

Final Report

Seeds, seed banks and cultivation of endangered coastal salt marsh plants under climate change



Coastal Saltmarsh, Ana Gracanin©

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PROJECT ORGANISATION

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Seeds, seed banks and cultivation of endangered coastal saltmarsh plants under climate change

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Atton, I.L. (2017) Reproductive biology of saltmarsh chenopods *Sarcocornia quinqueflora* and *Suaeda australis*: Multiple pollination mechanisms and germination success. Honours Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia. (Co-supervised by Dr Karen Sommerville, The Australian Botanic Garden Mount Annan and Dr Amy-Marie Gilpin, Western Sydney University)

Leahy, K.J (2017) Factors influencing the recruitment of coastal saltmarsh plants. Honours Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia. (Co-supervised by Dr Karen Sommerville, The Australian Botanic Garden Mount Annan and Dr Amy-Marie Gilpin, Western Sydney University)

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EXECUTIVE SUMMARY

Background and Significance of the Research

Coastal saltmarsh is an intertidal estuarine community comprised of plant species (succulent herbs, grasses, rushes, sedges, reeds, and shrubs) adapted to inundation by the tides and harsh environmental conditions in the air, water and soil (e.g. temperature, salinity, oxygen availability). Coastal saltmarsh is recognised in Australia and globally as an important ecological community, providing habitat and food for ecologically and economically important species (e.g. fish, shellfish), acting as a natural buffer and pollution filter, and storing carbon. Despite its ecological significance, coastal saltmarsh is one of the most highly impacted and threatened communities in New South Wales (with estimated losses across estuaries in New South Wales since 1950 ranging from 25 to 80%) and this has led the New South Wales government to list Coastal Salt Marsh as an Endangered ecological community (under the NSW Biodiversity Conservation Act 2016) and to protect Marine Vegetation, including saltmarsh plant species examined here (under the NSW Fisheries Management Act 1994). Moreover, the Commonwealth government has listed Subtropical and Temperate Coastal Saltmarsh as a Vulnerable ecological community (under the Environment Protection and Biodiversity Conservation Act 1999). Despite recognition of the ecological importance of coastal saltmarsh, we understand little about the resilience of the plant species that constitute the community, including their potential for natural regeneration through the production of viable seed following disturbance under varying environmental and climatic conditions or how to germinate and grow seed for restoration now and into the future.

Objectives and Approach

The overall objective of our research was to redress this deficiency of knowledge by examining the natural reproductive capacity, seed viability, and seed germination requirements for some of the dominant plants that constitute saltmarsh communities along the south and central coasts of New South Wales, Australia. An important and novel feature of our research was the examination of how variable salinity and temperature regimes, including extreme heat events, influence the viability and germination of seeds of these plant species.

To do this, we used field surveys and temperature-controlled cabinet experiments with a suite of the dominant plant species (the chenopod *Sarcocornia quinqueflora* – samphire or glasswort; the chenopod *Suaeda australis* – seablite; the grass *Sporobolus virginicus* – saltwater couch or sand couch; the rush *Juncus kraussii* – sea rush; and the herb *Samolus repens* – creeping brookweed) from saltmarshes in Lake Macquarie, Brisbane Water and Jervis Bay in New South Wales (see subsection 1.1 General Methods for details of plants and locations studied). This comparative approach across species and estuaries is advantageous because it allows the identification of local influences while permitting general conclusions to be made across the region. The report is divided into three parts: the first is a general introduction (**PART I**) and the remaining three address three specific project objectives:

Part II: To investigate the production and viability of seeds for a suite of dominant saltmarsh plant species across multiple saltmarshes (Lake Macquarie, Brisbane Water, Jervis Bay);

Part III: To quantify how variable temperature and soil salinity influence the germination success of seeds for a suite of dominant plant species; and

Part IV: To quantify the abundance, reproductive output, and seed germination of two key species of saltmarsh plants (the chenopods *Sarcocornia quinqueflora* and *Suaeda australis*) across multiple saltmarshes (Lake Macquarie, Brisbane Water, Jervis Bay).

The outcomes of this research will help to identify and cultivate key plant species for restoration of coastal saltmarshes in New South Wales. More broadly this research will inform climate change adaptation and conservation strategies for these important plant species in these endangered coastal saltmarsh communities.

Significant Findings, Implications and Practical Application of the Research

1. **Part II. Significant Findings.** All coastal saltmarsh plant species examined (*J. kraussii*, *S. repens*, *S. quinqueflora*, *S. virginicus* and *S. australis*) produced viable seed.

Implications and Application. The production of viable seed for all species is positive and likely confers some “resilience” to these species if aboveground vegetation is damaged. Seed is typically dispersed from adult plants into the local soil seed bank (or transported to other saltmarshes), which should lead to the production of new plants and persistence of these species (although little is known about the seed bank or seed dispersal of saltmarsh plants in Australia, and such investigations were beyond the scope here).

2. **Part III. Significant Findings.** (i) All species examined (*J. kraussii*, *S. repens*, *S. quinqueflora* and *S. australis*) had viable seed, but species differed in their germination success and in their response to temperature and salinity. (ii) Germination was greater under fluctuating day and night than constant temperatures for three species, but not for *Sarcocornia quinqueflora*. (iii) Germination decreased with increasing salinity for three species (and this was most pronounced for *Samolus repens*), but not for *Sarcocornia quinqueflora*. (iv) For all species, germination decreased with increasing temperature, but this was most pronounced for *Samolus repens*. (v) The negative effect of temperature on germination was generally enhanced at higher salinities, such that for all species germination was least at the highest temperatures and salinities.

Implications and Application. This study has demonstrated that species-specific differences and variations in temperature and salinity play important roles in the germination of four common coastal saltmarsh species. Such species-specific responses indicate that managing these plant species under varying environmental conditions will need to be considered on a species by species basis. Germination responses of seeds to temperatures and particularly salinity were, however, largely in line with the apparent tolerances of adult plants and their associated distributions in the field.

Importantly, there were generally consistent responses under some conditions, with fluctuating temperatures enhancing germination success and high temperatures and salinities negatively impacted germination. *Samolus repens* is identified as a particularly sensitive and vulnerable species to increases in temperature and particularly salinity. This research uncovering germination requirements under such variable temperature and salinity regimes for key saltmarsh plant species is a first in Australia. The outcomes will help to identify and cultivate plants for restoration under differing local conditions, as well as to conserve and manage these key plants into the future.

3. **Part IV. Significant Findings.** Plant cover, reproductive output and seed viability for the important and dominant plant species, the chenopod *Sarcocornia quinqueflora* (samphire) were generally high across all local sites and largely comparable among regions, although plants at Lake Macquarie and Brisbane Water saltmarshes were generally shorter than those at Jervis Bay and thus had less volume.

Implications and Application. This regional assessment of plant abundance, reproductive output and seed viability for the important and dominant plant species, the chenopod *Sarcocornia quinqueflora*, is one of only a few such quantitative assessments for any

saltmarsh plant in New South Wales, and thus provides baseline data for future comparisons.

Recommendations for Future Research

1. **Research should be done examining pollination, reproductive output and seed viability for a larger suite of plants across saltmarshes, particularly those that are less common or listed under NSW and Commonwealth Acts.**
2. **Research should be done on the soil seed bank of saltmarshes.** The seed bank can be an important source of new plants for regeneration if aboveground vegetation is damaged. Moreover, cues for germination of stored seed may be different to those of seed collected directly from the plant, and this can inform seed banking.
3. Along much of the coast of New South Wales, there appears to be relatively little quantitative data for plants constituting coastal saltmarshes. **Research is needed to quantify relative abundance, composition and diversity of the plant species in more coastal saltmarshes.** These data should then be integrated more broadly with information from other Local Councils in the region and New South Wales to identify gaps in knowledge and vulnerable species at local and regional levels. Species noted as vulnerable should receive greater investigation at the population level.
4. **More programs cultivating saltmarsh plants and rehabilitating and restoring saltmarsh plants and communities are needed in New South Wales.** For example, Lake Macquarie is one of only a few Local Councils with a pro-active program of cultivation, restoration and conservation of endangered saltmarsh plants.

List of Publications Generated by the Research

The project has contributed to two Honours theses in the School of Biological Sciences (now School of Earth, Atmospheric and Life Sciences) at the University of Wollongong. Some of these data are appropriate for publication in International scientific journals and these manuscripts are in preparation.

Atton, I.L. (2017) Reproductive biology of saltmarsh chenopods *Sarcocornia quinqueflora* and *Suaeda australis*: Multiple pollination mechanisms and germination success. Honours Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia.

Leahy, K.J. (2017) Factors influencing the recruitment of coastal saltmarsh plants. Honours Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia.

PART I

INTRODUCTION, OBJECTIVES, APPROACH AND GENERAL METHODS

1.1 Introduction

Coastal saltmarsh is an intertidal estuarine community comprised of plant species (succulent herbs, grasses, rushes, sedges, reeds, and shrubs) adapted to inundation by the tides and harsh conditions. Coastal saltmarsh occupies the highest tidal elevations on shore, with terrestrial habitats on their landward border and mangroves or mudflats on their seaward side.

Consequently, they are influenced and can be impacted by activities on land and in the sea.

Coastal saltmarsh is recognised in Australia and globally as an important ecological community (see Saintilan 2009). Saltmarsh plants provide habitat and food for a diverse array of species (crabs, mollusc, worms, insects, migratory birds), and serve as a nursery ground for larval and juvenile fish and shellfish. The plants can provide natural buffer against coastal erosion (so-called “green infrastructure”), as well as enhancing water quality by filtering nutrients and concentrating toxic substances. Finally, along with mangroves, saltmarsh plants are being increasingly recognised for their capacity to store atmospheric carbon (so-called “blue carbon”).

Despite their ecological significance, coastal saltmarsh is one of the most highly impacted and threatened communities in Australia and across the globe, with estimated losses across estuaries in New South Wales since 1950 ranging from 25 to 80% (see Daly 2013). Key threats in New South Wales and elsewhere in Australia include land reclamation, urban development, tidal and flood barriers, agriculture, damage by stock, human trampling and off-road vehicles, urban and agricultural run-off, rubbish dumping, mowing, weed invasion, illegal harvesting for human consumption (e.g. samphire *Sarcocornia quinqueflora*), mangrove encroachment, and potentially sea level rise (see Daly 2013).

The potential for future impacts and degradation has led the New South Wales government to list Coastal Salt Marsh as an Endangered ecological community (under the NSW Biodiversity Conservation Act 2016) and protect Marine Vegetation, including saltmarsh plant species examined here (under the NSW Fisheries Management Act 1994), and the Commonwealth government to list Subtropical and Temperate Coastal Saltmarsh as a Vulnerable ecological community (under the Environment Protection and Biodiversity Conservation Act 1999).

Despite recognition of the ecological importance of coastal saltmarsh, we understand little about the resilience of the plant species that constitute the community, including their potential for natural regeneration through the production of viable seed following disturbance or their response to varying environmental conditions under continued anthropogenic pressures or how to germinate and grow seed for restoration now and into the future.

1.2 Objectives and Approach

The overall objective of our research was to address this deficiency of knowledge and further our ability to cultivate the key plant species in endangered coastal saltmarsh communities in New South Wales. Our approach involved quantifying the reproductive capacity and seed germination requirements for some of the dominant plants that constitute coastal salt marsh communities in south and central New South Wales. A key feature of the research was to investigate how variable salinity and temperature regimes, including extreme heat events, might influence the germination of these plant species. The research thus assesses the resilience and capacity for natural regeneration of coastal saltmarsh plants threatened by increasing environmental change. Moreover, it can inform climate change adaptation and conservation strategies for key plant species in these ecological communities. The research also contributes to the identification of potential candidate species for plant restoration.

To do this, we used field surveys and temperature-controlled cabinet experiments with dominant plant species from saltmarshes in Lake Macquarie, Brisbane Water and Jervis Bay in New South Wales (see section 1.1 General Methods for details of plants and locations studied). The report is divided into three parts addressing three specific project objectives:

Part I: To investigate the production and viability of seeds for a suite of dominant plant species;

Part II: To quantify how variable temperature and salinity interact to influence the viability and germination success of seeds for a suite of dominant plant species; and

Part III: To quantify the abundance, reproductive output and seed viability and germination of two key species of saltmarsh plants (the chenopods *Sarcocornia quinqueflora* and *Suaeda australis*) across multiple saltmarshes (Lake Macquarie, Brisbane Water, Jervis Bay) in 2017.

1.3 Study System

The study was done from November 2015 to November 2017. Seeds were collected for two different reproductive seasons, spring 2015 to Autumn 2016 (designated as 2016) and spring 2016 to Autumn 2017 (designated as 2017). Field surveys were done from November 2015 to July 2017. Germination experiments were done from August 2016 to November 2017 at the Environmental Research Centre at the University of Wollongong and the Australian Botanic Garden Mount Annan in New South Wales, Australia.

1.3.1 Study locations and regions

This study was conducted at six saltmarsh locations within three different coastal regions of New South Wales, Australia (Fig. 3.1).

Jervis Bay

The southernmost locations were along Currumbene Creek, in the Jervis Bay region, approximately 180 km south of Sydney. The Woollamia (35°00'S, 150°40'E) and Myola (35°01'S, 150°40'E) saltmarshes are approximately 1.8 km apart, with Myola being closest to the entrance of the estuary. Both areas are dominated by the chenopod *Sarcocornia quinqueflora*, with regions in the upper marsh consisting of dense patches of *Juncus kraussii* interspersed with *Suaeda australis*. The grass *Sporobolus virginicus* is also widespread in these areas (Adam, 1990). At both locations, forests of *Casuarina glauca* form the terrestrial border along the banks of Currumbene Creek (Saintilan and Wilton, 2001). The mangrove *Avicennia marina* is also widely distributed throughout the marsh at both locations (Adam, 1990; Saintilan and Wilton, 2001), although is most dense on banks facing toward the opening of Currumbene Creek into Jervis Bay. The upper marsh in the Jervis Bay region is only inundated at the highest astronomical tide, however the lower marsh zones begin to be submerged from ~1.5m (Ricardo et al. 2014).

Brisbane Water

In the Brisbane Water region, the two saltmarsh locations at Empire Bay Wetlands (33°29'S, 151°21'E) and Cockle Bay (33°20'S, 151°22'E) are similar in community structure, with *S. quinqueflora* and *A. marina* widely distributed through the middle and lower marsh areas, and small fringes of *S. virginicus* and *J. kraussii* at the upper limits of the marsh (Harty and Cheng, 2003). Small patches of *S. australis* are situated amongst *J. kraussii* and frequently at the base of *A. marina* in the middle to upper marsh. Both Empire Bay and Cockle Bay

saltmarshes are accessible to vehicles, and the Empire Bay marsh area is separated by a central walking trail.

Lake Macquarie

The Teralba location (32°57'S, 151°36'E), at the northernmost point of Lake Macquarie, is nestled amongst highly urbanised areas and has undergone subsequent rehabilitation (Winning and MacFarlane, 2010). This saltmarsh site consists of *A. marina* mangrove zones adjacent to the lake, with open areas of *S. quinqueflora* and *S. virginicus* expanding between the roadway and water. The Swansea location (33°05'S, 151°39'E), at the opening of Lake Macquarie into the sea, is approximately 15 km away from Teralba. The area is dominated by *J. krausii* with interspersed patches of *S. quinqueflora* and sparse cover of *S. virginicus*. Mangrove stands of *A. marina* were only present along a central creek line. Being further from the entrance channel, the Teralba location has a restricted tidal range with few high tides inundating the marsh (Winning and MacFarlane, 2010). Although the Swansea location is located at the entrance channel, it is bordered by sand dunes which likely play a role in preventing frequent inundation by fluctuating tides.

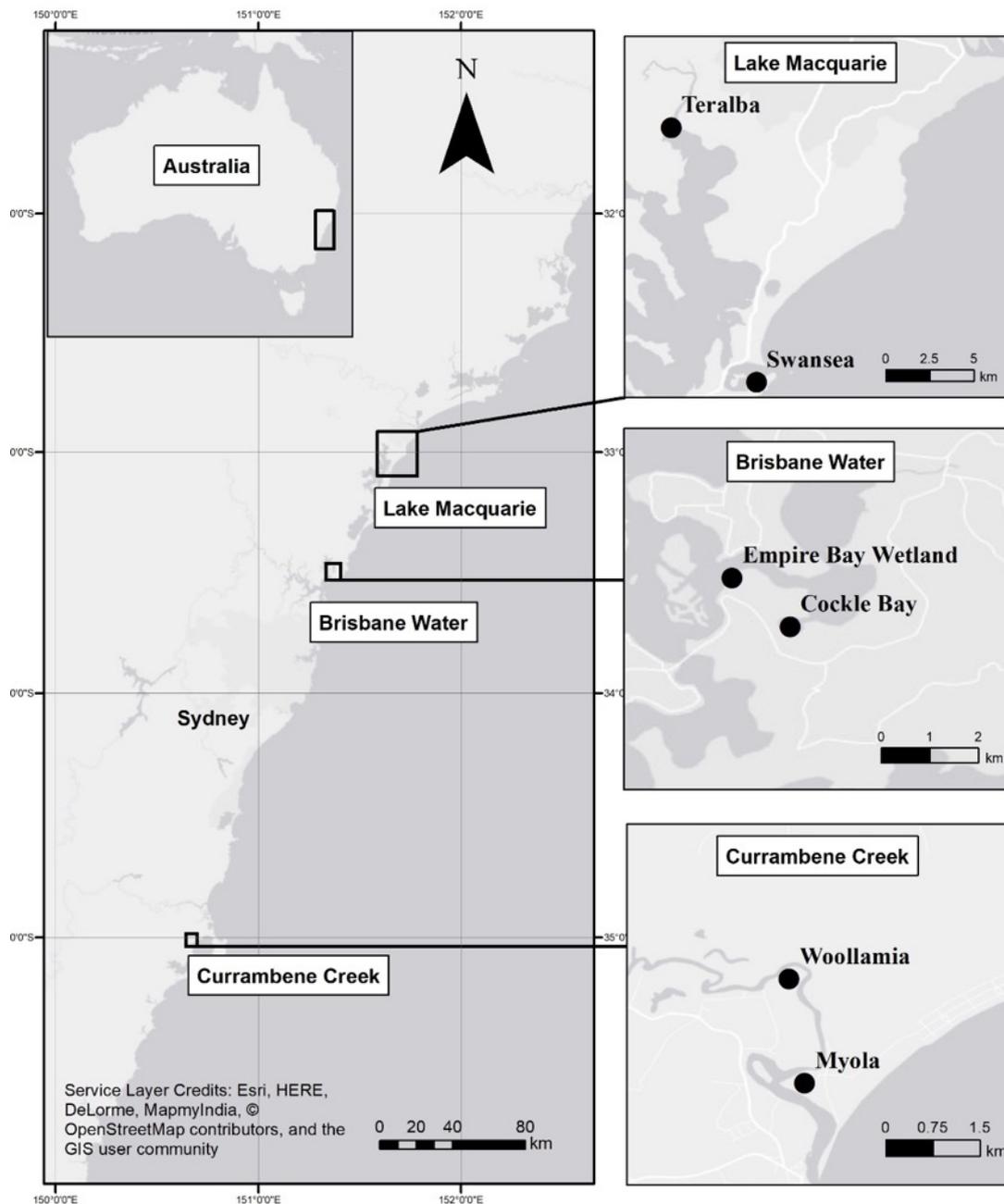


Figure 2.1. Saltmarsh study regions and locations on the New South Wales coast of Australia. Two locations were selected in each of the three regions: Jervis Bay ($35^{\circ}00'S$, $150^{\circ}40'E$), Brisbane Water ($33^{\circ}29'S$, $151^{\circ}22'E$) and Lake Macquarie ($33^{\circ}01'S$, $151^{\circ}37'E$) (I.L. Atton©).

1.3.2 Target saltmarsh plant species

Five species that are native, habitat-forming species that dominate coastal saltmarsh in New South Wales were targeted: the chenopod *Sarcocornia quinqueflora* (samphire, glasswort), the chenopod *Suaeda australis* (seablite), the grass *Sporobolus virginicus* (saltwater couch, sand couch), the rush *Juncus kraussii* (sea rush), and the herb *Samolus repens* (creeping brookweed) (see Fig. 2.2, 2.3 and 2.4). These species were chosen because they are common and widespread throughout New South Wales and are the dominant species from low to high tidal elevations across the saltmarsh landscape (see Saintilan 2009).

Two species, *J. kraussii* and *S. australis*, are typically found at higher tidal elevations, whereas *S. virginicus*, *S. repens* and *S. quinqueflora* are distributed at lower marsh elevations and receive more frequent tidal flushing (Saintilan, 2009) (Fig. 2.1). The chenopods *S. quinqueflora* and *S. australis* flower from summer to autumn and seeds remain attached to plant structures until early June (Raju and Kumar, 2016). The grass *S. virginicus* flowers from spring to summer with seed set generally in summer and autumn. *Samolus repens* flowers from September to February with a peak in November, and sets seed in March (Saintilan, 2009). The inflorescences of *J. kraussii* appear from October, and mature fruit are present from this time and remain attached to the rush until the next flowering period (Adam, 1990).



Figure 2.2. Species distribution in typical coastal saltmarsh ecosystems in New South Wales, Australia (K.J. Leahy©, I.L Atton©).

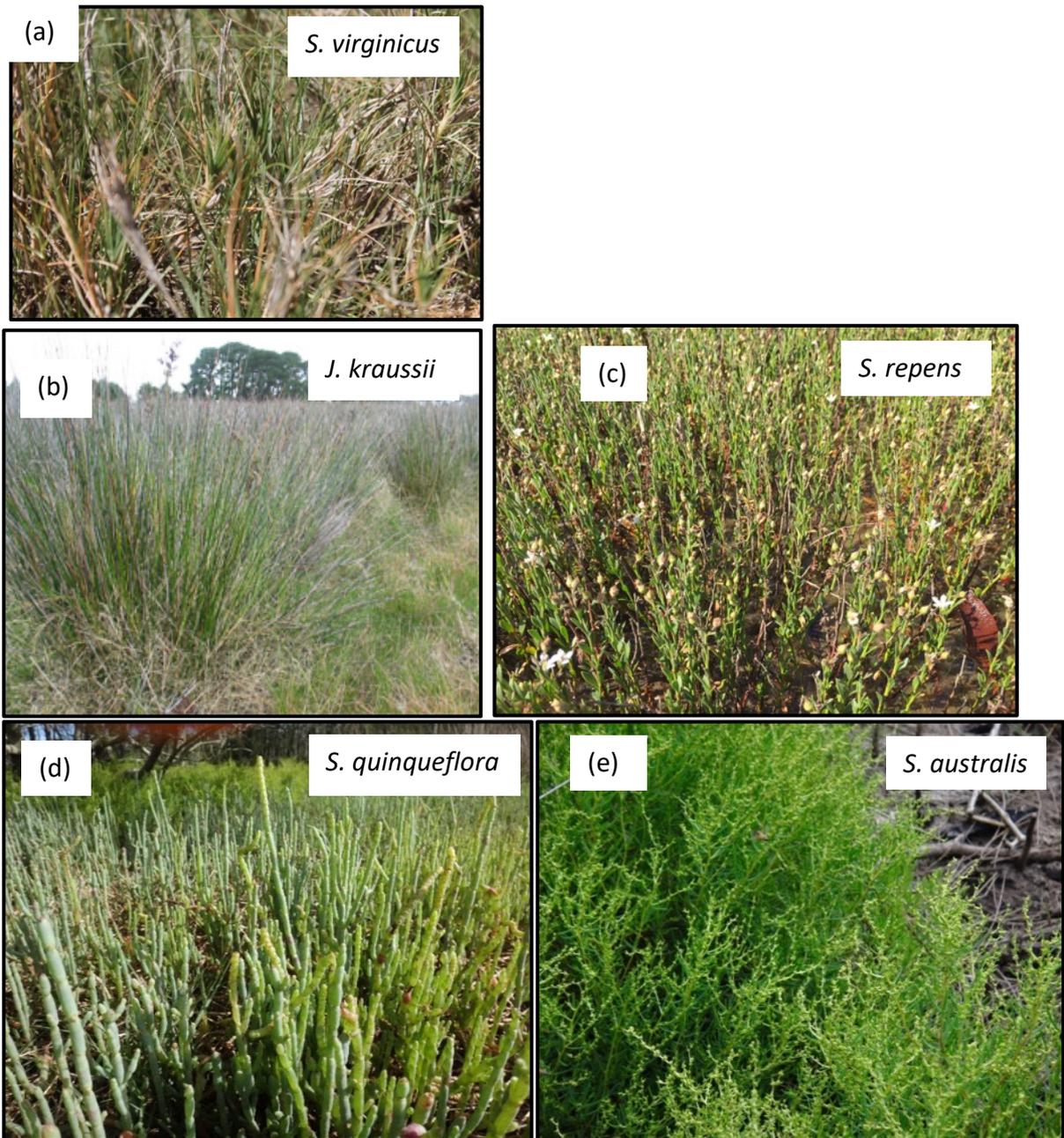


Figure 2.3. Close-up photographs of target species in typical coastal saltmarsh ecosystem: (a) the grass *Sporobolus virginicus*, (b) the rush *J. kraussii*, (c), the herb *S. repens*, (d) the chenopod *S. quinqueflora*, and (e), and the chenopod *S. australis* (K.J. Leahy©, I.L Atton©, T.E. Minchinton©).



Figure. 2.4. Microscopic photographs of seed (a, c, e, g) and fruit (b, d, f, h) of *J. kraussii* (a, b), *S. repens* (c, d), *S. quinqueflora* (e, f) and *S. australis* (g, h). Scale bar on left is in millimetres (K.J. Leahy©).

PART II

A SURVEY OF SEED PRODUCTION AND VIABILITY FOR A SUITE OF COMMON AND ABUNDANT COASTAL SALTMARSH PLANTS

2.1 Introduction

The reproductive biology of plants has been widely considered a crucial field of research for conservation, yielding valuable insight into the function and persistence of populations that constitute ecosystems worldwide (Barrett, 2010; Shivanna, 2014). The production of viable seed is an inherent measure of reproductive success in plants. Seed production requires effective pollination, which may be strongly associated with characteristics of the mating system (Faegri and van der Pijl, 1979), plant morphology (Kevan, 2001), and available vectors of pollination (Kearns and Inouye, 1997). Viable seed contributes to the persistence of a population through time (Shivanna, 2014), and by understanding viability we gain insight into plant regeneration in the face of widespread environmental change (Eckert et al. 2010; Gonzalez-Varo et al. 2013; Shivanna, 2014).

Given that the loss of species from any ecosystem can regularly be attributed to a failure in reproductive processes (Shivanna, 2014), determining the factors governing reproductive success will assist in maintaining biodiversity (Barrett, 2010; Gonzalez-Varo et al. 2013). Moreover, assessing the generality of such processes in space and time is beneficial in providing wider insight into patterns of reproductive output across many environmental contexts (Barrett, 2010). Although broad scale reproductive variation is an important consideration, there exists immense diversity in reproductive function across many related species (Eckert et al. 2010), and even intraspecific variation is commonplace (Michaels et al. 1988; Nathan and Muller-Landau, 2000). The reproductive traits and functionality of species therefore also differ on smaller scales (Galen, 1999).

In many studies primarily concerned with reproductive biology in plants, measures of flower production and fruit set are employed as primary indicators of reproduction (Campbell and Halama, 1993). As described in Larios et al. (2014), much of the previous research in reproductive biology distinguishes germination from reproductive success. Regardless, numerous investigations explicitly study demographic processes like germination and establishment and most comments about reproductive biology are generated from these processes (Clarke and Myerscough, 1993; Kolb, 2005). In some research cases, the final stage of seed production is considered the indicator of reproductive success (da Silva and

Pinheiro, 2009), although this may cause issue as there are a range of abiotic and biotic determinants of seed viability which will reduce both the quality and amount of seed produced.

Saltmarsh communities are frequently tidally inundated and are comprised of low growing halophytic vegetation dominated by succulent herbs, grasses, sedges and low shrubs (Adam, 1990). As Adam (1990) describes, present knowledge of the reproductive niche (which includes the requirements for production through to establishment of viable seed; Grubb, 1986) is significantly limited in saltmarsh plants. There is relatively little evidence, and mostly opportunistic observation, of potential vectors and mechanisms of pollination for saltmarsh plant species (Webb, 2011), regardless of the importance of pollination in initiating the process of seed development (Ollerton et al. 2011). Moreover, there is limited experimental data on the determining factors of seed production and viability in saltmarsh plants (Marks and Truscott, 1985). When considering the goal of restoration to be the active return of a population or community to a self-sustaining state (Vallee et al. 2004; Sommerville et al. 2012), it is fundamental that reproductive and ecological processes influencing the production of viable seed are given due consideration as a key component in long term reproductive functionality in saltmarsh communities (Sommerville et al. 2012).

Providing the deposition of pollen is successful (King et al. 2013), a seed will begin to develop. Seed production is widely used as the quantifiable measure of reproductive output, however gaining insight into the subsequent reproductive success of a species hinges on an understanding of seed development and viability. Currently, there is a lack of information on the other processes likely to interrupt seed development during the maturation stages of saltmarsh plants. Indeed, factors related to maternal (Bertness and Shumway, 1992) and paternal (Bertness and Shumway, 1992; Daehler and Strong, 1994) investment and floral development (Bertness and Shumway, 1992; Clarke, 1995) may be influential in reducing the reproductive success of marsh plants. Maternal resources may also not be adequate for seed production, influencing the viability of seed (Bertness and Shumway, 1992). As the seed matures there are external processes that may impact the seed set of that plant. In saltmarsh communities, there are known biotic factors, such as seed consumption (Ellison, 1987; Rand, 2002), that will inhibit the production of a viable seed or factors that will reduce viability during seed development (Bertness et al. 1987). Insight into the processes impacting the pre-emergent and post-emergent stages of seed development which may inhibit seed viability and

subsequent dispersal are essential for predicting population dynamics in saltmarsh communities.

Of the total number of seeds produced in saltmarsh communities, only some will develop and mature to a stage that will contribute to the persistence of the population (Bertness et al. 1987). In this sense, any evaluation of reproductive biology that may be used to supplement the understanding of population persistence through time should consider the potential for the total seed set to be reduced to a proportion of viable seed.

Here we present the results of (i) field surveys to assess the production of seeds and (ii) germination experiments to assess the viability of seeds for five common and abundant plant species in two saltmarsh locations in each of three estuaries in New South Wales, Australia in 2016 and 2017. The purpose of these investigations was not to provide detailed quantitative assessments, but to examine in a more preliminary way the reproductive output of these plants in a systematic manner and to explore a range of germination techniques to determine if seed were viable. Detailed, quantitative assessments of absolute germination success for most of these species are provided in Part III and IV, and results there confirm and add to results presented in this part of the study. Moreover, to avoid duplication, detailed discussion of the results for this Part of the study can be found in Parts III and IV.

The outcomes here provide a basis for comparison between saltmarsh located in other regions on the coast of New South Wales, therefore providing an indication of wider spatial patterns in reproductive success for these plant species. The findings contribute to current understanding of reproductive biology in saltmarsh plants and help to predict the persistence of populations of dominant saltmarsh species through time.

2.2 Methods

2.2.1 Study locations and regions

This study was conducted from November 2015 to November 2017 in six saltmarsh locations within three different coastal regions of New South Wales, Australia: Woollamai and Myola at Jervis Bay, Empire Bay and Cockle Bay at Brisbane Water, and Swansea and Teralba at Lake Macquarie (see subsection 1.3.1 and Fig. 3.1 for details).

2.2.2 Saltmarsh plant species

Five species that are native, habitat-forming species that dominate coastal saltmarsh in New South Wales were targeted: the chenopod *Sarcocornia quinqueflora* (samphire, glasswort),

the chenopod *Suaeda australis* (seablite), the grass *Sporobolus virginicus* (saltwater couch, sand couch), the rush *Juncus kraussii* (sea rush), and the herb *Samolus repens* (creeping Brookwood) (see Fig. 2.2, 2.3 and 2.4).

2.2.3 Field surveys to assess seed production

Field surveys to assess the production of seeds by plants were done (and seeds were collected) for two reproductive seasons, spring 2015 to Autumn 2016 (designated as 2016) and spring 2016 to Autumn 2017 (designated as 2017), although not all species were assessed in each year (see section 2.3 Results). For each location, seed was collected from >50 plants and combined.

2.2.4 Germination experiments to assess seed viability

To assess the viability of seeds, germination experiments were done from August 2016 to November 2017. A variety of techniques were trialled to assess seed viability and the outcomes presented in the results represent the combined outcomes of the various trials, as well as the results found in Parts III and IV of the study.

Seeds were examined and treated in a variety of ways to assess viability and trigger germination. Seeds were inspected for the presence of endosperm, suggesting the potential for germination. Seeds within or isolated from their fruit (see Fig. 2.3) were placed on filter paper in Petri dishes moistened with freshwater (RO water) and placed under ambient temperature and light conditions in the laboratory or incubated in temperature cycling cabinets (LABEC Laboratory Equipment P.L. Australia) with a fluctuating 12-h temperature (25°C/18°C) cycle corresponding to a 12-hour light/dark cycle. Twenty to 30 seeds from different collection sites were placed in replicate Petri dishes. Seed were sealed with clear plastic wrap or parafilm to prevent mould and fungus which can infect and destroy the seeds. The various germination trials ran for up to 30 days and seeds were monitored at least every three days. Germination was considered to have occurred when the radicle extended 2mm in length.

The goal of this part of the study was to determine if seeds were viable as indicated by their germination and, therefore, cut tests were not performed at the conclusion of the germination. Cut tests are typically done on seeds that do not germinate to determine if the reason is a lack of endosperm, and is important in quantifying germination success, and these were done in Parts III and IV where germination was quantified.

No statistical analysis was done for this part of the study, primarily because here we are reporting on questions about whether seeds are produced and whether they are viable, rather than how many are produced and which percent are viable. Again, detailed, quantitative assessments of reproductive output and germination successes were done in Parts III and IV.

2.3 Results and Interpretive Summary

Field surveys revealed that the majority of plants at all six locations were reproductive and producing seed. Observations in the laboratory showed that almost all seed sampled had endosperm, suggesting that they were potentially viable. Nevertheless, occasionally reproductive structures (e.g. fruit) lacked seed (particularly *S. virginicus*), and individual seeds lacked endosperm. There was some evidence of seed predation (particularly *S. quinqueflora* and *S. australis*), but the identity of predators is not known. Finally, all plant species germinated, indicating that seeds were viable. Germination success varied across species (and was least for *S. virginicus*) and was best under incubator conditions.

Investigations into how temperature and salinity influence seed germination are presented in Part III (but also see Part IV).

Table 2.1. Results of various germination trials for target saltmarsh species across two locations in each of three regions (Jervis Bay, Brisbane Water, Lake Macquarie). Seeds listed as 2016 (2017) were those collected from flowering and seeding period from spring 2015 (2016) to autumn 2016 (2017). Y = Yes, germinated; N = No, not germinated; * = no data.

Plant Species	Jervis Bay		Brisbane Water		Lake Macquarie	
	Woollamia	Myola	Empire Bay	Cockle Bay	Swansea	Teralba
<i>Sarcocornia quinqueflora</i>	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)
	Y (2017)	Y (2017)	Y (2017)	Y (2017)	Y (2017)	Y (2017)
<i>Suaeda australis</i>	Y (2016)	Y (2016)	Y (2016)	Y (2016)	* (2016)	* (2016)
	Y (2017)	Y (2017)	Y (2017)	Y (2017)	Y (2017)	Y (2017)
<i>Juncus krausii</i>	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)
	Y (2017)	Y (2017)	Y (2017)	Y (2017)	* (2017)	* (2017)
<i>Sporobolus virginicus</i>	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)
	* (2017)	* (2017)	* (2017)	* (2017)	* (2017)	* (2017)
<i>Samolus repens</i>	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)	Y (2016)
	* (2017)	* (2017)	* (2017)	* (2017)	* (2017)	* (2017)

PART III

GERMINATION RESPONSES OF COASTAL SALTMARSH PLANTS TO VARIABLE SALINITY AND TEMPERATURE REGIMES

3.1 Introduction

The transition from seed to seedling is the most vulnerable time of life for the majority of plant species, and examining how germination is influenced by abiotic conditions can aid in predicting range limits and distributions of species (Ungar, 1987). Although saltmarsh species commonly spread vegetatively, recruitment from seed is the primary means of colonisation of disturbed environments and is key for the persistence and expansion of a species' distribution (Adam, 1990). Most coastal saltmarsh plants are non-obligate halophytes and they are restricted to marsh ecosystems because they are unable to compete with non-halophytic species (Boorman, 1968). Consequently, they are expected to vary in their ability to withstand the harsh conditions of saltmarsh environments (Boorman, 1968). In particular, coastal marshes are subject to fluctuating temperature and salinity conditions, which are known to affect community structure and composition due to the variable responses of species to such abiotic factors (Howard and Mendelsohn, 1999a).

In coastal intertidal habitats, salinity regimes vary frequently due to constant tidal action, unpredictable storm events and anthropogenic hydrologic changes (Howard and Mendelsohn, 1999a). Consequently, the seeds of species dominating such ecosystems must be able to tolerate high salinities, particularly those inhabiting the low marsh where prolonged submersion in seawater is frequent (Adam, 1990). Most halophytes germinate best in non-saline conditions, with germination success negatively correlated with water salinity in many species (Gulzar et al. 2001). Moreover, seed of sensitive species can lose their viability due to exposure to salt (Vincente et al. 2007). In contrast, obligate halophytes occupying the lower tidal elevations of the marsh thrive in saline conditions, which are sometimes required to stimulate the germination of dormant seeds (Freitas and Costa, 2013). Seeds of many common saltmarsh species are viable following extended immersion in saltwater (Baskin and Baskin, 2001). Nevertheless, the response to salinity varies among species (Egan and Ungar 1999), and therefore it is important to determine individual tolerances of species to predict how the composition of plants is governed across the marsh landscape and at larger spatial scales.

Soil temperature can also affect germination by influencing the rates of biochemical reactions and water absorption (da Silva et al. 2016). Temperature can influence the rate and overall germination of coastal marsh plants because species have different temperature tolerance ranges according to their geographic location and reproductive season (Gulzar et al. 2001). Many species have demonstrated enhanced germination success under fluctuating temperatures, although this is not the case for all species (Fenner, 1985; Khan and Ungar, 1997). Testing seed germination at a range of constant and alternating temperatures is vital to determine the optimal temperature range for germination of saltmarsh plants within specific geographic localities.

Abiotic conditions do not occur in isolation, and the interaction of salinity and temperature is expected to have variable effects on seed germination of coastal marsh plants. Indeed, for some species the greatest inhibition of germination occurs at the extremes of temperature and salinity (Khan and Ungar 1997). With increased salinity, the osmotic potential of the soil narrows the range of temperatures in which a seed can successfully germinate (Khan et al. 2000). Studies have found that the germination of species that tolerate high salt stress is lower at high temperatures (Egan and Ungar, 1999; Ungar, 1987). Nevertheless, species will differ in their ability to tolerate a range of different abiotic cues, and the combination of variables imposed on a species creates a complex environment which needs to be examined to understand species distribution on a global scale and at different tidal elevations in the saltmarsh (Adam, 1990).

Here we assess the influence soil temperature and salinity within the post-dispersal environment on the germination of four common plant species dominant in south and central New South Wales and across many Australian saltmarsh ecosystems: the two chenopods *Sarcocornia quinqueflora* and *Suaeda australis*, the rush *Juncus kraussii* and the herb *Samolus repens*. More specifically, four key aims were investigated: (1) to determine if fluctuating day and night temperatures are required for germination, (2) to determine the range of temperatures suitable for germination, (3) to evaluate the influence of salinity on germination and (4) to investigate the influence of salinity on the maximum temperature at which germination can occur for each species.

It was hypothesised that germination would be highest at fluctuating and intermediate temperatures for all species. It was anticipated that increased salinity would reduce germination and that this would vary among species depending on their tidal elevation in the

marsh. It is expected that salinity will also restrict the range of temperatures at which germination would occur. Considering the endangered status of coastal saltmarsh ecosystems, understanding the impacts environmental drivers may have on recruitment processes, such as germination success, will contribute to our understanding of population dynamics and community connectivity in natural and restored saltmarshes.

3.2 Methods

The germination success of four saltmarsh species, *J. kraussii*, *S. repens*, *S. quinqueflora* and *S. australis*, was examined under variable temperature and salinity from August to September 2017 at The Australian PlantBank within The Australian Botanic Garden Mount Annan, NSW, Australia.

3.2.1 Seed collection and processing

For all experiments, seeds were collected from Myola and Woollamia, which are situated on opposite sides of Currumbene Creek in Jervis Bay NSW, Australia (35.0481° S, 150.7447° E) (Fig. 2.1). Seeds were only used from this region because they were readily available for the widest range of species and only a particular number of treatments could be accommodated using the thermogradient tables (see subsection 3.2.2. Experimental design). As this was the first study of its kind for these species, we opted for testing more temperatures and salinities rather than locations, but results would be expected to apply to the Brisbane Water and Lake Macquarie regions. Seed and fruit of the four study species were collected from Jervis Bay within their respective seeding periods from March 2016 to February 2017. This material was initially stored in dry conditions at ambient temperatures and then from March 2017 was transferred to a drying room at 15% relative humidity and 15°C at The Australian PlantBank where they remained until processing commenced. Due to a lack of seed, *S. australis* was sourced from the Australian PlantBank. This sample of *S. australis* seed was collected from Homebush Bay (33°51'17" S, 151°4'53" E) in May 2006 and stored at -18°C from June 2006. The collection was withdrawn from storage in April 2017 and was held at 15°C and 15% relative humidity until use.

Seed and fruit processing for germination experiments were conducted at The Australian PlantBank within The Australian Botanic Garden Mount Annan, New South Wales, Australia. For each species, empty fruits were first separated from fruits containing seed using a zig-zag aspirator (Selecta Machinefabriek BV, The Netherlands) which separates material based on weight. Subsequent seed extraction methods varied among species. Seeds

were extracted from the fruit of *J. kraussii* by upturning the inflorescence and tapping it to release the seeds. *Samolus repens* fruit were crushed between fingers to expose seeds. *Sarcocornia quinqueflora* and *S. australis* plant material was sieved through 1mm sieves prior to aspiration to obtain a sample of potentially viable fruit. Fruit were gently ground on a rubber mat and rolled between fingers to extract seeds. The majority of empty seed were then separated from seeds with an embryo using a zig-zag aspirator as noted above. Collections from multiple sites at Myola and Woollamia were combined and a random subsample was used for germination experiments.

3.2.2 Experiment design

Temperature was manipulated using two thermogradient tables. Each table generated six constant temperatures (12, 18, 24, 29, 34 and 40°C) and four fluctuating temperatures (24/12, 29/12, 35/12 and 40/12°C), all with a 12/12 h light/dark cycle. These temperatures encompass the range of mean maximum temperatures experienced by all four species throughout their distribution in Australia, and allowed an assessment of the temperature range in which species can successfully germinate. It also allowed for an interpretation of the role of fluctuating temperatures on germination success.

Individual temperatures were generated in separate cells of the thermogradient table (Fig. 3.1a). Each cell contained eight Petri dishes, two replicates of each of the four salinity levels (see below) per thermogradient table. This gave four replicates of each of the temperature and salinity treatments across the two tables, which were considered as random blocks in the design. Ten seeds of each species were sown into a Petri dish containing agar prepared with 0, 25, 50 or 75% seawater. Each dish was then sealed with two layers of plastic tape to prevent evaporation.

Because temperature varies horizontally and vertically within each cell on a thermogradient table, the position of each dish in a stack was reversed, and the position of each stack in a cell was rotated, twice per week. A 1cm strip of copper tape was wrapped around the top and bottom of each dish to facilitate the transfer of heat within a stack and limit temperature differences between the top and bottom dishes (deemed successful during a preliminary experiment) (Fig. 3.1b). This was necessary because the temperature at the agar surface varied by 3°C between the top and bottom plates at the lowest and highest temperatures tested, and varied by 1°C to 2°C in the middle range of temperatures. A data logger was positioned within the centre of each cell to monitor temperatures daily.

Due to the constraints of how temperatures are manipulated across the thermogradient tables, it was not possible to randomly allocate the two replicates of each temperature and salinity treatment within each table. Nevertheless, seeds were randomly selected for each salinity level and Petri dishes were randomly allocated to a temperature cells within the table.

Salinity was manipulated using the agar medium upon which seeds were germinated. The saline agar was prepared by dissolving 8 g L⁻¹ agar in freshwater and adding the required volume of sterile seawater to achieve salinity levels of 0, 25, 50 and 75% seawater (0, 9, 18 and 27ppt, respectively). The seawater was sterilised using a Sabac upright autoclave (Model T62, Sabac Autoclaves, Queensland) to inhibit the growth of bacteria, fungi or algae, which may influence seed germination. The pH of the saline agar solutions was adjusted to 7.2±0.1 using 1M HCl. Agar was used because preliminary trials revealed that agar maintained its water content for considerably longer than sand or filter paper (see Valencia-Diaz and Montana, 2003). Evaporation did occur at the highest temperatures, which was likely to increase salinity levels, so seeds were transferred onto new Petri dishes when the agar level within the plates was low (replating was necessary fortnightly for treatments at 40°C). This allowed the salinity to be readjusted to the required concentration (if necessary) to ensure validity of treatment manipulations. The range of salinities were chosen because they represent those that seeds might experience in the soil in the field (Saintilan, 2009).

Germination was examined two times each week for seven weeks. Germination was considered to have occurred when the radicle extended 2mm in length (as per subsection 2.2.6). Germinated seeds were removed from the plate weekly to prevent disturbance or competition due to the close proximity of seeds in the dishes. For each species, the experiment was concluded when no further germination had occurred for two weeks. At the end of the experiment, cut tests were conducted on the remaining non-germinated seeds. Seeds that did not have the potential of germinating (i.e. not viable) were excluded (see subsection 2.2.6). Germination success was determined by the percentage of seeds that had germinated, and this was calculated as the total number of germinated seeds per Petri dish, divided by the sum of the number of viable seeds (germinated, firm, unconsolidated and discoloured, as determined by cut tests, see subsection 2.2.6) per dish, multiplied by 100.

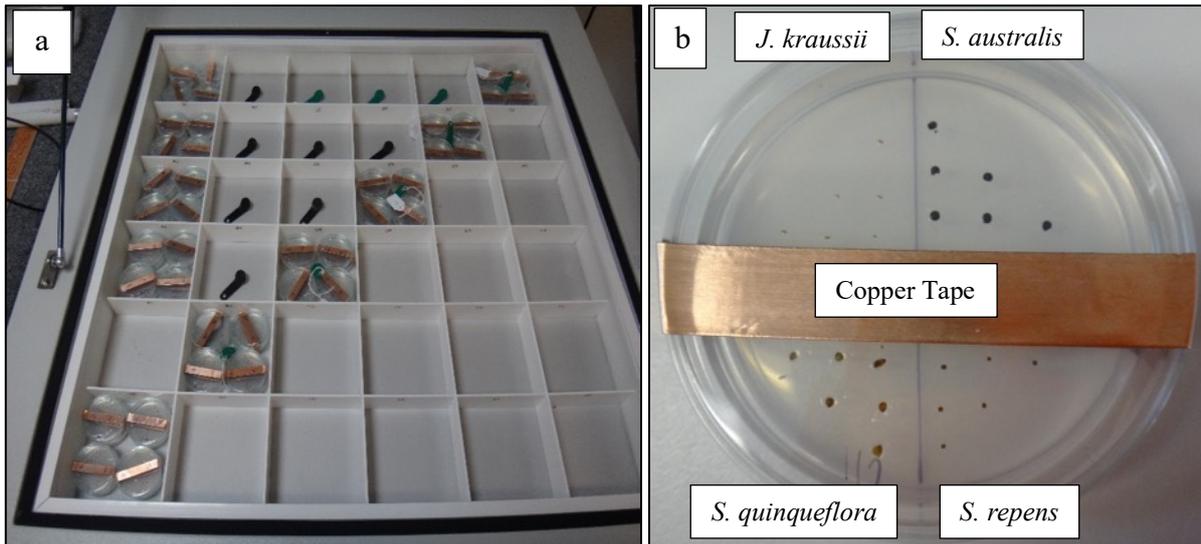


Figure 3.1. (a) Aerial view of the thermogradient table design with four dishes stacked two high in each of the ten temperature treatments (b) 60mm polystyrene petri dish including ten seeds per species, wrapped with copper.

3.2.3 Statistical analysis

Initially for each variable, a three-way, mixed model ANOVA of temperature, salinity and blocks (thermogradient tables) was conducted, and since no differences were detected between tables, the blocking factor was removed from the design. Analyses were then completed by pooling the data across the two tables for each treatment to produce $n=4$ Petri dishes with 10 seeds for each treatment combination for each species.

For each species, separate two-way ANOVA was used to identify differences in total seed germination for two temperature regimes (constant and fluctuating) and four temperatures (24°C, 29°C, 35°C and 40°C). Constant temperature regimes consisted of the given temperatures being maintained for 24h. Fluctuating temperature regimes held each of the temperatures constant for 12h, then alternated to a fixed temperature of 12°C for the following 12h. These analyses were conducted only for the salinity level of 0ppt salinity because this is the salinity at which germination was expected to be highest, and the focus here was to detect differences between the two temperature regimes.

Because the first analysis demonstrated that fluctuating and constant regimes produced significant differences in germination success, the two subsequent analyses examining temperature and salinity were conducted separately for fluctuating and constant temperatures. A two-way ANOVA was conducted to examine total germination in response to salinity level (0ppt, 9ppt, 18ppt and 27ppt) and four fluctuating temperatures (24/12°C, 29/12°C, 35/12°C and 40/12°C). A second analysis examined the total germination in response to salinity level

(0ppt, 9ppt, 18ppt and 27ppt) and five constant temperatures (12°C, 18°C, 24°C, 29°C and 35°C). For the analysis of salinity and constant temperature effects, 40°C was excluded because germination did not occur at this temperature for three species, and germination was minimal (on average below 4% in all treatments) for the fourth species, *S. quinqueflora*.

All data were transformed to the arcsine of their square root to satisfy the assumptions of homogeneity of variances and normality of residuals using Cochran's test and Shapiro-Wilks test, respectively. According to Cochran's test, homogeneity was improved by the transformation although the assumption was not always satisfied, but the analyses were continued due to the robustness of ANOVA to minor variance of heterogeneity (Underwood 1997). Analyses were conducted in JMP Pro 11. Tests were conducted at $\alpha=0.05$. All factors in the design were considered to be fixed factors.

3.3 Results

3.3.1 Seed germination responses to temperature regimes

The seeds of *J. kraussii*, *S. repens* and *S. australis* demonstrated similar patterns of germination in response to temperature regime (Fig. 3.2). For these three species, total germination was significantly higher under fluctuating temperatures compared to constant temperature regimes for most temperatures (Fig. 3.2; Table 3.1).

In the case of *J. kraussii*, total germination was greater than 95% at 24/12°C, 29/12°C and 35/12°C, but declined substantially to less than 20% at 40/12°C (Fig. 3.2). This resulted in a regime by temperature interaction, with significantly greater germination for fluctuating regimes at 24°C, 29°C and 35°C, but no difference in germination between fluctuating and constant temperatures at 40°C (Fig. 3.2a; Table 3.1).

Similarly, for *S. repens*, germination was greater for fluctuating than constant temperature regimes, but only at the lowest day time temperatures of 24°C and 29°C (Fig. 3.2b). This species did not germinate at constant or fluctuating temperatures above 29°C accounting for the significant interaction (Fig. 3.2b; Table 3.1).

Germination of *S. australis* seeds was greater than 85% for all fluctuating temperature regimes (Fig. 3.2d). At all temperatures, fluctuating regimes produced significantly higher germination for this species than constant regimes (Fig. 3.2d; Table 3.1). A significant interaction was observed because differences in germination between constant temperatures were greater at 24°C than at 40°C, where no seeds germinated (Fig. 3.2d; Table 3.1).

In contrast to the other species, there were no significant differences in germination for *S. quinqueflora* under constant versus fluctuating temperatures (Fig. 3.2c; Table 3.1).

Germination for this species was low and variable in comparison to the three other species, and did not reach 40% under any treatment (Fig. 3.2c).

3.3.2 Seed germination responses to salinity and temperature

For all species, except *S. quinqueflora*, an increase in salinity resulted in a reduction in total germination across all fluctuating and constant temperatures (Fig. 3.3, 3.4). For *J. kraussii* under fluctuating temperatures, the effect of salinity on seed germination was dependent on temperature (Fig. 3.3a, Table 3.2). At 40/12°C, little germination occurred and there were no differences among levels of salinity. In contrast, for all other fluctuating temperatures germination occurred at all salinities other than the highest level of 27ppt (Fig. 3.3a, Table 3.2). At 24/12°C and 35/12°C, *J. kraussii* germination at 0ppt salinity was significantly higher than all other treatments except 29/12°C at 0ppt (Fig. 3.3a, Table 3.2). Germination at 9ppt salinity under 24/12°C, 29/12°C and 35/12°C was significantly greater than germination under all temperatures at 18 and 27ppt and 40/12°C at 9ppt (Fig. 3.3a, Table 3.2).

Germination of *J. kraussii* was substantially higher at fluctuating temperatures than constant temperatures (Fig. 3.3a, Fig. 3.4a). Germination at 18°C, was significantly higher than all other constant temperatures other than 24°C and 35°C at 0ppt (Fig. 3.4a; Table 3.3).

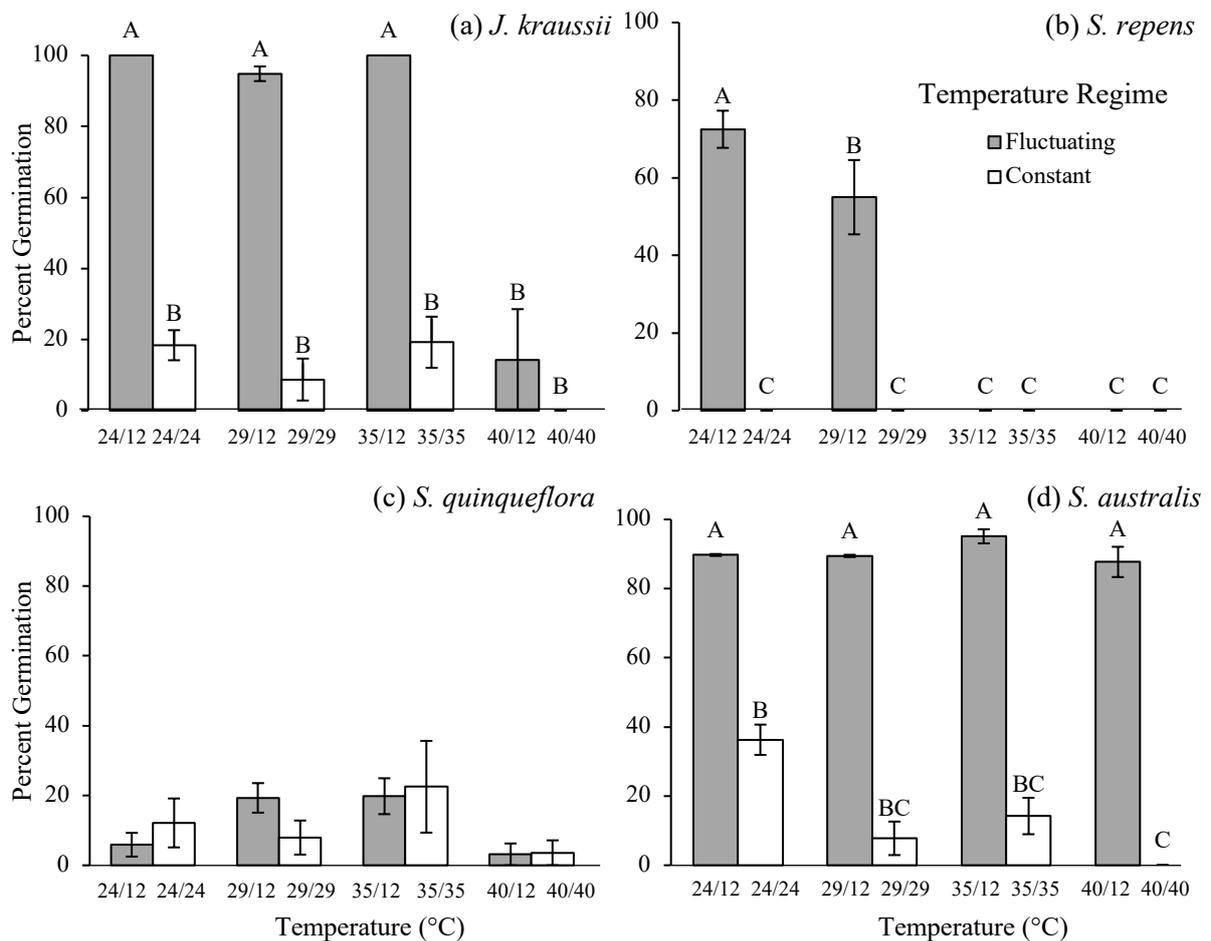


Figure 3.2. Mean (\pm SE) percent germination of seeds of *J. kraussii* (a), *S. repens* (b), *S. quinqueflora* (c), and *S. australis* (d) under fluctuating (24/12°C, 29/12°C, 35/12°C and 40/12°C) and constant (24/24°C, 29/29°C, 35/35°C and 40/40°C) temperature regimes. In each panel, treatments with different letters have significantly different means according to Tukeys HSD at $\alpha=0.05$. Error bars are always present, but small in some cases.

Table 3.1. Analyses of the effect of temperature regime and temperature on germination percent of seeds of *J. kraussii*, *S. repens*, *S. quinqueflora* and *S. australis*. Results are estimates of mean squares (MS), F statistic (F) and probability levels (P) from the two-way ANOVA. Values in bold signify a significant difference at $\alpha=0.05$. Data were transformed to the arcsine of their square roots. ns=not significant.

<i>Species</i> <i>Source</i>	<i>d.f.</i>	<i>J. kraussii</i>			<i>S. repens</i>			<i>S. quinqueflora</i>			<i>S. australis</i>		
		<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Regime: R	1	5.68	111.55	<0.0001	1.73	276.04	<0.0001	0.03	0.46	0.502	6.84	210.45	<0.0001
Temperature: T	3	1.27	24.94	<0.0001	0.59	93.86	<0.0001	0.10	1.58	0.220	0.08	2.45	0.088
R×T	3	0.55	10.85	0.0001	0.59	93.86	<0.0001	0.06	0.96	0.427	0.10	3.10	0.045
Error	24	0.05			0.01			0.06			0.03		
Cochran's C		0.512 ns			0.514 ns			0.427 ns			0.305 ns		

The seeds of *S. repens* had higher total germination at lower fluctuating and constant temperatures (Fig 3.3b, Fig. 3.4b). The highest salinity level of 27ppt prevented the occurrence of germination at all temperatures, under both fluctuating and constant regimes (Fig 3.3b, Fig. 3.4b). Germination occurred at fluctuating temperatures of 24/12°C and 29/12°C at 0ppt salinity, which were significantly higher than for other treatments that germinated at 9ppt salinity at 24/12°C, 29/12°C and 35/12°C (Fig. 3.3b; Table 3.2). Under constant temperatures, germination of *S. repens* was significantly higher at 0ppt, and 12°C than in any other treatment (Fig. 3.4b; Table 3.3).

Germination of the chenopod *S. quinqueflora* was on average less than 30% and highly variable across all treatments (Fig 3.3c, Fig. 3.4c). Under fluctuating temperature regimes, there was a significant effect of temperature on germination, with less germination at 40/12°C than at all other temperatures (Fig. 3.3c). In contrast, there was no effect of salinity on germination at any temperature and at 40/12°C germination only occurred at 0ppt (Fig. 3.3c, Table 3.2). Under constant temperature conditions, there was no effect of salinity of temperature on germination success of *S. quinqueflora* (Fig. 3.4c, Table 3.3).

Similarly, to the other chenopod *S. quinqueflora*, *S. australis* germinated at all salinity levels and the negative influence of salinity increased with temperature (Fig. 3.3d, Fig. 3.4d). Under fluctuating temperature regimes, the pattern of germination for *S. australis* was similar to that of *J. kraussii* and *S. repens*, with differences in the effect of salinity on germination dependent on temperature. Germination declined significantly when salinity rose above 9ppt and as temperatures rose above 29/12°C (Fig. 3.3d; Table 3.2). There was no germination for *S. australis* at 27ppt under the highest temperature of 40/12°C (Fig. 3.3d). Under constant temperature regimes, patterns were generally the same as those under fluctuating regimes but there was less germination overall. Germination was consistently higher at 0ppt salinity under all constant temperature treatments (Fig. 3.4d). Seed germination was the highest at the lowest constant temperatures of 12°C at all salinity levels (Fig. 3.4d; Table 3.3).

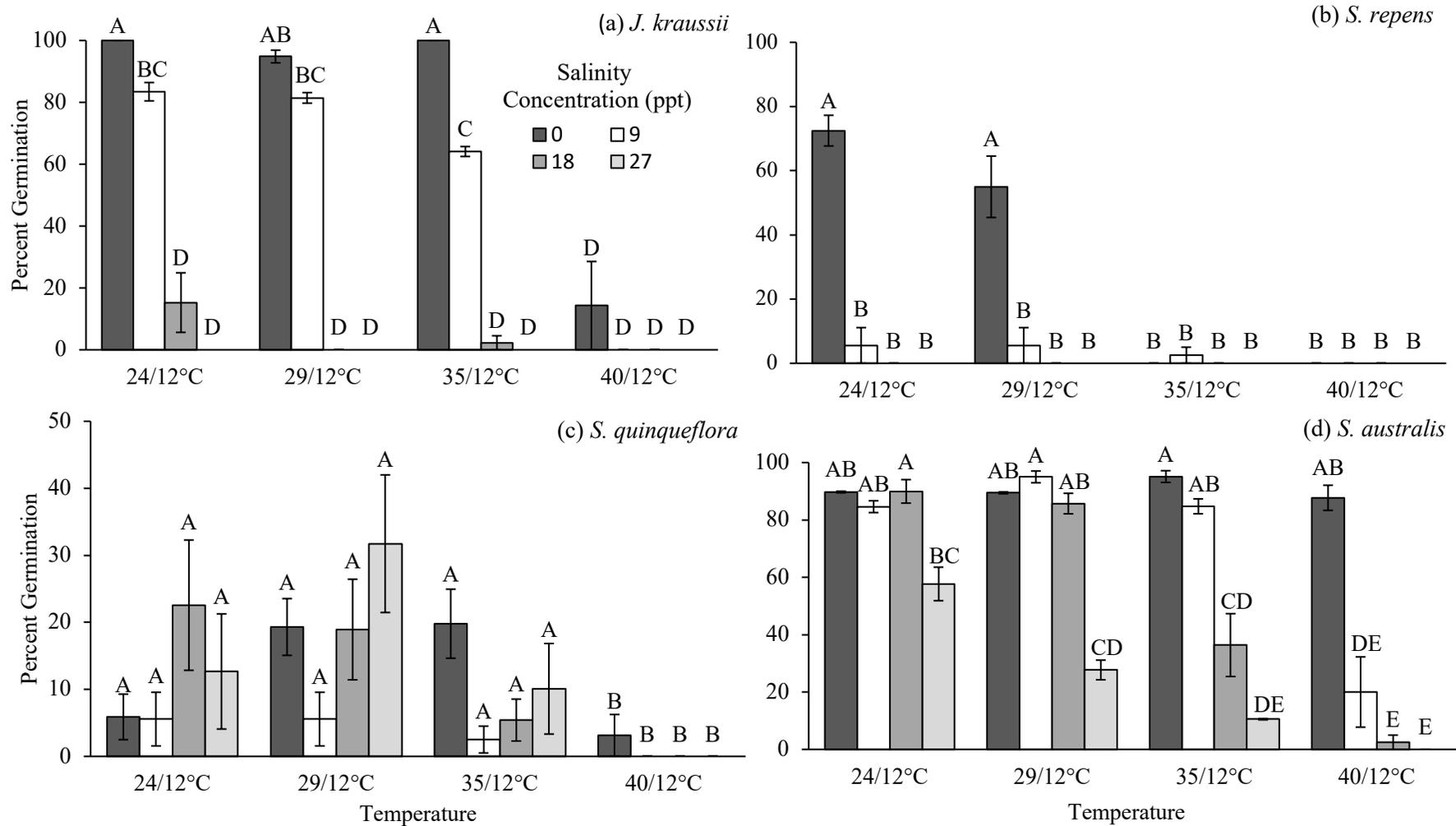


Figure 3.3. Mean (\pm SE) percent germination of seeds of *J. kraussii* (a), *S. repens* (b), *S. quinqueflora* (c), and *S. australis* (d) under fluctuating temperature regimes and four salinity levels (0, 9, 18 and 27ppt). In each panel, treatments with different letters have statistically similar means according to Tukeys HSD at $\alpha=0.05$. Error bars are present but small in some cases. Note different y-axis scales among panels.

Table 3.2. Analyses of the effect of fluctuating temperatures and salinity on germination percent of seeds of *J. kraussii*, *S. repens*, *S. quinqueflora* and *S. australis*. Results are estimates of mean squares (MS), F statistic (F) and probability levels (P) from the two-way ANOVA. Values in bold signify a significant difference at $\alpha=0.05$. Data were transformed to the arcsine of their square roots. ns= not significant, **= significance at $P<0.01$.

Species Source	d.f.	<i>J. kraussii</i>			<i>S. repens</i>			<i>S. quinqueflora</i>			<i>S. australis</i>		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Salinity: S	3	5.19	233.87	<0.0001	0.79	64.35	<0.0001	0.01	0.15	0.931	2.04	80.28	<0.0001
Temperature: T	3	1.59	71.86	<0.0001	0.35	28.38	<0.0001	0.49	10.07	<0.0001	1.73	68.17	<0.0001
S×T	9	0.46	20.57	<0.0001	0.28	22.82	<0.0001	0.08	1.55	0.158	0.31	12.06	<0.0001
Error	48	0.02			0.01			0.05			0.03		
Cochrans C		0.374	**		0.280	ns		0.134	ns		0.370	**	

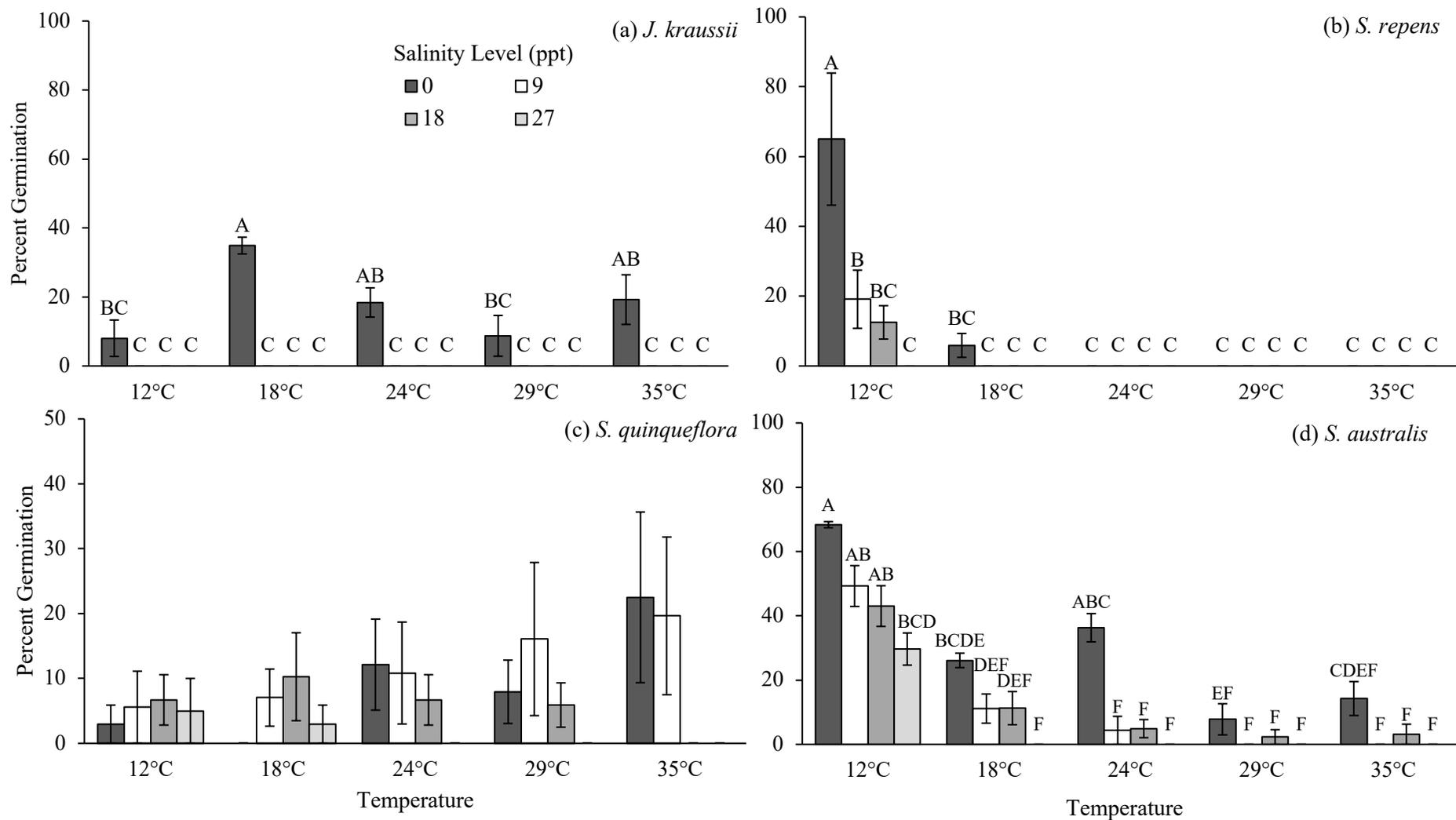


Figure 3.4. Mean (\pm SE) percent germination of seeds of *J. kraussii* (a), *S. repens* (b), *S. quinqueflora* (c), and *S. australis* (d) under constant temperature regimes and four salinity levels (0, 9, 18 and 27ppt). In each panel, treatments with different letters have statistically similar means according to Tukey's HSD at $\alpha=0.05$. Error bars are present but small in some cases. Note different y-axis scales among panels.

Table 3.3. Analyses of the effect of fluctuating temperatures and salinity on germination percent of seeds of *J. kraussii*, *S. repens*, *S. quinqueflora* and *S. australis*. Results are estimates of mean squares (MS), F statistic (F) and probability levels (P) from the two-way ANOVA. Values in bold signify a significant difference at $\alpha=0.05$. Data were transformed to the arcsine of their square roots. ns= not significant, **= significance at $P<0.01$.

<i>Species</i> <i>Source</i>	<i>d.f.</i>	<i>J. kraussii</i>			<i>S. repens</i>			<i>S. quinqueflora</i>			<i>S. australis</i>		
		<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Salinity: S	3	0.59	65.37	<0.0001	0.15	10.04	<0.0001	0.12	2.40	0.075	0.53	32.51	<0.0001
Temperature: T	5	0.05	5.42	0.0003	0.44	29.22	<0.0001	0.04	0.78	0.570	1.20	73.47	<0.0001
S×T	15	0.05	5.42	<0.0001	0.10	6.71	<0.0001	0.04	0.87	0.599	0.05	2.85	0.0015
Error	48	0.01			0.02			0.05			0.02		
Cochrans' C		0.348	**		0.527	**		0.149	ns		0.143	ns	

3.4 Discussion and Interpretive Summary

This study has demonstrated that temperature and salinity both play important roles in the germination of four common coastal saltmarsh species. In general, germination was optimal under fluctuating temperatures compared to constant regimes for all species except *S. quinqueflora*. Elevated temperatures reduced total germination, and germination also declined with an increase in salinity for all four study species. Under fluctuating and constant temperature regimes, an interaction between salinity and temperature was displayed for *J. kraussii*, *S. repens* and *S. australis*. Germination was greater at low temperatures under freshwater conditions for all species other than *S. quinqueflora*. Germination of *S. quinqueflora* was only influenced by temperature, with germination reduced at high temperatures.

3.4.1 Seed germination responses to temperature

The seeds of *J. kraussii*, *S. repens* and *S. australis* generally had higher total germination under fluctuating temperatures compared to constant temperatures, with an increase in 50% germination observed in all cases other than at 40°C in *J. kraussii* seeds. In another study with two saltmarsh grasses (*Aeluropus lagopoides* and *Sporobolus madraspatanus*) in India, fluctuating temperature regimes also produced substantially higher germination than constant temperatures (Joshi et al. 2005). Indeed, Thompson and Grime (1983) claimed that germination of numerous wetland species was enhanced by temperatures that fluctuated by as little as 1°C, compared to constant temperatures. In contrast, a study in California found that germination of the dicot *Lasthenia glabrata* and the grass *Parapholis incurva* displayed significantly higher germination in constant temperatures in November as opposed to fluctuating regimes (Noe and Zedler, 2000). Noe and Zedler (2000) proposed that these species showed different germination responses to temperature regimes than other studies because they are cued to variation present in a Mediterranean climate. The findings of the current study are ecologically important because fluctuating temperatures reflect the temperature conditions seeds would experience in the day and night during germination (Joshi et al. 2005). Results clearly demonstrate that, except at the most extreme temperatures where there was little germination, these natural temperature fluctuations promote seed germination for three of the four key saltmarsh plants.

Germination of *J. kraussii* was reduced substantially at 40/12°C compared to other temperatures. Furthermore, at constant temperatures there were significantly greater total

germination at 24°C compared to 40°C in seeds of *S. australis*. Temperatures above 35°C to 40°C have been shown to inhibit saltmarsh plant seed germination in several studies, matching observations in this study for *J. kraussii* and *S. australis* (Egan and Ungar 1999; Gulzar et al. 2001; Khan and Ungar 1997; da Silva et al. 2016). It has been hypothesised that temperature extremes above 40°C damage seed structure due to enzymatic alterations, a change in the rate of reactions, the prevention of embryo development and reduction in amino acids for RNA synthesis (da Silva et al. 2016).

Germination of the halophyte *S. quinqueflora* displayed a high tolerance to temperature. In contrast, other species demonstrated decreased germination as temperatures increased; for example, *S. repens* that did not germinate at temperatures above 29/12°C. Indeed, high variability in temperature tolerance ranges and temperature requirements for optimal germination exist among species (Egan and Ungar 1999; Khan and Ungar 1997). For example, seed germination of *Suaeda fruticosa* was not influenced by temperature (Khan and Ungar 1998). Similarly to the germination response of *S. repens* in this study, *Triglochin maritima* has also demonstrated a sensitivity to temperature (Khan and Ungar 1999). These differences among species in germination with temperature and variable tolerance of temperature extremes reflect variable timing of seasonal germination and distributions across tidal elevations and geographically (Baskin and Baskin 2001; Saintilan, 2009).

3.4.2 Seed germination responses to salinity

Increased salinity resulted in declines in germination of *J. kraussii*, *S. repens* and *S. australis* while the germination of *S. quinqueflora* was not significantly influenced by salinity. The trend of reduced germination with increased salinity has been observed in numerous studies (Cordazzo 1999; Engels et al. 2011; Joshi et al. 2005; Naidoo and Kift 2006; Redondo et al. 2004; Vincente et al. 2007), although Ungar (1991) has documented that species display various germination responses to saline environments. Nevertheless, it has been hypothesised that the zonation of plants with tidal elevation in saltmarsh ecosystems is primarily based on species-specific abilities to tolerate differences in salinity along a tidal gradient and compete with other species for the most favourable abiotic conditions (Egan and Ungar 1999).

Germination success of *J. kraussii* at 0ppt and 9ppt was 80 to 100% higher than at salinities of 18ppt and 27ppt. This result agrees with previous studies with the same species (Bosciau et al. 2011; Greenwood and MacFarlane 2006; Naidoo and Kift 2006). The germination of *J. kraussii* in response to salinity and its distribution at higher elevations of the marsh suggests

it is a facultative halophyte, where minimal to low salinity is required, as well as less frequent tidal immersion (Freitas and Costa 2013; Naidoo and Kift 2006). Indeed, moderate salinity levels inhibited seed germination of *J. kraussii*, potentially explaining its distribution at the higher tidal elevations of saltmarshes. In marshes with low salinities, *J. kraussii* is often replaced by more competitive grasses, such as *Phragmites australis* (Naidoo and Kift 2006), whereas in more saline marshes this competition is outweighed by the production of numerous seeds and superior tolerance of salinity at germination (Greenwood and MacFarlane 2006).

Germination of *S. repens* did not occur under in any treatment with brackish or saline conditions. This is a clear indication of the high sensitivity of *S. repens* seeds to even low salt concentrations. Similar responses have previously been recognized in the salt sensitive grasses, *Heleochoa setulosa* and *Aeluropus lagopoides* (Joshi et al. 2005). It is probable that the salt stress imposed upon *S. repens* is due to a combination of factors including the osmotic effects, toxicity and nutritional influences (Cordazzo 1999). The persistence of *S. repens* within depressions or pools in the mid marsh where there is frequent tidal inundation might be attributed to the reduction in competition with the few other species that can occupy these areas within saltmarsh ecosystems (Engels et al. 2011) or these might be areas that accumulate fresher water at the time of seed germination.

The seeds of *S. australis* germinated under all salinity treatments, although germination was substantially lowered at 18ppt and 27ppt in the higher temperature treatments. High germination under low salt concentrations and reduced germination success at increased salinities is a pattern observed in many halophytic plants (Baldwin et al. 1996; Cordazzo 1999; Freitas and Costa 2013). The same germination response to increasing salinity has been observed in a range of different coastal plants including the grass *Spartina ciliata* (Cordazzo 1999), various *Salicornia* species (Freitas and Costa 2013), the monocot *Sagittaria lancifolia* (Baldwin et al. 1996) and the shrub *Arthrocnemum macrostachyum* (Vincente et al. 2007). The advantage associated with the inability of these species to germinate in saline conditions enables seeds to endure hydrochory in seawater without undergoing premature germination (Adam, 1990). It also allows seed survival during extreme dry periods, characterised by salt pans within the marsh (Ungar and Hogan 1970).

Unlike the other species, *S. quinqueflora* germinated equally at all salinity levels. This is not surprising because *S. quinqueflora* is distributed low in the marsh and experiences ongoing

saltwater inundation (Adam, 1990). Due to the low germination responsiveness of *S. quinqueflora* to salt, it is possible that low to moderate salinity can stimulate germination (Engels et al. 2011). Alternatively, salinity might inhibit germination in *Sarcocornia* species and that no requirement of salinity is necessary for germination (Redondo et al. 2004). The low and variable germination response observed in this study made it difficult to detect differences among the various salinities and, consequently, further investigation is required to determine the exact explanation of the effects of salinity on the germination of *S. quinqueflora*.

3.4.3 Interactions between temperature and salinity

An interaction between temperature and salinity was observed in the germination of *J. kraussii*, *S. repens* and *S. australis* at both fluctuating and constant temperature regimes. This interaction was due to reduced germination at increased temperatures under moderate to high salinity, which is a common observation for saltmarsh plant species (Khan et al. 2000; Gulzar et al. 2001; Naidoo and Kift 2006; Ungar and Hogan 1970). The influence of osmotic soil potential on seed germination is well known, and studies have demonstrated that higher salinities reduce the temperature tolerance ranges for germination (Khan et al. 2000).

Previous research has provided plausible explanations for the considerable influences of salinity and temperature on seed germination. For many species, seed production and germination occurs in spring (Khan et al. 2000), similar to this several species in this study, where optimal temperature regimes for germination success of 24/12°C match those of mean spring time temperatures of Jervis Bay (Bureau of Meteorology, 2017). The spring season also coincides with increased rainfall, thus reducing soil surface salinity (Naidoo and Kift 2006). In contrast, reduced germination at increased temperatures corresponds to higher levels of evaporation resulting in rises in soil salinity and insufficient moisture for germination (Egan and Ungar 1999; Khan et al. 2000).

3.4.5 Conclusion

The four coastal saltmarsh plant species examined have distinct requirements for germination (and likely subsequent establishment) in relation to salinity and temperature. The tolerances of these plant species to these abiotic barriers likely explain in part their distributions across the tidal elevation gradient in the saltmarsh. There have been no studies examining germination over larger geographic scales to examine how tolerances to variations in temperature and salinity might explain broader species distributions for these species of

marsh plants. We might expect, however, that irrespective of disturbance regimes, the community structure of plants would develop from germination in response to environmental drivers including temperature and salinity (Baldwin et al. 1998). Indeed, understanding how germination and seed establishment respond to environmental variability is crucial for persistence, regeneration or restoration of plant communities under changing abiotic conditions.

PART IV

SPATIAL VARIATION IN REPRODUCTIVE OUTPUT AND GERMINATION OF THE DOMINANT SALTMARSH CHENOPODS *SARCOCORNIA QUINQUEFLORA* AND *SUAEDA AUSTRALIS*

4.1 Introduction

Examining spatial variation in reproductive output is beneficial in furthering our understanding of the patterns and environmental drivers of plant reproduction (Barrett, 2010). Variation in spatial context has previously revealed new insights into ecological processes (Moeller, 2006), as spatial scale is a fundamental component of the distribution of species. Nevertheless, investigations into specific aspects of plant reproductive biology are often limited in their spatial extent (Sork et al. 1999), with many specific to communities or populations in only one location (Bertness and Pennings, 2000). Sork et al. (1999) emphasise the need to consider a broader environmental context when investigating the reproductive success of a plant species through examining landscape scale gene flow via pollen and seed movement as a proxy for broad scale reproductive success. Generally, the difficulty in undertaking research projects in multiple locations contributes to the trend in extrapolating results to different communities with little to no direct investigation (Bertness and Pennings, 2000). There is an inherent risk in generalisation, where overstating the trends specific to one population may be detrimental to knowledge of another population in a different point in both space and time (Underwood and Denley, 1984).

Much of our current understanding of saltmarsh populations and communities in the past two decades is based on experimental studies conducted at few study sites (Bertness and Pennings, 2000; Saintilan 2009). This reduces thorough understanding of landscape scale variation in the reproductive biology of saltmarsh plant species and heightens the need for studies of spatial variation of saltmarsh plant reproductive success (Bertness and Pennings, 2000). Mechanisms of pollination in particular are subject to large variation due to fluctuations in pollinator abundance and community composition between different populations of saltmarsh plants (Moeller, 2006). In some cases, variation in pollination mechanisms parallel differences in mating systems at broad scales (Moeller, 2006). Such factors that influence reproductive success likely vary among marshes, and systematic studies of this spatial variation are necessary to provide a general understanding of the forces influencing saltmarsh plant reproduction and their persistence into the future (Bertness and Pennings, 2000).

This investigation examines spatial variability in plant abundance and associated reproductive success of *Sarcocornia quinqueflora* and *Suaeda australis* across different marshes along the central and south coast of New South Wales, Australia. Examining spatial variation in this investigation will provide broader context and widen the applicability of the detailed results. To do this, this study determined whether populations *S. quinqueflora* and *S. australis* occurring in different regions (Fig. 2.1) varied in (a) plant height, cover, volume, and floral abundance; and (b) viability of seed and subsequent germination success. Comparing these measures of reproductive output and seed viability across multiple locations will provide insight into the reproductive success of *S. quinqueflora* and *S. australis* at a broader scale. Understanding the variation in reproductive success between different populations in different environmental contexts will provide better foundations for future restoration projects aimed at re-establishing and protecting saltmarsh communities in developed areas (Saintilan and Williams, 2000; Green et al. 2009).

4.2 Methods

4.2.1 Study locations and regions

This study was conducted at six saltmarsh locations within three different coastal regions of New South Wales, Australia, from July to November 2017 (Fig. 2.1). The locations were Woollamai and Myola at Jervis Bay, Empire Bay and Cockle Bay at Brisbane Water, and Teralba and Swansea at Lake Macquarie.

4.2.2 Sampling plant population characteristics

Populations of *S. quinqueflora* and *S. australis* in the middle-upper marsh at all locations were sampled using ten randomly placed 0.25m² (0.5 m x 0.5 m) quadrats in patches of each species, where plant cover (%), height (cm) and volume (cm³) were quantified. Plant cover was calculated using a 0.25m² quadrat divided into 100 equal segments, where the number of segments containing the plant corresponded to the percent cover in the quadrat. Height was measured from soil surface to the tip of the reproductive spike, and was calculated as an average of ten random height measurements in each quadrat. Volume was calculated from these estimates but assuming a cubic shape for the vegetation and multiplying height (cm) by plant cover (cm² occupied in the 0.25m² quadrat).

4.2.3 Seed collection

Seed collection occurred throughout June and July 2017. Seed from *S. quinqueflora* was collected from all six locations. Seed of *S. australis* was only collected at Woollamia, Myola, Empire Bay Wetlands, and Cockle Bay because only these locations had patches of *S. australis* that had produced seed. Seeds were collected from the ten random quadrat samples used to measure plant abundance, where all *S. quinqueflora* and *S. australis* seeded spikes in each quadrat were collected and stored.

4.2.4 Germination experiments

To determine the germination success and viability of seed produced across all sampled locations, seeds were incubated in Temperature Cycling Chambers (LABEC Laboratory Equipment P.L. Australia) to monitor germination rate. Seeds were exposed to temperatures of 27°C and 12°C on 12-hour light and dark cycles. Seeds of *S. quinqueflora* and *S. australis* spikes were placed onto 90mm polystyrene agar plates containing 7.5% water agar. Seeds were distributed evenly over agar plates. In all germination experiments, the number of germinated seeds were counted at three day intervals for 12 days, and then weekly until no germination was recorded for two weeks. Seeds were considered to have germinated when the radicle length exceeded 2mm. All temperature and light conditions were monitored at 30 minute intervals by HOBO pendant data loggers for the duration of all germination experiments, and tested directly using thermometers.

At the conclusion of the germination experiments, cut tests were performed on seeds that did not germinate to determine the number of seeds that should be excluded from analysis due to lack of endosperm. Cut tests were conducted for a sample of 30 ungerminated seeds from each of the *S. quinqueflora* locations, and 20 from each of the *S. australis* locations to gain an estimate of seed viability at each location. From cut tests, the proportion of empty (no endosperm) seeds were subtracted from the total number of seeds produced in each replicate to give an indication of the number of viable seeds. This estimate was used to determine the overall germination success of viable seeds from each location, with percent germination used as a measure of the proportion of germinated seed from original potentially viable seed.

Germination experiments were conducted to compare the germination success of *S. quinqueflora* seeds produced at six different saltmarsh locations within three regions: Woollamia, Myola, Empire Bay, Cockle Bay, Swansea and Teralba (see Fig. 2.1). The germination success of *S. australis* was also compared across four saltmarsh locations in two

regions: Currambene Creek, Myola, Empire Bay and Cockle Bay (Fig. 3.1). For *S. quinqueflora*, 50 seeds from each of the ten random quadrats were randomly selected, generating a sample of 500 *S. quinqueflora* seeds for use in this experiment from each of Woollamia, Myola, Empire Bay, Cockle Bay, and Teralba. The Swansea location had fewer *S. quinqueflora* seeds than all others so all 229 seeds collected from quadrats at Swansea were used in this experiment. Seeds were distributed to fit 50 seeds on one 90mm petri dish. For *S. australis*, 20 seeds were randomly selected from each quadrat used to sample plant abundance, which generated a total of 200 seeds per location. Where location collections did not contain 200 seeds (Myola and Empire Bay) the total number of seeds collected at that location were used in this experiment (89 and 180 respectively).

4.2.4 Statistical analysis

Two-factor (locations, regions) nested analyses of variance were employed to identify spatial differences in plant height, cover, and volume, as well as number of seeded spikes produced and seed germination for populations of *S. quinqueflora* and *S. australis* at two saltmarsh locations in each of three regions. Locations were nested within regions in analysis, and both were considered random factors. Appropriate transformations to ensure normality and homogeneity of variances were conducted and are specified in results tables. Student-Newman-Keuls (SNK) multiple comparisons tests were used to detect differences among treatment means only where significant interactions were identified by ANOVA. For all tests, $\alpha = 0.05$.

4.3 Results

4.3.1 Plant abundance

At each saltmarsh location, *Sarcocornia quinqueflora* persisted in large open areas that dominated cover in the saltmarsh. In contrast, *Suaeda australis* tended to be found at the fringes of saltmarsh vegetation in small patches. At all locations, cover of *S. quinqueflora* was on average between 60-100% (Fig. 4.1a), and in no area was the cover of *S. quinqueflora* less than 50%. Although *S. quinqueflora* in Brisbane Water had the lowest average cover (Fig. 4.1a), this was not significantly different to that of other saltmarsh locations in the other regions at Currumbene Creek, or Lake Macquarie (Table 4.1).

Within saltmarsh at Swansea and Teralba, *S. quinqueflora* was shorter on average than other locations, however plant height did not differ significantly among regions (Fig. 4.1b; Table 4.1). At Teralba, 80% of spikes were less than 10 cm tall, with all other locations ranging between 10-30% of spikes below 10 cm. Interestingly, *S. quinqueflora* at Myola was significantly taller than at Woollamia where the pollination studies were conducted (Table 4.1, SNK tests).

There was a greater average volume of *S. quinqueflora* in saltmarsh at Currumbene Creek (Fig 4.1c). Unlike plant height, the volume of *S. quinqueflora* did significantly differ according to region (Fig. 4.1c; Table 4.1).

Populations of *S. australis* at Currumbene Creek ranged between 70-100% plant cover in 0.25m² patches, whereas populations in the Brisbane Water region had cover as low as 48%. However, on average, there was no significant difference in *S. australis* cover among regions (Fig. 4.2a; Table 4.2).

Plants ranged in height from 17-52 cm tall in populations of *S. australis* located in the Currumbene Creek region, and 15-29 cm tall in populations in the Brisbane Water region. There was no significant difference in *S. australis* height between regions (Fig. 4.2b; Table 4.2). The volume of *S. australis* in 0.25m² quadrats did differ significantly between regions (Fig 4.2c; Table 4.2), despite a greater volume at both Woollamia and Myola than Empire Bay and Cockle Bay.

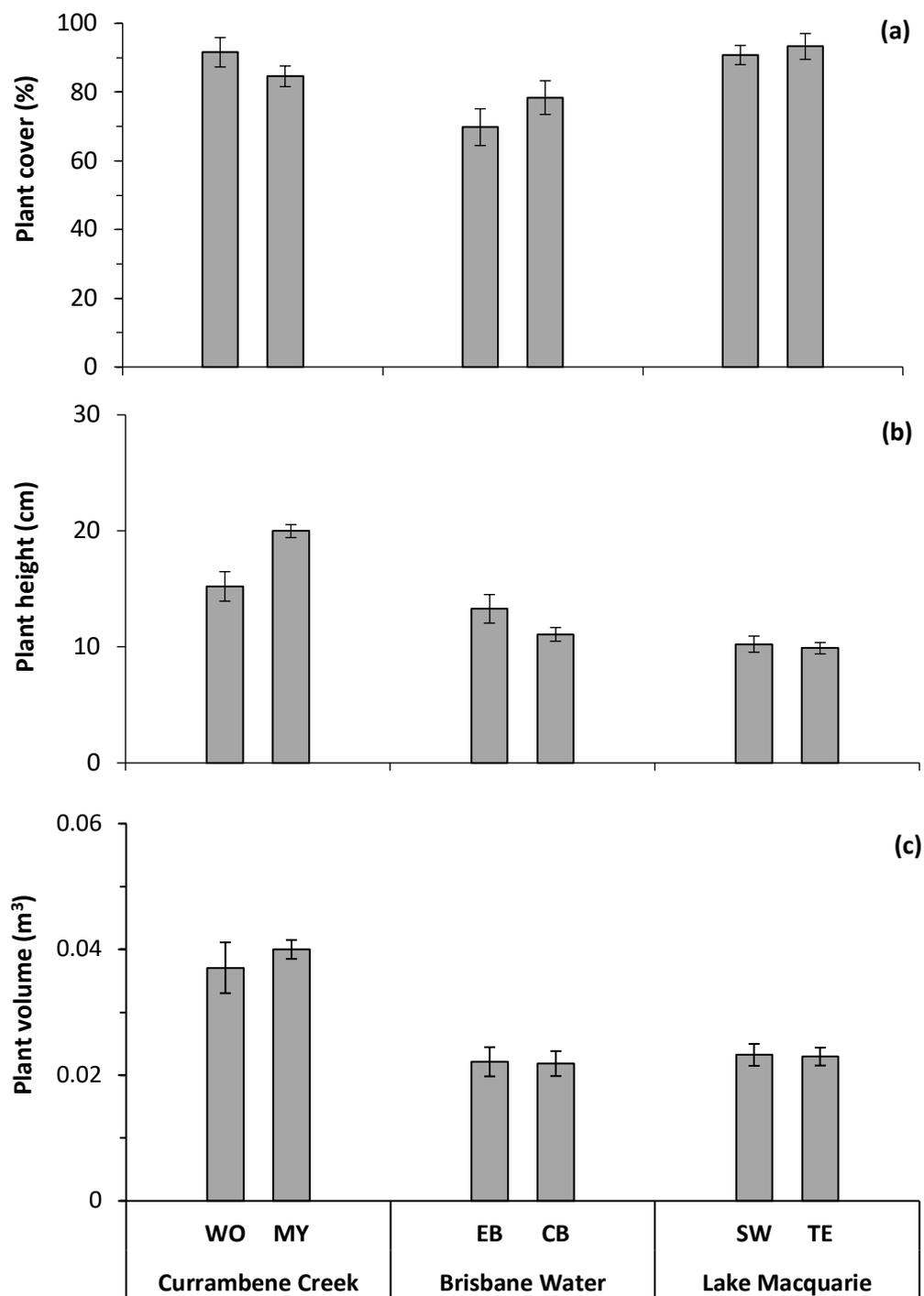


Figure 4.1. Mean (\pm SE) (a) percent cover (b) plant height and (c) plant volume of *Sarcocornia quinqueflora* at saltmarsh locations in the regions of Currambene Creek (Woollamia [WO], Myola [MY]), Brisbane Water (Empire Bay [EB], Cockle Bay [CB]), and Lake Macquarie (Swansea [SW], Teralba [TE]).

Table 4.1. Analyses of *Sarcocornia quinqueflora* cover (%), height (cm) and volume (m³) at the saltmarsh locations in each of three different regions of the New South Wales coast of Australia. Table includes results of analysis of variance, and transformations are specified. Asterisk corresponds to SNK results on footnote.

Source	d.f	MS	F	P	Transformation
Plant cover (%)					
Region	2	1779.3500	8.26	0.0602	None
Location (Region)	3	215.3500	1.24	0.3029	
Error	54	173.1204			
Plant height (cm)					
Region	2	261.0.331	8.15	0.0613	None
Location (Region)	3	32.0383	4.24	0.0092*	
Error	54	7.5497			
Plant volume (m ³)					
Region	2	0.0017	118.6	0.0014	None
Location (Region)	3	0.0000	0.26	0.8520	
Error	54	0.0001			

* Currumbene Creek: Myola > Woollamia (P<0.05).

Brisbane Water: Cockle Bay = Empire Bay.

Lake Macquarie: Swansea = Teralba.

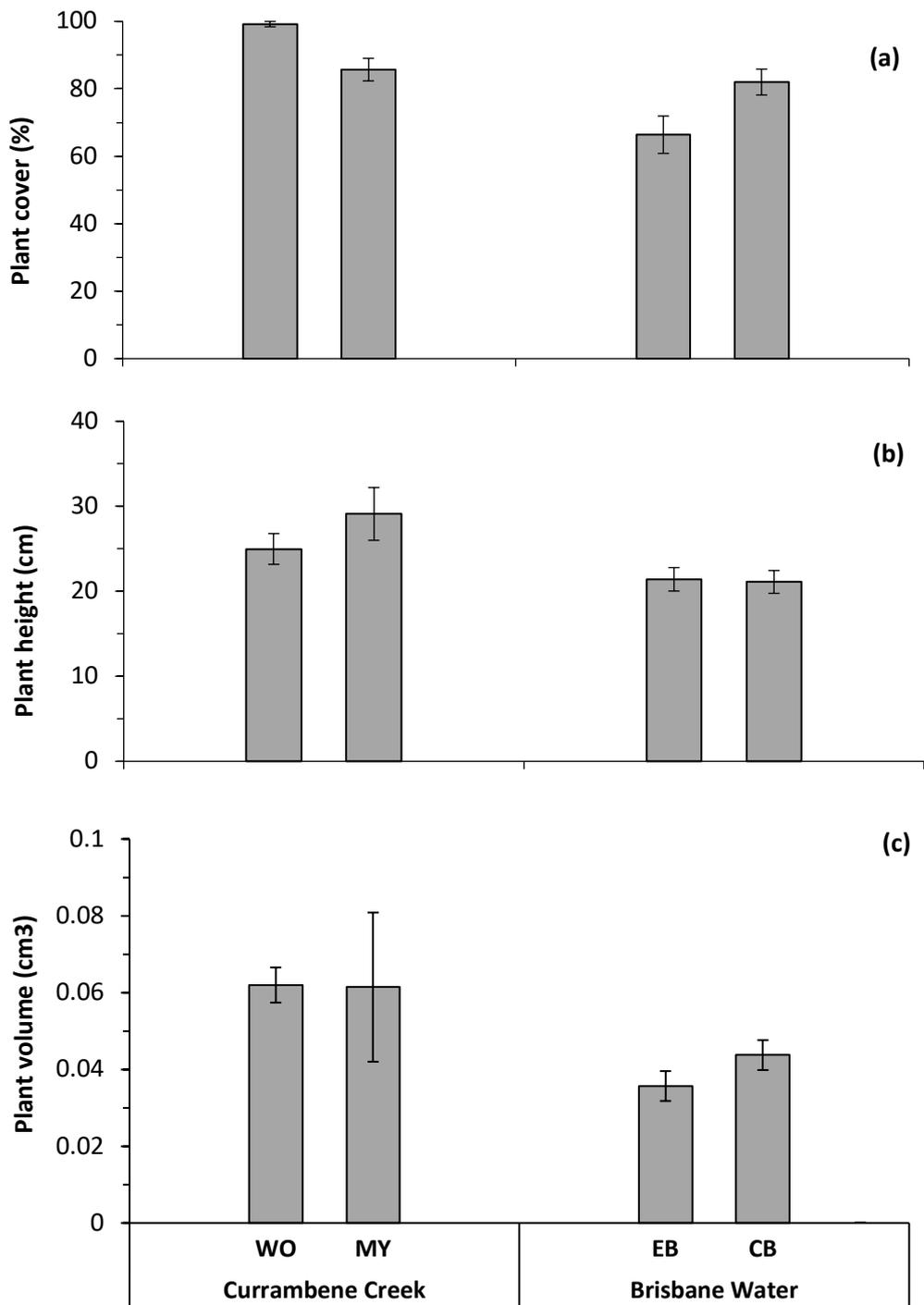


Figure 4.2. Mean (\pm SE) (a) percent cover, (b) plant height and (c) plant volume of *Suaeda australis* at saltmarsh locations in the regions of Currambene Creek (Woollamia [WO], Myola [MY]) and Brisbane Water (Empire Bay [EB], Cockle Bay [CB]).

Table 4.2. Analyses of *Suaeda australis* cover (%), height (cm) and volume (m³) at saltmarsh locations distributed in two different regions of the New South Wales coast of Australia. Table includes results of analysis of variance, and transformations are specified.

Source	d.f	MS	F	P	Transformation
Plant cover (%)					
Region	1	3203.0621	2.74	0.2397	ArcSine(%)
Location (Region)	2	1169.0693	12.73	0.0001	
Error	36	91.8607			
Plant height (cm)					
Region	1	0.4932	11.41	0.0776	Log(x)
Location (Region)	2	0.0433	0.75	0.4780	
Error	36	0.0574			
Plant volume (m ³)					
Region	1	0.0048	26.65	0.0321	None
Location (Region)	2	0.0002	0.78	0.4643	
Error	36	0.0002			

4.3.2 Seed production and viability

All quadrats contained reproductive spikes of *S. quinqueflora* that had set seed. The mean number of reproductive spikes that went to seed was lowest at Swansea, and highest at Teralba (Fig. 4.3a). There was no significant difference in the number of spikes that set seed among regions (Fig. 4.3a; Table 4.3). The number of seeded spikes at Teralba was significantly higher than at Swansea within Lake Macquarie (Table 4.3, SNK results).

All locations produced viable seed of *S. quinqueflora*, with between 80-100% germination after 30 days (Fig. 4.3b). There was no significant difference in the germination success of seeds among regions (Table 4.3), however the Teralba site produced significantly less seed than Swansea in the Lake Macquarie region (Fig. 4.3b; Table 4.3, SNK results).

At Woollamia, the average number of reproductive spikes of *S. australis* that set seed was approximately 15 times greater than at Swansea (Fig. 4.4a). There was no significant difference in the number of reproductive spikes that set seed between regions (Table 4.3), although there were differences among the two locations in each region (Table 4.3).

Woollamia and Swansea contained more seeded spikes than Myola and Teralba respectively (Fig. 4.4a; Table 4.3, SNK tests).

Germination of *S. australis* seeds was between 70-100% at every location (Fig. 4.4b). Myola had the lowest percent germination success on average (70.13 ± 13.56) of all locations. There was no significant difference however in percent germination success between regions (Fig. 4.4b; Table 4.4).

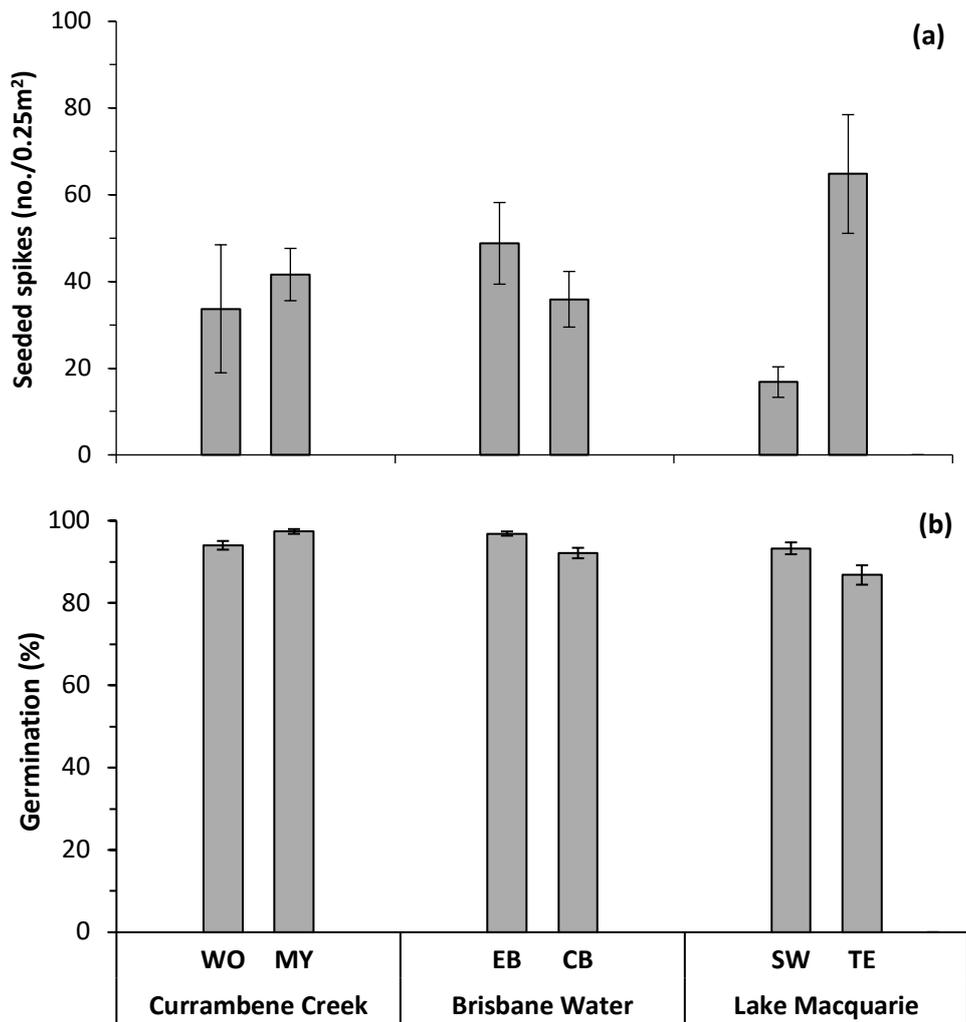


Figure 4.3. Mean (\pm SE) (a) number of seeded spikes per 0.25m² and (b) percent germination of *Sarcocornia quinqueflora* at saltmarsh locations in the regions of Currumbene Creek (Woollamia [WO], Myola [MY]), Brisbane Water (Empire Bay [EB], Cockle Bay [CB]), and Lake Macquarie (Swansea [SW], Teralba [TE]).

Table 4.3. Analyses of the number of *Sarcocornia quinqueflora* seeded spikes and germination success at saltmarsh locations distributed in three different regions of the New South Wales coast of Australia. Table includes results of analysis of variance, and transformations are specified. Asterisks corresponds to SNK results below.

Source	d.f	MS	F	P	Transformation
Seeded spikes (per 0.25m ²)					
Region	2	1.77872	0.07	0.9320	Sqrt(x+1)
Location (Region)	3	24.8001	5.53	0.0022*	
Error	54	4.4828			
Germination (%)					
Region	2	171.7942	0.93	0.4862	
Location (Region)	3	185.5629	6.83	0.0006**	Arcsine(x)
Error	54	27.1758			

* Currumbene Creek: Myola=Woollamia.

Brisbane Water: Cockle Bay=Empire Bay.

Lake Macquarie: Swansea < Teralba (P<0.05).

** Currumbene Creek: Myola=Woollamia.

Brisbane Water: Cockle Bay=Empire Bay.

Lake Macquarie: Swansea > Teralba (P<0.05).

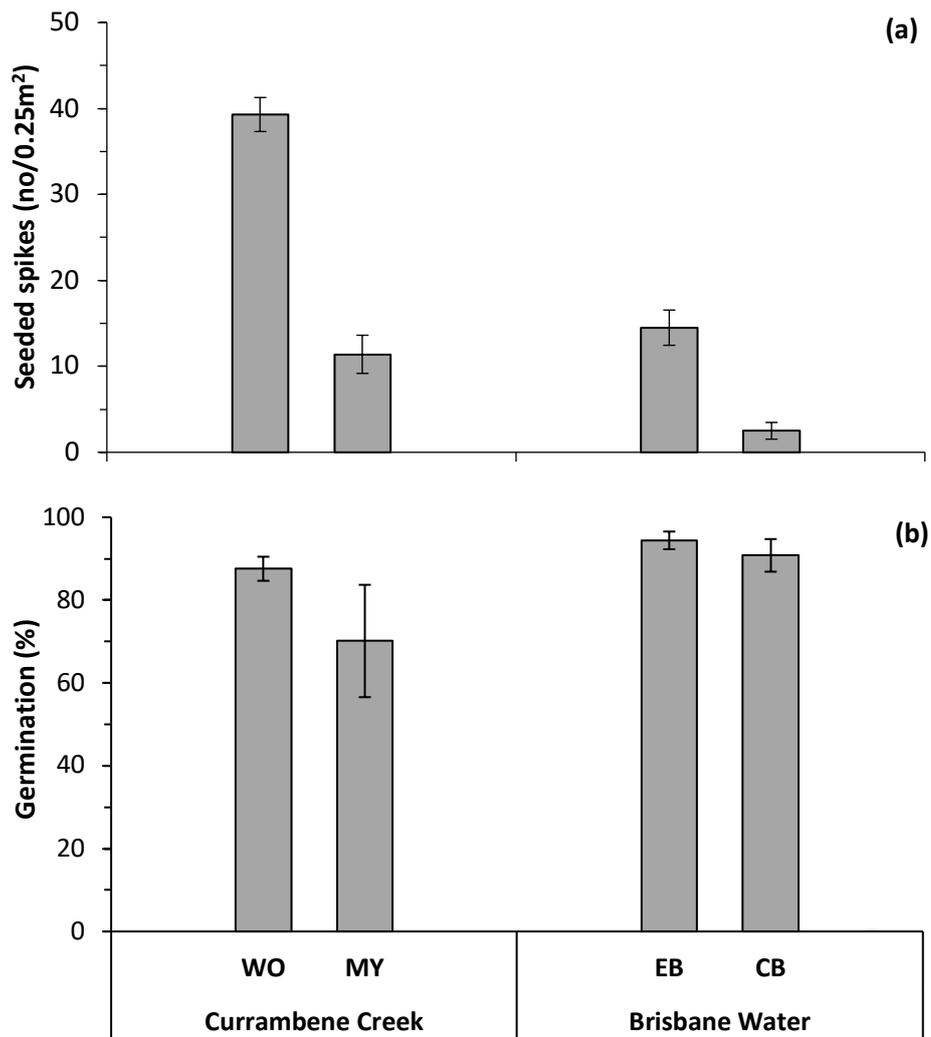


Figure 4.4. Mean (\pm SE) (a) number of seeded spikes per 0.25m² and (b) percent germination of *Suaeda australis* at saltmarsh locations in the regions of Currambene Creek (Woolamia [WO], Myola [MY]), and Brisbane Water (Empire Bay [EB], Cockle Bay [CB]).

Table 4.4. Analyses of the number of *Suaeda australis* seeded spikes and germination success of seeds collected at saltmarsh locations distributed in two different regions of the New South Wales coast of Australia. Table includes results of analysis of variance, and transformations are specified. Asterisks corresponds to SNK results below.

Source	d.f	MS	F	P	Transformation
Seeded spikes (per 0.25m ²)					
Region	1	2839.2250	1.23	0.3827	None
Location (Region)	2	2306.0250	66.02	<0.0001	
Error	36	34.9306			
Germination (%)					
Region	1	571.3837	5.43	0.1452	Arcsine(x)
Location (Region)	2	105.2736	0.57	0.5774	
Error	16	185.1849			

* Currumbene Creek: Woollamia > Myola (P<0.05).

Brisbane Water: Empire Bay > Cockle Bay (P<0.05).

4.4 Discussion and Interpretive Summary

The results of this investigation provide necessary spatial context for the reproductive output of both *Sarcocornia quinqueflora* and *Suaeda australis*. Although plant cover was high for both *S. quinqueflora* and *S. australis* at all locations, there was significant variation in the number of seed-producing spikes in both species. Regardless of this, all locations had a high rate of germination success for both saltmarsh species, which would indicate that local variation in reproductive processes do not significantly alter the viability of seed.

This study has provided spatial context for the reproductive output and germination saltmarsh of dominant saltmarsh plants at a regional spatial scale. High rates of germination success at all locations and amongst all regions suggests that processes involved in reproductive output are likely comparable across locations and regions, and that these processes are capable of producing viable seed. In this sense, the saltmarshes examined here potentially reflect broader trends in saltmarsh reproductive biology that may apply to other saltmarsh communities in other regions.

Although it is common to see spatial variation considered on small scales when community structure and zonation are the focus of saltmarsh research (Clarke, 1993; Hickey and Bruce, 2010), no known studies have exclusively examined spatial variation in the abundance of saltmarsh plants in relation to reproductive success in Australia. According to Roberts and Sainty (2005), *S. quinqueflora* cover in the Brisbane Water region is sparse due to the higher rate of disturbance in these saltmarsh locations. In this study, saltmarsh in Brisbane Water had the lowest *S. quinqueflora* cover and plant volume, along with the lowest *S. australis* cover, height and volume. Trampling and other direct physical disturbance such as vehicles (Adam et al., 1987) may cause reduced cover and growth of saltmarsh species (Laegdsgaard, 2002; Chapman and Robert, 2004). In relation to other regions in this study, the Brisbane Water region has also experienced significant urban development in foreshore areas (Harty, 1994), and damage from vehicle access (Roberts and Sainty, 2005). Currumbene Creek is part of the Jervis Bay Marine Park, where regulated foreshore development reduce disturbance to saltmarsh areas (Clarke, 1993). Generally, plant height and volume were higher at Currumbene Creek for both *S. quinqueflora* and *S. australis*. Although disturbances may impact plant growth and explain spatial variation in the abundance of *S. quinqueflora* and *S. australis* identified in this study, they do not appear to have impacted germination success of these populations but further localised investigations are necessary before conclusions may be drawn. Patterns of disturbance, and the relative impact of this on

Australian saltmarsh communities, have been considered at large scales (Saintilan and Williams, 2000), but if variation in seed production is widespread among saltmarsh locations at localised scales as this study has indicated, then identifying the impact of disturbance on smaller scales is essential for these saltmarsh populations (Laegdsgaard, 2002).

Reproductive output is an important driver of population dynamics in many terrestrial plants, although as this study reflects, reproductive output is not always an indication of relative success (Marks and Truscott, 1985). The germination success of both *S. quinqueflora* and *S. australis* was high at all sampled locations, regardless of plant abundance or the amount of seed initially produced. Marks and Truscott (1985) similarly identified variation in the seed production in British populations of *Spartina anglica*, a perennial saltmarsh grass, where even a greater volume of plant still produced fewer seeds. Marks and Truscott (1985) conclude that seed production is not solely correlated with the abundance of aerial vegetative parts. Reproductive output (amount of seed produced) should not be the sole indicator of the contribution a saltmarsh plant will make to successive generations, as this study reflects, even locations with fewer seed are still highly successful due to the high rate of germination (Marks and Truscott, 1985).

The similarities in germination success identified in this study indicate that both *S. quinqueflora* and *S. australis* within Australian saltmarsh may harness similar successful mechanisms of pollination to produce viable seed. These patterns in reproductive success indicate that local populations of *S. quinqueflora* and *S. australis* at across locations and regions are not driven by solely idiosyncratic factors unique to specific saltmarsh locations, which would create difficulty in predicting population behaviour amongst different marshes (Pennings et al. 2003). It is likely that saltmarsh distributed at greater distances across Australia will experience significant variation in abiotic conditions that may drive reproductive processes. Specifically, pollinator suites vary in different climatic zones and hence reliance on self-compatible or outcrossing mating systems shifts with latitudinal variation (Moeller, 2006). In the Australian saltmarsh context, there are no known studies that have quantified the potential spatial variation in pollination mechanisms amongst saltmarsh communities.

There has been very little consideration of reproductive variation across regions in saltmarsh species (Bertness and Pennings 2002; Saintilan 2009). This study has demonstrated similarities in the reproductive output and success of saltmarsh within the Currumbene Creek,

Brisbane Water and Lake Macquarie regions. This investigation across different regions provides the foundations of comparison in reproductive biology of dominant saltmarsh species *S. quinqueflora* and *S. australis*, initiating a wider understanding of the persistence of these species in saltmarsh communities. Local scale processes within saltmarsh locations are important in determining the structure and dynamics of these plant populations (Levine et al. 1981). This investigation identified variation on a local scale in the reproductive output and germination success of two saltmarsh species, indicating that it is necessary to understand smaller scale processes that may impact the production of viable seed in order to predict change in saltmarsh populations (Chapman and Underwood, 2000; Roberts and Sainty, 2005).

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