MW, Mendelssohn IA, McKee KL (2001) Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. Environmental and Experimental Botany 46: 277-297

Heugens EHW, Hendriks AJ, Dekker T, van Straalen NM, Admiraal W (2001) A review of the effects of multiple stressors on aquatic organisms and analysis of

uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31: 247-285

Higgins SI, Clark JS, Nathan R, Hovestadt T, Schurr F, Fragoso JMV, Aguiar MR, Ribbens E, Lavorel S (2003) Forecasting plant migration rates: managing uncertainty for risk assessment. Journal of Ecology 91: 341-347

Hill SJ, Tung PJ, Leishman MR (2005) Relationships between anthropogenic disturbance, soil properties and plant invasion in endangered Cumberland Plain Woodland, Australia. Austral Ecology 30: 775-788

Hogarth PJ (1999) Biology of Mangroves. Oxford University Press, Oxford, U.K.

Holaday AS, Chollet R (1984) Photosynthetic photorespiratory characteristics of C_3-C_4 intermediate species. Photosynthesis Research 5: 307-323

Hootsmans MJM, Wiegman F (1998) Four helophyte species growing under salt stress: their salt of life? Aquatic Botany 62: 81-94

Houlahan JE, Findlay CS (2004) Effect of invasive plant species on temperate wetland plant diversity. Conservation Biology 18: 1132-1138

Howard RJ, Mendelssohn IA (1999) Salinity as a constraint on growth of oligohaline marsh macrophytes. I. Species variation in stress tolerance. American Journal of Botany 86: 785-794

Howard RJ, Rafferty PS (2006) Clonal variation in response to salinity and flooding stress in four marsh macrophytes of the northern gulf of Mexico, USA. Environmental and Experimental Botany 56: 301-313

Hronec O, Hajduk J (1998) Remarkable resistance of *Phragmites australis* (Cav.) Trin. growing on soils intoxicated by magnesium emissions. Ekologia-Bratislava 17: 117-124

HTMC (1996) Ironbark Creek TCM Strategy, Volume 2: Technical Report. Hunter Catchment Management Trust, Patterson, NSW, Australia

Huckle JM, Potter JA, Marrs RH (2000) Influence of environmental factors on the growth and interactions between salt marsh plants: effects of salinity, sediment and waterlogging. Journal of Ecology 88: 492-505

Hughes CE (1998) Hydrology of a disturbed estuarine wetland, Hunter River, Australia: field investigation, process modelling and management implications. Doctor of Philosophy thesis. Department of Civil, Surveying and Environmental Engineering, Newcastle, NSW, Australia

Husain S, Munns R, Condon AG (2003) Effect of sodium exclusion trait on chlorophyll retention and growth of durum wheat in saline soil. Australian Journal of Agricultural Research 54: 589-597

Hwang YH, Chen SC (1995) Salt tolerance in seedlings of the mangrove *Kandelia-candel* (l) druce, rhizophoraceae. Botanical Bulletin of Academia Sinica 36: 25-31

Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80% acetone. Plant Physiology 77: 246-250

Isacch JP, Ricci L, Holz S, Martínez MM (2004) Post-fire vegetation change and bird use of a salt marsh in coastal Argentina. Wetlands 24: 235-243

Ishii J, Kadono Y (2002) Factors influencing seed production of *Phragmites australis*. Aquatic Botany 72: 129-141

Jiang C, Fan X, Cui G, Zhang Y (2007) Removal of agricultural non-point source pollutants by ditch wetlands: Implications for lake eutrophication control. Hydrobiologia 581: 319-327

Jimenez MS, Gonzalez-Rodriguez AM, Morales D, Cid MC, Socorro AR, Caballero M (1997) Evaluation of chlorophyll florescence as a tool for salt stress detection in roses. Photosynthetica 33: 291-301

Jones V, Richards PW (1954) *Juncus acutus* L. Journal of Ecology 42: 639-650

Jordan FL, Robin-Abbott M, Maier RM, Glenn EP (2002) A comparison of chelator-facilitated metal uptake by a halophyte and a glycophyte. Environmental Toxicology and Chemistry 21: 2698-2704

Kalaji MH, Pietkiewicz S (1993) Salinity effects on plant growth and other physiological processes. Acta Physiologiae Plantarum 15: 89-124

Karr JR, Chu EW (1999) Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington, DC, USA

Katembe WJ, Ungar IA, Mitchell JP (1998) Effect of salinity on germination and seedling growth of two *Atriplex species* (Chenopodiaceae). Annals of Botany 82: 167 - 175

Keddy PA (1999) Wetland restoration: the potential for assembly rules in the service of conservation. Wetlands 19: 716-732

Keiper FJ, Chen DM, DeFilippis LF (1998) Respiratory, photosynthetic and ultrastructural changes accompanying salt adaptation in culture of *Eucalyptus microcorys.* . Journal of Plant Physiology 152: 564-573

Keller BEM. (2000) Genetic variation among and within populations of *Phragmites australis* in the Charles River watershed. Aquatic Botany 66: 195-208

Kellogg CH, Bridgham SD, Leicht SA (2003) Effects of water level, shade and time on germination and growth of freshwater marsh plants along a simulated successional gradient. Journal of Ecology 91: 274-282

Khan MA, Gul B, Webber DJ (2000a) The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. Journal of Arid Environments 45: 73-84

Khan MA, Gul B, Webber DJ (2000b) Germination responses of *Salicornia rubra* to temperature and salinity. Journal of Arid Environments 45: 207-214

Khan MA, Gul B, Weber DJ (2001) Effect of salinity on the growth and ion content of *Salicornia rubra*. Communications in Soil Science and Plant Analysis 32: 2965-2977

Khan MA, Ungar IA (1984) The effect of salinity and temperature on germination of polymorphic seeds and growth of *Atriplex triangularis*. American Journal of Botany 71: 481 - 489

Khan MA, Ungar IA, Showalter AM (2000c) Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte, *Atriplex griffithii* var. *stocksii*. Annals of Botany 85: 225-232

Kong B, Zhang, SQ, Zhang B, Na XD, Li X, Lu XN (2007)Analysis of burn severity of wetlands in Zhalong Nature Reserve and impact of fire on red crowned crane habitat. Wetland Science 5: 348-355

Konisky RA, Burdick DM (2004) Effects of stressors on invasive and halophytic plants of New England salt marshes: a framework for predicting response to tidal restoration. Wetlands 24: 434–447

Koppitz H, Kuhl H, Hesse K, Kohl JG (1997) Some aspects of the importance of genetic diversity in *Phragmites australis* (cav) trin ex steudel for the development of reed stands. Botanica Acta 110: 217-223

Krishnamurty KV, Shpirt E, Reddy M (1976) Trace metal extraction of soils and sediments by nitric acid-hydrogen peroxide. Atomic Absorption Newsletter 15: 68-71

Ksenofontova T (1989) General changes in the Matsalu Bay reedbeds in this century and their present quality (Estonian SSR) General changes in the Matsalu Bay reedbeds in this century and their present quality (Estonian SSR) Aquatic Botany 35: 111-120

Kulmatiski A (2006) Exotic plants establish persistent communities. Plant Ecology 187: 1573-5052

Kulsler J (2003) Final report1: Wetland Assessment for Regulatory Purposes. Institute of Wetland Science and Public Policy, Association of State Wetland Managers Inc, Berne, NJ, USA

Kurtz JC, Jackson LE, Fisher WS (2001) Strategies for evaluating indicators based on guidelines from the Environmental Protection Agency's Office of Research and Development. Ecological Indicators 1: 49-60

Laegdsgaard P (2006) Ecology, disturbance and restoration of coastal saltmarsh in Australia: a review. Wetlands Ecology and Management 14: 379-399.

Lamp L, Collett F (1989) Field Guide to Weeds in Australia. Inkata Press, Melbourne, Australia

LCDC (2006) Weeds Toodyay Land Conservation District Committee. accessed 15/10/2006. http://www.toodyaylandcare.org.au/weeds.htm.

Lechno S, Zamski E, Telor E (1997) Salt stress-induced responses in cucumber plants. Journal of Plant Physiology 150: 206-211

Lenssen J P M, ten Dolle G E, Blom CWPM (1998) The effect of flooding on the recruitment of reed marsh and tall forb plant species. Plant Ecology 139: 13-23

Levine JM, Brewer JS, Bertness MD (1998a) Nutrients, competition and plant zonation in a New England salt marsh. Journal of Ecology 86: 285-292

Levine JM, Hacker SD, Harley CDG, Bertness MD (1998b) Nitrogen effects on an interaction chain in a salt marsh community. Oecologia 117: 266-272

Levitt J (1980) Responses of Plants to Environmental Stresses: Volume 11, Water, Radiation, Salt, and Other Stresses. Academic Press, New York

Li S, Pezeshki SR, Goodwin S (2004) Effects of soil moisture regimes on photosynthesis and growth in cattail (*Typha latifolia*). Acta Oecologica 25: 17-22

Liao FY, Li HM, He P (2004) Effect of high irradiance and high temperature on chloroplast composition and structure on *Dioscorea ziggiberensis*. Photosynthetica 42: 487-492

Lichtenthaler H (1987) Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. Journal of Plant Physiology 131: 101 - 110

Ling JE, Spencer J, Saintilan N, Rogers K, Jelbart J (2006) Ecological character of Kooragang Nature Reserve Wetland of International Importance. New South Wales Department of Environment and Conservation, Sydney, Australia

Lissner J, Schierup H-H (1997) Effects of salinity on the growth of *Phragmites australis*. Aquatic Botany 55: 247-260

Lissner J, Schierup HH, Comin FA, Astorga V (1999a) Effect of climate on the salt tolerance of two *Phragmites australis* populations. I. Growth, inorganic solutes, nitrogen relations and osmoregulation. Aquatic Botany 64: 317-333

Lissner J, Schierup HH, Comin FA, Astorga V (1999b) Effect of climate on the salt tolerance of two *Phragmites australis* populations. II. Diurnal CO2 exchange and transpiration. Aquatic Botany 64: 335-350

Long SP, Baker NR (1986) Saline Terrestrial Environments. In: Baker NR, Long SP (eds) Topics in Photosynthesis. Elsevier, Amsterdam, Netherlands, pp 63-102

Long SP, Mason CF (1983) Saltmarsh Ecology. Blackie, Glasgow, UK

Lopez CML, Takahashi H, Yamazaki S (2002) Plant-water relations of kidney bean plants treated with NaCl and foliarly applied glycinebetaine. Journal of Agronomy and Crop Science 188: 73-80

Lortie CJ, Callaway RM (2006) Re-analysis of meta-analysis: support for the stress-gradient hypothesis. Journal of Ecology 93: 7-16

Lu C, Qiu N, Wang B, Zhang J (2003) Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*. Journal of Experimental Botany 54: 85-860

Lukacs D, Beres I, Kazinczi G (2000) Herbicidal control of terrestrial reed *Phragmites australis* (Cav.) Trin ex Steudel. Zeitschrift fur

Pflanzenkrankheiten und Pflanzenschutz-Journal of Plant Diseases & Protection: 583-587

Lymbery AJ, Doupe RG, Bennett T, Starcevich MR (2006) Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. Aquacultural Engineering 34: 1-7

MacDonald TA (2001) Investigating the estuarine wetlands of the Lower Hunter River: Rehabilitation potential of tidal reinstatement following degradation caused by tidal restriction. PhD thesis. Department of Biological Sciences, Newcastle, Australia

MacDougall A, Turkington R (2004) Relative importance of suppression-based and tolerance-based competition in an invaded oak savannah. Journal of Ecology 92: 422-434

MacDougall AS, Boucher J, Turkington R, Bradfield GE (2006) Patterns of plant invasion along an environmental stress gradient. Journal of Vegetation Science 17: 47-56

MacFarlane GR, Burchett MD (2001) Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the Grey Mangrove, *Avicennia marina* (Forsk.) Vierh. Marine Pollution Bulletin 42: 233-240

Mack RN, D Simberloff, W. M Lonsdale, H Evans, M Clout, F A. Bazzazf (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications 10: 689–710.

Maggio A, Hasegawa PM, Bressan RA, Consiglio MF, Joly RJ (2001) Unravelling the functional relationship between root anatomy and stress tolerance. Functional Plant Biology 28: 999-1004

Mansour MMF, Salam KHA (2004) Cellular basis of salinity tolerance in plants. Environmental & Experimental Botany 52: 113-122

Marcum KB (2006) Salinity tolerance physiology in grasses. In: Khan MA, Webber DJ (eds) Ecophysiology of High Salinity Tolerant Plants. Springer, Dordrecht, The Netherlands, pp 157-172

Martindale J (1998) Kooragang Nature Reserve and Hexham Swamp Nature Reserve Plan of Management. NSW National Parks and Wildlife Service, Sydney, Australia

Martínez-Sánchez JJ, Conesaa E, Vicentea MJ, Jimeneza A, Francoa JA (2006) Germination responses of *Juncus acutus* (Juncaceae) and *Schoenus nigricans* (Cyperaceae) to light and temperature. Journal of Arid Environments 66: 187-191

Marwood CA, Solomon KR, Geenberg BM (2001) Chlorophyll fluorescence as a bioindicator of effects on growth in aquatic macrophytes from mixtures of polycyclic hydrocarbons. Environmental Toxicology and Chemistry 20: 890-898

Matoh T, Matsushita N, Takahashi E (1988) Salt tolerance of the reed plant *Phragmites communis*. Physiologia Plantarum 172: 8-14

Matsumura T, Kanechi M, Inagaki N, Maekawa S (1998) The effects of salt stress on ion uptake, accumulation of compatible solutes, and leaf osmotic potential in safflower, *Chrysanthemum paludosum* and sea aster. Journal of the Japanese Society for Horticultural Science 67: 426-431

Mauchamp A, Blanch S, Grillas P (2001) Effects of submergence on the growth of *Phragmites australis* seedlings. Aquatic Botany 69: 147-164

Mauchamp A, Mesleard F (2001) Salt tolerance in *Phragmites australis* populations from coastal Mediterranean marshes. Aquatic Botany 70: 39-52

Mauchamp A, Methy M (2004) Submergence-induced damage of photosynthetic apparatus in *Phragmites australis*. Environmental and Experimental Botany 51: 227-235

Mayer PM, Galatowitsch SM (2001) Assessing ecosystem integrity of restored prairie wetlands from species production-diversity relationships. Hydrobiologia 443: 177-185

McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA, Murray G (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415: 68-71

McNeely JA (2004) Strangers in our midst: the problem of invasive alien species. Environment, 46: p. 27

Mesleard F, Lepart J, Grillas P, Mauchamp A (1999) Effects of seasonal flooding and grazing on the vegetation of former ricefields in the Rhone delta (Southern France). Plant Ecology 145: 101-114

Meyerson LA, Saltonstall K, Windham L, Kiviat E, Findlay S (2000) A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. Wetlands Ecology and Management 8: 89-103

Minchinton TE, Bertness MD (2003) Disturbance-mediated competition and the spread of *Phragmites australis* in a coastal marsh. Ecological Applications 13: 1400-1416

Minello TJ (2000) Temporal development of salt marsh value for nekton and epifauna: utilization of dredged material marshes in Galveston Bay, Texas, USA. Wetlands Ecology and Management 8: 327-342

Mitsch WJ, Gosselink JG (2000) Wetlands. John Wiley, New York, NY, USA

Montalto A, Steenhuis TS (2004) The link between hydrology and restoration of tidal marshes in the New York/New Jersey estuary. Wetlands 24: 414-425

Moreira I, Monteiro A, Sousa E (1999) Chemical control of common reed (*Phragmites australis*) by foliar herbicides under different spray conditions. Hydrobiologia 415: 299-304

Morelock MM, Hunter EA, Moran TJ, Heynen S, Laris C, Thieleking M, Akong M, Mikic I, Callaway S, DeLeon RP, Goodacer A, Zacharias D, Price JH (2005) Statistics of Assay Validation in High Throughput Cell Imaging of Nuclear $_K$B Nuclear Translocation. Assay and Drug Development and Technologies 3: 483-499

Morris K, Ganf GG (2001) The response of an emergent sedge *Boloschoenus medianus* to salinity and nutrients. Aquatic Botany 70: 311-328

Morrison D (2001) Historical changes in land cover and predicted distribution of mangrove and saltmarsh in Hexham Swamp. Honours (unpublished). Discipline of Biological Sciences, Newcastle, Australia

Morton RM, Pollock BR, Beumer JP (1987) The occurrence and diet of fishes in a tidal inlet to a saltmarsh in southern Morton Bay, Queensland. Australian Journal of Ecology 12: 217-237

Mudroch J, Azcue JM, Murdroch P (1997) PhysicoChemical Analysis of Aquatic Sediments. Lewis, Boca Raton, Fl. USA

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell and Environment 25: 239-250

Munns R, Termatt A (1986) Whole plant response to salinity. Australian Journal of Plant Physiology 13: 143-160

Naidoo G, Kift J (2006) Responses of the saltmarsh rush *Juncus kraussii* to salinity and waterlogging. Aquatic Botany 84: 217-225

Navarroc A, Bañona S, Olmosb E, J S-BM (2007) Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. Plant Science 172: 473-480

Neckles HA, Dionne M, Burdick DM, Roman CT, Buchsbaum R, Hutchins E (2002) A monitoring protocol to assess tidal restoration of salt marshes on local and regional scales. Restoration Ecology 10: 556-563

Oceanics W (2001) Hexham Swamp Rehabilitation Project: Management Plan. WBM Pty Ltd, Broadmeadow, NSW, Australia

Odum WE (1988) Comparative ecology of tidal freshwater and salt marshes. Annual Review of Ecology and Systematics 19: 147-176

Onaindia M, Albizu L, Amezaqa I (2001) Effect of time on the natural regeneration of salt marsh. Applied Vegetation Science 4: 247-256

Ozdemir Z, Sagiroglu A (2000) *Salix acmophylla*, *Tamarix smyrnensis* and *Phragmites australis* as biogeochemical indicators for copper deposits in Elazig, Turkey. Journal of Asian Earth Sciences 18: 595-601

Page HN, Bork EW (2005) Effect of planting season, bunchgrass species, and neighbour control on the success of transplants for grassland restoration. Restoration Ecology 13: 651-658

Pagter M, Bragato C, Brix H (2005) Tolerance and physiological responses of *Phragmites australis* to water deficit. Aquatic Botany 81: 285-299

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60: 324-349

Parida AK, Das AB, Das P (2002) NaCl stress causes changes in photosynthetic pigmentation, proteins and other metabolic components in the leaves of a true mangrove, *Brugiera parviflora*, in hydroponic cultures. Journal of Plant Biology 45:28-36.

Parsons WT, Cuthbertson EG (2001) Noxious Weeds of Australia. CSIRO, Collingwood, Australia

Pastorok RA, MacDonald A, Sampson JR, Pace Wilber, Yozzo DJ, Titre JP (1997) An ecological decision framework for environmental restoration projects. Ecological Engineering 9: 89-107

Paul S, Young R, MacKay A (2007) Experimental control of the spiny rush *Juncus acutus* from Sydney Olympic Park: II Effects of treatment on other vegetation. Wetlands (Australia) 24, 1-13

Pennings SC, Grant MB, Bertness MD (2005) Plant zonation in low-latitude salt marshes: disentangling the roles of flooding, salinity and competition. Journal of Ecology 93: 159-167

Pennings SC, Selig ER, Houser LT, Bertness MD (2003) Geographic variation in positive and negative interactions among salt marsh plants. Ecology 84: 1527-1538

Pezeshki SR (2001) Wetland plant responses to soil flooding. Environmental & Experimental Botany 46: 299-312

Pujol JA, Calvo JF, Ramirez-Diaz L (2001) Seed germination, growth, and osmotic adjustment in response to NaCl in a rare succulent halophyte from southeastern Spain. Wetlands 21: 256-264

Pyke CR, Havens KJ (1999) Distribution of the invasive reed *Phragmites australis* relative to sediment depth in a created wetland. Wetlands 19: 283-287

Qu X-X, Huang Z-Y (2005) The adaptive strategies of halophyte seed germination. Acta Ecologica Sinica 25: 2389-2398

Ralph PJ (1998) Photosynthetic responses of *Halophila ovalis* (R. Br.) Hook. *f.* to osmotic stress. Journal of Experimental Marine Biology and Ecology 227: 203-220

Ralph PJ, Durako MJ, Ennrigues S, Collier CJ, Doblin MA (2007) Impact of light limitation on seagrasses. Journal of Experimental Marine Biology & Ecology 350: 176-193

Ranjan R (2008) Environmental restoration of invaded ecosystems: How much versus how often? Journal of Environmental Management 86: 616-626

Ravit B, Ehrenfeld JG, Haggblom MM (2003) A comparison of sediment microbial communities associated with *Phragmites australis* and *Spartina alterniflora* in two brackish wetlands of New Jersey. Estuaries 26: 465-474

Redondo-Gòmez S, Wharmby C, Castillo JM, Mateos-Naranjo E, Luque CJ, de Cires A, Luque T, Davey AJ, Figueroa ME (2006) Growth and photosynthetic responses to salinity in an extreme halophyte, *Sarcocornia fruticosa*. Physiologia Plantarum 128: 116-124

Richardson B (1983) Fishes. In: Briggs SV (ed) Wetlands in New South Wales. NSW Parks and Wildlife Service, Sydney, Australia

Ricky MA, Anderson RC (2004) Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. Journal of Applied Ecology 41: 888-896

Roberts J (2000) Changes in *Phragmites australis* in south-eastern Australia: A habitat assessment. Folia Geobotanica & Phytotaxonomica 35: 353-362

Rogers K, Saintilan N, Heijnis H (2005) Mangrove encroachment of salt marsh in Western Port Bay, Victoria: The role of sedimentation, subsidence, and sea level rise. Estuaries 28: 551-559

Rooth JE, Stevenson J, Cornwell JC (2003) Increased sediment accretion rates following invasion by Phragmites australis: The role of litter. Estuaries 26: 475-483

Russell IA (2003) Changes in the distribution of emergent aquatic plants in a brackish South African estuarine-lake system. African Journal of Aquatic Science 28: 103-122

Saintilan N (2004) Relationships between estuarine geomorphology, wetland extent and fish landings in New South Wales estuaries. Estuarine Coastal and Shelf Science 61: 591-601

Saintilan N, Williams RJ (1999) Mangrove transgression into saltmarsh environments in south-east Australia. Global Ecology and Biogeography 8: 117-124

Saintilan N, Wilton K (2001) Changes in the distribution of mangroves and saltmarshes in Jervis Bay, Australia. Wetlands Ecology and Management 9: 409-420

Sainty GR, Jacobs SWJ (1994) Waterplants in Australia: A Field Guide. Sainty and Associates, Sydney, Australia

Saltonstall K (2003) Genetic variation among North American populations of *Phragmites australis*: Implications for management. Estuaries 26: 444-451

Sanchez JM, Otero XL, Izco J (1998) Relationships between vegetation and environmental characteristics in a salt-marsh system on the coast of northwest Spain. Plant Ecology 136: 1-8

Santos CV (2004) Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Scientia Horticulturae 103: 93-99

Sayed OH (2003) Chlorophyll fluorescence as a tool in cereal crop research. Photosynthetica 41: 321-330

Schwenk CR (1982) Why sacrifice rigour for relevance? A proposal for combining laboratory and field research in strategic management. Strategic Management Journal 3: 213-225

Serrano R, Mulet JM, Rios G, Marquez JA, de Larrinoa IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft M, Ros R, Montesinos C (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. Journal of Experimental Botany 50: 1023-1036

Shaltout KH, Elkady HF, Alsodany YM (1995) Vegetation analysis of the Mediterranean region of Nile-Delta. Vegetatio 116: 73-83

Short FT, Burdick DM, Short CA, Davis RC, Morgan PA (2000) Developing success criteria for restored eelgrass, salt marsh and mud flat habitats. Ecological Engineering 15: 239-252

Shumway SW, Bertness MD (1992) Salt stress limitation of seedling recruitment in a salt-marsh plant community. Oecologia 92: 490-497

Silliman BR, Bertness MD (2002) Atrophic cascade regulates salt marsh primary production. Proceedings of the National Academy of Sciences of the United States of America 99: 10500-10505

Smith P (1991) The biology and management of Waders (Suborder Charadrii) in NSW. NSW National Parks and Wildlife Service, Hurstville, Australia

Snogerup S (1993) A revision of *Juncus subgen. Juncus* (Juncaceae). Willdenowia 23: 23-73

Snaydon RW (1991) Replacement or additive designs for competition studies? The Journal of Applied Ecology 28: 930-946

Srivastava AK, Bhargava P, Rai LC (2005) Salinity and copper-induced oxidative damage and changes in the antioxidative defence system. World Journal of Microbiology and Biotechnology 21: 1291-1298

Starr C, Taggert R (1995) Biology: The Unity and Diversity of Life. Wadsworth, Belmont, CA, USA

Stat-Soft (2005) Statistica 7.1. Stat Soft. Inc, Tulsa, OK, USA

Stoll P, Bergius E (2005) Pattern and process: competition causes regular spacing of individuals within plant populations. Journal of Ecology 93: 395-403

Straw P (2000) Hunter River Estuary Wader Habitat Investigation Stage 2. NSW National Parks and Wildlife Service, Hurstville, Australia

Streever WJ (1997) Trends in Australian wetland rehabilitation. Wetlands Ecology and Management 5: 5-18

Streever WJ, Genders AJ (1997) Effect of improved tidal flushing and competitive interactions at the boundary between salt marsh and pasture. Estuaries 20: 807-818

Streever WJ, Genders AJ (1998) Effect of improved tidal flushing and competitive interactions at the boundary between salt marsh and pasture. Oceanographic Literature Review 45: 999-1000

Streever WJ, Wiseman I, Turner P, Nelson P (1996) Short term changes in flushing of tidal creeks following culvert removal. Wetlands (Australia) 15: 21-29

Suarez N, Sobrado MA (2000) Adjustments in leaf water relations of mangrove (*Avicennia germinans*) seedlings grown in a salinity gradient. Tree Physiology 20: 277-282

Sudhir P, Murthy SDS (2004) Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42: 481-486

Sultana N, Ikeda T, Itoh R (1999) Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environment and Experimental Botany 42: 211-200

Svoboda P (2004) Kooragang Wetland Rehabilitation Project Management Plan. Hunter-Central Rivers Catchment Management Authority. Tocal, NSW, Australia

Systat (2004) SigmaPlot 9.01. Systat Software Inc

Taiz L, Zeiger E (2000) Plant physiology. Sinauer Associates, Sunderland, MA, USA

Tanaka A, Tanaka R (2006) Chlorophyll metabolism. Current Opinion in Biology 9: 248-255

Taylor N (1939) Salt tolerance of Long island salt marsh plants. New York State Museum, Circular 23, Albany, New York, USA

Teal JM, Weisharb L (2005) Ecological engineering, adaptive management, and restoration management in Delaware Bay salt marsh restoration. Ecological Engineering 25: 304-314

Templer P, Findlay S, Wigand C (1998) Sediment chemistry associated with native and non-native emergent macrophytes of a Hudson River marsh ecosystem. Wetlands 18: 70-78

Tester M, Davenport R (2003) Na$^+$ tolerance and Na$^+$ transport in higher plants. Annals of Botany 91: 503-527

Tewksbury L, Casagrande R, Blossey B, Hafliger P, Schwarzlander M (2002) Potential for biological control of *Phragmites australis* in North America. Biological Control 23: 191-212

Thom RM, Williams G, Borde A, Southard J, Sargeant S, Woodruff D, Laufle JC, Glasoe S (2005) Adaptively addressing uncertainty in estuarine and near coastal restoration projects. Journal of Coastal Research 21: 94-108

Thomas JC, Bohnert HJ (1993) Salt stress perception and plant growth regulators in the halophyte *Mesembryanthemum-crystallinum*. Plant Physiology 103: 1299-1304

Thompson K, Grime J P (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. The Journal of Applied Ecology 20: 141-156

Thomsen D, Marsden ID, Sparrow AD (2005) A field experiment to assess the transplant success of salt marsh plants into tidal wetlands. Wetlands Ecology and Management 13: 489-497

Tilman D, Lehman C (2001) Human-caused environmental change: Impacts on plant diversity and evolution Proceedings of the National Academy of Sciences of the United States of America, pp 5433-5440

Touchette BW (2006) Salt tolerance in a *Juncus roemerianus* brackish marsh: Spatial variations in plant water relations. Journal of Experimental Marine Biology and Ecology 337: 1-12

Thompson DJ, Shay JM (1989) First-year response of a *Phragmites* marsh community to seasonal burning. Canadian Journal of Botany 67: 1448-1455

Tremblin G, Ferard G (1994) Growth and salt accumulation of *Halopeplis-amplexicaulis* (vahl) cultivated under different salinity conditions. Acta Oecologica International Journal of Ecology 15: 355-364

Turner L, Creighton C, Heggie D, Harris P, Parslow J, Baird M, Dennison B, Tracey D (2001) An assessment of Australian estuaries 12th Biennial Coastal Zone Conference, July 15-19, Cleveland, OH, USA

Turner RE, Lewis RR (1997) Hydrologic restoration of coastal wetlands. Journal Wetlands Ecology and Management 4: 65-72

Turner PA, Streever WJ (1999) Changes in productivity of the saltmarsh mosquito, *Aedes vigilax* (Diptera: Culicidae), and vegetation cover following culvert removal. Australian Journal of Ecology 24: 240-248

Turner RE, Swenson EM, Milan CS, Lee JM, Oswald TA (2004) Below-ground biomass in healthy and impaired salt marshes. Ecological Research 19: 29-35

Underwood AJ (1997) Experiments in Ecology; Their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, U.K

Underwood AJ (2000) Importance of experimental design in detecting and measuring stresses in marine populations. Journal of Aquatic Ecosystem Stress and Recovery 7: 3-24

Ungar IA (1998) Are biotic factors significant in influencing the distribution of halophytes in saline habitats? The Botanical Review 64: 176-190

Van der Putten, W. H. (1997) Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Program on Reed Die-Back and Progression (1993-1994). Aquatic Botany 59: 263-275

Van Ruijven J, Berendse F (2005) Diversity–productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. Proceedings of the National Academy of Sciences 102: 695-700

Van Zandt PA, Tobler MA, Mouton E, Hasenstein KH, Mopper S (2003) Positive and negative consequences of salinity stress for the growth and reproduction of the clonal plant, *Iris hexagona*. Journal of Ecology 91: 837-846

Venter AJA, vanVuren JH (1997) Effect of gold-mine related operations on the physical and chemical characteristics of sediment texture. Water SA 23: 249-256

Villagra PE, Cavagnaro JB (2005) Effects of salinity on the establishment and early growth of *Prosopis argentina* and *Prosopis alpataco* seedlings in two contrasting soils: Implications for their ecological success. Austral Ecology 30: 325-335

Wackernagel M, Rees W (1996) Our ecological footprint: reducing Human Impact on the Earth. New Society, Gabriola Island, B.C. Canada

Waisel Y (1972) Biology of Halophytes. Academic Press Inc, New York, NY, USA

Wali MK (1999) Ecological succession and the rehabilitation of disturbed terrestrial ecosystems. Plant and Soil 213: 195-220

Walmsley J, Carden M, Revenga C, Sagona F, Smith M (2001) Indicators of sustainable development for catchment management in South Africa - Review of indicators from around the world. Water South Africa 27: 539-550

Wang L-W, Showalter AM, Ungar IA (2005) Effects of intraspecific competition on growth and photosynthesis of *Atriplex prostrata*. Aquatic Botany 83: 187-192

Weinstein MP, Litvin SY, Bosley KL, Fuller CM, Wainright SC (2000) The role of tidal salt marsh as an energy source for marine transient and resident finfishes: A stable isotope approach. Transactions of the American Fisheries Society 129: 797-810

Weisner SEB (1996) Effects of an organic sediment on performance of young *Phragmites australis* clones at different water depth treatments. Hydrobiologia 330: 189-194

Wellburn AR (1984) The spectral determination of chlorophylls a and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144: 307-313

Wijte A, Gallagher JL (1996) Effect of oxygen availability and salinity on early life history stages of salt marsh plants .II. early seedling development advantage of *Spartina alterniflora* over *Phragmites australis* (poaceae). American Journal of Botany 83: 1343-1350

Wilcox DA, Whillans TH (1999) Techniques for restoration of disturbed coastal wetlands of the Great Lakes. Wetlands 19: 835-857

Williams RJ, Meehan AJ (2004) Focusing management needs at the sub-catchment level via assessments of change in the cover of estuarine vegetation, Port Hacking, NSW, Australia. Wetlands Ecology and Management 12: 499-518

Williams RJ, Watford FA, Balashov V (2000) Kooragang Wetland Rehabilitation Project: History of changes to estuarine wetlands of the Lower Hunter River. NSW Fisheries, Port Stephens, Australia

Wills J (1962) A Handbook of Plants in Victoria. Melbourne University Press, Melbourne, Australia

Wilson C, Lesch SM, Grieve CM (2000) Growth stage modulates salinity tolerance of New Zealand spinach (*Tetragonia tetragonioides*, Pall.) and red orach (*Atriplex hortensis* L.). Annals of Botany 85: 501-509

Wilson SD, Keddy PA (1986) Species competitive ability and position along a natural stress/disturbance gradient. Ecology 67: 1236-1242

Wilton K (2002) Mangrove and saltmarsh habitat dynamics in selected NSW estuaries. PhD thesis, North Sydney, Australia

Winning G (1996) Vegetation of Kooragang Island Nature Reserve and Hexham Swamp Nature Reserve and adjoining land. NSW. NPWS, Sydney, Australia

Winning G (2006) Vegetation changes in a large estuarine wetland subsequent to construction of floodgates: Hexham Swamp in the Lower Hunter Valley, New South Wales. MPhil thesis. Arts and Science, Fitzory, Vic, Australia

Wolters M, Baker JP, Bertness MD, Jeffries RL, Moller I (2005) Saltmarsh erosion and restoration in south-east England: squeezing the evidence requires realignment. Journal of Applied Ecology 42: 844-851

Wolters M, Garbutt A, Bakker JP, Carey PD (2008) Restoration of salt-marsh vegetation in relation to site suitability, species pool and dispersal traits. Journal of Applied Ecology 45: 904–912

Woznzk AS, Roman CT, Wainright SC, McKinney RA, James-Pirri MJ (2006) Monitoring food web changes in tid-restored salt marshes: A carbon stable isotope approach. Estuaries and Coasts 29: 568-578

Wright DA, Wellbourn P. (2002) Environmental Toxicology. Cambridge University Press, Cambridge, UK

Xiong S, Johansson ME, Hughes FMR, Hayes A, Richards KS, Nilsson C (2003) Interactive effects of soil moisture, vegetation canopy, plant litter and seed addition on plant diversity in a wetland community. Journal of Ecology 91: 976-986

Ye Y, Tam NF-Y, Lu C-Y, Wong Y-S (2005) Effects of salinity on germination, seedling growth and physiology of three salt-secreting mangrove species. Aquatic Botany 83: 193-205

Yurkonis KA, Meiners SJ, Wachholder BE (2005) Invasion impacts diversity through altered community dynamics. Journal of Ecology 93: 1053-1061

Zar JH (1999) Biostatistical Analysis. Prentice Hall International Inc., New Jersey, USA

Zedler JB, Callaway JC (1999) Tracking wetland restoration: Do mitigation sites follow desired trajectories? Restoration Ecology 7: 69-73

Zedler JB, Callaway JC (2000) Evaluating the progress of engineered tidal wetlands. Ecological Engineering 15: 211-225

Zedler JB, Callaway JC, Sullivan G (2001) Declining biodiversity: Why species matter and how their functions might be restored in Californian tidal marshes. Bioscience 51: 1005-1017

Zedler JB, Morzaria-Luna H, Ward K (2003) The challenge of restoring vegetation on tidal, hypersaline substrates. Plant and Soil 253: 259-273

Zedler JB, Paling E, McComb A (1990) Differential responses to salinity help explain the replacement of native *Juncus kraussi*i by *Typha orientalis* in Western Australian salt marshes. Australian Journal of Ecology 15: 57-72

Zhao KF, Fan H, Zhou S, Song J (2003) Study on the salt and drought tolera*nce of Suaeda salsa* and *Kalanchoe claigremontiana* under iso-osmotic salt and water stress. Plant Science 165: 837 - 844

Zhao KF, Feng LT, Zhang SQ (1999) Study on the salinity-adaptation physiology in different ecotypes of *Phragmites australis* in the Yellow River Delta of China: Osmotica and their contribution to the osmotic adjustment. Estuarine Coastal & Shelf Science 49: 37-42

APPENDICES

A. Mean daily cumulative germination (± SE) of 25 seeds of *Phragmites australis*, *Juncus acutus* and *J. kraussii* (N = 4) under seven salinity treatments.

B. Average (N=3) metal concentrations (±SD) of potting mix (50% washed river sand, 25% loam soil and 25% organic material (coconut fibre), used to asses the effect of salinity on the survivorship of *Phragmites australis, Juncus acutus* and *J. kraussii*

C. Results of Na^+ concentration, respiration, net photosynthesis, photosynthetic pigments biomass, stem height and density of *Phragmites australis,* J*uncus acutus* and J. *kraussii* plants subjected to seven salinity treatments, over four months.

D. Vegetation sampling sheet

E. Average (± SE) results of soil salinity, leaf Na^+ concentration, photosynthetic pigments, density, percentage foliage cover and stem height of *Phragmites australis* stands (N = 5) within the Hunter Estuary and Lake Macquarie region (2003-2004).

F. Height and biomass results of *Juncus acutus* and *J. kraussii* plants grown at four densities and three salinity treatments

Appendix A.

Mean daily cumulative germination (± SE) of 25 seeds of *Phragmites australis*, *Juncus acutus* and *J. kraussii* (N = 4). Trial lasted 25 days with recovery period of 5 days Germination temperature 15-30°C. Trial commenced 14th October 2003

Phragmites australis

Time (Day)	0 (± SE)	5 (± SE)	10 (± SE)	15 (± SE)	20 (± SE)	25 (± SE)	30 (± SE)
1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
2	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
3	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
4	3.50 (0.96)	2.25 (0.75)	0.25 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
5	8.00 (1.35)	7.00 (1.08)	1.50 (0.65)	1.75 (0.85)	0.00 (0)	0.00 (0)	0.00 (0)
6	10.00 (1.29)	11.75 (1.18)	6.25 (1.89)	3.75 (1.31)	0.50 (0.19)	0.00 (0)	0.00 (0)
7	10.25 (1.25)	12.00 (1.08)	9.25 (1.49)	4.00 (1.22)	1.50 (0.65)	0.00 (0)	0.00 (0)
8	10.50 (1.44)	12.00 (1.08)	10.50 (1.26)	6.00 (1.47)	1.75 (0.85)	0.00 (0)	0.00 (0)
9	11.00 (1.08)	12.00 (1.08)	10.50 (1.26)	6.25 (1.65)	3.00 (1.47)	0.50 (0.5)	0.00 (0)
10	11.25 (0.95)	12.00 (1.08)	10.50 (1.26)	6.25 (1.65)	3.25 (1.14)	0.50 (0.5)	0.00 (0)
11	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.25 (1.65)	3.25 (1.14)	1.00 (0.41)	0.00 (0)
12	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.25 (1.65)	3.25 (1.14)	1.25 (0.25)	0.00 (0)
13	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.50 (1.55)	5.75 (2.53)	2.00 (0.71)	0.25 (0.25)
14	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.50 (1.55)	5.75 (2.53)	2.25 (0.63)	0.25 (0.25)
15	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.50 (1.55)	6.25 (2.39)	2.50 (0.87)	0.25 (0.25)
16	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.50 (1.55)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
17	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
18	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
19	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
20	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
21	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
22	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
23	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
24	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
25	11.25 (0.95)	12.00 (1.08)	10.75 (1.49)	6.75 (1.49)	6.25 (2.39)	2.75 (1.11)	0.25 (0.25)
30	11.25 (0.95)	12.00 (1.08)	12.75 (1.49)	7.00 (1.58)	8.25 (1.65)	3.50 (1.26)	4.00 (0.82)

Juncus acutus

Time (Day)	0 (± SE)	5 (± SE)	10 (± SE)	15 (± SE)	20 (± SE)	25 (± SE)	30(± SE)
1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
2	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
3	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
4	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
5	0.25 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
6	12.5 (1.32)	5 (3.32)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
7	22.75 (0.85)	16.25 (4.84)	0.25 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
8	23.25 (0.63)	22.25 (1.18)	3.5 (1.32)	0.25 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)
9	23.25 (0.63)	23.25 (0.75)	18 (2.74)	0.5 (0.29)	0.00 (0)	0.00 (0)	0.00 (0)
10	24 (0.71)	24 (0.71)	19 (2.35)	1.5 (0.96)	0.00 (0)	0.00 (0)	0.00 (0)
11	24 9 (071)	24.25 (0.75)	20.25 (2.5)	2.5 (1.89)	0.25 (0.25)	0.00 (0)	0.00 (0)
12	24.5 (0.5)	25 (0)	22.5 (1.55)	3.25 (1.93)	0.5 (0.29)	0.00 (0)	0.00 (0)
13	24.5 (0.5)	25 (0)	23.25 (1.11)	5.75 (4.13)	1.5 (0.65)	1.25 (0.48)	0.00 (0)
14	25 (0)	25 (0)	23.5 (1.12)	6.25 (3.92)	1.5 (0.65)	1.5 (0.29)	0.00 (0)
15	25 (0)	25 (0)	23.75 (.95)	6.75 (4.09)	1.5 (0.65)	1.5 (0.29)	0.00 (0)
16	25 (0)	25 (0)	24 (0.71)	7.5 (4.09)	2.5 (0.87)	1.5 (0.29)	0.00 (0)
17	25 (0)	25 (0)	24 (0.71)	8 (3.86)	2.5 (0.87)	1.5 (0.29)	0.00 (0)
18	25 (0)	25 (0)	24 (0.71)	8 (3.86)	3 (0.71)	1.5 (0.29)	0.00 (0)
19	25 (0)	25 (0)	24 (0.71)	8 (3.86)	3.75 (1.11)	1.75 (0.25)	0.00 (0)
20	25 (0)	25 (0)	24 (0.71)	10(3.11)	4.75 (1.03)	1.75 (0.25)	0.00 (0)
21	25 (0)	25 (0)	24 (0.71)	10.25 (3.84)	4.75 (1.03)	1.75 (0.25)	0.00 (0)
22	25 (0)	25 (0)	24 (0.71)	10.75 (3.77)	6.5 (1.94)	2.25 (0.63)	0.00 (0)
23	25 (0)	25 (0)	24 (0.71)	11 (3.77)	7 (1.96)	2.25 (0.63)	0.00 (0)
24	25 (0)	25 (0)	24 (0.71)	11 (3.77)	7 (1.96)	2.25 (0.63)	0.00 (0)
25	25 (0)	25 (0)	24 (0.71)	11 (3.77)	7 (1.96)	2.25 (0.63)	0.00 (0)
30	25 (0)	25 (0)	25 (0)	19.25 (2.1)	16.75 (2.25)	18.5 (1.32)	20.75 (1.49)

Juncus kraussii

Time (Day)	0 (± SE)	5 (± SE)	10 (± SE)	15 (± SE)	20 (± SE)	25 (± SE)	30 (± SE)
1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
2	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
3	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
4	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
5	23.5 (0.65)	19.5 (2.5)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
6	24 (0.71)	24 (0.58)	2.25 (0.85)	2.25 (1.11)	0.00 (0)	0.00 (0)	0.00 (0)
7	24.5 (0.29)	24.75 (0.25)	14.25 (4.73)	5.75 (2.78)	0.00 (0)	0.00 (0)	0.00 (0)
8	24.75 (0.25)	24.75 (0.25)	17.5 (5.27)	7 (3.58)	0.00 (0)	0.00 (0)	0.00 (0)
9	24.75 (0.25)	24.75 (0.25)	18.75 (4.63)	8.75 (3.4)	0.00 (0)	0.00 (0)	0.00 (0)
10	24.75 (0.25)	24.75 (0.25)	19.25 (4.13)	10.25 (3.2)	0.00 (0)	0.00 (0)	0.00 (0)
11	24.75 (0.25)	24.75 (0.25)	21 (2.41)	12.5 (2.87)	0.00 (0)	0.5 (0.29)	0.00 (0)
12	24.75 (0.25)	24.75 (0.25)	22 (2.04)	12.75 (2.66)	0.25 (0.25)	0.5 (0.29)	0.00 (0)
13	24.75 (0.25)	24.75 (0.25)	22 (2.04)	13.5 (2.79)	0.5 (0.29)	0.5 (0.29)	0.00 (0)
14	24.75 (0.25)	24.75 (0.25)	22.5 (1.55)	13.5 (2.79)	0.5 (0.29)	0.5 (0.29)	0.00 (0)
15	24.75 (0.25)	24.75 (0.25)	22.5 (1.55)	13.5 (2.79)	1.75 (1.11)	0.5 (0.29)	0.00 (0)
16	24.75 (0.25)	24.75 (0.25)	22.5 (1.55)	15.5 (3.4)	2 (1)	0.5 (0.29)	0.00 (0)
17	24.75 (0.25)	24.75 (0.25)	23 (1.08)	16 (3.4)	2 (1)	0.5 (0.29)	0.00 (0)
18	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17 (2.94)	5.5 (2.06)	0.5 (0.29)	0.00 (0)
19	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.5 (2.06)	0.5 (0.29)	0.00 (0)
20	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.5 (2.06)	0.5 (0.29)	0.00 (0)
21	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.5 (2.06)	0.5 (0.29)	0.00 (0)
22	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.5 (2.06)	0.5 (0.29)	0.00 (0)
23	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.75 (1.89)	0.5 (0.29)	0.00 (0)
24	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.75 (1.89)	0.5 (0.29)	0.00 (0)
25	24.75 (0.25)	24.75 (0.25)	23 (1.08)	17.5 (3.59)	5.75 (1.89)	0.5 (0.29)	0.00 (0)
30	24.75 (0.25)	24.75 (0)	25 (0.48)	25 (0.87)	25 (2.12)	24.75 (0.75)	24.25 (1.55)

Mean daily cumulative germination (± SE) of 25 seeds of *Phragmites australis*, *Juncus acutus* and *J. kraussii* (N = 4). Trial lasted 25 days with recovery period of 5 days Germination temperature 10-25°C. Trial commenced 14th October 2003

	Phragmites australis						
Time (Day)	0 (± SE)	5 (± SE)	10 (± SE)	15 (± SE)	20 (± SE)	25 (± SE)	30(± SE)
1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
2	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
3	1.75 (0.48)	0.25 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
4	7.50 (0.29)	3.25 (0.48)	1.50 (0.65)	0.50 (0.29)	0.00 (0)	0.00 (0)	0.00 (0)
5	11.25 (0.95)	9.25 (0.85)	6.25 (1.11)	2.75 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)
6	12.00 (1.08)	11.25 (1.25)	9.25 (1.93)	5.75 (1.55)	0.00 (0)	0.50 (0.50)	0.00 (0)
7	13.00 (1.47)	12.25 (1.44)	11.25 (2.06)	7.75 (1.25)	3.75 (0.95)	0.75 (0.48)	0.00 (0)
8	13.00 (1.47)	12.25 (1.44)	12.25 (2.29)	10.50 (1.85)	6.75 (1.25)	1.75 (0.25)	0.00 (0)
9	13.25 (1.65)	12.50 (1.26)	13.00 (2.38)	12.75 (1.75)	8.75 (1.18)	2.75 (0.25)	0.25 (0.25)
10	13.25 (1.65)	12.50 (1.26)	13.50 (2.4)	14.00 (1.78)	8.75 (1.18)	3.00 (0)	1.00 (0.81)
11	13.25 (1.65)	12.50 (1.26)	13.75 (2.25)	15.00 (1.08)	9.75 (0.85)	4.50 (0.29)	1.75 (0.85)
12	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	15.00 (1.08)	10.50 (0.65)	5.50 (0.65)	2.25 (1.03)
13	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	15.75 (0.95)	11.00 (0.91)	7.00 (1.08)	3.25 (0.63)
14	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.00 (0.91	7.00 (1.08)	3.25 (0.63)
15	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.00 (0.91	7.25 (0.95)	3.50 (0.87)
16	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.00 (0.91	7.75 (0.11)	3.75 (0.85)
17	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.00 (0.91	8.25 (0.95)	4.50 (0.87)
18	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	8.50 (0.87)	5.00 (1)
19	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	9.25 (1.11)	5.25 (0.95)
20	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	9.75 (0.85)	5.75 (1.11)
21	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	10.00 (0.95)	6.25 (1.31)
22	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	10.00 (0.91)	6.50 (1.19)
23	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	10.00 (0.95)	6.50 (1.19)
24	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	10.25 (0.75)	6.50 (1.19)
25	13.25 (1.65)	12.50 (1.26)	14.00 (2.12)	16.25 (0.48)	11.25 (0.75)	10.25 (0.75)	6.50 (1.19)
30	13.50 (1.85)	13.00 (1.08)	14.00 (2.12)	16.25 (0.48)	11.75 (1.03)	11.75 (1.03)	10.75 (1.65)

Juncus acutus

Time

(Day)	0 (± SE)	5 (± SE)	10 (± SE)	15 (± SE)	20 (± SE)	25 (± SE)	30(± SE)
1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
2	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
3	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
4	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
5	2.3 (0.63)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
6	13 (1.31)	3.3 (0.75)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
7	20 (0.95)	9.8 (1.11)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
8	24(0.48)	18 (1.5)	3.8 (1.89)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
9	25 (0.25)	22 (0.82)	8.8 (3.45)	0.25 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)
10	25 (0.25)	23 (0.83)	12 (3.04)	3 (0.71)	0.00 (0)	0.00 (0)	0.00 (0)
11	25 (0.25)	25 (0.25)	18 (2.66)	8.25 (1.38)	0.00 (0)	0.00 (0)	0.00 (0)
12	25 (0.25)	25 (0.25)	21 (2.04)	12.8 (1.38)	0.00 (0)	0.00 (0)	0.00 (0)
13	25 (0.25)	25 (0.25)	23 (1.31)	14 (1.78)	0.5 (0.29)	0.00 (0)	0.00 (0)
14	25 (0.25)	25 (0.25)	24 (1.19)	15.3 (2.06)	1.5 (0.5)	0.00 (0)	0.00 (0)
15	25 (0.25)	25 (0.25)	23 (1.03)	16.3 (1.7)	2 (0.41)	0.00 (0)	0.00 (0)
16	25 (0.25)	25 (0.25)	23 (1.03)	17.3 (1.44)	3.8 (1.03)	0.00 (0)	0.00 (0)
17	25 (0.25)	25 (0.25)	24 (1)	17.5 (1.5)	3.8 (1.03)	0.00 (0)	0.00 (0)
18	25 (0.25)	25 (0.25)	24 (1)	18 (1.68)	4.3 (1.25)	0.00 (0)	0.00 (0)
19	25 (0.25)	25 (0.25)	24 (1)	18.8 (1.93)	4.3 (1.25)	0.00 (0)	0.00 (0)
20	25 (0.25)	25 (0.25)	24 (1)	19 (2)	4.8 (1.03)	0.00 (0)	0.00 (0)
21	25 (0.25)	25 (0.25)	24 (1)	20 (2)	6.3 (1.89)	0.00 (0)	0.00 (0)
22	25 (0.25)	25 (0.25)	25 (0)	20 (2)	6.3 (1.89)	0.00 (0)	0.00 (0)
23	25 (0.25)	25 (0.25)	25 (0)	20 (2)	6.5 (1.71)	0.00 (0)	0.00 (0)
24	25 (0.25)	25 (0.25)	25 (0)	20 (2)	7.5 (1.44)	0.00 (0)	0.00 (0)
25	25 (0.25)	25 (0.25)	25 (0)	20 (2)	7.5 (1.44)	0.00 (0)	0.00 (0)
30	25 (0.25)	25 (0)	25 (0)	25 (0)	25 (0)	25 (0)	24.5 (0.29)

Juncus kraussii

Time (Day)	0 (± SE)	5 (± SE)	10 (± SE)	15 (± SE)	20 (± SE)	25 (± SE)	30(± SE)
1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
2	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
3	0.3 (0.25)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
4	3.5 (0.29)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
5	24 (0.58)	14 (.75)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
6	24 (0.48)	20 (1.03)	14 (1.93)	0.5 (0.5)	0.00 (0)	0.00 (0)	0.00 (0)
7	25 (0.03)	22 (1.08)	19 (2.36)	3.75 (1.03)	0.00 (0)	0.00 (0)	0.00 (0)
8	25 (0.25)	24 (0.48)	22 (1.49)	9 (1.78)	0.5 (0.29)	0.00 (0)	0.00 (0)
9	25 (0.25)	24 (0.48)	23 (1.68)	11 (1.47)	1.3 (0.48)	0.00 (0)	0.00 (0)
10	25 (0.25)	25 (0.29)	23 (1.44)	13.25 (0.48)	4.8 (1.93)	0.00 (0)	0.00 (0)
11	25 (0.25)	25 (0.29)	24 (0.95)	14 (0.41)	5.8 (2.17)	0.00 (0)	0.00 (0)
12	25 (0.25)	25 (0.25)	24 (0.95)	15 (0.71)	7.8 (1.89)	0.00 (0)	0.00 (0)
13	25 (0.25)	25 (0.25)	24 (0.75)	15.5 (0.65)	8 (1.78)	0.00 (0)	0.00 (0)
14	25 (0.25)	25 (0)	24 (0.75)	16 (0.91)	9.3 2.06)	0.3 (0.25)	0.00 (0)
15	25 (0.25)	25 (0)	24 (0.75)	17(1.08)	9.5 (1.85)	0.3 (0.25)	0.00 (0)
16	25 (0.25)	25 (0)	24 (0.75)	17 (1.08)	10 (2.12)	0.3 (0.25)	0.00 (0)
17	25 (0.25)	25 (0)	24 (0.75)	17 (1.08)	10 (2.06)	0.3 (0.25)	0.00 (0)
18	25 (0.25)	25 (0)	24 (0.75)	17.25 (1.11)	10 (2.06)	0.5 (0.29)	0.00 (0)
19	25 (0.25)	25 (0)	24 (0.75)	17.5 (1.19)	11 (2.22)	1 (0.41)	0.00 (0)
20	25 (0.25)	25 (0)	24 (0.75)	17.75(1.22)	12 (1.85)	1.3 (0.48)	0.00 (0)
21	25 (0.25)	25 (0)	24 (0.75)	18 (1.11)	13 (1.44)	1.5 (0.65)	0.00 (0)
22	25 (0.25)	25 (0)	24 (0.75)	18 (1.11)	13 (1.44)	1.5 (0.65)	0.00 (0)
23	25 (0.25)	25 (0)	24 (0.75)	18.25 (1.03)	14 (0.66)	1.5 (0.65)	0.00 (0)
24	25 (0.25)	25 (0)	24 (0.75)	18.25 (1.03)	14 (0.66)	1.5 (0.65)	0.00 (0)
25	25 (0.25)	25 (0)	24 (0.75)	18.25 (1.03)	14 (0.66)	1.5 (0.65)	0.00 (0)
30	24.75 (0.25)	25 (0)	24.5 (0.50)	24 (0.41)	25 (0)	24.75 (0.25)	24.75 (0.25)

Appendix B

Average (N=3) metal concentrations (±SD) of potting mix (50% washed river sand, 25% loam soil and 25% organic material (coconut fibre), used to asses the effect of salinity on the survivorship of *Phragmites australis*, *Juncus acutus* and *J. kraussii* (Chapter 4).

Compound	Ave (ppb)	Std Dev	Environmental Background Levels (ppb)	Compound	Ave (ppb)	Std Dev	Background Levels (ppb)
Ag	2.55	0.13	1400	Mn	3025.15	466.04	10000
As	22.73	1.73	2000	Ni	235.38	19.26	60000
Ba	1266	18.57	100000	Pb	50.48	2.82	10000
Bi*	0.39	0.04	48	Rb*	129.9	12.43	90000
Cd	0.46	0.05	1000	Se	3.43	1.7	200
Co	98.10	12.87	20000	Sn	0	0	50000
Cr	202.62	15.79	5000	Sr*	1171	90.02	370000
Cs*	13.57	0.82	300	Tl	0.08	0.08	600
Cu	162.26	54.38	65000	U	5.86	0.2	10
Fe	178505.9	13872.03	1000000	V	350.15	1	50000
Hg	0	0	300	Zn	349.96	58.26	15000
Mg	25629.1	1438.37	500000				

Analysis carried out by Inductively Coupled Plasma Mass Spectroscopy (Advanced Mass Spectrometry Spectrometer Unit, University of Newcastle).

Environmental background values referenced from:

ANZECC/NHMR, 1992. Australian and New Zealand Guidelines for the assessment and management of contaminated sites. Schedule B(1).

* Barbalace K.L. 1995-2007, *Periodic table of Elements* http://EnvironmentalChemistry.com//yogi/periodic Accessed January 2007.

Appendix C

Results of *P. australis*, *J. aucuts* and *J. kraussi* plants subjected to seven salinity treatments.

Trial conducted under controlled conditions, over four months.

Trial commenced January 13, 2003

Sodium concentration (mg/g dry weight)

      Root concentration

      Shoot concentration

Respiration (umol m-2 s-1)

Net photosynthesis (umol m-2 s-1)

Photosynthetic pigments (mg/g dry weight)

      Chlorophyll *a*

      Chlorophyll *b*

      Carotenoids

Biomass (g)

      Root

      Shoot

Stem height (percentage change from t^0)

Density (percentage change from t^0)

Data collected:

Root Na$^+$ concentration (mg/g dry weight)

	Phragmites Australis			*Juncus acutus*			*Juncus kraussii*		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	2.65	2.61	4.88	2.99	4.62	4.24	5.37	5.57	4.31
0	2.16	2.59	3.87	4.62	3.46	5.73	3.61	1.77	1.67
0	2.25	3.11	4.66	4.30	2.74	6.45	2.50	4.61	4.66
0	2.72	2.76	4.78	3.26	4.38	4.62	4.52	5.51	5.93
0	2.90	2.81	7.04	7.04	3.42	5.10	2.59	3.55	5.63
5	3.24	3.56	8.62	5.84	5.99	11.23	3.11	8.05	4.93
5	3.75	2.39	10.71	10.17	5.37	14.74	7.70	7.30	14.51
5	4.26	3.09	10.35	10.52	9.86	15.98	5.93	5.17	16.75
5	4.50	3.53	11.49	8.71	6.17	11.74	5.84	8.37	11.32
5	5.13	2.84	9.72	5.07	6.39	12.33	3.61	7.13	11.74
10	9.17	3.31	12.14	9.50	10.55	9.55	3.69	12.45	12.89
10	5.57	3.99	9.76	7.10	9.27	10.55	3.39	14.33	15.10
10	4.98	3.23	10.00	8.77	10.38	11.73	4.40	12.61	11.96
10	5.59	4.22	14.05	8.70	13.06	11.23	6.12	9.84	20.61
10	6.37	4.08	10.00	6.37	7.78	9.05	6.18	8.25	12.03
15	9.40	3.56	11.74	7.17	16.40	10.46	5.74	9.90	17.59
15	8.17	2.76	14.03	10.67	12.66	11.39	5.70	11.03	14.19
15	5.74	3.32	14.98	9.18	15.30	14.32	3.36	9.48	14.68
15	7.29	4.27	15.12	10.30	12.95	14.95	6.39	11.06	18.21
15	7.32	3.83	11.98	7.62	10.32	8.83	4.26	9.66	18.00
20	5.85	4.85	11.05	13.25	14.44	14.83	10.21	13.98	19.15
20	5.99	4.26	15.49	13.32	15.45	7.53	5.64	8.94	11.76
20	5.66	5.72	22.48	14.19	12.53	15.07	8.09	15.00	18.81
20	9.68	4.73	8.30	11.06	13.16	9.65	8.46	13.75	13.51
20	5.00	5.51	17.78	15.60	13.23	10.77	3.72	14.79	13.52
30	8.48	4.57	19.92	8.97	14.52	16.87	15.62	10.64	17.78
30	5.73	4.41	17.19	11.84	10.83	11.79	8.71	17.25	16.89
30	7.15	4.12	15.16	11.69	10.33	15.71	8.76	24.65	22.72
30	6.90	3.83	20.39	17.21	13.01	9.99	7.82	16.99	11.72
30	6.60	6.05	21.94	10.91	15.82	12.39	7.79	10.78	16.09
40	7.99	5.95	26.82	13.23	8.93	14.31	8.12	15.91	16.09
40	6.88	5.66	28.83	8.68	19.42	15.46	8.95	15.28	10.36
40	7.62	6.33	42.32	12.86	18.90	12.97	11.01	14.15	15.98
40	6.51	8.27	33.92	18.29	19.68	15.88	12.73	17.66	17.27
40	8.82	6.68	50.48	15.70	10.72	19.89	16.57	9.92	11.97

Shoot Na$^+$ concentration (mg/g dry weight)

	Phragmites australis			Juncus acutus			Juncus kraussii		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	0.36	1.24	0.92	4.80	3.68	1.53	4.48	4.43	3.57
0	0.53	0.75	0.56	3.98	2.87	1.60	5.19	4.64	3.49
0	0.42	1.35	0.94	4.47	4.23	2.25	3.96	5.27	2.47
0	0.58	1.52	0.72	3.51	3.00	1.91	4.34	4.72	4.21
0	0.70	1.23	0.72	5.44	4.32	2.24	3.89	5.95	1.74
5	0.32	1.49	0.70	7.02	8.60	5.29	5.62	9.13	6.24
5	0.51	1.16	1.16	6.06	6.05	3.49	6.47	12.62	8.99
5	0.51	1.07	0.55	11.16	9.45	3.42	10.13	10.62	6.96
5	0.44	1.29	1.09	8.73	10.60	4.43	7.01	9.91	6.38
5	0.49	0.95	0.95	6.23	7.91	5.02	6.78	10.17	10.44
10	0.66	1.36	2.01	7.28	10.87	5.90	9.57	15.75	8.71
10	0.53	1.69	1.12	8.87	12.06	3.76	10.86	12.73	7.24
10	0.70	1.22	1.51	12.20	12.65	3.20	8.74	11.72	9.33
10	0.51	1.12	2.58	10.62	14.24	5.24	8.88	15.43	11.81
10	0.61	1.34	2.55	9.89	12.15	4.21	7.65	11.49	11.19
15	0.78	1.86	1.25	10.46	13.49	7.68	11.07	15.70	9.32
15	0.70	1.24	0.63	8.69	17.24	3.99	8.97	13.87	9.97
15	1.04	1.10	0.96	10.33	12.70	5.15	8.41	12.59	10.01
15	0.54	1.56	3.01	8.62	11.73	7.97	10.30	13.29	11.25
15	0.46	1.44	1.98	9.33	13.10	8.36	7.26	13.42	9.92
20	0.51	1.12	1.03	10.92	14.18	6.70	11.54	14.98	12.99
20	0.63	1.93	2.85	9.97	13.05	4.94	12.57	15.60	13.06
20	0.92	1.21	1.90	12.59	14.66	7.85	14.86	13.60	10.38
20	0.70	1.49	1.60	10.88	10.77	6.46	12.25	18.02	10.30
20	0.87	1.24	1.35	8.70	15.34	7.90	10.86	13.02	8.96
30	1.45	2.02	4.56	10.02	15.90	6.91	11.62	17.71	13.02
30	0.68	1.30	5.93	14.18	17.76	8.95	16.04	17.48	12.99
30	0.84	1.63	4.33	10.47	13.99	6.76	14.92	14.87	11.71
30	1.28	3.43	5.23	8.15	16.49	7.13	14.18	18.96	9.07
30	0.54	1.04	8.26	7.45	11.93	7.66	10.88	15.92	13.29
40	0.77	1.74	5.86	10.06	16.73	6.19	14.50	18.29	8.08
40	0.87	3.31	6.13	12.94	15.74	5.80	13.72	18.06	13.45
40	1.44	3.25	6.44	12.26	12.00	5.07	17.86	17.58	11.82
40	1.04	4.08	4.37	12.35	12.29	5.41	13.43	16.74	9.36
40	0.99	2.78	7.10	12.27	13.62	8.99	15.35	17.67	9.63

Respiration (umol m-2 s-1)
Mean of three mid readings used.

	Phragmites australis				*Juncus acutus*				*Juncus kraussii*			
	24 h	48 h	96 h	1 week	24 h	48 h	96 h	1 week	24 h	48 h	96 h	1 week
0	2.34	2.48	1.86	1.65	3.85	5.21	4.34	3.24	2.77	3.93	2.99	2.24
0	2.90	1.52	0.84	1.31	3.95	3.90	2.23	3.40	3.25	4.03	2.41	2.40
0	2.49	2.15	1.41	1.60	4.05	4.10	2.21	4.77	2.76	3.99	1.99	2.77
0	2.09	2.06	1.29	2.29	3.46	4.60	3.97	3.58	2.97	5.01	3.09	2.41
0	1.64	1.48	1.44	1.49	6.29	4.10	6.96	2.74	3.20	5.37	2.25	3.08
5	1.94	0.84	1.19	1.73	2.54	4.11	1.87	4.56	1.49	9.73	2.14	4.56
5	2.16	1.76	1.48	1.26	3.07	3.98	4.04	3.39	2.48	9.72	2.12	3.39
5	2.29	1.61	1.98	1.65	5.21	4.72	1.85	3.20	2.40	3.96	2.08	3.20
5	1.65	1.40	1.36	1.04	4.10	4.81	3.97	3.71	2.38	3.67	2.11	3.00
5	1.55	1.18	1.24	2.21	3.18	3.94	2.97	3.43	1.50	3.67	2.31	3.06
10	1.11	1.26	1.16	2.25	4.59	5.55	1.60	3.23	3.28	4.25	2.68	3.23
10	1.66	1.82	1.78	1.44	2.60	4.55	1.69	1.83	2.60	4.08	1.89	1.83
10	1.18	1.27	0.85	1.74	3.36	4.03	2.41	2.43	3.36	4.46	2.19	2.43
10	1.40	1.49	0.96	1.74	4.96	6.97	3.89	2.50	3.16	4.17	2.86	3.32
10	1.33	1.45	1.37	1.36	3.91	6.49	1.99	2.24	2.57	4.28	2.13	1.81
15	1.07	2.17	1.23	1.07	2.53	5.27	2.10	2.62	2.53	4.17	1.98	2.62
15	1.39	2.26	0.78	1.46	3.99	5.49	2.56	2.60	2.10	3.88	4.25	2.60
15	1.79	1.16	2.00	1.29	4.97	5.45	1.50	2.34	2.44	2.93	2.01	2.34
15	0.79	2.31	0.73	0.58	4.20	3.94	3.90	2.52	2.72	3.84	4.66	2.59
15	0.95	1.88	1.34	1.69	3.91	4.79	3.40	2.44	1.74	2.28	1.77	2.51
20	1.45	1.11	1.13	2.28	5.90	5.72	2.94	4.52	4.10	4.94	1.86	4.52
20	1.19	1.30	0.90	1.35	4.91	3.98	2.13	2.61	3.16	4.79	2.16	2.61
20	1.06	1.10	1.23	1.32	5.59	4.44	2.49	2.32	4.14	4.65	1.82	2.32
20	0.69	1.73	1.05	1.10	4.95	4.91	2.96	3.15	4.00	4.83	1.79	2.64
20	1.32	1.45	1.46	1.47	3.94	5.10	4.97	3.92	3.10	5.09	1.72	2.22
30	1.86	2.03	1.49	1.25	3.91	5.77	2.34	4.09	2.41	3.67	2.21	4.09
30	1.26	1.38	1.65	1.38	2.99	4.98	2.05	4.36	2.06	3.95	2.25	3.36
30	1.39	1.50	2.03	1.22	4.92	6.45	1.50	2.46	2.86	4.50	1.94	2.46
30	1.26	1.38	1.02	1.44	3.98	5.49	2.99	3.97	2.32	3.27	2.33	3.87
30	1.58	1.69	2.62	0.93	3.97	4.94	3.96	2.05	2.25	3.30	1.97	4.42
40	1.47	1.46	0.89	1.18	5.90	6.01	3.91	3.48	5.07	8.15	1.64	3.48
40	1.07	1.20	0.43	0.74	5.02	6.97	1.78	3.54	4.93	8.40	2.76	3.54
40	1.22	1.31	0.75	1.15	6.91	5.81	2.24	2.56	6.91	5.26	1.95	1.56
40	1.41	1.47	0.96	1.43	5.82	6.94	4.96	2.86	4.81	5.51	2.86	3.70
40	1.72	1.86	0.45	1.57	3.99	4.99	3.15	2.84	3.03	5.34	1.78	3.18

Net photosynthesis (umol m-2 s-1)

Mean of three mid readings used.

	Phragmites australis				*Juncus acutus*				*Juncus kraussii*			
	24 h	48 h	96 h	1 week	24 h	48 h	96 h	1 week	24 h	48 h	96 h	1 week
0	12.11	20.76	19.32	15.29	8.75	9.87	14.34	8.59	6.04	9.53	6.11	7.59
0	15.04	20.42	21.06	11.92	6.90	10.39	6.58	6.49	6.27	9.29	4.30	5.49
0	16.55	19.13	18.19	17.29	7.95	8.12	6.17	8.66	6.85	8.97	5.77	7.66
0	16.86	22.08	15.31	20.09	7.33	9.88	8.90	7.73	6.45	8.01	6.01	6.67
0	12.97	15.21	10.90	18.82	9.98	9.85	10.37	8.01	6.51	10.56	4.39	7.37
5	13.11	12.79	13.74	13.71	10.35	9.47	8.61	4.69	6.36	2.21	7.39	4.69
5	15.84	14.69	14.56	14.98	8.46	10.85	4.60	5.41	5.72	2.19	3.53	5.41
5	10.37	10.44	15.41	14.77	9.88	9.61	7.24	8.18	5.65	9.09	8.81	7.18
5	8.72	10.79	16.34	12.57	8.49	6.60	6.57	6.09	5.80	8.92	7.40	5.54
5	7.05	11.06	9.00	18.19	7.96	7.88	9.84	5.30	6.36	8.63	3.40	8.34
10	7.62	10.51	8.29	20.88	6.79	9.00	7.81	6.60	3.77	3.79	3.17	6.60
10	19.14	14.36	21.04	13.58	6.50	9.88	3.09	6.93	4.58	3.54	4.13	6.93
10	11.11	11.78	12.73	13.87	9.48	9.14	6.22	6.10	3.55	8.01	6.12	6.10
10	12.69	13.98	14.65	15.14	4.90	9.96	7.50	6.54	2.80	8.08	2.51	6.21
10	10.77	14.14	12.21	11.14	5.96	8.85	5.49	5.27	4.62	7.70	6.21	6.83
15	11.80	13.71	15.81	10.04	4.89	7.62	8.89	6.92	4.07	5.59	5.64	6.92
15	9.21	10.62	13.08	11.57	8.46	9.90	6.34	4.06	4.54	6.64	2.60	4.06
15	7.85	11.46	8.08	5.95	6.48	7.84	7.02	5.59	4.32	5.99	4.12	5.59
15	4.85	8.95	4.48	7.35	7.97	8.86	4.94	5.52	4.03	4.56	2.28	3.85
15	5.98	8.63	9.70	8.90	5.09	10.87	3.96	4.85	4.82	6.31	4.58	5.46
20	5.34	9.26	10.13	12.84	3.88	6.61	2.96	3.16	1.86	2.03	3.63	3.16
20	6.49	10.01	12.83	9.42	3.85	7.58	4.44	3.36	3.13	1.61	4.97	3.36
20	7.91	10.42	8.59	7.10	3.91	8.93	4.29	5.59	2.32	1.53	5.29	5.59
20	7.99	7.60	8.59	7.17	5.91	8.85	5.95	4.04	2.16	6.22	3.67	3.52
20	10.11	12.44	10.38	6.57	4.79	4.97	3.90	3.86	3.36	6.22	6.70	5.70
30	7.17	6.49	8.57	8.65	5.95	3.07	4.10	1.95	2.41	3.98	3.20	1.95
30	4.46	7.84	-0.29	6.48	3.09	5.50	2.91	2.27	3.70	3.86	4.43	2.27
30	4.49	7.72	4.42	6.35	4.99	6.63	3.39	3.73	1.83	9.06	4.98	3.73
30	9.94	10.03	13.39	10.12	2.98	4.98	2.69	2.65	2.51	8.06	4.67	2.32
30	-1.37	6.51	1.13	9.12	4.60	2.96	3.29	2.38	3.38	7.94	4.88	2.41
40	-1.30	2.57	0.53	1.62	1.96	4.73	3.03	1.19	-0.44	3.89	3.79	1.19
40	-0.10	2.15	2.67	3.41	2.06	2.97	3.71	0.29	1.90	3.76	0.67	1.29
40	-1.51	0.62	-0.79	2.99	1.06	4.43	4.27	2.67	1.26	3.99	3.41	2.67
40	1.50	0.85	2.32	2.50	1.95	3.96	3.63	1.38	1.85	3.03	0.58	1.01
40	-2.20	-1.78	0.81	5.18	2.54	2.97	3.84	1.92	1.68	3.91	3.79	1.31

Chlorophyll *a* (mg/g dry weight)

	Phragmites australis			*Juncus acutus*			*Juncus kraussii*		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	1.04	1.67	1.20	0.89	0.73	0.88	0.50	0.47	0.60
0	1.22	1.02	0.76	0.94	0.69	0.75	0.48	0.42	0.58
0	0.89	1.23	0.92	1.04	0.85	1.00	0.57	0.60	0.57
0	0.90	1.00	1.18	1.03	0.80	1.04	0.57	0.51	0.41
0	0.98	1.59	3.10	0.79	0.66	0.83	0.57	0.50	0.48
5	0.10	0.95	0.92	0.88	0.77	0.86	0.55	0.53	0.48
5	0.99	1.15	1.18	0.89	0.87	0.88	0.50	0.51	0.52
5	0.88	1.04	1.03	0.94	0.94	0.96	0.49	0.44	0.38
5	0.12	0.96	1.25	0.91	0.93	0.91	0.44	0.49	0.46
5	0.88	1.31	1.12	0.89	0.75	0.86	0.55	0.50	0.51
10	0.90	1.41	0.99	0.79	0.74	0.77	0.47	0.57	0.53
10	0.10	1.10	1.04	0.88	1.02	0.87	0.47	0.52	0.49
10	0.61	1.32	1.23	0.84	0.81	0.83	0.58	0.37	0.46
10	0.92	1.23	1.01	0.96	0.84	0.87	0.51	0.60	0.61
10	0.90	1.23	1.14	0.94	0.80	0.77	0.46	0.35	0.36
15	0.11	1.17	1.12	0.85	0.80	0.83	0.57	0.70	0.76
15	0.11	0.95	1.23	0.97	0.75	0.76	0.57	0.40	0.34
15	0.93	0.90	0.98	0.86	0.78	0.86	0.60	0.44	0.45
15	0.76	0.86	1.25	0.84	0.87	0.84	0.53	0.49	0.43
15	0.92	1.06	1.09	0.90	0.62	0.87	0.55	0.52	0.51
20	0.89	1.29	0.97	0.58	0.76	0.58	0.55	0.72	0.82
20	0.80	1.08	1.05	0.56	0.97	0.56	0.53	0.52	0.58
20	0.78	1.26	1.04	0.83	0.76	0.76	0.47	0.22	0.61
20	0.12	1.04	0.98	0.74	0.54	0.74	0.76	0.42	0.80
20	0.91	1.40	0.93	0.55	0.89	0.59	0.63	0.56	0.57
30	0.11	0.93	1.36	0.85	0.70	0.84	0.51	0.56	0.55
30	0.85	1.08	0.77	0.75	0.91	0.80	0.52	0.58	0.54
30	0.11	0.97	0.90	0.72	0.87	0.73	0.52	0.71	0.67
30	0.10	0.91	0.67	0.76	0.84	0.76	0.52	0.50	0.47
30	0.74	1.12	0.04	0.89	0.85	0.88	0.52	0.42	0.49
40	0.76	0.82	0.72	0.83	0.96	0.81	0.45	0.45	0.49
40	0.76	0.75	0.03	0.69	0.78	0.63	0.45	0.34	0.52
40	0.85	0.71	0.49	0.89	0.96	0.76	0.46	0.60	0.56
40	0.11	0.72	0.60	0.91	0.88	0.67	0.50	0.46	0.54
40	0.84	0.49	0.41	0.66	0.87	0.65	0.52	0.41	0.53

Chlorophyll *b* (mg/g dry weight)

	Phragmites australis			*Juncus acutus*			*Juncus kraussii*		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	0.32	0.53	0.39	0.30	0.17	0.36	0.19	0.13	0.16
0	0.38	0.35	0.25	0.34	0.18	0.40	0.18	0.11	0.10
0	0.28	0.40	0.31	0.37	0.25	0.35	0.20	0.15	0.14
0	0.28	0.32	0.36	0.38	0.17	0.38	0.20	0.14	0.12
0	0.30	0.53	0.94	0.28	0.15	0.30	0.21	0.13	0.12
5	0.32	0.31	0.27	0.30	0.20	0.29	0.19	0.18	0.16
5	0.32	0.38	0.34	0.32	0.22	0.31	0.25	0.14	0.15
5	0.28	0.34	0.30	0.32	0.27	0.33	0.25	0.12	0.10
5	0.39	0.33	0.38	0.31	0.23	0.31	0.23	0.13	0.12
5	0.26	0.42	0.35	0.31	0.22	0.30	0.29	0.12	0.12
10	0.26	0.46	0.31	0.27	0.20	0.27	0.22	0.16	0.15
10	0.32	0.35	0.31	0.29	0.30	0.29	0.26	0.14	0.14
10	0.18	0.43	0.37	0.28	0.22	0.27	0.19	0.07	0.07
10	0.29	0.40	0.31	0.33	0.23	0.40	0.22	0.17	0.17
10	0.28	0.50	0.35	0.31	0.22	0.33	0.21	0.09	0.09
15	0.36	0.38	0.34	0.29	0.28	0.28	0.13	0.19	0.21
15	0.35	0.31	0.39	0.32	0.25	0.36	0.12	0.12	0.34
15	0.30	0.29	0.30	0.28	0.27	0.28	0.11	0.11	0.12
15	0.22	0.28	0.39	0.28	0.30	0.28	0.10	0.13	0.12
15	0.29	0.33	0.33	0.31	0.22	0.30	0.18	0.15	0.14
20	0.27	0.43	0.29	0.18	0.27	0.18	0.21	0.24	0.34
20	0.24	0.36	0.33	0.18	0.34	0.17	0.24	0.17	0.19
20	0.26	0.41	0.33	0.29	0.25	0.29	0.16	0.06	0.14
20	0.39	0.32	0.27	0.25	0.55	0.25	0.26	0.13	0.25
20	0.31	0.46	0.28	0.19	0.31	0.14	0.22	0.17	0.20
30	0.34	0.30	0.39	0.28	0.24	0.28	0.17	0.17	0.17
30	0.28	0.35	0.24	0.25	0.31	0.27	0.18	0.19	0.18
30	0.37	0.33	0.27	0.24	0.28	0.24	0.18	0.22	0.28
30	0.29	0.29	0.19	0.25	0.45	0.25	0.17	0.16	0.18
30	0.21	0.35	0.01	0.30	0.27	0.30	0.17	0.14	0.19
40	0.23	0.26	0.19	0.27	0.32	0.27	0.16	0.14	0.15
40	0.23	0.24	0.01	0.24	0.23	0.30	0.16	0.11	0.16
40	0.28	0.23	0.15	0.31	0.34	0.39	0.16	0.18	0.17
40	0.35	0.24	0.17	0.34	0.28	0.46	0.18	0.14	0.16
40	0.29	0.16	0.11	0.22	0.21	0.22	0.18	0.13	0.12

Carotenoids (mg/g dry weight)

	Phragmites australis			Juncus acutus			Juncus kraussii		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	0.19	0.34	0.24	0.18	0.14	0.17	0.10	0.08	0.09
0	0.20	0.19	0.16	0.19	0.13	0.17	0.10	0.09	0.09
0	0.16	0.22	0.19	0.21	0.15	0.21	0.12	0.11	0.11
0	0.16	0.20	0.24	0.21	0.15	0.21	0.12	0.08	0.07
0	0.16	0.28	0.71	0.16	0.14	0.17	0.12	0.09	0.08
5	0.18	0.19	0.21	0.18	0.14	0.17	0.10	0.10	0.09
5	0.18	0.22	0.25	0.18	0.09	0.18	0.08	0.09	0.09
5	0.17	0.20	0.21	0.19	0.18	0.19	0.08	0.07	0.06
5	0.21	0.17	0.27	0.18	0.16	0.18	0.07	0.09	0.08
5	0.16	0.24	0.25	0.18	0.14	0.17	0.09	0.09	0.09
10	0.17	0.28	0.20	0.16	0.13	0.16	0.07	0.09	0.09
10	0.13	0.20	0.24	0.17	0.18	0.17	0.07	0.08	0.08
10	0.12	0.23	0.26	0.17	0.14	0.17	0.11	0.07	0.08
10	0.17	0.23	0.22	0.19	0.15	0.15	0.09	0.10	0.10
10	0.17	0.23	0.24	0.19	0.15	0.16	0.08	0.06	0.06
15	0.19	0.23	0.24	0.17	0.15	0.17	0.09	0.13	0.08
15	0.19	0.23	0.27	0.19	0.14	0.17	0.13	0.06	0.00
15	0.17	0.18	0.21	0.17	0.15	0.17	0.12	0.08	0.08
15	0.14	0.21	0.26	0.17	0.16	0.17	0.10	0.08	0.07
15	0.17	0.20	0.23	0.18	0.13	0.18	0.10	0.09	0.09
20	0.16	0.25	0.20	0.11	0.15	0.11	0.08	0.13	0.18
20	0.15	0.20	0.23	0.11	0.18	0.11	0.08	0.09	0.10
20	0.14	0.23	0.24	0.17	0.15	0.16	0.09	0.03	0.10
20	0.21	0.18	0.22	0.15	0.14	0.15	0.15	0.06	0.12
20	0.16	0.26	0.21	0.10	0.17	0.12	0.12	0.11	0.11
30	0.19	0.18	0.25	0.10	0.13	0.10	0.11	0.10	0.10
30	0.15	0.21	0.16	0.15	0.17	0.16	0.11	0.10	0.10
30	0.20	0.18	0.19	0.14	0.18	0.14	0.11	0.13	0.13
30	0.17	0.18	0.14	0.15	0.14	0.15	0.11	0.09	0.08
30	0.13	0.21	0.01	0.18	0.17	0.18	0.11	0.08	0.10
40	0.14	0.16	0.16	0.17	0.17	0.16	0.10	0.09	0.10
40	0.14	0.14	0.01	0.14	0.15	0.11	0.09	0.07	0.10
40	0.15	0.14	0.12	0.18	0.19	0.17	0.10	0.12	0.11
40	0.20	0.13	0.14	0.18	0.16	0.16	0.11	0.09	0.11
40	0.11	0.12	0.10	0.14	0.18	0.14	0.11	0.08	0.10

Root Biomass (g)

| | *Phragmites australis* | | | *Juncus acutus* | | | *Juncus kraussii* | | |
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	8.15	9.58	40.96	4.83	2.46	7.94	1.56	1.63	7.15
0	7.57	10.65	38.05	4.35	2.49	8.37	1.37	1.74	8.49
0	8.66	8.48	41.51	2.40	2.89	8.05	1.31	1.84	7.97
0	6.76	9.97	39.18	3.61	2.83	7.70	1.42	0.88	8.16
0	8.12	11.03	51.90	6.67	2.84	8.37	1.69	0.97	7.00
5	6.85	13.28	51.90	3.29	2.60	8.06	1.91	0.92	7.61
5	10.35	10.51	45.47	3.98	2.97	8.29	1.54	1.18	7.36
5	9.03	12.56	31.79	3.88	2.44	7.86	1.30	1.49	8.36
5	7.47	11.03	37.68	6.00	2.75	4.92	2.14	1.62	8.13
5	8.80	14.13	52.12	3.79	2.58	8.55	1.66	0.87	7.71
10	6.86	9.38	28.90	4.22	2.39	7.57	1.80	1.55	8.22
10	8.44	14.90	27.86	1.63	2.85	7.52	1.58	0.95	7.83
10	9.69	14.88	37.06	3.99	2.17	7.77	1.89	0.99	7.37
10	6.69	14.78	44.99	5.29	2.64	7.63	1.40	1.28	7.69
10	7.82	11.32	42.09	2.96	2.44	7.88	1.40	1.26	7.70
15	8.57	14.30	39.15	3.22	2.81	7.70	1.72	1.30	7.38
15	8.88	11.43	31.37	2.86	2.76	7.47	1.28	1.14	7.90
15	7.54	10.26	22.58	3.45	2.35	7.46	1.92	1.18	7.69
15	8.00	11.34	24.96	3.42	2.44	8.04	1.49	1.13	7.83
15	9.80	14.93	33.70	3.58	2.54	7.60	1.38	1.19	7.37
20	8.32	11.67	27.97	8.45	2.67	7.61	1.66	1.22	7.65
20	10.65	9.39	16.55	3.27	2.91	7.46	1.71	1.16	7.62
20	10.71	12.96	19.15	8.30	2.46	7.82	1.78	1.19	7.41
20	6.98	10.69	18.63	3.38	2.58	7.66	1.81	1.07	7.98
20	9.77	11.79	29.28	2.81	2.70	6.60	1.65	1.08	7.66
30	8.52	13.31	6.62	2.76	2.72	7.25	1.30	1.11	7.24
30	12.12	10.20	10.42	3.98	2.71	7.31	1.48	1.05	7.30
30	10.28	8.57	7.82	3.66	2.59	7.55	1.65	1.21	7.50
30	8.09	11.96	7.81	5.83	2.40	7.16	1.96	1.08	7.33
30	11.09	12.66	2.64	3.16	2.65	7.28	1.77	0.83	7.19
40	8.78	9.15	6.84	4.60	2.40	7.20	1.30	0.95	6.98
40	8.85	10.89	5.41	3.56	2.60	7.19	1.26	1.17	7.11
40	6.78	8.17	6.76	6.31	2.42	7.37	1.72	1.07	7.14
40	10.77	9.87	9.24	7.80	2.52	7.47	1.86	1.11	7.26
40	8.14	10.86	8.91	5.28	2.39	7.45	1.76	1.19	7.05

Shoot biomass (g)

	Phragmites australis			Juncus acutus			Juncus kraussii		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	9.44	12.26	16.49	2.16	4.91	14.28	1.35	2.85	14.12
0	8.05	13.67	19.95	2.14	5.13	15.87	1.36	2.69	17.71
0	9.50	10.13	23.16	2.24	5.97	16.28	1.50	2.85	14.98
0	9.09	9.68	16.50	2.15	5.88	14.55	1.53	1.95	16.71
0	8.90	13.39	35.56	2.11	5.22	14.34	1.58	1.98	15.09
5	7.94	17.27	26.35	2.26	5.94	13.68	1.90	2.54	13.28
5	9.82	15.52	17.61	2.14	4.70	17.26	1.30	1.80	13.78
5	8.53	13.95	15.87	2.14	5.04	12.80	1.64	2.24	18.15
5	8.65	13.69	16.12	2.10	4.63	13.91	1.21	1.92	13.26
5	8.98	14.88	20.83	2.20	5.40	19.06	1.89	1.77	14.27
10	8.01	10.24	22.95	2.16	5.44	12.83	1.34	2.24	15.33
10	8.87	14.53	9.88	2.22	4.80	10.33	1.44	2.03	11.52
10	9.91	11.66	11.95	2.12	5.01	13.22	1.34	2.17	11.48
10	9.01	15.70	16.45	2.16	3.72	12.40	1.39	1.85	10.56
10	9.99	10.31	13.70	2.22	3.83	14.92	1.35	1.93	11.29
15	11.18	11.89	13.22	2.12	3.97	12.82	1.33	1.40	9.91
15	9.83	9.70	8.04	2.15	4.33	12.82	1.47	1.35	14.99
15	8.69	9.86	17.19	2.19	4.48	13.89	1.56	2.15	10.85
15	8.99	10.24	9.23	2.13	3.74	11.87	1.51	2.02	11.85
15	9.58	13.17	9.82	2.09	3.98	13.38	1.34	2.10	8.75
20	10.93	10.83	11.90	2.06	3.75	13.35	1.75	2.36	12.82
20	9.01	7.47	6.01	2.14	4.19	13.89	1.87	1.39	11.14
20	12.85	10.10	6.57	2.08	3.76	11.71	1.48	1.65	10.35
20	8.15	10.65	10.83	2.12	3.69	14.22	1.82	1.58	12.70
20	10.84	8.88	7.57	2.13	3.95	12.41	1.76	0.82	10.83
30	7.38	9.76	4.73	2.25	3.94	11.48	1.51	0.94	8.84
30	10.20	8.96	4.28	2.22	3.93	9.47	2.02	0.64	9.42
30	9.97	7.93	4.30	2.14	3.92	10.36	1.69	1.05	13.20
30	8.10	9.47	4.39	2.12	4.16	9.32	1.42	0.72	9.25
30	8.95	9.23	1.41	2.31	3.94	9.45	1.56	1.05	9.49
40	9.11	7.14	2.91	2.10	4.32	9.00	1.40	0.39	8.24
40	10.88	6.35	3.36	2.19	3.86	9.87	1.50	1.65	8.80
40	7.30	5.93	3.31	2.08	2.94	8.24	1.40	0.73	9.75
40	9.74	6.82	4.73	2.07	3.87	10.16	1.30	0.94	9.06
40	8.37	8.32	6.29	2.10	3.53	8.40	1.32	0.91	8.19

Height (percentage change from t^0)

	Phragmites australis			Juncus acutus			Juncus kraussii		
	1 week	1 month	4 month	1 week	1 month	4 month	1 week	1 month	4 month
0	0.07	1.55	2.04	0.12	0.28	0.92	0.37	0.45	0.94
0	0.16	1.91	2.60	0.14	0.19	1.05	0.28	0.48	1.32
0	0.08	1.74	1.41	0.19	0.30	1.58	0.27	0.69	0.77
0	0.08	2.24	0.95	0.30	0.28	1.10	0.28	0.93	1.07
0	0.23	1.22	0.96	0.06	0.03	0.71	0.26	0.99	1.11
5	0.08	1.36	2.66	0.17	0.07	0.53	0.36	0.93	1.05
5	0.15	1.72	1.86	0.15	0.13	0.48	0.14	0.33	0.71
5	0.23	0.95	1.68	0.08	0.26	0.33	0.39	0.50	0.81
5	0.18	1.90	1.79	0.23	0.20	0.55	0.15	0.59	0.75
5	0.18	2.12	2.61	0.21	0.32	0.78	0.28	0.24	0.91
10	0.21	1.59	2.19	0.21	0.25	0.16	0.23	0.20	0.85
10	0.14	2.78	1.47	0.08	0.21	0.26	0.20	0.44	0.76
10	0.07	1.75	2.25	0.11	0.31	0.42	0.08	0.26	0.62
10	0.36	1.25	1.88	0.13	0.30	0.45	0.24	0.45	1.03
10	0.07	1.40	2.47	0.28	0.33	0.38	0.26	0.14	0.76
15	0.23	0.26	1.47	0.28	0.14	0.13	0.31	0.69	0.64
15	0.10	1.09	2.39	0.29	0.14	0.55	0.24	0.61	0.86
15	0.08	1.36	2.02	0.15	0.26	0.34	0.14	0.59	0.57
15	0.03	1.37	1.88	0.28	0.39	0.48	0.16	0.48	0.45
15	0.14	1.68	1.61	0.08	0.14	0.27	0.37	0.35	0.64
20	0.03	0.51	0.88	0.22	0.31	0.88	0.22	0.39	0.29
20	0.30	0.29	1.34	0.19	0.15	0.28	0.05	0.20	0.21
20	0.15	1.40	0.87	0.22	0.24	0.48	0.13	0.75	0.48
20	0.07	1.24	0.53	0.16	0.25	0.14	0.24	0.25	0.25
20	0.47	1.95	0.43	0.32	0.10	0.31	0.09	0.26	0.36
30	0.06	-0.23	0.40	0.14	0.10	0.05	0.21	0.37	0.22
30	0.19	1.17	0.03	0.24	0.12	0.31	0.36	0.22	0.23
30	0.10	0.05	-0.12	0.16	0.24	0.26	0.15	0.15	0.28
30	0.13	0.50	0.42	0.12	0.10	0.12	0.27	0.26	0.31
30	0.11	0.39	-0.10	0.10	0.24	0.14	0.26	0.28	0.25
40	0.06	0.20	0.19	0.19	0.20	0.35	0.26	0.18	0.15
40	0.00	-0.18	0.50	0.14	0.13	0.03	0.21	0.14	0.20
40	0.16	0.13	0.16	0.26	0.11	0.03	0.17	0.31	0.24
40	0.13	-0.23	0.44	0.13	0.14	0.38	0.21	0.22	0.31
40	0.04	1.15	0.22	0.16	0.21	0.16	0.28	0.17	0.19

Density (percentage change from t^0)

	Phragmites australis			Juncus acutus			Juncus kraussii		
	1 week	1 month	4 month	1 week	1 month	4 months	1 week	1 month	4 month
0	0.13	0.75	2.20	0.00	0.40	0.80	0.25	0.11	1.25
0	0.00	0.67	1.80	0.13	0.29	0.93	0.00	0.33	2.25
0	0.00	0.67	2.40	0.13	0.67	0.83	0.25	0.38	1.75
0	0.17	0.20	1.71	0.13	0.50	0.60	0.50	0.40	1.40
0	0.00	0.50	2.83	0.29	0.33	0.76	0.25	0.25	1.50
5	0.33	0.57	1.17	0.29	0.33	0.62	0.20	0.71	0.75
5	0.00	0.67	2.44	0.14	0.29	0.71	0.33	0.13	2.00
5	0.14	0.40	1.83	0.00	0.10	0.80	0.13	0.22	0.75
5	0.25	1.75	1.89	0.13	0.33	0.56	0.00	0.33	1.00
5	0.20	0.86	2.00	0.60	0.43	0.71	0.11	0.43	0.80
10	0.14	0.86	1.86	0.00	0.50	0.33	0.00	0.71	1.00
10	0.00	0.29	1.49	0.13	0.45	0.50	0.33	0.33	1.00
10	0.00	0.27	1.64	0.11	0.33	0.71	0.33	0.33	1.00
10	0.00	0.45	2.80	0.00	0.38	0.50	0.25	0.10	0.50
10	0.10	0.43	1.69	0.13	0.40	0.60	0.20	0.40	0.50
15	0.09	0.63	2.67	0.13	0.29	0.29	0.00	0.50	1.00
15	0.00	0.20	1.23	0.00	0.33	0.43	0.50	0.33	0.75
15	0.00	0.43	1.83	0.22	0.20	0.34	0.33	0.33	0.50
15	0.50	0.67	1.56	0.13	0.17	0.50	0.33	0.17	1.33
15	0.00	1.50	1.56	0.20	0.33	0.40	0.14	0.33	0.25
20	0.25	1.00	2.17	0.00	0.20	0.29	0.20	0.20	0.30
20	0.00	0.50	1.50	0.40	0.57	0.29	0.20	0.22	0.30
20	0.00	0.20	1.83	0.20	0.17	0.50	0.17	0.56	0.50
20	0.20	0.60	1.28	0.33	0.33	0.67	0.40	0.33	0.47
20	0.13	0.80	1.44	0.00	0.25	0.60	0.17	0.25	0.30
30	0.00	1.00	0.75	0.40	0.20	0.29	0.40	0.38	0.20
30	0.00	0.18	0.11	0.29	0.40	0.47	0.40	0.40	0.33
30	0.18	0.00	0.08	0.33	0.50	0.50	0.20	0.50	0.50
30	0.00	0.60	0.20	0.40	0.33	0.17	0.33	0.24	0.25
30	0.25	0.63	0.25	0.29	0.14	0.25	0.20	0.33	0.36
40	0.00	0.33	0.11	0.17	0.33	0.20	0.00	0.33	0.56
40	0.00	-0.20	0.14	0.40	0.33	0.25	0.20	0.20	0.33
40	0.00	-0.17	0.06	0.00	0.40	0.33	0.25	0.17	0.33
40	0.50	0.00	0.55	0.20	0.25	0.19	0.25	0.25	0.25
40	0.50	-0.14	0.00	0.29	0.33	0.33	0.33	0.33	0.19

Appendix D

Example of vegetation sampling sheet used throughout the study

Vegetation Sampling Hunter Estuary, New South Wales 20023-04									SPECIES OF INTEREST			
SITE NAME: **SITE NO:**									PHRAGMITES AUSTRALIS			
LONG:				**LAT:**								
PHOTO NO:				**DATE:**					**INVESTIGATORS:**			**MG**
QUADRAT	**PFC**	**Den**	**Height**					**Ave. Height**	**NaCl:**			
			1	**2**	**3**	**4**	**5**					
1									**Rep**	**1**	**2**	**3**
2									**Quad 1**			
3									**Quad 2**			
4									**Quad 3**			
5									**Quad 4**			
									Quad 5			

COMMUNITY COMPOSITION AND ABUNDANCE

	Quad 1	**Quad 2**	**Quad 3**	**Quad 4**	**Quad 5**	**NOTES**
SPECIES	**%**	**%**	**%**	**%**	**%**	
Total						

Appendix E

Average ($\pm$ SE) results of *P. australis* stands (N = 5). Data collected in-situ at various sites within the Hunter Estuary and Lake Macquarie region. Sampling dates were June 2003, October 2003, January 2004 and April 2004.

Data collected:

Soil salinity (ppt). Average of three samples.

Leaf Na^+ concentration (mg/g dry weight). Average of three leaves.

Photosynthetic pigments (mg/g dry weight) Average of three leaves.

       Chlorophyll *a*

       Chlorophyll *b*

       Carotenoids

Density (m^2)

Percentage Foliage Cover

Stem height (m). Average of five stems.

Site No	Description	Site No	Description
1	Tomago, east levee bank	9	Newcastle Wetlands
2	Ash Island, Creek 6	10	Kooragang Island, Cormorant Rd
3	Kooragang Island, BHP pond	11	Ash Island, Creek 3
4	Kooragang Island, Tafe pond	12	Kooragang Island, Weighbridge
5	Kooragang Island, South Arm	13	Speers Pt., Five Island Bridge
6	Tomago, Nature Reserve	14	Teralba, Five Islands
7	Hexham, Floodgates	15	Cockle Creek
8	Pacific Hwy, Hexham	16	Tomago, west levee bank

June 2003

Site No	Soil (ppt)	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m²)	PCF (%)	Leaf NaCl (mg/g)
1	12	4.43	1.06	1.33	1.34	120	30	1.01
1	12	4.06	1.02	1.28	1.50	106	50	1.45
1	12	2.12	0.54	0.64	1.44	129	40	3.57
1	13	3.72	0.84	1.39	1.35	80	25	1.31
1	13	8.82	2.06	2.97	1.52	103	80	3.61
2	0.5	3.47	0.94	1.06	1.64	310	40	0.86
2	1	3.25	0.80	0.98	1.52	303	70	2.63
2	1	1.72	0.49	0.71	1.69	385	45	1.59
2	1.5	1.73	0.52	0.48	1.42	188	50	1.27
2	1.5	2.82	0.69	0.98	1.91	342	100	0.42
3	9.5	3.29	0.86	1.18	1.21	21	15	3.58
3	11.4	3.11	0.71	1.14	1.20	15	15	2.09
3	13.5	3.83	0.99	1.07	1.27	21	20	2.79
3	13.5	2.30	0.61	0.95	1.09	23	20	3.14
3	14.2	3.15	0.76	1.23	1.01	9	3	2.16
4	3	3.69	0.98	1.13	1.71	87	50	2.61
4	4	3.66	0.93	1.19	1.68	221	90	1.60
4	4.5	3.80	0.95	1.09	2.05	289	99	1.30
4	4.5	3.17	0.86	1.02	1.93	359	97	1.66
4	5	2.97	0.79	1.04	1.92	273	95	0.52
5	1	2.91	0.76	1.26	2.31	378	99	0.91
5	1.5	2.71	0.69	1.18	2.38	423	97	1.96
5	1.5	2.92	0.76	1.33	2.23	520	95	1.88
5	2.5	1.84	0.54	1.02	2.04	368	99	1.39
5	3	5.52	1.29	1.33	1.96	443	100	1.16
6	1.5	6.61	1.61	1.35	1.82	528	98	0.97
6	1.5	4.45	1.25	1.01	1.84	241	95	0.58
6	2.5	5.96	1.70	1.25	1.78	212	98	3.38
6	2.6	2.90	0.68	0.95	1.49	275	99	1.02
6	2.8	2.46	0.61	1.09	1.89	409	99	4.71
7	1.5	5.00	1.27	1.40	2.88	224	98	0.36
7	2	3.38	1.00	0.64	2.90	284	95	1.01
7	2.5	2.64	0.65	1.23	2.84	319	100	2.83
7	2.5	4.12	1.06	1.51	2.87	280	100	1.01
7	3	2.07	0.58	1.04	3.04	263	85	1.25
8	0.5	3.81	0.76	0.97	1.64	114	46	0.72
8	0.5	4.51	1.01	1.10	1.50	68	25	0.72
8	1	5.57	1.45	1.23	1.38	167	40	0.58

Site No	Soil (ppt)	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m^2)	PCF (%)	Leaf NaCl (mg/g)
8	1.5	5.30	2.30	1.34	1.54	226	83	0.80
8	2	7.45	1.92	1.23	1.67	208	80	0.50
9	5.5	2.78	0.60	0.98	1.85	310	100	2.14
9	6	2.84	0.75	0.81	1.81	328	100	0.89
9	6.5	3.64	0.82	0.89	1.58	315	100	1.55
9	7	3.23	0.87	0.78	1.77	345	100	1.45
9	7	4.16	1.14	0.95	1.95	322	99	0.64
10	8	3.65	0.92	1.09	2.60	191	99	0.87
10	9	4.65	1.18	1.24	2.57	246	99	0.79
10	10.5	1.87	0.40	0.60	2.65	229	100	0.78
10	13	3.35	0.67	1.30	2.47	215	99	0.58
10	14.5	3.76	0.93	1.50	2.58	268	100	0.50

October 2003

Site No	Soil (ppt)	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m²)	PCF (%)	Leaf NaCl (mg/g)
1	12.00	4.88	1.19	0.85	0.53	73	90	1.82
1	13.50	2.38	1.84	0.61	0.33	52	90	1.52
1	13.80	3.81	1.06	0.67	0.57	84	80	0.94
1	17.20	5.57	1.47	1.03	0.47	135	80	0.94
1	18.30	5.60	1.40	1.19	0.35	84	80	1.74
2	0.60	4.15	0.99	0.87	1.65	172	80	1.16
2	0.90	4.09	1.09	0.88	1.49	167	80	1.45
2	1.20	4.22	1.03	0.90	1.15	162	70	1.01
2	1.20	4.13	0.96	0.89	1.30	169	90	1.60
2	1.80	3.40	0.86	0.75	1.17	183	90	0.94
3	10.20	3.90	1.08	0.76	0.33	3	5	2.03
3	11.10	2.61	0.68	0.52	0.53	14	10	1.59
3	12.00	3.88	1.02	0.89	0.27	3	4	1.16
3	12.30	4.03	1.05	0.77	0.20	3	3	1.38
3	12.90	3.99	1.16	0.75	0.43	4	5	1.30
4	2.50	5.48	1.42	1.08	0.83	68	80	1.60
4	3.00	5.95	1.44	1.32	1.63	92	90	1.67
4	3.30	5.90	1.39	1.10	1.55	103	98	1.60
4	3.60	5.87	1.57	1.16	1.45	49	65	1.81
4	4.20	6.67	1.70	1.41	1.25	37	90	2.55
5	1.80	5.14	1.35	1.13	1.25	77	100	0.94
5	2.10	4.33	1.15	0.92	1.39	83	100	1.45
5	2.10	5.66	1.51	1.33	1.20	78	100	0.94
5	2.40	3.98	0.90	0.91	1.12	75	100	0.94
5	2.70	4.47	1.18	1.02	1.45	103	100	0.94
6	0.60	4.19	1.12	1.01	1.69	111	98	1.16
6	0.65	0.37	0.10	0.10	1.63	72	99	1.46
6	0.83	2.88	0.71	0.85	1.26	138	60	1.08
6	1.71	5.69	1.42	1.29	0.93	129	70	2.06
6	1.90	4.16	1.09	1.04	1.20	147	75	0.94
7	1.20	5.15	1.30	0.95	2.71	186	100	1.09
7	1.30	7.95	2.19	1.55	2.57	190	100	1.45
7	1.50	4.74	1.24	0.99	2.76	194	100	1.53
7	1.50	5.55	1.43	1.09	2.79	187	100	1.30
7	1.60	4.74	1.19	0.95	2.08	104	100	1.16
8	5.70	4.83	1.47	1.02	1.65	96	99	1.23
8	6.60	4.78	1.46	1.05	1.45	79	94	0.87
8	7.20	5.04	1.46	1.03	1.43	175	100	1.37
8	8.40	5.46	3.47	0.75	1.41	197	85	1.08

Site No	Soil (ppt)	Chl a (mg/g)	Chl b (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m^2)	PCF (%)	Leaf NaCl (mg/g)
8	9.00	5.40	1.80	1.08	1.18	171	80	0.87
9	9.60	7.80	1.97	1.69	1.65	116	100	1.16
9	10.60	6.96	1.81	1.62	0.97	126	92	1.09
9	10.90	2.59	0.69	0.63	1.37	158	95	1.16
9	10.90	10.63	2.72	2.20	1.27	139	96	1.01
9	11.60	5.56	1.39	1.40	1.17	78	80	0.72
10	0.90	3.79	0.99	0.92	1.22	165	49	1.82
10	1.20	3.88	1.00	0.87	1.17	169	90	2.83
10	1.20	6.74	1.59	1.98	1.39	191	100	2.25
10	1.60	2.50	0.63	0.63	1.28	183	100	2.55
10	1.60	3.20	0.75	0.96	1.37	204	100	2.98

January 2004

Site No	Soil (ppt)	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m^2)	PCF (%)	Leaf NaCl (mg/g)
1	10.60	3.25	0.86	0.91	0.72	123	90	2.98
1	12.00	4.34	1.16	1.05	0.80	54	95	2.75
1	13.50	5.50	1.50	1.16	0.65	71	100	2.25
1	13.80	3.91	0.99	1.26	1.12	81	100	1.82
1	17.20	3.38	0.80	1.21	0.47	143	70	1.74
2	10.80	3.83	1.02	0.76	0.55	10	10	1.96
2	11.20	2.98	0.79	0.69	0.72	8	8	4.87
2	15.60	3.70	1.00	1.02	0.83	11	10	1.96
2	17.20	3.36	0.92	0.92	0.60	5	5	1.63
2	18.90	2.82	0.78	0.70	0.60	5	5	1.89
3	9.90	2.90	0.80	0.78	2.50	89	70	1.30
3	10.20	4.38	1.09	1.01	2.33	103	80	1.96
3	11.10	4.02	1.05	1.01	2.30	114	90	2.25
3	12.00	4.36	1.17	1.12	1.80	106	100	1.09
3	12.30	4.18	1.12	1.07	2.03	88	80	1.95
4	3.00	4.50	1.29	1.15	0.97	92	98	2.68
4	3.30	4.20	1.25	1.01	0.80	198	99	2.69
4	3.50	4.42	1.20	1.09	0.70	120	80	2.97
4	3.60	3.96	1.22	0.96	0.70	143	100	2.62
4	4.20	5.05	1.47	1.15	0.60	168	100	1.82
5	11.80	4.63	1.31	1.05	2.10	96	80	2.68
5	12.10	4.67	1.36	1.10	1.90	103	90	2.25
5	12.10	4.86	1.36	1.06	1.77	92	100	2.17
5	12.40	5.86	1.63	1.26	2.13	112	100	2.03
5	12.70	5.01	1.44	1.15	2.17	108	95	1.96
6	1.65	3.02	0.81	0.77	0.97	236	80	2.32
6	1.71	2.83	0.90	0.77	0.80	198	40	1.74
6	1.90	3.20	0.88	0.78	0.70	103	70	2.03
6	2.83	3.63	0.94	0.95	0.70	120	95	1.89
6	2.90	3.50	0.92	0.84	0.60	196	100	2.03
7	12.50	5.77	1.63	1.34	1.17	52	50	2.04
7	12.50	4.16	1.12	0.97	1.32	64	70	2.18
7	12.60	3.93	1.05	0.99	0.95	59	60	2.69
7	13.20	4.44	1.13	1.06	1.37	81	70	2.61
7	13.28	0.35	0.09	0.08	0.75	71	60	2.40
8	9.90	4.57	1.22	0.93	0.93	147	50	1.38
8	12.90	4.94	1.29	1.04	0.85	178	50	1.82
8	13.60	4.51	1.23	1.00	1.22	168	60	1.88
8	12.70	4.14	1.00	0.90	0.97	163	65	1.38

	Soil (ppt)	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m^2)	PCF (%)	Leaf NaCl (mg/g)
8	13.40	5.00	1.45	1.00	1.28	163	55	1.59
9	10.60	4.59	1.15	0.94	1.28	126	100	2.48
9	13.40	5.02	1.39	0.98	1.13	62	45	1.67
9	15.70	5.42	1.53	1.13	0.83	28	50	1.89
9	16.60	5.04	1.41	1.06	0.87	39	40	1.38
9	17.20	3.43	1.00	0.69	1.00	114	100	1.67
10	8.30	3.18	0.97	0.47	0.62	141	50	1.74
10	9.30	2.54	0.71	0.51	0.70	148	45	1.31
10	8.60	2.65	0.79	0.64	0.68	152	50	1.67
10	8.60	7.07	1.97	1.28	0.47	143	40	2.62
10	9.90	4.95	1.28	1.04	0.72	138	70	1.72

April 2004

Site No	Soil (ppt)	Chl a (mg/g)	Chl b (mg/g)	Carotenoids (mg/g)	Height (m)	Density (m^2)	PCF (%)	Leaf NaCl (mg/g)
1	15.40	4.23	1.22	1.03	0.50	138	70	0.87
1	16.60	4.20	1.20	0.89	0.77	19	20	0.42
1	19.00	4.70	1.38	0.97	0.64	36	70	0.44
1	21.80	3.75	0.99	0.87	0.73	32	30	0.58
1	26.40	4.51	1.47	1.04	0.72	16	30	0.71
2	9.00	3.91	1.17	0.76	0.77	21	35	0.67
2	10.40	3.20	0.89	0.69	0.72	3	2	1.58
2	10.80	3.43	0.97	0.71	0.76	6	3	0.71
2	12.00	4.94	1.35	1.12	0.89	4	2	1.99
2	12.60	5.58	1.77	1.10	0.91	3	2	1.59
3	2.40	4.15	1.29	0.77	1.85	7	8	0.71
3	4.20	3.36	0.94	0.67	1.58	160	100	0.42
3	6.60	4.53	1.40	0.82	1.54	91	50	0.94
3	7.20	3.72	1.13	0.73	1.79	115	90	0.49
3	8.40	4.54	1.42	0.91	1.83	92	80	0.91
4	8.40	5.05	1.64	1.12	1.60	94	95	1.23
4	8.40	4.08	1.31	0.94	1.53	75	100	1.44
4	10.20	5.17	1.76	1.21	1.70	86	70	1.36
4	10.80	3.92	1.24	1.01	1.50	55	80	1.23
4	13.60	4.97	1.56	1.10	1.47	47	30	1.52
5	10.60	2.28	0.60	0.69	1.80	30	30	1.74
5	10.60	3.54	1.05	0.93	2.00	20	80	0.86
5	10.60	4.67	1.57	0.99	2.23	55	40	0.72
5	10.60	2.14	0.67	0.58	2.33	84	50	0.94
5	10.60	3.87	1.27	0.86	2.43	124	70	0.65

Appendix F

Height and biomass results of *Juncus acutus* and *J. kraussii* plants grown at four densities and three salinity treatments

Average (N = 5) individual plant height and weight of plants grown at four densities

Salinity	Species	Plants per pot	Average height (cm)	Ave shoot weight (g)	Average root weight(g)	Average Total Weight (g)
0	*acutus*	1	50.00	8.10	0.81	8.91
0	*acutus*	1	47.00	7.29	0.62	7.91
0	*acutus*	1	39.50	12.19	1.27	13.46
0	*acutus*	1	53.40	8.67	1.30	9.97
0	*acutus*	1	49.50	15.51	3.02	18.53
0	*acutus*	2	40.25	10.71	1.61	12.32
0	*acutus*	2	45.25	9.29	3.32	10.95
0	*acutus*	2	45.75	12.13	3.62	13.94
0	*acutus*	2	44.50	11.43	3.69	13.28
0	*acutus*	2	40.75	7.69	1.53	8.45
0	*acutus*	3	44.33	6.43	2.55	7.28
0	*acutus*	3	36.67	6.06	3.36	7.18
0	*acutus*	3	45.83	3.50	3.95	4.82
0	*acutus*	3	38.17	7.20	1.73	7.78
0	*acutus*	3	47.50	7.20	4.24	8.62
0	*acutus*	6	38.00	4.20	5.26	5.08
0	*acutus*	6	39.58	4.00	4.12	4.68
0	*acutus*	6	37.25	4.83	5.06	5.67
0	*acutus*	6	35.75	3.99	4.04	4.67
0	*acutus*	6	36.67	4.84	4.38	5.57

Average (N = 5) individual plant height and weight of *Juncus acutus* and *J. kraussii* plants grown at four densities (cont.)

Salinity	Species	Plants per pot	Average height (cm)	Ave shoot weight (g)	Average root weight (g)	Average Total Weight (g)
0	*kraussii*	1	81.00	13.04	1.81	14.85
0	*kraussii*	1	74.50	12.06	2.01	14.07
0	*kraussii*	1	71.50	13.57	2.43	16.00
0	*kraussii*	1	84.50	12.90	1.77	14.67
0	*kraussii*	1	77.00	7.75	0.56	8.31
0	*kraussii*	2	74.50	9.37	2.72	10.73
0	*kraussii*	2	73.75	5.43	1.66	6.26
0	*kraussii*	2	58.50	7.58	2.70	8.93
0	*kraussii*	2	75.25	4.64	3.53	6.40
0	*kraussii*	2	65.75	7.12	1.87	8.05
0	*kraussii*	3	63.67	4.13	1.40	4.59
0	*kraussii*	3	70.83	8.12	3.24	9.20
0	*kraussii*	3	57.33	8.16	2.49	8.99
0	*kraussii*	3	54.50	6.47	1.84	7.08
0	*kraussii*	3	56.00	6.55	3.34	7.66
0	*kraussii*	6	57.17	4.14	3.69	4.76
0	*kraussii*	6	55.17	4.35	3.18	4.88
0	*kraussii*	6	54.25	4.14	3.66	4.75
0	*kraussii*	6	51.42	3.91	3.51	4.49
0	*kraussii*	6	53.92	4.27	3.32	4.82

**Average (N = 5) individual plant height and weight of *Juncus acutus* and *J. kraussii*
plants grown at three salinities and under mono or mixed species treatments**

Salinity	Species	Plants per pot	Average height (cm)	Ave shoot weight (g)	Average root weight (g)	Average Total Weight (g)
0	*acutus*	6	38.00	4.20	0.88	5.08
0	*acutus*	6	39.58	4.00	0.69	4.68
0	*acutus*	6	37.25	4.83	0.84	5.67
0	*acutus*	6	35.75	3.99	0.67	4.67
0	*acutus*	6	36.67	4.84	0.73	5.57
0	*kraussii*	6	57.17	4.14	0.62	4.76
0	*kraussii*	6	55.17	4.35	0.53	4.88
0	*kraussii*	6	54.25	4.14	0.61	4.75
0	*kraussii*	6	51.42	3.91	0.59	4.49
0	*kraussii*	6	53.92	4.27	0.55	4.82
0	*acutus*	3	49.60	6.80	0.78	7.58
0	*acutus*	3	45.50	6.80	0.99	7.79
0	*acutus*	3	42.75	7.99	0.53	8.51
0	*acutus*	3	49.08	6.53	0.50	7.03
0	*acutus*	3	46.17	5.62	1.03	6.66
0	*kraussii*	3	62.50	3.81	0.62	4.44
0	*kraussii*	3	55.25	4.00	0.44	4.44
0	*kraussii*	3	62.00	5.74	0.44	6.18
0	*kraussii*	3	60.83	3.91	0.58	4.50
0	*kraussii*	3	53.00	4.55	0.73	5.27
5	*acutus*	6	36.00	4.86	0.42	5.28
5	*acutus*	6	35.53	4.12	0.38	4.50
5	*acutus*	6	34.83	3.54	0.42	3.96
5	*acutus*	6	36.17	1.88	0.37	2.25
5	*acutus*	6	38.42	2.35	0.47	2.82
5	*kraussii*	6	48.75	5.37	0.46	5.82
5	*kraussii*	6	51.00	4.88	0.48	5.36
5	*kraussii*	6	47.42	4.55	0.37	4.92
5	*kraussii*	6	50.33	5.11	0.32	5.43
5	*kraussii*	6	46.42	5.30	0.40	5.70
5	*acutus*	3	42.42	2.33	0.57	2.90
5	*acutus*	3	36.58	2.66	0.29	2.95
5	*acutus*	3	38.25	3.18	0.38	3.56
5	*acutus*	3	41.83	3.39	0.47	3.86
5	*acutus*	3	40.33	1.82	0.53	2.35

Salinity	Species	Plants per pot	Average height (cm)	Ave shoot weight (g)	Average root weight (g)	Average Total Weight (g)
5	*kraussii*	3	45.00	2.62	0.73	3.36
5	*kraussii*	3	38.67	2.58	0.64	3.23
5	*kraussii*	3	42.33	3.77	0.44	4.22
5	*kraussii*	3	51.17	2.83	0.43	3.27
5	*kraussii*	3	50.50	2.58	0.68	3.26
10	*acutus*	6	33.83	4.48	0.38	4.86
10	*acutus*	6	28.50	3.74	0.31	4.05
10	*acutus*	6	28.50	3.32	0.18	3.50
10	*acutus*	6	23.33	1.16	0.24	1.40
10	*acutus*	6	24.67	0.80	0.17	0.97
10	*kraussii*	6	31.17	0.60	0.16	0.76
10	*kraussii*	6	37.17	0.99	0.20	1.18
10	*kraussii*	6	34.33	2.86	0.12	2.98
10	*kraussii*	6	23.83	0.41	0.09	0.50
10	*kraussii*	6	41.42	3.49	0.20	3.69
10	*acutus*	3	25.17	0.77	0.19	0.96
10	*acutus*	3	28.17	1.14	0.27	1.41
10	*acutus*	3	21.33	1.98	0.30	2.28
10	*acutus*	3	28.00	1.25	0.12	1.38
10	*acutus*	3	20.67	1.16	0.26	1.41
10	*kraussii*	3	33.83	0.62	0.26	0.88
10	*kraussii*	3	35.00	1.07	0.18	1.25
10	*kraussii*	3	33.00	1.58	0.24	1.82
10	*kraussii*	3	29.67	0.98	0.14	1.12
10	*kraussii*	3	20.67	0.60	0.18	0.78