School of Science

The influence of morphological and physiological seed traits on oceanic dispersal and germination in saline coastal environments

Lydia K Guja

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

April 2014

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: 

Date: 12 April 2014

Declaration of candidate contribution

This thesis contains a mixture of sole-authored work, co-authored work that has been prepared for publication, and co-authored published work. Chapters 3 to 6 are presented as manuscripts prepared for publication in peer-reviewed journals. The contributions of co-authors to Chapters 3 to 6 are outlined below. Each chapter is self-contained, including tables, figures, references and appendices and follows the structure prescribed by the target or publishing journal.

Chapter 3. Sink or swim? A model to predict oceanic dispersal using seed morphological traits

Prepared for submission to *Oikos*.

Authors: **Lydia K Guja**, Mark J Wallace, Kingsley W Dixon, Grant Wardell-Johnson and David J Merritt.

LKG conceived the study, designed and performed the experiments, conducted analyses, prepared figures and was lead author. MJW provided guidance on study design, analyses, and interpretation of results and assisted with manuscript preparation. KWD, GWJ and DJM commented on study design and the manuscript. LKG addressed and incorporated co-authors' comments.

Chapter 4. Dispersal potential of *Scaevola crassifolia* (Goodeniaceae) is influenced by intraspecific variation in fruit morphology along a latitudinal environmental gradient

Published in *Australian Journal of Botany* (2014) **62**, 56-64.

Authors: **Lydia K Guja**, David J Merritt, Kingsley W Dixon and Grant Wardell-Johnson.

LKG conceived the study, designed and performed experiments, conducted analyses, prepared figures and was lead author. DJM provided guidance on experimental design, interpretation of results, and assisted with manuscript preparation. GWJ

commented on study design and GWJ and KWD commented on the manuscript. LKG addressed and incorporated co-authors' and reviewers' comments.

Chapter 5. Experimental manipulation of temperature, salinity and osmotic stress to determine the germination thresholds and oceanic dispersal capacity of coastal plant seeds

Prepared for submission to *Diversity and Distributions*.

Authors: **Lydia K Guja**, Grant Wardell-Johnson, Kingsley W Dixon and David J Merritt.

LKG conceived the study, designed and performed the experiments, conducted analyses, prepared figures and was lead author. DJM provided guidance on experimental design, interpretation of results and assisted with manuscript preparation. GWJ and KWD provided comments on study design and the manuscript. LKG addressed and incorporated co-authors' comments.

Chapter 6. Full spectrum X-ray mapping reveals differential localisation of salt in germinating seeds of differing salt tolerance

Published in *Botanical Journal of the Linnean Society* (2013) **173,** 129-142.

Authors: **Lydia K Guja**, Richard Wuhrer, Ken Moran, Kingsley W Dixon, Grant Wardell-Johnson and David J Merritt.

LKG conceived the study, collected and collated data, conducted analyses, prepared figures and was lead author. RW and KM provided training in X-ray mapping and analysis of results, provided facilities and software for X-ray mapping, assisted with data collection, and commented on the manuscript. DJM provided guidance on experimental design and assisted with manuscript preparation. GWJ and KWD provided comments on experimental design and the manuscript. LKG addressed and incorporated co-authors' and reviewers' comments.

Manuscripts and conference presentations based on this thesis

Peer reviewed manuscripts

Guja LK, Merritt DJ, Dixon KW, Wardell-Johnson, G (2014) Dispersal potential of *Scaevola crassifolia* (Goodeniaceae) is influenced by intraspecific variation in fruit morphology along a latitudinal gradient. *Australian Journal of Botany*, **62**, 56-64.

Guja LK, Wuhrer R, Moran K, Dixon KW, Wardell-Johnson G, Merritt DJ (2013) Full spectrum X-ray mapping reveals differential localisation of salt in germinating seeds of differing salt tolerance. *Botanical Journal of the Linnean Society*, **173,** 129-142.

Conference presentations

Guja, L, Wardell-Johnson G, Dixon K, Merritt D (2014) 'Experimental manipulation of temperature, salinity and osmotic stress to determine germination thresholds of coastal plant seeds.' *ComBio2014*, ASBMB, ASPS, ANZSCDB, Canberra, Australia.

Wuhrer R, **Guja L**, Merritt D, Moran K (2014) 'X-Ray mapping investigations of salt migration in seeds through use of window and windowless silicon drift detectors.' *Microscopy and Microanalysis*, Microscopy Society of America, Connecticut, USA, 20 (Suppl 3) pp. 364-365.

Guja L, Wuhrer R, Moran K, Dixon K, Wardell-Johnson G, Merritt D (2013) 'Full spectrum X-ray mapping reveals differential localisation of salt in germinating seeds of differing salt tolerance.' *Seed Ecology IV, Seeds and the Future, Conference Proceedings*, ed. Liu Z., Liu, B., Zhu, J., Yan Q., International Society for Seed Science, Shenyang, China, p. 6.

Guja L, Wuhrer R, Moran K, Phillips M, Dixon K, Wardell-Johnson G, Merritt D (2012) 'X-Ray mapping reveals mechanisms of salt tolerance in seeds of coastal plants *Ficinia nodosa* and *Spyridium globulosum*.' *APMC 10 / ICONN 2012 / ACMM 22*, *Conference Proceedings*, Perth, Australia, p. 88-1 – 88-2.

Guja L, Merritt D, Dixon K, Wardell-Johnson G (2011) 'Oceanic dispersal: can seeds survive and germinate under new environmental conditions?' *Biodiversity and People Symposium*, Curtin Institute for Biodiversity and Science, Perth, Australia.

Guja L, Merritt D, Wardell-Johnson G, Dixon K (2010) 'Oceanic dispersal: can seeds really float, survive and germinate?' *Seed Ecology III*, *Seeds and Change, Conference Proceedings*, ed. Pendelton, R., Meyer, S., Schultz, B., International Society for Seed Science, Utah, USA, p. 63.

Other research output during thesis preparation

Manuscripts

Guja L, Merritt D, Dixon K (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37,** 1175-1186.

Guja L, McAuliffe J, Taylor D (2013) Sowing seeds: bridging the gap between ex situ collections and reintroduction. *Australasian Plant Conservation* **21**, 14-17.

Conference presentations

Guja L, Huttner-Koros B (2013) 'Ecological drivers of seed germination in endangered Australian bog and fen communities.' *5th Global Botanic Gardens Congress*, Botanic Gardens Conservation International, Dunedin, New Zealand.

Cochrane A, **Guja L**, Hirst M, Hoyle G, Merritt D, North T, Wood J (2012) 'Applying seed science knowledge for better conservation and restoration outcomes - seed banking for conservation and restoration.' *9th National Conference. Plant Conservation in Australia: Achievements and Future Directions,* Australian Network for Plant Conservation, Canberra, Australia, p. 30.

Poster presentations

Guja L, Huttner-Koros B (2013) 'Ecological drivers of seed germination in endangered alpine bog and fen communities in Australia.' *Seed Ecology IV, Seeds and the Future, Conference Proceedings*, ed. Liu Z., Liu, B., Zhu, J., Yan Q. International Society for Seed Science, Shenyang, China, p. 120.

Guja L, North T, McAuliffe J, Taylor D (2012) 'Sowing seeds: bringing the gap between ex situ collections and reintroduction.' *9th National Conference. Plant Conservation in Australia: Achievements and Future Directions,* Australian Network for Plant Conservation, Canberra, Australia, pp. 27-28.

Acknowledgements

This thesis would not have been possible without the guidance, support, and encouragement of many individuals and organisations. To them I am sincerely thankful for contributing to my research.

My supervisor Prof Grant Wardell-Johnson and associate supervisors Dr David Merritt and Prof Kingsley Dixon are thanked for their guidance and support. Prof Wardell-Johnson is especially thanked for taking a risk on a new student not long after taking up his position at Curtin University. Dr Merritt has been a role model and mentor since my first introduction to seed biology during undergraduate study in 2006. I am fortunate to have worked with Dr Merritt who has helped me develop both as a scientist and person and am thankful for his constructive contributions to this thesis. Thanks to Prof Dixon for enthusiastically welcoming me to the Science Directorate at Kings Park and Botanic Garden.

Collaborators on Chapter 6, Dr Richard Wuhrer (UWS) and Ken Moran (Moran Scientific Pty Ltd), deserve special mention for taking up the challenge of X-ray mapping bulk, rough, biological samples. Thank you for teaching a novice the theory, methods and analysis techniques required to conduct the work.

Luke Sweedman is thanked for supplying seed from the Western Australian Botanic Gardens and Parks Authority seed bank and Dave Symons is thanked for preparing the seed images in Chapter 3. Brendan Lepschi (CSIRO) is thanked for advice on Goodeniaceae fruit anatomy. Dr Tim Colmer and Gary Cass (UWA) are thanked for their instruction in flame photometry.

Several people made valuable comments on earlier drafts of components of this thesis. In particular, Dr Rowena Long, Dr Renee Tuckett, Dr Lucy Commander, Dr Anja Kaczmarczyk and Mark Wallace formed the core of a regular writing group and are thanked for their constructive feedback. Dr Ryan Phillips is thanked for proof-reading the final draft.

I would not have achieved the field work required for this thesis, across hundreds of kilometres of the Western Australian coast, without the company and assistance of

Mark Wallace, Dr Michalie Foley, Dr Mark Bundock, Marie Jouault, Dr Rowena Long and Zoe Davies.

Thanks to Dave Symons, Jessica Taylor, Erin Griffiths, and Dr Markus Adamek who monitored germination in the lab at times when I was in the field or at conferences.

Arielle Fontaine and students of UWA's Conservation Biology unit, Tsz Shan Leung (April), Yi Zhai (Cherry) and Connor Weightman are thanked for laboratory assistance measuring seed traits and buoyancy.

My current employers the Australian National Botanic Gardens and the Centre for Australian National Biodiversity Research are thanked for support which helped me to balance full-time work and part-time study toward the end of my thesis. Despite numerous revised estimates of when this thesis would finally be complete the encouragement of colleagues was unwavering, particularly Dr Judy West, Dr Lucy Sutherland, Tom North, Peter Byron, Prof Andrew Young, Dr Linda Broadhurst and Dr Joe Miller.

I'd like to thank my family, Dura, Vesna, Megan and grandparents, for a lifetime of support and encouragement. Thanks also to friends including my dearest friend, Nicole Sabatino, for her positivity and for assisting with seed collection and processing just so we could spend time together. Finally, Mark Wallace is thanked for collaboration in science and life.

Funding

An Australian Postgraduate Award (2009) and Curtin Research Scholarship (SCH-SE-DEB-00335), as well as an initial Curtin University Postgraduate Scholarship (2008, ID2609) supported me during the course of this research.

The Australian Society for Plant Scientists (2011) 'RN Robertson Travelling Fellowship' was awarded for travel and equipment usage fees associated with conducting X-ray mapping in Sydney.

Curtin University's Centre for Ecosystem Diversity and Dynamics (2008) provided travel funding to conduct field work and seed collection.

Funding for travel and/or conference registration where results from this thesis have been presented was awarded by:

The Plant Nutrition Trust (2013) 'Travel award' allowed me to present data at the 4th Seed Ecology meeting in Shenyang, China.

The Australian Microscopy and Microanalysis Society (2012) 'Student and early career researcher bursary' covered registration for the APMC 10 / ICONN 2012 / ACMM 22 conference in Perth, Australia.

Some funding was also provided by Kings Park and Botanic Garden and Curtin University's Department of Environment and Agriculture.

Permits

Licences for seed (SW012612) and soil collection (CE002663) for scientific purposes were granted by the Western Australian Department of Environment and Conservation (now Department of Parks and Wildlife). The relevant land holders (Department of Parks and Wildlife, Shire of Dandaragan, Shire of Chapman Valley and Shire of Irwin) are also thanked for approval to conduct field work on their land.

Thesis summary

Seed dispersal is important for plants, because it links the life cycle of these sessile organisms with ecological processes at local, landscape and biogeographic scales. Plant seeds can be dispersed by many vectors including ants, animals, wind and water. Dispersal by water (hydrochory) has not been investigated as thoroughly as other dispersal modes, and oceanic hydrochory has received even less scientific attention despite increasing reliance on oceanic hydrochory to explain the biogeography of many plant species. For effective oceanic dispersal, seeds will most likely need to be buoyant in seawater, able to survive time in highly saline seawater, and be able to germinate in new environments where conditions such as temperature, salinity and osmotic stress may vary from conditions where the seed originated.

Oceanic hydrochory is likely to be a particularly prevalent mode of dispersal in coastal plant communities because of the close proximity of these communities to the ocean. This thesis includes a series of multidisciplinary experiments that relate to the ecology, biology and physiology of coastal seeds that may affect oceanic dispersal and germination in novel environments. Dispersal ability was first investigated by creating a model using interspecific variation in simple, easily measured morphological seed traits to predict buoyancy and dispersal potential (Chapter 3). Then, the role of intraspecific morphological and anatomical variation in modulating buoyancy of woody fruits was investigated (Chapter 4). These studies revealed that both interspecific and intraspecific morphological seed traits significantly affect seed buoyancy, and therefore dispersal potential.

Post-dispersal germination capacity was examined by quantifying not only the individual but more importantly the interactive effects of salinity, osmotic stress and temperature on the germination of seeds from different source populations (Chapter 5). In general, seeds exhibited broad physiological tolerances to varied temperature, salinity and water stress indicating that post-dispersal establishment in new coastal environments is likely. Finally, a novel application of X-ray mapping was used to investigate mechanisms of salt tolerance and to identify if, when, and where sodium chloride enters germinating seeds (Chapter 6). The salt tolerance of germinating

seeds was related to exclusion of sodium and chlorine from seed embryos, whereas salt sensitivity was associated with the accumulation of sodium in seed embryos.

This thesis identifies the morphological and physiological seed traits that influence the capacity of coastal plant seeds to be dispersed by the ocean and germinate under stress or in new post-dispersal environments. The high buoyancy and broad germination thresholds of the tested seeds indicates that oceanic dispersal is indeed possible and may contribute to the broad peri-continental distribution of many Australian coastal plant species.

Table of contents

Chapter 1

General introduction

Introduction

Dispersal of terrestrial plant seeds

Dispersal is critical for plants because they are sessile organisms and many rely on dispersal of seeds to move offspring into suitable habitat away from the immediate vicinity of the parent plant. Dispersal patterns affect distribution and abundance at local, landscape and biogeographic scales (Bullock and Nathan 2008; Cousens *et al.* 2008; Nilsson *et al.* 2010). Understanding seed dispersal is a prerequisite to addressing many ecological questions, as is reflected by the large increase in publications that have examined seed dispersal over the past 20 years (Schupp *et al.* 2010). Fields of ecological study for which seed dispersal has been central include plant population dynamics, community structure, recruitment, gene flow, plant migration in response to historic and future climate change, maintenance of biodiversity, consequences of habitat fragmentation, ecological restoration, and the effectiveness of corridors for conservation (reviewed by Schupp *et al.* 2010).

Given the significance of seed dispersal, many dispersal modes have been closely investigated in an attempt to elucidate mechanistic relationships between seed traits and dispersal. For example, dispersal by wind (anemochory) and animals (zoochory) have received significant attention and investigations have revealed the particular seed morphologies conducive to dispersal via those syndromes (Augspurger and Franson 1987; Matlack 1987; Greene and Johnson 1989; Andersen 1991; Andersen 1992; Greene and Johnson 1992; Andersen 1993; Greene and Johnson 1993; Hensen and Müller 1997; Jongejans and Schippers 1999; Tackenberg 2003; Tackenberg *et al.* 2003; Römermann *et al.* 2005; Kuparinen 2006; Tackenberg *et al.* 2006; de Pablos and Peco 2007; Will *et al.* 2007; D'hondt *et al.* 2012; Horn *et al.* 2012).. Investigations of dispersal of seeds by water (hydrochory) in fresh water systems have also identified the seed traits and environmental factors that affect dispersal in

riparian systems (Hroudova *et al.* 1997; Williamson *et al.* 1999; Lopez 2001; Pettit and Froend 2001; Merritt and Wohl 2002; van den Broek *et al.* 2005; Leyer 2006; Leyer and Pross 2009; Nilsson *et al.* 2010). However, mechanistic investigations of plant dispersal via oceanic hydrochory remain scarce, even though it may be a significant process for coastal plants.

Coastal plants

Plants require a number of adaptations to survive in coastal habitats. Air-borne salinity, sand abrasion and accretion, drought, and low nutrient availability are some of the factors that limit seedling establishment and plant growth in coastal environments (Rozema 1985; Maun 1994; Greaver and Sternberg 2007). Seed germination in coastal environments is further determined by factors such as temperature, depth and light (Woodell 1985; Mariko *et al.* 1992; Martinez *et al.* 1992; Necajeva and Ievinsh 2008). In particular, high salinity can reduce or inhibit germination and has therefore been investigated to determine the capacity of some coastal seeds to be dispersed by highly saline seawater (c. 500 mM NaCl) (Appendix 1; Stephens 1958; Lesko and Walker 1969; Quilichini and Debussche 2000; Guja *et al.* 2010). There is limited knowledge of dispersal syndromes among coastal species. Laboratory and field research have identified that wind (Darling *et al.* 2008), rafting (Minchinton 2006), ants (Quilichini and Debussche 2000) and seawater (Yang *et al.* 2012) are dispersal syndromes of coastal species around the world. Dispersal via seawater may be more common than presumed, as evidenced by growing biogeographic and phylogenetic literature citing long-distance oceanic dispersal to explain evolutionary history and plant distributions (e.g. Howarth *et al.* 2003; Cowie and Holland 2006). For example, dispersal across the 1500 – 2000 km Tasman Sea appears to have occurred more commonly among coastal and wetland species than among species from dry, inland environments (Jordan 2001). This suggests that coastal species are more likely to undergo long-distance trans-oceanic dispersal.

Given their proximity to the ocean, coastal plants are the most likely plants to be dispersed by ocean currents. Therefore, oceanic dispersal may play a significant role in shaping the biogeography, evolution and persistence of coastal plant species. To persist through future climate changes coastal plants will need to be adaptable or

resilient to ever increasing changes in what is already a dynamic environment (Greaver and Sternberg 2007; Travis *et al.* 2013). Climate change predictions for sandy beaches include changes in temperature and precipitation, raised sea level and shoreline retreat, increases in storm events, and alteration of circulation, upwelling and wave regimes (Harley *et al.* 2006; Schlacher *et al.* 2008). These changes will likely affect physiological performance, tolerance and survival of many coastal organisms, drive geographical shifts in species' ranges, alter community structure and dynamics, and ultimately result in modified dune vegetation communities (Greaver and Sternberg 2007; Schlacher *et al.* 2008). More importantly, the increased erosion and more frequent severe weather events are likely to result in increased oceanic seed loads (Travis *et al.* 2013). Oceanic dispersal of coastal plants to new environments might provide access to new niches in coastal environments and, therefore, may become increasingly important for persistence under changing climates.

Oceanic seed dispersal

Long-distance dispersal via oceanic hydrochory is increasingly recognised as a rare, but important, mode of dispersal for many organisms (Higgins *et al.* 2003; de Queiroz 2005; Cowie and Holland 2006; Nathan 2006; Nathan *et al.* 2008; Gillespie *et al.* 2011). There are ongoing debates about the significance of oceanic dispersal versus vicariance (de Queiroz 2005), and there is growing evidence that both processes have played significant roles in shaping biogeographic patterns (Cowie and Holland 2006; Crisp and Cook 2007; Christenhusz and Chase 2013). The advent of molecular techniques has resulted in much phylogeographic evidence that suggests oceanic hydrochory may have been fundamental to the radiation of many plant species (Howarth *et al.* 2003; de Queiroz 2005; Cowie and Holland 2006; Kokubugata *et al.* 2012; Christenhusz and Chase 2013). Yet the mechanisms of oceanic dispersal in plants have received relatively little attention since the 19th century when Darwin was among the first to systematically investigate the ability of seeds to be dispersed by the ocean (Darwin 1856; Darwin 1859; Guppy 1906; Black 2009). There have been few recent investigations of seeds to determine whether they are physically capable of surviving injurious oceanic conditions (Appendix 1; Quilichini and Debussche 2000; Atia *et al.* 2010b; Guja *et al.* 2010).

Furthermore, long-distance dispersal is often likely to take seeds into new environments with different climate or substrate to their source population. More research is required to understand the capacity of seeds to successfully disperse via the ocean and germinate in new environments (i.e. seed dispersal effectiveness; Schupp *et al.* 2010).

Successful oceanic seed dispersal

Dispersal by oceanic hydrochory can enable seeds to reach distant sites. However, the capacity to germinate under new environmental conditions is critical for the successful establishment of plants following dispersal (Nilsson *et al.* 2010; Gillespie *et al.* 2011; van Loon *et al.* 2011). Effective oceanic dispersal involves progression through many stages that are affected by morphological and physiological seed traits. Seeds need to be buoyant in seawater, be able to survive high salinity, and be capable of germinating in new environments where conditions such as temperature, salinity and osmotic stress may vary from the conditions where the seed originated.

Determinants of oceanic seed dispersal

Seed buoyancy

Seed dispersal potential is a continuous variable that can be obtained from direct measurements in the field or measurements/models of traits related to a particular dispersal syndrome (Pérez-Harguindeguy *et al.* 2013). The potential for hydrochory is correlated with the buoyancy of propagules because the longer propagules remain buoyant the further they are carried by currents (Kleyer *et al.* 2008; Pérez-Harguindeguy *et al.* 2013). The significance of buoyancy is evident from its use to determine the dispersal potential (percentage of propagules remaining buoyant over time) of many plant species in water (Appendix 1; Hroudova *et al.* 1997; Lopez 2001; van den Broek *et al.* 2005; Leyer and Pross 2009; Guja *et al.* 2010; Yang *et al.* 2012). However, the precise morphological seed traits or mechanisms of buoyancy are rarely studied.

Interspecific seed variation

It remains unclear, and difficult to determine, whether particular seed morphological traits are conducive to hydrochorous dispersal. Certainly some coastal species such as coconuts and mangrove trees produce propagules that are well adapted to oceanic dispersal (De Ryck *et al.* 2012; Van der Stocken *et al.* 2013). However, oceanic dispersal may also be important for species that are not obviously adapted for hydrochorous dispersal. There is increasing recognition of the significance of non-standard modes of dispersal, where dispersal is achieved by vectors other than the 'classic' syndrome inferred from seed morphology (Higgins *et al.* 2003; Nathan 2006; Nathan *et al.* 2008). Further, secondary dispersal (movement by a second dispersal mode after initial dispersal; Cousens *et al.* 2008) and/or polychory (one seed type dispersed by multiple modes; Thomson *et al.* 2010) are also significant processes. Many species, with various seed morphologies, are likely to be dispersed by non-standard modes, including oceanic hydrochory.

A variety of seed morphological traits are likely to influence seed buoyancy, but these are poorly defined because of the tendency to associate distinctive seed morphologies with only the primary/standard mode of dispersal ('classic' morphological syndrome; Higgins *et al.* 2003), rather than also considering non-standard means of dispersal. For example, arils are often assumed to promote ant dispersal (myrmecochory), fleshy fruits are generally associated with animal ingestion (endozoochory), and awns and pappus can facilitate wind dispersal (anemochory) (Werker 1997; Higgins *et al.* 2003), yet all of these seed morphological types may also be capable of hydrochorous dispersal. Seeds that are apparently wind-dispersed are frequently buoyant and remain viable in water and may therefore be dispersed longer distances by water than by wind (Higgins *et al.* 2003). The contribution of seed morphological traits to the dispersal ability of seeds via well-studied dispersal syndromes such as anemochory and zoochory has been investigated in some depth. Results of these studies have been used to produce models that predict dispersal capacity. For example, the relationship between seed morphology and attachment to animal fur has been modelled to reveal that elongated seeds and seeds with appendages or rough surface structure have the highest attachment potential, while light seeds are retained most effectively by sheep and

cattle fur (Römermann *et al.* 2005; Tackenberg *et al.* 2006; de Pablos and Peco 2007; Will *et al.* 2007).

Some seed characteristics such as air chambers or cork-like tissue have been predicted to be associated with extended seed buoyancy (Lopez 2001; Higgins *et al.* 2003; Cousens *et al.* 2008; Vargas *et al.* 2014). However, the association is often speculative and not supported experimentally. Evidently, a large knowledge gap exists in relation to the seed traits that are associated with buoyancy and this lack of knowledge is particularly acute for the Australian flora (Thomson *et al.* 2010). Previous research on 13 Australian coastal plant species concluded that oceanic dispersal may be an effective means of inter- and peri-continental dispersal as seeds of various morphological types were capable of floating and surviving in seawater, often for ≥ 70 days (Appendix 1; Guja *et al.* 2010). The study suggested that seed morphology type affected buoyancy (woody fruits and awned achenes floated well) (Appendix 1; Guja *et al.* 2010), however, there were too few representatives of each seed morphology type to adequately describe any relationships between buoyancy and morphological seed traits. Consequently, the relationships between interspecific seed variation and buoyancy remain poorly understood for species that are not obviously adapted for oceanic dispersal.

Intraspecific seed variation

Although research on the effects of within-species variation includes only a limited number of seed morphology types and dispersal modes, it is clear that within-species variation can significantly affect dispersal (Telenius and Torstensson 1989; Hroudova *et al.* 1997; Darling *et al.* 2008). Within-species (intra- and infraspecific) variation can arise from genetic variation, the location of fruits in the infructescence, or the maternal environment during fruit development and maturation (Matilla *et al.* 2005; Donohue 2009). For example, anemochorous dispersal distances can be affected by variation among winged seeds from a single parent plant (Telenius and Torstensson 1989). Further, differences between the thickness of the exocarp in two subspecies of *Bolboschoenus* was related to buoyancy duration and therefore the potential for hydrochorous dispersal (Hroudova *et al.* 1997). While the magnitude of morphological differences within a species is often less than differences between

species, the effects of intraspecific variation on dispersal distance may be of particular significance for long-distance dispersal. Long-distance dispersal is often the result of rare events and is associated with seeds that fall within the tails of the 'dispersal kernel' (the probability of dispersal to different distances; Nathan 2006). Intraspecific variation can have a large impact by altering the tails of the dispersal kernel, which results in changes to the probability of rare long-distance dispersal events (Nathan 2006; Nathan *et al.* 2008). The generation of data that quantify the effects of intraspecific variation on seed dispersal would facilitate the inclusion of intraspecific variation in dispersal models and may enhance predictions of the probability of dispersal.

Survival of seeds in seawater

Seeds may be buoyant and therefore capable of oceanic dispersal, but survival in potentially injurious seawater is also critical for successful dispersal. Seeds must be capable of surviving the highly saline conditions (c. 500 mM sodium chloride) during transport by ocean currents. Darwin was among the first to examine the capacity of seeds to float and survive in seawater (Darwin 1856; Darwin 1859; Black 2009). Since these early investigations there has been little consideration of the physiological seed traits required for successful oceanic dispersal (however, see Stephens 1958; Lesko and Walker 1969). In a recent study of Australian coastal species, the majority (9/10 species) had a proportion of seeds that were capable of floating and surviving in seawater for $\geq$ 70 days. Although most species survived extended exposure to seawater, subsequent germination was significantly reduced in highly saline conditions when compared with germination in non-saline conditions (Appendix 1; Guja *et al.* 2010). This demonstrates that seeds require salt tolerance during exposure to seawater, and may also require salt tolerance during germination in new post-dispersal environments, to achieve successful oceanic dispersal.

Germination in new environments

Salinity, water stress and temperature

Seeds are generally adapted to germinate in response to particular environmental conditions and if those requirements are not met they do not germinate (Vleeshouwers *et al.* 1995; Baskin and Baskin 1998). After long-distance dispersal it is likely that a seed will arrive in a coastal environment that differs to that from which it originated. This new coastal environment may be characterised by substantially different soil salinity, water availability, and temperature (Greaver and Sternberg 2007), which may not meet the germination requirements of the dispersed seed. The germination requirements of only 7% of the world's coastal flora are known (Baskin and Baskin 1998), making predictions of post-dispersal recruitment challenging, and in many cases, impossible.

Ocean-dispersed seeds will likely be deposited in coastal areas characterised by elevated salinity. Salt tolerance during germination is generally high for coastal plant species (Woodell 1985; Mariko *et al.* 1992; Martinez *et al.* 1992; Walmsley and Dowey 1997; Guja *et al.* 2010; Gul *et al.* 2013). However, salt tolerance during germination is often only assessed at a single temperature. For example, a study of 10 Australian coastal plant species found that the salt tolerance of seeds, and the ability of seeds to recover from salt exposure, was high at 15 °C (Guja *et al.* 2010). Interactions between temperature and salinity affect germination of halophytes (Ungar 1978; Baskin and Baskin 1998; Gul *et al.* 2013), however many ecological and dispersal studies overlook the potential impact of this interaction. Without knowledge of the combined effect of temperature, salinity, and water stress on germination the capacity of seeds to germinate in new environments cannot be reliably predicted.

Intraspecific variation in seed germination traits may affect the ability of a species to germinate under new salinity and temperature conditions (Woodell 1985; Hanslin and Eggen 2005; Donohue 2009). For example, some coastal halophytes show between population variation in salinity tolerance during germination (Woodell 1985; Hanslin and Eggen 2005). In the context of oceanic dispersal, and particularly under the influence of changing climates, differences between populations may

render some more capable of successful dispersal and, therefore, increase the resilience of the species to climate change. However, the magnitude of intraspecific variation in germination thresholds and salinity tolerance of coastal seeds is largely unknown.

Salt tolerance mechanisms

High salinity has a detrimental effect on the growth and survival of many plant species (Greenway and Munns 1980; Flowers *et al.* 1986; Carillo *et al.* 2011). The effects of salinity include reduced ability to take up water, metabolic changes, and leaf senescence, all of which can lead to plant death (Munns 2002). For many plant species, the effect of salinity is similar to the effect of water stress and distinguishing between these two stresses can be difficult (Munns 2002). While the effect of salinity on mature plants is relatively well-documented, there is limited understanding of seed germination under saline conditions. As with mature plants, elevated salinity can affect seed germination through both osmotic and toxic effects. Assessing the relative contribution of osmotic and toxic effects on germination is often a goal of research that aims to determine a species' salt-sensitivity (Redmann 1974; Berger 1985; Hardegree and Emmerich 1990; Katembe *et al.* 1998; Tobe *et al.* 2000). Salt tolerance in mature plants relies on an active balance between sodium, potassium, and chlorine (Greenway and Munns 1980; Flowers *et al.* 1986). This same balance may be required during seed germination in saline conditions, however, little is known about mechanisms of salt tolerance during germination.

In saline conditions, maturing seeds of some salt-tolerant species avoid seed damage by localising salt within external seed or fruit coat tissues (Hocking 1982; Khan *et al.* 1985; Atia *et al.* 2010a). However, it is unknown whether salt is imbibed by germinating seeds and if so, where it is localised and how this relates to salt tolerance. Further research to determine how salt affects seeds during imbibition (water uptake) and early germination, and whether seeds exclude or compartmentalise salt, is required. Salt tolerance mechanisms may have significant effects on the capability of seeds to germinate after dispersal by the ocean and will also have implications in the fields of plant physiology and agriculture.

Rationale of thesis

Following previous work that investigated the capacity of coastal seeds to disperse via the ocean (Appendix 1; Guja *et al.* 2010), this thesis investigates mechanistic aspects of oceanic seed dispersal and germination in post-dispersal environments. Using seed morphological and physiological traits, this thesis aims to quantify the oceanic dispersal capacity and germination ability of seeds by addressing four major areas of oceanic hydrochory where scientific knowledge is currently lacking:

Dispersal

- The effect of interspecific morphological seed variation on buoyancy
- The effect of intraspecific morphological seed variation on buoyancy

Germination

- The effect of temperature, salinity and water stress on germination
- The physiological mechanisms of salt tolerance during germination

The research addresses these critical questions by:

Dispersal

- Creating a model for the prediction of seed buoyancy using well defined, easily measured morphological seed traits
- Identifying intraspecific variation in seed traits, their influence on buoyancy, and the specific seed and fruit tissues that increase buoyancy

Germination

- Describing the individual, and more importantly the interactive effects of salinity, osmotic stress and temperature on seed germination and the implications for dispersal and germination of seeds from different source populations
- Investigating the salt tolerance mechanisms of seeds of coastal species of contrasting salt sensitivity to identify if, when, and where sodium chloride enters germinating seeds

Each of these complementary research areas are addressed in a chapter. The chapters are related conceptually as indicated in **Figure 1**.

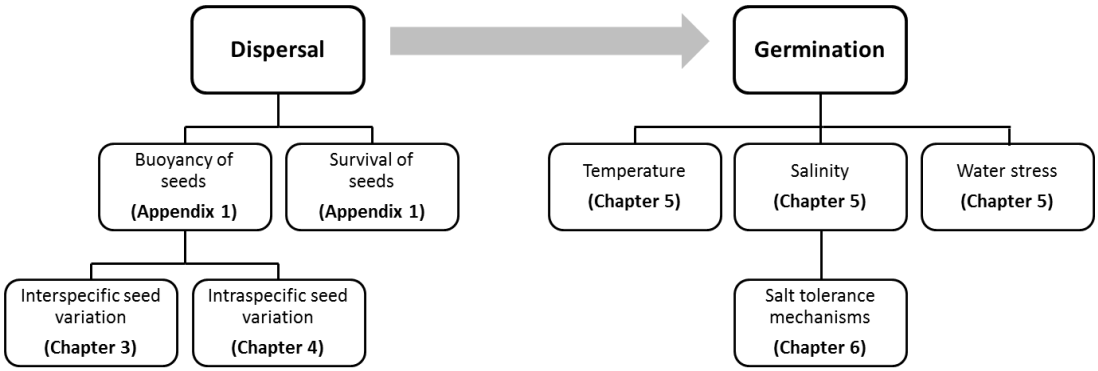


Figure 1. Diagrammatic representation of a dispersal framework identifying key research areas and the chapters in this thesis where they are addressed.

Thesis outline

As outlined below, this thesis consists of a general introduction, description of the study system, four experimental chapters, a synthesis of the main results, and appendices.

Chapter 1: Highlights key research areas and knowledge gaps relating to oceanic seed dispersal and recruitment capacity.

Chapter 2: Provides an ecological description of the study system.

Chapter 3: Investigates the ability to predict dispersal of coastal seeds with differing seed morphologies. Simple morphological seed traits are identified that can be used to predict how long seeds will remain buoyant and therefore, their dispersal potential. This chapter aims to provide the first model for prediction of oceanic hydrochory using seed traits.

Chapter 4: Examines intraspecific variation in fruits of *Scaevola crassifolia* (Goodeniaceae) to determine how this variation affects dispersal potential. Variation in fruit morphology and anatomy along a latitudinal environmental gradient is used to identify traits that increase dispersal capacity and determine differences in the dispersal ability of populations. The aim is to provide the first report of the effects of intraspecific variation in woody fruits on buoyancy.

Chapter 5: Uses experimental manipulation to explore the effects of salinity, osmotic potential, temperature, and their interactions, on seed germination. Variation between seeds from different source populations is also considered. The experimental design is novel in dispersal ecology and aims to provide a new understanding of the seed ecology of coastal plants.

Chapter 6: Investigates mechanisms of salt tolerance in germinating seeds by comparing a salt-tolerant and salt-sensitive coastal plant species. A novel approach that combines X-ray mapping and flame photometry is used in an attempt to locate and quantify sodium and chlorine in internal tissues of germinating seeds.

Chapter 7: Synthesises the results reported in the preceding chapters and discusses the implications of these results for the understanding of oceanic dispersal.

Appendices: Include previous research, published manuscripts from this thesis, and evidence of the right to reproduce the manuscripts.

Chapters 3 to 6 are prepared as stand-alone manuscripts for publication in peer-reviewed journals. These manuscripts complement one another as outlined in Chapter 1 and **Figure1**. Each chapter is self-contained. Therefore, the reference list and the numbering of tables and figures are specific to each chapter. Chapters 3 to 6 are prepared as co-authored manuscripts for which the thesis candidate was the lead author. The contributions of co-authors to each chapter are listed in the thesis preface. Published or accepted manuscripts have been modified slightly for inclusion in the thesis.

References

Andersen M (1991) Mechanistic models for the seed shadows of wind-dispersed plants. *The American Naturalist* **137**, 476-497.

Andersen MC (1992) An analysis of variability in seed settling velocities of several wind-dispersed Asteraceae. *American Journal of Botany* **79**, 1087-1091.

Andersen MC (1993) Diaspore morphology and seed dispersal in several wind-dispersed Asteraceae. *American Journal of Botany* **80**, 487-492.

Atia A, Debez A, Abdelly C, Smaoui A (2010a) Relationship between ion content in seed and spongy coat of the medicinal halophyte *Crithmum maritimum* L. and germination capacity. *Notulae Scientia Biologicae* **2**, 72-74.

Atia A, Debez A, Barhoumi Z, Pacini E, Abdelly C, Smaoui A (2010b) The mericarp of the halophyte *Crithmum maritimum* (Apiaceae): structural features, germination, and salt distribution. *Biologia* **65**, 489-495.

Augspurger CK, Franson SE (1987) Wind dispersal of artificial fruits varying in mass, area, and morphology. *Ecology* **68**, 27-42.

Baskin CC, Baskin JM (1998) 'Seeds: ecology, biogeography, and evolution of dormancy and germination.' (Academic Press: California).

Berger A (1985) Seed dimorphism and germination behaviour in *Salicornia patula*. *Vegetatio* **61**, 137-143.

Black M (2009) Darwin and seeds. *Seed Science Research* **19**, 193-199.

Bullock JM, Nathan R (2008) Plant dispersal across multiple scales: linking models and reality. *Journal of Ecology* **96**, 567-568.

Carillo P, Annunziata MG, Pontecorvo G, Fuggi A, Woodrow P (2011) Salinity stress and salt tolerance. In 'Abiotic stress in plants - mechanisms and adaptations. (Eds A Shanker and B Venkateswarlu). (Intech: Online).

Christenhusz MJM, Chase MW (2013) Biogeographical patterns of plants in the neotropics – dispersal rather than plate tectonics is most explanatory. *Botanical Journal of the Linnean Society* **171**, 277-286.

Cousens R, Dytham C, Law R (2008) 'Dispersal in plants: a population perspective.' (Oxford University Press: New York).

Cowie RH, Holland BS (2006) Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. *Journal of Biogeography* **33**, 193-198.

Crisp MD, Cook LG (2007) A congruent molecular signature of vicariance across multiple plant lineages. *Molecular Phylogenetics and Evolution* **43**, 1106-1117.

D'hondt B, D'hondt S, Bonte D, Brys R, Hoffmann M (2012) A data-driven simulation of endozoochory by ungulates illustrates directed dispersal. *Ecological Modelling* **230**, 114-122.

Darling E, Samis KE, Eckert CG (2008) Increased seed dispersal potential towards geographic range limits in a Pacific coast dune plant. *New Phytologist* **178**, 424-435.

Darwin C (1856) On the action of sea-water on the germination of seeds. *Journal of the Proceedings of the Linnean Society of London. Botany* **1**, 130-140.

Darwin C (1859) ' The origin of species.' (Oxford University Press: Oxford).

de Pablos I, Peco B (2007) Diaspore morphology and the potential for attachment to animal coats in Mediterranean species: an experiment with sheep and cattle coats. *Seed Science Research* **17**, 109-114.

de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution* **20**, 68-73.

De Ryck DJR, Robert EMR, Schmitz N, Van der Stocken T, Di Nitto D, Dahdouh-Guebas F, Koedam N (2012) Size does matter, but not only size: two alternative dispersal strategies for viviparous mangrove propagules. *Aquatic Botany* **103**, 66-73.

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1059-1074.

Flowers TJ, Hajibagheri MA, Clipson NJW (1986) Halophytes. *The Quarterly Review of Biology* **61**, 313-337.

Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK (2011) Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology & Evolution* **27**, 1-10.

Greaver TL, Sternberg LSL (2007) Fluctuating deposition of ocean water drives plant function on coastal sand dunes. *Global Change Biology* **13**, 216-223.

Greene DF, Johnson EA (1989) A model of wind dispersal of winged or plumed seeds. *Ecology* **70**, 339-347.

Greene DF, Johnson EA (1992) Can the variation in samara mass and terminal velocity on an individual plant affect the distribution of dispersal distances? *The American Naturalist* **139**, 825-838.

Greene DF, Johnson EA (1993) Seed mass and dispersal capacity in wind-dispersed diaspores. *Oikos* **67**, 69-74.

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* **31**, 149-90.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**, 1175-1186.

Gul B, Ansari R, Flowers TJ, Khan MA (2013) Germination strategies of halophyte seeds under salinity. *Environmental and Experimental Botany* **92**, 4-18.

Guppy HB (1906) 'Observations of a naturalist in the Pacific between 1896 and 1899, volume 2 plant dispersal.' (Macmillan and Co.: London).

Hanslin HM, Eggen T (2005) Salinity tolerance during germination of seashore halophytes and salt tolerant grass cultivars. *Seed Science Research* **15**, 43-50.

Hardegree SP, Emmerich WE (1990) Partitioning water potential and specific salt effects on seed germination of four grasses. *Annals of Botany* **66**, 587-595.

Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. *Ecology Letters* **9**, 228-241.

Hensen I, Müller C (1997) Experimental and structural investigations of anemochorous dispersal. *Plant Ecology* **133**, 169-180.

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* **84**, 1945-1956.

Hocking PJ (1982) Salt and mineral nutrient levels in fruits of two strand species, *Cakile maritima* and *Arctotheca populifolia*, with special reference to the effect of salt on the germination of *Cakile*. *Annals of Botany* **50**, 335-343.

Horn S, Raabe A, Will H, Tackenberg O (2012) TurbSeed - a model for wind dispersal of seeds in turbulent currents based on publicly available climate data. *Ecological Modelling* **237–238**, 1-10.

Howarth DG, Gustafsson MHG, Baum DA, Motley TJ (2003) Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacifc Basin and colonization of the Hawaiian Islands. *American Journal of Botany* **90**, 915-923.

Hroudova Z, Moravcova L, Zakravsky P (1997) Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. *Folia Geobotanica et Phytotaxonomica* **32**, 377-390.

Jongejans E, Schippers P (1999) Modeling seed dispersal by wind in herbaceous species. *Oikos* **87**, 362-372.

Jordan GJ (2001) An investigation of long-distance dispersal based on species native to both Tasmania and New Zealand. *Australian Journal of Botany* **49**, 333-340.

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

Khan MA, Webber DJ, Hess WM (1985) Elemental distribution in seeds of the halophytes *Salicornia pacifica* var. *utahensis* and *Atriplex canescens*. *American Journal of Botany* **72**, 1672-1675.

Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, Van Groenendael JM, Klimeš L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel AK, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E, Peco B (2008) The LEDA Traitbase: a database of life-history traits of the northwest European flora. *Journal of Ecology* **96**, 1266-1274.

Kokubugata G, Nakamura K, Forster PI, Hirayama Y, Yokota M (2012) Antitropical distribution of *Lobelia* species (Campanulaceae) between the Ryukyu Archipelago of Japan and Oceania as indicated by molecular data. *Australian Journal of Botany* **60**, 417-428.

Kuparinen A (2006) Mechanistic models for wind dispersal. *Trends in Plant Science* **11**, 296-301.

Lesko GL, Walker RB (1969) Effect of sea water on seed germination in two Pacific atoll beach species. *Ecology* **50**, 730-734.

Leyer I (2006) Dispersal, diversity and distribution patterns in pioneer vegetation: the role of river floodplain connectivity. *Journal of Vegetation Science* **17**, 407-416.

Leyer I, Pross S (2009) Do seed and germination traits determine plant distribution patterns in riparian landscapes? *Basic and Applied Ecology* **10**, 113-121.

Lopez OR (2001) Seed flotation and postflooding germination in tropical *terra firme* and seasonally flooded forest species. *Functional Ecology* **15**, 763-771.

Mariko S, Kachi N, Ishikawa S, Furukawa A (1992) Germination ecology of coastal plants in relation to salt environment. *Ecological Research* **7**, 225-233.

Martinez ML, Valverde T, Moreno-Casasola P (1992) Germination response to temperature, salinity, light and depth of sowing of ten tropical dune species. *Oecologia* **92**, 343-353.

Matilla A, Gallardo M, Puga-Hermida MI (2005) Structural, physiological and molecular aspects of heterogeneity in seeds: a review. *Seed Science Research* **15**, 63-76.

Matlack GR (1987) Diaspore size, shape, and fall behavior in wind-dispersed plant species. *American Journal of Botany* **74**, 1150-1160.

Maun MA (1994) Adaptations enhancing survival and establishment of seedlings on coastal dune systems. *Vegetatio* **111**, 59-70.

Merritt DM, Wohl EE (2002) Processes governing hydrochory along rivers: hydraulics, hydrology, and dispersal phenology. *Ecological Applications* **12**, 1071-1087.

Minchinton TE (2006) Rafting on wrack as a mode of dispersal for plants in coastal marshes. *Aquatic Botany* **84**, 372-376.

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

Nathan R (2006) Long-distance dispersal of plants. *Science* **313**, 786-788.

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. *Trends in Ecology and Evolution* **23**, 638-647.

Necajeva J, Ievinsh G (2008) Seed germination of six coastal plant species of the Baltic region: effect of salinity and dormancy-breaking treatments. *Seed Science Research* **18**, 173-177.

Nilsson C, Brown RL, Jansson R, Merritt DM (2010) The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews* **85**, 837-858.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**, 167-234.

Pettit NE, Froend RH (2001) Availability of seed for recruitment of riparian vegetation: a comparison of a tropical and a temperate river ecosystem in Australia. *Australian Journal of Botany* **49**, 515-528.

Quilichini A, Debussche M (2000) Seed dispersal and germination patterns in a rare Mediterranean island endemic (*Anchusa crispa* Viv., Boraginaceae). *Acta Oecologica* **21**, 303-313.

Redmann RE (1974) Osmotic and specific ion effects on the germination of alfalfa. *Canadian Journal of Botany* **52**, 803-808.

Römermann C, Tackenberg O, Poschlod P (2005) How to predict attachment potential of seeds to sheep and cattle coat from simple morphological seed traits. *Oikos* **110**, 219-230.

Rozema J, Bijwaard, P., Prast, G., & Broeckman, R. (1985) Ecophysiological adaptation of coastal halophytes from foredunes and salt marshes. *Vegetatio* **62**, 499-521.

Schlacher TA, Schoeman DS, Dugan J, Lastra M, Jones A, Scapini F, McLachlan A (2008) Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. *Marine Ecology* **29**, 70-90.

Schupp EW, Jordano P, Gómez JM (2010) Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* **188**, 333-353.

Stephens SG (1958) Salt water tolerance of seeds of *Gossypium* species as a possible factor in seed dispersal. *The American Naturalist* **92**, 83-92.

Tackenberg O (2003) Modeling long-distance dispersal of plant diaspores by wind. *Ecological Monographs* **73**, 173-189.

Tackenberg O, Poschlod P, Bonn S (2003) Assessment of wind dispersal potential in plant species *Ecological Monographs* **73**, 191-205.

Tackenberg O, Romermann C, Thompson K, Poschlod P (2006) What does diaspore morphology tell us about external animal dispersal? Evidence from standardized experiments measuring seed retention on animal-coats. *Basic and Applied Ecology* **7**, 45-58.

Telenius A, Torstensson P (1989) The seed dimorphism of *Spergularia marina* in relation to dispersal by wind and water. *Oecologia* **80**, 206-210.

Thomson FJ, Moles AT, Auld TD, Ramp D, Ren S, Kingsford RT (2010) Chasing the unknown: predicting seed dispersal mechanisms from plant traits. *Journal of Ecology* **98**, 1310-1318.

Tobe K, Li X, Omasa K (2000) Seed germination and radicle growth of a halophyte, *Kalidium caspicum* (Chenopodiaceae). *Annals of Botany* **85**, 391-396.

Travis JMJ, Delgado M, Bocedi G, Baguette M, Bartoń K, Bonte D, Boulangeat I, Hodgson JA, Kubisch A, Penteriani V, Saastamoinen M, Stevens VM, Bullock JM (2013) Dispersal and species' responses to climate change. *Oikos* **122**, 1532-1540.

Ungar IA (1978) Halophyte seed germination. *Botanical Review* **44**, 233-264.

van den Broek T, van Diggelen R, Bobbink R (2005) Variation in seed buoyancy of species in wetland ecosystems with different flooding dynamics. *Journal of Vegetation Science* **16**, 579-586.

Van der Stocken T, De Ryck DJR, Balke T, Bouma TJ, Dahdouh-Guebas F, Koedam N (2013) The role of wind in hydrochorous mangrove propagule dispersal. *Biogeosciences* **10**, 3635-3647.

van Loon AH, Soomers H, Schot PP, Bierkens MFP, Griffioen J, Wassen MJ (2011) Linking habitat suitability and seed dispersal models in order to analyse the effectiveness of hydrological fen restoration strategies. *Biological Conservation* **144**, 1025-1035.

Vargas P, Nogales M, Jaramillo P, Olesen JM, Traveset A, Heleno R (2014) Plant colonization across the Galápagos Islands: success of the sea dispersal syndrome. *Botanical Journal of the Linnean Society* **174**, 349-358.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**, 1031-1037.

Walmsley CA, Dowey AJ (1997) Germination characteristics of shingle beach species, effects of seed ageing and their implications for vegetation restoration. *Journal of Applied Ecology* **34**, 131-142.

Werker E (1997) 'Seed anatomy.' (Stuttgart: Borntraeger, Berlin).

Will H, Maussner S, Tackenberg O (2007) Experimental studies of diaspore attachment to animal coats: predicting epizoochorous dispersal potential. *Oecologia* **153**, 331-339.

Williamson GB, Costa F, Vera CVM (1999) Dispersal of Amazonian trees: hydrochory in *Swartzia polyphylla*. *Biotropica* **31**, 460-465.

Woodell SRJ (1985) Salinity and seed germination patterns in coastal plants. *Vegetatio* **61**, 223-229.

Yang H, Lu Q, Wu B, Zhang J (2012) Seed dispersal of East Asian coastal dune plants via seawater – short and long distance dispersal. *Flora* **207**, 701-706.

Chapter 2

Description of study system

Introduction

The south west of Western Australia (SWWA) includes one of the richest floras in the world and is recognised as a biodiversity hotspot (Myers *et al.* 2000). Many endemic and relictual species occur in expanses of SWWA that are old and infertile (Hopper 2009). Whereas much of SWWA contains some of the oldest landscapes in the world, the coastal fringe is young and dynamic (Sauer 1965). The number of species occurring in the Perth coastal dunes (147) is low compared with inland areas of the Swan Coastal Plain (Sauer 1965; Dixon 2011), perhaps because these plant species must be specialised to survive in difficult coastal conditions. Coastal plants must endure seasonal cycles of strong winds, salt-laden air, drought, sand accretion and erosion in combination with the low soil nutrient content and high alkalinity of coastal sands (Sauer 1965; Dixon 2011). Many species occurring along the SWWA coast, as in coastal areas around the world, exhibit adaptations to salinity, aridity, wind and sand abrasion, high pH, a wide range of nutrient levels, and a range of light intensities (Oosting and Billings 1942; Barbour 1978; Rozema 1985; Maun 1994; Greaver and Sternberg 2007). This chapter presents environmental data and background information concerning the study system.

Coastal geomorphology

The SWWA coast predominantly consists of coastal sand dunes interspersed with exposed limestone or granitic formations and estuary systems. The coastal dune systems are relatively young (Holocene and Pleistocene, c. 10,000-14,000 years old) and consist of two geologically recent deposits, the Quindalup and Spearwood dunes (Rippey and Rowland 2004). The Spearwood dunes, the oldest of the two dune systems, form the foundation of the coast and islands and consist of consolidated limestone. The youngest dune formation, the Quindalup dunes, consists of white lime-sand, which has eroded from coastal islands and reefs and washed on-shore.

This process of sand deposition has occurred in association with sea level changes over the past 10,000 years (Rippey and Rowland 2004; Dixon 2011). The geomorphology of the Quindalup dunes is characterised by a linear series of north-south dunes that can be up to several kilometres wide (Semeniuk and Johnson 1982; Semeniuk 1995). The Quindalup dunes are characterised by highly mobile beach sands, frontal dunes subject to episodic erosion, and stable secondary dunes that generally contain continuous vegetative cover (Sauer 1965; Semeniuk and Johnson 1982). SWWA beaches are wave-dominated environments, differentiated by variation in the wave energy levels at each beach (Semeniuk and Johnson 1982; Department of Planning 2003) (**Table 1**).

Environmental conditions

The SWWA coast runs approximately north-south along most of its length (**Figure 1**). In this thesis, eight beaches approximately 100 km apart were selected as study sites (**Figures 1** and **2**). Most of the region is characterised by a Mediterranean climate of cool wet winters (rain originates from east-moving depressions) and warm to hot dry summers (with very little rain over a 5-6 month period) (Li *et al.* 2005; Timbal *et al.* 2006). Unlike other regions at the same latitude, which tend to be arid, the Perth region has much higher rainfall due to the effects of the Leeuwin Current, which brings warm tropical waters south, particularly during winter when the current is strongest (Rippey and Rowland 2004; Waite *et al.* 2007).

Figure 1. Map of SWWA indicating the eight coastal study sites where seed and environmental data were collected. Perth is marked for reference.

Figure 2. Images of coastal study sites, from North to South. ***a.*** Coronation Beach, ***b.*** Dongara, ***c.*** Sandy Cape, ***d.*** Breton Bay, ***e.*** Scarborough, ***f.*** Tim's Thicket, ***g.*** Injidup Beach, ***h.*** Skippy Rock. Images: LK Guja, MJ Wallace and RL Long.

Table 1. Environmental data and beach and dune characteristics for eight coastal study sites.

Site	GPS coordinates (WGS 84)	Annual mean[a]			Climate[b] classification	IBRA sub-region[c]	Wave action[d]	Distance from high tide to:[e]			pH[f] (0-1 cm)	pH[f] (1-10 cm)
		Rainfall (mm)	Temperature max (°C)	Temperature min (°C)				Pioneer plants (m)	Dune top (m)	Swale (m)		
Coronation Beach	28°33'31"S 114°33'58"E	441.8	25.9	13.6	Semi arid warm	Geraldton Sandplains	Low to moderate	8	14	18	9.19	9.27
Dongara	29°17'51"S 114°55'32"E	401.5	na	na	Semi arid warm	Geraldton Sandplains	Low to moderate	32	46	51	9.23	9.33
Sandy Cape	30°10'44"S 115°00'03"E	533.9	24.9	13.0	Warm	Swan Coastal Plain	Low	11	14	17	8.59	8.51
Breton Bay	31°10'24"S 115°23'53"E	590.9	24.1	13.5	Warm	Swan Coastal Plain	Low	9	16	19	8.47	8.37
Scarborough	31°54'32"S 115°45'24"E	716.6	24.0	13.9	Warm	Swan Coastal Plain	Low to moderate	38	49	55	9.43	9.29
Tim's Thicket	32°39'50"S 115°36'33"E	649.2	23.2	14.7	Warm	Swan Coastal Plain	Low to moderate	32	39	48	9.18	9.19
Injidup	33°41'32"S 114°59'34"E	806.0	20.7	12.7	Moderate	Warren Region	High	27	40	53	8.59	8.67
Skippy Rock	34°21'22"S 115°07'44"E	1048.4	19.7	14.1	Moderate	Warren Region	High	13	23	29	9.19	9.32

na = not available

[a] Annual rainfall and temperature means for all years of record (Bureau of Meteorology 2013)

[b] Classification of mediterranean climate type (Department of the Environment 2013)

[c] Bioregion classification (Interim Biogeographic Regionalisation for Australia (IBRA)) (Department of the Environment 2013)

[d] Beach energy and wave action description (Semeniuk and Johnson 1982; Department of Planning 2003)

[e] Distance from the high tide mark to the first pioneer vegetation, top of the first dune, and swale behind the dune.

[f] pH (mean of 9 samples) was determined using air dried (40 °C) soil samples collected from three transects 10 m apart that ran perpendicular to the shoreline. Along each transect soil was collected from within three 30 × 30 cm plots. Samples were taken from the surface (0-1 cm) and sub-surface (1-10 cm) in each plot. Samples were diluted 1:5 in deionised water, tumbled for 1 hour and left to settle before measurement with a Cyberscan pH 300 meter (Entech Instruments) calibrated with pH 4, 7 and 10 standards.

Although the Holocene sandy beaches of SWWA can be regarded as relatively homogenous, there are a number of environmental gradients that result in differences among beaches along the coast (**Table 1** and **Figure 2**) (Sauer 1965). For example, there are strong temperature and rainfall gradients with a pattern of decreasing average temperature and increasing average rainfall from north to south (**Table 1**) (Bureau of Meteorology 2013). The annual mean maximum temperature is 25.9 °C in the north of the study area and 19.7 °C in the south. Annual mean rainfall ranges between 443.2 mm in the north and 967.6 mm in the south (Bureau of Meteorology 2013). This variation along the SWWA coast is reflected in the three distinct biogeographical sub-regions (Geraldton Sandplains, Swan Coastal Plain and Warren Region) that span the study area (**Table 1**) (Department of the Environment 2013). These biogeographical regions are defined based on common climate, geology, landform, and native vegetation (Department of the Environment 2013).

Coastal plant distributions

There are many common coastal plant species with broad distributions that span the strong climatic gradients and geological formations along the SWWA coast (Sauer 1965). Even some Western Australian endemic species such as *Spyridium globulosum* (Rhamnaceae) have wide distributions that span hundreds of kilometres of coastline (**Figure 3**). Many common coastal species have distributions that extend around the south of the continent (e.g. *Scaevola crassifolia* (Goodeniaceae)), to the eastern states, including as far north as Queensland (Sauer 1965; Rippey and Rowland 2004), and occasionally to New Zealand (e.g. *Ficinia nodosa* (Cyperaceae) (Sauer 1965; CSIRO 2013)) (**Figure 3**).

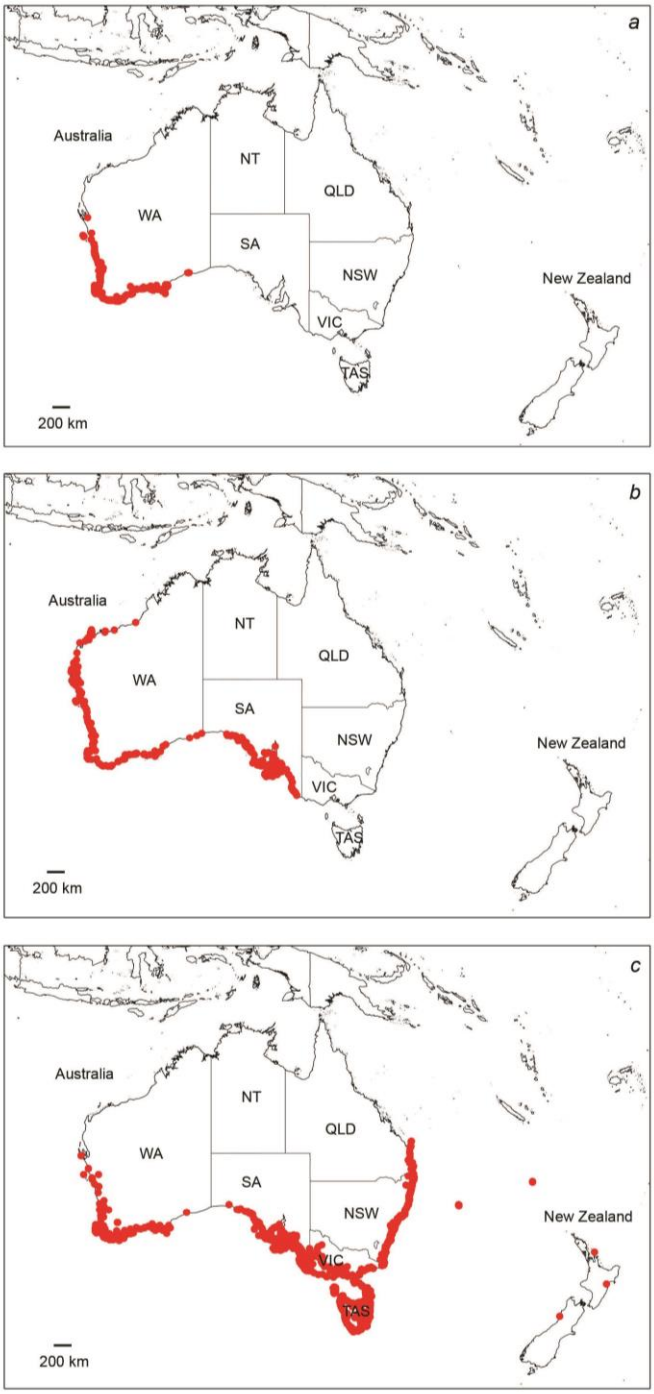


Figure 3. Wide coastal distributions of ***a.*** *Spyridium globulosum,* **b.** *Scaevola crassifolia* and ***c.*** *Ficinia nodosa*. Derived from Australian herbarium records (modified from CSIRO 2013). These species are the focus of **Chapters 4**, **5**, and **6**.

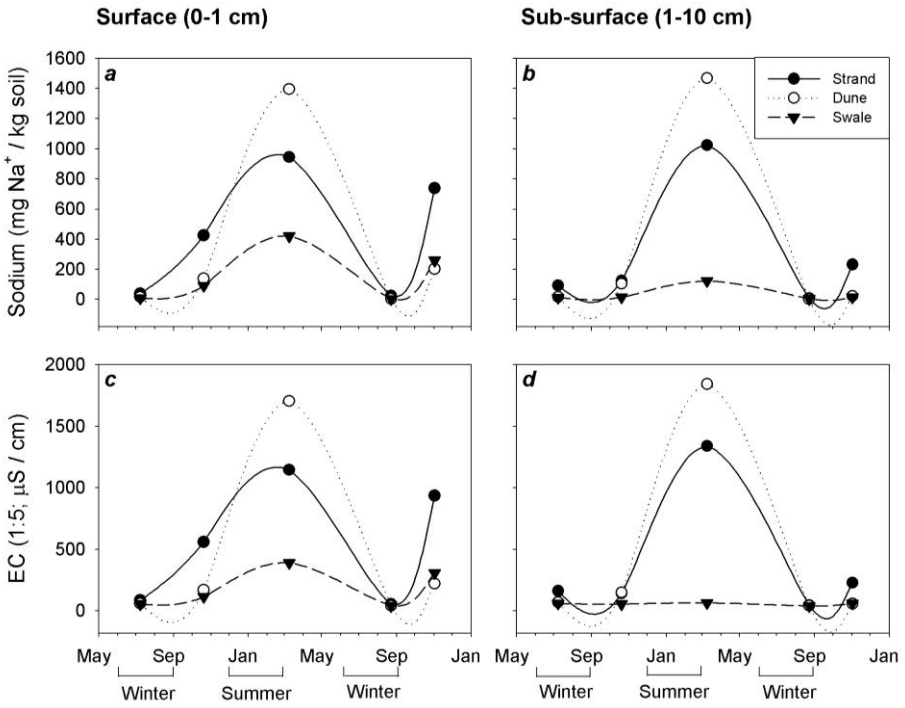


Figure 4. Mean soil salinity (sodium) and electrical conductivity (EC) (n = 3) at Scarborough Beach between July 2009 and November 2011. Error bars omitted for clarity, but did not exceed ± 163 for sodium and ± 219 for EC. The maximum variation was observed in the strand samples from November 2011. Three replicate transects spaced 10 m apart were run perpendicular to the high tide mark. Soil samples were taken from the strand (the point where the first pioneer vegetation began; 38 m from the high tide mark), dune (top of the first dune; 49 m from the high tide mark), and the swale (depression at the back of the first dune; 55 m from the high tide mark). At each of the three positions a soil sample was taken from within a 30 × 30 cm area on the soil surface (0-1 cm deep) and a composite sub-surface sample was taken from 1-10 cm deep.

a – b. Flame photometry was used to determine sodium content. Air dried (40 °C) soil samples were diluted 1:100 (0.5 g soil: 50 ml deionised water). Samples were tumbled end over end for 1 hour then centrifuged for 10 min at 2000 rpm. Approximately 30 ml of solution was removed by syringe and filtered using a 25 mm bulk acrodisc syringe filter with 0.45 um supor membrane (PALL Life Sciences). The filtered samples were then analysed using either a Sherwood Flame Photometer 410 or a Jenway PFP7 Flame Photometer. Standard NaCl curves were used to calculate the amount of sodium contained in each sample.

c – d. To determine electrical conductivity (EC), air dried (40 °C) soil samples were first diluted 1:5 in deionised water (4g soil: 20 ml deionised water). Samples were tumbled end over end for 1 h then left to settle between 15 min and 1 hour. Measurements were made with a Cyperscan Con 20 conductivity meter (Activon) calibrated with 0.6 M KCl.

Using conversion tables (CRC Press 1974) it was possible to estimate that the maximum EC recorded here is approximately equivalent to 100 mM NaCl.

Vegetation zonation

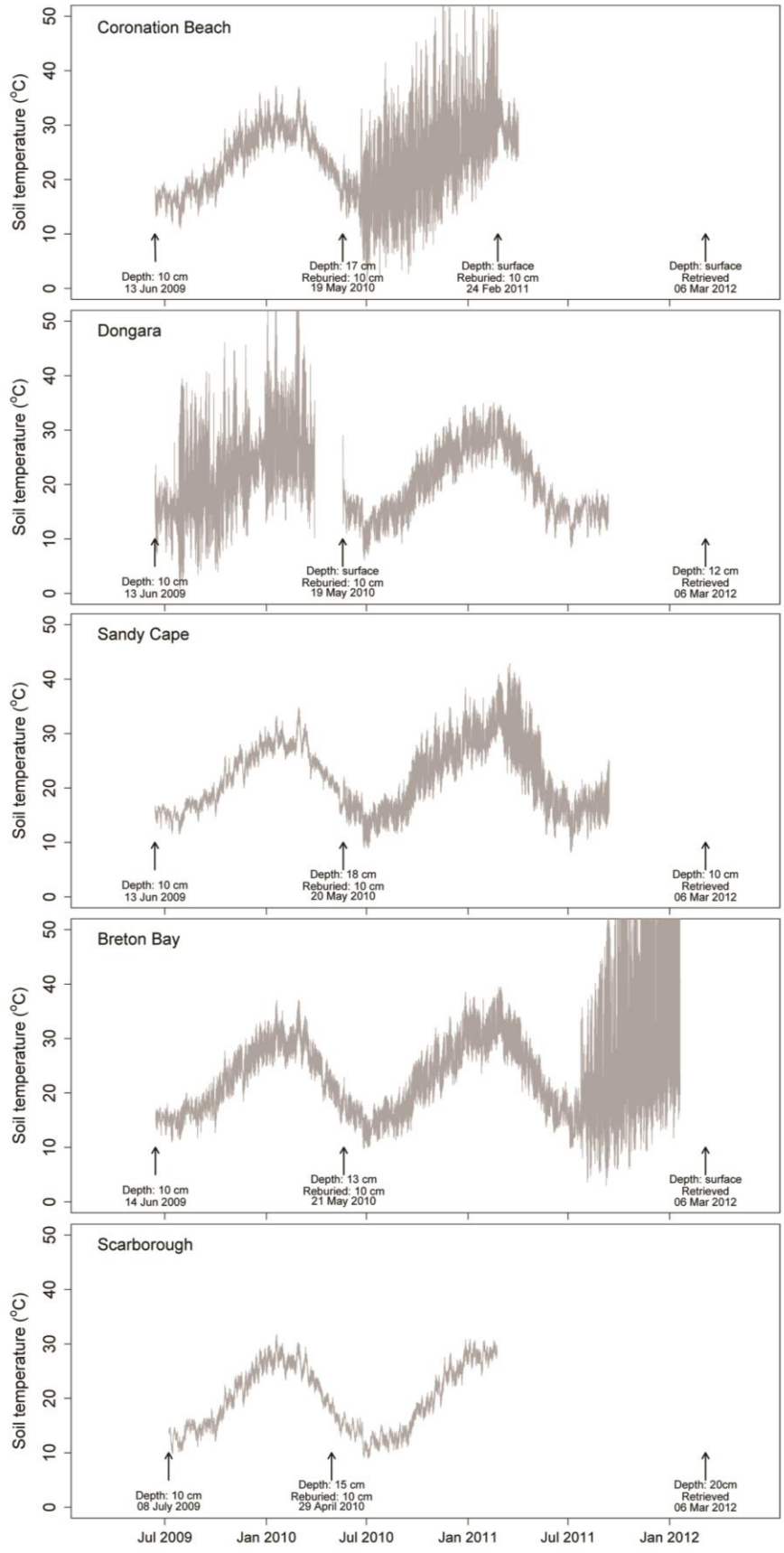
Plant species often occur within a specific zone of the dune system. These zones are parallel to the high tide mark and change with distance from the ocean (Oosting and Billings 1942; Sauer 1965; Barbour 1978; Doing 1985). The pioneer plants and heathlands of the SWWA Quindalup dunes occur in areas affected by salt spray, or in areas with shallow soils over calcareous rocks. As the amount of salt spray decreases with distance from the ocean (**Figure 4**) (Barbour 1978; Rozema 1985; Mariko *et al.* 1992; Greaver and Sternberg 2007), and the sand deepens, a zone of shrubland and thickets occurs on primary and secondary dunes (Rippey and Rowland 2004). Further inland, this zone gives way to *Eucalyptus* woodland (Marchant *et al.* 1987; Rippey and Rowland 2004). Within this general pattern of zonation, multiple microhabitats form that are the result of topographic variation, the interaction between sand movement and vegetation, salt spray and depth of the water table (Doing 1985; Martinez *et al.* 1992). This thesis focuses primarily on plants from the foredune and primary dunes.

Differences in species composition are associated with the structural zonation. Bare sand above the beach is generally colonised by pioneer strand plants, less than 1 m high, including native spinifex (*Spinifex hirsutus* and *S. longifolius* (Poaceae) and exotic species such as *Cakile maritima* (Brassicaceae) and *Euphorbia paralias* (Euphorbiaceae)). The foredunes often have a low cover of *Spinifex* species, *Carpobrotus virescens* (Aizoaceae), *Ficinia nodosa* (Cyperaceae), *Lepidosperma gladiatum* (Cyperaceae) and the naturalised exotic *Tetragonia decumbens* (Aizoaceae) (Rippey and Rowland 2004). The shrub community is more sheltered and stable, sometimes richer in nutrients, up to 2 m in height, and comprises a more diverse range of species (Sauer 1965; Rippey and Rowland 2004). These shrub communities are dominated by *Acacia cyclops* (Fabaceae), *Acanthocarpus preissii* (Dasypogonaceae), *Myoporum insulare* (Schrophulariaceae), *Olearia axillaris* (Asteraceae), *Scaevola crassifolia* (Goodeniaceae) and *Spyridum globulosum* (Rhamnaceae) (Sauer 1965; Rippey and Rowland 2004).

Temporal variation in environmental conditions

Most plants of SWWA coastal communities flower in spring, with peak flowering occurring during September (Marchant *et al.* 1987). In late spring, October to November, days become warmer and drier, flowering progresses to seed set and the sandy coastal soils dry out rapidly (Marchant *et al.* 1987). As in many coastal dune systems, salt accumulation occurs on the soil surface during dry periods (Barbour 1978; Baskin and Baskin 1998; Rippey and Rowland 2004; Greaver and Sternberg 2007). In SWWA, seed of coastal plants is generally mature and released from plants between November and March, and is therefore shed onto highly saline topsoil (**Figure 4**). The maximum salinity (EC) measured over time at Scarborough was approximately equivalent to 100 mM NaCl (c.f. CRC Press 1974), on the top of the first dune, 49 m from the high tide mark (**Figure 4**). During late summer, strong easterly winds are common in the morning and ripe fruits, particularly conspicuous diaspores of *Spinifex*, are seen tumbling into the water (L K Guja pers. obs.) and drifting out to sea (salinity of approximately 500 mM NaCl). Given the large number of putatively wind adapted seeds in the coastal flora (Dixon 2011), deposition in the ocean is also likely to occur for many other species, but may not be as readily observed for small-seeded species. By late afternoon a cool south-westerly breeze is often present and can wash drifting seeds or fruits ashore (L K Guja pers. obs.) (**Figure 5**).

Figure 5. Stranded *Spinifex* diaspores washed ashore at Sandy Cape after floating in seawater. Image: L K Guja.


Coronation Beach
Depth: 10 cm
13 Jun 2009
Depth: 17 cm
Reburied: 10 cm
19 May 2010
Depth: surface
Reburied: 10 cm
24 Feb 2011
Depth: surface
Retrieved
06 Mar 2012
Dongara
Depth: 10 cm
13 Jun 2009
Depth: surface
Reburied: 10 cm
19 May 2010
Depth: 12 cm
Retrieved
06 Mar 2012
Sandy Cape
Depth: 10 cm
13 Jun 2009
Depth: 18 cm
Reburied: 10 cm
20 May 2010
Depth: 10 cm
Retrieved
06 Mar 2012
Breton Bay
Depth: 10 cm
14 Jun 2009
Depth: 13 cm
Reburied: 10 cm
21 May 2010
Depth: surface
Retrieved
06 Mar 2012
Scarborough
Depth: 10 cm
08 July 2009
Depth: 15 cm
Reburied: 10 cm
29 April 2010
Depth: 20cm
Retrieved
06 Mar 2012
Soil temperature (°C)
0
10
20
30
40
50
Jul 2009
Jan 2010
Jul 2010
Jan 2011
Jul 2011
Jan 2012

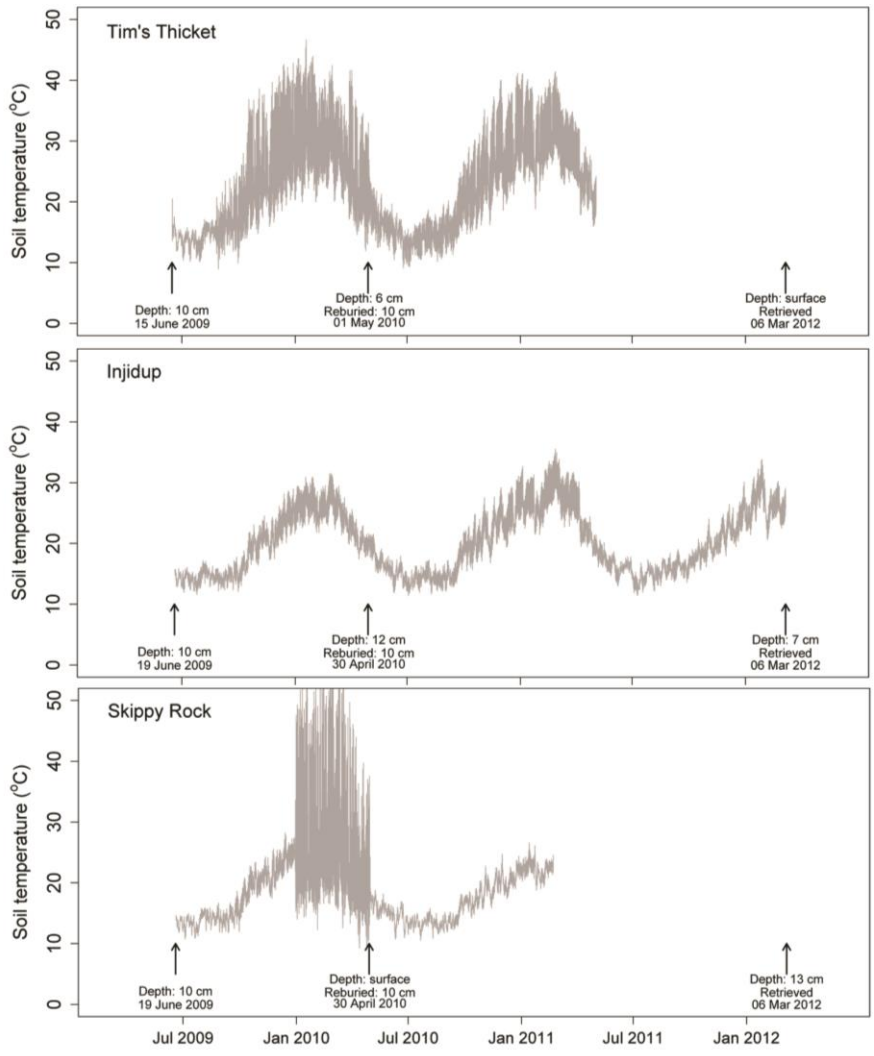


Figure 6. Soil temperature at each of the eight study sites (from north to south) over time. July indicates the middle of winter while January indicates the middle of summer in SWWA. Data loggers (Hobo Microstation H21, OneTemp) were deployed in June or July 2009 to monitor soil temperature. The data loggers were generally deployed in slightly sheltered locations beyond the primary dune in an attempt to avoid sand burial or erosion. The data loggers were mounted to a metal stake and elevated at least 15 cm. Temperature probes (Smart Sensor S-TMB) were buried at least 25 cm from the stake and the cables were covered in plastic conduit for protection. Temperature probes were buried 10 cm below the soil surface. The seed emergence depth in the study system is unknown and 10 cm was selected for practical reasons to help avoid probe exposure. Nonetheless, the depth of the probes changed over time due to the dynamic nature of coastal dune systems. Probes were therefore reburied to 10 cm when possible. Missing data are the result of file corruption, battery depletion or vandalism.

Significant rainfall events usually begin around April or May and vegetation responds with an onset of growth and some flowering (Marchant *et al.* 1987). Seed germination of the SWWA coastal flora also generally begins at this time, in response to increased rainfall and reduced temperatures (Turner *et al.* 2006). During the cooler months, soil temperature at the depth of the soil seed bank is approximately 10 - 15 °C (**Figure 6**). Germination at this time of year increases the probability of seedling survival because there is high moisture availability (Maun 1994) and low to no salinity (c. 0 mM NaCl; **Figure 4**) because the salt that accumulates on the soil surface during summer has been leached through the soil by winter rain (Hocking 1982; Woodell 1985). Over the winter months of June to August half of SWWA's rainfall is received (Marchant *et al.* 1987) via fronts of rainfall moving eastward and bringing moisture from the warm ocean (Li *et al.* 2005; Timbal *et al.* 2006). These weather fronts cause significant beach erosion over the winter months (Sauer 1965; Dixon 2011), which may result in soil-stored seed being washed out to sea.

Summary

Coastal plants grow under difficult and dynamic conditions. The portion of the SWWA coast investigated in this thesis spans a large temperature and rainfall gradient. Salinity in the coastal environment can vary with seasonally, and/or with distance from the high tide mark. These environmental conditions are likely to have a significant effect on seeds during oceanic dispersal and post-dispersal germination and this is investigated in this thesis.

References

Barbour MG (1978) Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes California. *Oecologia* **32**, 213-224.

Baskin CC, Baskin JM (1998) 'Seeds: ecology, biogeography, and evolution of dormancy and germination.' (Academic Press: California).

Bureau of Meteorology (2013) Maps of average conditions. (Commonwealth of Australia) Available at http://www.bom.gov.au/jsp/ncc/climate_averages

CRC Press (1974) 'CRC handbook of chemistry and physics.' (CRC Press: Cleveland).

CSIRO (2013) The atlas of living australia. Available at http://www.ala.org.au

Department of Planning (2003) 'Coastal planning and management manual.' (Government of Western Australia: Perth).

Department of the Environment (2013) Interim Biogeographic Regionalisation for Australia (IBRA) version 7. (Commonwealth of Australia) Available at http://www.environment.gov.au/topics/land/nrs/science-maps-and-data/australias-bioregions-ibra#ibra

Dixon KW (2011) 'Coastal plants: a guide to the identification and restoration of plants of the Perth region.' (CSIRO Publishing: Melbourne).

Doing H (1985) Coastal fore-dune zonation and succession in various parts of the world. *Vegetatio* **61**, 65-75.

Greaver TL, Sternberg LSL (2007) Fluctuating deposition of ocean water drives plant function on coastal sand dunes. *Global Change Biology* **13**, 216-223.

Hocking PJ (1982) Salt and mineral nutrient levels in fruits of two strand species, *Cakile maritima* and *Arctotheca populifolia*, with special reference to the effect of salt on the germination of *Cakile*. *Annals of Botany* **50**, 335-343.

Hopper S (2009) OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and Soil* **322**, 49-86.

Li Y, Cai W, Campbell EP (2005) Statistical modeling of extreme rainfall in southwest Western Australia. *Journal of Climate* **18**, 852-863.

Marchant NG, Wheeler JR, Rye BL, Bennett EM, Lander NS, Macfarlane TD (1987) 'Flora of the Perth region.' (Western Australian Herbarium: Perth).

Mariko S, Kachi N, Ishikawa S, Furukawa A (1992) Germination ecology of coastal plants in relation to salt environment. *Ecological Research* **7**, 225-233.

Martinez ML, Valverde T, Moreno-Casasola P (1992) Germination response to temperature, salinity, light and depth of sowing of ten tropical dune species. *Oecologia* **92**, 343-353.

Maun MA (1994) Adaptations enhancing survival and establishment of seedlings on coastal dune systems. *Vegetatio* **111**, 59-70.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**, 853-858.

Oosting HJ, Billings WD (1942) Factors effecting vegetational zonation on coastal dunes. *Ecology* **23**, 131-142.

Rippey E, Rowland B (2004) 'Coastal plants: Perth and the south-west region.' 2nd edn. (University of Western Australia Press: Perth).

Rozema J, Bijwaard, P., Prast, G., & Broeckman, R. (1985) Ecophysiological adaptation of coastal halophytes from foredunes and salt marshes. *Vegetatio* **62**, 499-521.

Sauer J (1965) Geographic reconnaissance of Western Australian seashore vegetation. *Australian Journal of Botany* **13**, 39-69.

Semeniuk V (1995) New Pleistocene and Holocene stratigraphic units in the Yalgorup Plain area, southern Swan Coastal Plain. *Journal of the Royal Society of Western Australia* **78**, 67-79.

Semeniuk V, Johnson DP (1982) Recent and Pleistocene beach/dune sequences, Western Australia. *Sedimentary Geology* **32**, 301-328.

Timbal B, Arblaster J, Power S (2006) Attribution of the late-twentieth-century rainfall decline in southwest Australia. *Journal of Climate* **19**, 2046-2062.

Turner SR, Merritt DJ, Ridley EC, Commander LE, Baskin JM, Baskin CC, Dixon KW (2006) Ecophysiology of seed dormancy in the Australian endemic species *Acanthrocarpus preissii* (Dasypogonaceae). *Annals of Botany* **98**, 1137-1144.

Waite AM, Thompson PA, Pesant S, Feng M, Beckley LE, Domingues CM, Gaughan D, Hanson CE, Holl CM, Koslow T, Meuleners M, Montoya JP, Moore T, Muhling BA, Paterson H, Rennie S, Strzelecki J, Twomey L (2007) The Leeuwin Current and its eddies: an introductory overview. *Deep Sea Research Part II: Topical Studies in Oceanography* **54**, 789-796.

Woodell SRJ (1985) Salinity and seed germination patterns in coastal plants. *Vegetatio* **61**, 223-229.

Chapter 3

Sink or swim? A model to predict oceanic dispersal using seed morphological traits

Abstract

Oceanic dispersal is the topic of much current research, yet the seed traits that facilitate oceanic dispersal in plants have received little empirical investigation. As a result, the potential for dispersal by water (hydrochory; as both a standard and non-standard mode of dispersal) is underappreciated. The aim of this research was to determine the morphological seed traits of multiple species that best predict the key determinant of oceanic dispersal, seed buoyancy. Using buoyancy as a surrogate for hydrochory we created five simple linear models to predict seed buoyancy over time using easily measured seed morphological traits. Seed morphology type and specific weight were most strongly associated with buoyancy. However, morphology type was not associated with buoyancy beyond three days indicating that morphology type is not a good predictor of long-distance hydrochorous dispersal. For short term buoyancy the combination of seed specific weight and seed morphology type explained 56% (0 day) and 55% (3 days) of the variation in buoyancy. For long term buoyancy (likely to be indicative of long-distance dispersal), specific weight alone was the strongest predictor of buoyancy but explained only 30% (8 days), 20% (15 days) and 13% (21 days) of the variation, suggesting further investigation is required. These simple models provide a practical tool for predicting seed dispersal of multiple species via the ocean and a framework for understanding the ocean as an important dispersal vector of plant seeds.

Introduction

Long-distance plant dispersal is the topic of much current research (Nathan 2006; Cousens *et al.* 2008; Nathan *et al.* 2008; Gillespie *et al.* 2011). This is because many important research questions in pure and applied ecological research require a clear understanding of dispersal (Cousens *et al.* 2008; Thomson *et al.* 2010; van Loon *et al.* 2011). Charles Darwin was one of the first to propose that oceanic hydrochory is an important means of plant dispersal (Darwin 1859; Black 2009). However, his theories of long-distance dispersal by the ocean fell out of favour due to scientific advances in fields such as plate tectonics and cladistics, which strongly favoured theories of vicariance (de Queiroz 2005). Recently, long-distance dispersal has become an equal contender with the theory of vicariance, and both are now considered important drivers of plant radiations (Howarth *et al.* 2003; de Queiroz 2005; Dawson and Hammer 2008; Kokubugata *et al.* 2012). Despite this resurgence, there have been relatively few empirical investigations of multiple species to determine whether seeds are physically capable of dispersal by the ocean (Appendix 1;Quilichini and Debussche 2000; Atia *et al.* 2010; Guja *et al.* 2010). Dispersal of individual diaspores involves interactions between a number of processes affecting removal from the parent plant, movement to a substrate, movement along a substrate, and arrival at the final location (Cousens *et al.* 2008). The capacity for movement along a substrate, or the 'buoyancy potential', is the crucial factor that determines how far a seed may be dispersed via the ocean. Predictions and/or descriptions of oceanic dispersal require the identification of seed traits associated with buoyancy coupled with quantitative measurement of buoyancy.

Dispersal potential is often investigated via two approaches; addressing either seed traits, or environmental conditions. The hydraulic processes that affect hydrochory have been investigated in rivers (Merritt and Wohl 2002), but for oceanic dispersal neither the environmental conditions or seed traits that determine dispersal have been investigated. Seed traits associated with dispersal could be used to create predictive models as demonstrated for many other dispersal modes. For example, the influence of multiple seed types on attachment to vertebrates has been modelled to determine which morphological seed traits can predict the potential for epizoochorous dispersal (Römermann *et al.* 2005; Tackenberg *et al.* 2006; de Pablos and Peco 2007; Will *et*

al. 2007). For anemochorous (wind) dispersal the effect of interspecific variation in diaspore weight, plume area, and wing shape on fall rate has been investigated (Matlack 1987). In pappus-bearing Asteraceae seeds, the relationship between settling velocity and the ratio of a falling seed's mass to its projected area has been identified (Andersen 1993). These models have demonstrated that, at least for these commonly-studied dispersal syndromes, there are significant relationships between seed morphology and dispersal potential.

Because some distinctive seed morphologies can imply the standard dispersal mechanism of a species (Werker 1997), morphology type is often confused with standard dispersal mode. The possibility of dispersal by nonstandard means (e.g. arrilate seeds by a vector other than ants) has rarely been investigated (however, see Appendix 1; Higgins *et al.* 2003; Guja *et al.* 2010; Thomson *et al.* 2010). It remains unclear, and difficult to determine, whether particular seed traits are conducive to hydrochorous dispersal. A variety of seed morphologies may influence buoyancy potential, but have perhaps been overlooked because of the tendency to associate distinctive seed morphologies with only one mode of dispersal. Some seed characteristics, such as air chambers (e.g. air pockets between cotyledons or between exterior fibres, spongy mesocarps, or cork-like pericarps), have been predicted to be associated with a high buoyancy potential (Lopez 2001; Higgins *et al.* 2003; Cousens *et al.* 2008). However, the association between buoyancy and particular seed characteristics (such as air chambers) is often speculative and not supported experimentally.

Seed traits that influence buoyancy potential have been examined in a small number of plant species. Seed traits that have been correlated with prolonged buoyancy are aeriforous tissue in the exocarp of *Bolboschoenus maritimus* (Hroudova *et al.* 1997), low specific weight (mass/volume) of 12 tropical rainforest species (Lopez 2001), and the volume of an air pocket between cotyledons in *Swartzia polyphylla* (Williamson *et al.* 1999). However, the applicability of these findings to other species or seed types is unclear. For example, an examination of taxa that colonised Surtsey, a volcanic island south of Iceland, determined that only one quarter of 78% of taxa that arrived by sea currents were classed as morphologically adapted to water dispersal (i.e. contained airspaces) (Higgins *et al.* 2003). Evidently, a large

knowledge gap exists in relation to the seed traits that are associated with buoyancy and the potential for a variety of seed types to be dispersed by the ocean. Furthermore, Thompson *et al*. (2010) highlight the lack of knowledge relating to hydrochory in the Australian flora.

There are inherent difficulties in measuring dispersal, particularly long-distance dispersal. However, models based on quantitative data are more likely to accurately predict dispersal than are theoretical models. Here seed buoyancy is used as an indicator of hydrochorous dispersal and is used to examine whether easily measurable morphological traits can be used to predict buoyancy of a wide range of seeds over time. This research aims to identify the morphological seed traits that best predict potential dispersal in a range of species. Based on the available literature it is hypothesised that high buoyancy will be related to: (1) morphology type, (2) low specific weight; (3) low seed mass or low seed fill; (4) the coastal dune habitat where the species occur with those closest to the high tide mark having the most buoyant seeds.

Method

Species selection

The buoyancy potential was measured (**Supplementary Table 1**) for 60 common species of the foredune, primary, secondary, and tertiary dunes of the Swan Coastal Plain in southwest Australia (Rippey and Rowland 2004; Dixon 2011). A variety of plant families, genera and species were selected to include a wide range of seed characteristics (e.g. size, mass, shape, and appendages). The most represented families were Fabaceae: Mimosoideae (8), Fabaceae: Faboideae (2), Myrtaceae (6), Goodeniaceae (5), and Chenopodiaceae (4). The most represented genera were *Acacia* (8), *Melaleuca* (4), and *Scaevola* (4). Seeds were collected at the natural point of dispersal between November 2008 and February 2009 from natural populations, or sourced from the Botanic Gardens and Parks Authority seed bank in Western Australia.

Seed traits

Measurements were conducted on natural dispersal units i.e. seeds with appendages intact or non-dehiscent fruits (**Supplementary Table 1** and **Figure 1**). All measurements were made on seeds dried for ≥ 1 month in a controlled environment room at 15 °C and 15% relative humidity. Seeds were imaged using a Leica M205 C microscope (Leica, North Ryde, NSW, Australia) (**Figure 1**). For simplicity, dispersal units will be referred to as seeds throughout the text unless further detail is required.

Morphological traits hypothesised to affect buoyancy were investigated. The average seed mass (mg) was obtained gravimetrically. Seeds were X-rayed (Faxitron MX-20, Faxitron X-ray, Lincolnshire, IL, USA) to determine the percentage of filled seeds (or for fruits, the percentage of fruits that contained at least one filled seed) in the experimental sample used for each species.

To determine mean projected area (mm^2; the two dimensional area occupied by seeds in a scanned image), four replicates of 25 seeds were scanned using an Epson flatbed scanner (Epson Perfection 4990, Regent Instruments, QC, Canada). Scanned images were used to measure projected area using WinRhizo software (WinRhizo Pro 2007d, Regent Instruments, QC, Canada). These images were also used to measure the length (mm) and width (mm) of 5 seeds per species using Image J software (Version 1.44p with Java 1.6.0, National Institute of Health, MD, USA). The height (mm) of 5 seeds per species was measured using digital callipers. The three seed dimensions were measured at the maximal extent of the dimension including all appendages.

To express the extent to which shape differed from sphericity, the variance of diaspore length, width, and height was calculated after first dividing all values by length (Thompson *et al.* 1993). The minimum variance of zero represents perfectly spherical seeds and the maximum of approximately 0.3 indicates elongated or flattened seeds (e.g. seeds with long awns or flat wings).

Figure 1. Images of seeds (experimental unit) of study species. White scale bars represent 50 µm. From left to right species are *Acacia cochlearis, A. cyclops, A. lasiocarpa, A. littorea, A. pulchella, A. saligna, A. truncata, A. xanthina, Allocasuarina lehmanniana, Alyogyne huegelii, Alyxia buxifolia, Atriplex isatidea, Austrostipa elegantissima, Boronia alata, Brachyscome iberidifolia, Cakile maritima, Callitris preissii, Calothamnus quadrifidus, Conostylis candicans, Diplolaena dampieri, Diplopeltis huegelii, Dodonaea aptera, Enchylaena tomentosa, Eremophila glabra, Eucalyptus gomphocephala, Exocarpos sparteus, Ficinia nodosa, Guichenotia ledifolia, Hakea prostrata, Hardenbergia comptoniana, Hemiandra pungens, Jacksonia furcellata, Lechenaultia linariodes, Lepidosperma gladiatum, Leucopogon parviflorus, Melaleuca cardiophylla, M. huegelii, M. lanceolata, M. systena, Muehlenbeckia adpressa, Myoporum insulare, Olax benthamiana, Opercularia benthamiana, Ozothamnus cordatus, Phyllanthus calycinus, Pimelea ferruginea, Rhagodia baccata, Scaevola anchusifolia, S. crassifolia, S. nitida, S. thesioides, Schoenus grandiflorus, Senecio pinnatifolius var. pinnatifolius, Solanum symonii, Spinifex longifolius, Spyridium globulosum, Tetragonia decumbens, Thomasia triphylla, Threlkeldia diffusa,* and *Trachymene coerulea.*

Seed volume (µl) was determined by placing 25-100 seeds in various graduated vessels (relative to seed size) and recording the displacement of water. The specific weight (mg μl^{-1}) was calculated by dividing seed weight by seed volume (Lopez 2001).

Diaspores were classed as either seeds or fruits. For some species, appendages or fruit coats were removed during seed cleaning and all classifications are based on the dispersal unit used in experiments (**Supplementary Table 1** and **Figure 1**). The presence or absence of elongated appendages that extended beyond the seed coat was recorded (e.g. an aril, awn, hook or wing) (de Pablos and Peco 2007). Seed descriptions (Marchant *et al.* 1987; Western Australian Herbarium 1998-2014) and images (**Figure 1**) were used to assign seeds to one of seven morphological categories: 'arillate', 'flattened', 'fleshy', 'membranous', 'rough', 'woody'; or 'no' if seeds did not have distinctive features that fit the aforementioned categories (c.f. Römermann *et al.* 2005; Tackenberg *et al.* 2006; Will *et al.* 2007; Pérez-Harguindeguy *et al.* 2013).

The type 'arillate' refers to seeds with appendages, generally fleshy and brightly coloured, (e.g. arils, elaisomes, caruncles or hypogynous scales/discs) arising from the hilum or funiculus of the seed, but not completely enveloping the seed. The 'flattened' category includes flat, thin structures extending from the seed that at least double the seed surface of the respective dimension of the seed (e.g. wings). The type 'fleshy' refers to seeds completely enveloped by a thick layer of fleshy or succulent tissue. Seeds enveloped by thin membranous tissue or glumes were termed 'membranous'. The type 'rough' refers to seeds or appendages with a raised surface such as hairs, pappus or a rugose exterior. The type 'woody' refers to indehiscent dry fruits with woody or cork-like tissue surrounding seeds. All seeds not belonging to one of the above categories were classified as 'no'.

For seeds that fit two or more morphological categories, only the dominant structure (i.e. likely to have the most effect on buoyancy; Pérez-Harguindeguy *et al.* 2013) was considered such that each species was only assigned to one category (**Supplementary Table 1**). For example, a woody fruit with fine hairs would be described as 'woody' rather than 'rough'. For *Cakile maritima* each fruit naturally dehisces into two parts that are capable of individual dispersal (Rippey and Rowland

2004), and these two parts were treated as separate dispersal units in experiments. However, at the end of the floating experiment there was no significant difference between buoyancy of the two parts (data not shown) and data were combined.

Habitat was classified into four groups to describe the minimum distance from the ocean that each species commonly grows. The categories, from closest to furthest from the high tide mark were foredune, primary dune, secondary dune, and tertiary dune (Rippey and Rowland 2004; Dixon 2011).

Buoyancy measurements

To assess buoyancy potential, four replicates of 25 seeds of each species were placed in plastic containers (11 cm diameter by 4 cm high, Genfac Plastics, Melbourne) that contained 250 mL seawater. The position of containers was randomised and remained under ambient laboratory conditions (approx. 23 °C and 50% relative humidity) for the duration of the experiment. To disturb surface tension, the water in each container was initially stirred for 30 s before the number of floating seeds was recorded. This is referred to throughout the manuscript as ‘day 0’. Water levels were maintained with the addition of deionised water, ensuring that during each addition the surface tension was disrupted and all seeds were agitated before counting the number of buoyant seeds (Appendix 1; Guja *et al.* 2010). The number of buoyant seeds in each container was recorded on day 0, 3, 8, 15, and 21, upon which the experiment ended. Neutrally buoyant seeds were recorded as floating. If any part of a seed or appendage was in contact with the bottom of the container it was recorded as non-buoyant. Buoyancy upon initial contact with seawater (0 days) and after 3 days was considered to represent general hydrochorous dispersal, buoyancy for 8 to 15 days was considered to represent intermediate dispersal and extended buoyancy of 21 days or greater was considered to represent long-distance dispersal via hydrochory.

Table 1. Relationships between seed traits and buoyancy between 0 and 21 days in seawater. Continuous variables were analysed using Spearman rank correlations. Relationships between categorical variables and buoyancy were determined by Kruskal-Wallis tests. Variables that are significantly related (P < 0.05) to buoyancy are marked in bold.

		Day 0			Day 3			Day 8			Day 15			Day 21		
Seed trait	**N**	**Rho**	**Chi**	**P-value**	**Rho**	**Chi**	**P-value**	**Rho**	**Chi**	**P-value**	**Rho**	**Chi**	**P-value**	**Rho**	**Chi**	**P-value**
Mass	60	-0.102		0.440	-0.147		0.261	-0.350		**0.006**	-0.333		**0.009**	-0.274		**0.034**
Fill	60	-0.140		0.286	-0.074		0.576	0.046		0.727	-0.054		0.684	0.054		0.680
Length	60	0.028		0.832	-0.008		0.954	-0.140		0.286	-0.119		0.364	-0.141		0.283
Width	60	0.079		0.550	-0.013		0.923	-0.221		0.09	-0.236		0.069	-0.195		0.136
Height	60	0.164		0.209	0.146		0.265	-0.087		0.508	-0.08		0.541	-0.04		0.763
Shape	60	-0.176		0.179	-0.225		0.084	-0.067		0.611	-0.081		0.536	-0.156		0.234
Projected area	60	-0.003		0.980	-0.062		0.639	-0.252		0.053	-0.242		0.062	-0.235		0.070
Volume	60	0.067		0.610	0.028		0.835	-0.231		0.075	-0.237		0.068	-0.192		0.141
Specific weight	60	-0.575		**<0.001**	-0.602		**<0.001**	-0.554		**<0.001**	-0.457		**<0.001**	-0.384		**0.002**
Morphology	7		-0.156	**<0.001**		20.376	**0.002**		12.803	0.046		9.363	0.154		4.614	0.594
Appendage	2		1.248	0.264		0.639	0.424		0.441	0.507		0.204	0.652		0.000	0.994
Diaspore	2		6.818	**0.009**		6.434	**0.011**		0.000	0.994		0.166	0.684		0.002	0.961
Habitat	4		5.480	0.140		3.730	0.292		4.333	0.228		3.018	0.389		4.489	0.213

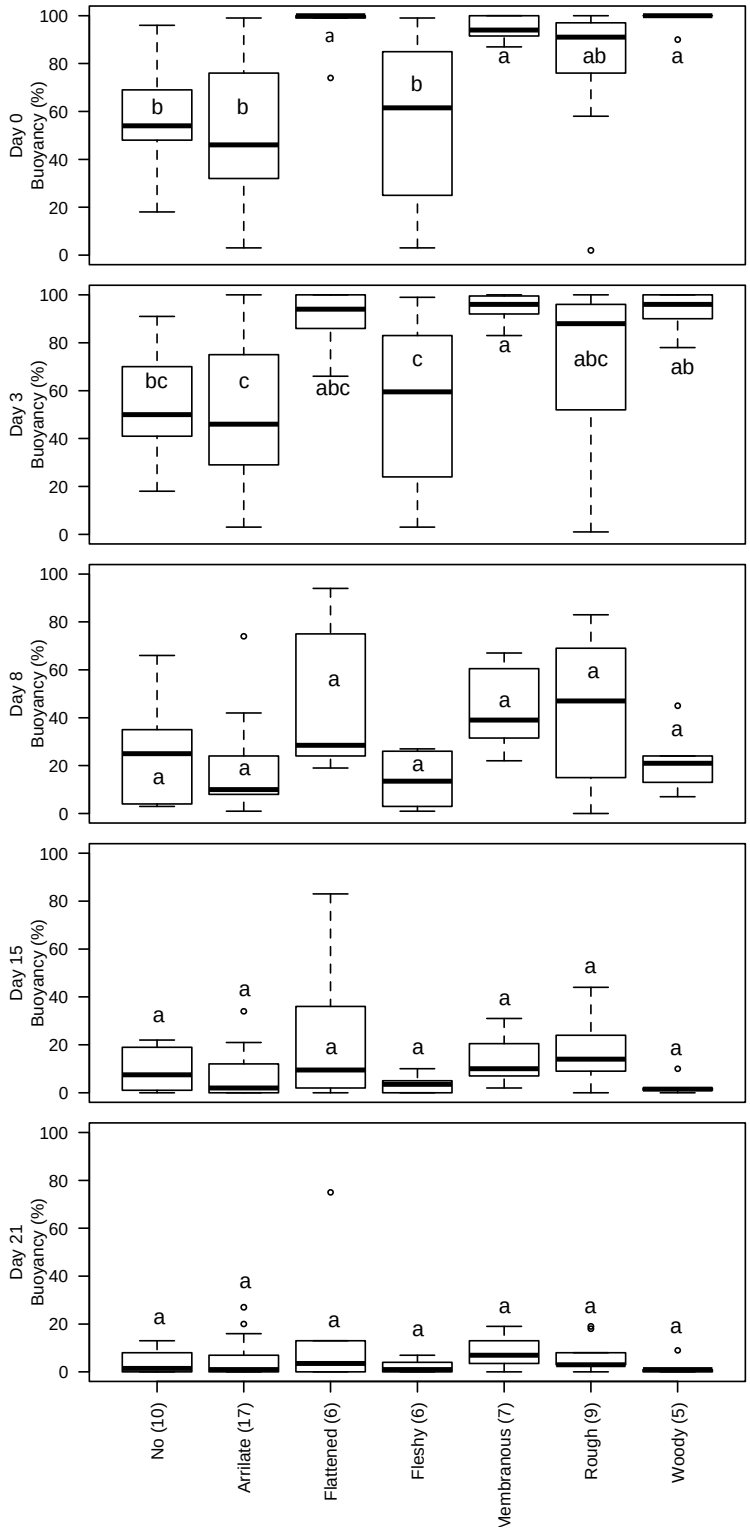


Figure 2. Buoyancy (%) of seeds of each morphology type at 0, 3, 8, 15 and 21 days. Sample size for each morphology type is indicated in parentheses on the x axis. Morphology types marked with the same letter were not significantly different ($P < 0.01$) at each time (post-hoc LSD test of arcsin square-root transformed buoyancy data).

Analysis

All analyses were performed using R version 2.15.1 (R Foundation for Statistical Computing). Buoyancy data at each time point were analysed separately. To determine relationships between morphological seed traits and seed buoyancy, and between the seed traits themselves, nonparametric tests (Spearman rank correlations, Kruskal Wallis or Pearson's Chi Squared tests) were used.

Buoyancy percentage data were arcsin-square root transformed before analysis. Relevant seed trait data were also transformed, as required, to achieve a normal distribution. For all statistical tests alpha was set to 0.05, except post-hoc LSD tests for categorical traits where alpha was set to 0.01 to account for multiple comparisons.

Seed traits found to be correlated with buoyancy, but not with each other, were included in linear models. Separate regression models were created for all five time points to better understand buoyancy from initial contact with seawater i.e. general hydrochorous dispersal (0 and 3 days), intermediate floating durations (8 and 15 days), and extended floating durations (21 days). Interaction terms were initially included and if not significant they were removed to simplify the models.

Table 2. Tests of relationship (Spearman Rank, Kruskal Wallis and Pearson's Chi Squared) between seed traits that were significantly related to buoyancy. Variables with P > 0.05 (in bold) were not correlated with each other and could be used in models.

Trait 1	Trait 2	Test statistic	P-value
Mass	Specific weight	0.459[a]	<0.001
Mass	Morphology	21.302[b]	0.002
Mass	Diaspore	10.357[b]	0.001
Specific weight	Morphology	9.036[b]	**0.172**
Specific weight	Diaspore	0.234[b]	**0.628**
Morphology	Diaspore	46.848[c]	<0.001

[a] Spearman Rank Correlation

[b] Kruskal Wallis

[c] Pearson's Chi Squared

Table 3. Estimated generalised linear model parameters for morphology types at day 0 and day 3. Intercept was calculated using a slope calculated from a linear model including all morphology types (fixed slope). Morphologies that were significantly different ($P < 0.05$) to seeds with no distinctive morphological features are marked in bold. Based on this analysis, morphology types were grouped into two sets (FMW = flattened, membranous and woody seeds and Other = no distinctive features, arillate, fleshy and rough seed morphologies). The two morphology sets were used to derive a final model, which was used to calculate the derived intercept for each morphology set.

	Day 0					Day 3				
Morphology	**Fixed Slope**	**Intercept**	**T**	**P-value**	**Derived intercept**	**Fixed slope**	**Intercept**	**T**	**P-value**	**Derived Intercept**
No	-0.504	1.448			1.466	-0.532	1.434			1.449
Arillate		1.432	-0.165	0.87	1.466		1.452	0.185	0.854	1.449
Flattened		1.755	2.53	**0.014**	1.779		1.668	1.889	**0.064**	1.73
Fleshy		1.477	0.243	0.809	1.466		1.492	0.471	0.64	1.449
Membranous		1.786	2.913	**0.005**	1.779		1.795	3.059	**0.003**	1.73
Rough		1.542	0.869	0.389	1.466		1.43	-0.037	0.971	1.449
Woody		1.797	2.709	**0.009**	1.779		1.714	2.133	**0.038**	1.73

Results

Relationships between buoyancy and seed traits

Of the continuous morphological variables (mass, fill, size, shape, projected area, volume and specific weight), specific weight correlated most strongly with buoyancy and this negative relationship was observed at all sampling times (0, 3, 8, 15 and 21 days) (**Table 1**). Although mass was not initially correlated with buoyancy, the relationship changed over time and lower mass was associated with sustained seed buoyancy (≥ 8 days) (**Table 1**). Seed fill, and the other continuous variables, were not significantly related to buoyancy at any time. The categorical variable diaspore (whether the dispersal unit was a seed or fruit) was significantly related to buoyancy at 0 and 3 days, but did not affect buoyancy after 3 days (**Table 1**). At day 0 and day 3 fruits were more buoyant than seeds. Appendage (yes or no) and habitat (proximity to the high tide mark) were not significantly related to buoyancy at any time (**Table 1**).

Effect of morphology type on seed buoyancy

Of the categorical variables, seed morphology type had the greatest effect on seed buoyancy at day 0 and day 3 (**Table 1**). For example, at day 0 mean buoyancy differed significantly between morphology types ($F_{6,53} = 5.60$, $P < 0.001$): Flattened (95%), membranous (95%), and woody (98%) seeds were most buoyant; rough (78%) seeds had intermediate buoyancy; and arillate (53%), fleshy (56%), and seeds with no distinctive features (58%) were least buoyant (**Figure 2**). Buoyancy of all morphology types decreased over time and from day 8 to day 21 there were no significant differences between the mean buoyancy of the seven morphology types.

Relationships between seed traits

As expected, many of the seed traits related to buoyancy were also correlated with each other. However, there were no significant relationships between morphology type and specific weight; or diaspore type and specific weight (**Table 2**). There was, however, a significant relationship between morphology type and diaspore type,

indicating that morphology type and diaspore type were not independent and only one should be included in the model. Overall, across the five time points the relationship between morphology type and buoyancy was stronger than the relationship between diaspore type and buoyancy (**Table 1**). Therefore, as specific weight and morphology were correlated with buoyancy but not with each other, only those traits were used in regression models for 0 and 3 days. Regression models for ≥ 8 days only included the variable specific weight. Mass and specific weight were both significantly related to buoyancy at ≥ 8 days, but were correlated with each other. Of the two, the correlation between buoyancy and specific weight was stronger than buoyancy and mass.

Table 4. Slopes and intercepts for the five final linear models for the two morphology groups at 0 and 3 days (FMW = flattened, membranous and woody seeds and Other = no distinctive features, arillate, fleshy and rough seed morphologies), and for specific weight of all morphologies at 8, 15 and 21 days.

		FWM	Other	All
Day 0	Slope	-0.504	-0.504	
	Intercept	1.779	1.466	
	T	59.59	36.71	
	P-value	<0.001	<0.001	
Day 3	Slope	-0.532	-0.532	
	Intercept	1.73	1.449	
	T	48.02	36.45	
	P-value	<0.001	<0.001	
Day 8	Slope			-0.44
	Intercept			1.072
	T			-4.927
	P-value			<0.001
Day 15	Slope			-0.398
	Intercept			0.738
	T			-3.803
	P-value			<0.001
Day 21	Slope			-0.298
	Intercept			0.562
	T			-2.977
	P-value			0.004

Regression models for buoyancy at day 0

Morphology and specific weight variables were included in a linear model to determine their relationship with buoyancy at day 0. This linear model revealed significant main effects of morphology ($F_{6,46}$ = 4.39, P = 0.001) and specific weight ($F_{1,46}$ = 17.02, P < 0.001). There was not a significant interaction between morphology and specific weight ($F_{6,46}$ = 1.09, P = 0.380), indicating that the slope of the regression for each seed morphology type did not differ. Because the slope for each morphology type did not differ statistically, the effect of specific weight alone on buoyancy was modelled to obtain a common slope of -0.5035 ($F_{1,58}$ = 25.60, P < 0.001). The common slope was then used to determine the relative buoyancy (intercepts) for each of the morphology types (**Table 3**). Based on the intercepts for each of the morphology types, data were aggregated into two groups and a new final model created ($F_{1,58}$ = 73.18, P < 0.001). The first group of morphology types (characterised by relatively buoyant seeds), included flattened, membranous and woody morphologies (FMW), and had an intercept of 1.779. The remaining seed morphologies (characterised by less buoyant seeds), included no distinctive features, arillate, fleshy or rough morphologies (Other), and had an intercept of 1.466 (**Table 4** and **Figure 3**).

The model obtained for buoyancy at day 0 was:

y = (-0.504) specific weight + 1.779 where morphology = FMW (1)

y = (-0.504) specific weight + 1.466 where morphology = Other (2)

where y = arcsin (sqrt(Buoyant seeds at day 0 (%)/100))

and specific weight is measured in mg μl^{-1}.

Regression models for buoyancy at day 3

The procedure followed for day 0 was repeated for day 3. A linear model revealed that morphology ($F_{6,46}$ = 3.43, P = 0.007) affected buoyancy, and specific weight was negatively related to buoyancy ($F_{1,46}$ = 25.14, P < 0.001) at 3 days. There was no interaction between morphology and specific weight ($F_{6,46}$ = 1.66, P = 0.1524). An overall model revealed that the common slope for all seed morphologies was -0.5319 ($F_{1,58}$ = 29.50, P < 0.001). The slope was then fixed to determine the relative buoyancy (intercepts) for each morphology type. Based on significant differences between the intercepts of the morphology types, the dataset was split into two morphology subsets and a final model created ($F_{1,58}$ = 69.90, P < 0.001). Although flattened seeds were not significantly different to those with no distinctive morphological features (P = 0.064), they were not similar enough to be included in the 'Other' subset, and not different enough to justify a third subset. The value of the intercept, and the magnitude of the difference in P values, indicated flattened seeds were better grouped with membranous and woody seeds, than with the 'Other' morphologies (**Table 3**). The model for the relatively buoyant subset, those seeds with flattened, membranous and woody morphologies (FMW), had an intercept of 1.730. The model for the remaining seed morphologies, those with no distinctive morphological features, arillate, fleshy or rough morphologies (Other), had an intercept of 1.449 (**Table 4** and **Figure 3**).

The model obtained for buoyancy at day 3 was:

y = (-0.532) specific weight + 1.730 where morphology = FMW (3)

y = (-0.532) specific weight + 1.449 where morphology = Other (4)

where y = arcsin (sqrt(Buoyant seeds at day 3 (%)/100))

and specific weight is measured in mg μl^{-1}.

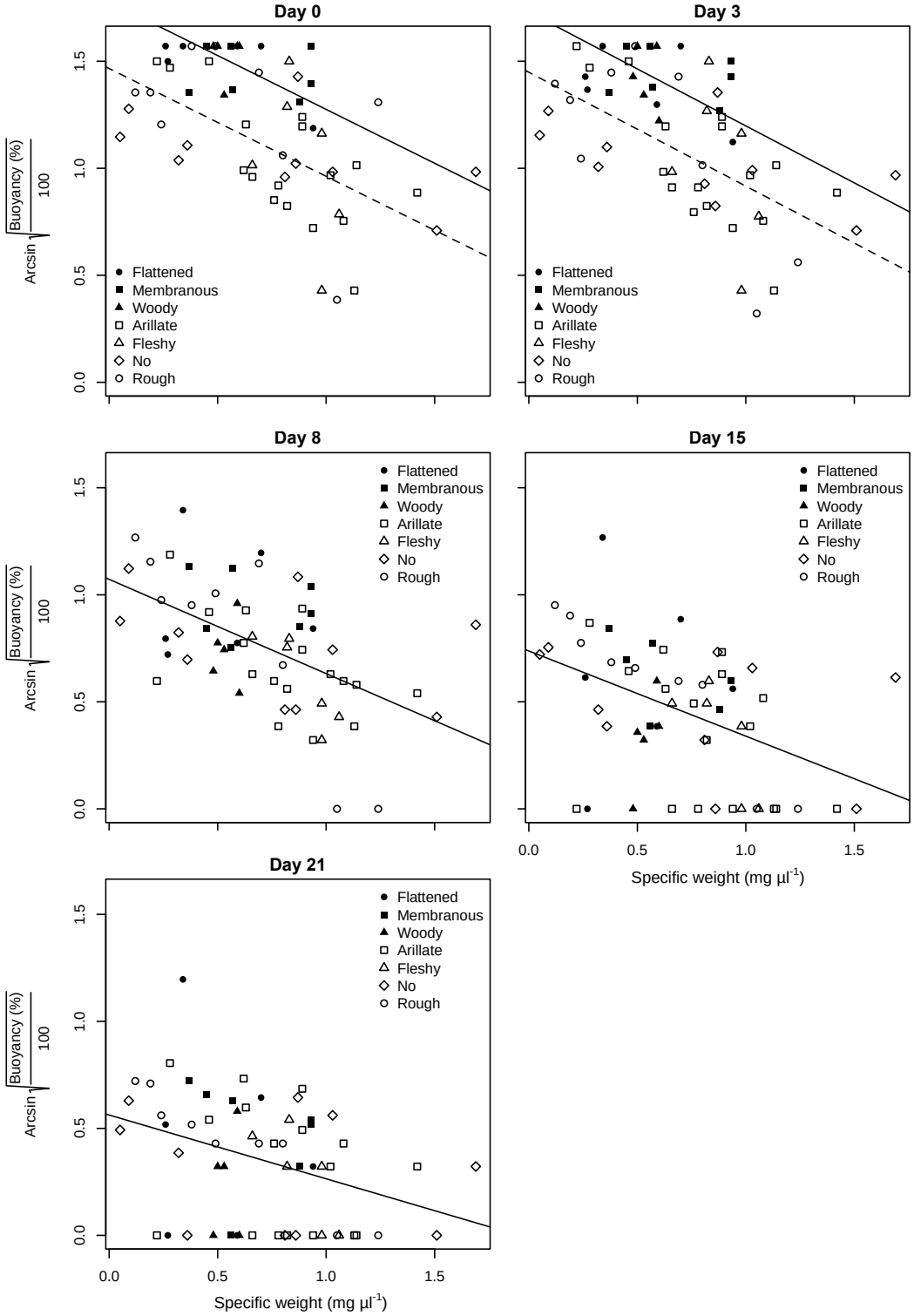


Figure 3. Regression models for arcsin square-root transformed buoyancy (%) data and specific weight (mg μl^{-1}) at 0, 3, 8, 15 and 21 days. For 0 and 3 days the solid line represents the regression model for the subset that includes flattened, membranous and woody seed morphologies, and the dashed line represents the regression model for the subset that includes no distinctive features, arillate, fleshy and rough seed morphologies.

Regression models for buoyancy at day 8 to day 21

Morphology type did not significantly affect buoyancy at ≥ 8 days. Specific weight was the variable that best predicted buoyancy at 8, 15 and 21 days and was thus included in the models (**Tables 2** and **3**). The models revealed that low specific weight resulted in higher buoyancy (negative correlation) at day 8 ($T = -4.93$, $F_{1,58} = 24.27$, $P < 0.001$), day 15 ($T = -3.80$, $F_{1,58} = 14.47$, $P < 0.001$), and day 21 ($T = -2.98$, $F_{1,58} = 8.86$, $P = 0.004$) (**Figure 3**).

The models obtained for buoyancy at day 8, 15 and 21 were:

$$y_{8d} = (-0.440) \text{ specific weight} + 1.072 \quad (5)$$

$$y_{15d} = (-0.398) \text{ specific weight} + 0.738 \quad (6)$$

$$y_{21d} = (-0.298) \text{ specific weight} + 0.562 \quad (7)$$

where y_x = arcsin (sqrt(Buoyant seeds at day x (%)/100))

x = number of days

and specific weight is measured in mg μl^{-1}.

Model accuracy

Analysis of the observed versus the predicted buoyancy for each species, at each time, revealed that the regression model for day 0 was highly significant and explained 56% of the variation in buoyancy ($r^2 = 0.56$, $F_{1,58} = 73.18$, $P < 0.001$). The regression model for day 3 was highly significant and explained 55% of the variation in buoyancy ($r^2 = 0.55$, $F_{1,58} = 69.90$, $P < 0.001$). Although the regression models for other times were also significant, they only explained 30% of the variation in buoyancy at day 8 ($r^2 = 0.30$, $F_{1,58} = 24.27$, $P < 0.001$), 20% at day 15 ($r^2 = 0.20$,

$F_{1,58}$ = 14.47, P < 0.001), and 13% at day 21 (r^2 = 0.13, $F_{1,58}$ = 8.86, P =0.004) (**Figure 4**). Although all the models described above were significantly better than their respective null models, the low r^2 values for ≥ 8 days reflect the low predictive power of these models for extended buoyancy, which may reflect long-distance dispersal (**Figure 4**).

Discussion

Here we present five simple models that use easily determined seed traits, specific weight and morphology type, to predict seed buoyancy, an indicator of hydrochorous dispersal potential. Using simple seed morphological traits the models can predict between 56% (at 0 days) and 13% (at 21 days) of variation in seed buoyancy of coastal species. These are the first models created specifically for the prediction of hydrochorous dispersal for a range of species, and the first models to address buoyancy over time. While most other seed dispersal vectors have been modelled in the past, this research has addressed a gap in dispersal ecology by improving understanding of the seed traits that influence hydrochorous dispersal. The predicted and observed data show a strong linear relationship for seed buoyancy between 0 and 8 days. The models predict seed buoyancy with sufficient accuracy to investigate oceanic seed dispersal potential for multiple species upon initial contact with water, but are limited in their ability to predict extended buoyancy, which may have the largest effect on true long-distance dispersal.

Morphological seed traits

The hypothesis that high buoyancy would be related to morphology type was supported. However, no individual morphology type was indicative of high buoyancy and the relative buoyancy of each morphology type was not as hypothesised. Flattened, woody and membranous seeds were equally, and significantly, more buoyant than seeds with other morphologies at 0 and 3 days, but this relationship did not extend ≥ 8 days. This research confirms that the common methodology of using seed morphological characteristics to infer dispersal vectors is most applicable to general dispersal and may not be applicable for polychory or

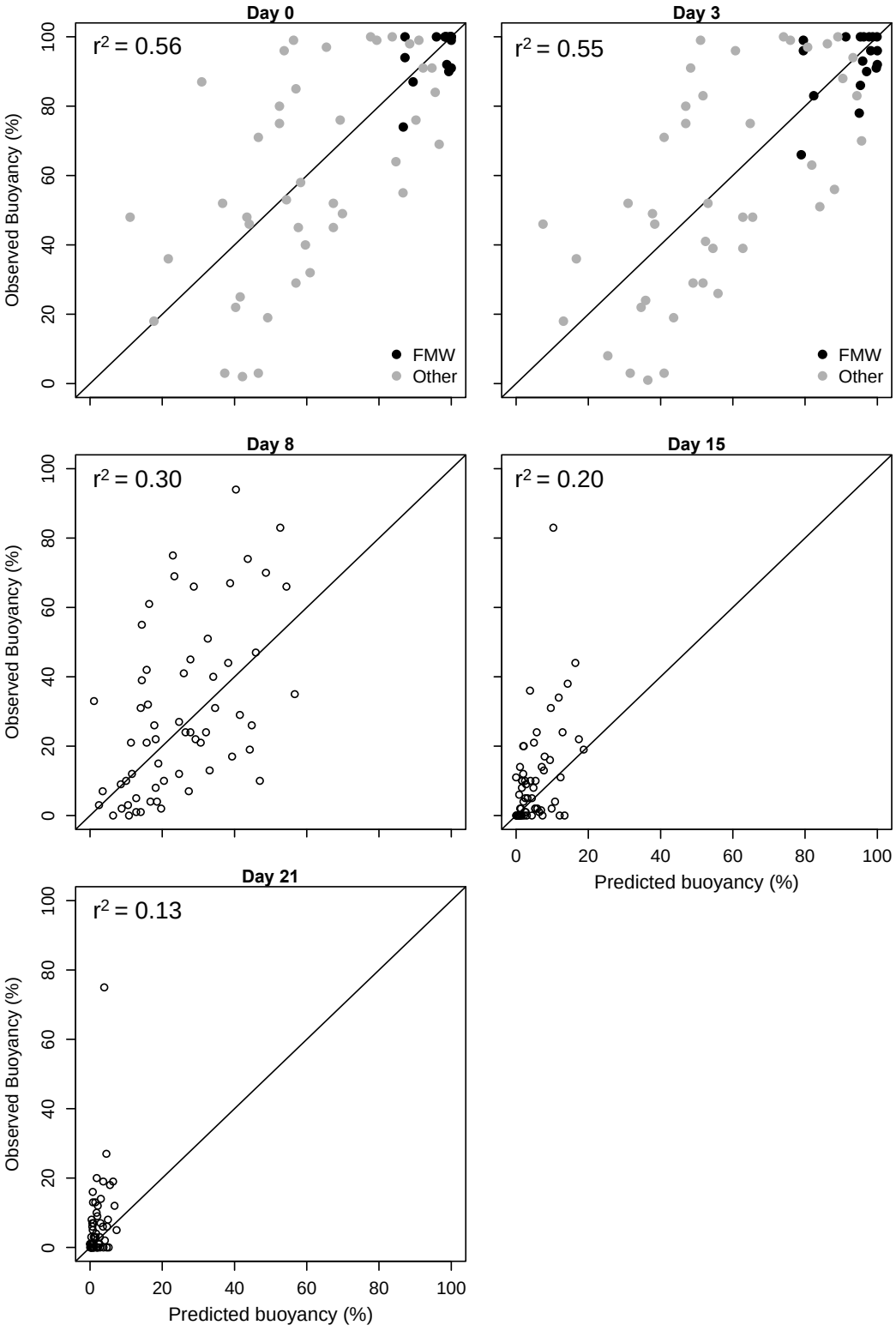


Figure 4. Correlation between the predicted (x) and observed (y) buoyancy (%) at 0, 3, 8, 15 and 21 days. At 0 and 3 days black circles represent the subset of flattened, membranous and woody seed morphologies (FMW) and grey circles represent the subset of no distinctive features, arillate, fleshy and rough seeds (Other). A line showing a 1:1 relationship is also included in each plot.

long-distance dispersal (Nathan *et al.* 2008). Indeed, existing data on dispersal distances, colonisation rates, and migration rates have been used to demonstrate that dispersal processes suggested by the morphology of the dispersal unit are poorly related to long-distance dispersal because of the complex relationship between the morphology of dispersal units and the multiple processes that move seeds (Higgins *et al.* 2003). Our data suggest that for the coastal species tested one specialised morphology has not evolved for hydrochorous dispersal, but that multiple morphology types do facilitate hydrochorous dispersal, and perhaps long-distance dispersal.

The greater initial buoyancy of flattened, membranous and woody morphologies does not necessarily indicate that those morphologies are specialised for hydrochorous dispersal. The results suggest that polychory and non-standard means of dispersal may be of greater influence (Higgins *et al.* 2003; Nathan 2006; Nathan *et al.* 2008). Seeds with various non-specialised morphologies may still achieve long-distance dispersal (Higgins *et al.* 2003; de Pablos and Peco 2007) through chance events such as oceanic hydrochory. For example, ultra-low seed mass (e.g. dust-like seed) and flattened appendages are generally regarded as adaptations that enhance wind dispersal (Werker 1997; Cousens *et al.* 2008). However, despite not being an adaptation specifically for hydrochorous dispersal, the wings of flattened seeds increase the probability of diaspores reaching the ocean, as well as increasing their buoyancy (due to the large surface area and low mass of a wing resulting in a low specific weight). Similarly, transoceanic dispersal may occur via multiple vectors, particularly for species such as *Scaevola taccada* where the fleshy outer mesocarp facilitates endozoochory (ingestion by birds) and the woody inner mesocarp may facilitate hydrochory (Howarth *et al.* 2003). With polychory likely to be more frequent than generally realised, non-standard dispersal such as oceanic hydrochory may often be facilitated by the same seed features that govern the standard mode of dispersal (Higgins *et al.* 2003).

Specific weight was significantly correlated with buoyancy at all times in these multi-species models, supporting both our hypothesis and the findings of other investigators (Lopez 2001; Cousens *et al.* 2008). However, the correlations between specific weight and buoyancy became weaker as time progressed. Extended seed

buoyancy is likely to be affected by a seed's ability to retain a low specific weight. Imbibition (water uptake) causes an increase in seed mass and, without a corresponding increase in seed volume, the specific weight of a seed may eventually exceed the density of water, causing it to sink. Previous assessments of buoyancy have shown that the diaspores of *Olearia axillaris*, *Rhagodia baccata*, and *Spyridium globulosum* were initially more buoyant than seeds, but then sank at a faster rate than seeds (Appendix 1; Guja *et al.* 2010). This suggests that wetting or waterlogging of appendages reduces dispersal potential, and that surface wetting and imbibition require further consideration as factors influencing hydrochory. Future investigations of extended buoyancy may benefit from measurement of relative imbibition and the morphological traits of waterlogged seeds over time. Adjusting for the effect of imbibition at each time may refine models, particularly for long-distance dispersal.

The hypothesis that species with low seed mass would exhibit extended buoyancy was supported. Within species, seed fill was not correlated with buoyancy indicating that the relationship between buoyancy and low seed mass was not caused by empty seeds. Variables such as diaspore (0 and 3 days) and mass (8, 15 and 21 days) were also significantly correlated with buoyancy but could not be used in the linear models because they were correlated with other variables and their inclusion would violate assumptions of independence. The variables that were related to buoyancy differed between $\leq$ 3 days and $\geq$ 8 days, suggesting that the variables governing general buoyancy on contact with water (0 to 3 days) may differ from the variables governing intermediate ($\geq$ 8 days) and long-distance dispersal ($\geq$ 21 days) via hydrochory. The model derived for 21 days explains only a small proportion of the variation in the data, indicating that another variable significant to extended buoyancy may remain undiscovered. If the seed traits governing long-distance hydrochorous dispersal are to be understood in greater detail, statistical approaches capable of analysing correlated variables may be required.

Chance events

Because the relationships between the morphology of dispersal units and the multiple processes that move seeds are complex (Higgins *et al.* 2003) it remains difficult to predict rare or chance events that cause long-distance dispersal. For each species, the seeds in the tails of the distributions (buoyant for extended time periods) represent unusual behaviour of a seed relative to the mean of the sample and it is these seeds that are most likely to undergo long-distance dispersal (Andersen 1991; Higgins *et al.* 2003; Nathan *et al.* 2008). Some dispersal processes such as rafting are truly chance events that can occasionally be observed, but remain inherently difficult to predict (Higgins *et al.* 2003; Minchinton 2006; Luiz *et al.* 2012). Despite the low probability of rafting and other chance events, over evolutionary time periods, these phenomena may be significant drivers of plant distributions.

Chance events such as oceanic dispersal may exert only weak selection pressure when compared with better studied dispersal modes such as myrmecochory and zoochory. As a result, the potential for hydrochory is often difficult to predict from a single seed trait when compared with other dispersal vectors. However, with an understanding of the multiple significant traits identified here, predictions can now be made with some confidence. Because there is not one single morphology type or seed trait predictive of high buoyancy, empirical data can be gathered and used with these models to estimate the probability that hydrochory is a potential explanation for phylogeographic and biogeographic patterns. Use of these models may allow greater confidence in biogeographic hypotheses, provided that there have not been significant changes in seed morphology over the evolutionary timelines considered.

Environmental variables

Although proximity to the ocean was not related to buoyancy in this study, this may be because all species investigated were coastal plants. Perhaps comparison with species from other habitats may have revealed higher buoyancy in coastal species as has been demonstrated for riparian species, which have greater seed buoyancy than species from non-flooded areas (Lopez 2001; van den Broek *et al.* 2005). Alternatively, the rarity of oceanic hydrochory, particularly for non-adapted species,

and the consequent low selection pressure may result in relatively equal buoyancy between coastal and non-coastal species.

Unusual behaviour of the dispersal vector also requires consideration. The current models were built from buoyancy data collected in a homogenous laboratory environment and, despite disruption of surface tension, do not capture the turbulent nature of the ocean. Understanding turbulence has greatly improved models of wind dispersal (Greene and Johnson 1989; Higgins *et al.* 2003) and may also improve predictions of oceanic dispersal. Incorporating environmental variables in models of oceanic dispersal, however, will remain difficult because of the many different scales that need to be considered. For example in hydrochorous dispersal, surface movements are dictated by local prevailing winds, the medium-range effects of swell, and the larger scale effects of ocean currents. Application of the knowledge about the physical and biological dynamics of currents and eddies (e.g. investigation of the Leeuwin Current; Waite *et al.* 2007) would be useful in dispersal modelling. Ultimately, mixed models (de Pablos and Peco 2007) that account for factors other than buoyancy and incorporate environmental effects may predict hydrochorous and long-distance dispersal more accurately.

Concluding remarks

These models should only be used to infer buoyancy in species with seed traits and morphologies in the same range as the study species (Römermann *et al.* 2005; de Pablos and Peco 2007). Although this model has not been tested outside the study system, other dispersal models based on Australian flora adequately predicted dispersal mechanisms for species of other temperate regions (Thomson *et al.* 2010). Field studies that investigate oceanic dispersal could also be used to validate these models, which were derived from laboratory data.

The models presented here provide practical tools and a step toward better prediction of seed dispersal via the ocean. Specific weight and seed morphology type are demonstrated as the best predictive traits for buoyancy. Predictions using these models may facilitate interpretation of phylogeographic results and improve understanding of dispersal potential and range shifts under climate change. Mixed models with a greater regard for variation in the dispersal unit (either inherent or due

to imbibition), and the dispersal vector, would revolutionise our capacity to quantitatively describe hydrochorous seed dispersal. Extended buoyancy, which promotes long-distance seed dispersal by the ocean, remains difficult to predict accurately. However, this research has created simple models that can be used to predict medium-term buoyancy and identified future research directions that may improve prediction of long-distance dispersal.

References

Andersen M (1991) Mechanistic models for the seed shadows of wind-dispersed plants. *The American Naturalist* **137**, 476-497.

Andersen MC (1993) Diaspore morphology and seed dispersal in several wind-dispersed Asteraceae. *American Journal of Botany* **80**, 487-492.

Atia A, Debez A, Barhoumi Z, Pacini E, Abdelly C, Smaoui A (2010) The mericarp of the halophyte *Crithmum maritimum* (Apiaceae): structural features, germination, and salt distribution. *Biologia* **65**, 489-495.

Black M (2009) Darwin and seeds. *Seed Science Research* **19**, 193-199.

Cousens R, Dytham C, Law R (2008) 'Dispersal in plants: a population perspective.' (Oxford University Press: New York).

Darwin C (1859) ' The origin of species.' (Oxford University Press: Oxford).

Dawson M, Hammer W (2008) A biophysical perspective on dispersal and geography of evolution in the sea. *Journal of the Royal Society Interface* **5**, 135-150.

de Pablos I, Peco B (2007) Diaspore morphology and the potential for attachment to animal coats in Mediterranean species: an experiment with sheep and cattle coats. *Seed Science Research* **17**, 109-114.

de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution* **20**, 68-73.

Dixon KW (2011) 'Coastal plants: a guide to the identification and restoration of plants of the Perth region.' (CSIRO Publishing: Melbourne).

Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK (2011) Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology & Evolution* **27**, 1-10.

Greene DF, Johnson EA (1989) A model of wind dispersal of winged or plumed seeds. *Ecology* **70**, 339-347.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**, 1175-1186.

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* **84**, 1945-1956.

Howarth DG, Gustafsson MHG, Baum DA, Motley TJ (2003) Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacifc Basin and colonization of the Hawaiian Islands. *American Journal of Botany* **90**, 915-923.

Hroudova Z, Moravcova L, Zakravsky P (1997) Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. *Folia Geobotanica et Phytotaxonomica* **32**, 377-390.

Kokubugata G, Nakamura K, Forster PI, Hirayama Y, Yokota M (2012) Antitropical distribution of *Lobelia* species (Campanulaceae) between the Ryukyu Archipelago of Japan and Oceania as indicated by molecular data. *Australian Journal of Botany* **60**, 417-428.

Lopez OR (2001) Seed flotation and postflooding germination in tropical *terra firme* and seasonally flooded forest species. *Functional Ecology* **15**, 763-771.

Luiz OJ, Madin JS, Robertson DR, Rocha LA, Wirtz P, Floeter SR (2012) Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society of London B* **279**, 1033-1040.

Marchant NG, Wheeler JR, Rye BL, Bennett EM, Lander NS, Macfarlane TD (1987) 'Flora of the Perth region.' (Western Australian Herbarium: Perth).

Matlack GR (1987) Diaspore size, shape, and fall behavior in wind-dispersed plant species. *American Journal of Botany* **74**, 1150-1160.

Merritt DM, Wohl EE (2002) Processes governing hydrochory along rivers: hydraulics, hydrology, and dispersal phenology. *Ecological Applications* **12**, 1071-1087.

Minchinton TE (2006) Rafting on wrack as a mode of dispersal for plants in coastal marshes. *Aquatic Botany* **84**, 372-376.

Nathan R (2006) Long-distance dispersal of plants. *Science* **313**, 786-788.

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. *Trends in Ecology and Evolution* **23**, 638-647.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**, 167-234.

Quilichini A, Debussche M (2000) Seed dispersal and germination patterns in a rare Mediterranean island endemic (*Anchusa crispa* Viv., Boraginaceae). *Acta Oecologica* **21**, 303-313.

Rippey E, Rowland B (2004) 'Coastal plants: Perth and the south-west region.' 2nd edn. (University of Western Australia Press: Perth).

Römermann C, Tackenberg O, Poschlod P (2005) How to predict attachment potential of seeds to sheep and cattle coat from simple morphological seed traits. *Oikos* **110**, 219-230.

Tackenberg O, Romermann C, Thompson K, Poschlod P (2006) What does diaspore morphology tell us about external animal dispersal? Evidence from standardized experiments measuring seed retention on animal-coats. *Basic and Applied Ecology* **7**, 45-58.

Thompson K, Band SR, Hodgson JG (1993) Seed size and shape predict persistence in soil. *Functional Ecology* **7**, 236-241.

Thomson FJ, Moles AT, Auld TD, Ramp D, Ren S, Kingsford RT (2010) Chasing the unknown: predicting seed dispersal mechanisms from plant traits. *Journal of Ecology* **98**, 1310-1318.

van den Broek T, van Diggelen R, Bobbink R (2005) Variation in seed buoyancy of species in wetland ecosystems with different flooding dynamics. *Journal of Vegetation Science* **16**, 579-586.

van Loon AH, Soomers H, Schot PP, Bierkens MFP, Griffioen J, Wassen MJ (2011) Linking habitat suitability and seed dispersal models in order to analyse the effectiveness of hydrological fen restoration strategies. *Biological Conservation* **144**, 1025-1035.

Waite AM, Thompson PA, Pesant S, Feng M, Beckley LE, Domingues CM, Gaughan D, Hanson CE, Holl CM, Koslow T, Meuleners M, Montoya JP, Moore T, Muhling BA, Paterson H, Rennie S, Strzelecki J, Twomey L (2007) The Leeuwin Current and its eddies: an introductory overview. *Deep Sea Research Part II: Topical Studies in Oceanography* **54**, 789-796.

Werker E (1997) 'Seed anatomy.' (Stuttgart: Borntraeger, Berlin).

Western Australian Herbarium (1998-2014) FloraBase—the Western Australian flora. (Department of Parks and Wildlife) Available at http://florabase.dpaw.wa.gov.au/

Will H, Maussner S, Tackenberg O (2007) Experimental studies of diaspore attachment to animal coats: predicting epizoochorous dispersal potential. *Oecologia* **153**, 331-339.

Williamson GB, Costa F, Vera CVM (1999) Dispersal of Amazonian trees: hydrochory in *Swartzia polyphylla*. *Biotropica* **31**, 460-465.

Supplementary Table 1. Seed traits analysed to determine their effect on mean seed buoyancy over time. Continuous variables: seed mass, seed fill, two dimensional projected area, seed size (length, width and height), variance in seed shape (calculated as the variance of the three dimensions length width and height, first divided by length) (Thompson *et al.* 1993), volume, and specific weight. Categorical variables: diaspore type (seed (S) or fruit (F)), appendage (yes (Y) or no (N)), morphology type (arillate, flattened, fleshy, membranous, rough, woody or no) (sensu. Marchant *et al.* 1987; Western Australian Herbarium 1998-2014; Römermann *et al.* 2005; Tackenberg *et al.* 2006; Will *et al.* 2007) and the dune habitat where the species grows closest to the water line (Rippey and Rowland 2004; Dixon 2011). Where applicable, standard errors are given in parentheses, - indicates that standard error could not be calculated due to sampling method.

Species	Family	Mass (mg)	Fill (%)	Projected Area (mm^2)	Size (mm)			Shape variance	Volume (µ l)	Spec. Wt. (mg µ l^{-1})
					Length	Width	Height			
Acacia cochlearis	Fabaceae: Mimosoideae	6.24 (0.13)	99	13.24 (2.54)	3.77 (0.13)	2.14 (0.12)	1.28 (0.08)	0.11	8.00	0.78
Acacia cyclops	Fabaceae: Mimosoideae	59.81 -	100	59.40 (1.72)	12.79 (0.24)	6.83 (0.40)	2.22 (0.12)	0.17	90.00	0.66
Acacia lasiocarpa	Fabaceae: Mimosoideae	3.70 (0.13)	82	11.50 (2.43)	2.64 (0.12)	1.84 (0.08)	1.28 (0.04)	0.07	4.50	0.82
Acacia littorea	Fabaceae: Mimosoideae	3.58 (0.20)	82	7.49 (1.54)	3.49 (0.13)	1.69 (0.07)	1.11 (0.04)	0.13	4.00	0.89
Acacia pulchella	Fabaceae: Mimosoideae	9.13 (0.21)	89	9.55 (1.40)	3.61 (0.15)	2.15 (0.04)	1.37 (0.07)	0.10	8.00	1.14
Acacia saligna	Fabaceae: Mimosoideae	14.15 (0.53)	88	12.80 (0.86)	4.68 (0.12)	2.67 (0.05)	1.27 (0.10)	0.13	10.00	1.42
Acacia truncata	Fabaceae: Mimosoideae	4.09 (0.07)	91	6.52 (0.93)	2.92 (0.06)	2.44 (0.28)	1.11 (0.06)	0.10	4.00	1.02
Acacia xanthina	Fabaceae: Mimosoideae	23.71 (1.45)	74	16.17 (0.95)	5.61 (0.15)	3.74 (0.20)	1.99 (0.07)	0.10	22.00	1.08
Allocasuarina lehmanniana	Casuarinaceae	1.40 (0.03)	60	10.99 (0.87)	5.20 (0.20)	2.03 (0.14)	0.72 (0.04)	0.20	2.00	0.70
Alyogyne huegelii	Malvaceae	5.53 (0.08)	78	6.15 (0.53)	3.06 (0.12)	2.14 (0.05)	1.79 (0.12)	0.05	8.00	0.69
Alyxia buxifolia	Apocynaceae	46.54 (1.84)	92	18.46 (0.29)	6.80 (0.28)	4.99 (0.07)	4.99 (0.07)	0.02	56.00	0.83
Atriplex isatidea	Chenopodiaceae	11.28 (0.26)	83	31.17 (0.58)	7.34 (0.48)	6.91 (0.29)	3.31 (0.22)	0.09	44.00	0.26
Austrostipa elegantissima	Poaceae	0.85 (0.03)	91	3.63 (0.09)	4.84 (0.30)	0.59 (0.01)	0.45 (0.02)	0.27	0.50	1.69
Boronia alata	Rutaceae	4.55 (0.08)	71	5.40 (0.11)	2.89 (0.05)	2.08 (0.06)	1.45 (0.03)	0.06	6.00	0.76
Brachyscome iberidifolia	Asteraceae	0.10 (0.01)	48	1.11 (0.03)	1.58 (0.08)	0.59 (0.01)	0.59 (0.01)	0.13	0.50	0.19
Cakile maritima	Brassicaceae	14.17 -	100	22.68 (0.37)	9.55 (0.63)	4.73 (0.35)	3.80 (0.19)	1.10	28.50	0.50
Callitris preissii	Cupressaceae	7.50 (0.52)	60	25.44 (0.65)	7.17 (0.20)	4.64 (0.39)	1.54 (0.15)	0.15	8.00	0.94
Calothamnus quadrifidus	Myrtaceae	0.12 (0.01)	58	1.46 (0.17)	1.58 (0.02)	0.51 (0.04)	0.51 (0.04)	0.15	0.50	0.24
Conostylis candicans	Haemodoraceae	0.52 -	100	1.04 (0.01)	1.59 (0.04)	0.70 (0.03)	0.68 (0.03)	0.11	0.50	1.05
Diplolaena dampieri	Rutaceae	8.00 (0.14)	68	8.58 (0.08)	4.40 (0.15)	1.89 (0.08)	1.48 (0.10)	0.13	9.000	0.89
Diplopeltis huegelii	Sapindaceae	2.49 (0.06)	56	3.20 (0.04)	1.99 (0.09)	1.68 (0.08)	1.43 (0.04)	0.02	4.00	0.62
Dodonaea aptera	Sapindaceae	9.43 (0.11)	85	7.78 (0.04)	3.87 (0.21)	2.46 (0.13)	1.85 (0.05)	0.07	10.00	0.94
Enchylaena tomentosa	Chenopodiaceae	2.68 (0.14)	85	5.12 (0.17)	2.57 (0.19)	2.29 (0.17)	1.95 (0.11)	0.01	6.00	0.45
Eremophila glabra	Scrophulariaceae	127.79 (3.23)	66	46.43 (2.05)	8.91 (0.61)	6.18 (0.17)	6.18 (0.17)	0.03	130.00	0.98
Eucalyptus gomphocephala	Myrtaceae	1.62 (0.07)	92	4.52 (0.14)	2.98 (0.07)	2.04 (0.11)	0.78 (0.03)	0.14	2.00	0.81
Exocarpos sparteus	Santalaceae	21.17 (0.54)	85	13.01 (0.24)	5.94 (0.30)	2.98 (0.04)	2.98 (0.04)	0.08	20.00	1.06
Ficinia nodosa	Cyperaceae	0.32 -	100	0.88 (0.02)	1.41 (0.07)	0.78 (0.02)	0.51 (0.02)	0.11	0.50	0.63
Guichenotia ledifolia	Malvaceae	1.12 (0.05)	74	2.49 (0.04)	1.95 (0.10)	1.16 (0.03)	0.96 (0.02)	0.07	4.00	0.28
Hakea prostrata	Proteaceae	41.54 (1.03)	94	70.13 (0.95)	15.62 (2.63)	7.92 (0.47)	2.86 (0.22)	0.17	70.00	0.59
Hardenbergia comptoniana	Fabaceae: Faboideae	48.72 -	100	19.16 (0.25)	5.79 (0.16)	4.49 (0.08)	2.73 (0.05)	0.07	43.00	1.13
Hemiandra pungens	Lamiaceae	3.96 (0.11)	92	5.68 (0.09)	2.78 (0.09)	2.25 (0.21)	1.76 (0.12)	0.03	8.00	0.49
Jacksonia furcellata	Fabaceae: Faboideae	6.04 (0.15)	76	6.07 (0.12)	3.54 (0.06)	1.87 (0.06)	1.36 (0.08)	0.10	4.00	1.51
Lechenaultia linariodes	Goodeniaceae	6.86 (0.52)	37	8.64 (0.44)	3.53 (0.15)	2.69 (0.28)	1.55 (0.05)	0.08	8.00	0.86
Lepidosperma gladiatum	Cyperaceae	2.74 (0.11)	10	5.58 (0.14)	3.73 (0.08)	1.66 (0.04)	1.66 (0.04)	0.10	6.00	0.46
Leucopogon parviflorus	Ericaceae	13.14 (0.32)	18	9.18 (0.04)	4.11 (0.17)	2.99 (0.09)	2.99 (0.09)	0.03	16.00	0.82
Melaleuca cardiophylla	Myrtaceae	0.18 (0.01)	81	1.66 (0.15)	1.57 (0.08)	0.64 (0.06)	0.64 (0.06)	0.12	0.50	0.36
Melaleuca huegelii	Myrtaceae	0.16 (0.01)	87	1.16 (0.09)	1.19 (0.05)	0.59 (0.06)	0.59 (0.06)	0.09	0.50	0.32
Melaleuca lanceolata	Myrtaceae	0.03 -	68	0.87 (0.10)	0.85 (0.05)	0.42 (0.02)	0.42 (0.02)	0.09	0.50	0.05
Melaleuca systena	Myrtaceae	0.05 -	80	0.68 (0.23)	0.98 (0.02)	0.48 (0.03)	0.48 (0.03)	0.09	0.50	0.09
Muehlenbeckia adpressa	Polygonaceae	3.41 (0.17)	53	5.05 (0.05)	3.22 (0.14)	2.19 (0.03)	2.19 (0.03)	0.03	6.00	0.57
Myoporum insulare	Scrophulariaceae	68.91 -	100	22.27 (0.56)	4.93 (0.32)	4.60 (0.29)	4.49 (0.42)	0.00	70.00	0.98
Olax benthamiana	Olacaceae	35.11 (1.17)	65	18.77 (0.17)	7.20 (0.46)	3.76 (0.05)	3.76 (0.05)	0.08	40.00	0.88
Opercularia benthamiana	Rubiaceae	0.53 (0.02)	37	2.51 (0.10)	1.90 (0.04)	1.41 (0.11)	0.72 (0.03)	0.10	2.00	0.27
Ozothamnus cordatus	Asteraceae	0.06 (0.00)	98	4.33 (0.09)	3.57 (0.09)	4.11 (0.37)	4.11 (0.37)	0.01	0.50	0.12
Phyllanthus calycinus	Phyllanthaceae	5.22 (0.09)	86	5.65 (0.03)	2.77 (0.11)	2.11 (0.07)	1.81 (0.06)	0.03	6.00	0.87
Pimelea ferruginea	Thymelaeaceae	3.70 (0.10)	85	5.23 (0.05)	3.62 (0.06)	1.77 (0.04)	1.77 (0.04)	0.09	4.00	0.93
Rhagodia baccata	Chenopodiaceae	3.93 -	100	4.48 (0.16)	2.65 (0.07)	2.65 (0.07)	1.98 (0.08)	0.02	6.00	0.66
Scaevola anchusifolia	Goodeniaceae	19.19 (0.25)	43	13.79 (0.19)	4.43 (0.12)	3.33 (0.12)	3.33 (0.12)	0.02	32.00	0.60
Scaevola crassifolia	Goodeniaceae	5.94 -	100	7.21 (0.26)	3.11 (0.37)	2.24 (0.25)	2.55 (0.17)	0.02	10.00	0.59
Scaevola nitida	Goodeniaceae	7.67 (0.35)	60	6.92 (2.33)	3.50 (0.05)	2.60 (0.08)	2.04 (0.18)	0.04	16.00	0.48
Scaevola thesioides	Goodeniaceae	2.10 (0.05)	76	4.01 (0.08)	2.37 (0.08)	1.94 (0.12)	1.06 (0.06)	0.08	4.00	0.53
Schoenus grandiflorus	Cyperaceae	8.23 (0.30)	74	7.18 (0.16)	3.94 (0.12)	2.18 (0.04)	2.18 (0.04)	0.07	8.00	1.03
Senecio pinnatifolius	Asteraceae	0.40 (0.01)	70	2.04 (0.03)	2.64 (0.16)	0.72 (0.03)	0.72 (0.03)	0.18	0.50	0.80
Solanum symonii	Solanaceae	2.49 (0.20)	34	5.35 (0.05)	2.84 (0.07)	2.38 (0.07)	0.82 (0.05)	0.14	2.00	1.24
Spinifex longifolius	Poaceae	11.41 -	100	13.54 (0.33)	8.32 (0.39)	1.96 (0.06)	1.49 (0.10)	0.21	31.00	0.37
Spyridium globulosum	Rhamnaceae	1.86 -	100	2.80 (0.03)	2.34 (0.08)	1.68 (0.20)	1.26 (0.06)	0.05	2.00	0.93
Tetragonia decumbens	Aizoaceae	118.63 -	100	144.17 (5.81)	15.51 (1.39)	16.20 (0.91)	15.51 (1.39)	0.00	350.00	0.34
Thomasia triphylla	Malvaceae (Sterculiaceae)	0.46 (0.02)	39	2.04 (0.03)	1.79 (0.03)	1.27 (0.05)	0.72 (0.02)	0.09	2.08	0.22
Threlkeldia diffusa	Chenopodiaceae	3.35 (0.20)	28	5.34 (0.19)	3.09 (0.06)	1.73 (0.10)	1.73 (0.10)	0.06	6.00	0.56
Trachymene coerulea	Araliaceae	1.53 (0.03)	93	6.06 (0.08)	3.80 (0.31)	2.47 (0.10)	0.71 (0.06)	0.17	4.00	0.38

Supplementary Table 1 continued

Species	Diaspore	Appendage	Morphology	Habitat	Buoyancy (%)				
					Day 0	Day 3	Day 8	Day 15	Day 21
Acacia cochlearis	S	Y	Arillate	Secondary	40.00 (1.63)	39.00 (1.91)	2.00 (1.15)	0.00 (0.00)	0.00 (0.00)
Acacia cyclops	S	Y	Arillate	Primary	45.00 (3.00)	39.00 (3.00)	12.00 (4.32)	0.00 (0.00)	0.00 (0.00)
Acacia lasiocarpa	S	Y	Arillate	Primary	29.00 (4.12)	29.00 (4.12)	8.00 (1.63)	1.00 (1.00)	0.00 (0.00)
Acacia littorea	S	Y	Arillate	Secondary	80.00 (3.27)	80.00 (3.27)	42.00 (2.00)	20.00 (2.83)	16.00 (1.63)
Acacia pulchella	S	Y	Arillate	Secondary	52.00 (10.07)	52.00 (10.07)	9.00 (2.52)	0.00 (0.00)	0.00 (0.00)
Acacia saligna	S	Y	Arillate	Tertiary	36.00 (6.93)	36.00 (6.93)	7.00 (3.00)	0.00 (0.00)	1.00 (1.00)
Acacia truncata	S	Y	Arillate	Secondary	46.00 (6.00)	46.00 (6.00)	12.00 (4.90)	2.00 (2.00)	1.00 (1.00)
Acacia xanthina	S	Y	Arillate	Primary	22.00 (3.46)	22.00 (3.46)	10.00 (2.58)	6.00 (1.15)	3.00 (1.00)
Allocasuarina lehmanniana	S	Y	Flattened	Tertiary	100.00 (0.00)	100.00 (0.00)	75.00 (7.55)	36.00 (8.16)	13.00 (2.52)
Alyogyne huegelii	S	N	Rough	Secondary	97.00 (1.91)	96.00 (4.00)	69.00 (3.79)	10.00 (4.16)	3.00 (1.91)
Alyxia buxifolia	F	N	Fleshy	Primary	98.61 (1.39)	98.61 (1.39)	26.39 (1.39)	9.72 (3.50)	6.94 (2.66)
Atriplex isatidea	F	Y	Flattened	Foredune	100.00 (0.00)	96.00 (2.31)	26.00 (5.03)	11.00 (4.43)	6.00 (2.58)
Austrostipa elegantissima	S	N	No	Secondary	48.00 (12.33)	46.00 (11.83)	33.00 (7.19)	11.00 (3.00)	1.00 (1.00)
Boronia alata	S	Y	Arillate	Secondary	32.00 (4.32)	26.00 (2.58)	10.00 (2.58)	5.00 (1.91)	3.00 (1.00)
Brachyscome iberidifolia	S	N	Rough	Secondary	91.00 (3.00)	88.00 (4.32)	70.00 (7.02)	38.00 (3.83)	18.00 (3.46)
Cakile maritima	F	N	Woody	Foredune	100.00 (0.00)	100.00 (0.00)	24.00 (6.27)	1.50 (0.96)	1.00 (1.00)
Callitris preissii	S	Y	Flattened	Secondary	74.00 (4.76)	66.00 (6.22)	31.00 (5.51)	8.00 (2.31)	1.00 (1.00)
Calothamnus quadrifidus	S	N	Rough	Secondary	76.00 (5.89)	56.00 (12.33)	47.00 (6.40)	24.00 (4.32)	8.00 (4.32)
Conostylis candicans	S	N	Rough	Secondary	2.00 (1.15)	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Diplolaena dampieri	S	Y	Arillate	Secondary	75.00 (8.06)	75.00 (8.06)	21.00 (5.97)	12.00 (1.63)	5.00 (2.52)
Diplopeltis huegelii	S	Y	Arillate	Secondary	49.00 (3.79)	48.00 (5.66)	24.00 (4.00)	21.00 (3.79)	20.00 (4.00)
Dodonaea aptera	S	Y	Arillate	Tertiary	19.00 (5.97)	19.00 (5.97)	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)
Enchylaena tomentosa	F	N	Membranous	Primary	100.00 (0.00)	100.00 (0.00)	31.00 (6.40)	17.00 (8.06)	14.00 (9.02)
Eremophila glabra	F	N	Fleshy	Primary	71.00 (5.97)	71.00 (5.97)	5.00 (3.00)	2.00 (1.15)	1.00 (1.00)
Eucalyptus gomphocephala	S	N	No	Tertiary	45.00 (8.23)	41.00 (7.00)	4.00 (1.63)	1.00 (1.00)	0.00 (0.00)
Exocarpos sparteus	F	Y	Fleshy	Secondary	25.00 (6.81)	24.00 (7.12)	3.00 (1.91)	0.00 (0.00)	0.00 (0.00)
Ficinia nodosa	S	Y	Arillate	Foredune	76.00 (5.16)	75.00 (4.43)	41.00 (14.27)	8.00 (2.83)	10.00 (2.00)
Guichenotia ledifolia	S	Y	Arillate	Secondary	98.00 (1.15)	98.00 (1.15)	74.00 (4.16)	34.00 (4.76)	27.00 (5.00)
Hakea prostrata	S	Y	Flattened	Secondary	100.00 (0.00)	86.00 (5.03)	24.00 (9.52)	2.00 (2.00)	0.00 (0.00)
Hardenbergia comptoniana	S	Y	Arillate	Secondary	3.00 (1.91)	3.00 (1.91)	2.00 (2.00)	0.00 (0.00)	0.00 (0.00)
Hemiandra pungens	S	N	Rough	Primary	100.00 (0.00)	100.00 (0.00)	51.00 (9.00)	14.00 (2.00)	3.00 (1.91)
Jacksonia furcellata	S	N	No	Tertiary	18.00 (4.76)	18.00 (4.76)	3.00 (1.00)	0.00 (0.00)	0.00 (0.00)
Lechenaultia linariodes	S	N	No	Secondary	53.00 (8.23)	29.00 (11.59)	4.00 (2.83)	0.00 (0.00)	0.00 (0.00)
Lepidosperma gladiatum	S	Y	Arillate	Primary	99.00 (1.00)	99.00 (1.00)	40.00 (5.42)	13.00 (3.79)	7.00 (3.00)
Leucopogon parviflorus	F	N	Fleshy	Secondary	85.00 (2.52)	83.00 (3.00)	22.00 (6.63)	5.00 (3.00)	1.00 (1.00)
Melaleuca cardiophylla	S	N	No	Secondary	64.00 (5.89)	63.00 (5.26)	17.00 (1.00)	2.00 (1.15)	0.00 (0.00)
Melaleuca huegelii	S	N	No	Secondary	55.00 (11.7)	51.00 (13.89)	29.00 (7.72)	4.00 (2.31)	2.00 (2.00)
Melaleuca lanceolata	S	N	No	Primary	69.00 (3.42)	70.00 (4.16)	35.00 (3.79)	19.00 (2.52)	5.00 (2.52)
Melaleuca systena	S	N	No	Secondary	84.00 (4.32)	83.00 (5.00)	66.00 (7.02)	22.00 (4.76)	12.00 (4.32)
Muehlenbeckia adpressa	F	N	Membranous	Secondary	92.00 (3.27)	93.00 (2.52)	66.00 (2.58)	24.00 (5.42)	12.00 (4.90)
Myoporum insulare	F	N	Fleshy	Primary	3.00 (1.91)	3.00 (1.91)	1.00 (1.00)	0.00 (0.00)	0.00 (0.00)
Olax benthamiana	F	N	Membranous	Secondary	87.00 (3.42)	83.00 (4.12)	32.00 (5.89)	4.00 (1.63)	1.00 (1.00)
Opercularia benthamiana	S	Y	Flattened	Primary	99.00 (1.00)	92.00 (2.31)	19.00 (7.55)	0.00 (0.00)	0.00 (0.00)
Ozothamnus cordatus	S	Y	Rough	Secondary	91.00 (6.61)	94.00 (6.00)	83.00 (7.19)	44.00 (10.71)	19.00 (4.43)
Phyllanthus calycinus	S	N	No	Secondary	96.00 (1.63)	91.00 (4.12)	61.00 (6.61)	20.00 (4.90)	13.00 (4.43)
Pimelea ferruginea	F	N	Membranous	Secondary	94.00 (2.58)	96.00 (1.63)	39.00 (9.85)	10.00 (2.00)	6.00 (1.15)
Rhagodia baccata	F	N	Fleshy	Foredune	52.00 (7.83)	48.00 (8.79)	27.00 (4.73)	5.00 (3.00)	4.00 (2.83)
Scaevola anchusifolia	F	N	Woody	Secondary	100.00 (0.00)	78.00 (9.02)	7.00 (4.43)	2.00 (1.15)	0.00 (0.00)
Scaevola crassifolia	F	N	Woody	Foredune	100.00 (0.00)	100.00 (0.00)	45.00 (10.63)	10.00 (5.03)	9.00 (5.26)
Scaevola nitida	F	N	Woody	Secondary	100.00 (0.00)	96.00 (1.63)	13.00 (5.26)	0.00 (0.00)	0.00 (0.00)
Scaevola thesioides	F	N	Woody	Secondary	90.00 (1.15)	90.00 (1.15)	21.00 (11.00)	1.00 (1.00)	1.00 (1.00)
Schoenus grandiflorus	S	N	No	Secondary	48.00 (5.89)	49.00 (6.40)	21.00 (1.91)	14.00 (2.58)	8.00 (2.83)
Senecio pinnatifolius	S	N	Rough	Foredune	58.00 (10.00)	52.00 (10.71)	15.00 (5.00)	9.00 (5.26)	3.00 (1.91)
Solanum symonii	S	N	Rough	Foredune	87.00 (5.26)	8.00 (2.31)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Spinifex longifolius	S	N	Membranous	Foredune	91.00 (4.12)	91.00 (4.12)	67.00 (17.08)	31.00 (7.72)	19.00 (7.72)
Spyridium globulosum	S	N	Membranous	Secondary	100.00 (0.00)	99.00 (1.00)	55.00 (16.36)	10.00 (5.29)	7.00 (4.43)
Tetragonia decumbens	F	Y	Flattened	Foredune	100.00 (0.00)	100.00 (0.00)	94.00 (2.00)	83.00 (1.91)	75.00 (1.00)
Thomasia triphylla	S	Y	Arillate	Secondary	99.00 (1.00)	100.00 (0.00)	10.00 (3.46)	0.00 (0.00)	0.00 (0.00)
Threlkeldia diffusa	F	N	Membranous	Primary	100.00 (0.00)	100.00 (0.00)	22.00 (4.76)	2.00 (1.15)	0.00 (0.00)
Trachymene coerulea	S	Y	Rough	Secondary	100.00 (0.00)	97.00 (1.91)	44.00 (2.83)	16.00 (5.89)	6.00 (2.00)

Chapter 4

Dispersal potential of *Scaevola crassifolia* (Goodeniaceae) is influenced by intraspecific variation in fruit morphology along a latitudinal environmental gradient

Abstract

Dispersal of plant propagules by ocean currents can result in long-distance dispersal and is important for the persistence of coastal species. However, the ability of such species to disperse via the ocean is often unknown because there is relatively little evidence that demonstrates that seeds or fruits can float and survive for extended periods in seawater. Furthermore, the seed or fruit traits that facilitate buoyancy, and their intraspecific variation, remain largely unidentified. The genus *Scaevola* (L.) contains several widespread coastal species that may be capable of oceanic dispersal, such as *S. crassifolia* (Labill). We collected fruits of *S. crassifolia* along 700 km of a latitudinal environmental gradient. These fruits were used to determine the influence of fruit morphology and anatomy on fruit buoyancy. Morphological and anatomical variation in *S. crassifolia* was associated with dispersal potential. Our empirical data demonstrated that fruits with larger aeriferous mesocarp layers have greater buoyancy and, therefore, enhanced capacity for long-range oceanic dispersal. Of three characters hypothesised to affect buoyancy (aeriferous mesocarp, air pockets in empty locules and number of vascular cavities), only the properties of the mesocarp were significant. Intraspecific variation can significantly affect dispersal potential, and should not be overlooked in dispersal ecology.

Introduction

Seed dispersal is crucial to the formation and persistence of plant populations and hydrochory (dispersal by water) is increasingly recognised as an important dispersal mechanism. Hydrochory, as with other dispersal mechanisms, significantly affects patterns and processes at genetic (e.g. gene flow and diversity), community (e.g. species richness and arrival of new species), and population (e.g. longevity and range size) levels (Nilsson *et al.* 2010). Capacity to undertake long-distance dispersal is likely to be increasingly important for the persistence of plant species as their ranges shift in response to continuing global climate change (Hughes 2000; Travis *et al.* 2013). Dispersal via ocean currents can potentially result in long-distance dispersal, with recent phylogenetic and biogeographic studies leading to a resurgence of support for oceanic hydrochory as a means of long-distance dispersal of plants (Howarth *et al.* 2003; de Queiroz 2005; Cousens *et al.* 2008; Dawson and Hamner 2008; Kokubugata *et al.* 2012). However, unlike Darwin's seminal studies of oceanic hydrochory (Darwin 1856; Darwin 1859), recent hypotheses are generally not supported by evidence that fruits or seeds are capable of remaining buoyant and surviving in seawater for the extended periods required to achieve effective long-distance dispersal.

Buoyancy is a key factor that governs hydrochorous dispersal of seeds, which may be associated with interspecific variation in morphological and anatomical traits of the dispersal unit (Lopez 2001; Leyer and Pross 2009; Vargas *et al.* 2014). However, differences in the morphology of seeds or fruits within a species may also influence dispersal capacity (Darling *et al.* 2008). Such variation may arise through genetic variation, the location of fruits in the infructescence, or the maternal environment during fruit development and maturation (Matilla *et al.* 2005; Donohue 2009). This variation in seed or fruit morphology may greatly affect the dispersal potential of seeds (Darling *et al.* 2008). For example, infraspecific variation in subspecies of *Bolboschoenus maritimus* (L.) (Cyperaceae) affects the duration of achene buoyancy (Hroudova *et al.* 1997). *Bolboschoenus maritimus* subsp. *compactus* possesses well-developed aeriferous tissue in the exocarp resulting in greater buoyancy than the achenes of *B. maritimus* subsp. *maritmus*, which have thin or no aeriferous tissue in the exocarp (Hroudova *et al.* 1997). Further, intraspecific variation is evident in

seeds from the same parent plant. For example, at low wind speeds, the dispersal distance of winged seeds from a single *Spergularia marina* (L.) (Caryophyllaceae) plant was greater than that of seeds without wings, whereas in water, winged seeds were more frequently trapped in vegetation than seeds without wings (Telenius and Torstensson 1989). At a time when metadata analysis and multispecies models are increasingly common, intraspecific variation is often overlooked despite the potential to influence the frequency of rare events such as long-distance dispersal.

Intraspecific variation in seeds and fruits has been linked to differences in dispersal capacity and used to identify specific traits associated with large dispersal distances. Manipulation of seeds or fruits, or the construction of mimics (artificial seeds or fruits), is one method of investigating relationships between dispersal and intraspecific variation in seed or fruit traits such as mass, area and the presence of appendages (Augspurger and Franson 1987; Hughes and Westoby 1992; Yang *et al.* 2012). Although many seed and fruit traits are frequently proposed to be associated with particular dispersal vectors, the traits associated with hydrochorous dispersal have rarely been demonstrated experimentally. Seed characteristics associated with air chambers are commonly assumed to result in extended buoyancy (Higgins *et al.* 2003; Cousens *et al.* 2008) and some investigations have determined the particular traits that influence buoyancy in a small number of plant species. For example, aeriferous tissue in the exocarp of *Bolboschoenus* (Cyperaceae) (Hroudova *et al.* 1997), low specific weight (mass/volume) of twelve species from Panama (Lopez 2001), and the volume of an air pocket between embryonic cotyledons in *Swartzia* (Fabaceae) seeds (Williamson *et al.* 1999) are all associated with prolonged buoyancy. The effect of morphological traits on buoyancy requires further investigation, particularly for species that have diaspore morphologies different from those of the few species that have been studied.

The majority of Goodeniaceae genera are confined to the Australian continent, except for *Scaevola*, which is pantropical; 40 of 130 species are found outside Australia (Howarth *et al.* 2003). These species occur throughout the coastal regions of the Pacific and Indian Oceans, including the tropical Americas, Africa, Philippines, China, Marquesas and Hawaiian Islands (Howarth *et al.* 2003). Phylogenetic relationships within *Scaevola* suggest three independent colonisation

events from the Australian continent to the isolated Hawaiian Islands (Howarth *et al.* 2003). This finding, combined with the widespread distribution of several *Scaevola* species, implies a capacity for long-distance dispersal in *Scaevola*. This capacity may result from or be enhanced by fruit morphology. For example, it has been hypothesised that the widespread distribution of *Scaevola taccada* may be due to its fleshy exocarp (facilitating bird dispersal), or its corky mesocarp (facilitating buoyancy) (Lesko and Walker 1969; Howarth *et al.* 2003). Certainly, the fruits of the widely distributed *S. crassifolia* and *S. taccada* are buoyant and survive in seawater for 42 and 120 days, respectively, without adverse effects on seed germination, demonstrating a capacity for oceanic dispersal (Appendix 1; Lesko and Walker 1969; Guja *et al.* 2010).

The aeriferous, cork-like fruit coat of *Scaevola* species is often identified as a key trait that may determine oceanic dispersal ability (Lesko and Walker 1969; Howarth *et al.* 2003). However, there have been no experimental investigations of the capacity of the aeriferous fruit coat, or intraspecific variation in fruit morphology, to influence buoyancy. Fruits of all *Scaevola* species are indehiscent and drupe-like with a hard endocarp towards the locules (Carolin 1966). Three layers are visible in most species and are referred to as epicarp, mesocarp and endocarp, although they do not correspond exactly to the layers as named in true drupes because the epicarp is likely to be derived from outer floral whorls rather than ovary tissue (drupe-like, c.f. (Carolin 1966)). In fruits of *S. crassifolia*, the endocarp and mesocarp are not differentiated and are henceforth referred to as mesocarp. Unlike some *Scaevola* species that have succulent or fleshy epicarp and mesocarp layers, *S. crassifolia* fruits are dry with gradation to unthickened cells on the outermost part of the fruit (Carolin 1966). The outer mesocarp consists of air-filled parenchyma cells, which in some species is presumed to be the feature most likely to promote buoyancy (Lesko and Walker 1969; Howarth *et al.* 2003). Fruits of *S. crassifolia* have two (Carolin 1966), or occasionally three locules (pers. obs.). Often only one locule is filled (pers. obs.) and the resultant air pocket in the empty locule, surrounded by hard, dry mesocarp, may increase fruit buoyancy. False locules, and small cavities in the mesocarp, are formed by disintegration of vascular bundles (Carolin 1966) and these air pockets may also influence fruit buoyancy.

In the present study we aimed to identify the anatomical features of *Scaevola* fruits that are most strongly associated with buoyancy, and to test for relationships between buoyancy and intraspecific morphological variation in fruits. We reasoned that the fruits of *S. crassifolia* possess the following three main anatomical traits that may be related to buoyancy: 1) aeriferous mesocarp, 2) air pockets in empty locules, and 3) vascular cavities. We quantified anatomical and morphological variation of fruits along a latitudinal environmental gradient and investigated how this variation affected dispersal potential. Specifically, we hypothesised that: 1) the anatomical structure of fruits would affect buoyancy, such that fruits with large aeriferous mesocarp and many vascular cavities would be most buoyant, and 2) fruits containing one seed and one empty locule would be more buoyant than fruits containing two filled locules.

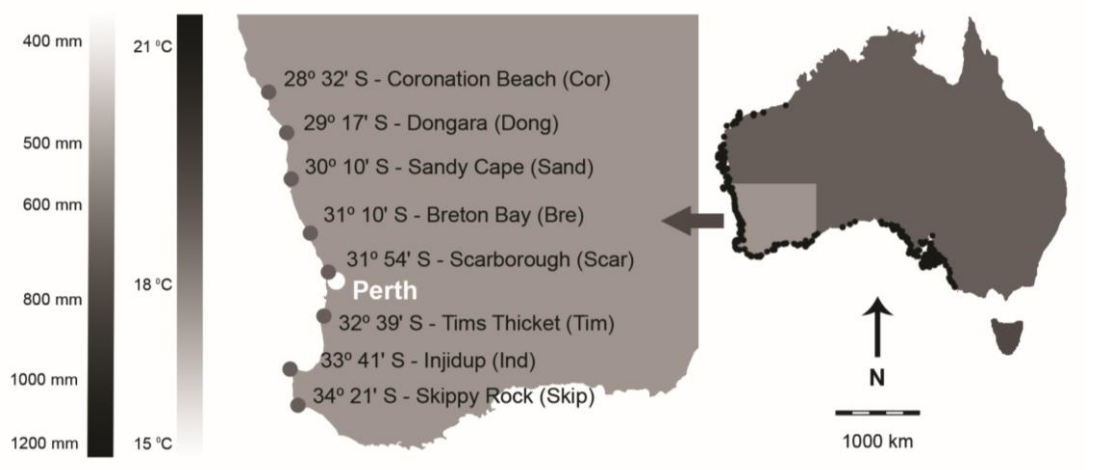


Figure 1. Map of south-western Australia, showing the study sites for *Scaevola crassifolia*, and the annual average daily mean temperature and mean annual precipitation for coastal localities, as derived from the Bureau of Meteorology (2013). The capital city, Perth, is marked for reference. Abbreviations (in parentheses) are used in reference to each collection location throughout the text. The peri-continental distribution of *Scaevola crassifolia* is represented by black circles in the map on the right (CSIRO 2013).

Materials and Methods

Study species and sites

Scaevola crassifolia is a shrub 0.1-1.5 m high occurring on frontal coastal sand dunes and limestone cliffs in western and southern Australia (Western Australian Herbarium 1998-2014; Rippey and Rowland 2004; Dixon 2011). The inflorescence is a terminal to sub-terminal spike of blue/mauve/white flowers present from July to February (Western Australian Herbarium 1998-2014; Rippey and Rowland 2004; Dixon 2011). Fruits are more or less globular/spherical, slightly compressed, hard, woody and minutely hairy (Marchant *et al.* 1987). The north-south distribution of *S. crassifolia* along the south-western Australian coastline extends for approximately 2000 km along a strong temperature and rainfall gradient – dry and hot in the north, to wet and cool in the south (Chapter 2; Bureau of Meteorology 2013; CSIRO 2013). To examine morphological variation of fruits, collections of *S. crassifolia* fruits were made at eight distant localities along 700 km of the latitudinal temperature and rainfall gradient (**Figure 1**). Average annual rainfall at the study sites is 400 mm in the north and 1200 mm in the south, and average annual daily-mean-temperature ranges from 21 °C in the north to 15 °C in the south (Bureau of Meteorology 2013). Climate data are based on 30-year climatology (1961-1990).

Fruit collection

Mature fruits (browned, woody and dry) were collected at the natural point of dispersal (when easily detached with minimal force) along the length of multiple infructescences from at least 10 plants at each site between January and April 2009. The average number of fruits collected per site was 23000 (min 9750 at Breton Bay and max 39000 at Tim's Thicket). Herbarium vouchers for each collection were lodged at the Kings Park and Botanic Garden Herbarium (KPBG). Collector's field numbers are: Coronation Beach LKG003, Dongara LKG024, Scarborough LKG107, Sandy Cape LKG031, Breton Bay LKG060, Tim's Thicket LKG066, Injidup LKG078, Skippy Rock LKG090. At the northern-most site, Coronation Beach, a number of fruits at the basal end of infructescences had already been released at the time of collection and only some fruits remained at the distal end. Fruits were stored

within two days of collection in a controlled-environment room at 15 ºC and 15% relative humidity (RH). After five months of storage, when all fruits had equilibrated to 15% RH, fruits were sealed in laminated aluminium foil bags and stored at -18 ºC until used in experiments.

Sample selection

To identify fruits that comprised zero, one, or two seeds, fruits were imaged using a digital X-ray (Faxitron MX-20, Faxitron X-ray, Lincolnshire, IL, USA). Fruits from each collection site were mounted upright on double-sided tape so that both locules were visible in X-ray images. For buoyancy experiments and subsequent morphological and anatomical measurements, 30 single-seeded fruits (three replicates of 10) were selected randomly from each collection. For the second experiment, a comparison of buoyancy of one- and two-seeded fruits, samples from all sites were pooled, X-rayed as above, and one- and two- seeded fruits (three replicates of 20 each) were randomly selected. Fruits containing more than two locules were rare and were not used in experiments.

Buoyancy of fruits

To determine buoyancy of fruits from each collection site, three replicates of 10 dry fruits were placed in 250 ml of seawater in plastic containers (11 cm diameter, 4 cm high, Genfac Plastics, Melbourne, Victoria). To release surface tension, the water in each container was initially stirred for 30 seconds (day 0). During the experiment, the container was topped up with de-ionised water to balance losses from evaporation and during this process all fruits were agitated (modified from Appendix 1; Guja *et al*. 2010). The same procedure was followed to determine buoyancy of fruits containing one or two seeds for each replicate of 20 fruits (pooled across all sites). For both experiments, the number of buoyant fruits was recorded at 0, 3, 6, and 8 days, after stirring. After eight days, approximately 50% of fruits remained buoyant and the experiment was terminated so that the buoyant and non-buoyant sample sizes were approximately equal for analysis.

Fruit morphology and anatomy

To study variation in the anatomical features of fruits from each site, sunken and buoyant fruits were removed from seawater, separated, and rinsed in deionised water to remove external salt. Fruits from each replicate were then dried by being placed in 10 g of silica gel for two days. Each fruit was weighed, height (**Figure 2**) was measured with digital callipers, and each fruit was then cut transversely to expose both locules. One half of the section was mounted, with the cut surface facing upwards, on carbon tape on an aluminium stub. Before examination in a scanning electron microscope (SEM) stubs were sputter-coated with gold at 25 mA for 2 min (Emitech K550X, Quorum Technologies Ltd, Ashford, Kent, UK). Fruits were imaged using a Jeol JCM6000 SEM under vacuum with an accelerating voltage of 10 kV and magnification between 28 and 40x, as required (Jeol Ltd, Sydney, NSW, Australia).

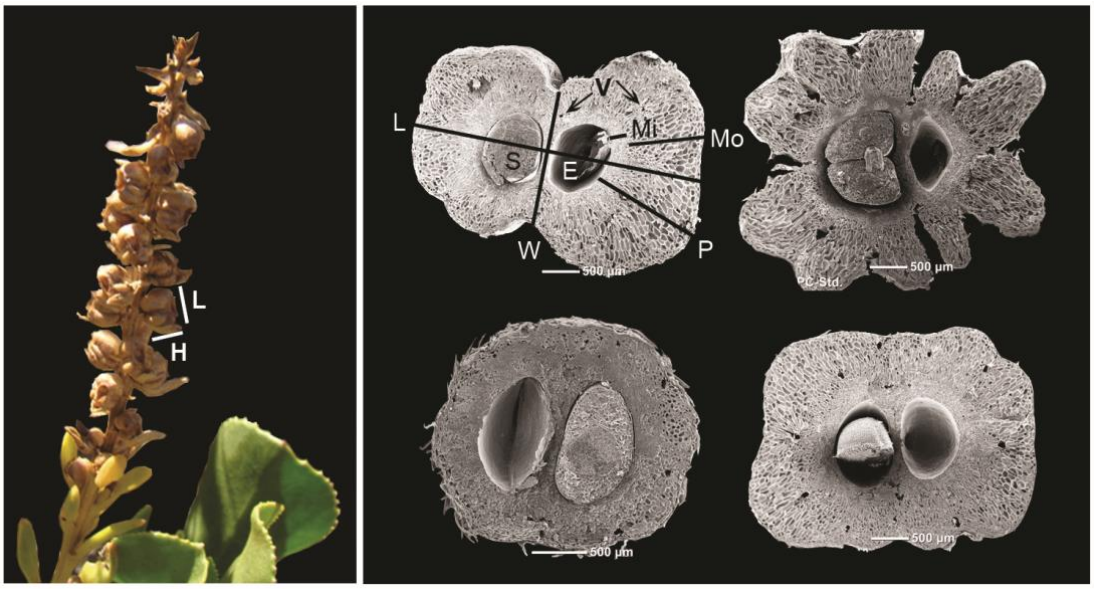


Figure 2. Left: Infructescence of *Scaevola crassifolia,* identifying the measured height (H) and length (L) dimensions of a fruit. Right: Transverse sections of *S. crassifolia* fruits. Morphological and anatomical variables measured for each fruit were length (L), width (W), pericarp (P), outer mesocarp (Mo), inner mesocarp (Mi), and vascular cavities (V). Each fruit generally contained one seed (S) and one empty locule (E). A typical fruit is shown top left. Extremes of variation in fruit morphology and anatomy are also shown; ridges in the pericarp (top right), small fruits with minimal outer mesocarp layers (bottom left), and large fruits with large outer mesocarp layers and many vascular cavities (bottom right).

Table 1. Morphology and anatomy of fruits collected from each site. Values are means followed by standard deviations in parentheses. For each variable, means with the same superscript letters are not significantly different (P > 0.05).The data shown for each of the fruit layers (pericarp, outer mesocarp and inner mesocarp) are the mean calculated from the maximum and minimum widths of the respective layer. Refer to **Figure 1** for explanation of collection site abbreviations.

		Mass (mg)	Height (mm)	Length (mm)	Volume (mm^3)	Specific weight (mg μl^{-1})	Pericarp (mm)	Mesocarp			Day 08 Buoyancy (%)
								Outer (mm)	Inner (mm)	Outer : Inner	
Site	Cor	4.66 (1.2) ^{bd}	2.12 (0.21) ^{be}	2.16 (0.22) ^{f}	4.43 (1.26) ^{e}	1.09 (0.24) ^{d}	0.43 (0.09) ^{e}	0.23 (0.09) ^{e}	0.20 (0.06) ^{ab}	1.26 (0.52) ^{e}	46.67 (23.09) ^{ab}
	Dong	7.22 (2.53) ^{a}	2.72 (0.39) ^{a}	3.14 (0.44) ^{ab}	9.93 (3.97) ^{ab}	0.75 (0.16) ^{ab}	0.89 (0.18) ^{a}	0.69 (0.16) ^{a}	0.19 (0.05) ^{ab}	3.95 (1.69) ^{a}	34.44 (5.09) ^{ab}
	Sand	7.15 (2.35) ^{a}	2.65 (0.30) ^{a}	3.39 (0.55) ^{a}	10.28 (4.87) ^{a}	0.74 (0.19) ^{ab}	0.89 (0.24) ^{a}	0.63 (0.20) ^{ab}	0.21 (0.04) ^{b}	2.97 (0.94) ^{abc}	76.67 (15.28) ^{a}
	Bre	5.12 (1.12) ^{bc}	2.33 (0.31) ^{bc}	2.75 (0.35) ^{cd}	6.12 (1.91) ^{cd}	0.89 (0.21) ^{bc}	0.62 (0.13) ^{bc}	0.47 (0.13) ^{cd}	0.17 (0.04) ^{ac}	2.99 (1.20) ^{abc}	70.00 (10.00) ^{ab}
	Scar	6.00 (1.53) ^{ac}	2.57 (0.30) ^{ad}	2.94 (0.47) ^{bc}	7.36 (2.89) ^{bd}	0.87 (0.20) ^{bc}	0.72 (0.23) ^{cd}	0.52 (0.23) ^{bc}	0.20 (0.06) ^{bc}	2.81 (1.43) ^{cd}	26.67 (20.82) ^{ab}
	Tim	5.82 (1.52) ^{acd}	2.36 (0.23) ^{cd}	2.61 (0.43) ^{de}	6.70 (2.80) ^{d}	0.94 (0.25) ^{cd}	0.65 (0.20) ^{c}	0.43 (0.21) ^{cd}	0.22 (0.04) ^{b}	2.06 (0.98) ^{d}	66.67 (15.28) ^{ab}
	Ind	4.12 (1.09) ^{b}	2.05 (0.22) ^{e}	2.34 (0.37) ^{ef}	4.56 (1.40) ^{ce}	0.94 (0.21) ^{cd}	0.51 (0.12) ^{be}	0.35 (0.12) ^{de}	0.16 (0.04) ^{a}	2.35 (0.97) ^{bd}	16.67 (20.82) ^{b}
	Skip	5.95 (1.82) ^{ac}	2.50 (0.27) ^{ac}	3.28 (0.39) ^{ab}	8.97 (3.59) ^{ab}	0.70 (0.17) ^{a}	0.84 (0.16) ^{ad}	0.64 (0.17) ^{ab}	0.19 (0.05) ^{ab}	3.72 (1.29) ^{ac}	48.15 (30.17) ^{ab}
	Mean	**5.76 (1.99)**	**2.41 (0.36)**	**2.83 (0.58)**	**7.30 (3.71)**	**0.86 (0.23)**	**0.69 (0.24)**	**0.50 (0.22)**	**0.19 (0.05)**	**2.76 (1.41)**	**48.24 (26.07)**
ANOVA	Df	7	7	7	7	7	7	7	7	7	7
	F value	12.58	21.31	33.86	21.25	11.04	28.99	24.61	5.60	17.92	3.87
	Pr(>F)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0119**

Measurements of the length (perpendicular to locules) and width (parallel to locules) (**Figure 2**) of the fruit cross-section were made using the scaler function on the graphical user interface of the Jeol JCM6000 licensed software. In cross-section, measurements were taken at both the minimum and maximum to record the variation in the width of layers. The maximum and minimum width of the pericarp (from the inside edge of the locule to the outer edge of the epicarp), outer mesocarp (aeriferous cells), and inner mesocarp (hard layer) were measured (**Figure 2**). Approximate total fruit volume (mm^3) was calculated using the formula for an ellipsoid sphere ($4/3\pi \times$ length $\times$ width $\times$ height). Specific weight (mg μl^{-1}) was calculated as mass/volume. Average layer width was calculated as (max + min)/2 for each layer. The ratio of outer to inner mesocarp was calculated by dividing the mean of the outer by the mean of the inner mesocarp. The number of distinct vascular cavities in each fruit was counted.

Statistical analysis

To investigate the extent of intraspecific variation in *S. crassifolia* fruits from different collection locations, each morphological and anatomical variable was analysed with ANOVA with post-hoc Tukey's multiple comparisons of means (R version 2.15.1, R Foundation for Statistical Computing). Differences in mean buoyancy (% after 8 days) of fruits from each collection site were also compared with one-way ANOVA with post-hoc Tukey's multiple comparison of means. Where required, data were transformed and checked to ensure they met assumptions of normality and homogeneity of variance (Shapiro-Wilk test). Fruit width and the number of vascular cavities could not be transformed to normal and were therefore not included in the ANOVAs. Non-transformed data are shown in figures and tables.

To determine the effect of morphological and anatomical variables on buoyancy at 8 days, the two groups (buoyant versus sunken) were compared using binomial generalised linear models (GLM) with a logit link function. All variables were analysed because normality was not prerequisite. Binomial GLMs with a logit link function were also used to compare buoyancy of one- versus two-seeded fruits at each time.

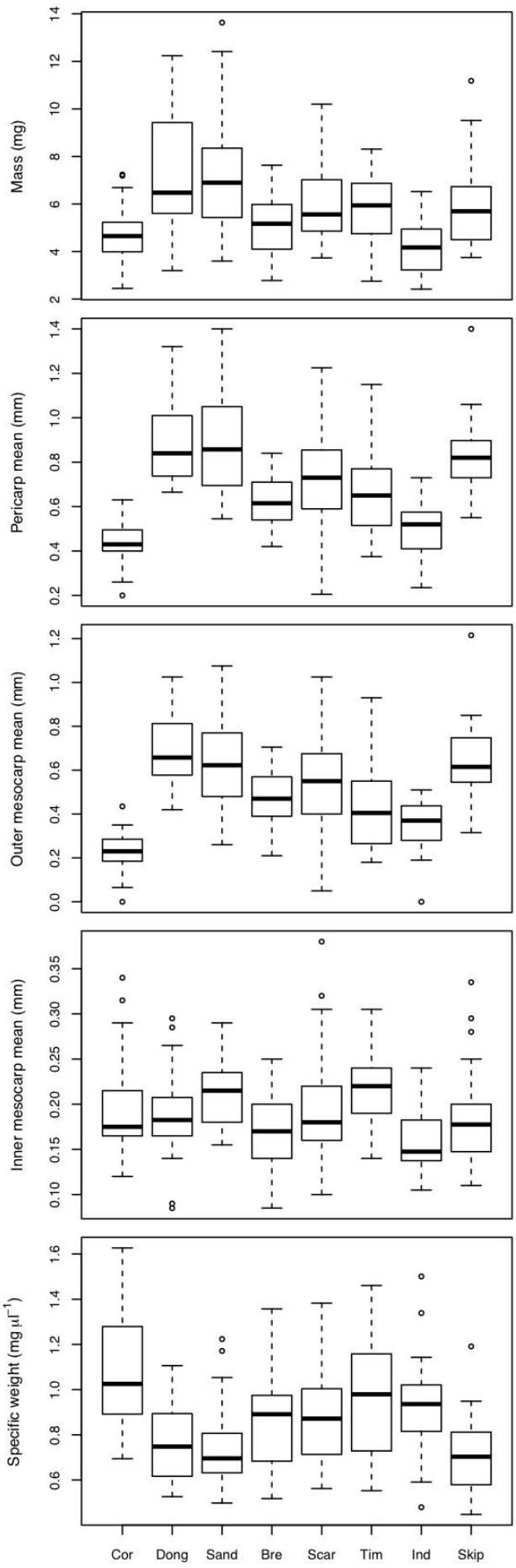


Figure 3. Box plots of morphological (specific weight and mass) and anatomical variables (pericarp, outer mesocarp, inner mesocarp) of fruits by collection site from left, North (Cor), to right, South (Skip). Refer to **Figure 1** for collection site abbreviations.

Results

There were significant differences among all morphological and anatomical traits (that could be transformed to normal) of fruits from different collection locations (**Figure 2** and **Table 1**). Post-hoc tests revealed that fruits from Coronation Beach (northern-most study site) were generally the smallest and had the smallest length (2.16 ± 0.22 mm), volume (4.43 ± 1.26 mm^3), pericarp (0.43 ± 0.09 mm), outer mesocarp (0.23 ± 0.09 mm) and mesocarp outer inner ratio (1.26 ±0.52). These fruits were also the densest (highest specific weight 1.09 ± 0.24 mg μl^{-1}) among all sites. Fruits from Dongara (c. 100 km south of Coronation Beach) were generally largest and had the greatest mass (7.22 ± 2.53 mg), height (2.72 ± 0.39 mm), pericarp (0.89 ± 0.18 mm), outer mesocarp (0.69 ± 0.16 mm), and mesocarp outer inner ratio (3.95 ± 1.69) among all collections. Variation in fruit morphological and anatomical traits (i.e. mass, height, length, volume, specific weight, pericarp mean, outer mesocarp mean, and mesocarp outer inner ratio, but not inner mesocarp mean) varied according to collection site, and south from Dongara there was a general trend of smaller and denser fruits (to Injidup) (**Table 1**, **Figure 3** selected variables). However, this trend did not extend to the most northern (Coronation Beach) and southern (Skippy Rock) study sites.

Table 2. Means of morphological and anatomical variables of buoyant and sunken fruits. Standard deviations are given in parentheses. Significant differences between buoyant and sunken fruits ($P < 0.05$) are identified in bold type and were determined by binomial generalised linear modelling with a logit link function. For all models there were 228 degrees of freedom.

Morphological/anatomical variable		Buoyant	Sunken	Test statistic	P
Mass (mg)		5.93 (2.01)	5.60 (1.95)	1.27	0.204
Height (mm)		2.44 (0.34)	2.39 (0.38)	1.091	0.275
Length (mm)		2.95 (0.61)	2.71 (0.53)	2.971	**0.00297**
Width (mm)		1.98 (0.46)	1.90 (0.46)	1.288	0.198
Volume (mm^3)		7.84 (3.97)	6.79 (3.39)	2.094	**0.0363**
Specific weight (mg μl^{-1})		0.83 (0.23)	0.90 (0.24)	-2.191	**0.0285**
Pericarp (mm)	Mean	0.74 (0.24)	0.65 (0.23)	2.602	**0.00926**
	Min	0.39 (0.20)	0.33 (0.14)	2.693	**0.00708**
	Max	1.08 (0.35)	0.98 (0.37)	2.087	**0.0369**
Outer mesocarp (mm)	Mean	0.53 (0.22)	0.46 (0.22)	2.505	**0.0123**
	Min	0.18 (0.18)	0.12 (0.14)	2.469	**0.0136**
	Max	0.89 (0.33)	0.80 (0.36)	1.992	**0.0464**
Inner mesocarp (mm)	Mean	0.19 (0.05)	0.19 (0.06)	-0.559	0.576
	Min	0.13 (0.04)	0.13 (0.04)	0.343	0.732
	Max	0.25 (0.06)	0.26 (0.08)	-0.969	0.333
Mesocarp Outer : Inner		2.95 (1.30)	2.58 (1.49)	1.962	**0.0498**
Vascular cavities		2.71 (2.65)	2.52 (2.57)	0.574	0.566

Initially, an average of 98% of all fruits were buoyant in seawater. After eight days, an average of 48% of all fruits remained buoyant. The rate at which fruits sank varied with collection site (**Figure 4**). After eight days 77% of fruits from Sandy Cape remained buoyant, being the highest percentage among all collection sites, whereas only 17% of fruits from Injidup remained buoyant, being the lowest percentage among all collection sites (**Figure 4**). There was no significant difference between buoyancy of single-seeded versus double-seeded fruits at any of the time points investigated (**Table 3**).

Across all collection locations, many morphological and anatomical variables were significantly different between buoyant and sunken fruits (as separated after eight days in seawater). Length, volume, pericarp (mean, max and min), outer mesocarp (mean, max and min) and mesocarp outer inner ratio were significantly greater in buoyant fruits than in sunken fruits (**Table 2**). Mean mesocarp thickness was significantly greater for buoyant than for sunken fruits. The 25% and 75% quartiles for the outer mesocarp of buoyant fruits were 0.381 and 0.665 mm (n = 118), and 0.281 and 0.600 mm (n = 112) for sunken fruits. Buoyant fruits also had a lower specific weight than did sunken fruits (**Table 2** and **Figure 5**). Mass, height, width, inner mesocarp and vascular cavities did not significantly affect fruit buoyancy at eight days (**Table 2**).

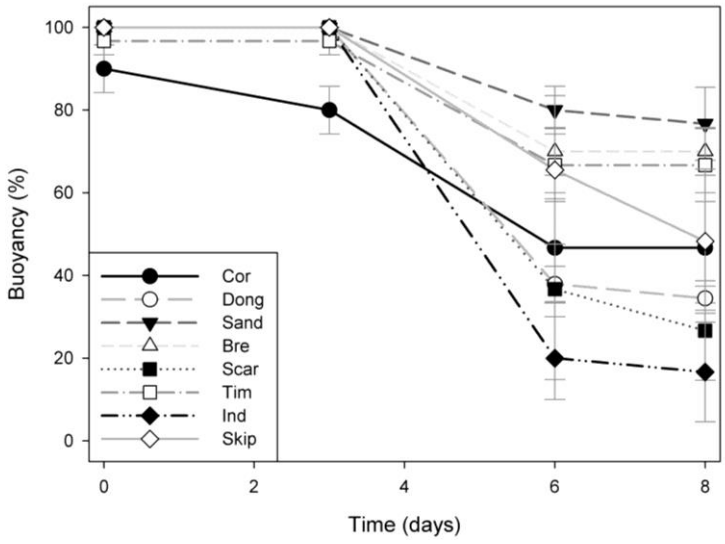


Figure 4. Buoyancy in seawater of fruits (%) from each collection site (n = 3 x 10) over time (days). Error bars represent ± 1 standard error.

Discussion

The present study has described large intraspecific variation in the morphology and anatomy of *Scaevola crassifolia* fruits along a latitudinal gradient, and resultant differences in dispersal potential of fruits from each collection site. The basic morphology and anatomical character of fruits was consistent. Yet, as hypothesised, intraspecific variation in the anatomical structure of fruits, particularly the size of the aeriferous outer mesocarp, was related to buoyancy. Here we demonstrated empirically, for the first time, that larger aeriferous mesocarp increases fruit buoyancy and can, therefore, increase the probability and distance of hydrochorous dispersal. These data empirically support the proposition made in other studies that aeriferous or cork-like fruit and seed coats increase buoyancy potential (Appendix 1; Lopez 2001; Cousens *et al.* 2008; Guja *et al.* 2010; Nilsson *et al.* 2010; Vargas *et al.* 2014). These data also demonstrated that the relationship between aeriferous tissue in the exocarp and buoyancy described for achenes (Hroudova *et al.* 1997) is similar for drupe-like woody fruits with multiple locules. Of the three fruit characteristics expected to affect buoyancy of *S. crassifolia* fruits (aeriferous mesocarp, empty locules and vascular cavities), only the mesocarp differed significantly between buoyant and non-buoyant fruits. A well-developed aeriferous outer mesocarp extended the duration of fruit buoyancy, whereas small outer mesocarp resulted in denser fruits (high specific weight) that sank rapidly.

Table 3. Mean buoyancy (%) of one- and two-seeded fruits (n = 3 x 20) in seawater at 0, 3, 6 and 8 days. Standard deviations follow in parentheses. Buoyancy of one-seeded and two-seeded fruits was not significantly different (P > 0.05) as determined by binomial generalised linear modelling with a logit link function. For all models there were 4 degrees of freedom.

Time	Buoyancy (%)		Test	P
	One seed	Two seeds	statistic	
Day 0	94.82 (5.27)	98.33 (2.89)	0.984	0.325
Day 3	93.07 (8.07)	98.33 (2.89)	1.285	0.199
Day 6	59.12 (11.34)	56.67 (28.43)	-0.293	0.769
Day 8	55.79 (8.84)	56.67 (28.43)	0.081	0.936

Specific weight is often reported to affect buoyancy (Lopez 2001; Cousens *et al.* 2008) and in a multi-species model it was the only variable that consistently influenced buoyancy over time (Chapter 3). In the present study, we have reported for the first time that a larger proportion of aeriferous tissue resulted in *Scaevola* fruits with lower specific weight and this was associated with prolonged buoyancy. The initial high buoyancy of fruits (98%) was likely to be due to their average specific weight being lower (0.9 ± 0.2 mg μl^{-1}) than that of seawater (1.03 mg μl^{-1}). Over extended periods it is possible that fruits sink because of an increase in mass caused by water uptake. Water may be absorbed by the woody fruit coat, or by the seeds imbibing water that has entered through the woody fruit (Turner *et al.* 2009). Future investigations in dispersal ecology should quantify relationships between specific weights of imbibed and dry fruits, and their buoyancy relative to the specific weight of seawater.

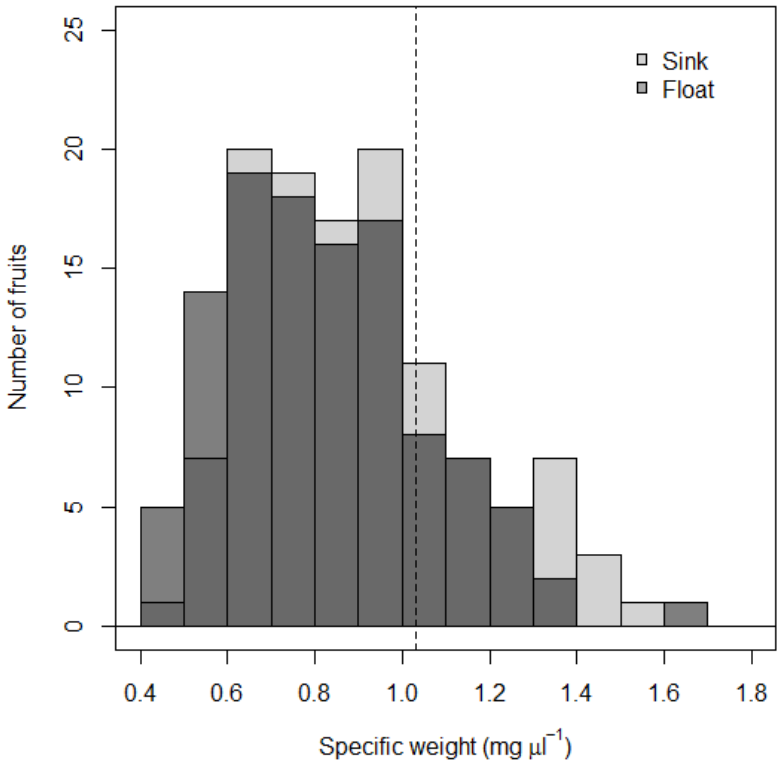


Figure 5. Histogram showing specific weight (mg μl^{-1}) of *S. crassifolia* fruits after eight days in seawater. The two series, float and sink, indicate the buoyant and sunken fruits. The vertical line at 1.03 marks the density of seawater. Floating fruits are shown in light grey, sunken fruits are shown in intermediate grey, and overlap is shown in dark grey.

Often investigations of dispersal ecology aim to predict dispersal ability, potential, or distance by using simple plant, fruit or seed traits (Römermann *et al.* 2005; Will *et al.* 2007; Thomson *et al.* 2010). Hydrochory has received less attention than other dispersal modes with knowledge of hydrochory in the Australian flora particularly lacking (Thomson *et al.* 2010), even though the continent has a large coastal fringe and numerous wetlands. While we have previously shown that fruits of *S. crassifolia* can survive up to 42 days in seawater and are therefore capable of oceanic dispersal (Appendix 1; Guja *et al.* 2010), here we show that a number of morphological variables differ significantly between buoyant and sunken fruits. However, the quantified variables are not all independent. This dependence limited the analysis and required each variable to be assessed individually, preventing the creation of models that account for the relative contributions of multiple correlated variables to buoyancy potential. Further, the differences between buoyant and sunken fruits do not allow simple prediction of whether a fruit will sink or float. For example, the mean mesocarp thickness of buoyant fruits was significantly larger than that of sinking fruits, although there was also considerable overlap between the mesocarp thickness of the two groups. Easily identifiable features of seeds and fruits that are indicative of hydrochorous dispersal remain to be discovered, as do diagnostic traits that would allow identification of buoyant and non-buoyant seeds.

Intraspecific variation can increase dispersal (Augspurger and Franson 1987; Greene and Johnson 1992; Hroudova *et al.* 1997; Higgins *et al.* 2003), affect patterns and processes at genetic, community and population levels (Darling *et al.* 2008; Nilsson *et al.* 2010) and may have ecological consequences such as risk-spreading, enhancement of population stability and an increase in fitness in unpredictable habitats (Andersen 1992). A combination of variation in both seeds (intraspecific variation) and dispersal vectors (environmental variability) affects dispersal distance (Greene and Johnson 1992) and is likely to result in very important but uncommon, long-distance dispersal events (Nathan 2006; Nathan *et al.* 2008). In the present study, even though intraspecific variation is described along an environmental gradient, it is uncertain whether variation in fruit morphology was driven by genetic or environmental effects or, more likely, by a combination of the two. Environmental variation (Chapter 2) influencing the maternal environment of the developing seed can affect seed germination, dormancy and response to environmental conditions or

stress (Matilla *et al.* 2005; Donohue 2009). Such effects on germination are critical in a dispersal context because germination in new environmental conditions or under stress will be required for establishment of seedlings post-dispersal (Chapter 5). Future research focussed on the effects of intraspecific variation on dispersal potential, and germination or stress tolerance, will provide important insights for dispersal ecology. Intraspecific variation that imparts greater buoyancy and establishment potential may facilitate range shifts and assist coastal species to persist through disturbance or other significant changes to local climate.

References

Andersen MC (1992) An analysis of variability in seed settling velocities of several wind-dispersed Asteraceae. *American Journal of Botany* **79**, 1087-1091.

Augspurger CK, Franson SE (1987) Wind dispersal of artificial fruits varying in mass, area, and morphology. *Ecology* **68**, 27-42.

Bureau of Meteorology (2013) Maps of average conditions. (Commonwealth of Australia) Available at http://www.bom.gov.au/jsp/ncc/climate_averages

Carolin RC (1966) Seeds and fruit of the Goodeniaceae. *Proceedings of The Linnean Society of New South Wales* **91**, 58-83.

Cousens R, Dytham C, Law R (2008) 'Dispersal in plants: a population perspective.' (Oxford University Press: New York).

CSIRO (2013) The atlas of living australia. Available at http://www.ala.org.au

Darling E, Samis KE, Eckert CG (2008) Increased seed dispersal potential towards geographic range limits in a Pacific coast dune plant. *New Phytologist* **178**, 424-435.

Darwin C (1856) On the action of sea-water on the germination of seeds. *Journal of the Proceedings of the Linnean Society of London. Botany* **1**, 130-140.

Darwin C (1859) ' The origin of species.' (Oxford University Press: Oxford).

Dawson MN, Hamner WM (2008) A biophysical perspective on dispersal and the geography of evolution in marine and terrestrial systems. *Journal of the Royal Society Interface* **5**, 135-150.

de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution* **20**, 68-73.

Dixon KW (2011) 'Coastal plants: a guide to the identification and restoration of plants of the Perth region.' (CSIRO Publishing: Melbourne).

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1059-1074.

Greene DF, Johnson EA (1992) Can the variation in samara mass and terminal velocity on an individual plant affect the distribution of dispersal distances? *The American Naturalist* **139**, 825-838.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**, 1175-1186.

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* **84**, 1945-1956.

Howarth DG, Gustafsson MHG, Baum DA, Motley TJ (2003) Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacifc Basin and colonization of the Hawaiian Islands. *American Journal of Botany* **90**, 915-923.

Hroudova Z, Moravcova L, Zakravsky P (1997) Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. *Folia Geobotanica et Phytotaxonomica* **32**, 377-390.

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution* **15**, 56-61.

Hughes L, Westoby M (1992) Effect of diaspore characteristics on removal of seeds adapted for dispersal by ants. *Ecology* **73**, 1300-1312.

Kokubugata G, Nakamura K, Forster PI, Hirayama Y, Yokota M (2012) Antitropical distribution of *Lobelia* species (Campanulaceae) between the Ryukyu Archipelago of Japan and Oceania as indicated by molecular data. *Australian Journal of Botany* **60**, 417-428.

Lesko GL, Walker RB (1969) Effect of sea water on seed germination in two Pacific atoll beach species. *Ecology* **50**, 730-734.

Leyer I, Pross S (2009) Do seed and germination traits determine plant distribution patterns in riparian landscapes? *Basic and Applied Ecology* **10**, 113-121.

Lopez OR (2001) Seed flotation and postflooding germination in tropical *terra firme* and seasonally flooded forest species. *Functional Ecology* **15**, 763-771.

Marchant NG, Wheeler JR, Rye BL, Bennett EM, Lander NS, Macfarlane TD (1987) 'Flora of the Perth region.' (Western Australian Herbarium: Perth).

Matilla A, Gallardo M, Puga-Hermida MI (2005) Structural, physiological and molecular aspects of heterogeneity in seeds: a review. *Seed Science Research* **15**, 63-76.

Nathan R (2006) Long-distance dispersal of plants. *Science* **313**, 786-788.

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. *Trends in Ecology and Evolution* **23**, 638-647.

Nilsson C, Brown RL, Jansson R, Merritt DM (2010) The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews* **85**, 837-858.

Rippey E, Rowland B (2004) 'Coastal plants: Perth and the south-west region.' 2nd edn. (University of Western Australia Press: Perth).

Römermann C, Tackenberg O, Poschlod P (2005) How to predict attachment potential of seeds to sheep and cattle coat from simple morphological seed traits. *Oikos* **110**, 219-230.

Telenius A, Torstensson P (1989) The seed dimorphism of *Spergularia marina* in relation to dispersal by wind and water. *Oecologia* **80**, 206-210.

Thomson FJ, Moles AT, Auld TD, Ramp D, Ren S, Kingsford RT (2010) Chasing the unknown: predicting seed dispersal mechanisms from plant traits. *Journal of Ecology* **98**, 1310-1318.

Travis JMJ, Delgado M, Bocedi G, Baguette M, Bartoń K, Bonte D, Boulangeat I, Hodgson JA, Kubisch A, Penteriani V, Saastamoinen M, Stevens VM, Bullock JM (2013) Dispersal and species' responses to climate change. *Oikos* **122**, 1532-1540.

Turner SR, Commander LE, Baskin JM, Baskin CC, Dixon KW (2009) Germination behaviour of *Astroloma xerophyllum* (Ericaceae), a species with woody indehiscent endocarps. *Botanical Journal of the Linnean Society* **160**, 299-311.

Vargas P, Nogales M, Jaramillo P, Olesen JM, Traveset A, Heleno R (2014) Plant colonization across the Galápagos Islands: success of the sea dispersal syndrome. *Botanical Journal of the Linnean Society* **174**, 349-358.

Western Australian Herbarium (1998-2014) FloraBase—the Western Australian flora. (Department of Parks and Wildlife) Available at http://florabase.dpaw.wa.gov.au/

Will H, Maussner S, Tackenberg O (2007) Experimental studies of diaspore attachment to animal coats: predicting epizoochorous dispersal potential. *Oecologia* **153**, 331-339.

Williamson GB, Costa F, Vera CVM (1999) Dispersal of Amazonian trees: hydrochory in *Swartzia polyphylla*. *Biotropica* **31**, 460-465.

Yang H, Lu Q, Wu B, Zhang J (2012) Seed dispersal of East Asian coastal dune plants via seawater – short and long distance dispersal. *Flora* **207**, 701-706.

Chapter 5

Experimental manipulation of temperature, salinity and osmotic stress to determine the germination thresholds and oceanic dispersal capacity of coastal plant seeds

Abstract

Seed-mediated colonisation into new environments depends not only on the dispersal capacity of plant seeds, but also the ability of seeds to germinate and form reproducing populations under new environmental conditions. Coastal plants may achieve mid- to long-distance dispersal through oceanic transport of seeds, a process that may be significant for the persistence of coastal species. Here, manipulation of temperature, salinity and osmotic stress was used to investigate the post-dispersal germination capacity of seeds collected from multiple populations of two coastal plants in Western Australia. Seeds were most sensitive to salinity (exhibited reduced germination in, or recovery from, saline conditions) at non-optimal temperatures (5 and 20 °C for *Spyridium globulosum*; 10 and 25 °C for *Ficinia nodosa*). However, salt tolerance of seeds of both species varied between populations, suggesting that some populations are likely to have greater recruitment capacity than others in new environments. Furthermore, investigation of the relative reduction in germination caused by water stress and ion toxicity revealed that the impact of these stressors is also modulated by temperature. Collectively, these results indicate that the interaction between temperature, ion toxicity, osmotic stress and within-species variation can affect germination in new, post-dispersal environments. In particular, salt sensitivity may be intensified as species disperse to, or experience, environments of non-optimal temperature. This has implications for the dispersal capacity and germination of seeds in dispersal and climate change contexts.

Introduction

Coastal systems are among the ecosystems most threatened by climate change. Climate change predictions for sandy beaches include changes in temperature and precipitation, raised sea level and shoreline retreat, increases in storm events, and alteration of circulation, upwelling and wave regimes (Harley *et al.* 2006; Schlacher *et al.* 2008). These changes will likely affect physiological tolerance, drive geographical shifts in species' ranges, alter community structure and dynamics, and ultimately result in modified dune vegetation and dune stability with a tendency for landward retraction (Greaver and Sternberg 2007; Schlacher *et al.* 2008). Coastal plants generally display considerable physiological plasticity in response to spatially heterogeneous and temporally fluctuating dune environments (Greaver and Sternberg 2007). For future persistence, coastal plants may need to be resilient to significant changes in what is already a dynamic environment.

Both dispersal and colonisation potential are important considerations when assessing the resilience of species to climate change (Williams *et al.* 2008; Travis *et al.* 2013). For plants, dispersal by water (hydrochory) plays an important ecological role in structuring riparian and wetland vegetation communities, and many species growing in or near fresh water have propagules capable of water dispersal (Nilsson *et al.* 2010). Although less often considered, it has also been demonstrated that many coastal and oceanic island plants have seeds capable of oceanic dispersal (Appendix 1; Stephens 1958; Lesko and Walker 1969; Hroudova *et al.* 1997; Quilichini and Debussche 2000; Guja *et al.* 2010). Oceanic hydrochory is increasingly recognised as a nonstandard, yet influential, dispersal vector (Higgins *et al.* 2003), and is frequently used to explain phylogenetic and biogeographic patterns (Howarth *et al.* 2003; de Queiroz 2005; Kokubugata *et al.* 2012). The potential of coastal plants to disperse via the ocean may be an important component of resilience in the context of climate change.

It is generally accepted that climate change is likely to have direct and indirect impacts on the dispersal of species (Williams *et al.* 2008; Travis *et al.* 2013). However, it is less widely recognised that climate change is also likely to affect dispersal itself by exerting new selection pressures which may lead to evolution of traits related to dispersal (Travis *et al.* 2013). Furthermore, given the predicted

increases in beach erosion and sea level rise (Schlacher *et al.* 2008) it is possible that there will be increased oceanic seed loads and therefore greater incidence of oceanic dispersal. The changes to coastal environments and dispersal vectors will likely have synergistic effects on coastal plant populations. Dispersal, like climate change, often requires organisms to survive in environments that are different to those from which they originated and are acclimated or adapted. Therefore, the interface of dispersal and climate change provides an interesting experimental framework. Hydrochory alone can enable plants to reach distant sites. However, the timing of dispersal and the capacity for germination and growth under new environmental conditions are also essential for successful colonisation (Nilsson *et al.* 2010; Schupp *et al.* 2010; Gillespie *et al.* 2011).

Seeds are a fundamental unit of plant dispersal and colonisation, yet the seed ecology of only 7% of the world's coastal flora has been investigated (Baskin and Baskin 1998). Consequently, the environmental conditions that alleviate seed dormancy, stimulate germination and therefore influence colonisation capacity remain unknown for the majority of coastal species. Likely environmental differences that may be encountered post-dispersal include combinations of altered temperature, salinity, and soil moisture. In coastal environments plants are generally tolerant of high soil salinity and salt spray, particularly during dry seasons (Baskin and Baskin 1998; Greaver and Sternberg 2007). Whereas many coastal and dune plants are known to survive and germinate under relatively high concentrations of salt (Appendix 1; Schat and Scholten 1985; Woodell 1985; Mariko *et al.* 1992; Walmsley and Dowey 1997; Necajeva and Ievinsh 2008; Guja *et al.* 2010), germination under osmotic stress has received relatively little attention (Berger 1985), and there has been limited investigation of the effects of temperature on salt tolerance (Berger 1985; Schat and Scholten 1985; Martinez *et al.* 1992; Naidoo and Naicker 1992). Most studies of salinity have focused on germination of halophytic species from hypersaline lakes and marshes or inland deserts (reviewed by Ungar 1978; Gul *et al.* 2013). These studies suggest that salt tolerance is correlated with soil salinity and that habitats of greater salinity are occupied by species that exhibit the greatest relative salt tolerance during seed germination (Woodell 1985; Mariko *et al.* 1992). Without similar ecological data for coastal plant communities the sensitivity,

capacity for resilience, physiological tolerance, and germination responses of coastal seeds in new environments remain largely unknown.

Successful seed germination in high salinity and/or recovery from salt exposure is critical for oceanic seed dispersal and the subsequent colonisation of new sites. As for whole plants, the survival and germination of seeds under saline conditions is influenced by avoidance or tolerance of sodium chloride (Appendix 2; Guja *et al.* 2013). Plants that are intolerant of salt (glycophytes) or tolerant of salt (halophytes) have various mechanisms of salt avoidance and/or tolerance that facilitate growth in high salinity (Greenway and Munns 1980; Munns 2002). At the level of the whole plant, these mechanisms can include excretion, osmotic adjustment using organic solutes, and compartmentalisation of sodium ions (Zhang *et al.* 2012). Less is known about the mechanism of salt tolerance of seeds, but the ability of seeds to avoid germination until external salinity is reduced (Ungar 1978; Woodell 1985; Hanslin and Eggen 2005), or to successfully germinate under saline conditions, appear to be common traits of halophytic species (Keiffer and Ungar 1997; Katembe *et al.* 1998; Tobe *et al.* 2000). Although both halophytes and glycophytes germinate best in nonsaline conditions, it is the ability of halophytes to survive and germinate in moderate to high salinity (albeit at reduced levels), and germinate fully when salinity is reduced, that characterises halophyte germination (Gul *et al.* 2013).

Many coastal plants are halophytes and many have seeds that are known to survive or germinate in saline conditions (Appendix 1; Lesko and Walker 1969; Harty and McDonald 1972; Berger 1985; Schat and Scholten 1985; Woodell 1985; Mariko *et al.* 1992; Martinez *et al.* 1992; Naidoo and Naicker 1992; Walmsley and Dowey 1997; Hanslin and Eggen 2005; Necajeva and Ievinsh 2008; Guja *et al.* 2010). The capacity of seeds to germinate under saline conditions is influenced by temperature (Ungar 1978; Baskin and Baskin 1998) and therefore germination in saline conditions may vary between seasons, in different locations, or under climate change. Although high salinity inhibits germination, the detrimental effects of salinity are greatest when seeds germinate in sub- and/or super-optimal temperatures (Ungar 1978; Gulzar *et al.* 2001; Khan and Gulzar 2003; El-Keblawy and Al-Rawai 2005; Qu *et al.* 2008; Tlig *et al.* 2008; Zhang *et al.* 2012). Therefore, it appears that

Table 1. Collection information for each species. The localities and coordinates where seed was collected including environmental data (mean annual maximum temperature and mean annual rainfall of the nearest weather station (Bureau of Meteorology 2013)). Initial seed fill (%) was determined from X-ray of a sub-sample of 100 seeds. X-ray was used to identify and remove empty seeds from all collections prior to the experiment.

Species	Population	Locality in Western Australia	Coordinates (WGS84)	Mean maximum (°C)	Mean rainfall (mm)	Herbarium voucher	Seed collection date	Initial seed fill (%)[a]
Ficinia nodosa	North	Coronation Beach Drive, Howatharra	28° 32' S 114° 33' E	25.9	443.2	LKG021	20/01/2009	95
	Central	Peasholm Street, Scarborough	31° 54' S 115° 45' E	24.0	725.3	LKG101	26/02/2009	98
	South	Skippy Rock Road, Leeuwin	34° 21' S 115° 07' E	19.7	967.6	LKG087	22/03/2009	79
Spyridium globulosum	North	Dongara Airport, Port Denison	29° 17' S 114° 55' E	24.8	535.9	LKG026	21/01/2009	77
	Central	Peasholm Street, Scarborough	31° 54' S 115° 45' E	24.0	725.3	LKG111	21/11/2008	90
	South	Injidup Spring Road, Yallingup	33° 41' S 114° 59' E	20.7	807.5	na	22/03/2009	82

[a] After assessing initial seed fill, X-ray analysis was then used to identify empty seeds in each collection and they were removed. i.e. all experiments were conducted with only filled (likely viable) seeds.

non-optimal temperatures not only reduce germination, but also salt tolerance thresholds (Ungar 1978). Despite the implications of these interactions for oceanic dispersal and germination in new environments, the relationship between salinity and temperature is rarely considered in an ecological context.

Elevated soil salinity can influence seed germination by reducing soil water potential and/or causing ionic imbalances (Munns 2002). Decreased soil water potential results in osmotic stress that limits water and nutrient uptake, and ionic imbalances (e.g. sodium) result in toxicity that can further decrease seed germination or plant growth (Munns 2002). Because osmotic stress may cause the same physiological responses as salinity, careful experimental design is required to distinguish tolerance of salt from tolerance of water stress (Munns 2002). For example, comparison of germination in saline conditions with germination in isosmotic polyethylene glycol (PEG) solutions can distinguish the toxic and osmotic effects of salinity (Berger 1985; Katembe *et al.* 1998; Tobe *et al.* 2000). Such investigations can reveal thresholds of seed tolerance to salinity and osmotic stress. Species-specific variation in these thresholds may be important determinants of dispersal and recruitment ability in new environments and in a changing climate.

Whereas interspecific differences in salinity tolerance are well documented, intraspecific differences are less often considered. Within-species variation that can affect germination can arise from genetic variation, the location of maturing fruits in the infructescence, or the maternal environment during fruit development and maturation (Donohue 2009; Matilla et al. 2005). For example, variation in salinity tolerance occurs between populations of coastal halophytes (Woodell 1985), desert shrubs (Bazzaz 1973), and salt-tolerant grass cultivars (Hanslin and Eggen 2005).

Table 2. Significance of model terms for analysis of germination of each species in salt, or after recovery from salt exposure, using binomial generalised linear models (GLM). Two models were created, one for each time (i.e. salt = day 56, recovery = day 84). Statistical significance was determined using chi-squared tests. First order terms are not present because all were present in significant second order interactions; ns = not significant at $P < 0.05$.

	Ficinia nodosa		***Spyridium globulosum***	
	NaCl	**Recovery**	**NaCl**	**Recovery**
Temperature: Salinity	0.0005	<0.0001	0.0482	<0.0001
Temperature: Population	<0.0001	<0.0001	<0.0001	<0.0001
Population: Salinity	0.0026	<0.0001	ns	<0.0001
Temperature: Population: Salinity	ns	ns	ns	ns

In the context of oceanic dispersal, and particularly under the influence of changing climate, these differences between populations may render some populations more capable of successful dispersal and therefore more resilient to change.

Studies of seed germination can provide novel insights into the adaptation of early life history traits to specific environments (Kim and Donohue 2013). In this study, experiments were conducted to investigate how seed germination thresholds may determine seedling recruitment of coastal species following seed dispersal to new environments. To determine the effects of temperature, osmotic stress and source population on seed germination, and interactions between these factors, a three-way factorial experiment was conducted. *Ficinia nodosa* (Rottb.) Goetgh., Muasya & D.A.Simpson (Cyperaceae) and *Spryidium globulosum* (Labill.) Benth. (Rhamnaceae) were selected for study. Both species are common and widely distributed through the coastal dunes of southwest Australia and produce buoyant seeds capable of surviving exposure to seawater (Appendix 1; Guja *et al.* 2010). Specifically, three hypotheses were tested: 1) an interaction between salinity and temperature will influence germination, 2) salt tolerance will vary between seeds collected from different plant populations along a climatic gradient, 3) due to ion toxicity, exposure to salt will have a greater detrimental effect on seed germination compared with equivalent levels of osmotic stress.

Methods

Seed collection and processing

Seeds of two coastal species, *Ficinia nodosa* and *Spyridium globulosum*, were collected from three natural plant populations (located ≥ 250 km apart) in Holocene sand-dune communities in Western Australia. Populations were selected to represent northern, central and southern localities within the distribution of each species along a latitudinal gradient (Chapter 2). Seeds were collected between November 2008 and March 2009 at the time of natural of dispersal. From each population, seeds were collected from ≥ 10 plants and pooled. Locality and collection information are summarised in **Table 1**. Within 2 days of collection, seeds were placed into dry storage in a controlled environment room at 15 ºC and 15% relative humidity. After

at least 5 months of dry storage (on 19/08/09), seeds were hermetically sealed in laminated aluminium foil bags and stored at -18 ºC until they were used in experiments between February and June 2011. Voucher specimens were lodged at the Kings Park and Botanic Garden Herbarium (KPBG) (**Table 1**). The seed fill (%) of a sub-sample of 100 seeds from each population was assessed via digital X-ray imaging (Faxitron MX-20, Faxitron X-ray, Lincolnshire, IL, USA) (**Table 1**). X-ray analysis was used to remove empty seeds from each collection so that all experiments were conducted with only filled (likely viable) seeds.

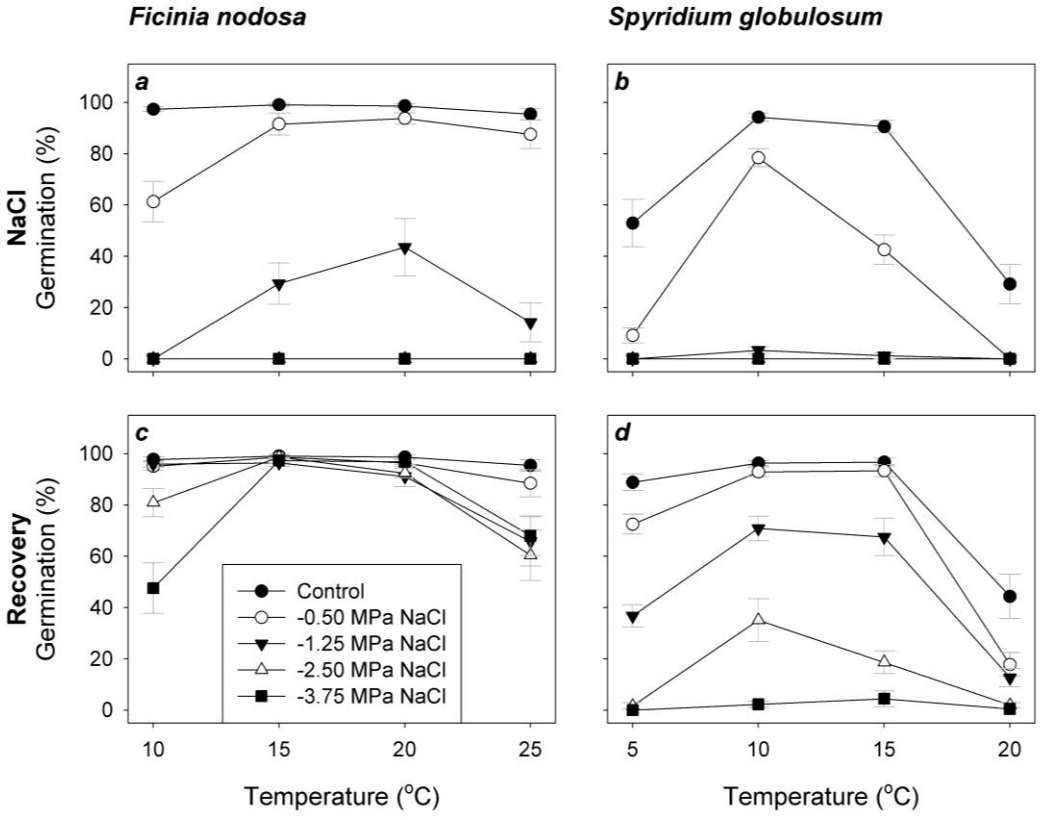


Figure 1. Mean germination (% ± standard error; n = 9) at day 56 of (**a**) *Ficinia nodosa* and (**b**) *Spyridium globulosum* seeds in saline (NaCl) solutions at various temperatures. Means are the aggregate of three populations of each species. Final recovery (mean germination at day 84, after transfer from saline solutions to deionised water) of (**c**) *F. nodosa* and (**d**) *S. globulosum* at each temperature.

Seed germination of different populations under manipulated temperature, salinity and osmotic stress

A three-way factorial design was used to assess the effect of temperature, osmoticum and source population on germination of two species. A preliminary germination experiment determined that *F. nodosa* seeds germinated at constant temperatures of 10 - 25 °C (no germination at 5 °C after 45 days), and that *S. globulosum* seeds germinated at temperatures of 5 - 20 °C (no germination at 25 °C after 84 days). Germination was assessed in isosmotic solutions of sodium chloride (NaCl) and polyethylene glycol 8000 (PEG) to investigate whether reduction in seed germination in saline solutions was due to toxic or osmotic effects of NaCl. Constant temperatures were required to ensure that the osmotic potentials of the two solutions remained equal and comparable during the experiment. The osmotic potentials used in experiments were 0, -0.50, -1.25, -2.50, and -3.75 MPa. These osmotic potentials are approximately equal to 0, 100, 250, 500 and 750 mM NaCl at 25 °C; or, 0, 0.2, 0.5, 1 and 1.5 times the NaCl concentration of seawater, respectively. NaCl was used in experiments because it is the salt that is most abundant in seawater and coastal environments. Because osmotic potential changes with temperature, 20 solutions each of NaCl and PEG were prepared to achieve the desired osmotic potential at each experimental temperature. The concentration of NaCl required to achieve each osmotic potential, at each temperature, was calculated using the formula

$$\text{Osmotic potential (MPa)} = -\,MiRT$$

where M = molarity of the solute, i = osmotic coefficient (the ratio of particles in solution to formula units dissolved), R = ideal gas constant, and T = absolute temperature (°K). Solutions of PEG were prepared following Michel (1983).

Seeds of *S. globulosum* are physically dormant at maturity and were treated prior to germination experiments with hot water at 95 ºC for 2 min to rupture the water-gap (Turner *et al.* 2005). Seeds of *F. nodosa* are non-dormant at maturity and did not require pre-treatment (Appendix 1; Guja *et al.* 2010). To avoid contamination of dishes, including from fungal endophytes that may confound salt- or water- stress tolerance (Rodriguez *et al.* 2009), each seed collection was treated with a standard sterilising regime of 30 min (alternating 10 min intervals under vacuum) in 2%

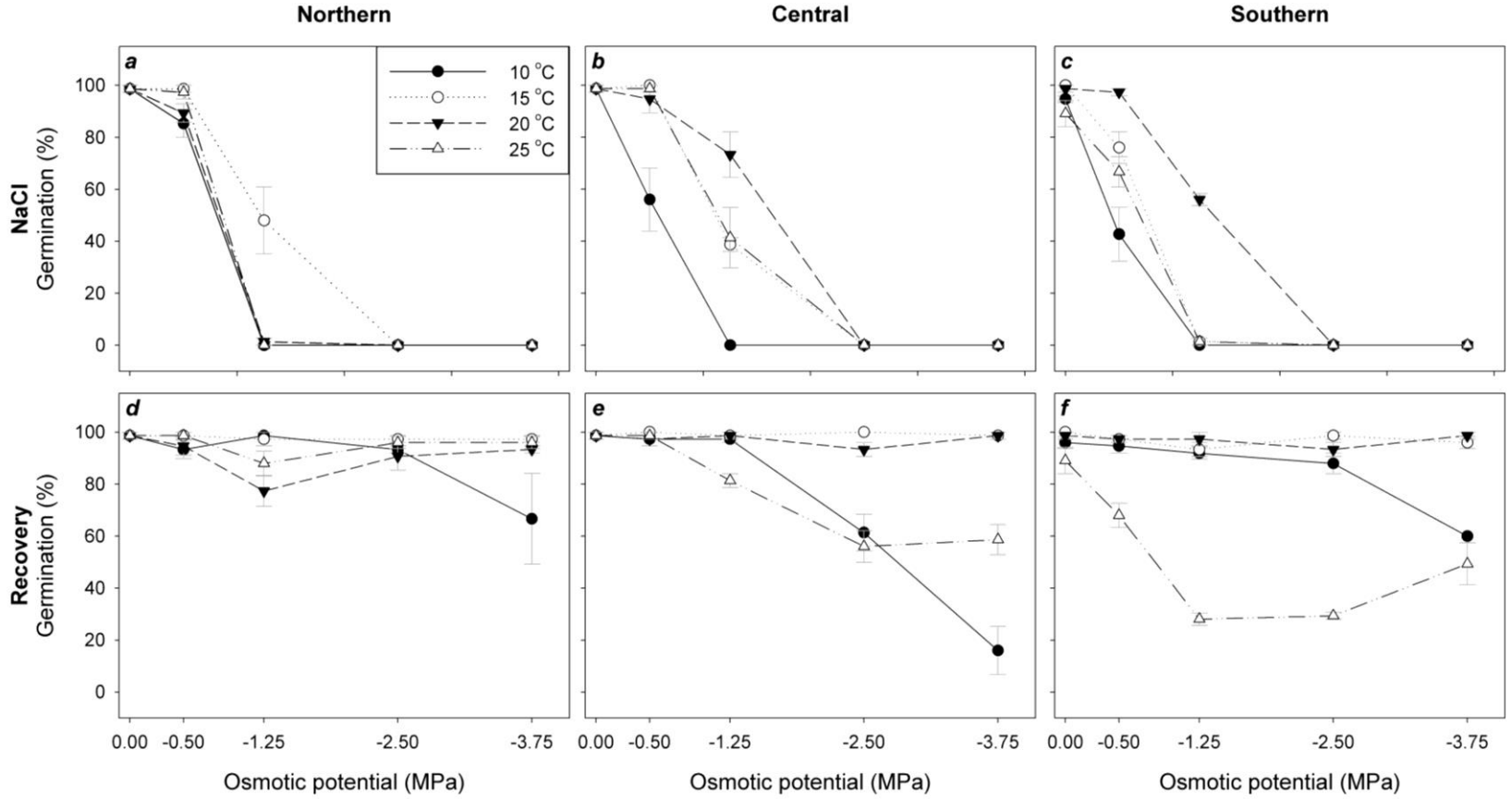


Figure 2. Effects of temperature and salinity on mean germination (% ± standard error; n = 3) of *Ficinia nodosa* seeds from three populations. (**a**-**c**) Germination in saline (NaCl) solutions (0, -0.50, -1.25, -2.50, -3.75 MPa) at day 56. (**d**-**f**) Final recovery (mean germination at day 84, after transfer from saline solutions to deionised water).

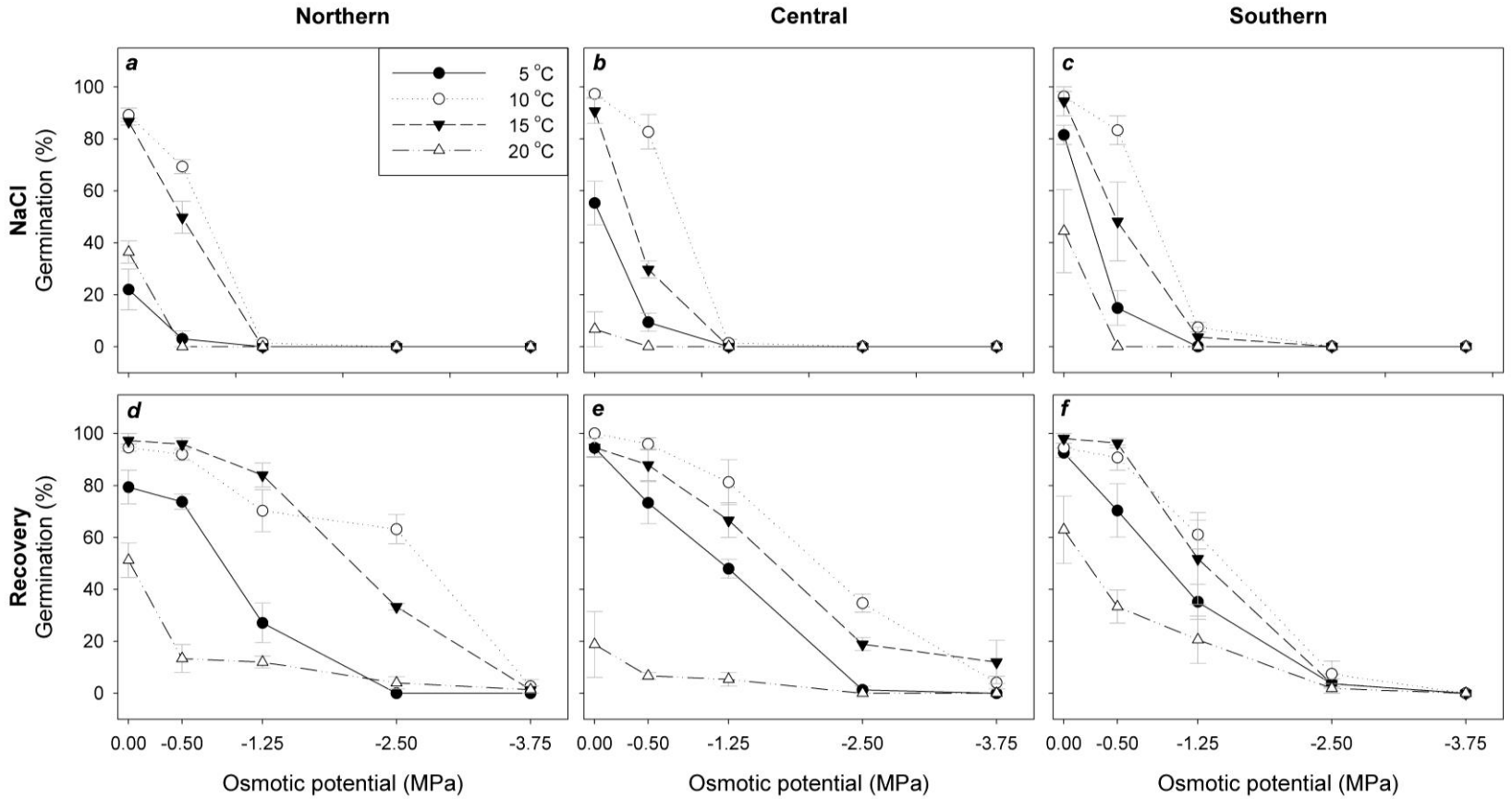


Figure 3. Effects of temperature and salinity on mean germination (% ± standard error; n = 3) of *Spyridium globulosum* seeds from three populations. (**a**-**c**) Germination in saline (NaCl) solutions (0, -0.50, -1.25, -2.50, -3.75 MPa) at day 56. (**d**-**f**) Final recovery (mean germination at day 84, after transfer from saline solutions to deionised water).

calcium hypochlorite solution. Seeds were then rinsed three times for 2 min in sterile deionised water immediately before placement in Petri dishes.

The factorial experiment was replicated three times with each replicate established on separate days. For each replicate of each treatment combination 25 seeds were used, except for the southern population of *S. globulosum* where replicates contained 18 seeds. Seeds were placed in 90 mm plastic Petri dishes lined with two glass fibre filter papers (1.1 µm , grade 483, 82 mm), irrigated with 7 ml of solution and sealed in plastic wrap to prevent evaporation. Dishes were incubated under a 12:12 h light:dark photoperiod (15.4 µmol m^{-2} s^{-1}, 400–700 nm). Germination (extension of the radicle ≥ 2 mm) was scored every 2-3 days for 8 weeks (56 days).

Germination recovery after exposure to treatments

To assess recovery of germination after exposure to NaCl or PEG, all non-germinated seeds were transferred from osmotic solutions to water after 56 days. Seeds from each replicate were placed in 50 mL deionised water for 15 mins, followed by 1 min on an orbital shaker, and a further 15 mins standing in deionised water before decanting. Seeds were then placed in new Petri dishes lined with two filter papers and irrigated with 7 mL deionised water, sealed, and incubated as described above. Germination was monitored every 2-3 days for a further 4 weeks (28 days). In total, experiments were terminated after 12 weeks (84 days).

Statistical analysis

At the conclusion of the experiment any *S. globulosum* seeds that had not imbibed (i.e. physical dormancy had not been broken by the hot water treatment) were subtracted from the sample before calculating germination proportions. Data presented in **Figures 1** and **4** aggregate the three populations and show mean ± standard error (n = 9). **Figures 2** and **3** and **Supplementary Figures 1** and **2** show data for each population (mean ± standard error; n = 3).

To address hypotheses 1 and 2 the germination (proportion) of seeds of each species in NaCl (day 56) and recovery in water (day 84) were analysed using binomial generalised linear models (GLMs) with a logit link function in R (version 3.0.2;

R Development Core Team). Factors considered in the models were: temperature (4 levels); NaCl salinity (5 levels); and population (3 levels). The full model included main effects, second-order, and third-order interactions. Stepwise elimination was used to simplify full models. Models were compared using Chi-squared tests to determine whether the reduction in explained deviance with each term removed was statistically significant at $P < 0.05$. When terms were statistically significant they were retained and the p-values reported.

Models were also created to address hypothesis 3 and compare, at the species level (aggregated data for the three populations), the overall effects of isosmotic NaCl or PEG on germination (proportions) at each temperature. Germination in NaCl or PEG (day 56) and recovery (day 84) were analysed using binomial GLMs with a logit link function. Factors considered in the models for each species at each temperature were concentration (5 levels) and osmoticum (NaCl or PEG). The full model included main effects and interactions and terms were considered significant when $P < 0.001$ to account for multiple comparisons.

Results

Germination response to temperature and salinity

For both species there was a significant interaction (*F. nodosa*, $P = 0.0005$; *S. globulosum*, $P = 0.0482$) between the effects of temperature and salinity on seed germination in NaCl (day 56) (**Table 2**). Across all populations, both species exhibited reduced germination percentages as salinity increased. These reductions in germination were greatest at high and low temperatures (**Figure 1a** and **1b**). Germination of *F. nodosa* in -0.50 MPa NaCl was 5% lower than germination of controls in deionised water at 20 °C, 7% lower at 15 °C and 25 °C, and 36% lower at 10 °C (**Figure 1a**). Germination in -1.25 MPa NaCl was further reduced compared with germination in deionised water and was 70% lower than water at 15 °C, 55% lower at 20 °C, 81% lower at 25 °C, and no germination occurred at 10 °C (**Figure 1a**).

For *S. globulosum*, germination in -0.50 MPa NaCl was 16% lower than in water at 10 °C, 48% lower at 15 °C, 44% lower at 5 °C, and there was no germination at 20 °C (**Figure 1b**). In -1.25 MPa NaCl, germination did not occur at 5 °C and 20 °C, and germination was only 3% and 1% in 10 °C and 15 °C, respectively (**Figure 1b**). In higher salinities (-2.50 and -3.75 MPa NaCl), seeds of neither species germinated (**Figure 1a** and **1b**).

Germination recovery

Recovery (final germination at day 84) was affected by an interaction between temperature and salinity (*F. nodosa* $P < 0.0001$, *S. globulosum* $P < 0.0001$) (**Table 2**). For *F. nodosa*, at 15 °C, all seeds recovered and germinated to a similar final percentage as the control seeds in water (all ≥ 96%) (**Figure 1c**). At 20 °C, final recovery was also high for seeds that had been exposed to all salinities (germination ≥ 91% after transfer to deionised water) (**Figure 1c**). At 10 °C, recovery from -0.50 and -1.25 MPa NaCl was equivalent to germination in water (≥ 95%), recovery from -2.50 MPa was 18% lower than germination in water, and seeds exposed to -3.75 MPa NaCl had the greatest decline with final germination 51% lower than seeds in water (**Figure 1c**). At 25 °C germination of seeds exposed to -0.50 MPa NaCl was 7% less than germination of seeds in water, whereas, seeds exposed to all higher salinities had final germination about 30% lower than seeds in water (**Figure 1c**).

For *S. globulosum*, recovery from -0.50 MPa NaCl was only slightly lower than germination of seeds in water at 10 °C and 15 °C (**Figure 1d**). Seeds exposed to -1.25 MPa NaCl germinated 25% less than controls at 10 °C, 29% less at 15 °C, 31% less at 20 °C and 52% less at 5 °C (**Figure 1d**). Seeds exposed to -2.50 MPa NaCl germinated 61% less than controls at 10 °C, 78% less at 15 °C, and seeds showed very little recovery from exposure to -2.50 MPa NaCl at 5 °C and 20 °C (final germination after transfer to deionised water < 2%) (**Figure 1d**). No recovery of *S. globulosum* germination was observed in seeds exposed to -3.75 MPa NaCl at 5 °C, and there was little recovery from exposure to this high salinity at the remaining temperatures (all germination < 5%). For both species recovery from high salinities was lowest at sub- and super-optimal temperatures (**Figure 1**).

Table 3. Significance of model (GLM) terms for germination of *Ficinia nodosa* in isosmotic NaCl and PEG at each tested temperature. Separate models were used for each time (exposure to osmoticum = day 56, recovery from exposure = day 84); ns = not significant at P < 0.001.

Ficinia nodosa	**10 °C**		**15 °C**		**20 °C**		**25 °C**	
	Exposure	**Recovery**	**Exposure**	**Recovery**	**Exposure**	**Recovery**	**Exposure**	**Recovery**
Osmoticum (NaCl or PEG)	<0.0001	<0.0001	<0.0001	ns	<0.0001	ns	<0.0001	<0.0001
Concentration	<0.0001	<0.0001	<0.0001	ns	<0.0001	ns	<0.0001	<0.0001
Osmoticum: Concentration	ns	<0.0001	<0.0001	ns	<0.0001	ns	<0.0001	<0.0001

Table 4. Significance of model (GLM) terms for germination of *Spyridium globulosum* in isosmotic NaCl and PEG at each tested temperature. Separate models were used for each time (exposure to osmoticum = day 56, recovery from exposure = day 84); ns = not significant at P < 0.001; na = not applicable because no germination occurred.

Spyridium globulosum	**5 °C**		**10 °C**		**15 °C**		**20 °C**	
	Exposure	**Recovery**	**Exposure**	**Recovery**	**Exposure**	**Recovery**	**Exposure**	**Recovery**
Osmoticum (NaCl or PEG)	ns	ns	ns	<0.0001	ns	<0.0001	na	ns
Concentration	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	na	<0.0001
Osmoticum: Concentration	ns	ns	ns	<0.0001	ns	<0.0001	na	ns

Germination of seeds from different populations

Salt tolerance thresholds (germination at day 56) varied between populations of both species (**Figures 2** and **3**). There were interactions between population and temperature, and population and the level of salinity, on the germination of *F. nodosa* seeds (both $P < 0.05$) (**Table 2**). At -0.50 MPa NaCl germination of seeds from the northern population (**Figure 2a**) was high (> 85%) at all temperatures, whereas seeds from the central population (**Figure 2b**) had lower germination at 10 °C (56%), and seeds from the southern population (**Figure 2c**) had lower germination at 10 °C, 15 °C and 25 °C (43%, 76% and 67%, respectively). At -1.25 MPa NaCl, germination (> 1%) was only observed at 15 °C in the northern population (48%) (**Figure 2a**), and only at 20 °C in the southern population (56%) (**Figure 2c**). In contrast, germination of seeds from the central population at -1.25 MPa NaCl occurred at most temperatures and at 15 °C, 20 °C, and 25 °C was 39%, 73%, and 41%, respectively. For all populations of *F. nodosa* germination did not occur at ≥ - 2.5 MPa NaCl.

For *S. globulosum* seeds in NaCl there was a significant interaction between temperature and population ($P < 0.0001$) (**Table 2**). For *S. globulosum* at day 56 germination in water (0 MPa NaCl) differed between populations at sub- and super-optimal temperatures with the highest germination occurring in seeds from the southern population (**Figure 3**). Seeds from all populations exhibited only low germination (< 7%) in -1.25 MPa NaCl and no germination occurred in ≥ -2.50 MPa NaCl.

Recovery of seeds from different populations

The recovery of germination (final germination percentage at day 84) differed significantly between populations of both species (**Figures 2** and **3**). Recovery of *F. nodosa* seeds was affected by interactions between population and temperature, and population and salinity (both $P < 0.05$) (**Table 2**). The recovery of northern *F. nodosa* seeds (**Figure 2d**) from exposure to all concentrations of NaCl was high at all temperatures (germination of > 88%), except for seeds exposed to -3.75 MPa NaCl at 10 °C (67%) and seeds exposed to -1.25 MPa NaCl at 20 °C (77%). Recovery of the central population (**Figure 2e**) from moderate to high salinities

(-2.50 and -3.75 MPa NaCl) was significantly reduced at 10 °C (61% and 16% final germination, respectively), and 25 °C (56% and 59% final germination, respectively). The southern population (**Figure 2f**) also recovered poorly from exposure to -3.75 MPa NaCl at 10 °C (60% germination), and recovery was lowest in all salt solutions at 25 °C (between 28 and 68% final germination).

Recovery of *S. globulosum* seeds was also affected by interactions between population and temperature, and population and salinity (both $P < 0.05$) (**Table 2**). Seeds exposed to low salinity (-0.5 MPa) generally recovered well at 10 and 15 °C, recovered less at 5 °C, and germinated least at 20 °C. Across all temperatures seeds from the southern population recovered the most from exposure to -0.5 MPa NaCl. For seeds exposed to moderate salinities recovery varied between populations. At -1.25 and -2.50 MPa NaCl the northern population had the greatest sensitivity to temperature (i.e. the range of germination (%) was large), the central population had a moderate sensitivity to temperature, and the southern population was least sensitive to temperature (i.e. more similar germination (%) at all temperatures). At -2.50 MPa NaCl all populations did not recover (all < 4%) at 5 °C and 20 °C. However, after exposure to -2.50 MPa NaCl at 10 °C and 15 °C, germination recovery of the northern population (**Figure 3d**) (63% and 33%, respectively) was greater than the central population (**Figure 3e**) (35% and 19%, respectively), while the southern population (**Figure 3f**) had the lowest recovery (7% and 4%, respectively).

Germination in isosmotic solutions of NaCl and PEG

For seeds of *F. nodosa*, germination was generally greater in NaCl than PEG (**Table 3** and **Figure 4**). This was particularly evident at -0.50 MPa at 10 °C, and -1.25 MPa at 15 °C, 20 °C, and 25 °C. For *S. globulosum*, germination in -0.50 MPa NaCl and PEG was equivalent at 5 °C, 10 °C and 15 °C, and no germination was observed at 20 °C (**Table 4** and **Figure 4**). At -1.25 MPa at 10 °C and 15 °C very low germination was observed in NaCl and no germination occurred in PEG (**Figure 4d** and **4f**).

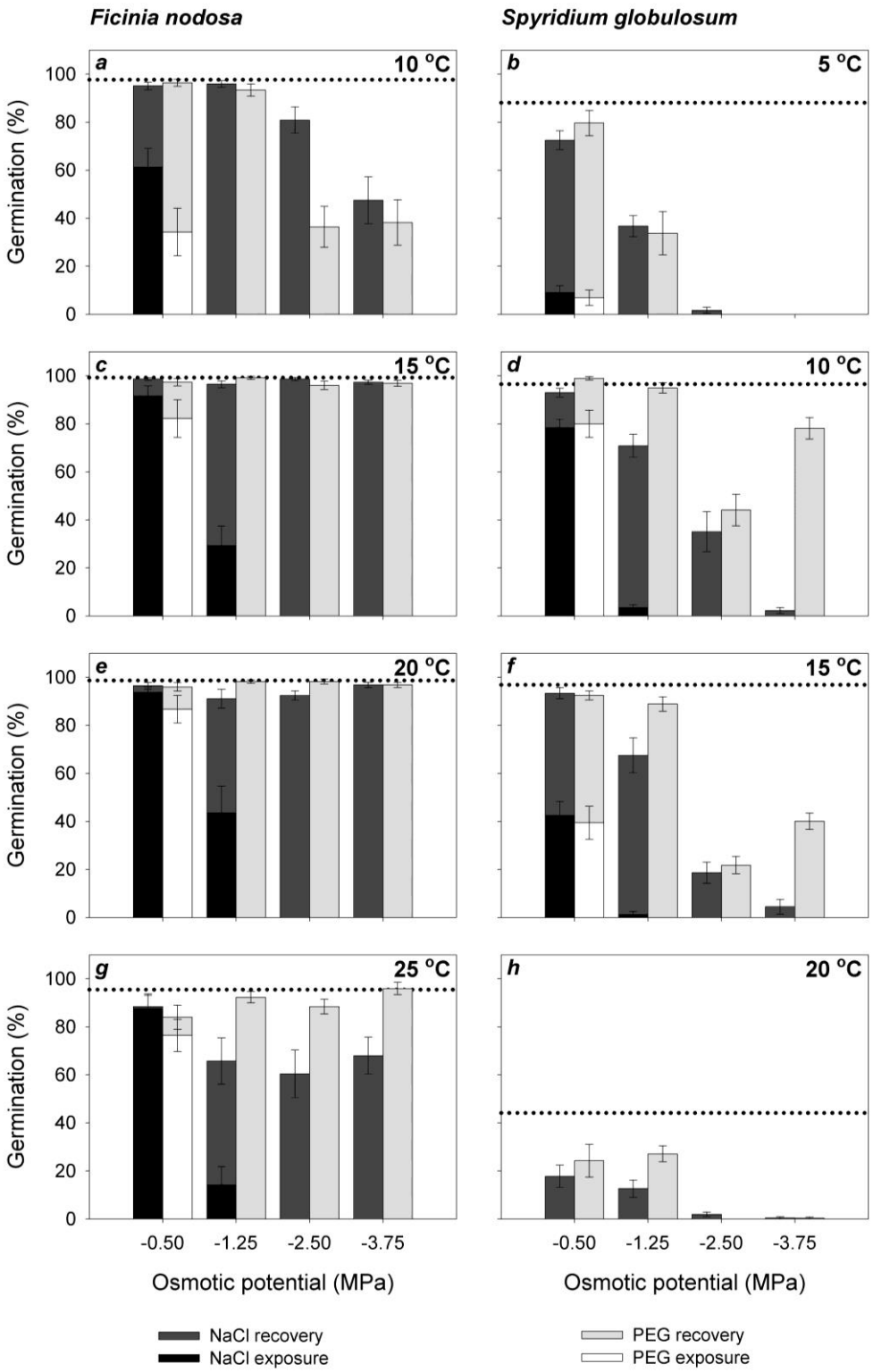


Figure 4. Mean germination at day 56 and recovery at day 84 (% ± standard error; n = 9) of *Ficinia nodosa* (left) and *Spyridium globulosum* (right). Seeds were incubated in isosmotic sodium chloride (NaCl) and polyethylene glycol (PEG) solutions at various temperatures for 56 days. Means are the aggregate of three populations of each species. To measure recovery from exposure to NaCl (salinity) and PEG (water stress) seeds were transferred to deionised water and germination at day 84 (recovery) was recorded. The dotted line represents final germination of seeds after 84 days in deionised water.

Recovery from isosmotic solutions of NaCl and PEG

For seeds of *F. nodosa*, despite some differences in germination during exposure to osmotic solutions, seeds at 15 °C and 20 °C recovered well from all osmotic potentials (all ≥ 91% germination) (**Figure 4c** and **4e**). Recovery from the two osmotic solutions was most disparate at non-optimal temperatures. At 10 °C recovery from NaCl was generally greater than PEG ($P < 0.0001$), particularly at an osmotic potential equivalent to seawater (-2.50 MPa) (**Figure 4a**). In contrast, at 25 °C recovery from PEG was greater than recovery from NaCl ($P < 0.0001$) when MPa was ≤ -1.25 (**Figure 4g**). For seeds of *S. globulosum*, recovery from PEG was always equal to (5 °C and 20 °C, $P > 0.05$) or greater than (10 °C and 15 °C, $P < 0.0001$) recovery from NaCl (**Table 4**). Recovery from high osmotic potentials (-3.75 MPa NaCl and PEG) did not occur (all < 1%) at 5 °C and 20 °C **(Figure 4b** and **4h**). At 10 °C and 15 °C recovery from PEG was significantly greater than NaCl (**Figure 4d** and **4f**). Some minor differences in stress tolerance thresholds (germination in, and recovery from, NaCl and PEG) were observed between populations but were not analysed (**Supplementary Figures 1** and **2**).

Discussion

Germination response to temperature and salinity

This study revealed that temperature and salinity interact to influence the germination of two coastal plant species. This interaction affected the sensitivity of seeds (germination thresholds) during exposure to salinity, and the proportion of seeds that recovered from salt exposure (final germination percentages at the end of the recovery phase). Therefore, the salinity tolerance threshold of a species is not constant, and can be significantly altered by environmental conditions such as temperature (Gulzar *et al.* 2001; Khan and Gulzar 2003; El-Keblawy and Al-Rawai 2005; Qu *et al.* 2008; Tlig *et al.* 2008; Zhang *et al.* 2012). Generally, salt-sensitive seeds are unable to recover from salt exposure during germination because of the toxic effects of sodium uptake (Chapter 6; Appendix 1; Appendix 2; Rehman *et al.* 1998; Guja *et al.* 2010; Guja *et al.* 2013). Sodium uptake may differ with

temperature and it has been suggested that more ions may enter seeds at non optimal temperatures (Ungar 1978). The effects of salinity on seed germination are generally investigated at the species level to compare the tolerance of different species (for example Woodell 1985; Hardegree and Emmerich 1990; Necajeva and Ievinsh 2008; Elsey-Quirk *et al.* 2009). However, a significant and novel result of this investigation was that even seed from a single population exhibited different salt responses under different experimental conditions. For example, the southern population of *F. nodosa* was salt-tolerant at 15 °C and 20 °C, but salt-sensitive at 25 °C. This is the first example of seed from a single population exhibiting different types of salt response and highlights the significant effects of temperature and salinity interactions on seed germination.

The reduction in salt tolerance (either germination in salt or recovery from salt) at non-optimal temperatures (i.e. the temperatures at which germination is significantly reduced in water) suggests that as the difference between environments increases, fewer seeds will germinate following dispersal. If seeds disperse very long distances they are more likely to encounter new conditions, potentially necessitating germination in non-optimal temperatures - conditions where the seeds are more vulnerable to salinity. Indeed, in Western Australia the northern edges of the distributions of both study species (*F. nododsa* and *S. globulosum*) extend only to the northern limits of the temperate south-west, and not further north into the warmer sub-tropical climate (Chapter 2). Perhaps the greater sensitivity of seeds to salinity at higher temperatures has prevented these species from extending further north along the coast. It is conceivable that under particular environmental conditions the germination of a salt-tolerant species in non-optimal temperatures may be more sensitive to salinity than a salt-sensitive species germinating in optimal temperatures. This study indicates that new environmental conditions are likely to have a significant effect on the germination success of seeds that have been dispersed long distances by the ocean.

Germination only occurs within a germination niche that is the product of interactions between environmental factors. In obligate seeding species, the germination niche is crucial for colonisation, population persistence, distribution and abundance patterns and is directly regulated by environment (Cochrane *et al.* 2011),

particularly temperature and moisture (Baskin and Baskin 1998). One key determinant of the breadth of the post-dispersal establishment niche for coastal species is likely to be salt tolerance. Species that germinate well across a range of temperatures and salinities are likely to have a greater probability of establishment in a wider range of new post-dispersal environments than species with a narrow germination niche. In this study *F. nodosa* had a broader germination niche than *S. globulosum* (*F. nodosa*, broad temperature window and high overall salinity tolerance; *S. globulosum*, narrower temperature window and higher sensitivity to salinity and water stress) and may therefore be more capable of successful oceanic dispersal.

In new environments it is likely that temporal factors also act as filters to recruitment. New environments may cause changes in phenology such as timing of germination (Kimball *et al.* 2010), and this is particularly significant for germination and survival in saline environments (Ungar 1978; Gul *et al.* 2013). The timing of halophyte germination, as for most seeds, generally coincides with periods of optimal temperature, moisture and light in the habitat of the species (Ungar 1978; Baskin and Baskin 1998; Gul *et al.* 2013). However, in new environments there may be a disconnect between the optimal temperature for germination and the time when salinity is lowest. For example, seeds adapted to germinate in cool, wet winters (e.g. Mediterranean climate) may be dispersed to a climate characterised by summer rainfall (e.g. sub-tropical climate). If germination in this new environment occurs in summer during the rainfall period when salinity is lowest, the temperature may be at the upper limits for germination and only some seeds would be capable of germination. Conversely, if germination occurs in winter when temperature is optimal, salinity may be high and there may be little rainfall, which would also reduce germination, recruitment and survival. Therefore, it is not only the combined effects of temperature and salinity on germination, but also the changed germination phenology and decreased germination rate observed under non-optimal conditions (**Supplementary Figures 1** and **2**) that are likely to determine germination and survival in new environments. Physical dormancy may also regulate germination timing and therefore the capacity of *S. globulosum* seeds to avoid salinity, however, it was alleviated prior to these germination experiments.

Germination of seeds from different populations

Although the physical environment can limit germination, the strength of environmental filters can be modified by phenotypic plasticity (Gillespie *et al.* 2011). High phenotypic plasticity may allow some seed recruitment in sub-optimal habitats or environments, giving populations the opportunity to adapt under selection (Gillespie *et al.* 2011). Mature coastal plants generally have flexible physiological responses (Greaver and Sternberg 2007) to temperature, salinity and osmotic stress; the present study has shown that flexibility in physiological responses are also evident in germinating seeds. The differences between the germination and salinity tolerance of populations of the study species suggest that seeds from some populations may have a greater probability of dispersing successfully i.e. germinating and persisting in new environments. A combination of broad germination niche and variation among populations contributes to overall species plasticity suggesting that successful oceanic dispersal may be possible, and could facilitate range tracking under projected climate change.

Differences in the germination behaviour of seeds from different populations could be due to several genetic factors. For example, germination under salinity and water stress may be affected by population genetic parameters including heterozygosity or total diversity (Kochankova and Mandak 2009) or cytological differences between populations (Jiang *et al.* 2013). As well as these genetic effects, the maternal environment can also cause variation in the germination behaviour of seeds (Donohue 2009). Given that the populations sampled in the present study span an environmental temperature and rainfall gradient we might expect to see gradients in seed salt-tolerance. Indeed, at the super-optimal temperature of 25 °C the seeds of *F. nodosa* from the northern (drier and warmer) population were salt-tolerant and recovered well from exposure to most salinities, while seeds from the central population showed salt injury and the southern (cooler) population was salt-sensitive at this high temperature (**Figure 2** and **Supplementary Figure 1**). Further investigations using common garden or reciprocal transplant experiments (Kim and Donohue 2013) are required to reveal adaptations and elucidate the relative influence of genetic and environmental determinants of salinity tolerance.

Germination in isosmotic solutions of NaCl and PEG

Only during oceanic dispersal, or deposition close to the high tide line, are seeds of these coastal dune species likely to encounter salinities and water potentials as extreme as those examined here (Woodell 1985). The capacity of seeds to recover from salt exposure during laboratory experiments reflects the capacity for post-dispersal germination to occur during seasonal changes in salinity. Changes in soil salinity occur because salt accumulates in the soil during dry periods and is leached away when rain falls (Woodell 1985; Greaver and Sternberg 2007). Germination in periods of high moisture is particularly important for coastal dune plants and other halophytes which generally germinate to the highest percentages when salinity is low or absent in the environment (Ungar 1978; Woodell 1985; Maun 1994). In the natural environment of the study species the change from high to low salinity is most likely to occur at the start of the winter rainfall season. Overall, the majority of *F. nodosa* seeds recovered from exposure to salinity and water stress (there was high recovery in most treatment combinations). In contrast, *S. globulosum* only recovered well under optimal temperatures. The relatively high recovery from low to moderate levels of salinity in optimal temperatures indicates that it is likely many seeds could survive exposure to unfavourable conditions in new environments and then germinate once salt stress is alleviated.

Germination responses to salinity and isosmotic water stress were investigated to distinguish between the osmotic and toxic effects of salt on germination (Munns 2002). If seeds germinate in or recover from NaCl and PEG exposure equivalently then it can be concluded that only osmotic stress affects germination. Conversely, if recovery from PEG is significantly higher than from NaCl it can be concluded that there is an added effect of ion toxicity. Overall, at optimal temperatures *S. globulosum* was more sensitive to both NaCl and PEG exposure than *F. nodosa*. However, in non-optimal temperatures, the direction and magnitude of differences between germination in NaCl and PEG were unexpected. The higher germination of *F. nodosa* seeds in NaCl compared with PEG during initial exposure to the osmotic solutions indicates that seeds can tolerate salt exposure during germination. However, after transfer to deionised water the expected recovery pattern (equal germination in NaCl and PEG) was only observed at optimal temperatures, 15 and

20 °C. At a sub-optimal temperature, seeds germinated better after NaCl than PEG exposure, while at a super-optimal temperature, seeds germinated better after PEG than NaCl exposure, indicating that these seeds experienced ion toxicity. For *S. globulosum*, germination in and recovery from NaCl and PEG was equal at non-optimal temperatures (but significantly less than germination in water only). At optimal temperatures (10 °C and 15 °C), the higher recovery from PEG than NaCl, indicates that many *S. globulosum* seeds were killed by NaCl. The mechanisms of salt tolerance remain unknown, but it is likely that NaCl enters some seeds and if the ionic balance is compromised the seeds perish (Rehman *et al.* 1998; Nichols *et al.* 2009). However, moderate salt uptake by a seed may actually promote germination in some salt-tolerant species by maintaining the osmotic balance between the seed and the external solution (Zhang *et al.* 2010). This mechanism may be responsible for the better germination of *F. nodosa* in NaCl compared with PEG in moderate salinity at optimal temperatures (-1.25 MPa; 15 and 20 °C). Investigation of whether Na is imbibed by germinating *F. nodosa* and *S. globulosum* seeds would further our understanding of salt tolerance mechanisms in these two coastal species and is addressed in Chapter 6 (Guja *et al.* 2013).

For the salt-sensitive species *S. globulosum*, some seeds were killed by salinity; however, some were also unexpectedly killed by osmotic stress. Physical dormancy was removed by hot water treatment before *S. globulosum* seeds were exposed to NaCl and PEG, and perhaps without the physical barrier to water uptake the non-dormant seeds do not have any mechanisms for excluding toxic ions or tolerating severe water stress. Physical dormancy (hardseededness) prevents imbibition and therefore protects seeds from water stress or uptake of sodium while dormant in the soil (Nichols *et al.* 2009) or during dispersal by the ocean (Appendix 1; Guja *et al.* 2010). Physical dormancy also prevents the internal tissues of *S. globulosum* seeds from experiencing NaCl exposure during hot dry summers when soil salinity is highest (Chapter 2). In contrast, *F. nodosa* seeds are non-dormant at dispersal and have likely developed active mechanisms of NaCl exclusion to prevent germination in unfavourable conditions, potentially facilitating their greater salt tolerance. As with vegetative plants, germinating seeds may be sensitive to water stress. This sensitivity may account for the death of *S. globulosum* seeds exposed to severe osmotic stress (in PEG treatments) but requires further examination.

Conclusion

Oceanic dispersal is likely to result in a seed being deposited in a saline coastal habitat that may differ significantly from its maternal environment. Therefore, post-dispersal germination and establishment are critical steps that determine the success of long-distance dispersal events. The present study investigated the capacity of two common coastal species to germinate under new environmental conditions, representative of those that may be encountered after oceanic dispersal. Results demonstrated that salt sensitivity is intensified when species germinate in non-optimal temperatures and can vary between populations to a larger extent than previously recognised. Ultimately, this research has shown that the capacity to successfully germinate in a new post-dispersal environment depends on temperature, salinity, and moisture conditions, and, more importantly, the interaction between these environmental variables.

References

Baskin CC, Baskin JM (1998) 'Seeds: ecology, biogeography, and evolution of dormancy and germination.' (Academic Press: California).

Bazzaz FA (1973) Seed germination in relation to salt concentration in three populations of *Presopis farcta*. *Oecologia* **13**, 73-80.

Berger A (1985) Seed dimorphism and germination behaviour in *Salicornia patula*. *Vegetatio* **61**, 137-143.

Bureau of Meteorology (2013) Maps of average conditions. (Commonwealth of Australia) Available at http://www.bom.gov.au/jsp/ncc/climate_averages

Cochrane A, Daws MI, Hay FR (2011) Seed-based approach for identifying flora at risk from climate warming. *Austral Ecology* **36**, 923-935.

de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution* **20**, 68-73.

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1059-1074.

El-Keblawy A, Al-Rawai A (2005) Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *Journal of Arid Environments* **61**, 555-565.

Elsey-Quirk T, Middleton BA, Proffitt CE (2009) Seed flotation and germination of salt marsh plants: the effects of stratification, salinity, and/or inundation regime. *Aquatic Botany* **91**, 40-46.

Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK (2011) Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology & Evolution* **27**, 1-10.

Greaver TL, Sternberg LSL (2007) Fluctuating deposition of ocean water drives plant function on coastal sand dunes. *Global Change Biology* **13**, 216-223.

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* **31**, 149-90.

Guja L, Wuhrer R, Moran K, Dixon KW, Wardell-Johnson G, Merritt DJ (2013) Full spectrum X-ray mapping reveals differential localization of salt in germinating seeds of differing salt tolerance. *Botanical Journal of the Linnean Society* **173**, 129-142.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**, 1175-1186.

Gul B, Ansari R, Flowers TJ, Khan MA (2013) Germination strategies of halophyte seeds under salinity. *Environmental and Experimental Botany* **92**, 4-18.

Gulzar S, Khan MA, Ungar IA (2001) Effect of salinity and temperature on the germination of *Urochondra setulosa* (Trin.) C. E. Hubbard. *Seed Science and Technology* **29**, 21-29.

Hanslin HM, Eggen T (2005) Salinity tolerance during germination of seashore halophytes and salt tolerant grass cultivars. *Seed Science Research* **15**, 43-50.

Hardegree SP, Emmerich WE (1990) Partitioning water potential and specific salt effects on seed germination of four grasses. *Annals of Botany* **66**, 587-595.

Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. *Ecology Letters* **9**, 228-241.

Harty RL, McDonald TJ (1972) Germination behaviour in beach spinifex (*Spinifex hirsutus* Labill.). *Australian Journal of Botany* **20**, 241-251.

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* **84**, 1945-1956.

Howarth DG, Gustafsson MHG, Baum DA, Motley TJ (2003) Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacifc Basin and colonization of the Hawaiian Islands. *American Journal of Botany* **90**, 915-923.

Hroudova Z, Moravcova L, Zakravsky P (1997) Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. *Folia Geobotanica et Phytotaxonomica* **32**, 377-390.

Jiang A, Gan L, Tu Y, Ma H, Zhang J, Song Z, He Y, Cai D, Xue X (2013) The effect of genome duplication on seed germination and seedling growth of rice under salt stress. *Australian Journal of Crop Science* **7**, 1814-1821.

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

Keiffer CH, Ungar IA (1997) The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *American Journal of Botany* **84**, 104-111.

Khan MA, Gulzar S (2003) Light, salinity and temperature effects on the seed germination of perennial grasses. *American Journal of Botany* **90**, 131-134.

Kim E, Donohue K (2013) Local adaptation and plasticity of *Erysimum capitatum* to altitude: its implications for responses to climate change. *Journal of Ecology* **101**, 796-805.

Kimball S, Angert AL, Huxman TE, Venable DL (2010) Contemporary climate change in the Sonoran Desert favors cold-adapted species. *Global Change Biology* **16**, 1555-1565.

Kochankova J, Mandak B (2009) How do population genetic parameters affect germination of the heterocarpic species *Atriplex tatarica* (Amaranthaceae)? *Annals of Botany* **103**, 1303-1313.

Kokubugata G, Nakamura K, Forster PI, Hirayama Y, Yokota M (2012) Antitropical distribution of *Lobelia* species (Campanulaceae) between the Ryukyu Archipelago of Japan and Oceania as indicated by molecular data. *Australian Journal of Botany* **60**, 417-428.

Lesko GL, Walker RB (1969) Effect of sea water on seed germination in two Pacific atoll beach species. *Ecology* **50**, 730-734.

Mariko S, Kachi N, Ishikawa S, Furukawa A (1992) Germination ecology of coastal plants in relation to salt environment. *Ecological Research* **7**, 225-233.

Martinez ML, Valverde T, Moreno-Casasola P (1992) Germination response to temperature, salinity, light and depth of sowing of ten tropical dune species. *Oecologia* **92**, 343-353.

Maun MA (1994) Adaptations enhancing survival and establishment of seedlings on coastal dune systems. *Vegetatio* **111**, 59-70.

Michel BE (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology* **72**, 66-70.

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

Naidoo G, Naicker K (1992) Seed germination in the coastal halophytes *Triglochin bulbosa* and *Triglochin striata*. *Aquatic Botany* **42**, 217-229.

Necajeva J, Ievinsh G (2008) Seed germination of six coastal plant species of the Baltic region: effect of salinity and dormancy-breaking treatments. *Seed Science Research* **18**, 173-177.

Nichols PGH, Malik AI, Stockdale M, Colmer TD (2009) Salt tolerance and avoidance mechanisms at germination of annual pasture legumes: importance for adaptation to saline environments. *Plant Soil* **315**, 241-255.

Nilsson C, Brown RL, Jansson R, Merritt DM (2010) The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews* **85**, 837-858.

Qu X-X, Huang Z-Y, Baskin JM, Baskin CC (2008) Effect of temperature, light and salinity on seed germination and radicle growth of the geographically widespread halophyte shrub *Halocnemum strobilaceum*. *Annals of Botany* **101**, 293-299.

Quilichini A, Debussche M (2000) Seed dispersal and germination patterns in a rare Mediterranean island endemic (*Anchusa crispa* Viv., Boraginaceae). *Acta Oecologica* **21**, 303-313.

Rehman S, Harris PJC, Bourne WF (1998) The effect of sodium chloride on the Ca^{2+}, K^{+} and Na^{+} concentrations of the seed coat and embryo of *Acacia tortilis* and *A. coriacea*. *Annals of Applied Biology* **133**, 269-279.

Rodriguez RJ, White Jr JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. *New Phytologist* **182**, 314-330.

Schat H, Scholten M (1985) Comparative population ecology of dune slack species: the relation between population stability and germination behaviour in brackish environments. *Vegetatio* **61**, 189-195.

Schlacher TA, Schoeman DS, Dugan J, Lastra M, Jones A, Scapini F, McLachlan A (2008) Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. *Marine Ecology* **29**, 70-90.

Schupp EW, Jordano P, Gómez JM (2010) Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* **188**, 333-353.

Stephens SG (1958) Salt water tolerance of seeds of *Gossypium* species as a possible factor in seed dispersal. *The American Naturalist* **92**, 83-92.

Tlig T, Gorai M, Neffati M (2008) Germination responses of *Diplotaxis harra* to temperature and salinity. *Flora* **203**, 421-428.

Tobe K, Li X, Omasa K (2000) Seed germination and radicle growth of a halophyte, *Kalidium caspicum* (Chenopodiaceae). *Annals of Botany* **85**, 391-396.

Travis JMJ, Delgado M, Bocedi G, Baguette M, Bartoń K, Bonte D, Boulangeat I, Hodgson JA, Kubisch A, Penteriani V, Saastamoinen M, Stevens VM, Bullock JM (2013) Dispersal and species' responses to climate change. *Oikos* **122**, 1532-1540.

Turner SR, Merritt DJ, Baskin CC, Dixon KW, Baskin JM (2005) Physical dormancy in seeds of six genera of Australian Rhamnaceae. *Seed Science Research* **15**, 51-58.

Ungar IA (1978) Halophyte seed germination. *Botanical Review* **44**, 233-264.

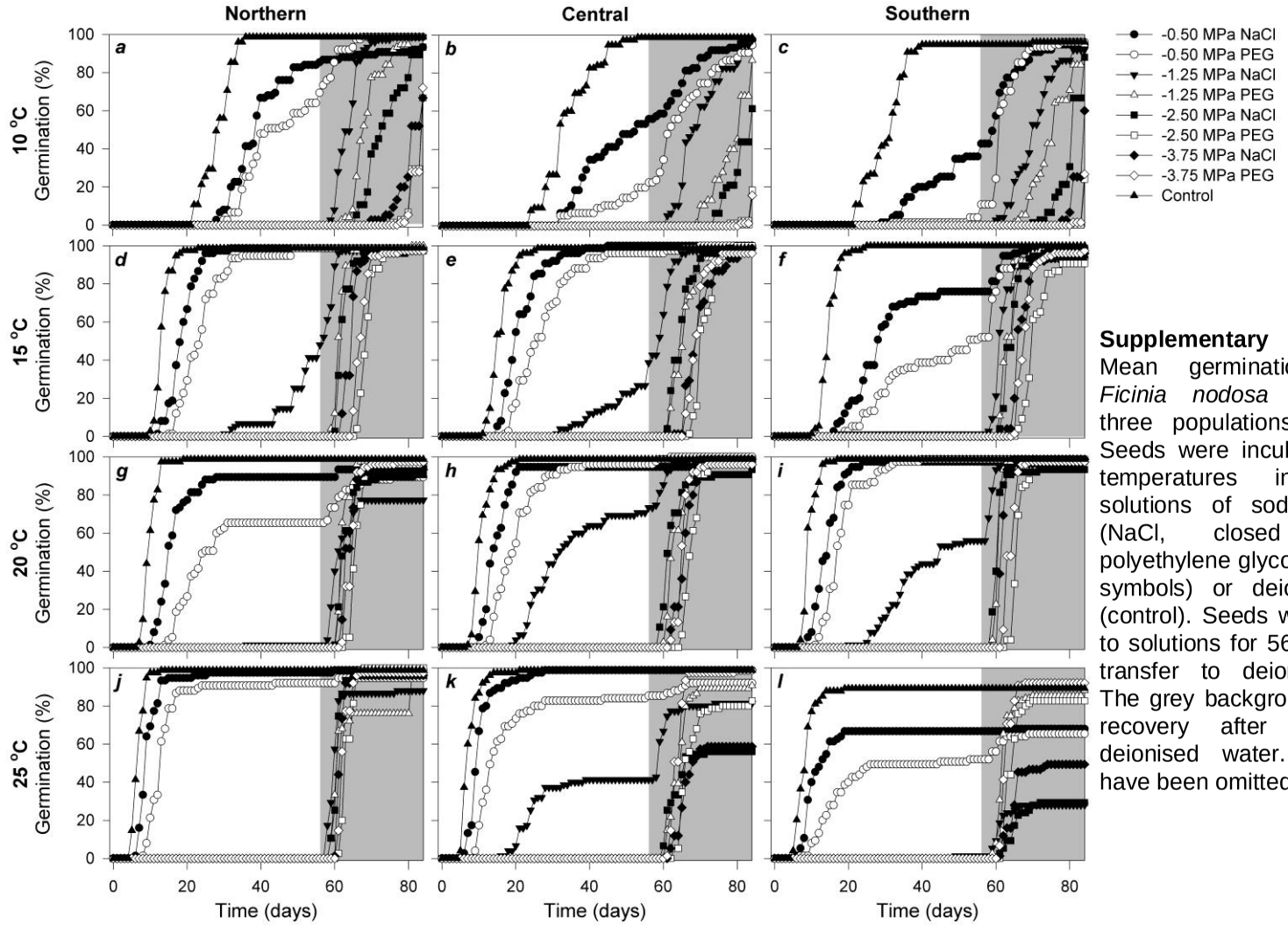
Walmsley CA, Dowey AJ (1997) Germination characteristics of shingle beach species, effects of seed ageing and their implications for vegetation restoration. *Journal of Applied Ecology* **34**, 131-142.

Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology* **6**, 2621-2626.

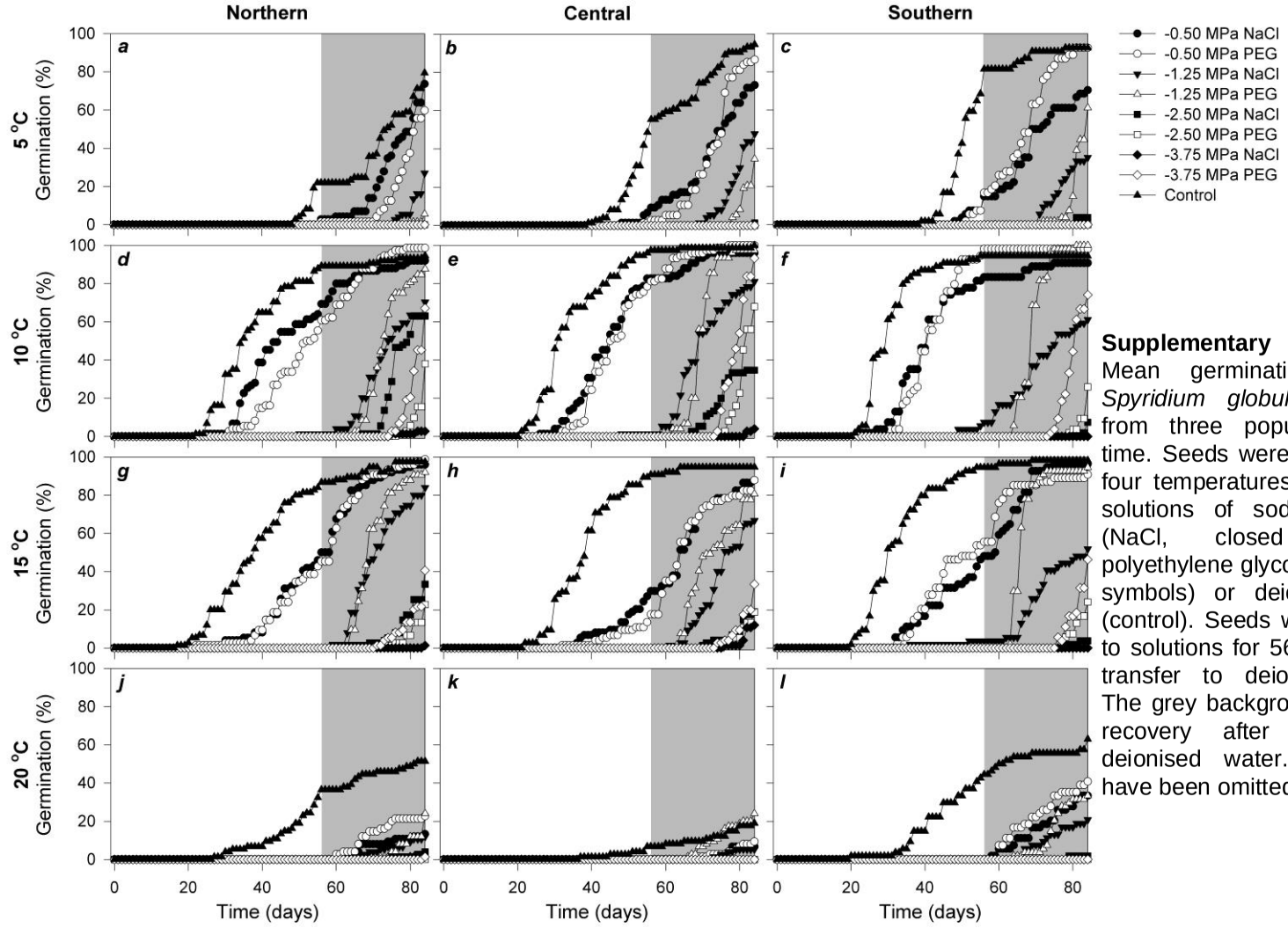
Woodell SRJ (1985) Salinity and seed germination patterns in coastal plants. *Vegetatio* **61**, 223-229.

Zhang H, Irving LJ, McGill C, Matthew C, Zhou D, Kemp P (2010) The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Annals of Botany* **106**, 1027-1035.

Zhang H, Irving LJ, Tian Y, Zhou D (2012) Influence of salinity and temperature on seed germination rate and the hydrotime model parameters for the halophyte, *Chloris virgata*, and the glycophyte, *Digitaria sanguinalis*. *South African Journal of Botany* **78**, 203-210.



Supplementary Figure 1. Mean germination (%) of *Ficinia nodosa* seeds from three populations over time. Seeds were incubated at four temperatures in isosmotic solutions of sodium chloride (NaCl, closed symbols), polyethylene glycol (PEG, open symbols) or deionised water (control). Seeds were exposed to solutions for 56 days before transfer to deionised water. The grey background indicates recovery after transfer to deionised water. Error bars have been omitted for clarity.



Supplementary Figure 2. Mean germination (%) of *Spyridium globulosum* seeds from three populations over time. Seeds were incubated at four temperatures in isosmotic solutions of sodium chloride (NaCl, closed symbols), polyethylene glycol (PEG, open symbols) or deionised water (control). Seeds were exposed to solutions for 56 days before transfer to deionised water. The grey background indicates recovery after transfer to deionised water. Error bars have been omitted for clarity.

Chapter 6

Full spectrum X-ray mapping reveals differential localisation of salt in germinating seeds of differing salt tolerance

Abstract

Seeds of many coastal plants can survive exposure to seawater and may be dispersed long distances by the ocean. The salt tolerance or avoidance strategies of seeds are poorly understood, even though these traits may fundamentally influence dispersal and recruitment in coastal dunes. This research aimed to demonstrate how salt exclusion or localisation within germinating seeds may affect salt tolerance. To determine the response of seeds to external salinity during imbibition (water uptake), it was necessary to quantify uptake and spatially resolve the internal distribution of salt. Flame photometry was used to quantify salt concentration in imbibing seeds and a new application of full spectrum X-ray mapping allowed visualisation of the spatial distribution and relative abundance of salt. As external salinity increased, salt-sensitive *Spyridium globulosum* (Rhamnaceae) seeds accumulated sodium and chlorine in the seed embryo, while potassium was increasingly displaced and germination was reduced. Conversely, salt-tolerant *Ficinia nodosa* (Cyperaceae) seeds avoided ion uptake and germination was not affected by imbibition in high sodium chloride (NaCl) concentrations. These results provide insight into mechanisms of salt tolerance/avoidance during imbibition and early germination and suggest that oceanic dispersal can be a viable explanation for the distribution of some plant species.

Introduction

Seeds form a crucial part of the life cycle of seed-bearing plants. Seeds provide a reproductive advantage by dispersing plants through time and space, avoiding periods of adverse environmental conditions unsuitable to plant growth. Seeds have many adaptations that contribute to their persistence in hostile environments and that ensure germination only occurs when the environmental conditions are most conducive to seedling establishment. For coastal plants, buoyant seeds can be dispersed long distances via the ocean (Darwin 1859; Nathan 2006) and contribute to the wide distribution of coastal species (Howarth *et al.* 2003). Seeds of many species can survive exposure to seawater [or equivalent salinities of approximately 500 mM sodium chloride (NaCl)] for days, weeks, or even months (Appendix 1; Darwin 1856; Darwin 1859; Nathan 2006; Guja *et al.* 2010; Gillespie *et al.* 2011), and subsequently germinate under saline conditions or following transfer to pure water (Appendix 1; Debez *et al.* 2004; Necajeva and Ievinsh 2008; Guja *et al.* 2010). How seeds are able to survive and germinate following exposure to salt is a question that has been considered since Darwin's first experiments examining the possibility of long-distance dispersal of seeds via the ocean (Darwin 1856; Darwin 1859; Black 2009).

In temperate- and mediterranean-type coastal environments, such as south-western Australia, soil salinity fluctuates seasonally as a result of drought and the accumulation of saline aerosols, interspersed with episodes of rainfall (Chapter 2; Greaver and Sternberg 2007). Nevertheless, plants are capable of growing and reproducing in such an environment. Seeds of coastal plants are likely to have evolved mechanisms for avoiding or tolerating, and surviving, the highly variable saline conditions to which they are periodically exposed. High concentrations of sodium (Na) and/or chloride (Cl) ions are detrimental to plant organs at a number of developmental stages because of a combination of osmotic effects, and ion toxicity or ion imbalance (Greenway and Munns 1980; Katembe *et al.* 1998). Ion balance is influenced by potassium (K) and calcium (Ca) ions, which are involved in the modulation of univalent cation influx (e.g. sodium) (Zhang *et al.* 2007), enzyme activation (Rehman *et al.* 1998; Hasegawa *et al.* 2000), protein synthesis for growth (Rehman *et al.* 1998), and membrane permeability of cells to ions (Greenway and

Munns 1980; Cramer *et al.* 1985). Therefore, these cations (potassium and calcium) generally occur in all plants and are found in relatively high concentrations in plants that grow successfully in saline environments (Greenway and Munns 1980; Rehman *et al.* 1998).

Salinity negatively affects seed germination and, as in the vegetative stage, an active balance between sodium, potassium, and chlorine may be imperative for successful seed germination under saline conditions. Slowed imbibition (Katembe *et al.* 1998) and delayed or inhibited germination (Appendix 1; Woodell 1985; Katembe *et al.* 1998; Guja *et al.* 2010) are characteristics of seeds able to survive highly saline conditions. However, it is unclear whether salt avoidance and/or tolerance mechanisms in seeds mirror the well-documented salt responses of whole-plants (Greenway and Munns 1980; Flowers *et al.* 1986).

A germinating seed is an independent unit, with germination exclusively controlled by the seed and its response to external environmental cues. Germination begins with water uptake (imbibition) and ends with elongation of the embryonic axis (usually the radicle) through the surrounding structure(s) (the testa or pericarp) (Bewley 1997; Shabala *et al.* 2000). Salt uptake or exclusion in seeds, particularly during the transition from imbibition (water uptake) to the completion of germination (radicle emergence), is critical to seedling establishment in saline environments. Imbibition is triphasic (Bewley and Black 1994; Bewley 1997; Nonogaki *et al.* 2010). The first phase involves rapid movement of water into the apoplast until all of the matrices and cell contents are fully hydrated (Bewley and Black 1994; Nonogaki *et al.* 2010). During phase II water uptake slows and is balanced between the negative osmotic potential of the external solution and the positive pressure potential of the cells (Bewley and Black 1994). Imbibition at more negative external water potentials (e.g. in high salt) generally lowers the seed water content, extends the length of phase II, and delays and/or blocks entry into phase III if a minimum seed water content, specific to each species, is not reached (Bewley 1997). The completion of germination is marked by radicle emergence and signifies that seeds have entered phase III (Bewley and Black 1994).

During the imbibition and germination process, tolerance of external salt appears to be variable between species. It has been suggested that seed dormancy type or seed

coat properties can affect the survival of seeds by influencing salt uptake (Appendix 1; Nichols *et al.* 2009; Guja *et al.* 2010). However, there is little empirical data concerning the uptake of salt by germinating seeds. Salt may enter seeds continuously or during a particular phase of imbibition and accumulate uniformly or be actively redirected to particular seed tissues. For example, differences in salt accumulation between the embryo and seed coat following imbibition in saline conditions have been described for seeds of Fabaceae (Rehman *et al.* 1998; Nichols *et al.* 2009). This suggests that salt may be partitioned in certain seed tissues and the seed coat may regulate ion exchange and uptake, meaning seed tissues should be considered separately when investigating ion exchange (Rehman *et al.* 1998). If seeds germinating under saline conditions do indeed actively distribute salts between seed tissues, it is unclear during which phase of imbibition or germination such partitioning occurs.

To determine how seeds respond to external salinity during imbibition and germination it is necessary to quantify the uptake and spatially resolve the distribution of salt in a seed. Changes in the distribution of salt among seed tissues during imbibition and germination are difficult to visualise. X-ray mapping (XRM) is an established microscopy technique applicable to biological samples that involves the collection of characteristic X-rays as a function of the position of the scanning electron beam on the specimen (Goldstein *et al.* 2003). The resultant high magnification image reveals the distribution and relative abundance of the elements within a specimen (Moran and Wuhrer 2006). Energy dispersive X-ray microanalysis has been utilised to identify elements such as sodium and chlorine present in mature, dry seeds of halophytes (Khan *et al.* 1985; Atia *et al.* 2010), and wheat (*Triticum aestivum*) (Lott and Spitzer 1980). However, this technique has not yet been applied to germinating seeds or to investigate the dynamics of salt uptake during imbibition.

Although the salt tolerance and/or avoidance strategies of imbibing and germinating seeds are poorly known, these traits may fundamentally influence the composition of vegetation communities in coastal environments. S

eeds of common species of Australian coastal Holocene dune communities exhibit different responses to salt during germination and plants occupy different niches

within these dunes. For example, *Ficinia nodosa* (Rottb.) Goetgh., Muasya & D.A.Simpson (Cyperaceae), a common sedge, extends close to the frontal edges of foredunes and occurs in highly exposed sites. Seeds of *F. nodosa* are salt-tolerant and can germinate in salt concentrations of ≤ 200 mM sodium chloride (Appendix 1; Guja *et al.* 2010). In higher salt concentrations (up to 500 mM sodium chloride) the seeds do not germinate, but survive for extended periods and subsequently germinate if salinity is alleviated (Appendix 1; Guja *et al.* 2010). This response suggests that in higher sodium chloride concentrations seeds may not imbibe and, although germination is inhibited, salt uptake may be avoided. Alternatively, these seeds may be capable of exporting/leaching salt when transferred to pure water. Conversely, plants of the woody shrub *Spyridium globulosum* (Labill.) Benth. (Rhamnaceae) occur in the protected swales of secondary dunes, and further inland. Seeds of *S. globulosum* are less able to survive in high salt concentrations. Whereas seeds can germinate in ≤ 300 mM sodium chloride they do not germinate in, and many do not survive exposure to, higher concentrations ≥ 400 mM sodium chloride (Appendix 1; Guja *et al.* 2010), suggesting that salt may enter seeds and adversely affect survival. In addition, there is generally a positive relationship between seed death in *S. globulosum* and external salt concentration.

In this study we utilised full spectrum X-ray mapping for the identification of elements, including sodium, potassium, and chlorine, to visualise the spatial distribution and relative abundance of salt-related ions during imbibition of seeds of coastal species. We selected *F. nodosa*, and *S. globulosum*, sympatric species of Holocene dune communities in south-western Australia, to address the following key questions regarding salt exclusion or transport within seeds of differing salt tolerance: (1) what is the relationship between increased external sodium chloride concentrations (≥ 100 mM), exposure time, and seed mortality for the two species?; (2) does the rate of water uptake (imbibition) at increased external sodium chloride (≥ 100 mM) concentrations vary differentially between the two species?; (3) how does seed mortality relate to the uptake and accumulation of sodium chloride or changes in potassium?; and (4) how do patterns of sodium, potassium and chlorine localisation within seed tissues differ between the species? We hypothesised (1) that the salt-tolerant seeds of *F. nodosa* would have slowed imbibition under high sodium chloride concentrations, potassium concentration in the seed tissues would

remain constant, and that sodium and chlorine would be excluded from internal seed tissues, or partitioned away from the embryonic axis; and (2) that seeds of salt-sensitive *S. globulosum* would also have slowed imbibition in the presence of high sodium chloride concentrations, but that sodium and chlorine would accumulate in the seed and be equally distributed among all seed tissues, resulting in seed death.

Materials and methods

Seed collection

Mature seeds of *F. nodosa* and *S. globulosum* were collected from natural plant populations in coastal Holocene sand-dune communities near Perth, Western Australia (-31° 54' 31.358" S 115° 45' 25.673" E) in November 2008 (*S. globulosum*) and February 2009 (*F. nodosa*). Immediately following collection, seeds were cleaned and dried at 15% relative humidity (RH) and 15 ºC in a controlled environment room. Seeds were then hermetically sealed in laminated aluminium foil bags, and stored at -18 °C until required. Seeds of *S. globulosum* are physically dormant at maturity and were treated prior to imbibition and germination experiments by hot water at 95 ºC for 2 min to rupture the water-gap (Turner *et al.* 2005). Seeds of *F. nodosa* are non-dormant at maturity and did not require pretreatment.

Seed samples

To assess the effect of salt solutions on seed germination and internal ion concentrations three replicates per treatment were used. During imbibition (prior to radicle emergence) seeds were sealed in individual nylon mesh bags and incubated in 90 mm Petri dishes to which 15 mL of liquid was added. Dishes were irrigated with saline solution (NaCl, Sigma Aldrich) with calculated osmotic potentials of 0 (deionised water), -0.5, -1.25, -2.5 and -3.75 MPa (equivalent to 0, 104, 261, 522, 783 mM sodium chloride, respectively) at 15 °C. Note that for simplicity, approximate salt concentrations of 0, 100, 250, 500 and 750 mM will be referred to henceforth. The concentration of sodium chloride required to achieve each calculated osmotic potential was determined following the formula

$$\text{Osmotic potential (MPa)} = -\text{MiRT}$$

where M = concentration (molarity) of the solute, i = osmotic coefficient (the ratio of amount of particles in solution to amount of formula units dissolved), R = ideal gas constant, and T = absolute temperature (°K).

Imbibition properties of seeds

To measure the rate of water uptake, first the mass (to four decimal places) of three replicates of air-dried seeds (n = 400, c. 0.12 g, *F. nodosa*; and n = 100, c. 0.13 g, *S. globulosum*) was determined. These seeds were then placed in Petri dishes lined with nylon mesh and irrigated as described above. Seeds were periodically re-weighed after removing the mesh and seeds from dishes and patted dry with paper towel. Water uptake was measured as the change in seed mass:

$$\%\ \text{Increase in mass} = [(W_1 - W_d)/W_d] \times 100$$

where W_1 = mass of imbibed seeds and W_d = mass of air-dry seeds.

Effects of sodium chloride on seed germination

To quantify seed germination and survival after salt exposure, seeds were imbibed under saline conditions. Seeds were sealed in nylon mesh bags (*F. nodosa* n = 25, *S. globulosum* n = 25), three replicate bags per treatment, and irrigated, sealed and incubated as above for up to 21 days. To measure the effect of time of exposure to saline conditions on subsequent germination (i.e. from the commencement of imbibition through to radicle emergence), bags of seeds were retrieved after 1, 5, and 10 days for *F. nodosa*, and after 1, 7, 14, and 21 days for *S. globulosum*. Exposure times were based on imbibition in fresh water and the retrieval times for each species coincided with late phase I, mid phase II, and the onset of phase III of imbibition for each species. Two other exposure times were investigated, early phase I (1 day) for *S. globulosum* seeds due to the sensitivity of these seeds to sodium chloride, and late phase III (15 days) for the more salt-tolerant *F. nodosa* seeds.

Bags of imbibed seeds were removed from sodium chloride solutions and rinsed three times for 1 min each in 40 mL deionised water. Seeds were removed from bags

and placed in Petri dishes containing 0.7% (w/v) water agar. Petri dishes were sealed and incubated as above, and final germination was scored after 42 days based on radicle emergence > 1/3 of seed length. After 42 days the viability of any non-germinated seeds was assessed via a cut test. Only seeds with an intact, turgid, white embryo were classified as viable.

Quantification of sodium and potassium ions during seed imbibition

Flame photometry was used to quantify total fluctuations of sodium and potassium ions in seeds in relation to imbibition phases and germination response. Seeds were placed in three replicate nylon mesh bags (*F. nodosa* n = 50, *S. globulosum* n = 25), irrigated, sealed and incubated as above. Seeds were retrieved after 1, 5, 10, and 15 days for *F. nodosa*, and after 1, 7, 14, and 21 days for *S. globulosum*, and fresh mass was recorded. To remove surface ions and avoid internal osmotic changes, extracted seeds were washed three times for 1 min each in 40 mL of iso-osmotic solution (0, -0.5, -1.25, -2.5, or -3.75 MPa) of polyethylene glycol 8000 (PEG), then blotted dry. Solutions of polyethylene glycol 8000 were prepared following Michel (1983). The dry weight of seeds was determined gravimetrically following oven drying at 103 ºC

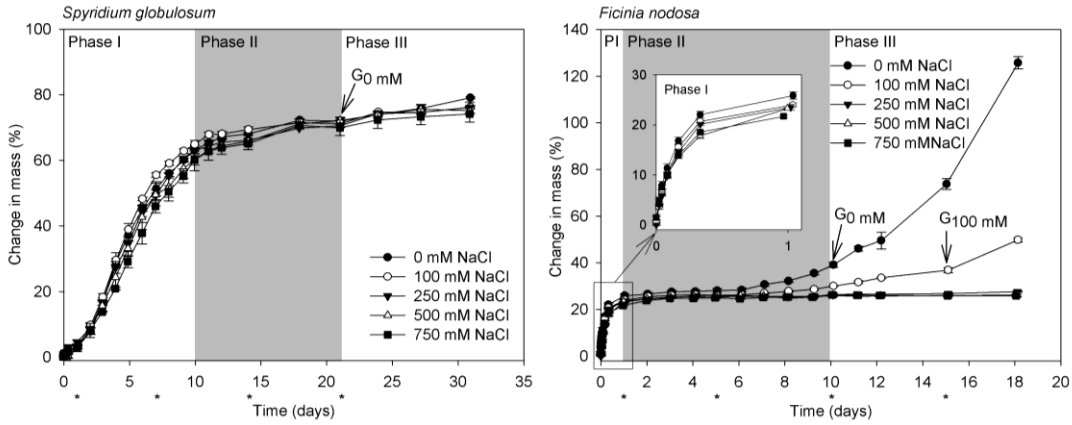


Figure 1. Imbibition (percentage change in seed mass) over time (days). Data points represent the mean ± SE of three replicates (n = 400 for *Ficinia nodosa*, n = 100 for *Spyridium globulosum*) of seeds imbibed in various concentrations of sodium chloride (NaCl) at 15 ºC. Shading indicates transition between the three imbibition phases for seeds imbibing water. $G_{0\ mM}$ = first visible radicle emergence in pure water, $G_{100\ mM}$ = first visible radicle emergence in 100 mM sodium chloride (*F. nodosa* seeds only). Sampling times for later experiments are labelled by an asterisk (*) on the x-axis.

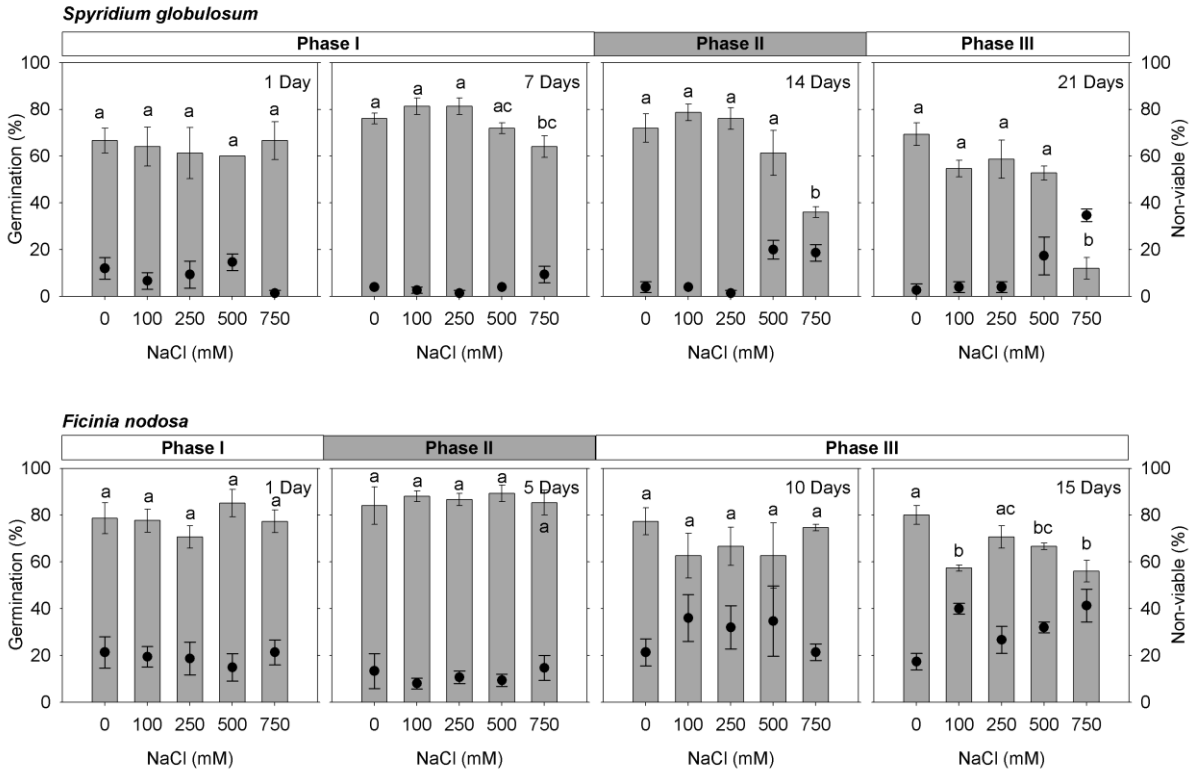


Figure 2. Germination of seeds (three replicates of n = 25) following exposure to a range of sodium chloride (NaCl) concentrations for time periods equivalent to phase I, II, and III of imbibition in pure water. Bars represent mean germination (%) ± SE at 15 ºC, 42 days after transfer from saline conditions to pure water agar. Black circles indicate the proportion of non-germinated seeds that were determined via cut-test to be non-viable (%) ± SE, 42 days after transfer from saline conditions to pure water agar. Letters (a, b, c) denote significant differences in germination attributable to the external salt concentration.

for 17 h (International Seed Testing Association (ISTA) 1999). Ions were extracted by grinding seeds in a 1.5 mL tube with a ceramic bead and 1 mL of 0.5 M nitric acid (HNO_3) for 60 s at a speed of 4 m s^{-1} using a fast prep machine (FastPrep (R)-24 Instrument V4.0, MP Biomedicals, NSW, Australia). After grinding, samples and beads were transferred to 10 mL plastic tubes and 9 mL 0.5 M nitric acid was then added before shaking on an orbital shaker for 48 h at room temperature (c. 23 ºC). Samples were filtered using 0.45 µm Supor membrane syringe filters, and the concentration of sodium and potassium ions determined by flame photometry (PFP7, Jenway, Essex, UK) using standards of 0-30 mg L^{-1} Na^+ in 0.5 M nitric acid and 0-10 mg L^{-1} K^+ in 0.5 M nitric acid.

Localisation of sodium, potassium, and chlorine in seed tissues

To visualise the relative partitioning of sodium, potassium, and chlorine within seed tissues during imbibition in a variety of sodium chloride concentrations, seeds of *F. nodosa* (n = 50) and, *S. globulosum* (n = 25) were placed in nylon mesh bags in Petri dishes and irrigated, sealed and incubated as above for up to 21 days. Bags were removed from each saline solution after 15 days for *F. nodosa*, and after 1, 7, 14, and 21 days for *S. globulosum*, rinsed in isosmotic polyethylene glycol 8000 and patted dry. Seeds were then removed from bags, spread out on aluminium foil, and air-dried at 15 ºC and 15% relative humidity for at least 6 days to achieve a very low moisture content of approximately 5%. Dried seeds were dissected longitudinally using a scalpel or double-edged razor blade. One half of each seed was mounted (cut-surface facing upwards) on an aluminium scanning electron microscope (SEM) stub covered with carbon-tape. The stub and samples were then coated with carbon to make them conductive for X-ray analysis. Maps were created on a Jeol 35CF scanning electron microscope with dual Noran and Oxford energy dispersive spectrometers (EDS). When a small sodium peak close to detection limits was detected for a sample, the seed was mapped again using a Jeol 733 microprobe column equipped with two Amptek silicon drift detectors, and four Jeol wavelength dispersive spectrometers (WDS) for improved detection. All SEM, energy and wavelength dispersive spectrometer hardware was controlled using a Moran Scientific hardware control system (Moran Scientific, NSW, Australia). X-ray maps were performed with an accelerating voltage of 25 kV with collection conditions of

150 ms point^{-1} and 512 x 512 pixel resolution. Moran Scientific quantitative X-ray mapping software was used to process and visualise results (Moran Scientific, NSW, Australia). All maps were background and overlap corrected so that true elemental composition, and not density change, was obtained. The EDS spectrum for each sample was examined to confirm the presence of sodium, potassium and chloride ions in each seed tissue (data not shown). For each sodium chloride exposure treatment at least two seeds were mapped to confirm localisation of elements in seed tissues (McCully *et al.* 2010).

Statistical analysis

The effects of time of exposure to sodium chloride solution, and sodium chloride concentration, on seed germination and the internal sodium and potassium ion concentration were analysed for statistical significance by analysis of variance (ANOVA) using StatView (SAS Institute Inc.) Percentage values for germination were arcsin-transformed prior to one-way ANOVA (non-transformed data appear in all figures). Fisher's least significant difference at the 5% level ($P < 0.05$) was used to compare means and determine significant differences between treatments.

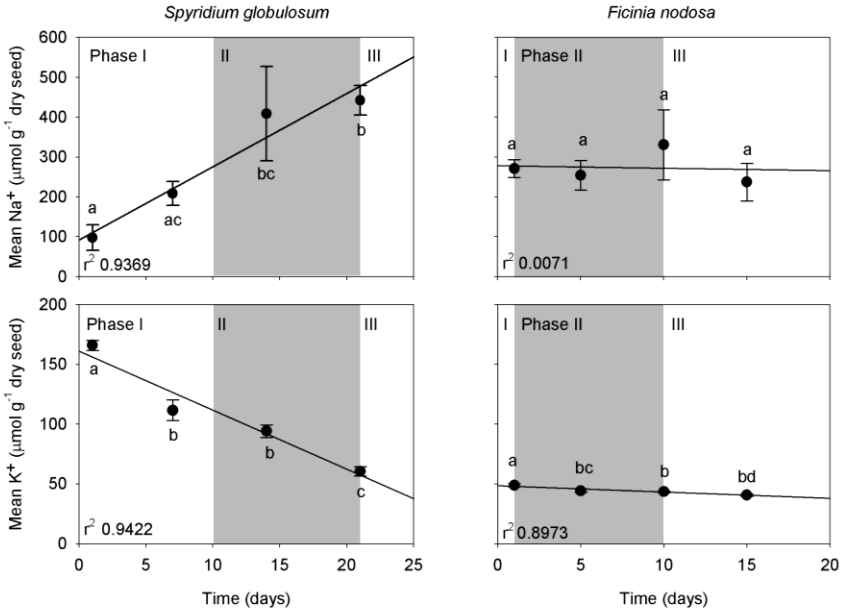


Figure 3. Effect of time in 750 mM sodium chloride (NaCl) on internal sodium (Na)(top) and potassium (K)(bottom) ion concentrations of seeds. Points represent the mean of three replicates ± SE (n = 50 *F. nodosa*, n = 25 *S. globulosum*). Shading represents imbibition phases and indicates the time required for seeds in water to reach phase I, II and III. Letters (a, b, c, d) denote significant differences in sodium or potassium content of seeds as a result of time in 750 mM sodium chloride.

Results

Imbibition properties of seeds

Imbibition curves of both species were sigmoidal in shape, indicative of the triphasic progression of water uptake (**Figure 1**). Water uptake tended to decrease as the concentration of sodium chloride in the external solution increased, except for *S. globulosum* where seeds in 100 mM sodium chloride had the greatest uptake during phase I. The rate of imbibition also slowed as sodium chloride concentration increased. These effects of increasing sodium chloride were most prominent in *F. nodosa* seeds as they approached the latter stages of phase II, and entered phase III of imbibition. Regardless of sodium chloride concentration, seeds of *F. nodosa* imbibed more rapidly than seeds of *S. globulosum*. In pure water, seeds of *F. nodosa* completed phase I of imbibition after 1 day, whereas *S. globulosum* seeds required 10 days to complete phase I of imbibition. However, in relative terms, seeds of *S. globulosum* imbibed more water than seeds of *F. nodosa* (c. 60% increase in mass for *S. globulosum* seeds, compared with c. 23% increase in mass for *F. nodosa* seeds at the completion of phase I). The duration of phase II of imbibition (for seeds imbibed in pure water) was similar for both species, being 9 – 11 days, and during phase II there was no marked net increase in water uptake above that of phase I. Progression to phase III of imbibition, and radicle emergence, only occurred in seeds of *S. globulosum* incubated in pure water, and in seeds of *F. nodosa* incubated in pure water or 100 mM sodium chloride. For seeds of *F. nodosa* the conclusion of phase II of imbibition was characterised by an increase in water uptake prior to radicle emergence, and water uptake continued during phase III, after radicle emergence. Radicle emergence occurred after 10 days in pure water, and after 15 days in 100 mM sodium chloride. Conversely, for seeds of *S. globulosum*, an increase in water uptake coincident with radicle emergence at the onset of phase III (21 days) was not observed.

Effects of sodium chloride on seed germination

Seeds of both species were able to survive imbibition in solutions of 100 - 750 mM sodium chloride to varying degrees, for a time period equivalent to the onset of phase III of imbibition in pure water (10 days for *F. nodosa* and 21 days for *S. globulosum*). Following transfer to pure water, germination of both species was c. 80 – 90% (**Figure 2**). However, after imbibition in 750 mM sodium chloride, differences in salt tolerance between species were apparent. For seeds of *S. globulosum*, after 7 days' imbibition (the point at which seeds were in the later stages of phase I in pure water), germination after imbibition in 750 mM sodium chloride (64.0 ± 4%) was significantly lower ($P = 0.04$) than germination of seeds that were imbibed in water for 7 days (76.0 ± 2%). Germination of seeds continued to decline progressively after 14 days (36.0 ± 2%) and 21 days (12.0 ± 4%) imbibition in 750 mM sodium chloride (equivalent to mid phase II, and the onset of phase III of imbibition in pure water, respectively). Extended periods of imbibition (14 and 21 days) in higher salt concentrations (500 mM and 750 mM) also resulted in a significant decrease ($P < 0.05$) in viable *S. globulosum* seeds, as determined by a cut test. In contrast, a significant reduction in germination of *F. nodosa* seeds was noted only after 15 days imbibition in 750 mM sodium chloride; a time period equivalent to well past the onset of phase III of imbibition, and 5 days after radicle emergence was first evident in pure water. In fact, imbibition of *F. nodosa* seeds for 15 days significantly reduced ($P < 0.05$) germination and seed viability at almost all sodium chloride concentrations, but lesser periods of imbibition in salt had no effect on viability or germination.

Quantification of sodium and potassium ions during seed imbibition

The concentration of Na^+ and K^+ in seeds was dependent on imbibition time and the concentration of sodium chloride in the external solution (significant interaction $P < 0.05$). It was apparent that seeds of *F. nodosa* were able to maintain a relatively constant internal Na^+ and K^+ concentration, in contrast to seeds of *S. globulosum*, for which concentrations of Na^+ and K^+ changed substantially. For example, the internal Na^+ concentration of *S. globulosum* seeds imbibed in 750 mM sodium chloride increased linearly with time, from 97 µmol Na^+ g^{-1} dry seed (1 day), to 442 µmol

Na^+ g^{-1} dry seed (21 days), r^2 = 0.94, P = 0.0059 while the K^+ concentration decreased linearly with time from 166 µmol K^+ g^{-1} dry seed (1 day), to 61 µmol K^+ g^{-1} dry seed (21 days), r^2 = 0.94, P < 0.0001 (**Figure 3**). Seeds of *F. nodosa* maintained a relatively constant internal sodium concentration (approximately 270 µmol Na^+ g^{-1} dry seed), and a slightly but significantly decreased K^+ concentration over time (from 1 to 15 days P = 0.0006, and concentration fell from 48.9 ± 1 to 40.7 ± 1 µmol K^+ g^{-1} dry seed) (**Figure 3**).

Contrasting patterns of Na^+ and K^+ concentration in seeds were also evident for seeds imbibed in various concentrations of sodium chloride (**Figure 4**). For example, after 21 days imbibition of *S. globulosum* seeds, there was a positive linear relationship (r^2 = 0.96) between external sodium chloride concentration and internal Na^+ concentration, with Na^+ increasing significantly (P < 0.0001) from 113 µmol Na^+ g^{-1} dry seed (0 mM sodium chloride) to 442 µmol Na^+ g^{-1} dry seed (750 mM sodium chloride). There was also a negative linear relationship (r^2 = 0.87) between external sodium chloride concentration and internal K^+ concentration, with K^+ decreasing significantly (P < 0.0001) from 110 µmol K^+ g^{-1} dry seed (0 mM sodium chloride) to 61 µmol K^+ g^{-1} dry seed (750 mM sodium chloride). For seeds of *F. nodosa* imbibed for 15 days, a relatively constant internal concentration of Na^+ (c. 200 µmol Na^+ g^{-1} dry seed) and K^+ (c. 45 µmol K^+ g^{-1} dry seed) was evident, regardless of the external sodium chloride concentration.

Localisation of sodium, potassium, and chloride within seed tissues during imbibition

The quantified changes in internal sodium and potassium concentration were greatest for *S. globulosum* seeds imbibed in 750 mM sodium chloride for up to 21 days (as shown in **Figure 3** and discussed above). Therefore, *S. globulosum* seeds that had imbibed in 750 mM sodium chloride were further investigated to map visually the movement of sodium, potassium and chlorine within seeds during imbibition (**Figure 5**). All seed tissues absorbed an increasing amount of sodium and chlorine over time, which corresponded with the displacement of potassium (**Figure 5**). Sodium was localised in the embryo after 14 and 21 days' imbibition. Prior to imbibition, potassium was located in all seed tissues, being least concentrated in the

seed coat < endosperm < embryo. After 14 days' imbibition the distribution of potassium had changed and it was localised in the endosperm > embryo > seed coat. After 21 days' imbibition most potassium had migrated from the embryo and was present predominantly in the endosperm. After 1 day of imbibition, chlorine was present in the seed coat, and had migrated to all tissues by 7 days of imbibition. After 14 days and 21 days' imbibition, chlorine was still present in the endosperm, but was mostly localised in the embryo. As flame photometry did not reveal a significant change in the internal Na^+ concentration of *F. nodosa* seeds because of time in 750 mM sodium chloride these seeds were not investigated via X-ray mapping.

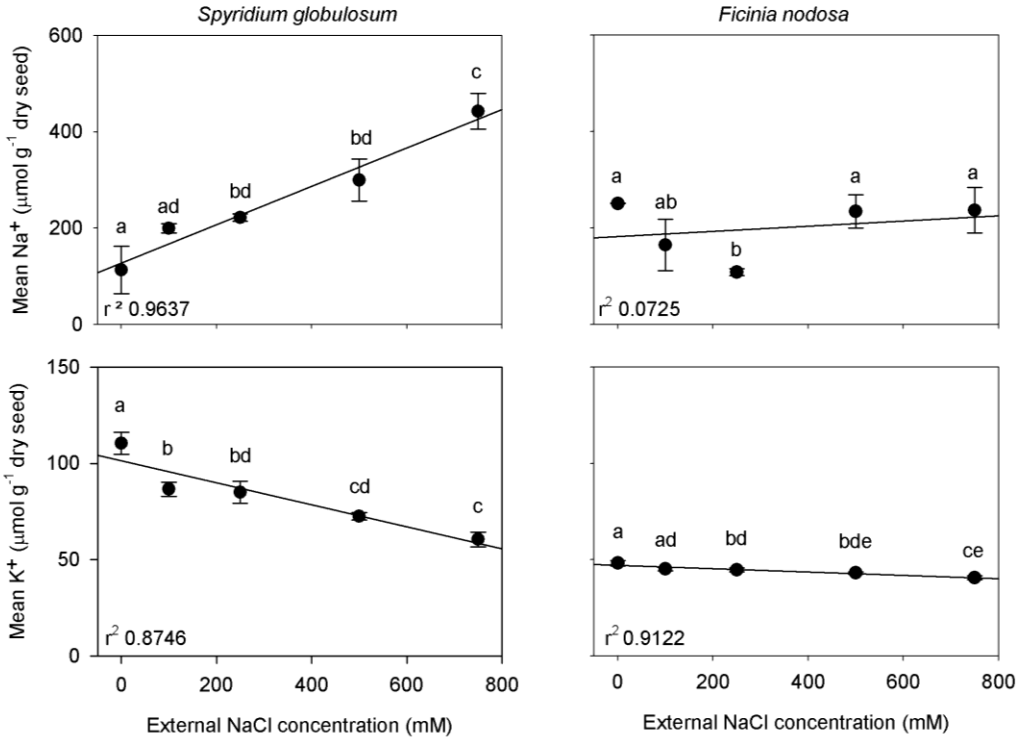


Figure 4. Effect of external salt concentration on internal seed Na^+ and K^+ content at phase III, radicle emergence in water. Points represent the mean of three replicates ± SE (n = 50 *F. nodosa*, n = 25 *S. globulosum*). Letters (a, b, c, d, e) denote significant changes in internal sodium or potassium content of seeds attributable to external sodium chloride (NaCl) concentration.

Localisation of sodium, potassium, and chlorine within seed tissues due to external sodium chloride concentration

To visualise changes in ion localisation as a result of the external sodium chloride concentration, *S. globulosum* seeds were sampled prior to imbibition, and after 21 days' imbibition in all sodium chloride concentrations. The selection of maps presented in **Figure 6** best exemplifies the internal localisation of elements in relation to external sodium chloride concentration. Prior to imbibition (**Figure 6a**) seed tissues of *S. globulosum* contained no detectable sodium (determined from EDS spectrum, data not shown). After 21 days' imbibition in 100 mM sodium chloride (**Figure 6b**), sodium was visible, localised within the inner edge of the seed coat. Following 21 days imbibition in 750 mM sodium chloride (**Figure 6c**) some sodium remained in the seed coat, but had also accumulated in the embryo. Prior to imbibition, potassium was present in the seed coat and all seed tissues, but most concentrated in the endosperm. After imbibition in 750 mM sodium chloride, potassium had migrated from the embryo into the endosperm and was no longer detectable in the seed coat. Small amounts of chlorine were present in all seed tissues at dispersal. Following imbibition in 100 mM sodium chloride for 21 days, chlorine had accumulated mostly in the endosperm and was also present in the embryo. Conversely, imbibition in 750 mM sodium chloride resulted in chlorine accumulating predominantly in the embryo, with only small amounts detected in the endosperm.

Regardless of the sodium chloride concentration of the imbibing solution [0 mM sodium chloride (**Figure 6d**), 250 mM sodium chloride (**Figure 6e**) or 500 mM sodium chloride (**Figure 6f**)], seeds of *F. nodosa* excluded sodium from internal seed tissues and sodium was only present on the exterior of the seed coat. The embryo contained potassium, a layer of potassium-rich cells surrounded the endosperm in all samples, and the concentration of the imbibing solution did not alter potassium localisation. However, some chlorine was imbibed and was detected within the embryo after imbibition in 250 mM sodium chloride for 15 days. At both 500 mM (**Figure 6f**) and 750 mM sodium chloride (data not shown), chlorine appeared to be compartmentalised into cells of the endosperm.

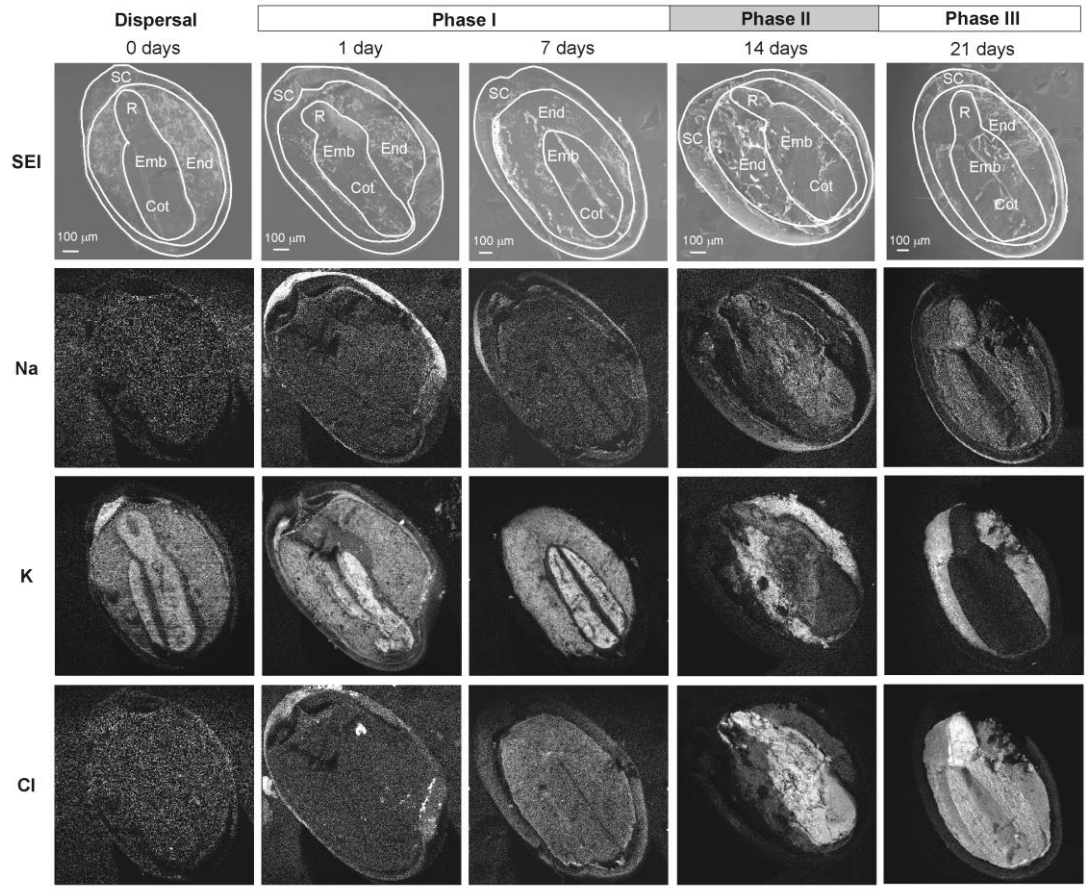


Figure 5. Secondary electron images (SEI) of seeds annotated to identify seed tissues. SC = seed coat, End = endosperm, Emb = embryo, R = radicle, Cot = cotyledons (embryo = cotyledons + radicle). X-ray maps indicate localisation of sodium (Na), chlorine (Cl), and potassium (K) in seeds of *S. globulosum* exposed to 750 mM sodium chloride (NaCl) for increasing time (0 days exposure to salt = seed as at dispersal). Dark areas in X-ray maps indicate the absence of the specified element and bright white areas indicate where the element is most concentrated. X-ray maps were performed on a Jeol 35CF scanning electron microscope with a dual energy dispersive spectrometer, accelerating voltage of 25 kV, collection conditions of 150 ms point^{-1}, and 512 x 512 pixel resolution. All maps are background and overlap corrected.

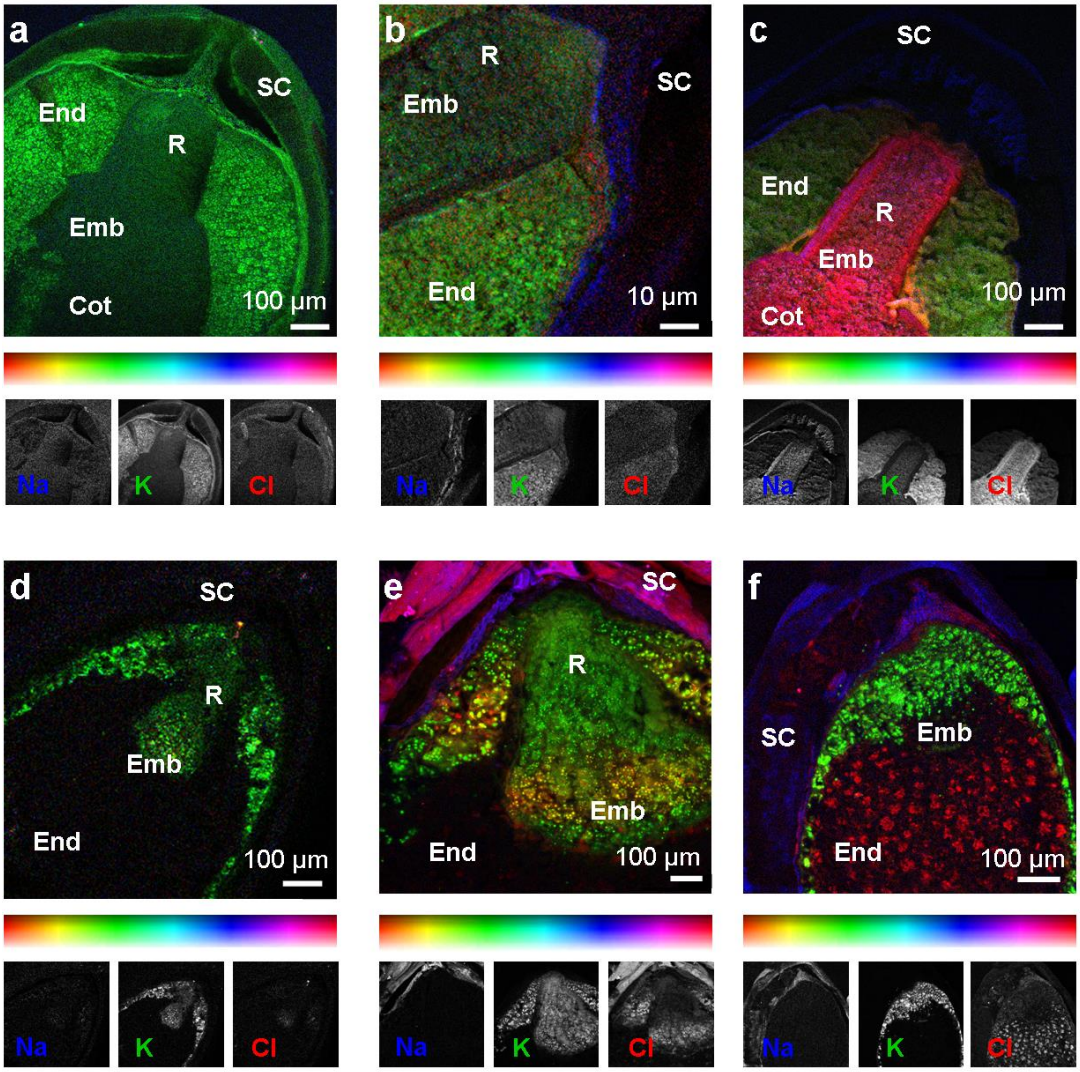


Figure 6. Pseudo-colour images and X-ray maps of *S. globulosum* seeds (**a-c**). **a.** prior to imbibition, **b**. exposed to 100 mM sodium chloride, **c**. exposed to 750 mM sodium chloride for 21 days, and (**d-f**) *F. nodosa* seeds exposed to **d**. water, **e**. 250 mM sodium chloride, and **f**. 500 mM sodium chloride for 15 days. Three background and overlap corrected X-ray maps are given below each pseudo colour image and show the localisation of each element (sodium, potassium and chlorine) within a sample. Dark areas indicate the element was not detected, and bright white represents the areas of highest concentration (as for **Figure 5**.). In pseudo colour images sodium (Na) is shown in blue, chlorine (Cl) red, and potassium (K) green. Areas where elements co-occur can be identified using the colour gradient [e.g. between red (chlorine) and green (potassium) on the gradient is yellow, therefore, yellow areas in pseudo colour images indicate equal co-occurrence of potassium and chlorine]. X-ray maps were performed with a Jeol 35CF scanning electron microscope with a dual energy dispersive spectrometer (**a, c, d**), or Jeol 733 microprobe with silicon drift detectors, and four wavelength dispersive spectrometers (**b, e, f**), with an accelerating voltage of 25 kV with collection conditions of 150 ms point $^{-1}$ and 512 x 512 pixel resolution. SC, seed coat; End, endosperm; Emb, embryo; R, radicle; Cot, cotyledons (embryo = cotyledons + radicle).

Discussion

In coastal habitats, salt tolerance is an important factor in the germination, survival, and establishment of plants, but also plays a role in the oceanic dispersal of seeds. The dynamics of salt movement within seed tissues during imbibition are poorly understood and the novel application of X-ray mapping employed in this study has provided insights into the spatial distribution, ion exchange, and regulation of sodium, potassium, and chlorine within and between seed tissues for two coastal species during the course of imbibition. Further, the patterns of ion localisation can be linked to the germinability and viability of seeds following exposure to saline conditions during imbibition. Coupled with germination experiments and flame photometry, X-ray mapping has revealed that seeds of a salt-tolerant species (*Ficinia nodosa*) exclude sodium. The seeds may compartmentalise, or selectively fail to exclude, chlorine into endosperm cells when exposed to high sodium chloride for extended periods. These data indicate that *F. nodosa* seeds are well adapted to survival in saline environments. In contrast, seeds of the salt-sensitive species (*S. globulosum*) imbibe sodium and chlorine. Although *S. globulosum* seeds are able to tolerate sodium and chlorine uptake when exposed to lower concentrations or for shorter durations, the cumulative effects over time and the increased uptake with increasing external concentration reduce germinability and viability.

In seeds of *S. globulosum* the harmful elements of salt (sodium and chlorine) displace potassium and accumulate to toxic levels in the embryo. The negative effects of sodium on plant growth are often attributed to ionic imbalance (Greenway and Munns 1980) and this is probably the cause of seed death, given that increased mortality corresponded with the accumulation of sodium within seed tissues, which increased proportionally as sodium chloride concentration increased. It was apparent through X-ray mapping that, during the first 7 days of imbibition, some sodium entered the seed at lower sodium chloride concentrations, but it was not localised to any specific internal tissues and there was no reduction in germination. At 750 mM sodium chloride, while germination declined somewhat, sodium appeared localised to the inner edge of the seed coat. After 14 or 21 days' imbibition at 750 mM, when germination was most severely impacted and the concentration of sodium was

approximately four times higher than that in dry seeds, sodium had accumulated predominately within the embryo. Sodium is likely to be most toxic to seeds when accumulated within the embryo.

As predicted, chlorine also became localised and most concentrated in the embryo, rather than being distributed throughout all seed tissues. However, X-ray mapping showed that sodium and chlorine did not merely co-occur throughout seed tissues, as sodium was present in the seed coat after exposure to 750 mM sodium chloride for all treatment times, but chlorine was not detected in the seed coat during the early phases of imbibition (i.e. in the first 7 days). Imbibition of *F. nodosa* seeds for extended periods (15 days; equal to late phase III of imbibition for seeds in pure water) in any sodium chloride concentration resulted in reduced germination upon transfer to pure water, but this loss in germinability was not associated with any increase in internal sodium. Rather, it appears the reduction in germination may have been caused by chlorine that unexpectedly moved through the seed embryo at moderate concentrations of external sodium chloride (250 mM). However, at high concentrations (500-750 mM sodium chloride), chlorine appeared to be compartmentalised in endosperm tissue. Further investigation of compartmentalisation via mapping of individual endosperm cells was not attempted. Samples were mapped and interpreted at the tissue level only as some movement of ions within or between cells may have occurred during preparation. Future investigations using CryoSEM methods would probably provide further insight by describing the detailed localisation or compartmentalisation of ions within cells. The ability to compartmentalise chlorine away from the embryo may explain why *F. nodosa* seeds tolerate higher exposure to salt compared with *S. globulosum* seeds. In transpiring plants chlorine has been implicated in root injury and even slight damage to roots can cause large increases in the uptake of ions to the shoot (Greenway and Munns 1980). The implication for seeds is unclear, however, the presence of excess chloride ions in the embryo and particularly the radicle (embryonic root), is perhaps as detrimental to imbibing and germinating seeds as sodium ions.

In *S. globulosum* seeds, the decrease in internal potassium was proportional to the external sodium chloride concentration and the time of imbibition. Similar results were found for salt-sensitive seeds of *Acacia coriacea* DC. Rehman *et al.* (1998), in

which potassium leakage was associated with greater sodium uptake. In plants, the maintenance of a high internal potassium concentration, or a high potassium/sodium ratio, has been associated with salt tolerance and some similar investigations have been made for seeds (Greenway and Munns 1980; Rehman *et al.* 1998; Rehman *et al.* 2000). Here the internal potassium content of the more salt-tolerant *F. nodosa* seeds decreased only minimally, whereas in salt-sensitive *S. globulosum* seeds potassium was reduced by more than half. Thus, the patterns of sodium, potassium and chlorine movement in seeds of *F. nodosa* and *S. globulosum* are consistent with those seen for salt-tolerant and sensitive *Acacia* spp. (Rehman *et al.* 1998; Rehman *et al.* 2000). Salt-tolerant seeds of *F. nodosa* did not take up sodium or leak potassium, and X-ray mapping showed that potassium was higher in the embryo than the endosperm. The salt-sensitive seeds of *S. globulosum* showed high sodium uptake and potassium leakage, with potassium more abundant in the endosperm than the embryo. Similarly, Rheman *et al.* (1998; 2000) found that salt tolerance in *Acacia* was related to low sodium uptake, low potassium leakage and initially higher potassium in the embryo. *Ficinia nodosa* seeds had half the internal potassium of *S. globulosum* seeds (on a whole-seed basis) in a similar way that salt-tolerant *Acacia tortilis* Hayne seeds had a lower initial concentration of potassium on a whole-seed basis than the salt-sensitive *A. coriacea* (Rehman *et al.* 1998). These trends in sodium and chlorine uptake, and potassium leakage from seed embryos appear indicative of salt sensitivity during seed germination.

For both study species, an increase in external sodium chloride concentration not only affected germination and ion localisation, but also somewhat reduced the rate of imbibition. Reductions in water uptake are likely to be osmotic effects in phase I and the chemical properties of the apoplast are likely to have the greatest effect during this stage. Imbibition in solutions of lower salinity delayed entry into phase III (radicle emergence) and this was particularly evident in seeds of *F. nodosa* where seeds exposed to 100 mM sodium chloride entered phase III later (after 15 days in solution) than seeds imbibed in fresh water (after 10 days in solution). Imbibition in salt solutions of > 100 mM, inhibited entry into phase III but, contrary to the assumption that salinity inhibits germination by preventing water uptake (Debez *et al.* 2004), seeds exposed to all experimental salt concentrations did imbibe. Increasing sodium chloride concentration in the imbibing solution generally resulted

in a progressive decrease in total water uptake by the seeds, probably because of a decrease in the water potential gradient between the seeds and the external solution (Katembe *et al.* 1998). Salt may affect water uptake during phase III by preventing cell division in a number of ways. It is possible that high external sodium chloride concentrations, which reduce the osmotic gradient, prevent the cells from achieving sufficient turgor pressure to expand and enter phase III. Some species survive salt exposure by taking up solutes, for example Na^+ and K^+, to maintain turgor (Greenway and Munns 1980). The uptake of sodium by *S. globulosum* seeds in lower external salt concentrations, and during the early stages of imbibition, may assist in the maintenance of turgor and may be effective at lower external sodium chloride concentrations.

Seeds of *S. globulosum* imbibe slowly compared with *F. nodosa* and require comparatively greater amounts of water for germination. Time to germination and the amount of sodium imbibed by *S. globulosum* seeds are traits that may result in a greater potential for the accumulation of detrimental ions. Salt-sensitive plants grown under highly saline conditions have been described as having intrinsically lower relative growth rates than salt-tolerant taxa, and as accumulating higher chloride ion concentrations in leaves (Greenway and Munns 1980). This may also be true for seeds with lower relative growth rates reflecting lower salt tolerance. The fast imbibition and germination rate of *F. nodosa* seeds may facilitate sodium exclusion and maintenance of low internal chlorine. A relationship between rapid germination and salt tolerance has been suggested for other salt-tolerant species (Rehman *et al.* 1998; Rehman *et al.* 2000) but the mechanism remains to be investigated.

The mechanisms by which salt-tolerant seeds excluded sodium are unresolved. Cellular channels and pumps are responsible for ion movement in other plant or seed tissues. The results of this investigation provide some indication of how ions may move between seed tissues during germination. Two types of non-selective channels that are permeable to K^+ and Na^+ may play a similar role in the uptake of ions during seed germination as they do in the release of ions from seed coats to the apoplasm of developing seeds (Zhang *et al.* 2007). In *F. nodosa* seeds, chlorine uptake is not associated with the uptake of a positive ion (e.g. sodium), or a decrease of another

element (e.g. potassium), suggesting other mechanisms may be involved. Pore size cannot be responsible for exclusion as ionic chloride is larger (0.181 nm) than sodium (0.102 nm), and reported pore sizes for various plant tissues are 3.5-5.2 nm, 20-50 times larger than sodium and chloride ions (Carpita *et al.* 1979). Therefore, it is likely that sodium is excluded from *F. nodosa* seeds via a selective channel or an ionic pump. During seed development in a number of species, large amounts of mineral nutrients, notably potassium and chloride ions, are released from seed coats to the seed apoplasm (Zhang *et al.* 2007). A pulsing chloride permeable anion efflux channel was recently characterised and is suggested to be the route for chloride release into the apoplasm, and a non-selective membrane pore present in seed coats has also been suggested for seeds of *Phaseolus vulgaris* (Zhang *et al.* 2007). The internal localisation of chloride in *F. nodosa* seeds may be facilitated by similar anion pores or channels. The movement of ions between seed tissues during germination has not been researched and future investigations of transport are likely to reveal the precise mechanisms by which seeds are able to tolerate germination in saline conditions. Studies of this type will provide a guide for development of seeds that are resistant to salt during the early phases of germination and will have implications in many fields.

References

Atia A, Debez A, Barhoumi Z, Pacini E, Abdelly C, Smaoui A (2010) The mericarp of the halophyte *Crithmum maritimum* (Apiaceae): structural features, germination, and salt distribution. *Biologia* **65**, 489-495.

Bewley DJ (1997) Seed germination and dormancy. *The Plant Cell* **9**, 1055-1066.

Bewley DJ, Black M (1994) 'Seeds: physiology of development and germination.' (Plenum Press: New York).

Black M (2009) Darwin and seeds. *Seed Science Research* **19**, 193-199.

Carpita N, Sabularse D, Montezinos D, Delmer DP (1979) Determination of the pore size of cell walls of living plant cells. *Science* **205**, 1144-1147.

Cramer GR, Lauchli A, Polito VS (1985) Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells: a primary response to salt stress? *Plant Physiology* **79**, 207-211.

Darwin C (1856) On the action of sea-water on the germination of seeds. *Journal of the Proceedings of the Linnean Society of London. Botany* **1**, 130-140.

Darwin C (1859) ' The origin of species.' (Oxford University Press: Oxford).

Debez A, Hamed KB, Grignon C, Abdelly C (2004) Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. *Plant and Soil* **262**, 179-189.

Flowers TJ, Hajibagheri MA, Clipson NJW (1986) Halophytes. *The Quarterly Review of Biology* **61**, 313-337.

Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK (2011) Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology & Evolution* **27**, 1-10.

Goldstein J, Newbury D, Joy D, Lyman C, Echlin P, Lifshin E, Sawyer L, Michael J (2003) 'Scanning Electron Microscopy and X-Ray Microanalysis.' (Kluwer Academic/Plenum Publishers: New York).

Greaver TL, Sternberg LSL (2007) Fluctuating deposition of ocean water drives plant function on coastal sand dunes. *Global Change Biology* **13**, 216-223.

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* **31**, 149-90.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**, 1175-1186.

Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 463-499.

Howarth DG, Gustafsson MHG, Baum DA, Motley TJ (2003) Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacifc Basin and colonization of the Hawaiian Islands. *American Journal of Botany* **90**, 915-923.

International Seed Testing Association (ISTA) (1999) International rules for seed testing. *Seed science and technology* **27 (Suppl)**, 1-35.

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

Khan MA, Webber DJ, Hess WM (1985) Elemental distribution in seeds of the halophytes *Salicornia pacifica* var. *utahensis* and *Atriplex canescens*. *American Journal of Botany* **72**, 1672-1675.

Lott JNA, Spitzer E (1980) X-Ray analysis studies of elements stored in protein body globoid crystals of *Triticum* grains. *Plant Physiology* **66**, 494-499.

McCully ME, Canny MJ, Huang CX, Miller C, Brink F (2010) Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: energy dispersive X-ray microanalysis (CEDX) applications. *Functional Plant Biology* **37**, 1011-1040.

Michel BE (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology* **72**, 66-70.

Moran K, Wuhrer R (2006) Quantitative bulk and trace element X-ray mapping using multiple detectors. *Microchimica Acta* **155**, 59-66.

Nathan R (2006) Long-distance dispersal of plants. *Science* **313**, 786-788.

Necajeva J, Ievinsh G (2008) Seed germination of six coastal plant species of the Baltic region: effect of salinity and dormancy-breaking treatments. *Seed Science Research* **18**, 173-177.

Nichols PGH, Malik AI, Stockdale M, Colmer TD (2009) Salt tolerance and avoidance mechanisms at germination of annual pasture legumes: importance for adaptation to saline environments. *Plant Soil* **315**, 241-255.

Nonogaki H, Bassel GW, Bewley JD (2010) Germination - still a mystery. *Plant Science* **179**, 574-581.

Rehman S, Harris PJC, Bourne WF (1998) The effect of sodium chloride on the Ca^{2+}, K^{+} and Na^{+} concentrations of the seed coat and embryo of *Acacia tortilis* and *A. coriacea*. *Annals of Applied Biology* **133**, 269-279.

Rehman S, Harris PJC, Bourne WF, Wilkin J (2000) The relationship between ions, vigour and salinity tolerance of *Acacia* seeds. *Plant and Soil* **220**, 229-233.

Shabala S, Newman I, Wilson S, Clark R (2000) Nutrient uptake patterns over the surface of germinating wheat seeds. *Australian Journal of Plant Physiology* **27**, 89-97.

Turner SR, Merritt DJ, Baskin CC, Dixon KW, Baskin JM (2005) Physical dormancy in seeds of six genera of Australian Rhamnaceae. *Seed Science Research* **15**, 51-58.

Woodell SRJ (1985) Salinity and seed germination patterns in coastal plants. *Vegetatio* **61**, 223-229.

Zhang WH, Zhou Y, Dibley KE, Tyerman SD, Furbank RT, Patrick JW (2007) Nutrient loading of developing seeds. *Functional Plant Biology* **34**, 314-331.

Chapter 7

General discussion

Introduction

Dispersal is critical for plants because they are sessile organisms that rely on dispersal of seeds or other propagules to move offspring to suitable habitat away from the parent plant. Dispersal patterns affect distribution, abundance and genetic processes at local, landscape and biogeographic scales (Bullock and Nathan 2008; Cousens *et al.* 2008; Nilsson *et al.* 2010). This thesis examined one of the least investigated modes of dispersal, oceanic hydrochory, and provides the first mechanistic investigation of critical processes required for successful oceanic seed dispersal. Chapter 2 described the system selected for study, a coastal plant community in Western Australia. The following chapters addressed questions in two broad areas: seed dispersal and germination. Chapters 3 and 4 focussed on seed dispersal by investigating the effect of interspecific and intraspecific seed variation on buoyancy. Chapter 5 assessed seed germination thresholds by investigating germination under a broad range of temperature, salinity and water stress conditions. Finally, Chapter 6 investigated the biological mechanisms of salt tolerance in germinating seeds. The research presented in this thesis addressed these two areas (dispersal and germination) by:

Dispersal

- Creating a model for the prediction of seed buoyancy using well defined, easily measured, morphological seed traits

- Identifying intraspecific variation in seed traits, their influence on buoyancy, and the specific seed and fruit tissues that increase buoyancy

Germination

- Describing the individual and interactive effects of salinity, osmotic stress and temperature on seed germination and the implications for dispersal and germination of seeds from different source populations

- Investigating the salt tolerance mechanisms of seeds of coastal species with contrasting salt sensitivity to identify if, when, and where sodium chloride enters germinating seeds

The four main experiments described in this thesis are complementary and collectively addressed processes that determine successful oceanic dispersal. This final chapter synthesises the research findings and highlights the significance and contribution of this work to relevant fields of study. Finally, future research opportunities in oceanic seed dispersal are discussed at the conclusion of this chapter.

Morphological seed traits conducive to oceanic seed dispersal

Seed dispersal involves a number of processes including removal from the parent plant, movement to a substrate, movement along a substrate, and arrival at the final location (Cousens *et al.* 2008). For hydrochorous dispersal, it is the buoyancy potential of a seed that determines movement along the substrate and therefore the distance that a seed may be dispersed. The relationship between seed morphology and buoyancy has been investigated in freshwater systems (Lopez 2001; van den Broek *et al.* 2005; Leyer and Pross 2009), but had not been investigated for oceanic dispersal. This thesis determined the morphological traits of coastal plant seeds that affect buoyancy in seawater and, therefore, the traits that increase the potential for seeds to be dispersed by ocean currents.

Interspecific variation

Chapter 3 presented the first models created specifically for the prediction of oceanic hydrochorous dispersal for a range of seed morphology types from a coastal plant community. Researchers now have access to models that allow prediction of oceanic

hydrochory based on easily measured seed morphological traits. These new models for hydrochorous dispersal will complement those available for other dispersal syndromes, such as epizoochorous dispersal (Römermann *et al.* 2005; Tackenberg *et al.* 2006; de Pablos and Peco 2007; Will *et al.* 2007), and anemochorous dispersal (Matlack 1987; Andersen 1993). Although proximity to the ocean was not related to buoyancy in this study, this may be because there was not a wide degree of variation in distance from the coastline because all studied species were coastal plants. Perhaps comparison of SWWA coastal species with species from other habitats may reveal higher buoyancy in coastal species. A similar relationship has been demonstrated for riparian species, which have greater seed buoyancy than species from non-flooded areas (Lopez 2001; van den Broek *et al.* 2005). Alternatively, the rarity of oceanic hydrochory for non-adapted species and the consequent low selection pressure may result in relatively equal buoyancy between coastal and non-coastal species and it would be interesting to explore this further. Testing of our models outside the study system would also be worthwhile. Other dispersal models based on Australian flora adequately predicted dispersal mechanisms for species of other temperate regions (Thomson *et al.* 2010).

For the species studied, morphology type had a significant effect on buoyancy; however, this relationship was only evident for up to 3 days in seawater. Beyond 3 days the best predictor of buoyancy was specific weight, which has been identified as a strong predictor of seed buoyancy in fresh water systems (Lopez 2001). During the first 3 days of exposure to seawater, flattened, woody and membranous seeds were significantly more buoyant than other seed morphologies. These results support the hypothesis that woody or cork-like seeds/fruits have a high buoyancy potential (Werker 1997; Cousens *et al.* 2008; Vargas *et al.* 2014). However, the identification of membranous and flattened seeds as highly buoyant is novel in dispersal ecology. It had also been hypothesised that flattened or winged appendages may increase the buoyancy potential of seeds (Higgins *et al.* 2003), a prediction that was supported by our models. The similarly high buoyancy of various morphology types (flattened, membranous and woody seeds) in our hydrochory models was in contrast to other dispersal syndromes which are generally associated specifically with a single morphology e.g. arillate seeds are dispersed by ants (Hughes and Westoby 1992b;

Hughes and Westoby 1992a; Werker 1997) and elongated/flattened seeds are dispersed by wind (Matlack 1987; Hensen and Müller 1997).

Polychory (one seed type dispersed by multiple modes), and dispersal by nonstandard modes (e.g. flattened wind-adapted seeds also dispersed by water) may be a significant factor for oceanic dispersal of non-adapted seeds. Certainly, only one quarter of 78% of taxa that arrived by sea currents and colonised a recently exposed volcanic island were classed as morphologically adapted to water dispersal (i.e. contained airspaces) (Higgins *et al.* 2003). Evidently, a large knowledge gap exists in relation to the seed traits that are associated with buoyancy. Several morphology types displayed equally high buoyancy in this study, demonstrating that oceanic hydrochory may be a non-standard mode of dispersal for many seed types and may be more common than commonly thought. Significantly, polychory and non-standard dispersal may increase the probability of long-distance dispersal (Higgins *et al.* 2003; de Pablos and Peco 2007) through chance events such as oceanic hydrochory. The potential for non-standard dispersal of coastal seeds by the ocean is important given the relatively high frequency with which coastal seeds may be deposited in the ocean. By focussing on seeds that are not clearly adapted for dispersal by the ocean, this research allowed mechanistic assessment of the buoyancy potential of 'non-adapted' seeds. The finding that many seeds were buoyant supports phylogenetic evidence that suggests that the distribution of many 'non-adapted' lineages is the result of oceanic dispersal (e.g. the disjunct distributions of Fabaceae and Lauraceae, which have large, seemingly non-adapted seeds; Christenhusz and Chase 2013).

The simple models presented in Chapter 3 were designed to estimate buoyancy using easily obtainable seed trait measurements to complement any suggestion that hydrochory is a likely explanation for phylogeographic and biogeographic patterns. These models allow greater confidence in conclusions about the drivers of biogeography, provided that there have not been significant changes in seed morphology over the evolutionary timelines considered. The ability to more accurately predict oceanic dispersal, especially up to 8 days of buoyancy, rather than continuing the current practice of explicitly or implicitly assuming either no or unlimited dispersal as in most species distribution modelling methods (Matzke 2013)

will benefit many researchers including phylogeneticists, biogeographers and species distribution modellers.

Intraspecific variation

While Chapter 3 demonstrated that differences in morphological seed traits between species have a significant effect on the duration of buoyancy and the proportion of buoyant seeds in seawater, Chapter 4 examined fruit morphology and anatomy in one species to determine the features that have the greatest effect on the buoyancy of woody fruits. Woody fruits are generally considered to be one of the most buoyant fruit types, but the mechanism of buoyancy is often unknown (Werker 1997; Cousens *et al.* 2008; Vargas *et al.* 2014). In Chapter 4 it was empirically demonstrated that larger aeriferous mesocarp increases fruit buoyancy and can, therefore, increase the probability and distance of hydrochorous dispersal. These data support propositions made in other studies that aeriferous fruit/seed coats increase buoyancy (Lopez 2001; Cousens *et al.* 2008; Guja *et al.* 2010; Nilsson *et al.* 2010) and demonstrate that the positive relationship described between pericarp thickness and buoyancy of achenes (Hroudova *et al.* 1997) is similar for drupe-like woody fruits with multiple locules.

It remains unclear whether intraspecific variation in fruit morphology (Chapter 4) and germination behaviour (Chapter 5) described in this thesis is driven by genetic or environmental effects or, most likely, an interaction between them. Environmental conditions can influence the maternal environment of the seed, which can in turn affect germination, seed dormancy, viability, and responses to environmental stress (Donohue 2009). Further investigation using common garden or reciprocal transplant experiments (Kim and Donohue 2013) could reveal the relative contribution of genetic and environmental factors to the observed variability in seed morphology and germination traits.

Investigation of within-population variation would provide complimentary and powerful results that may help tease apart genetic and environmental effects. Study of maternal families would allow exploration of the biology and heritability of seed morphological and dispersal traits, and may reveal selective pressure exerted on these traits. While within-population investigations may address differences between

individual plants/maternal lines it would also be beneficial to better understand the effects of variation within individuals e.g. the variation between basal and distal end seeds and how those variances contribute to within and between population patterns. For all of these experiments the sampling would be more robust if bags were placed over infructescences prior to dispersal, so that all seeds are released naturally and unintentional sampling for early or late seeds can be avoided.

Physiological seed traits that affect seed germination after oceanic dispersal

The success of oceanic dispersal is determined by a seed's buoyancy potential as well as the synergy between its germination thresholds or establishment niche and the new environment. Long-distance oceanic dispersal is likely to result in a seed being deposited in an environment that differs from its maternal environment. Therefore, a combination of broad germination niche and variation between populations, which contribute to species adaptability or evolutionary potential, may facilitate germination and, therefore, successful oceanic dispersal. This thesis explored germination thresholds under different temperature, salinity and water stress conditions for two common coastal species (Chapter 5) and the mechanisms of salt tolerance during imbibition and germination (Chapter 6).

Germination thresholds in saline environments

Chapter 5 demonstrated that seeds are most sensitive to salinity at non-optimal temperatures (5 and 20 °C for *Spyridium globulosum*; 10 and 25 °C for *Ficinia nodosa*). Therefore, salt sensitivity is likely to be intensified as species disperse to, or experience, environments of non-optimal temperature during germination. Furthermore, the effects of salinity on germination phenology may cause germination at a time of year that is not conducive to survival. For example, the reduction in germination rate caused by high salinity may result in seeds only completing germination at the end of the rainfall period, which would likely reduce their probability of survival. Such interaction between temperature and salinity (Ungar 1978; Gul *et al.* 2013) demonstrates that recruitment is limited by a number of environmental conditions. A combination of various abiotic factors typical of

dynamic coastal environments (Maun 1994) may have a greater combined effect on germination and recruitment than the effects of temperature thresholds alone (e.g. Cochrane *et al.* 2011). The combined effect of factors such as temperature, salinity, water stress and other environmental variables will likely affect germination and establishment of dispersed seeds along coastlines, which can ultimately influence community composition and vegetation zonation (Coops and van der Velde 1995; Rand 2000).

The germination niche of seeds of both study species varied between populations. Therefore, some populations may have greater recruitment capacity than others in new environments. A significant and novel result of this investigation was that seed from a single population exhibited different salt responses under different experimental conditions. To our knowledge this is the first example of seed from a single population exhibiting more than one type of salt response (c.f. Guja *et al.* 2010; Appendix 1) and highlights the hitherto underappreciated effect of interactions between environmental variables, such as temperature and salinity on seed germination. Collectively, these results indicate that the interaction between temperature, ion toxicity, water stress, and within-species variation will affect germination in new, post-dispersal, environments. However, in most experimental combinations a proportion of seeds were able to germinate, which suggests that some seeds can survive the process of oceanic dispersal and germinate in new environments.

Mechanisms of salinity tolerance

In coastal habitats, salt tolerance is an important factor for the germination, survival, and establishment of plants (Maun 1994; Greaver and Sternberg 2007). The dynamics of salt movement within seed tissues during imbibition and germination are poorly understood although they may shed light on the differing salt tolerance of species. The novel application of X-ray mapping employed in this study (Chapter 6) provided insights into the spatial distribution of sodium, potassium, and chlorine within and between seed tissues of two coastal species during the course of imbibition. Salt-tolerant *F. nodosa* seeds excluded sodium, but surprisingly some chlorine did migrate through the seed embryo. Following exposure to high

concentrations of sodium chloride (500-750 mM NaCl) or extended exposure over the entire course of imbibition, chlorine appeared to be compartmentalised in the endosperm. Compartmentalisation is a common salt tolerance mechanism in halophytic plants (Flowers *et al.* 1986), yet this study may provide the first evidence of compartmentalisation in seeds. Conversely, seeds of the salt-sensitive *S. globulosum* absorbed both sodium and chlorine without evidence of compartmentalisation. While these seeds could tolerate high concentrations of external sodium chloride (500-750 mM NaCl) for short durations (1-7 days), greater exposure time resulted in accumulation of sodium in the growing embryo which led to potassium displacement, ion toxicity and death of *S. globulosum* seeds. This finding supports suggestions that sodium is most toxic to seeds when accumulated within the embryo (Rehman *et al.* 1998). The patterns of ion localisation were linked to the survival and germination of seeds following exposure to saline conditions during imbibition. Patterns of sodium and chlorine uptake, and potassium leakage from seed embryos, appear indicative of salt sensitivity during seed germination. Salinity response is likely to significantly affect the dispersal ability of seeds in seawater and also their germination and recruitment following dispersal to new environments.

Summary

As outlined above, this thesis has elucidated the capacity for, and mechanisms of, coastal seed dispersal via the ocean. Overall, I have demonstrated that many non-adapted seeds do in fact have a high buoyancy potential and can germinate across a relatively broad range of environmental conditions. These key findings are integrated into a broader conception of the process of oceanic hydrochory in **Figure 1**.

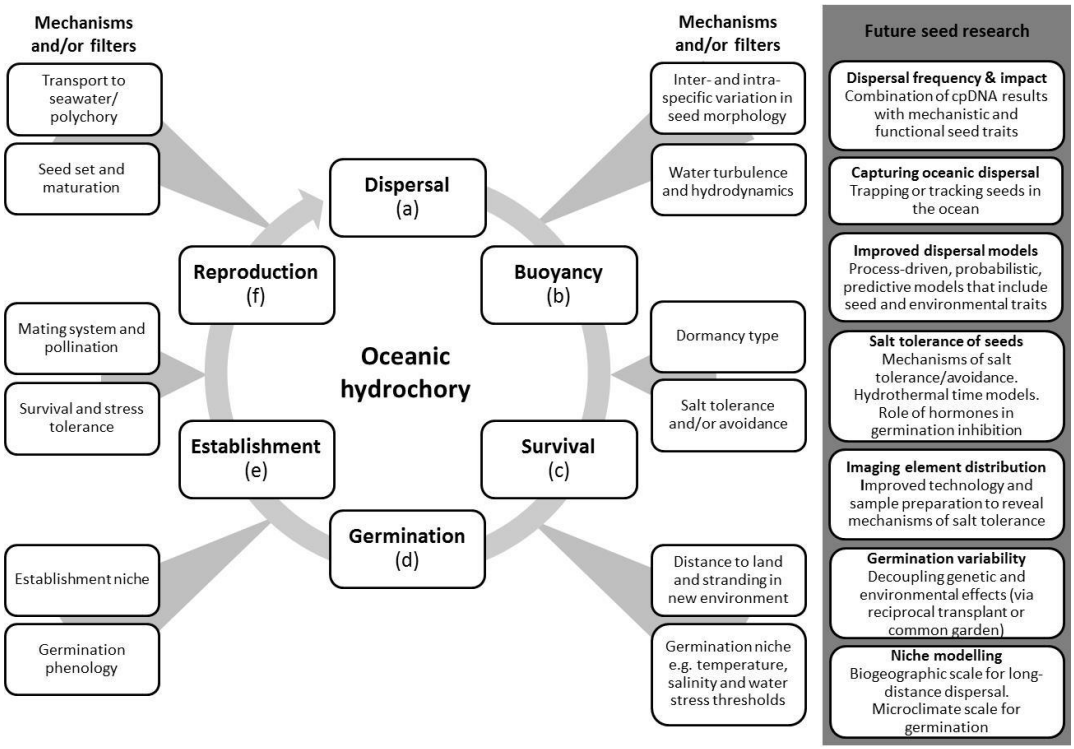


Figure 1. Overview of phases of the oceanic dispersal process (a-f). The mechanisms that allow, or filters that select against, seeds progressing through each phase are summarised in columns alongside the central oceanic dispersal process. Suggested directions for future research are presented in the panel on the right. The ultimate goal of future research should be to identify the probability of, and major drivers for, successful dispersal by quantitatively linking the mechanisms and/or filters to the phases of the dispersal process.

Future research

Although this thesis describes significant progress in advancing knowledge of the capacity for, and mechanisms of, oceanic seed dispersal, I now draw attention to several areas of potentially fruitful research in the field of seed biology (**Figure 1**).

Quantifying the frequency and impact of oceanic dispersal

Phylogenetic studies frequently posit that oceanic seed dispersal is a possible explanation for the extant distribution of plant lineages (e.g. Howarth *et al.* 2003; Kokubugata *et al.* 2012; Vargas *et al.* 2014). The research presented in this thesis and Appendix 1 (Guja *et al.* 2010) has shown that many coastal species are in fact capable of dispersal by the ocean. However, it would be informative to directly investigate the frequency of oceanic dispersal to truly understand its impact on

species distributions and genetic structure. Strategies spanning a range of disciplines are suggested to address this aim.

Research combining genetic techniques with supporting investigation of mechanistic and functional seed traits could greatly improve understanding of oceanic dispersal. While seed functional traits reveal capacity for dispersal, genetic research can reveal patterns and frequency of realised dispersal. For example, comprehensive phylogenetic studies can help identify how many times a lineage may have been dispersed between landmasses and thereby provide indirect estimates of the frequency of successful oceanic dispersal within a lineage (Howarth *et al.* 2003). Similarly, population genetic or phylogeographic studies could be used to estimate gene flow between disjunct coastal populations or between islands (Cowie and Holland 2006). Estimates of gene flow, particularly if based on maternally inherited chloroplast DNA (cpDNA) that is transmitted only via seed (Birky 1995), would give an estimate of the frequency with which seeds are transported between disjunct populations. It would be instructive to combine these genetic results with measurements of buoyancy potential, which is accumulating in online databases (e.g. Kleyer *et al.* 2008), to test for correlation between high buoyancy and estimated gene flow, or high buoyancy and large disjunctions within species' distributions.

While the suggestions in the above paragraph can strengthen inferences about the frequency and evolutionary significance of oceanic hydrochory, it is also possible that long-distance dispersal across oceans occurs via other means e.g. rafting or adhesion/ingestion by migratory birds (Higgins *et al.* 2003; Minchinton 2006; Luiz *et al.* 2012). Ultimately, to resolve these uncertainties, it is necessary to accumulate evidence of seeds physically undergoing oceanic dispersal. Seed traps and marked and recaptured seeds (Wolters *et al.* 2004) have been used in riparian systems that are inherently contained and often result in unidirectional and predictable dispersal direction. In contrast, the wide expanse of the ocean and the multiple currents, eddies and effects of surface winds on propagule movement greatly reduce the likelihood of recovering experimental seeds (Van der Stocken *et al.* 2013). Some investigators have attempted dispersal studies using seed mimics released in the ocean but the recovery rate decreased greatly with dispersal distance so that conclusions about long-distance dispersal were not possible (Yang *et al.* 2012). However, technology

has progressed and sophisticated tracking of seeds is, or will shortly be, possible. An exciting new innovation is the proposed development of seed analogues, which mimic the buoyancy and morphology of real seeds (Smith *et al.* 2011). These analogues will be fitted with wireless sensor technology (which could presumably also be attached to large seeds of some species) that will allow tracking of the analogues as they are transported by the ocean. Data collected from these analogues will allow realistic and accurate dispersal models over vast geographic scales (Smith *et al.* 2011).

Improving models of oceanic dispersal

The buoyancy models derived from seed morphology and presented in this thesis are the only predictive models for oceanic hydrochory to date. However, several improvements could be made in the future. Future models would benefit from inclusions of factors beyond seed traits and buoyancy including environmental variables such as current direction and speed, the effects of swell, and the movement of surface water and buoyant seeds due to wind (Van der Stocken *et al.* 2013). Detailed environmental information could greatly improve dispersal models in a similar way to the improvements gained through the inclusion of environmental variables such as turbulence in models of wind dispersal (Greene and Johnson 1989; Horn *et al.* 2012) or hydraulics and hydrology in models of hydrochory in rivers (Merritt and Wohl 2002).

Process-driven probabilistic models of oceanic dispersal would also improve predictions by including data on dispersal and germination, because both are necessary factors for successful colonisation of a new environment. Ideally, future models of oceanic dispersal would take into account intraspecific variation in dispersal units, particularly because intraspecific variation can ‘fatten’ the tails of a dispersal kernel, which is of particular relevance to long-distance dispersal (Chapter 4; Nathan 2006; Nathan *et al.* 2008). The inclusion of germination probability data in models of oceanic dispersal is important as seeds may often be dispersed to environments that do not meet their optimal germination requirements. This is critical in saline conditions where non-optimal temperature regimes can significantly reduce germination (Chapter 5). Ultimately, data-driven, probability-based models

that incorporate as many aspects of the dispersal processes as possible will allow the accurate description and prediction of oceanic dispersal.

Niche modelling

Future germination research would benefit from the assessment of the specific microclimates that would be experienced by seeds during post-dispersal germination. The data presented in Chapter 2 are a step forward in this direction, but better resolution of different soil depths would be beneficial. A focus on microclimates has enabled the accurate and mechanistic prediction of hourly local microclimates across continental scales, which has created new opportunities for understanding how organisms respond to changes in climate (Kearney *et al.* 2014). However, these models require detailed data on physiological responses to the environment. It is now possible to assess germination microclimates in the field with the more widespread use of data loggers such as those used in this thesis (Chapter 2). Further, the increased adoption of thermo-gradient tables allows the simultaneous testing of multiple levels of temperature to elucidate seed germination thresholds under controlled conditions (Cochrane *et al.* 2011). When combined with additional experiments to determine physiological thresholds for other environmental factors significant for coastal plants (e.g. salinity), thermo-gradient tables will allow detailed germination niche mapping. Ultimately, environment-matched experimental conditions will allow for fine-scale mapping of the germination niche of coastal plants, and this can be used to estimate the probability of post-dispersal germination.

Germination in saline conditions

Although the germination of several plant species in saline conditions has been studied (e.g. reviewed by Gul *et al.* 2013), variation between and within species makes it difficult to predict seed germination under numerous combinations of temperature and salinity. Future research aiming to predict germination under saline conditions may benefit from the adoption of hydrothermal time models. These models allow an understanding of osmotic thresholds for germination under a range of temperatures by using data from controlled experiments to predict how and when germination will be reduced under field conditions (Allen 2003). Although these

models can only be used to understand germination thresholds, rather than distinguish salt toxicity from water stress, they have recently been applied to predict/describe germination patterns under different salinity and temperature regimes (Zhang *et al.* 2012). Hydrothermal time models can easily incorporate germination rate data and may therefore be beneficial for investigating observations that salt-sensitive plants grown under highly saline conditions have intrinsically lower relative growth rates than salt-tolerant plants (Greenway and Munns 1980). Using hydrothermal time analysis it would be interesting to test whether salt-sensitive seeds also have lower relative germination rates than salt-tolerant seeds, as has been documented for some species (Rehman *et al.* 1998; Rehman *et al.* 2000).

Commonly, salt-tolerant seeds survive saline conditions through inhibition of germination, maintenance of viability, and germination once salinity is alleviated (e.g. *Ficinia nodosa*). However, the methods by which salt-tolerant seeds are able to inhibit germination in saline conditions remain unknown. In salt-tolerant plants the levels of abscisic acid (ABA) increase under salt stress (Carillo *et al.* 2011). It may be helpful to determine whether a similar accumulation of ABA also occurs in seeds during exposure to salinity. If ABA does accumulate in seeds it would have a major effect on seed germination because it is the removal or deactivation of ABA, and the interaction of ABA with gibberellin (GA), that regulates seed germination (Nonogaki *et al.* 2010). This line of enquiry is particularly interesting because GA is known to alleviate some of the effects of salinity on germination (i.e. increase salt tolerance), and because the magnitude of this effect is larger in halophytes than in glycophytes (Ungar 1978; Hanslin and Eggen 2005).

The imaging of the distribution of elements in plants is a field of research that is rapidly growing (reviewed by Zhao *et al.* 2014). Further work using advanced microscopy techniques could increase our understanding of germination in saline conditions. In this thesis, salt tolerance and uptake by germinating seeds were analysed at the tissue level because some movement of ions within or between cells may have occurred during sample preparation. Future investigations could employ CryoSEM techniques that have been optimised for use with biological samples and would prevent ionic movement during sample preparation (McCully *et al.* 2010). Such methods could therefore be used to describe the specific localisation or

compartmentalisation of ions within cells (McCully *et al.* 2010; Zhao *et al.* 2014). Most previous work on the distribution of elements has focused on the movement of ions between seed tissues during maturation because of its significance in agricultural research (Lott *et al.* 1995; Zhang *et al.* 2007). Mature seeds and fruits have also been analysed in an attempt to determine whether inhibition of seed germination is related to the salt content of mature fruit coats at dispersal (Hocking 1982; Atia *et al.* 2010a; Atia *et al.* 2010b). It would be even more informative, however, if future microscopy work focussed on ion transport during germination (rather than ion content at seed maturation and dispersal) because this is more likely to reveal mechanisms by which seeds are able to germinate in saline conditions (Chapter 6; Shabala *et al.* 2000; Guja *et al.* 2013). Understanding salinity tolerance during germination may provide a guide for the development of crop species that are resistant to salt during the early stages of germination.

Summary

The research directions suggested above will further our understanding of oceanic hydrochory. The ultimate goal of future research should be to identify the probability and major drivers of successful dispersal by quantitatively linking the mechanisms of dispersal to the phases of the dispersal process. The most efficient approach to achieve these research goals will be to foster collaborations with experts in fields such as physiology, hydrology, oceanography and climatology.

Conclusion

This thesis has demonstrated how morphological and physiological seed traits influence the potential for oceanic seed dispersal. This research has contributed to dispersal ecology and seed biology by developing dispersal models, determining germination behaviour in new environments, and studying the mechanisms of salt tolerance in seeds. There is increasing evidence that oceanic dispersal is a significant ecological process and this thesis presents novel experimental findings that demonstrate that oceanic hydrochory may be more common among non-adapted species than generally appreciated.

References

Allen P (2003) When and how many? hydrothermal models and the prediction of seed germination. *New Phytologist* **158**, 1-3.

Andersen MC (1993) Diaspore morphology and seed dispersal in several wind-dispersed Asteraceae. *American Journal of Botany* **80**, 487-492.

Atia A, Debez A, Abdelly C, Smaoui A (2010a) Relationship between ion content in seed and spongy coat of the medicinal halophyte *Crithmum maritimum* L. and germination capacity. *Notulae Scientia Biologicae* **2**, 72-74.

Atia A, Debez A, Barhoumi Z, Pacini E, Abdelly C, Smaoui A (2010b) The mericarp of the halophyte *Crithmum maritimum* (Apiaceae): structural features, germination, and salt distribution. *Biologia* **65**, 489-495.

Birky CW (1995) Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 11331-11338.

Bullock JM, Nathan R (2008) Plant dispersal across multiple scales: linking models and reality. *Journal of Ecology* **96**, 567-568.

Carillo P, Annunziata MG, Pontecorvo G, Fuggi A, Woodrow P (2011) Salinity stress and salt tolerance. In 'Abiotic stress in plants - mechanisms and adaptations. (Eds A Shanker and B Venkateswarlu). (Intech: Online).

Christenhusz MJM, Chase MW (2013) Biogeographical patterns of plants in the neotropics – dispersal rather than plate tectonics is most explanatory. *Botanical Journal of the Linnean Society* **171**, 277-286.

Cochrane A, Daws MI, Hay FR (2011) Seed-based approach for identifying flora at risk from climate warming. *Austral Ecology* **36**, 923-935.

Coops H, van der Velde G (1995) Seed dispersal, germination and seedling growth of six helophyte species in relation to water-level zonation. *Freshwater Biology* **34**, 13-20.

Cousens R, Dytham C, Law R (2008) 'Dispersal in plants: a population perspective.' (Oxford University Press: New York).

Cowie RH, Holland BS (2006) Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. *Journal of Biogeography* **33**, 193-198.

de Pablos I, Peco B (2007) Diaspore morphology and the potential for attachment to animal coats in Mediterranean species: an experiment with sheep and cattle coats. *Seed Science Research* **17**, 109-114.

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1059-1074.

Flowers TJ, Hajibagheri MA, Clipson NJW (1986) Halophytes. *The Quarterly Review of Biology* **61**, 313-337.

Greaver TL, Sternberg LSL (2007) Fluctuating deposition of ocean water drives plant function on coastal sand dunes. *Global Change Biology* **13**, 216-223.

Greene DF, Johnson EA (1989) A model of wind dispersal of winged or plumed seeds. *Ecology* **70**, 339-347.

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* **31**, 149-90.

Guja L, Wuhrer R, Moran K, Dixon KW, Wardell-Johnson G, Merritt DJ (2013) Full spectrum X-ray mapping reveals differential localization of salt in germinating seeds of differing salt tolerance. *Botanical Journal of the Linnean Society* **173**, 129-142.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**, 1175-1186.

Gul B, Ansari R, Flowers TJ, Khan MA (2013) Germination strategies of halophyte seeds under salinity. *Environmental and Experimental Botany* **92**, 4-18.

Hanslin HM, Eggen T (2005) Salinity tolerance during germination of seashore halophytes and salt tolerant grass cultivars. *Seed Science Research* **15**, 43-50.

Hensen I, Müller C (1997) Experimental and structural investigations of anemochorous dispersal. *Plant Ecology* **133**, 169-180.

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* **84**, 1945-1956.

Hocking PJ (1982) Salt and mineral nutrient levels in fruits of two strand species, *Cakile maritima* and *Arctotheca populifolia*, with special reference to the effect of salt on the germination of *Cakile*. *Annals of Botany* **50**, 335-343.

Horn S, Raabe A, Will H, Tackenberg O (2012) TurbSeed - a model for wind dispersal of seeds in turbulent currents based on publicly available climate data. *Ecological Modelling* **237–238**, 1-10.

Howarth DG, Gustafsson MHG, Baum DA, Motley TJ (2003) Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacifc Basin and colonization of the Hawaiian Islands. *American Journal of Botany* **90**, 915-923.

Hroudova Z, Moravcova L, Zakravsky P (1997) Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. *Folia Geobotanica et Phytotaxonomica* **32**, 377-390.

Hughes L, Westoby M (1992a) Effect of diaspore characteristics on removal of seeds adapted for dispersal by ants. *Ecology* **73**, 1300-1312.

Hughes L, Westoby M (1992b) Fate of seeds adapted for dispersal by ants in Australian sclerophyll vegetation. *Ecology* **73**, 1285-1299.

Kearney MR, Shamakhy A, Tingley R, Karoly DJ, Hoffmann AA, Briggs PR, Porter WP (2014) Microclimate modelling at macro scales: a test of a general microclimate model integrated with gridded continental-scale soil and weather data. *Methods in Ecology and Evolution* **5**, 273-286.

Kim E, Donohue K (2013) Local adaptation and plasticity of *Erysimum capitatum* to altitude: its implications for responses to climate change. *Journal of Ecology* **101**, 796-805.

Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, Van Groenendael JM, Klimeš L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel AK, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E, Peco B (2008) The LEDA Traitbase: a database of life-history traits of the northwest European flora. *Journal of Ecology* **96**, 1266-1274.

Kokubugata G, Nakamura K, Forster PI, Hirayama Y, Yokota M (2012) Antitropical distribution of *Lobelia* species (Campanulaceae) between the Ryukyu Archipelago of Japan and Oceania as indicated by molecular data. *Australian Journal of Botany* **60**, 417-428.

Leyer I, Pross S (2009) Do seed and germination traits determine plant distribution patterns in riparian landscapes? *Basic and Applied Ecology* **10**, 113-121.

Lopez OR (2001) Seed flotation and postflooding germination in tropical *terra firme* and seasonally flooded forest species. *Functional Ecology* **15**, 763-771.

Lott JNA, Greenwood JS, Batten GD (1995) Mechanisms and regulation of mineral nutrient storage during seed development. In 'Seed development and germination. (Eds J Kigel and G Galili). (Marcel Dekker: New York).

Luiz OJ, Madin JS, Robertson DR, Rocha LA, Wirtz P, Floeter SR (2012) Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society of London B* **279**, 1033-1040.

Matlack GR (1987) Diaspore size, shape, and fall behavior in wind-dispersed plant species. *American Journal of Botany* **74**, 1150-1160.

Matzke NJ (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* **5**, 242-248.

Maun MA (1994) Adaptations enhancing survival and establishment of seedlings on coastal dune systems. *Vegetatio* **111**, 59-70.

McCully ME, Canny MJ, Huang CX, Miller C, Brink F (2010) Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: energy dispersive X-ray microanalysis (CEDX) applications. *Functional Plant Biology* **37**, 1011-1040.

Merritt DM, Wohl EE (2002) Processes governing hydrochory along rivers: hydraulics, hydrology, and dispersal phenology. *Ecological Applications* **12**, 1071-1087.

Minchinton TE (2006) Rafting on wrack as a mode of dispersal for plants in coastal marshes. *Aquatic Botany* **84**, 372-376.

Nathan R (2006) Long-distance dispersal of plants. *Science* **313**, 786-788.

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. *Trends in Ecology and Evolution* **23**, 638-647.

Nilsson C, Brown RL, Jansson R, Merritt DM (2010) The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews* **85**, 837-858.

Nonogaki H, Bassel GW, Bewley JD (2010) Germination - still a mystery. *Plant Science* **179**, 574-581.

Rand TA (2000) Seed dispersal, habitat suitability and the distribution of halophytes across a salt marsh tidal gradient. *Journal of Ecology* **88**, 608-621.

Rehman S, Harris PJC, Bourne WF (1998) The effect of sodium chloride on the Ca^{2+}, K^{+} and Na^{+} concentrations of the seed coat and embryo of *Acacia tortilis* and *A. coriacea*. *Annals of Applied Biology* **133**, 269-279.

Rehman S, Harris PJC, Bourne WF, Wilkin J (2000) The relationship between ions, vigour and salinity tolerance of *Acacia* seeds. *Plant and Soil* **220**, 229-233.

Römermann C, Tackenberg O, Poschlod P (2005) How to predict attachment potential of seeds to sheep and cattle coat from simple morphological seed traits. *Oikos* **110**, 219-230.

Shabala S, Newman I, Wilson S, Clark R (2000) Nutrient uptake patterns over the surface of germinating wheat seeds. *Australian Journal of Plant Physiology* **27**, 89-97.

Smith RN, Prentis P, Langendoen K, Corke P (2011) Pseudoseeds: investigating long-distance, ocean seed dispersal with wireless sensors. In 'Workshop on Robotics for Environmental Monitoring (WREM). ' pp. 1–4. (IEEE)

Tackenberg O, Romermann C, Thompson K, Poschlod P (2006) What does diaspore morphology tell us about external animal dispersal? Evidence from standardized experiments measuring seed retention on animal-coats. *Basic and Applied Ecology* **7**, 45-58.

Thomson FJ, Moles AT, Auld TD, Ramp D, Ren S, Kingsford RT (2010) Chasing the unknown: predicting seed dispersal mechanisms from plant traits. *Journal of Ecology* **98**, 1310-1318.

Ungar IA (1978) Halophyte seed germination. *Botanical Review* **44**, 233-264.

van den Broek T, van Diggelen R, Bobbink R (2005) Variation in seed buoyancy of species in wetland ecosystems with different flooding dynamics. *Journal of Vegetation Science* **16**, 579-586.

Van der Stocken T, De Ryck DJR, Balke T, Bouma TJ, Dahdouh-Guebas F, Koedam N (2013) The role of wind in hydrochorous mangrove propagule dispersal. *Biogeosciences* **10**, 3635-3647.

Vargas P, Nogales M, Jaramillo P, Olesen JM, Traveset A, Heleno R (2014) Plant colonization across the Galápagos Islands: success of the sea dispersal syndrome. *Botanical Journal of the Linnean Society* **174**, 349-358.

Werker E (1997) 'Seed anatomy.' (Stuttgart: Borntraeger, Berlin).

Will H, Maussner S, Tackenberg O (2007) Experimental studies of diaspore attachment to animal coats: predicting epizoochorous dispersal potential. *Oecologia* **153**, 331-339.

Wolters M, Geertsema J, Chang ER, Veeneklaas RM, Carey PD, Bakker JP (2004) Astroturf seed traps for studying hydrochory. *Functional Ecology* **18**, 141-147.

Yang H, Lu Q, Wu B, Zhang J (2012) Seed dispersal of East Asian coastal dune plants via seawater – short and long distance dispersal. *Flora* **207**, 701-706.

Zhang H, Irving LJ, Tian Y, Zhou D (2012) Influence of salinity and temperature on seed germination rate and the hydrotime model parameters for the halophyte, *Chloris virgata*, and the glycophyte, *Digitaria sanguinalis*. *South African Journal of Botany* **78**, 203-210.

Zhang WH, Zhou Y, Dibley KE, Tyerman SD, Furbank RT, Patrick JW (2007) Nutrient loading of developing seeds. *Functional Plant Biology* **34**, 314-331.

Zhao F-J, Moore KL, Lombi E, Zhu Y-G (2014) Imaging element distribution and speciation in plant cells. *Trends in Plant Science* **19**, 183-192.

Appendices 1 - 3 have been removed from this version
for copyright reasons

Appendix 4

Evidence of right to include Appendix 1 in this thesis

CSIRO PUBLISHING

Licence to Publish

Journal FUNCTIONAL PLANT BIOLOGY **Manuscript No.** FP10166

Title of the paper (the 'Work') BUOYANCY, SALT TOLERANCE AND GERMINATION OF COASTAL SEEDS: IMPLICATIONS FOR OCEANIC HYDROCHOROUS DISPERSAL.

Corresponding Author LYDIA GUJA

By submitting this paper, the Author (Authors if a multi-authored paper) has the right or has obtained authorisation to enter into this Licence and agrees to its submission by signing below.

The Author warrants that the Work:

- Has not been published before
- Is not presently being considered for publication elsewhere
- Does not violate any intellectual property right of any person or entity
- Does not contain any subject matter that contravenes any laws (including defamatory material and misleading and deceptive material) and
- Meets ethical standards applicable to the research discipline.

Terms of the Licence to Publish

1. In consideration for publication of the Work, the Author grants to CSIRO, through **CSIRO** PUBLISHING (the 'Publisher'), an exclusive worldwide licence to:
   - Reproduce, publish and communicate the Work, or any part of the Work, to the public in any and all media, whether existing before or after the date of this Licence, for commercial purposes for the full remaining term of the copyright subsisting in the Work in each applicable jurisdiction
   - Approve fair and reasonable permission requests from third parties to reuse material contained in the Work for the purpose of study, research or subsidiary publication.
2. In addition to the Author's moral rights in respect of the Work, the Author retains the right to:
   - Use the Work for non-commercial purposes within his/her institution
   - Use the Work for further research and spoken presentations (including those that are accompanied by visual materials such as slides, overheads and computer projections)
   - Use the Abstract of the Work for any purpose
   - Use the illustrations (line art, photographs, figures, plates) and research data in his/her own future Works
   - Use the Work within his/her institution for teaching purposes, including course packs and in electronic archives accessible to students
   - Share print or digital copies of the Work with colleagues for their personal use or study.
3. The Author agrees to:
   - Include a link and/or reference to the Work as published by the Publisher on all digital copies used within his/her institution
   - Not reproduce or authorise others to reproduce adaptations of the Work that are substantially identical to the Work for any commercial publication
   - Not permit digital copies of the Work as published by the Publisher to be systematically networked to external users
   - Not use the Work in any way that implies that the Publisher, the Journal or the Editorial Advisory Committee endorses any product or procedure described in the Work.
4. When exercising any of the rights assigned or granted, each party is required to give sufficient acknowledgement of the contribution made by the other party to the published material including a citation to the Journal.
5. If the paper is rejected by the Publisher in writing, all rights under this licence revert to the Author.

All authors are requested to sign this form. If not signed by all authors, the corresponding author acknowledges that he/she is signing on behalf of all the authors and with their authorisation. Faxed signatures and multiple forms are acceptable provided the corresponding author collates all the material and submits it in one batch.

(1) Author signature _____ Print name LYDIA GUJA Date 19/10/10

(2) Author signature _____ Print name DAVID MERRITT Date 19/10/10

(3) Author signature _____ Print name K. Dixon Date 20/10/10

(4) Author signature _____ Print name _____ Date _____

(5) Author signature _____ Print name _____ Date _____

(6) Author signature _____ Print name _____ Date _____

Authorisation (if required) _____ Print name _____ Date _____

Copies of this form are available from www.publish.csiro.au/media/client/CopyrightAssign.pdf or by contacting:

CSIRO PUBLISHING (Journals), PO Box 1139, Collingwood, Victoria 3066, Australia.
Telephone: +61 3 9662 7610 Fax: +61 3 9662 7611 Email: publishing.journals@csiro.au

Appendix 5

Permission to include Chapter 6 and Appendix 2 in this thesis

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Feb 12, 2014

This is a License Agreement between Lydia Guja ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3326691309731
License date	Feb 12, 2014
Licensed content publisher	John Wiley and Sons
Licensed content publication	Botanical Journal of the Linnean Society
Licensed content title	Full spectrum X-ray mapping reveals differential localization of salt in germinating seeds of differing salt tolerance
Licensed copyright line	© 2013 The Linnean Society of London
Licensed content author	Lydia Guja,Richard Wuhrer,Ken Moran,Kingsley W. Dixon,Grant Wardell-Johnson,David J. Merritt
Licensed content date	Aug 21, 2013
Start page	129
End page	142
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	Oceanic dispersal of coastal plant species
Expected completion date	Apr 2014
Expected size (number of pages)	100
Total	0.00 USD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or a society for whom a Wiley Company has exclusive publishing rights in relation to a particular journal (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions

established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

1. The materials you have requested permission to reproduce (the "Materials") are protected by copyright.

2.You are hereby granted a personal, non-exclusive, non-sublicensable, non-transferable, worldwide, limited license to reproduce the Materials for the purpose specified in the licensing process. This license is for a one-time use only with a maximum distribution equal to the number that you identified in the licensing process. Any form of republication granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before may be distributed thereafter). The Materials shall not be used in any other manner or for any other purpose. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Material. Any third party material is expressly excluded from this permission.

3. With respect to the Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Materials without the prior permission of the respective copyright owner. You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Materials, or any of the rights granted to you hereunder to any other person.

4. The Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc or one of its related companies (WILEY) or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto.

5. NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

6. WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

7. You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

8. IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

9. Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

10. The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

12. Any fee required for this permission shall be non-refundable after thirty (30) days from receipt

13. These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

14. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

15. WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

16. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

17. This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

Wiley Open Access Terms and Conditions

Wiley publishes Open Access articles in both its Wiley Open Access Journals program [http://www.wileyopenaccess.com/view/index.html] and as Online Open articles in its subscription journals. The majority of Wiley Open Access Journals have adopted the Creative Commons Attribution License (CC BY) which permits the unrestricted use, distribution, reproduction, adaptation and commercial exploitation of the article in any medium. No permission is required to use the article in this way provided that the article is properly cited and other license terms are observed. A small number of Wiley Open Access journals have retained the Creative Commons Attribution Non Commercial License (CC BY-NC), which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Online Open articles - Authors selecting Online Open are, unless particular exceptions apply, offered a choice of Creative Commons licenses. They may therefore select from the CC BY, the CC BY-NC and the Attribution-NoDerivatives (CC BY-NC-ND). The CC BY-NC-ND is more restrictive than the CC BY-NC as it does not permit adaptations or modifications without rights holder consent.

Wiley Open Access articles are protected by copyright and are posted to repositories and websites in accordance with the terms of the applicable Creative Commons license referenced on the article. At the time of deposit, Wiley Open Access articles include all changes made during peer review, copyediting, and publishing. Repositories and websites that host the article are responsible for incorporating any publisher-supplied amendments or retractions issued subsequently.
Wiley Open Access articles are also available without charge on Wiley's publishing platform, **Wiley Online Library** or any successor sites.

Conditions applicable to all Wiley Open Access articles:

- The authors' moral rights must not be compromised. These rights include the right of "paternity" (also known as "attribution" - the right for the author to be identified as such) and "integrity" (the right for the author not to have the work altered in such a way that the author's reputation or integrity may be damaged).

- Where content in the article is identified as belonging to a third party, it is the obligation of the user to ensure that any reuse complies with the copyright policies of the owner of that content.

- If article content is copied, downloaded or otherwise reused for research and other purposes as permitted, a link to the appropriate bibliographic citation (authors, journal, article title, volume, issue, page numbers, DOI and the link to the definitive published version on Wiley Online Library) should be maintained. Copyright notices and disclaimers must not be deleted.
    - Creative Commons licenses are copyright licenses and do not confer any other rights, including but not limited to trademark or patent rights.

- Any translations, for which a prior translation agreement with Wiley has not been agreed, must prominently display the statement: "This is an unofficial translation of an article that appeared in a Wiley publication. The publisher has not endorsed this translation."

Conditions applicable to non-commercial licenses (CC BY-NC and CC BY-NC-ND)

For non-commercial and non-promotional purposes individual non-commercial users may access, download, copy, display and redistribute to colleagues Wiley Open Access articles. In addition, articles adopting the CC BY-NC may be adapted, translated, and text- and data-mined subject to the conditions above.

Use by commercial "for-profit" organizations

Use of non-commercial Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee. Commercial purposes include:

- Copying or downloading of articles, or linking to such articles for further redistribution, sale or licensing;
- Copying, downloading or posting by a site or service that incorporates advertising with such content;
- The inclusion or incorporation of article content in other works or services (other than normal quotations with an appropriate citation) that is then available for sale or licensing, for a fee (for example, a compilation produced for marketing purposes, inclusion in a sales pack)
- Use of article content (other than normal quotations with appropriate citation) by for-profit organizations for promotional purposes
- Linking to article content in e-mails redistributed for promotional, marketing or educational purposes;
- Use for the purposes of monetary reward by means of sale, resale, license, loan, transfer or other form of commercial exploitation such as marketing products
- Print reprints of Wiley Open Access articles can be purchased from: corporatesales@wiley.com

  The modification or adaptation for any purpose of an article referencing the CC BY-NC-ND License requires consent which can be requested from RightsLink@wiley.com .

  Other Terms and Conditions:

  BY CLICKING ON THE "I AGREE..." BOX, YOU ACKNOWLEDGE THAT YOU HAVE READ AND FULLY UNDERSTAND EACH OF THE SECTIONS OF AND PROVISIONS SET FORTH IN THIS AGREEMENT AND THAT YOU ARE IN AGREEMENT WITH AND ARE WILLING TO ACCEPT ALL OF YOUR OBLIGATIONS AS SET FORTH IN THIS AGREEMENT.

  v1.8

Appendix 6

Evidence of right to include Chapter 4 and Appendix 3 in this thesis

CSIRO PUBLISHING

Licence to Publish

Australian Journal of Botany **Manuscript No.** BT13290

Title of the paper (the 'Work') Dispersal potential of Scaevola crassifolia (Goodeniaceae) is influenced by intraspecific variation in fruit morphology along a latitudinal environmental gradient

Author(s) Guja, Lydia; Merritt, David; Dixon, Kingsley; Wardell, Grant

By submitting this paper, the Author (Authors if a multi-authored paper) warrants that the Author has the right or has obtained authorisation to enter into this Licence.

The Author warrants that the Work:

- Has not been published before (except if included in an institutional pre-print server)
- Is not presently being considered for publication elsewhere
- Does not infringe any intellectual property right of any person and its use in accordance with this Licence will not infringe any intellectual property right of any person
- Does not contain any subject matter that contravenes any laws (including defamatory material and misleading and deceptive material) or breaches any obligation to a third person (including confidentiality and contractual obligations) and
- Meets ethical standards applicable to the research discipline.

Terms of the Licence to Publish

1. In consideration for publication of the Work, the Author grants to CSIRO, through **CSIRO** PUBLISHING (the 'Publisher'), an exclusive and royalty-free worldwide licence to:
   - Reproduce, publish and communicate the Work, or any part of the Work, to the public in any and all media, whether existing before or after the date of this Licence, for commercial, educational and all other purposes for the full remaining term of the copyright subsisting in the Work in each applicable jurisdiction.
   - Approve fair and reasonable permission requests from third parties to reuse material contained in the Work for the purpose of study, research or subsidiary publication.
2. In addition to the Author's moral rights in respect of the Work, the Author retains the right to:
   - Use copies of the work for non-commercial purposes within his/her institution subject to the usual copyright licencing agency arrangements
   - Use the work for further research and presentations at meetings and conferences
   - Use the illustrations (line art, photographs, figures, plates) and research data in his/her own future works
   - Share print or digital copies of his/her work with colleagues for personal use or study
   - Include the work in part or in full in a thesis provided it is not published for commercial gain
   - Place his/her pre-publication version of the work on a pre-print server
   - Place his/her pre-publication version of the work on a personal website or institutional repository on condition that there is a link to the definitive version on the **CSIRO** PUBLISHING website.
3. The Author agrees to:
   - Include a link and/or reference to the Work as published by the Publisher on all digital copies used within his/her institution
   - Not reproduce or authorise others to reproduce adaptations of the Work that are substantially identical to the Work for any commercial publication
   - Not permit digital copies of the Work as published by the Publisher to be systematically networked to external users
   - Not use the Work in any way that implies that the Publisher, the Journal or the Editors endorse any product or procedure described in the Work.
4. When exercising any of the rights assigned or granted, each party is required to give sufficient acknowledgement of the contribution made by the other party to the published material including a citation to the Journal.
5. If the paper is rejected, all rights under this licence revert to the Author and, where relevant, to the Author's employer.

Works made in the course of employment

6. Where the Work has been made by the Author (or in the case of multiple Authors, by any of them) in the course of employment and the employer owns copyright in the Work, that employer must sign this Licence. The employer has the same rights and obligations and gives the same warranties and licence rights as an Author under this Licence.
7. Works made in the course of employment by United States Government employees:

   (a) Where the Work has been made by all Authors in the course of employment by the United States Government then there is no United States copyright to license and no licence in respect of that copyright is necessary. Such United States Government Authors must still sign this form to affirm the warranties in it (which are of general application whether or not there is copyright in the Work) and to enable the Publisher to exercise its copyright rights in jurisdictions other than the United States. Without limiting rights that the United States Government may have to use the Work, such United States Government employees may also exercise the Authors' rights and will observe the Authors' obligations set out in this form.

   (b) Where the Work has been made by United States Government employee(s) in the course of their employment together with other Authors, then the form must be signed by those United States Government employees as well as by or on behalf of the non–United States Government employees indicating licensing of rights to the Publisher.

This form may be printed, signed and reuploaded. All Authors and where relevant their employers (see 'Works made in the course of employment' above) are requested to sign this form. If not signed by all Authors, the corresponding Author represents that he/she is signing on his/her own behalf and on behalf of all the Authors and with their authorisation. Where an Author signs this form on behalf of his/her employer or any other person, the Author warrants that he/she is authorised to sign on behalf of the employer and grant the rights and accept the obligations in this Licence on behalf of that person.

(1) Author/Authorised Agent for all Authors

Signature _____ _____ Print name ___Lydia Guja_____ Date __06/01/2014___

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

(2) Author signature _____ Print name _____ Date _____

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

(3) Author signature _____ Print name _____ Date _____

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

(4) Author signature _____ Print name _____ Date _____

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

(5) Author signature _____ Print name _____ Date _____

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

(6) Author signature _____ Print name _____ Date _____

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

(7) Author signature _____ Print name _____ Date _____

Employer signature _____ Print name _____ Date _____
(if required)
for and on behalf of _____ (Institution)

United States Government Employee certification (where relevant)

This will certify that the Authors listed below are United States Government employees who prepared the work in the course of their official duties.

Authorised signatory _____

United States Government Authors: _____

_____