The pollination biology and reproductive ecology of selected plants in South Australian saltmarshes, with a focus on the impact of the introduced plant genus *Limonium*

Melissa Schlein

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School of Biological Sciences, Faculty of Science and Engineering
Flinders University, Adelaide, South Australia

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Contents

Chapter five: Reproductive biology of the native saltmarsh plant species *Samolus repens* (Primulaceae) and the introduced *Limonium hyblaeum* agg. and *Limonium binervosum* (Plumbaginaceae) in a South Australian saltmarsh……………………………………………………………………172

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Thesis abstract

Saltmarshes are highly productive ecosystems, serving as habitat for terrestrial and marine fauna and flora, as well as areas of nutrient import and export, flood and erosion control, and carbon sequestration. Despite their importance, studies on the ecology of saltmarshes have been somewhat neglected, especially in southern Australia, and the pollination biology and ecology of saltmarsh plants has been particularly under-studied. This study aims to provide insight into not just the pollination biology and ecology of the two most common, native, insect-pollinated plant species within South Australian saltmarshes, *Frankenia pauciflora* DC. var. *gunnii* Summerh. (Frankeniaceae) and *Samolus repens* (J.R.Forst. & G.Forst.) Pers. (Primulaceae), but also to investigate the effects of introduced plants in the genus *Limonium* Mill. Studies were conducted primarily within the Goolwa saltmarsh in South Australia with a manipulated density study conducted in the Torrens Island saltmarsh. Comparisons were made between the saltmarsh study sites and the Port Elliott cliffs, which had many plant species in common.

Flowering phenologies were examined for both the native *F. pauciflora* and *S. repens* and the introduced *Limonium* at the Goolwa saltmarsh and Port Elliott cliffs, and it was determined that all overlap considerably in their flowering period. A series of experiments was then conducted to examine the interactions between these plant species. Observations of insect floral visitors were conducted and showed that all of the study species were visited by a common group of insect visitors. Floral preferences were determined for the main insect visitors. Syrphids and *Zizina labradus* Godart preferentially selected flowers of *F. pauciflora*, but neither of these two groups, or the introduced *Apis mellifera* L. preferentially visited flowers of *S. repens*. The effects of conspecific and heterospecific floral densities on per-flower visitation rates to *F. pauciflora* and *S. repens* were examined, showing that for both species an increase in conspecific floral density resulted in reduced visitation rates to flowers, suggesting that at high densities, some flowers may not receive sufficient visitation to ensure pollination. An increased floral display of *Limonium* also resulted in lower visitation rates to flowers of *S. repens*. Pollen loads were determined within the Goolwa saltmarsh and Port Elliott cliffs: while the majority of insects did not carry any measureable pollen, most of the pollen that was detected was that of the introduced *L. hyblaeum* agg.

Pollen limitation experiments were conducted within the Goolwa saltmarsh for the two native study species, and these experiments revealed that both species were pollen-limited during some seasons, and likely to be resource-limited during others. Density studies, both naturally occurring and manipulated, were conducted within the Goolwa saltmarsh and the Torrens Island saltmarsh. These revealed that at a high density, the presence of *Limonium* significantly reduced the fruit set and seed set of *F. pauciflora*. Breeding system studies were then carried out for the two *Limonium* taxa present at the study sites and revealed that both taxa are able to reproduce via apomixis in these South Australian habitats.

Overall, these studies demonstrate that introduced plants in the genus *Limonium* have the capacity to spread clonally and rapidly increase in numbers in South Australian saltmarshes and at high densities, to negatively affect the pollination and reproductive biology of the two most common insect-pollinated native species, *F. pauciflora* and *S. repens,* in these habitats.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Melissa Jane Schlein

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Overview of thesis content

This thesis is comprised of a general introduction, followed by four independent chapters written in the format of journal articles, each with an independent reference list and with figures and tables presented after the text. These four independent papers will be published in the future. The four papers are followed by a general discussion.

I wrote all chapters. My supervisors, Dr. Molly Whalen and Dr. Duncan Mackay are co-authors on each paper in view of their significant and invaluable advice and input into the project.

Chapter two: The impact of the introduced genus *Limonium* on the reproductive biology of *Samolus repens* (Primulaceae) and *Frankenia pauciflora* (Frankeniaceae) within a South Australian saltmarsh and cliff habitat

Schlein, M.J., Mackay, D.A. and Whalen, M.A.

Chapter three: Pollen limitation in two native plant species, *Samolus repens* (Primulaceae) and *Frankenia pauciflora* (Frankeniaceae) within a South Australian saltmarsh

Schlein, M.J., Whalen, M.A. and Mackay, D.A.

Chapter four: The effect of plant density on the facilitative and competitive effects of introduced *Limonium* species in two South Australian saltmarshes

Schlein, M.J., Mackay, D.A. and Whalen, M.A.

Chapter five: Reproductive biology of the native saltmarsh plant species *Samolus repens* (Primulaceae) and the introduced *Limonium hyblaeum* agg. and *Limonium binervosum* (Plumbaginaceae) within a South Australian saltmarsh

Schlein, M.J., Whalen, M.A. and Mackay, D.A.

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1. General introduction

Coastal saltmarshes are transitional zones that connect the land to the sea, and are usually found on protected, low-energy coasts (Adam 1990; 2002; 2009; Barbier *et al.* 2011; Saintilan 2009). These saltmarshes form a transitional zone between terrestrial and marine environments, and although they are exposed to air for the majority of the time, they are also subject to periodic flooding and waterlogging (Adam 1990; Foster *et al.* 2013; Laegdsgaard 2006). These habitats are highly productive and serve as habitat for terrestrial and marine fauna and flora, as well as areas of nutrient import and export, flood and erosion control, and carbon sequestration (Adam 1990; Foster *et al.* 2013; Tabot and Adams 2013).

To be considered coastal saltmarsh, the water inundating the saltmarsh must be saline, although the water can be less saline than seawater, thus differentiating coastal saltmarsh from other tidal marshes, such as fresh water marshes, which border fresh water bodies (Adam 1990). Inland saline areas are differentiated from coastal saltmarsh by being located away from the coast, by having their sodium and chloride derived from groundwater, and often having evapotranspiration exceed precipitation (Williams 2002). The vegetation found in coastal saltmarshes is mostly halophytic and plants can not only withstand high levels of salinity but also continual waterlogging and periodic tidal inundation (Saintilan 2009). Lower areas in coastal saltmarshes are inundated twice daily by the tide whilst areas in the upper marsh are subject to sporadic tidal inundation and usually have highly saline soils (Adam 1990; Boon *et al.* 2011). Strong zonation is present in most saltmarshes, with a clear sign of where the tide reaches (Adam 1990; Boon *et al.* 2011).

Saltmarsh plant diversity increases in temperate latitudes and the saltmarshes of southern Australia have a relatively high species diversity (Adam 2009; Saintilan and Rogers 2013), usually containing dozens of plant species, compared with saltmarshes in more northerly parts of Australia, which can have only three or four plant species (Adam 1990; Boon *et al.* 2011; Rogers *et al.* 2006). Saltmarsh plant species are predominantly pollinated by wind and insects (Adam 1990; Friess *et al.* 2012; Mackay and Whalen 2009; Pojar 1973); however, conditions often present in saltmarshes can affect insect flight and foraging, making insect pollination potentially variable (Adam 1990). Research into insect-pollination and pollinator assemblages, as well as the pollination and reproductive biology of insect-pollinated saltmarsh plants is generally lacking (Adam 1990; Boon *et al.* 2011).

Coastal saltmarshes are important ecosystems yet some aspects, such as plant-pollinator interactions, are poorly studied, especially in the southern temperate regions of Australia (Adam 1990). This lack of research may be because of their apparent unattractiveness as a study site and the small percentage of land occupied by saltmarshes both locally and globally (Adam 1990; Chapman 1974). It is estimated that Australia has 13,595 km^2 of saltmarsh, with South Australia having 84 km^2 (Zann 1995). It is also estimated that approximately 50% of all saltmarshes have been destroyed or at least disturbed globally, and in some areas, such as the west coast of the United States of America, up to 90% of saltmarshes have been destroyed (Barbier *et al.* 2011; Bromberg Gedan *et al.* 2009). Areas of saltmarsh in the north eastern Gulf St Vincent, South Australia, have been greatly reduced, with approximately 80% of all saltmarshes lost already in this region through industrial and urban developments (Department for Environment and Heritage 2007; Department of Environment, Water and Natural Resources 2007; Edyvane 1999).

Some locations in the metropolitan area of the South Australian coast have lost all their areas of saltmarsh since 1954 (Edyvane 1999).

Major threats to saltmarsh habitats along the South Australian coast include introduced plant and animal species, land clearing, industrial activity and development, off-road vehicle use and climate change with its subsequent sea-level rises (City of Port Adelaide Enfield 2007; Department of Environment, Water and Natural Resources 2007; Zann 1995). The harsh and extreme environment of saltmarshes was once thought to protect saltmarshes from invasion by introduced plant species (Daehler 2003); however, many introduced plant species have successfully invaded saltmarshes both locally and globally (Adam 2009). *Spartina anglica* has invaded numerous saltmarshes throughout Victoria and Tasmania, with smaller invasions present in South Australia and New South Wales (Adam 1995; Saintilan and Rogers 2013). *Cortaderia selloana* has invaded saltmarshes in southern Australia, and *Juncus acutus* has invaded saltmarshes across the south-east of Australia, often displacing the native *Juncus kraussii* (Adam 1995; Harvey *et al.* 2014).

Coastal cliff communities are also considered to be extreme environments, where plants are affected by a range of microclimatic factors, such as reduced moisture availability, low soil nutrient levels, and high winds, usually carrying salt-spray with them (Adam 2009; Boyce 1954; Coates and Kirkpatrick 1992; Larson *et al.* 2000; Nuzzo 1996; Wilson and Cullen 1986). Few studies have examined coastal cliff communities and their vegetation, even though many of the world's coasts are backed with coastal cliffs (Larson *et al.* 2000; Malloch 1971; Wilson and Cullen 1986), and even fewer studies have looked at pollination systems of plant species in these communities. The majority of studies conducted on temperate coastal cliff communities have been from the UK (Larson *et al.* 2000). Quite often cliff communities share plant species with nearby saltmarshes (Adam 2002; Adam 2009) as is the case in southern Australia. Many plant species of coastal cliff communities may be lithophytes; however, in some cases, these communities can also contain outlying populations of normally non-lithophytic plant species, such as the species included in this study (Adam *et al.* 1989; Coates and Kirkpatrick 1992).

Although cliff populations do not experience waterlogging like saltmarsh populations, plants are still exposed to sea spray with a high salt content (Adam 2009; Adam *et al.* 1989; Boyce 1954; Spanò *et al.* 2013). Cliff-dwelling plants must tolerate drought conditions and varying levels of salinity (Wilson and Cullen 1986) whilst saltmarsh populations must tolerate tidal inundation and elevated levels of salinity (Adam 1990). The comparison of plants in these habitats, which differ in water availability, is interesting in the context of the broader distribution of plants.

Aims and objectives

The primary aim of this study was to gain an understanding of the impact that the introduced plant genus *Limonium* was having on the pollination and reproductive biology of two native saltmarsh species, *Frankenia pauciflora* DC var. *gunnii* Summerh. and *Samolus repens* (J.R.Forst. & G.Forst.) Pers. in South Australian saltmarshes (Fig. 1.1). Both *F. pauciflora* and *S. repens* share a flowering period of late spring to early autumn with *Limonium* spp. thus creating the opportunity for competitive or facilitative interactions among these species for pollination services. In this context, the breeding systems of the native and introduced species were compared (Chapter five), the extent of pollen limitation in the native species (Chapter three), the extent of phenological overlap among the plant species (Chapter two), and the extent of overlap among the plant species in pollinators and in pollinator visitation (Chapters two and four) were all compared. The effect of density was also examined (Chapter four). This study therefore provides novel information on the pollination and reproductive biologies of the two main, native insect-pollinated saltmarsh plant species about which relatively little was previously known.

The results of this study will be of practical conservation significance, as saltmarsh plants such as *F. pauciflora* and *S. repens* are likely to be substantially altered in the future as a result of coastal development, introduced flora and fauna, and climate change. The understanding of breeding systems and pollination biology gained in this study should greatly enhance our ability to predict which species will be threatened by such changes and which species are likely to persist or expand their ranges.

Study sites

Goolwa saltmarsh

The Goolwa saltmarsh (-35° 31' 55.69"S, +138° 49' 36.13"E) is in the Coorong National Park on the Fleurieu Peninsula of South Australia, Australia and is a part of the Murray-Darling Basin (Fig. 1.2). The saltmarsh is situated approximately 2 km below the Goolwa Barrage on the Murray River and 6 km above the Murray Mouth, on the Sir Richard Peninsula. The surrounding area, known as the Coorong and Murray Mouth, or The Coorong and Lakes Alexandrina and Albert wetland, is a Ramsar Wetland of International Importance (Department of the Environment, Water, Heritage and the Arts 2010; Gawne *et al*. 2011).

The Goolwa Barrage is located 8 km upstream from the Murray Mouth. Construction on the Goolwa Barrage, along with four other barrages in the area, began in 1935 and was completed in 1940 (Eaton 1945; Murray-Darling Basin Authority 2011; River Murray Commission 1940). The barrages were built to maintain fresh water upstream for drinking and irrigation, and to prevent saltwater from travelling upstream when water levels in the river were low (Eaton 1945; River Murray Commission 1940). Barrage construction has significantly altered hydrology in the Coorong and the Goolwa saltmarsh (Adam 2002; Eaton 1945; Gippel and Blackham 2002; Murray-Darling Basin Authority 2011; River Murray Commission 1940). Mean flow volume through the Goolwa barrage and River Murray mouth have been reduced by 62% of natural flows, with median flows reduced by 80%, the frequency of ‘cease-to-flow’ events at the mouth of the River Murray have gone from 1 in 20 years to 1 in 2 years and minor to medium sized floods have been reduced from every one to seven years to never (Gippel and Blackham 2002). The River Murray now has a distinct break from freshwater to saltwater with estuarine habitat now reduced to 11% of it’s naturally occurring area (Gippel and Blackham 2002). The increasing occurrence and frequency of low-flow events has contributed greatly to increased levels of sedimentation and salinity throughout the lower Murray and mouth, turbidity has increased and contradictory and confusing hydrological and geomorphic changes have interfered with breeding cycles of fish, invertebrates and macroinvertebrates (Gippel and Blackham 2002).

The saltmarsh area used in this study is approximately 2 km long, 50-100 m wide and borders the northern edge of the Younghusband Peninsula near the town of Goolwa. Fieldwork took place over four flowering seasons, from late October to early March, from 2007 to 2011.

The saltmarsh has four main insect-pollinated plant species with showy flowers: the native *Samolus repens* and *Frankenia pauciflora* and the introduced *Limonium binervosum* (G.E.Sm.) C.E.Salmon and *Limonium hyblaeum* agg. Brullo, all of which were included in this study. The upper marsh at Goolwa is dominated by *Juncus kraussii* Hochst., growing alongside *Sarcocornia blackiana* (Ulbr.) A.J.Scott, *S. repens* and *F. pauciflora* (pers. obs) (Fig. 1.3). The mid and lower marshes are dominated by *Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J.Scott and *Suaeda australis* (R.Br.) Moq. (pers. obs.), and host significant stands of the introduced *L. binervosum* and *L. hyblaeum* agg. Brullo.

Torrens Island saltmarsh

The Torrens Island saltmarsh (-34° 47' 46.90"S, +138° 31' 23.13"E) is situated on the west side of Torrens Island, inland of mangroves and mudflats, and is a part of Torrens Island Conservation Park (City of Port Adelaide Enfield 2007) (Fig. 1.4). The island is situated in between LeFevre Peninsula and Barker Inlet, near Adelaide in South Australia, Australia with saltmarsh habitat making up approximately 376 ha, or 13%, of the Port River Barker Inlet Estuary (Baker 2004; Department for Environment and Heritage 2007; Department of Environment, Water and Natural Resources 2007). The area used in this study is approximately 1 km by 1 km. Fieldwork at Torrens Island was conducted over the summer of 2010/2011. The Torrens Island saltmarsh is dominated by *S. quinqueflora*, *Tecticornia* and *Atriplex* species. Large areas are dominated by the introduced plant *L. hyblaeum* agg. The Torrens Island saltmarsh has many creeks and pans throughout the marsh (Fig. 1.5).

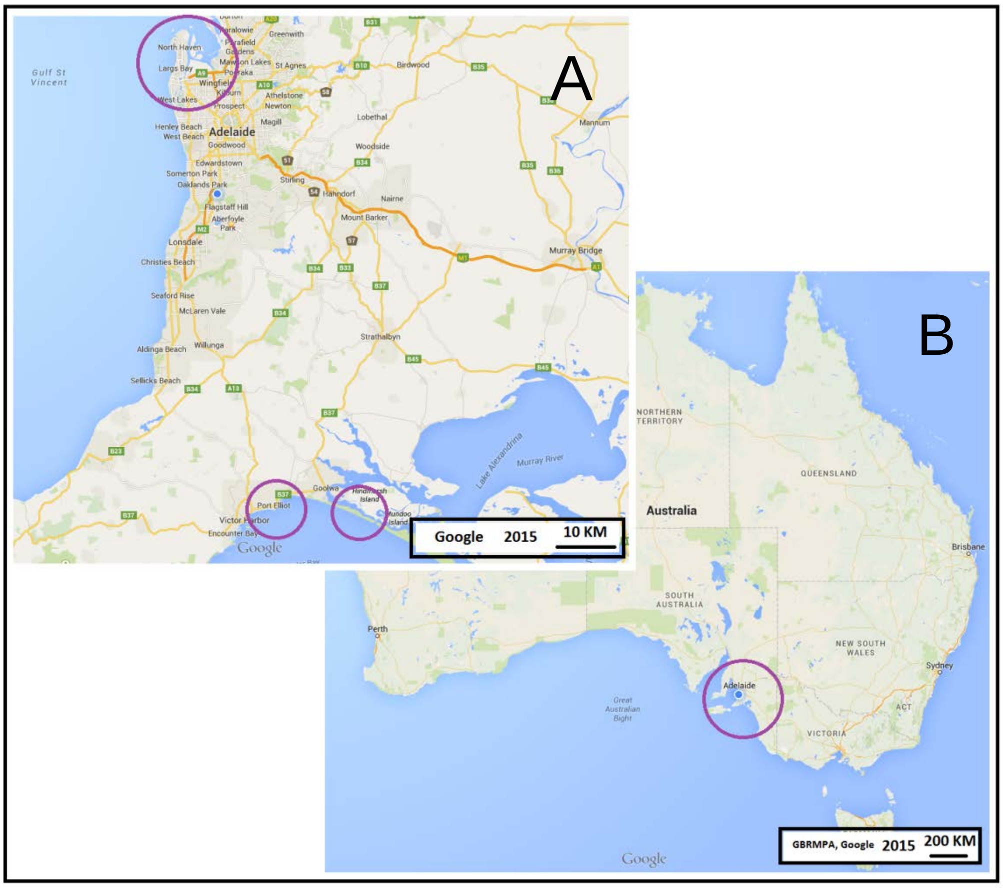

Figure 1.1 Location of field sites in South Australia, Australia. (A) Adelaide, South Australia and surrounds with the locations of the three field sites indicated (Google Maps 2015a). (B) Location of Adelaide, South Australia in Australia (GBRMPA and Google Maps 2015).

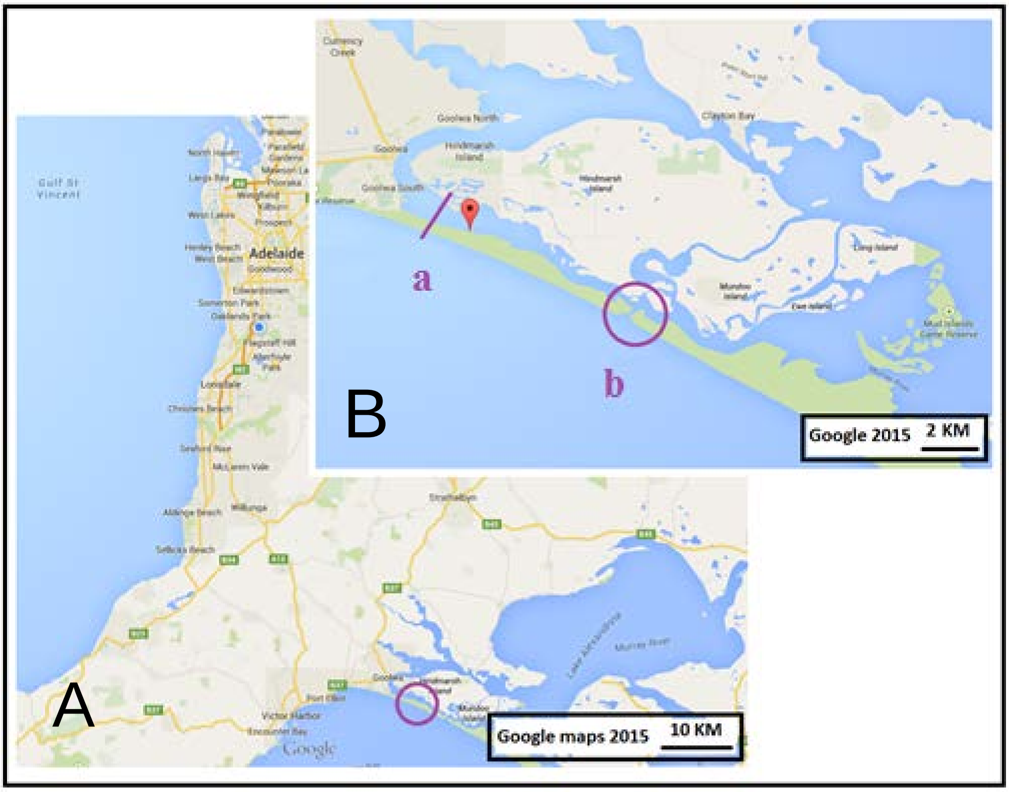

Figure 1.2 Location of the Goolwa saltmarsh in South Australia, Australia. (A) Adelaide, South Australia and surrounds with South Goolwa highlighted (Google Maps 2015a). (B) Goolwa and surrounds, with the Goolwa saltmarsh indicated by the red pin, the Goolwa Barrage (point a) and the Murray River mouth (point b) (Google Maps 2015b).

Figure 1.3 Views of the Goolwa saltmarsh, Goolwa, South Australia. (Photos: M. Schlein). Top: view looking north-east towards the River Murray. Mid-marsh visible in foreground with distinct zonation showing lower-marsh in the background. Bottom: view looking south-west towards the Southern Ocean. The border between the mid- and upper-marsh is visible with the sand dunes in the background. Note the purple tinge in the marsh is from the purple flowers of *Limonium binervosum.*

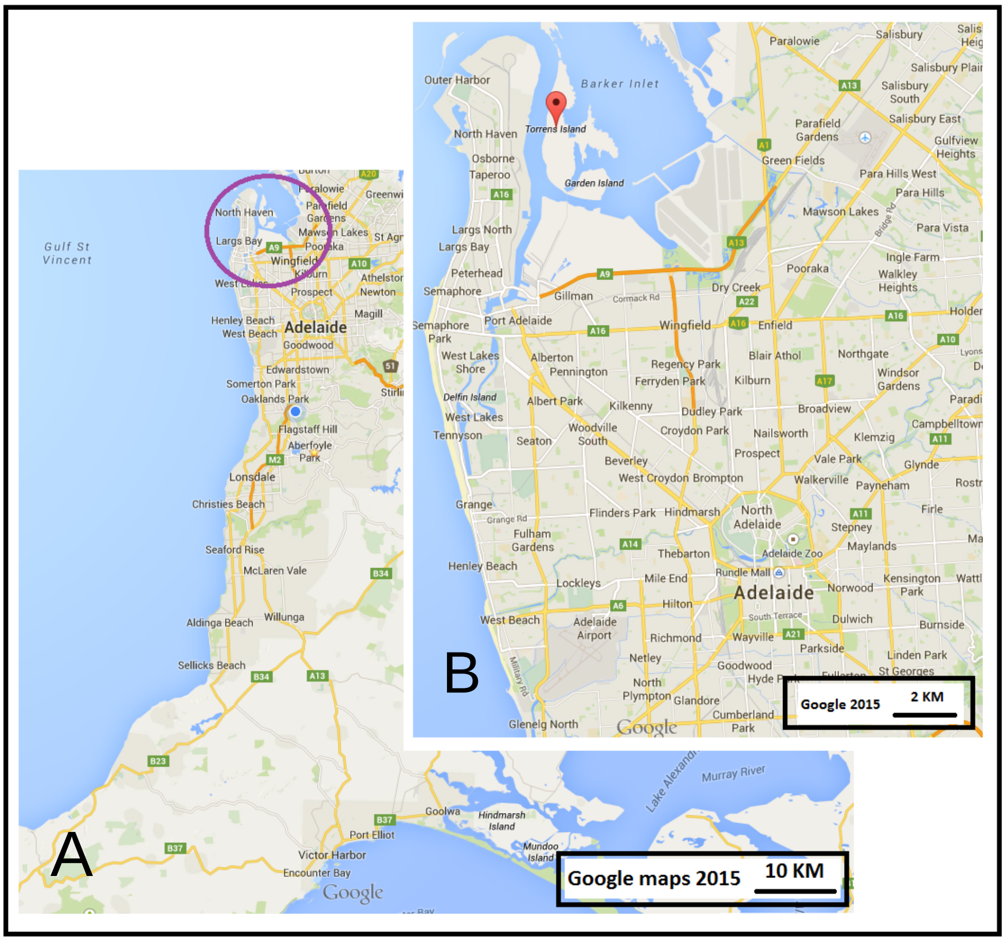

Figure 1.4 Location of the Torrens Island saltmarsh in South Australia, Australia. (A) Adelaide, South Australia and surrounds with Torrens Island highlighted (Google Maps 2015a). (B) Torrens Island and the surrounding Barker Inlet (Google Maps 2015c).

Figure 1.5 Views of the Torrens Island saltmarsh, Torrens Island, South Australia. (Photos: M. Schlein). Top: view looking east towards the Barker Inlet, towards the mangroves with saltmarsh transitioning to dunes in foreground. Bottom: view looking south-west showing the many waterways passing through the saltmarsh.

Port Elliott cliffs

The Port Elliott cliffs (-35° 32' 15.75"S, +138° 41' 1.81"E) are located on the Fleurieu Peninsula of South Australia, near the town of Port Elliott below the Commodore Reserve (Fig. 1.6), and are located 11.5 km to the west of the Goolwa saltmarsh. The area used in this study is approximately 200 m long by 50 m wide. Fieldwork took place from October to March from 2007 to 2011. Three main insect-pollinated plant species with showy flowers occur at this site and all were used in this study. Plant species are limited at the Port Elliott cliffs; only a few species are present amongst the rocks, although many species are found above the rock face (Fig. 1.7).

Seasonal and climatic influences at the study sites

Goolwa and Port Elliott are 11.5 km apart and share similar weather conditions because of their proximity to one another. Over the multiple years of this study, different weather extremes were recorded at Goolwa and Port Elliott. Mean maximum temperatures for Goolwa from 2007/2008 until 2010/2011 (data were taken from the Hindmarsh Island weather station, located less than 5 km from the Goolwa saltmarsh) and the average monthly rainfall at Goolwa by season from 2007/2008 until 2010/2011 (data were taken from three different weather stations at Goolwa and averaged as none of these weather stations had complete data sets; however, at least two rainfall measurements for each period were present) are shown in Figures 1.8 and 1.9 respectively. The Goolwa saltmarsh has a Mediterranean climate with the majority of rainfall falling over winter.

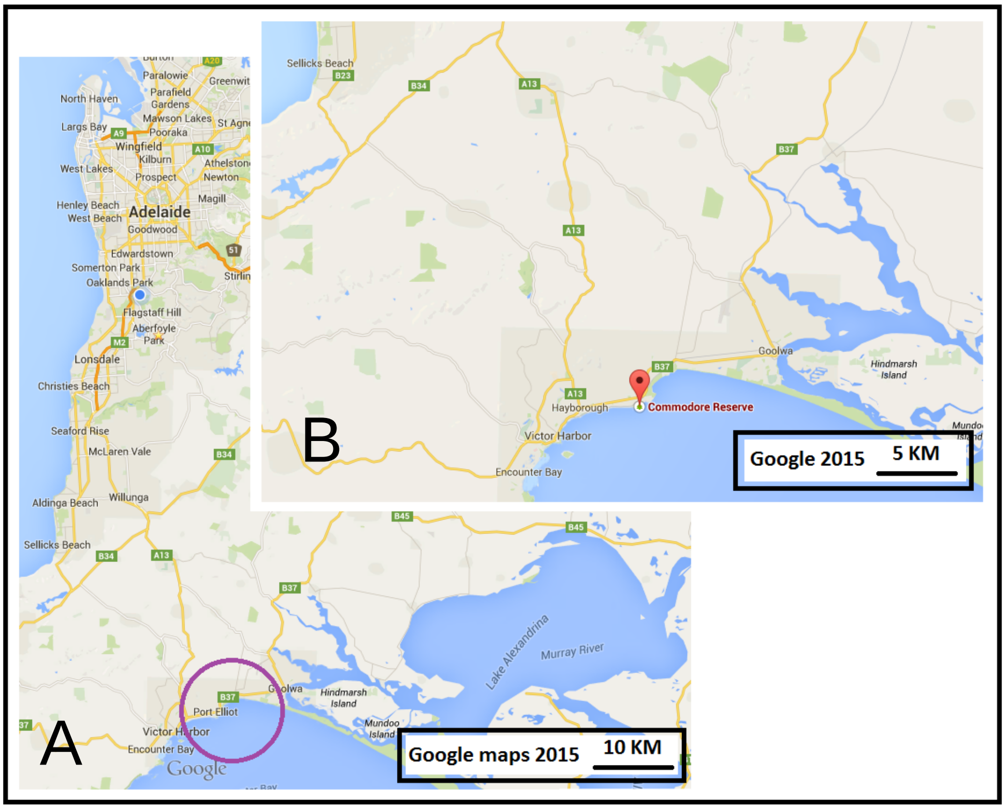

Figure 1.6 Location of the Port Elliott cliffs in South Australia, Australia. (A) Adelaide, South Australia and surrounds with Port Elliott highlighted (Google Maps 2015a). (B) Port Elliott and surrounds, with the Commodore Reserve indicated by the red pin; this reserve is directly above the cliffs (Google Maps 2015d).

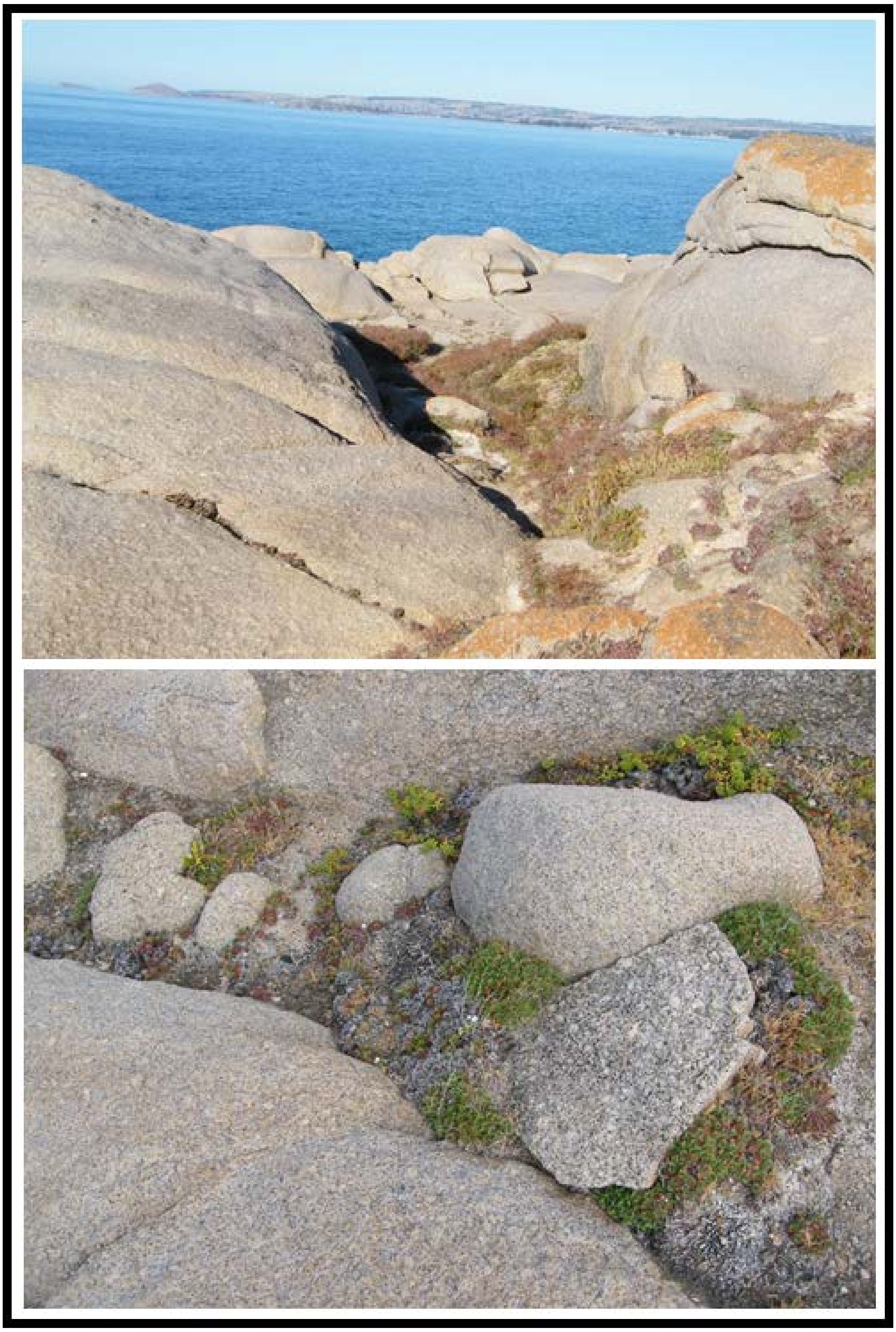

Figure 1.7 Views of the Port Elliott cliffs, Port Elliott, South Australia (Photos: M. Schlein). Top: view looking southeast showing plants growing amongst the rocks. Bottom: close up view of the low growth form of the plants growing amongst the rocks forming the Port Elliott cliffs.

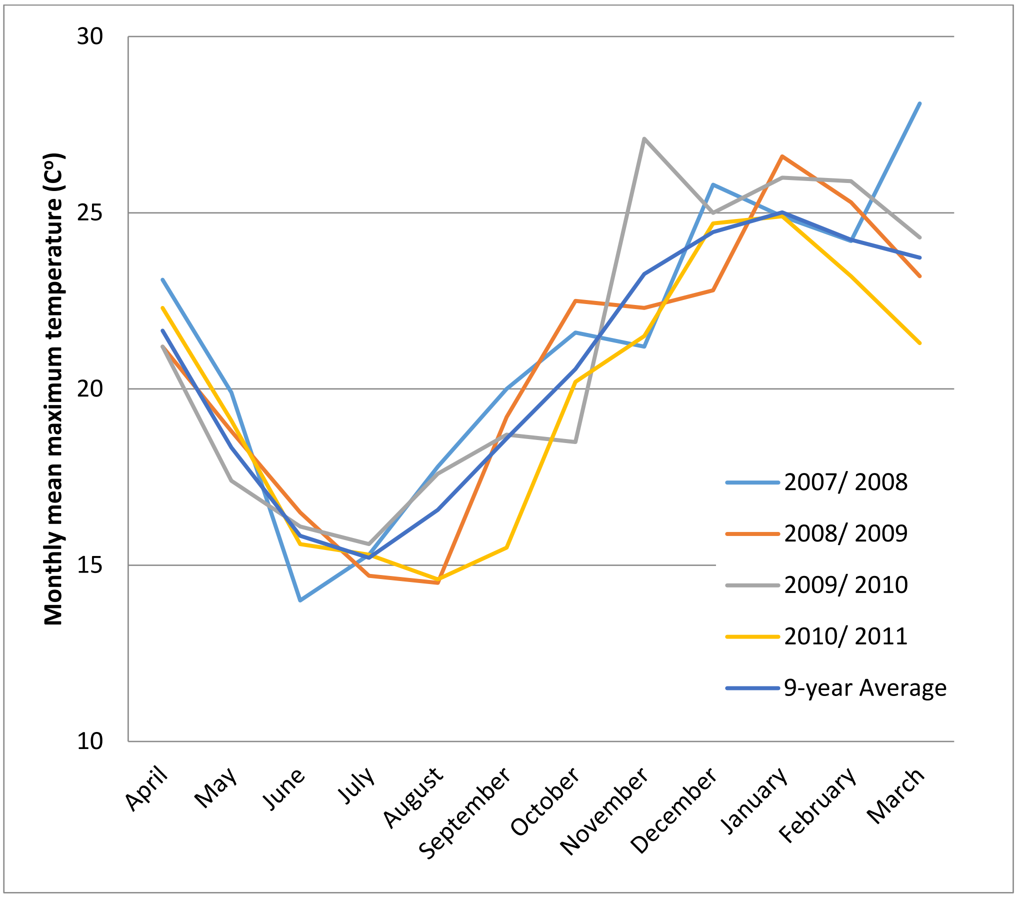

Figure 1.8 Monthly mean maximum temperatures at Goolwa from 2007/2008 to 2010/2011, including the 9-year average from 2003 to 2012. Bureau of Meteorology weather station used:

Hindmarsh Island, ID 023894, Latitude 35°31'10"S Longitude 138°49'4"E

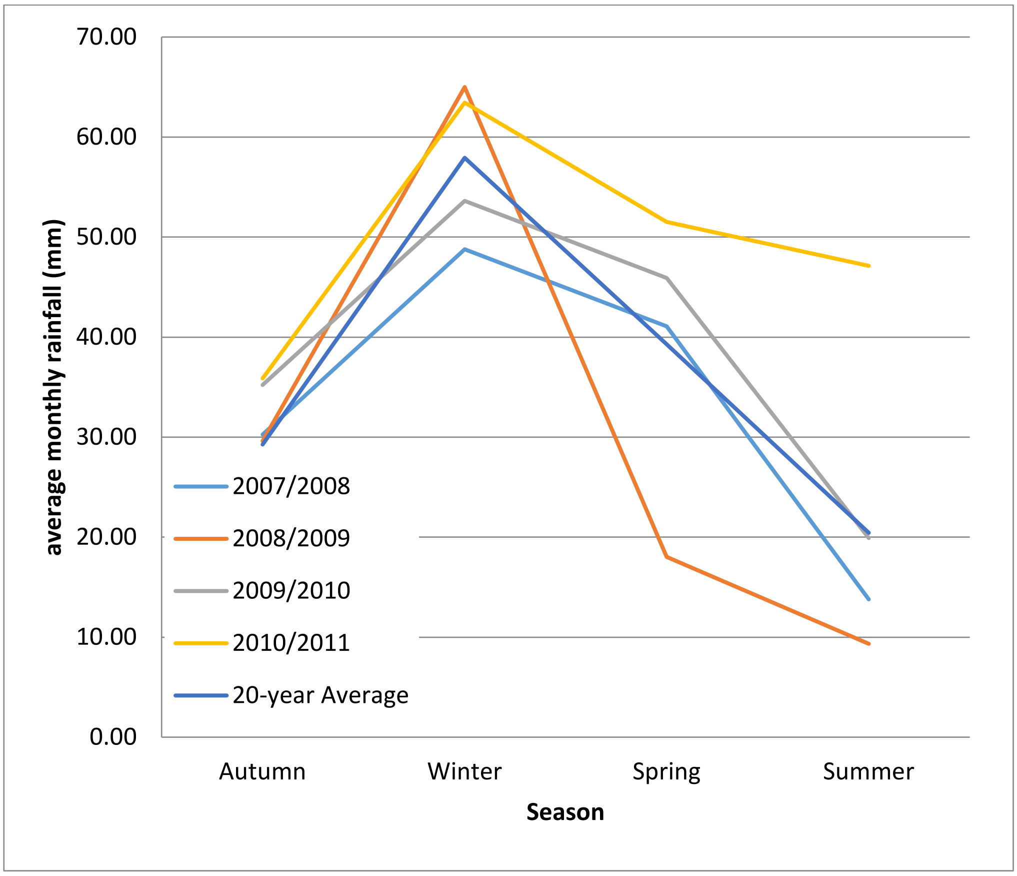

Figure 1.9 Average monthly rainfall by season at the Goolwa saltmarsh from 2007/2008 to 2010/2011, including 20-year average from 1991/1992 to 2010/2011. Data were taken from three different weather stations around Goolwa and averaged as none of these weather stations had complete data sets, however, there were always at least two rainfall measurements for each period with most having three. Bureau of Meteorology weather stations used were:

Goolwa barrage, ID 023825, Latitude 35°31'44"S Longitude 138°48'24"E;
Hindmarsh Island, ID 023894, Latitude 35°31'10"S Longitude 138°49'4"E;
Goolwa council depot, ID 023718, Latitude 35°29'54"S Longitude 138°45'53"E

The Millennium Drought is said to be the worst drought on record for southeast Australia and took place from 2001 to 2009, ending with a strong La Niña event in 2010 (van Dijk *et al.* 2013). Goolwa and Port Elliott were in areas of serious to severe rainfall deficiency, meaning that the rainfall from April 1997 to March 2010 was in the lowest 5% to 10% of historical totals (Department of Sustainability, Environment, Water, Population and Communities 2011; National Climate Centre 2011). The Millennium Drought severely affected river ecosystems across the southeast of Australia, especially the Murray-Darling Basin (van Dijk *et al.* 2013) which suffered from the equal driest six-year period on record from November 2001 to October 2007, with 20% less rainfall than the long-term average of 480 mm/year (National Climate Centre 2007; National Climate Centre 2008).

Mean daily temperatures for the Murray- Darling Basin were the highest on record, and four out of the ten hottest years in the Murray-Darling Basin took place after 2001 (National Climate Centre 2007). To counteract low water levels in the River Murray, temporary flow regulators were built in 2009 between Hindmarsh Island and Clayton to increase water levels in the Goolwa Channel (Government of South Australia 2009), which had dropped to minus 1.05 m Australian Height Datum (AHD) in April 2009 (Department of Water, Land and Biodiversity Conservation 2009).

After March 2010, however, large areas of Australia experienced above-average rainfall caused by an extremely strong La Niña event in the Pacific Ocean (Department of Sustainability, Environment, Water, Population and Communities 2011). From March 2010 to February 2011, Goolwa and Port Elliott were in areas with the highest 10% to 30% of historical totals (Department of Sustainability, Environment, Water, Population and Communities 2011; National Climate Centre 2011). December 2010 was the wettest on record for eastern Australia as a whole, and the second wettest for the Murray-Darling Basin (National Climate Centre 2011). This high-rainfall December followed a high-rainfall spring, the wettest on record for the Murray-Darling Basin (National Climate Centre 2011). The Murray-Darling Basin recorded its seventh wettest year since records began in 1900 (Department of Sustainability, Environment, Water, Population and Communities 2011).

As well as increases to rainfall, 2010 saw increases to River Murray flows, associated with the Goolwa saltmarsh. High river flows caused flooding upstream in Victoria and caused rising flows downstream from August 2010, with peaks projected to reach 25,000 to 35,000 ML / day in October 2010 (Department for Water 2010; Murray-Darling Freshwater Research Centre 2013). In comparison, daily flows into South Australia in May 2008 were 1,600 ML/ day (Department of Water, Land and Biodiversity Conservation 2009). Controlled water releases were being managed from the barrages across the River Murray at a rate of 31,400 ML/ day, with the average water level at the Goolwa Channel plus 0.75 m AHD (Department for Water 2010). River Murray flows peaked at around 80,000 ML/ day in late January/ early February 2011 (Department for Water 2011). In October 2011 work began on the removal of the Goolwa Channel temporary flow regulator and was completed in 2012 (Government of South Australia 2011).

By conducting studies over multiple years and multiple flowering seasons, seasonal variation can be taken into account, enabling a more accurate understanding of what is taking place in communities over time. This study was able to do that.

Study species

Frankenia pauciflora

Frankenia pauciflora DC. var. *gunnii* Summerh. (Frankeniaceae) henceforth referred to as *F. pauciflora*, is also known as common sea-heath. It is a native small shrub growing up to half a metre in height, and can spread along the ground or have a more upright growth form (Whalen 1986) (Fig. 1.10). It is halophytic and shows markedly reduced seed set following self-pollination (Whalen 1986, Mackay and Whalen 2009). *Frankenia pauciflora* naturally occurs in South Australian saltmarshes and on coastal cliffs as well as in inland saline areas (Barnsley 1982). *Frankenia pauciflora* flowers are small and actinomorphic with a tubular calyx (Fig. 1.11) (Whalen 1986). They are insect-pollinated and range from white to pale pink in colour (Dixon 2011; Whalen 1986).

Figure 1.10 Plants of *Frankenia pauciflora* showing different growth forms. (Photos: M. Schlein). Top: in the Goolwa saltmarsh (upper-marsh). Bottom left: at the Port Elliott cliffs. Bottom right: in the Goolwa saltmarsh (lower-marsh).

Figure 1.11 Flowers of *Frankenia pauciflora* showing actinomorphic shape and pale pink to white colour. (Photos: M. Schlein).

Samolus repens

Samolus repens (J.R.Forst & G.Forst) Pers., commonly referred to as creeping brookweed, is traditionally placed in the family Primulaceae (Stevens 2014; Toelken 2007b), although some sources place the genus *Samolus* in its own family (Samolaceae) (Stahl 2004). *Samolus repens* is a native, perennial small herb commonly found forming dense clusters of branches up to 60 cm high, although plants can also be found growing low to the ground with runners (Fig. 1.12) (Dixon 2011; Saintilan 2009; Toelken 1986b). *Samolus repens* is halophytic and occurs along the coast of southern Australia in saltmarsh, cliff and mangrove communities (Saintilan 2009). *Samolus repens* flowers are small and actinomorphic with a tubular corolla (Fig. 1.13) (Toelken 1986b). They are insect-pollinated and range from white to pink in colour (Dixon 2011; Toelken 1986b). *Samolus repens* appears to prefer growing in low-lying areas with moist soil (pers. obs.) when compared to *F. pauciflora*, which can grow in higher areas with less soil moisture. For example, at Port Elliott, plants of *S. repens* grow deep in the crevices of rocks where water collects as opposed to plants of *F. pauciflora*, which can grow on top of the rocks in more exposed areas. In addition, at Torrens Island, *Samolus repens* grows alongside waterways, but not amongst *F. pauciflora* plants further away from water (pers. obs). It was also noted by Jones *et al.* (2012) and Stahl (2004), that members of the genus *Samolus* prefer to grow in temporarily inundated areas like around waterways, or in damp saline habitats such as brackish pools near the seashore or in saltmarshes. Chapman (1974) noted that plants of *S. repens* can tolerate periods of waterlogging.

Figure 1.12 Plants of *Samolus repens* showing different growth forms. (Photos: M. Schlein). Top: in the Goolwa saltmarsh (upper-marsh). Bottom left: in the mid-marsh at the Goolwa saltmarsh. Bottom right: amongst the cliffs at Port Elliott.

Figure 1.13 Flowers of *Samolus repens* showing actinomorphic shape and white colour, although flowers can uncommonly be pink. (top left) (Photos: M. Schlein).

Limonium

Limonium P. Miller is an introduced genus in South Australia in the family Plumbaginaceae (Kubitzki 1993; Stevens 2014) although Toelken (1986a; 2007a) places the genus in the family Limoniaceae. The genus *Limonium* is cosmopolitan and found in saltmarshes and coastal cliffs, as well as on inland salt steppes and along rocky and sandy seashores (Palop-Esteban *et al.* 2011; Rois *et al.* 2013). *Limonium* occurs naturally in saltmarshes in Europe and the Mediterranean with *Frankenia* L. and *Samolus* L. species (Boira 1995; Chapman 1974) and has spread quite extensively across the coast of Australia, through South Australia, Victoria and New South Wales, as well as into some inland areas (Laegdsgaard *et al.* 2009). More than 150 taxa of *Limonium* occur worldwide (Rois *et al.* 2013). Members of the genus *Limonium* can produce seeds sexually and asexually (Rois *et al.* 2012), and many species of *Limonium* are triploid or tetraploid and capable of agamospermy (Cowan *et al.* 1998; Palop-Esteban *et al.* 2011). Species of the genus *Limonium* have also invaded other saltmarshes across the world including San Francisco Bay (Archbald and Boyer 2014).

Some members of the family Plumbaginaceae, and the genus *Limonium,* have well-developed incompatibility systems (Adam 1990; Baker 1966; Burge and Morgan 1993; Palop-Esteban *et al.* 2011). Often members of the genus *Limonium* are monomorphic and self-compatible, others are monomorphic and apomictic, for example the *L. binervosum* aggregate (Adam 1990; Baker 1966; Cowan *et al.* 1998). Some members of the *L. binervosum* aggregate have anthers that do not produce pollen (male sterile) (Adam 1990; Ingrouille and Stace 1986).

Species of the genus *Limonium* in South Australian saltmarshes are small plants with rosettes of leaves, with one to multiple, long flowering stems protruding above the leaves, consisting of branches bearing variable numbers of spikelets, with one to multiple flowers per spikelet (Toelken 1986a; Toelken 2007a). Calyces of flowers are cone to funnel-shaped (Toelken 1986b). *Limonium binervosum* and plants of the *L. hyblaeum* agg. are found growing alongside *F. pauciflora* and *S. repens* in South Australian saltmarshes.

Limonium binervosum

Limonium binervosum (G.E. Sm.) C.E. Salmon, (Plumbaginaceae), also known as dwarf sea lavender, is an introduced *Limonium* species found at Goolwa, it is a weedy species in Australia and in other locations globally, and has been naturalized in Australia (Randall 2007). *Limonium binervosum* naturally occurs as a coastal species along the Atlantic coast of Europe, from Portugal to Britain, growing on cliffs, exposed rocks and slopes and also colonises bare ground (Ingrouille and Stace 1985). It has purple flowers in an inflorescence with one to multiple flowering stems protruding from a rosette of leaves (Toelken 1986a) (Fig. 1.14 and 1.15). Some members of the *L. binervosum* aggregate are apomictic, with some populations entirely male-sterile (Baker 1948; Ingrouille and Stace 1986). Some taxa lack pollen altogether or have entire colonies lacking pollen (Ingrouille and Stace 1986). Ingrouille and Stace (1985) believed *L. binervosum* to be obligately agamospermous.

Figure 1.14 Plants of *Limonium binervosum* at the Goolwa saltmarsh showing growth forms. (Photos: M. Schlein). Photographs illustrate the spread of the introduced plant species, across the saltmarsh and its co-occurrence with other plant species.

Figure 1.15 Flowers of *Limonium binervosum* showing actinomorphic shape and purple colour. (Photos: M. Schlein).

It is highly likely that the population of *L. binervosum* at Goolwa is male-sterile (pers. obs.). Members of the *L. binervosum* aggregate are mainly tetraploid although some are triploid (Cowan *et al.* 1998).

Limonium hyblaeum

Limonium hyblaeum Brullo (Plumbaginaceae), also known as Sicilian sea lavender, is becoming a serious threat to saltmarsh biodiversity and functioning across the Victorian and South Australian coasts (Caton *et al.* 2011). *Limonium hyblaeum*, a member of the *Limonium albidum* group, originated from the coast of Sicily, where it is endemic with a very localised distribution (Brullo and Pavone 1981). It is a weedy species in Australia and in other locations globally, and has been naturalised in Australia (Randall 2007). It has become a weed in the natural environment and has escaped from cultivation (Randall 2007). This species has pink/ purple flowers in an inflorescence with one to multiple flowering stems protruding from a dense rosette of leaves (Toelken 1986a; Toelken 2007a). *Limonium hyblaeum* is tetraploid (Brullo and Pavone 1981; Cowan *et al.* 1998).

Limonium companyonis

Limonium companyonis (Gren. & Billot) Kuntze (Plumbaginaceae), also known as blue mist, is an introduced species from the genus *Limonium*, originally from southern France and the Balearic Islands (Toelken 1986a), and now found across the south coast of Australia as well as in New Zealand (Weedbusters NZ 2004). *Limonium companyonis* has a small rosette of leaves with one to several flowering stems (Toelken 1986a).

***Limonium hyblaeum* agg.**

The most common *Limonium* species at the Goolwa saltmarsh is *Limonium binervosum*, which is readily distinguished in the field from a less common *Limonium*. This less common *Limonium* is referred to here as the *Limonium hyblaeum*/ *Limonium companyonis* complex or *L. hyblaeum* agg. (*L. hyblaeum* aggregate) (Fig. 1.16 and 1.17). Adair (2012) noted the close similarity between *L. companyonis* and *L. hyblaeum* and that they can be problematic to distinguish in New Zealand.

In the Flora of South Australia (Toelken 2007a) *Limonium hyblaeum* is distinguished from *Limonium companyonis* by "Midrib of the sepals extending half to two-thirds the length of the lobes; spikelets usually touching one another with 2-5 flowers" versus "Midrib of the sepals extending into the apex of the lobes; spikelets c. 2 mm apart and with 1 to 2 flowers" (Toelken 2007a). However, at the study sites, variation within plants, in the degree of contact or separation of spikelets was observed, and flowering branches of separated spikelets were frequently observed on plants in which the midrib does not extend to the tip of the lobes. All individuals examined had pollen present. *Limonium* is a taxonomically difficult group, and as with many agamospermous species, has resulted in numerous microspecies, distinguished only by small differences (Cowan *et al.* 1998).

Thus, given the similarities between *L. companyonis* and *L. hyblaeum*, and issues in distinguishing them in the study sites in South Australia, these *Limonium* plants are referred to here as the *Limonium hyblaeum*/ *Limonium companyonis* complex or *L. hyblaeum* aggregate.

Limonium hyblaeum, as well as *L. companyonis* and an unknown *Limonium* species (possibly *L. binervosum*), are classified as red alert priority weeds in the Limestone Coast and Coorong Coastal Action Plan and Conservation Priority Study, published by the South East Natural Resource Management Board and Department of Environment and Natural Resources (Caton *et al.* 2011). Red alert priority weeds are species that are already present and have the potential to become a major threat; each of the *Limonium* taxa mentioned was classified as threat value 4, meaning that they should receive immediate attention (Caton *et al.* 2011).

Impact of introduced plant species on plant pollinator interactions

Many flowering plants can reproduce both sexually and asexually. Not only are some plants only able to reproduce sexually, but many plant species rely on insect visitors to serve as pollinators (Traveset and Richardson 2006). If this process of sexual reproduction is interfered with, for example by the introduction of introduced plant species, the reproduction of some native plant species could be hindered, leading to reduced recruitment and community size. Several studies have examined seed germination in saltmarsh plants and found that germination rates are often very low because of high soil salinity and waterlogging (Boon *et al.* 2011; Kellogg *et al.* 2003; Robinson *et al.* 2006). By determining the breeding systems of native plants, ecologists can hope to predict potential impacts of introduced plant species on the reproductive and pollination biology of native plant species.

Figure 1.16 Plants of *Limonium hyblaeum* agg. showing growth form. (Photos: M. Schlein). Top left: plants at the Goolwa saltmarsh growing amongst *Limonium binervosum*. Top right: plants growing amongst the cliffs at Port Elliott, and Bottom: an isolated plant at Torrens Island.

Figure 1.17 Flowers of *Limonium hyblaeum* agg. showing actinomorphic shape and pink/ purple colour. (Photos: M. Schlein).

Introduced plant species are plants whose presence in an area is the result of human involvement but that can successfully reproduce without human intervention (Pyšek and Richardson 2008; Traveset and Richardson 2006). In Australia, counts suggest that naturalised, introduced plant species have reached nearly 3000 species (Randall 2007; Ward *et al.* 2012). At Torrens Island, 81 introduced plant species are present, representing 43% of all plant species on the island (Caton *et al.* 2009). Introduced plant species can alter ecosystem functioning and can have positive, negative or neutral effects. Introduced plant species can interact with natives by competing for light, space, nutrients and water and by modifying the structure and composition of communities (Baumberger *et al.* 2012; Richardson *et al.* 2000; Tang *et al.* 2013; Traveset and Richardson 2006). They can also interfere with plant-animal interactions such as pollination and seed dispersal, potentially leading to long term changes in community dynamics and species composition (Traveset and Richardson 2006).

Introduced plant species may disrupt existing interactions between native flora and fauna (Bjerknes *et al.* 2007; Morales and Traveset 2009; Valdovinos *et al.* 2009) but less understood is the way that these introduced plant species interact with native flora and fauna and with the existing pollination systems etc. (Lopezaraiza–Mikel *et al.* 2007; Morales and Traveset 2009). These interactions can range from competitive to facilitative and often change over time (Morales and Traveset 2009; Rathcke 1988).

This study aims to further understand the impact of introduced plant species on pollinator networks in South Australian saltmarsh plant communities. Understanding the breeding systems of introduced plant species is important to gauge the effects that they may have on native plant species and to better organise management efforts (Phillips *et al.* 2010; Randall 2007). Although introduced plant species are a major threat to biodiversity, relatively little work has been conducted examining the reproductive biology of saltmarsh plants in general or introduced plant species in Australia (Rambuda and Johnson 2004; Randall 2007; Richardson *et al.* 2000; Rodger and Johnson 2013)**.**

A major factor influencing whether an introduced plant species shows competitive or facilitative effects in relation to pollination, is its density (Grindeland *et al.* 2005; Zorn-Arnold and Howe 2007). Many studies have examined the role of density on the impact of introduced plant species. Muñoz and Cavieres (2008) studied the effects of flower density on alpine species in Chile. They found that while high densities of introduced species can negatively affect native plants by reducing the visitation rates of their pollinators, at lower densities, the introduced species can attract pollinators and increase pollinator visitation rates. By taking pollinator observations and comparing sites with high and low densities of introduced species, the overall importance of density can be determined. Kitamoto *et al.* (2008) examined the effects of density on the vulnerable herb *Primula sieboldii*. They found that in areas with high densities of other plants, *Primula sieboldii* had a reduced seed set as well as a lower number of pollen donors (Kitamoto *et al.* 2008). Density manipulations have also been conducted where the different densities of the study species have been manipulated to complement studies using naturally occurring densities. Brown *et al*. (2002) conducted a study examining the effect of the varying densities of the introduced *Lythrum salicaria* on the native *Lythrum alatum*. They found that by manipulating the density, factors such as location and the possible influences of microclimate were removed as confounding factors. The most important factors thought to influence the success of an introduced plant species are population size and density, as well as similarity in floral morphologies (Bjerknes *et al.* 2007).

Thesis layout

This thesis comprises a general introduction, followed by four independent chapters written in the format of journal articles, and then concludes with a general discussion. Chapter two examines the impact of the introduced genus *Limonium* on the reproductive biology of *F. pauciflora* and *S. repens*. A phenological study was conducted, and confirmed that the flowering period for *Limonium* coincided with the flowering periods of *F. pauciflora* and *S. repens* so additional studies examined pollinator visitation rates, pollinator movement and flower preference. Chapter three examines the presence of pollen limitation in *F. pauciflora* and *S. repens* across several flowering seasons at the Goolwa saltmarsh and Chapter four looks at the role that density plays in influencing whether the introduced plant species *Limonium* has a facilitative, competitive or neutral impact on native saltmarsh species. Chapter five includes glasshouse studies examining the breeding systems of *S. repens* and *Limonium*. The final chapter of this thesis is a general discussion of each of the chapters.

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2. The pollination biology of the native plant species *Samolus repens* (Primulaceae) and *Frankenia pauciflora* (Frankeniaceae), and the introduced genus *Limonium* (Plumbaginaceae), in a South Australian saltmarsh and cliff habitat

Schlein, M.J., Mackay, D.A. and Whalen, M.A.

Flinders University, School of Biological Sciences,

GPO Box 2100, Adelaide, SA, 5001, Australia

Abstract

The impact of plants of the introduced genus *Limonium* P. Miller in South Australian saltmarshes and cliff communities was investigated through a series of experiments conducted over several flowering seasons, focusing on the reproductive and pollination biology of two native saltmarsh species with flowers of similar size and overall appearance, *Frankenia pauciflora* DC. var. *gunnii* Summerh. and *Samolus repens* (J.R.Forst & G.Forst) Pers. Overlap in flowering seasons was observed for the study species which also shared many insect floral visitors. The most common visitors at these sites were *Eristalinus aeneus* (Scopoli) (Syrphidae: Diptera), *Apis mellifera* Linnaeus (Apidae: Hymenoptera) and *Zizina labradus* (Godart, [1824]) (Lycaenidae: Lepidoptera). The number of visitors per observation period varied as did the impact of other plant species on visitation rates to flowers of the study species. Insect visitors caught at Goolwa carried similar numbers of pollen grains from the three study species whilst at Port Elliott insect visitors predominantly carried pollen of plants of the *Limonium hyblaeum* Brullo agg. and *F. pauciflora*. These results show a highly generalist pollination community, at both the Goolwa saltmarsh and the Port Elliott cliffs, and highlight the complexity of saltmarsh and cliff communities. Pollinator sharing is taking place at both sites, not only between the two native species, but also with the introduced *Limonium*. *Limonium* has become integrated into these communities, and is having positive and negative effects on the naturally occurring flora and fauna and management of the introduced *Limonium* needs to take this into account.

Introduction

Saltmarshes are important ecosystems, yet they face major threats from introduced flora and fauna, land clearing and rising sea levels (Laegdsgaard 2006, Adam 2009, Bromberg Gedan *et al*. 2009). It was thought that the difficult environmental conditions found in saltmarshes would help to protect them from introduced plant species; however, plant invasions have now been noted in saltmarshes around the world (Adam 2009). Introduced plant species from Europe and elsewhere have caused significant damage to saltmarsh ecosystems and habitats in Australia (Boon *et al*. 2011).

The effects of introduced plant species on the pollination biology and reproduction of native plant species range from competitive to facilitative (Choler *et al*. 2001, Bjerknes *et al*. 2007, Muñoz and Cavieres 2008, Thijs *et al*. 2012). The majority of flowering plants around the world depend on animals as pollinators (Lomov *et al*. 2010, Ollerton *et al*. 2011) including many insect species (Ghazoul 2006, Lopezaraiza–Mikel *et al*. 2007, Blažytė-Čereškienė *et al*. 2012) and introduced plant species can not only compete with native plant species for water and nutrients, but also for floral visitors (Brown *et al*. 2002). Introduced plant species can create monocultures, reduce densities of native plant species, and cause fragmentation (Jakobsson *et al*. 2009, Flanagan *et al*. 2011), and these changes can then go on to interfere with pollen transfer and pollination (Brown and Mitchell 2001, Mitchell *et al*. 2009, Thijs *et al*. 2012). Changes in visitation rates and pollen receipt from pollinators, may then lead to pollen limitation, which can alter species diversity and composition (Knight *et al*. 2005) (Chapter three).

Facilitation occurs when an introduced plant species has a positive effect on native plant species (Feldman *et al*. 2004) often by creating a greater floral display (Bjerknes *et al*. 2007) or by providing resources for a more diverse insect community, leading to increased visitation rates to native plant species (Gurevitch and Padilla 2004, Bjerknes *et al*. 2007).

Floral density often influences whether an introduced plant species will have a competitive or facilitative effect on native plant species (Flanagan *et al*. 2010) (see Chapter four). Sun *et al.* (2013) examined the impact of the introduced plant *Solidago canadensis* on the plant-pollinator interactions of two native plant species with similar morphologies. For one of the plant species, *Ixeris chinensis,* an increased density of *S. canadensis* resulted in fewer visits from insect visitors and reduced seed set, suggesting competition. For the other native plant species in the study, S*onchus arvensis,* however, increased densities of *S. canadensis* resulted in increased visitation rates and a trend towards increased seed set, suggesting facilitation. Their study looked at two native plant species with similar floral morphologies, as was done in this study, and it is surprising that the two different species had different responses, given that flower morphology is thought to be an important factor in the response to introduced plant species. These studies highlight how complex plant-plant and plant-pollinator interactions are, and how difficult it is to predict the effects of introduced plant species on these native plant-pollinator interactions.

The present study examined the impact of the introduced plant species *Limonium binervosum* (G.E.Sm.) C.E. Salmon and the *Limonium hyblaeum* agg. on the pollination biology and ecology of two native saltmarsh plant species with showy flowers, *F. pauciflora* and *S. repens,* which are insect-pollinated. Relatively few studies have been conducted on the pollination biology of flowers of saltmarsh plants, or the interactions between these plants and their insect visitors (Adam *et al*. 1987). The plant species used in this saltmarsh study are also present in a nearby cliff population and so the study was extended to provide a comparison.

We asked five main questions:

- Do *F. pauciflora*, *S. repens* and *Limonium* species share a common flowering period?
- Do *F. pauciflora*, *S. repens* and *Limonium* species share floral visitors?
- Does the presence of conspecific and heterospecific flowers affect visitation rates to *F. pauciflora* or *S. repens* flowers?
- Do insect visitors show a preference for *F. pauciflora* or *S. repens*?
- Do insect visitors carry pollen from all of the study species?

It is predicted that *Limonium* species will have a competitive effect on plant-pollinator interactions for *F. pauciflora* and *S. repens*. The saltmarsh and cliff communities included in this study both have harsh environmental conditions and are quite secluded from other plant communities. There is low plant diversity at both sites as well as low insect diversity and the ability of *Limonium* species to not only produce thousands of showy flowers but to also thrive in harsh conditions could make *Limonium* a strong competitor to existing plant species.

Both of the native plant species included in this study have a similar floral appearance, so it could be assumed that both will be affected by *Limonium* in a similar manner.

Materials and methods

Study sites

The sites used in this study are both to the south-east of Adelaide, 11.5 km from one another on the coast of South Australia. Due to their proximity to one another, the two sites share similar weather patterns. The weather conditions at these sites varied over the years of the study, with the Millennium Drought causing below-average rainfall and above-average temperatures (2008-2009) and then later years (2009-2011) experienced above-average rainfall and increased flows in the River Murray (see Chapter one).

Goolwa saltmarsh

The Goolwa saltmarsh (-35° 31' 55.69"S, +138° 49' 36.13"E) is in the Coorong National Park on the Fleurieu Peninsula of South Australia, Australia and falls within the boundaries of the Murray-Darling Basin. The saltmarsh is located 1.6 km below Goolwa Barrage on the Murray River and 6 km above the Murray Mouth, on the Sir Richard Peninsula. Fieldwork took place from October to March from 2008 to 2011. All four common insect-pollinated plant species at this site were examined in this study, including species of *Limonium* that have been introduced into this saltmarsh, *L. binervosum* and the *L. hyblaeum* agg.

Port Elliott cliffs

The Port Elliott cliffs (-35° 32' 15.75"S, +138° 41' 1.81"E) are located on the Fleurieu Peninsula of South Australia, near the town of Port Elliott, approximately 10 km west of the Goolwa saltmarsh. Fieldwork took place from October to March from 2008 to 2011. Only three common insect-pollinated plant species occur amongst the Port Elliott cliffs; *F. pauciflora, S. repens* and the *L. hyblaeum* agg. and all were used in this study.

Study species

Frankenia pauciflora

Frankenia pauciflora DC. var. *gunnii* Summerh. (Frankeniaceae) is a native, small shrub that can be low-growing with spreading branches or form more upright bushes (Whalen 1986). *Frankenia pauciflora* DC. var. *gunnii*, subsequently referred to as *F. pauciflora*, is found in southern Australia in saltmarshes, on coastal cliffs and saline flats (Barnsley 1982). It has actinomorphic flowers around 1 cm in diameter with petals that can vary from white to pale pink (Whalen 1986, Dashorst and Jessop 1998).

Samolus repens

Samolus repens (J.R.Forst & G.Forst) Pers., which is traditionally placed in the family Primulaceae (Toelken 2007, Stevens 2014), is a native, small perennial herb (Toelken 1986b) that occurs along the coast of southern Australia in saltmarshes and on coastal cliffs. *Samolus repens* has actinomorphic flowers around 1 cm in diameter, which vary from white to pale pink in colour (Toelken 1986b, Saintilan 2009).

Introduced species of *Limonium*

Limonium, which is in the family Plumbaginaceae (following Stevens 2007), is an introduced genus in South Australia although *Limonium* occurs naturally alongside *Frankenia a*nd *Samolus* species in Europe and the Mediterranean (Primack 1985). *Limonium* taxa have spread extensively across southern coastal regions of South Australia and Victoria other members of the genus have invaded saltmarshes in other countries, including that of the San Francisco Bay (Archbald and Boyer 2014). *Limonium* species at the study sites are small plants with one to multiple flowering stems projecting above the rosette of leaves (Toelken 1986a).

Limonium binervosum is the most common *Limonium* species at the Goolwa saltmarsh and is readily distinguished in the field from plants of the *Limonium hyblaeum* agg. which are less common. The most obvious difference between the two *Limonium* species is the colour of their calyx and corolla, with *L. binervosum* flowers being purple, whilst flowers of *L. hyblaeum* agg. are purple/ pink in colour. The *Limonium hyblaeum* agg. refers to plants of the *L. hyblaeum* Brullo/ *Limonium companyonis* (Gren. & Billot) Kuntze complex, as these species are difficult to distinguish at the field sites in South Australia (see Chapter one for details). In the Goolwa saltmarsh, plants of the introduced *L. binervosum* (G.E.Sm.) C.E.Salmon and of the *L. hyblaeum* agg. are present, whilst only the latter is present on the Port Elliott cliffs.

Methods

Phenology

To determine the degree of overlap in the flowering times of *F. pauciflora*, *S. repens* and *Limonium* taxa, phenology studies were conducted over three flowering seasons at the Goolwa saltmarsh (2008/2009, 2009/2010 and 2010/2011) and two at the Port Elliott cliffs (2009/2010 and 2010/2011). Thirty healthy individuals of each study species were selected across each study site. Each plant was tagged and the number of open flowers was recorded every two weeks from before the summer flowering period commenced, in October, until after summer flowering had finished in March. The most common *Limonium* found at the Goolwa saltmarsh was *L. binervosum,* which was included in the phenology studies at Goolwa from 2008/2009 to 2010/2011. Plants of the *L. hyblaeum* agg. were included in the study at Goolwa in the 2010/2011 flowering season, to determine if their flowering phenology differed from that of the more common *L. binervosum* in the same saltmarsh, or if the phenology of plants of the *L. hyblaeum* agg. at Goolwa differed from the phenology of plants of the *L. hyblaeum* agg. at the Port Elliott cliffs.

Observations of floral visitors

Observations of floral visitors, and potential pollinators, were carried out at both Goolwa and Port Elliott. One m^2 plots were observed for 15 minutes, after five minutes of settling time, across varying densities and combinations of the study species. Seventy-seven observation periods of floral visitor activity were undertaken in 2008/2009, 266 in 2009/2010 and 276 in 2010/2011 giving 706 observation periods, 463 at Goolwa and 243 at Port Elliott.

An insect observed in a plot was considered a floral visitor if it came into contact with the reproductive parts of a flower. Insect floral visitors were recorded and foraging patterns identified, including the species of the visited flowers, the species the visitors travelled to, the time spent at each flower and the density of flowers present. When possible, insect visitors were caught for pollen load analysis. These field observations of floral visitation rates and behaviours were used to assess the diversity of insects that visited the different study species. Since it was not always possible to distinguish between similar insect visitors in the field, some families were combined for analyses into functional family groups. Moths in the Arctiidae, Geometridae and Tineidae were combined, as were wasps in the Braconidae and Chalcididae, and flies in the Muscidae and Tachinidae, as visitors from these families were not easily differentiated from one another in the field because of their size for example. These functional groups were then used in the analysis where comparisons were made between families and/ or functional groups to determine patterns in visitation.

Pollen loads

When possible, insect floral visitors were caught and examined for pollen loads. Insects were collected in the field and brought back to the laboratory at Flinders University to analyse the pollen loads. Insects were kept in individual vials, 70% ethanol was added and vials were shaken on a mechanical mixer for 30 seconds and shaken by hand for 30 seconds. The liquid was then removed from the vial and placed in a centrifuge tube where it was centrifuged for two minutes at 1500 rpm. The supernatant was removed and the remaining pollen was then placed on a microscope slide for identification. Pollen baskets were removed from bees before processing to help restrict counts of pollen loads to pollen that could have been used in pollination. Insects were then pinned and identified to family, wherever possible, chiefly by Dr. Gabriel Neve, and by specialists Scott Groom, Dr. Michael Schwarz, and Dr. Robert Lavigne and Mike Moore at the SA Museum. This analysis of pollen loads is only preliminary, with some insects proving more difficult than others to catch and thus likely not being represented in proportion to their abundance.

Data analyses

The effects of conspecific and heterospecific floral densities on numbers of floral visits to flowers of *F. pauciflora* and *S. repens* at Goolwa were examined using linear mixed-effects multiple regression models with the densities of flowers of different species treated as fixed factors, and year of study treated as a random factor. For these analyses, only observation periods with flowers of *F. pauciflora* or *S. repens* present in observation plots were used to test the effect of conspecific and heterospecific densities on visitation rates to flowers of these species. Both the independent (conspecific and heterospecific floral densities) and dependent (number of visits per flower) variables were log-transformed before analysis.

Analyses could not be conducted for Port Elliott because of low rates of floral visitation.

Floral preferences were examined for the main insect visitors, by comparing the proportion of visits made to flowers of the study species with the proportional availability (abundance) of flowers of the study species in observation plots. The statistical significance of differences in these proportions was assessed with a binomial test. The SPSS statistical package (version 20) was used for all analyses.

Results

Do *Frankenia pauciflora, Samolus repens* and *Limonium* species share a common flowering period?

Goolwa

The number of open flowers was recorded per censused plant, from October to March at the Goolwa saltmarsh for 2008/2009, 2009/2010 and 2010/2011 (Fig. 2.1) revealing overlap in flowering for all of the study species for all three flowering seasons. Variation in flowering peak was present over the three flowering seasons, with the greatest floral display present during the 2010/2011 flowering season, which occurred after the breaking of the Millennium Drought and the influx of flood waters from the Murray River. The total number of flowers counted during the phenology study differed across species and flowering season, as did the greatest number of flowers open at any one recording period (Table 2.1). There was also variation amongst plants for all three study species at Goolwa.

At Goolwa, flowering peaks for *F. pauciflora* in late January to early February. Although most *F. pauciflora* plants finish flowering in late March, as with the censused plants, a few plants flower all year round. Flowering of *S. repens* at

Goolwa peaked in December although mean numbers of flowers per plant varied markedly across the different flowering seasons. During the flowering season of 2010/2011, *S. repens* showed a reduction in floral display in January/February, as it had in previous flowering seasons; however, flowering increased again in late February/early March.

Limonium binervosum plants at the Goolwa saltmarsh also tended to peak in flowering from December to February and exhibited variation between years in the timing, duration and intensity of flowering. The *Limonium hyblaeum* agg. which is also found at Goolwa but in lower numbers than *L. binervosum*, was included in the phenology study in 2010/2011. Plants of the *L. hyblaeum* agg. commenced and finished flowering at similar times as *L. binervosum* at the Goolwa saltmarsh in 2010/2011, however *L. hyblaeum* agg. did have a greater floral display.

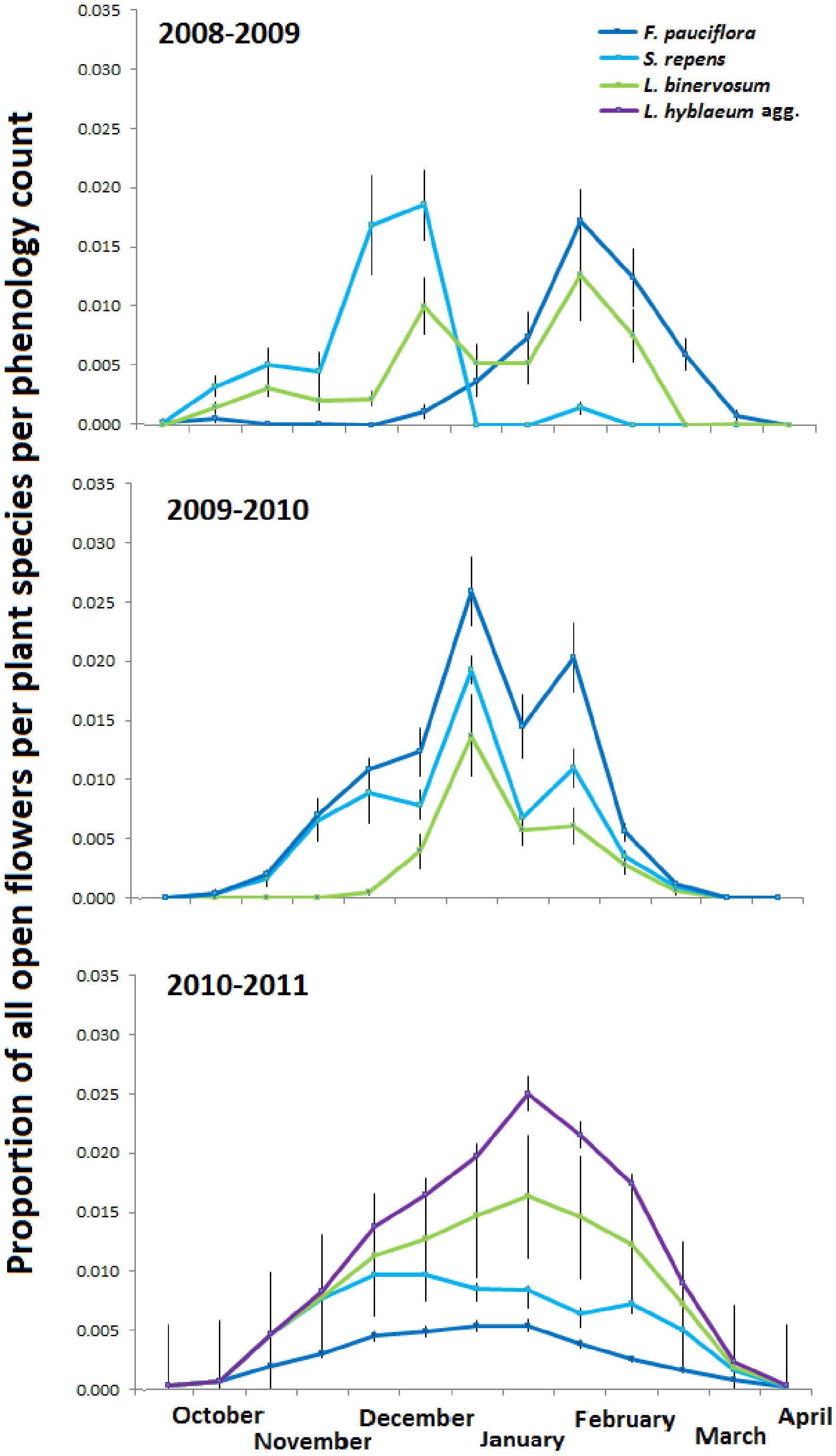

Figure 2.1 The proportion of open flowers per plant species across flowering seasons at the Goolwa saltmarsh from 2008/2009 to 2010/2011 (30 individual plants per plant species). Error bars represent 95% confidence intervals.

Goolwa	*F. pauciflora*	*S. repens*	*L. binervosum*	*L. hyblaeum*
2008/2009	2379 **(100)**	197 **(13)**	2142 **(102)**	NA
2009/2010	3407 **(210)**	102 **(4)**	1577 **(100)**	NA
2010/2011	13072 **(135)**	1040 **(15)**	4905 **(120)**	2895 **(65)**
Port Elliott				
2009/2010	536 **(37)**	113 **(10)**	NP	3604 **(120)**
2010/2011	268 **(6)**	425 **(9)**	NP	3560 **(60)**

Table 2.1 Total number of open flowers recorded during phenology studies at the Goolwa saltmarsh and Port Elliott cliffs from 2008/2009 to 2010/2011, with the greatest number of open flowers on an individual plant per count in parentheses. NA: *L. hyblaeum* agg. was included in the study at Goolwa in 2010/2011 to see if its phenology was similar to that of the more common *L. binervosum*; NP: *Limonium binervosum* is not present at the Port Elliott cliffs.

Port Elliott

Phenology studies were also conducted at the Port Elliott cliffs during the 2009/2010 and 2010/2011 flowering seasons (Fig. 2.2) revealing overlap in flowering periods for all three of the study species. The number of open flowers per plant is considerably lower at Port Elliott than at Goolwa for *F. pauciflora* (Table 2.1). All three study species at Port Elliott had similar peaks for each flowering season, although 2009/2010 saw a quick increase in flower numbers, whereas the 2010/2011 flowering season was spread out more evenly across the summer months. There was much less variation amongst plants for the study species at Port Elliott than at Goolwa.

Do *Frankenia pauciflora, Samolus repens* and *Limonium* species share floral visitors?

Goolwa

Insects from several different orders, including Diptera, Hymenoptera and Lepidoptera, visited the study species at Goolwa (Table 2.2). The most frequent visitors to flowers of the study species at Goolwa during the 2008/2009 and 2009/2010 flowering seasons were insects from the Syrphidae family (Table 2.3) whilst members of the family Apidae were most frequent in 2010/2011. The flowers visited most commonly at Goolwa were those of *F. pauciflora* although the number of *L. binervosum* flowers visited increased during the 2010/2011 flowering season (Fig. 2.3).

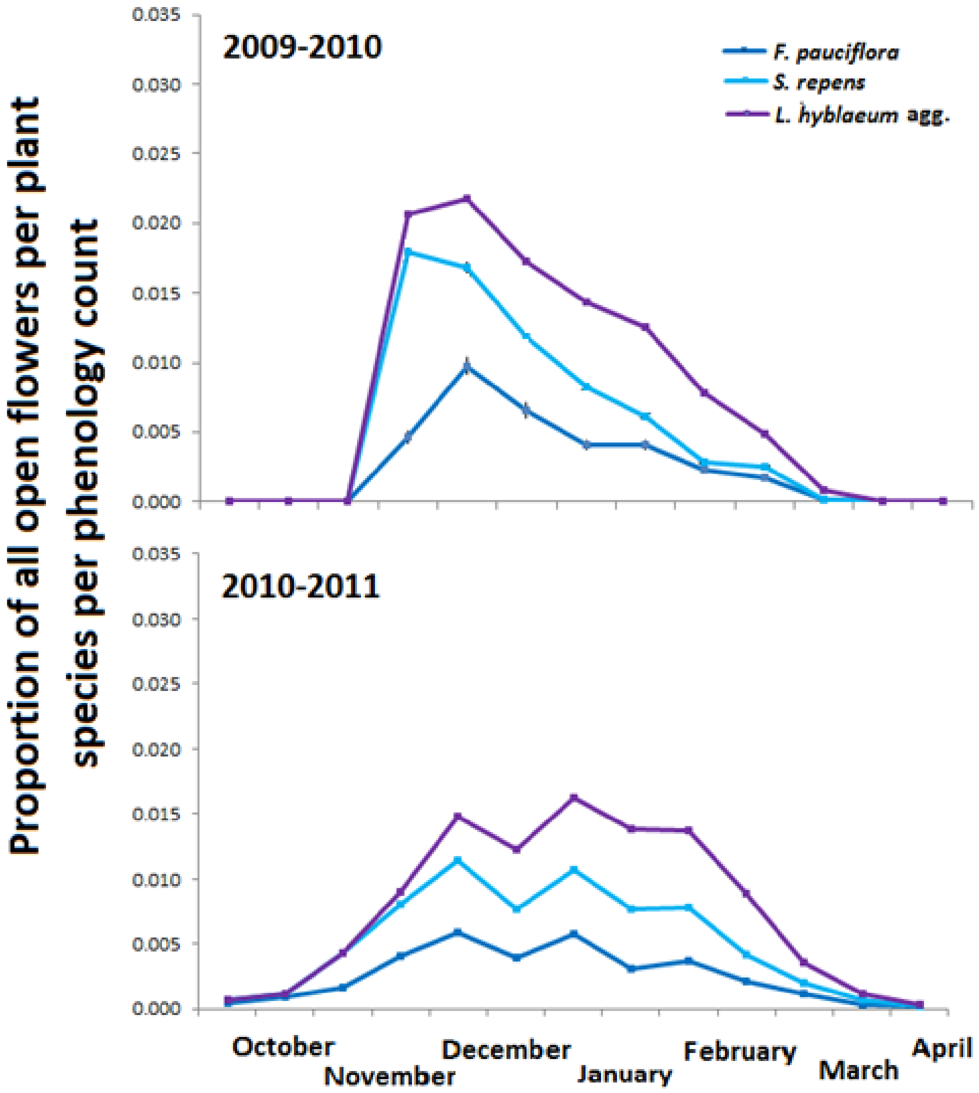

Figure 2.2 The proportion of open flowers per plant species across flowering seasons at the Port Elliott cliffs from 2009/2010 to 2010/2011 (30 individual plants per plant species). Error bars represent 95% confidence intervals.

The most common insect visitors to *F. pauciflora* flowers at Goolwa over all three study periods were syrphids (Table 2.3). Syrphids were also the most common floral visitors to *S. repens* flowers in 2008/2009 and 2009/2010. In 2010/2011 syrphids were only the third most common floral visitors to *S. repens*, representing 17% of all floral visits (six individuals visiting 31 flowers), after lycaenid butterflies (28% of all floral visits) and flies in the Muscidae and Tachinidae representing 19% of all floral visits (five individuals visiting 13 flowers). For *L. binervosum*, the most common floral visitors were lycaenids in 2008/2009, syrphids in 2009/2010 and honeybees (Apidae) in 2010/2011.

Two species of hoverflies (Diptera: Syrphidae) were identified visiting flowers at Goolwa and Port Elliott (see below). The most common species was *Eristalinus aeneus* (Scopoli, 1763), with some individuals of *Eristalinus hervebazini* (Klocker, 1924) also visiting flowers of the study species. Two species of Lycaenidae were recorded visiting flowers at Goolwa and Port Elliott (see below). The most common species was *Zizina labradus* although a few individuals of *Theclinesthes miskini* Lucas 1889 were also recorded.

Apis mellifera, also known as the European, common or western honeybee, was recorded visiting all study species at Goolwa (Table 2.3). At Goolwa an average of 20 flowers from the study species per 15 minute observation period in 2008/2009 was visited by *A. mellifera*, with the corresponding averages in 2009/2010 and 2010/2011 being 51 flowers per observation period and 100 flowers per observation period.

Table 2.2 Insect families recorded at flowers of the study species at Goolwa, during 463 15-minute pollinator observation periods, across the three years of the study with the number of individuals per family observed per year in parentheses. * denotes presence at both Goolwa and Port Elliott.

F = *Frankenia pauciflora*, S = *Samolus repens* and L = *Limonium.*

Family	**2008/2009**	**2009/2010**	**2010/2011**
Anthophoridae		F, S, L (4)	L (1)
Apidae (*Apis mellifera* only) *	F, L (5)	F (6)	F, S, L (29)
Arctiidae, Geometridae & Tineidae		F (4)	F, S, L (9)
Asilidae		L (1)	F, S, L (19)
Bombyliidae			F, S, L (19)
Braconidae & Chalcididae	F (3)	F (5)	
Formicidae	F, S (3)	S (1)	
Lycaenidae *	F, L (10)	F, S, L (36)	F, S, L (27)
Lygaeoidea		S (2)	
Muscidae & Tachinidae *		F, S, L (27)	F, S (9)
Oecophoridae	F (2)	F, S, L (3)	
Pompillidae *		F (2)	
Stratiomyidae			S (2)
Syrphidae *	F, S (17)	F, S, L (174)	F, S, L (28)
Tabanidae		F (2)	
Thysanoptera		S (4)	

Port Elliott

As at Goolwa, insects in several orders, including Diptera, Hymenoptera and Lepidoptera, visited flowers of the study species at Port Elliott (Table 2.4). The most frequent visitors to flowers of the different study species together were *A. mellifera* (Table 2.3) with the most common flowers visited being those of the *L. hyblaeum* agg. The only insect visitors to flowers of *F. pauciflora* observed during 15 minute observation periods at Port Elliott were syrphids in 2009/2010 and native bees (Halictidae) and lycaenid butterflies in 2010/2011 (Table 2.3). No insect visitors were recorded visiting flowers of *S. repens* during 15 minute observation periods in 2009/2010 while in 2010/2011 the most common insect visitors were halictids. The most common insect floral visitor of plants of the *L. hyblaeum* agg. in 2009/2010 and 2010/2011 was *Apis mellifera*.

Apis mellifera was recorded visiting flowers of the *L. hyblaeum* agg. and *S. repens* at Port Elliott with most flowers visited being those *of L.* hyblaeum agg. The number of flowers visited by *A. mellifera* during 15 minute observation periods for the different study species varied between Goolwa and Port Elliott. At Port Elliott an average of 178 flowers of the study species, per 15 minute observation period in 2009/20010 was visited by *A. mellifera*, with an average of 52 flowers per observation period visited in 2010/2011.

Table 2.3 Most common insect floral visitors observed at the Goolwa saltmarsh and Port Elliott cliffs during 706 15-minute pollinator observation periods (463 at Goolwa, 243 at Port Elliott), with the percentage of all visits to flowers of each plant species that were made by different visitors by flowering season, with the number of flowers visited followed by the number of insect visitors of that type. Note: *Limonium binervosum* was observed at Goolwa, whilst *L. hyblaeum* agg. was observed at Port Elliott.

		% of all visits to *F. pauciflora* flowers within flowering season		**% of all visits to *S. repens* flowers within flowering season**		**% of all visits to *Limonium* flowers within flowering season**	
		Number of flowers visited	Number of individuals	Number of flowers visited	Number of individuals	Number of flowers visited	Number of individuals
Goolwa	2008/2009	**Syrphidae 58%**		**Syrphidae 50%**		**Lycaenidae 67%**	
		105	12	3	2	111	4
	2009/2010	**Syrphidae 64%**		**Syrphidae 68%**		**Syrphidae 28%**	
		763	134	127	37	15	3
	2010/2011	**Syrphidae 26%**		**Lycaenidae 28%**		**Apidae *(Apis mellifera) 33%***	
		313	18	34	10	567	13
Port Elliott	2009/2010	**Syrphidae 100%**		no visits recorded		**Apidae *(Apis mellifera) 94%***	
		18	1			9954	58
	2010/2011	**Halictidae 58%**		**Halictidae 52%**		**Apidae *(Apis mellifera) 95%***	
		11	7	21	13	2694	114

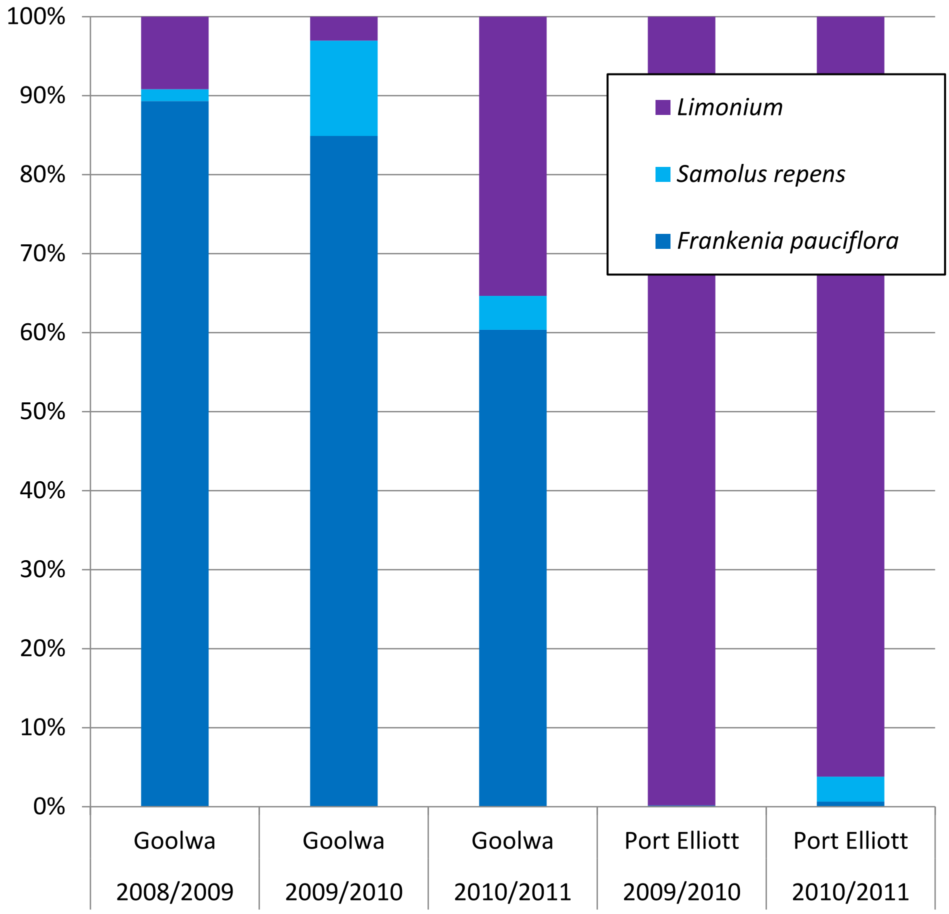

Figure 2.3 Percentages of insect visits to flowers of the different plant species across all flowering seasons at the Goolwa saltmarsh and Port Elliott cliffs observed over 706 pollinator observation periods periods (463 at Goolwa, 243 at Port Elliott). Note: only *Limonium binervosum* was included in 15-minute observations at Goolwa and only *Limonium hyblaeum* agg. at Port Elliott.

Table 2.4 Insect families recorded at flowers of the study species at Port Elliott, during 243 15-minute pollinator observation periods, across the two years of the study with the number of individuals per family observed per year in parentheses. * denotes presence at both Goolwa and Port Elliott.

F = *Frankenia pauciflora,* S = *Samolus repens* and L = *Limonium*.

Family	**2009/2010**	**2010/2011**
Apidae (*Apis mellifera* only) *	L (302)	S, L (117)
Halictidae	L (1)	F, S, L (27)
Lycaenidae *	L (4)	F, S, L (25)
Muscidae & Tachinidae *	L (3)	
Pompillidae *	L (8)	S, L (3)
Syrphidae *	F, L (2)	S, L (4)

Does the presence of conspecific and heterospecific flowers influence visitation rates to other plant species?

Frankenia pauciflora

Over the course of the study, the visitation rates to flowers of *F. pauciflora* in 15-minute observation periods (defined as the log of the ratios of the number of visits to *F. pauciflora* flowers to the number of *F. pauciflora* flowers available) were negatively influenced by conspecific floral densities (t = -4.1, $P < 0.001$) but not significantly by the floral densities of heterospecific species *S. repens* and *L. binervosum* ($P > 0.05$) (Fig. 2.4; Table 2.5). The standard deviation due to differences amongst years (0.02) was less than the residual or error standard deviation (0.29).

Samolus repens

The mixed effects model showed that rates of visitation to flowers of *S. repens* were not affected by floral densities of *F. pauciflora* but were negatively affected by increasing conspecific flower densities and, to a lesser extent, increasing densities of *L. binervosum* flowers (Fig. 2.5; Table 2.6). The standard deviation due to among-year variation was again small (0.006) relative to the residual variation (0.06) in the mixed-effects model.

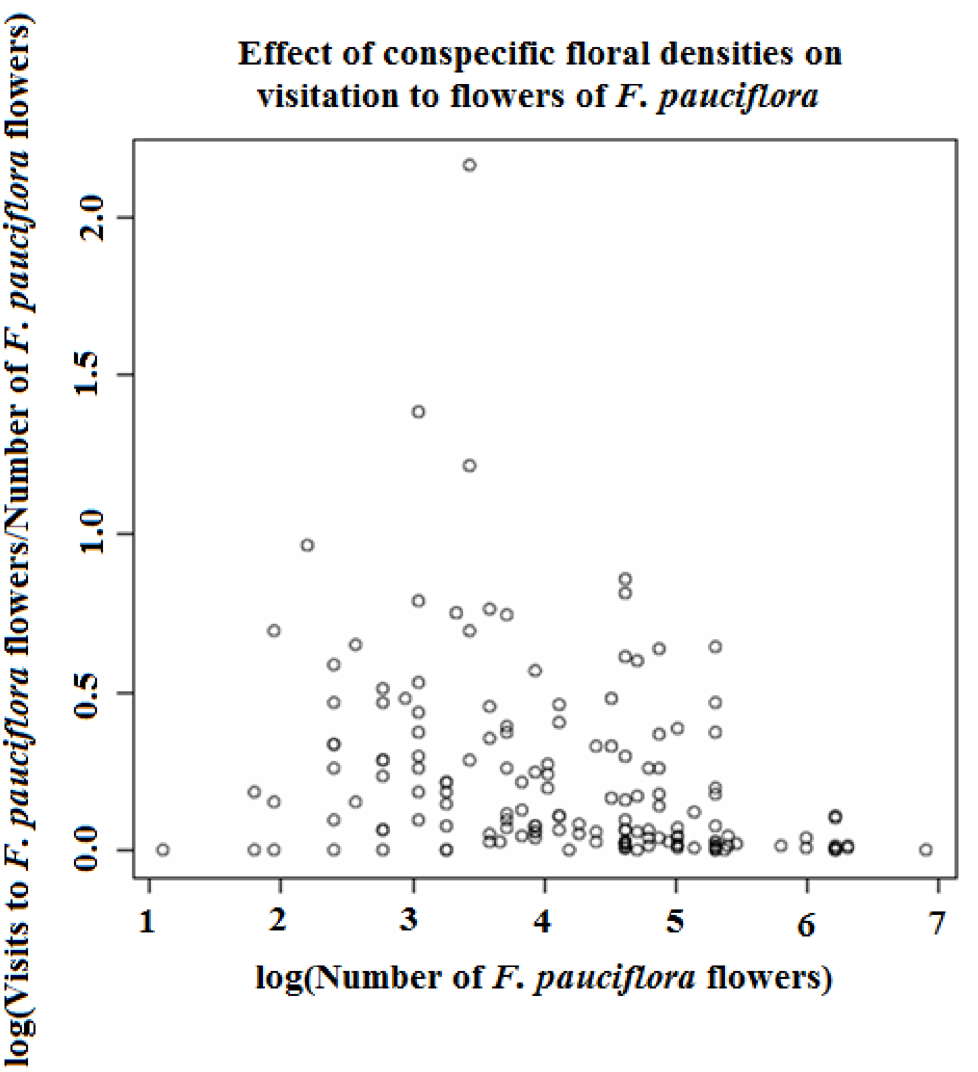

Figure 2.4 Effect of conspecific floral densities on visitation rates to flowers of *Frankenia pauciflora* observed during 463 pollinator observation periods at the Goolwa saltmarsh.

Table 2.5 Linear mixed-effects regression model for the effects of numbers of conspecific and heterospecific flowers on visitation to flowers of *Frankenia pauciflora* observed during 463 pollinator observation periods at the Goolwa saltmarsh.

F = *Frankenia pauciflora*, S = *Samolus repens*, L = *Limonium binervosum*.

F. pauciflora	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.6376	0.106	149	5.985	0.000
log1p(no.F)	-0.0927	0.022	149	-4.099	0.000
log1p(no.S)	-0.0249	0.023	149	-1.054	0.293
log1p(no.L)	-0.0116	0.015	149	-0.771	0.442

	(Intercept)	Residual
StdDev:	0.0164	0.2869

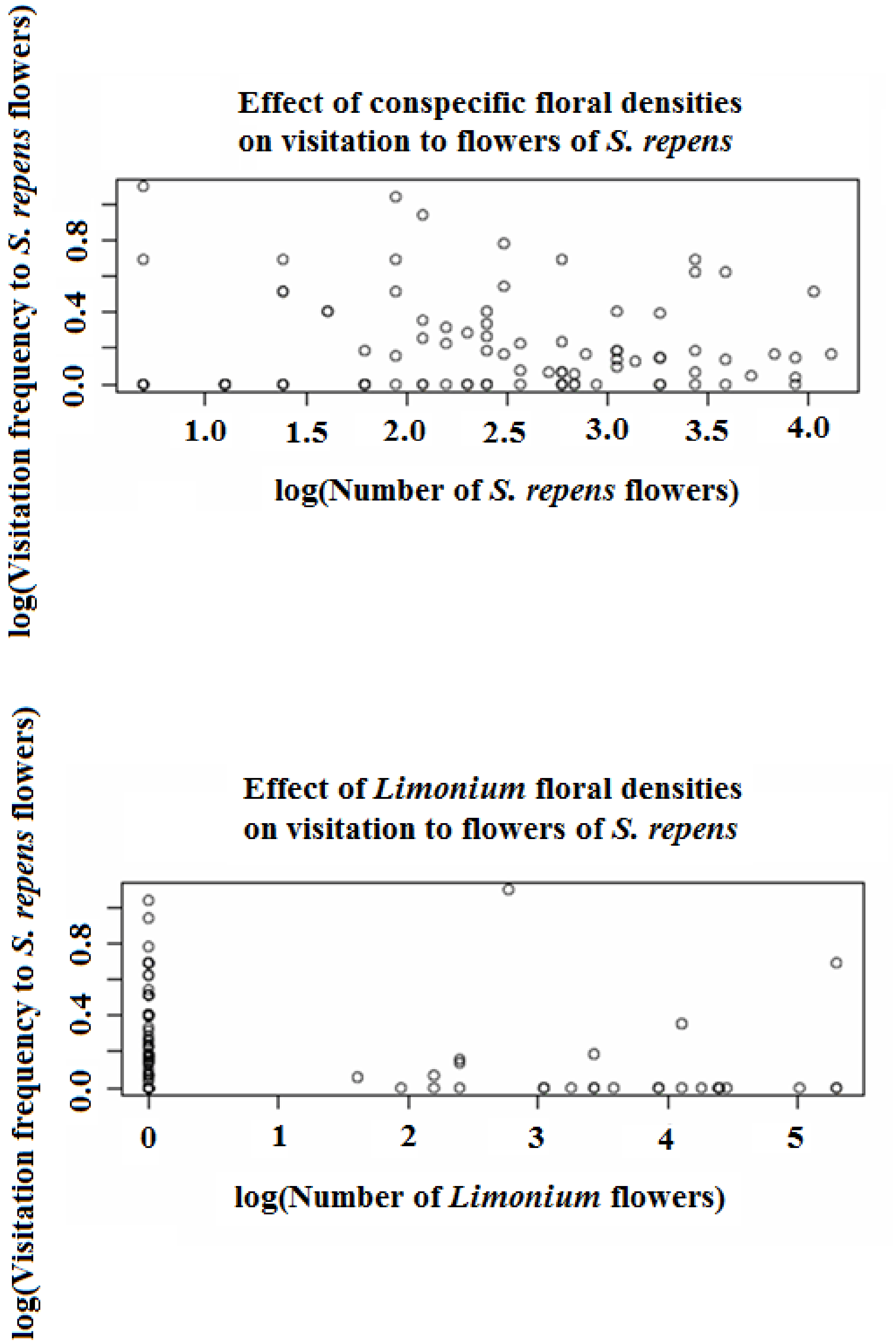

Figure 2.5 Effect of conspecific floral densities (top) and *Limonium binervosum* floral densities (bottom) on visitation rates to flowers *Samolus repens* observed during 463 pollinator observation periods at the Goolwa saltmarsh.

Table 2.6 Linear mixed-effects regression model for the effects of numbers of conspecific and heterospecific flowers on visitation to flowers of *Samolus repens* observed during 463 pollinator observation periods at the Goolwa saltmarsh.

F = *Frankenia pauciflora*, S = *Samolus repens*, L = *Limonium binervosum*.

S. repens	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.5576	0.134	83	4.159	0.000
log1p(no.F)	-0.0270	0.017	83	-1.550	0.125
log1p(no.S)	-0.0983	0.038	83	-2.548	0.013
log1p(no.L)	-0.0428	0.016	83	-2.642	0.010

	(Intercept)	Residual
StdDev:	0.0762	0.2486

Do insect visitors show a preference for *Frankenia pauciflora* or *Samolus repens*?

Visitation rates were examined to determine if the three main insect visitors, syrphids, *A. mellifera* and *Z. labradus*, showed a preference for either *F. pauciflora* or *S. repens* at the Goolwa saltmarsh. Analyses could only be conducted for Goolwa visitation rates because of the low number of visits at Port Elliott.

Frankenia pauciflora

The proportion of floral visits by syrphids to *F. pauciflora* flowers exceeded the proportional availability of *F. pauciflora* flowers in 51 of 80 observations, indicating that syrphids showed a significant preference for *F. pauciflora* flowers (*P* = 0.018). *Zizina labradus* also showed a significant preference for *F. pauciflora* flowers, with proportional visitation exceeding proportional availability in 15 of 20 observations (*P* = 0.041). *Apis mellifera* visits to *F. pauciflora* flowers were proportional to availability (*P* = 0.263).

Samolus repens

In 45 of 71 observations, proportional visitation to *S. repens* flowers by syrphids was less than the proportional availability of *S. repens* flowers, showing a significant avoidance for *S. repens* flowers by syrphids (*P* = 0.032). *Zizina labradus* visits to *S. repens* flowers were proportional to availability (*P* = 0.179). *Apis mellifera* showed a significant avoidance for *S. repens* flowers, with proportional visitation being less than proportional availability in 12 out of 13 observations (*P* = 0.003).

Do insect visitors carry pollen from all of the study species?

A brief examination of pollen loads was conducted, with 162 insects caught using sweep nets (135 at Goolwa and 27 at Port Elliott) (Fig. 2.6). Of all insects caught, only 36% had detectable pollen present.

Goolwa

Of the 135 insects caught at Goolwa, only 41 individuals (31%) carried measurable amounts of pollen (not including pollen baskets from honeybees). The number of pollen grains on individual insects of different orders varied (Fig. 2.7). The insects caught represent 16 families in the Diptera, Hymenoptera, Lepidoptera and Odonata. Of the insects with pollen present, 31 individuals had pollen from plants of *L. hyblaeum* agg. present (76%), seven had pollen of *S. repens* present (17%) and 15 had *F. pauciflora* pollen present (36%). Eleven individuals were carrying more than one species of pollen, with one individual, a Lycaenidae, having all three species of pollen present.

Port Elliott

Of the 27 insects caught at Port Elliott, 18 individuals (67%) carried measureable amounts of pollen (not including pollen baskets from honeybees) (Fig. 2.6). The insects caught represent 7 families in the Diptera, Hymenoptera, Lepidoptera and Hemiptera. The number of pollen grains on individual insects of different orders varied (Fig. 2.7). Of the insects with pollen present, 18 individuals had pollen from plants of *L. hyblaeum* agg. present (100%), zero had pollen of *S. repens* present (0%) and 12 had *F. pauciflora* pollen present (66%). Twelve individuals were carrying more than one species of pollen.

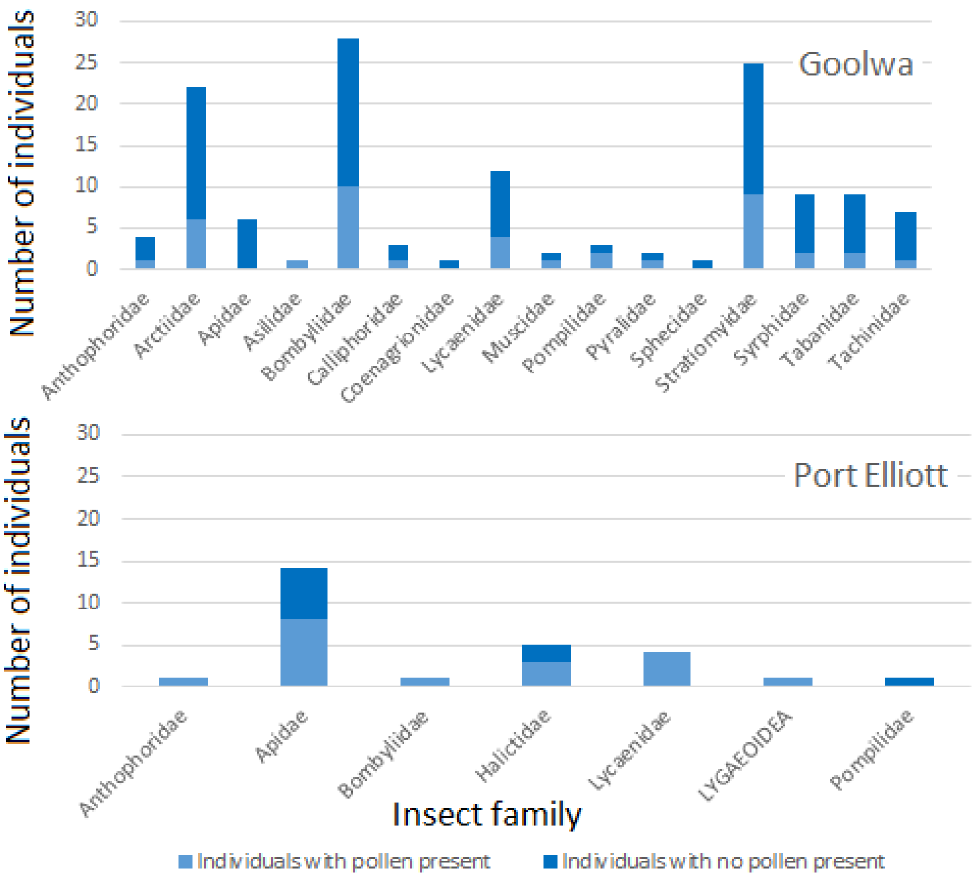

Figure 2.6 The number of insects by family, with pollen present or not present from the Goolwa saltmarsh and Port Elliott cliffs. In total 162 insects were caught, 135 at Goolwa and 27 at Port Elliott, with 41 individuals found to be carrying pollen at Goolwa (31%) and 18 individuals at Port Elliott (67%).

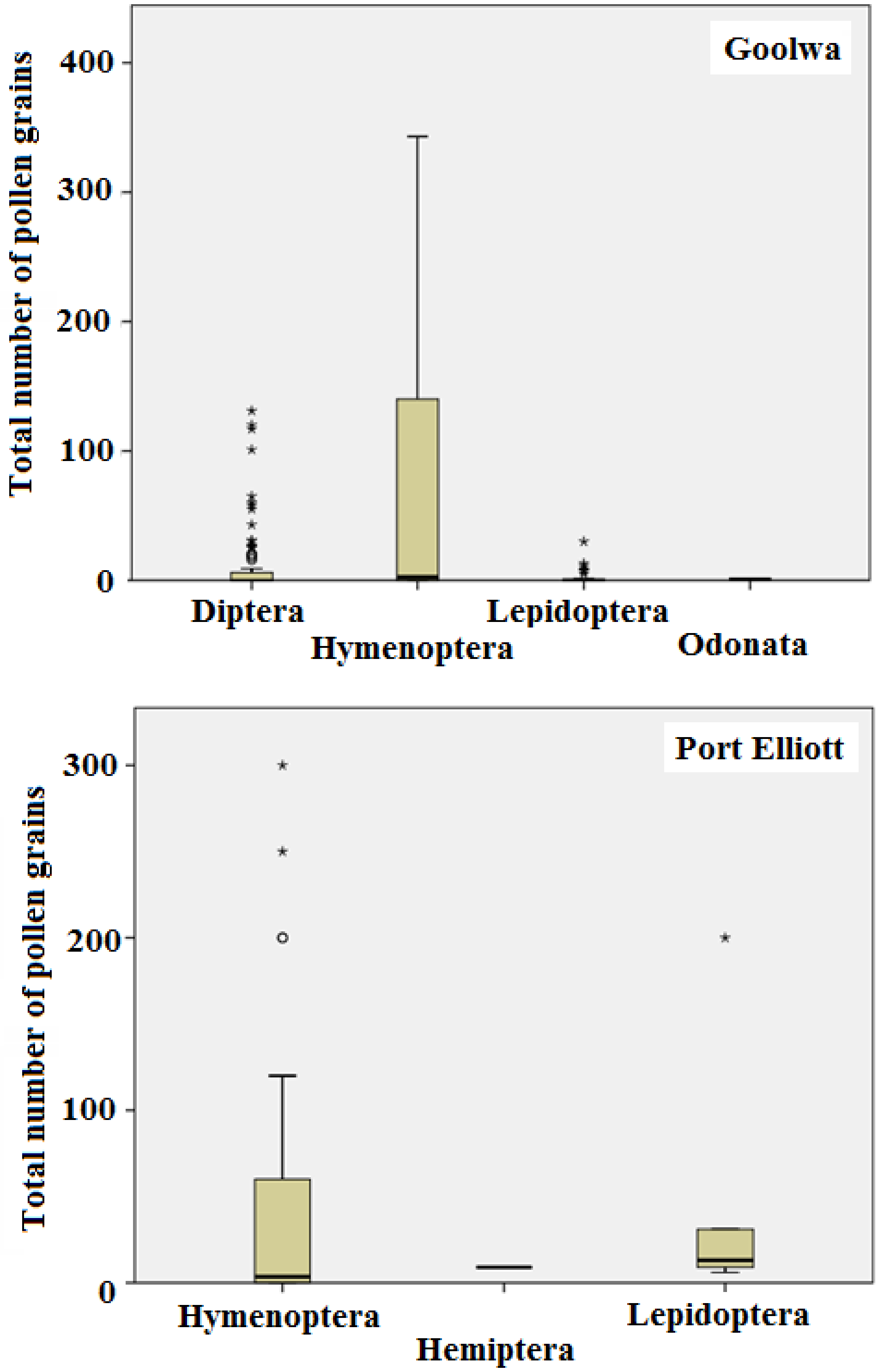

Figure 2.7 Total number of pollen grains on individual insects, by order, at Goolwa and Port Elliott**.** In total 162 insects were caught, 135 at Goolwa and 27 at Port Elliott, with 41 individuals found to be carrying pollen at Goolwa (31%) and 18 individuals at Port Elliott (67%).

Discussion

Flowering phenology and visitation

The aim of this study was to compare the phenology and pollination biology of introduced *Limonium* taxa and two co-occurring native plant species, *F. pauciflora* and *S. repens*, and to examine the impact of the introduced *Limonium* taxa on the pollination biology of these two native species. The phenology studies conducted at Goolwa and Port Elliott confirmed that the two native study species share a common flowering period with the introduced *Limonium* taxa.

A few plants of *F. pauciflora* at Goolwa flower all year round and this may provide a more consistently available floral resource, available when other species are not flowering. *Frankenia pauciflora* would be the only insect pollinated plant species flowering from autumn to spring, so if insects were visiting *F. pauciflora* flowers, there would be no other species to compete with, potentially leading to high rates of floral visitation and high levels of fruit and seed set. This year round flowering could prove wasted however, as the Goolwa saltmarsh has cold, wet and extremely windy conditions over the winter months and it would be difficult for insects, especially flying insects, to withstand these conditions. Syrphids are known to be year round floral visitors at other sites (Sajjad *et al*. 2010), so it would be interesting to examine what floral visitors, if any, are utilising the floral resources of *F. pauciflora* throughout the entire year, not just during the peak flowering times and to see the fruit and seed set for *F. pauciflora* over this time period.

Environmental variables may influence the nature of phenologies, with temperature (Hegland *et al*. 2009) and water availability (Ma *et al*. 2013) able to influence the timing of flowering (Inouye *et al*. 2002, McCall and Irwin 2006), often causing spatial and temporal variation. Temporal variation was present across the flowering seasons at Goolwa and Port Elliott, with peaks in flower numbers for the study species generally reduced during the drier flowering seasons, such as 2008/2009 and increased during the flowering seasons with higher rainfall, such as 2010/2011.

Few studies have been conducted on the pollination biology of saltmarsh plant species, and even fewer studies have looked at insect visitation to saltmarsh plants (Adam *et al*. 1987). This study looked at the pollination biology of both native and introduced saltmarsh species. Floral traits such as odour, colour and morphological features, as well as floral display and density, will determine what floral visitors visit flowers of introduced plant species, which will affect reproductive output and invasiveness (Sunny *et al*. 2015). One of the most important factors for an introduced plant species is to gain pollinators (Pyšek *et al*. 2011, Sunny *et al*. 2015). In this study, observations of floral visitors were conducted, where it was found that many different insect species visit the flowers of both the native *F. pauciflora* and *S. repens* and the introduced *Limonium* at the Goolwa saltmarsh and Port Elliott cliffs.

Rathcke (1988) studied competition for pollinators for four co-flowering plant species, which found that all four plant species shared pollinators. It was hypothesised that the reason these species could all flower together and successfully share pollinators was because of their generalist pollination assemblages, extended floral longevity and ability to self-pollinate (Rathcke 1988). Other studies have also found that these three traits can lead to reproductive success, such as Motten (1986) who studied co-occurring plant species for pollen limitation in a wildflower community. Motten found that some of the species studied were not pollen limited, whilst others were, and concluded that for those species not pollen limited, a number of traits were present, including an extended flowering period and the ability to be pollinated by a wide range of floral visitors. Motten also noted that the ability to self-pollinate can increase the likelihood for successful reproduction. For plant of *F. pauciflora* and *S. repens*, although they are visited by generalist pollinators, there is no distinct time where one flowers without the other, and both have been shown to be self-incompatible (Chapter five). Gross and Werner (1983) studied four co-occurring plant species in abandoned agricultural fields in North America and found varying results. It was concluded that the key to successful reproduction in these fields, was to have a flowering period with as little overlap with other plant species as possible, to reduce competition for pollinators. In a 2003 study by Rathcke on *Kalmia latifolia* in the Great Swamp in North America, floral longevity was found to be the key to reproductive success with an increase in seed set and a reduction in pollen limitation. Floral longevity is especially important in locations where pollinators are scares, or where environmental conditions make pollination uncertain (Rathcke 2003). The previously mentioned traits are also beneficial for plant species who occur in harsh environments, or areas with unfavourable weather conditions for pollinators, such as the Goolwa saltmarsh and Port Elliott cliffs (Motten 1986, Rathcke 1988). With regard to the study at Goolwa and Port Elliott, the presence of pollen limitation was examined for plants of *F. pauciflora* and *S. repens* (Chapter three) to determine if these differences in visitation patterns were expressed through pollen limitation.

Hegland and Totland (2005) examined the role of different floral traits on visitation rates to wildflowers in grasslands in Norway. Of the various traits investigated, the strongest relationships were between visitation frequency and the visual display area and date of peak flowering. And although flower morphology was thought to be important in flower selection for insects, it was only important to Diptera, a finding which corresponds with other studies on Diptera (see below). The grassland study found that areas with large floral displays, or high floral density attracted the most insect visitors as costs for searching for flowers are reduced for insect floral visitors. This could explain why flowers of *S. repens* are visited less by insect visitors in the Goolwa saltmarsh than flowers of *F. pauciflora* and *Limonium* despite sharing a similar size and shape (Hegland and Totland 2005). Both *F. pauciflora* and *Limonium* plants can have more than 100 open flowers at any one time, whist *S. repens* was only recorded with a maximum of 15 open flowers at any one time (Table 2.1). The role of density was examined for *F. pauciflora* and *Limonium* in Chapter four.

Most common visitors: Syrphidae, Apidae, and Lycaenidae

Syrphidae

Hoverflies (Diptera: Syrphidae), also known as flower flies or syrphids, were the most common visitors at Goolwa, and were recorded visiting all of the study species across the different flowering seasons. Two species of hoverflies were identified visiting flowers of the study species at Goolwa and Port Elliott, the most common being *Eristalinus aeneus* (Scopoli, 1763). *Eristalinus aeneus* was the most commonly sighted syrphid at Goolwa, and is widely distributed over many parts of the world with a large host plant range (Ghahari *et al*. 2008, Sajjad *et al*. 2010). Sajjad *et al.* (2010) examined seasonal variation and the abundance of hoverflies in Multan, Pakistan. *Eristalinus aeneus* was one of the most abundant and frequent floral visitors and was also one of only four hoverfly species present all year round (Sajjad *et al*. 2010). In another study by Sajjad and Saeed (2010) looking at the host plant ranges of hoverfly species in southern Punjab, Pakistan, *E. aeneus* was one of the most frequent floral visitors to a large number of plant species. This study found that *E. aeneus* is a frequent and abundant visitor to multiple species at both the Goolwa saltmarsh and the Port Elliott cliffs, and if present all year round at the Goolwa saltmarsh, could visit the flowers of *F. pauciflora* all year round.

Syrphids showed a significant preference for flowers of *F. pauciflora*, with visitation rates being higher than expected from the availability of flowers. Members of the Syrphidae family were the most common visitors to *F. pauciflora* across all three flowering seasons at Goolwa. Mackay and Whalen (2009) also found syrphids to be visitors to flowers of *F. pauciflora* at Goolwa, and Brightmore (1979) found that syrphids visit flowers of *Frankenia* species in western European saltmarshes.

With regards to *S. repens*, members of the Syrphidae family avoided visiting *S. repens* flowers, with visitation rates being significantly less than expected from the proportional availability. Nevertheless, syrphids were still found to visit some *S. repens* flowers, and whilst syrphids do not prefer *S. repens* to *F. pauciflora*, they may still visit some *S. repens* flowers. These visits may actually contribute a large proportion of visits to *S. repens* flowers, and syrphids could prove to be very important to the reproductive output of *S. repens*. Syrphids are a very important group of pollinators both locally and globally (Tooker *et al*. 2006, Ghahari *et al*. 2008, Ssymank *et al*. 2008, Sajjad and Saeed 2010, Sajjad *et al*. 2010, Petanidou *et al*. 2011); however, research into their role as pollinators is lacking (Ssymank *et al*. 2008, Sajjad *et al*. 2010, Petanidou *et al*. 2011). Hoverflies visit hundreds of different plant species and use the nectar from these flowers for energy and pollen for proteins, lipids and vitamins (Faegri and van der Pijl 1979, Tooker *et al*. 2006, Sajjad *et al*. 2010).

Multiple studies have found that syrphids preferentially select white or yellow actinomorphic flowers over other flowers for food and ovipositioning (Faegri and van der Pijl 1979, Sutherland *et al*. 1999, Sajjad and Saeed 2010, Sajjad *et al*. 2010, Xie *et al*. 2013), with colour known to be one of the most important floral signals for floral visitors (Jersáková *et al*. 2012). Day *et al.*'s study in 2015 found that flower colour was of 'paramount importance' for syrphid flower selection. In fact, syrphids selected yellow flowers even when there were no rewards available, highlighting the importance of colour to syrphids (Day *et al*. 2015). When olfactory cues were tested, syrphids preferentially selected yellow flowers, indicating that olfactory cues, from pollen or nectar for example, are still less attractive to syrphids than colour (Day *et al*. 2015). Campbell *et al*. (2010) conducted an alpine study where flower colour was manipulated, finding that 80% of syrphid visits were to white flowers painted yellow over un-painted white flowers, and UV markings or patterns had no effect on visitation (Campbell *et al*. 2010). McGimpsey and Lord (2015) found in their alpine study of floral preference, that syrphids and bees both avoided purple flowers and preferentially selected white flowers of *Euphrasia dyeri*. Interestingly, even though the purple and white flowers have the same floral morphology, purple flowers no longer have pollen or nectar available whilst the white flowers do, which poses the question, of whether white flowers are selected because of their colour or because of the rewards available.

This colour preference is confirmed by a preference for *F. pauciflora* flowers, which are usually quite pale or white at the study sites, although they can also be pink. However, preference for certain colours does not explain why syrphids preferentially select flowers of *F. pauciflora* but not those of *S. repens*, which have flowers that are remarkably similar in size and overall appearance. De Groot and Bevk (2012) found that syrphids in a Slovenian forest mainly visited herbs and shrubs and showed a preference towards members of the family Asteraceae and poison ivy. They concluded that syrphids use flower morphology when choosing flowers during foraging bouts and prefer open shaped flowers (de Groot and Bevk 2012). Floral morphology, or floral architecture as Hogg *et al.* (2011) referred to it, was also found to be important in their study of flower attractiveness to beneficial insects. The study found that syrphids were consistently attracted to swe*et al*yssum, possibly due to its short corolla, which increases the availability of nectar (Hogg *et al*. 2011). Sajjad and Saeed commented that many syrphid species actually have relatively short mouth parts, which would make flowers with open or short flowers easier for syrphids to get to nectar and pollen etc. (Sajjad and Saeed 2010). *Frankenia pauciflora* flowers may have slightly shorter corollas than *S. repens*, which although may not be apparently obvious, but could be important to syrphids when selecting flowers to visit. It may also be possible that floral density plays a part in flower selection for syrphids in the Goolwa saltmarsh, as *F. pauciflora* tends to have a greater floral density than *S. repens*. The role of floral density is discussed in more detail in Chapter four.

***Apis mellifera* (Hymenoptera: Apidae)**

Apis mellifera, or European honeybees, were introduced to Australia in 1821 to produce honey and to aid in crop pollination (Horskins and Turner 1999, Pyke 1999). Individuals escaped from domestic or agricultural hives and established wild hives, spreading across most of Australia, being absent only from colder areas like the Great Dividing Range (Paton 1993, Horskins and Turner 1999, Pyke 1999). It was initially thought that *A. mellifera* would be beneficial to native pollination networks; however, introduced honeybees are now known to cause significant damage to native pollination networks (Paton 1993, Horskins and Turner 1999, Pyke 1999).

The major concern with the introduced *A. mellifera* is that although it may be an efficient pollen remover, it may be less efficient at pollen deposition and may simply be removing pollen from the pollination network and acting as a pollen and/ or nectar robber (Carbonari *et al*. 2009, Ollerton *et al*. 2012, Polatto and Chaud-Netto 2013, Zych *et al*. 2013). *Apis mellifera* uses many Australian native plants for nectar and pollen and many of the insect visitors of those plant species may be affected by the presence of *A. mellifera* (Paton 1993, Horskins and Turner 1999, Pyke 1999), in fact, *A. mellifera* is known to visit more than 200 Australian plant genera, is often the most frequent visitor to these plant genera, and often consumes more than half of the floral resources available (Paton 1996). Paton (1993) studied *A. mellifera* visitation in Adelaide, South Australia, and found that 80 genera from 32 families were visited, including species which were wind, insect and bird pollinated. The effects of *A. mellifera* vary however, with some plant species showing increases in seed set and others showing a reduction in seed set (Paton 1996). Plant species who saw an increase in seed set may likely have been suffering from pollen limitation due to an inadequate rate of visitation and pollen transfer from native pollinators, whilst *A. mellifera* may be causing pollen limitation in other plant species (Paton 1996). Pollen limitation, discussed in more detail in Chapter three, can occur through the loss of floral visitors etc. and some Australian plants now depend on *Apis mellifera* as floral visitors.

Webb (2011) found that *A. mellifera* visits flowers of *Sarcocornia quinqueflora* in a saltmarsh at Sydney Olympic Park, a species present at the Goolwa saltmarsh. The removal of large quantities of pollen by the introduced *A. mellifera*, meant that there was less available pollen for native or more efficient pollinators (Webb 2011). Adam *et al.* (1987) found that *A. mellifera* was collecting pollen from *S. quinqueflora* flowers in New South Wales, whilst Keighery (1979) found that *A. mellifera* and syrphids were collecting pollen from *Suaeda australis*, another species also present at the Goolwa saltmarsh. Keighery went on to state, that insect pollination may be significant for at least some populations of *S. australis*, a species, along with *S. quinqueflora*, traditionally thought to be wind pollinated. Hermansen *et al.* (2013) identified *A. mellifera* as the most dominant visitor to the mangrove species *Avicennia marina* in Australian temperate mangroves, and it was concluded that *A. mellifera* had likely displaced one or more native pollinators, especially since only three insect species carried *A. marina* pollen (Hermansen *et al*. 2013). It would be insightful to examine *A. mellifera* visitation further at the study sites to see what other plant species are being visited.

Apis mellifera showed no significant preference or avoidance for *F. pauciflora* flowers at the Goolwa saltmarsh, suggesting that *F. pauciflora* flowers were visited if present, but were not sought out. If *F. pauciflora* flowers are visited by *A. mellifera* only when they are come across, and not preferentially selected, plants of *F. pauciflora* could potentially have a great enough floral display to be able to withstand nectar and pollen loss without pollen limitation taking place. Interestingly, whilst *F. pauciflora* flowers were visited by *A. mellifera* at the Goolwa saltmarsh across all years of the study, none were visited at Port Elliott across any of the study years, where *F. pauciflora* plants are much smaller, with a greatly reduced floral display.

In contrast to *F. pauciflora*, *A. mellifera* specifically avoided flowers of *S. repens*. This could prove important to *S. repens* if *A. mellifera* is acting as a pollen and/or nectar thief. It may be that the density of *S. repens* flowers is too low to attract individuals of *A. mellifera* and if *A. mellifera* is not visiting flowers of *S. repens*, then it leaves pollen available for native insect visitors and pollinators. This, along with a lack of visitation to *F. pauciflora* flowers at Port Elliott indicates that density is very important to *A. mellifera* in flower selection.

Plant species composition differs quite significantly between Goolwa and Port Elliott, with large stands of *F. pauciflora*, *S. repens* and *L. binervosum* plants at Goolwa, but only large stands of the *L. hyblaeum* agg. plants at Port Elliott. Differences in plant composition could account for the large difference in floral visitors between the two sites. It may be that because the plants of *L. binervosum* found at Goolwa appear to be male sterile and have no pollen, not enough resources are present to maintain the *A. mellifera* populations similar to those found at Port Elliott, where plants of the *L. hyblaeum* agg. do have pollen present. In Australia, many introduced plant species from Europe are now being visited by introduced insect species from Europe (Goulson 2003), which was the case with the introduced plant genus *Limonium* and *A. mellifera* at Goolwa, and most prominently at the Port Elliott cliffs and the Torrens Island saltmarsh, discussed in Chapter four.

Zizina labradus **(Lepidoptera: Lycaenidae)**

Two species of Lycaenidae were recorded visiting flowers at Goolwa and Port Elliott, with the most common species being *Zizina labradus*. *Zizina labradus*, or the common grass-blue butterfly, is a member of the family Lycaenidae, the largest butterfly family in the Australian subregion (Braby 2000, Yago *et al*. 2008). The genus *Zizina* is small, with only one species in the Australian faunal subregion, but has a wide distribution across tropical to temperate zones from Africa to Japan and is most commonly found in open grasslands, riversides and coastlines (Braby 2000, Yago *et al*. 2008, New 2011). Very little is known about this species or its effectiveness as a pollinator. In this study, although it was a common visitor to *S. repens*, none of the fourteen individuals caught carried *S. repens* pollen. Of the six individuals caught that carried *F. pauciflora* pollen, all of them also carried pollen from plants of *L. hyblaeum* agg.

Does the presence of conspecific and heterospecific flowers influence visitation rates to the other plant species?

Rates of visitation to flowers of native species, *F. pauciflora* and *S. repens,* were reduced as conspecific floral densities increased. This result suggests that at high densities, some flowers may not receive sufficient visitation to ensure pollination, a finding consistent with the demonstrated pollen limitation experiments discussed in Chapter three. Whilst a greater floral display per plant may be more attractive to insect visitors, on an individual flower level, this can increase within-plant competition, which potentially eliminates any benefit of an increased floral display (Toräng *et al*. 2006, Yang *et al*. 2011).

Visitation rates to flowers of *S. repens* declined as floral densities of *L. binervosum* increased, indicating possible competition for pollinators with *L. binervosum* flowers. The possibility of such a competitive effect is increased by the considerable overlap in flowering period and the number of shared floral visitors.

It is not uncommon for multiple flowering plants to share a common flowering period and to also share pollinators (Ghazoul 2006, Lopezaraiza–Mikel *et al*. 2007, Blažytė-Čereškienė *et al*. 2012), as was the case for these study sites and species. Such overlap can lead to interactions between and amongst plant species and their pollinators within a community (Yang *et al*. 2011). It is assumed that when sharing occurs, competition or facilitation will take place, with the flowers that are most attractive receiving the most visits (Yang *et al*. 2011, Blažytė-Čereškienė *et al*. 2012). Different groups of pollinators show preferences for different floral traits, such as larger flowers, larger floral display or greater nectar rewards (Fenster *et al*. 2006, Ishii and Harder 2006, Celedon-Neghme *et al*. 2007).

The numbers of *S. repens* and *F. pauciflora* flowers open at any one time are typically very different, with *S. repens* flowers peaking at an average of five open flowers per plant in 2010/2011 compared to *F. pauciflora* with a peak average of 60 open flowers per plant during the same flowering season. For *S. repens*, flowers appear to be self-incompatible, so by lowering the floral density of individual plants, the likelihood of pollen movement between flowers of the same plant is reduced. Because *S. repens* has a much smaller floral display than *F. pauciflora*, it may be more susceptible to competition from the introduced *Limonium*.

Vanparys *et al.* (2008) examined differences in insect visitation and seed set between an introduced species and two native species of *Senecio*, who share morphological similarities with overlapping phenologies. It is known that floral display and phenology are important factors in attracting insect visitors, Vanparys *et al.* (2008) found that *Senecio inadequidens* had a higher visitation rate than *Senecio jacobaea* due to differences in floral display, although insects visited more capitula per plant on *S. jacobaea*. It is thought then, that although *S. jacobaea* plants have lower visitation, more capitula per plant are visited, due their proximity to one another. It was concluded that it was differences in floral display which caused differing responses from floral visitors. The study also found that syrphids represented more than half of all visits to the study species making them of major importance to the pollination of the two *Senecio* species as they appeared to be pollinators.

There can also be variation of time and across populations. Moragues and Traveset (2005) examined the effect of introduced *Carpobrotus* species on a number of co-occurring native plant species who share a common flowering period. One of the species showed a competitive effect for pollinators, two species showed facilitative effects and the last species had a neutral interaction with the introduced plant species (Moragues and Traveset 2005). The study also found that these effects varied across time. Interestingly, although two of the species share similar appearances, the effects of the introduced plant species were different, with one showing negative and neutral effects whilst the other showed facilitative and neutral effects. The study highlights the variability in responses to introduced plant species and the need to conduct observations and studies over multiple years and preferably, across multiple populations.

It seems most likely, that the reason why *F. pauciflora* and *S. repens* respond differently to *Limonium* is because of their differences in floral display. It is possible that less obvious differences are present between *S. repens* and *F. pauciflora*, such as differences in floral odour, differences in ultraviolet markings that are not visible to the human eye, or differences in the amount of nectar or pollen available, and these warrant further investigation.

Do insect visitors carry pollen from all of the study species?

At Goolwa, the majority of insects examined for pollen loads had no measureable pollen present. Of the insects that did, 35% had pollen from more than one plant species of the study present and 2% had pollen from all three plant species present, confirming the pollinator sharing observed in the floral observations.

Members of the Bombyllidae family, also known as bee flies, carried large amounts of *F. pauciflora* and *L. hyblaeum* agg. pollen. Adult bombyliids are nectar and pollen feeders, with females obligate pollen feeders (Evenhuis and Greathead 2003, Evenhuis 2011). Because of their need for nectar and pollen, many bombyliids serve as the main pollinators for many flowering plants, and many flowering plant species are dependent on these bombyliids for pollination (Evenhuis and Greathead 2003, Evenhuis 2011, Fernández *et al*. 2012).

One individual from the genus *Scolia* (Hymenoptera) was caught at Goolwa and carried large amounts of both *S. repens* and *L. hyblaeum* agg. pollen, with *F. pauciflora* pollen also present, but in lower amounts. This individual was the only insect examined for pollen that carried all three species of pollen. Scoliid wasps, also known as flower wasps, feed on nectar and honeydew and are important pollinators for some plant species (Hurd Jr. 1952, Barratt 2003). Members of the genus *Scolia* are visitors of many flowering plants (Hurd Jr. 1952), although none were observed visiting flowers of the three study species during floral observation periods.

Other families with floral visitors bearing considerable pollen loads were the Stratiomyidae (Diptera), Pompilidae (Hymenoptera) and Tabanidae (Diptera), all known to be floral visitors feeding on nectar and pollen, and subsequently acting as pollinators for certain flowering species (Shuttleworth and Johnson 2006, Lessard and Yeates 2011, Woodley 2011, Kurczewski and Edwards 2012, Daniels 2013).

Syrphids were one of the most common visitors to flowers at the two study sites, although very few individuals carried detectable pollen. Adult syrphids are major pollinators of numerous flowering plants globally (Ghahari *et al*. 2008, Sajjad and Saeed 2010, Petanidou *et al*. 2011, Thompson and Vockeroth 2011); however, most individuals caught did not carry detectable pollen.

At Port Elliott, the majority of insects caught carried measureable pollen, and of these, 17% had pollen from more than one of the study species, five individuals carried pollen from one of the native plant species. None of the insects carried pollen from all three plant species. At Port Elliott, the main floral visitor was *A. mellifera*, predominantly to flowers of plants of *L. hyblaeum* agg. and of the floral visitors with detectable pollen, most were *A. mellifera* bearing *L. hyblaeum* agg. pollen.

The insects caught at these two sites do not represent an exhaustive collection of the insect floral visitors, but represent what could be caught on certain days throughout the flowering season, and the relative representations of these visitors in the study samples are likely to be biased by the relative difficulty of catching several of the visitor types.

Conclusion

This study examined the effects of the introduced plant species *L. binervosum* and *L. hyblaeum* agg. on *F. pauciflora* and *S. repens* in the Goolwa saltmarsh and Port Elliott cliffs. It was confirmed that *F. pauciflora*, *S. repens* and the two introduced *Limonium* species all share a common flowering period and floral observations revealed that all four of the study species share floral visitors. Visitation rates to *F. pauciflora* were found to be negatively affected by increasing conspecific floral density whilst *S. repens* and *Limonium* floral densities had no effect. Visitation rates to *S. repens* were also negatively affected by increasing conspecific floral density, *F. pauciflora* density had no effect however increasing density of *Limonium* had a negative effect. Of the three main insect visitors, syrphids and *Z. labradus* both preferentially visited flowers of *F. pauciflora* whilst *A. mellifera* visits to *F. pauciflora* flowers were proportional to availability. For *S. repens,* none of the three main insect visitors preferentially selected *S. repens* flowers, *Z. labradus* visits were proportional to availability and syrphids and *A. mellifera* avoided flowers of *S. repens*. Pollen was only present on 31% of insects examined at the Goolwa saltmarsh and 67% at Port Elliott.

These results have illustrated a range of effects of the introduced *Limonium* species on the pollination biology of the native plant species *F. pauciflora* and *S. repens*. For *F. pauciflora* and *S. repens*, which share a common flowering period with *Limonium,* outcomes are likely to differ. Both *F. pauciflora* and *S. repens* share pollinators with *Limonium*; however, visitation rates to *F. pauciflora* are not affected by the presence of *Limonium*, whereas visitation rates to flowers of *S. repens* are reduced in the presence of high densities of *Limonium.* Two of the most common insect visitors, syrphids and lycaenids (*Z. labradus),* both preferentially select flowers of *F. pauciflora*: however, no insect visitor does so for *S. repens*. In fact, syrphids and *A. mellifera* both tended to avoid *S. repens* flowers.

For two plant species that share very similar flower size and overall appearance, these differences are unexpected; however, the floral displays (number of open flowers at any given point per plant) are considerably different for the two plant species, and this could explain these differences in interactions with insect visitors.

Few studies have been conducted on the pollination biology of saltmarsh plants, and this study has added to our knowledge of not only saltmarsh plant species but on the impacts of introduced plant species in saltmarshes.

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3. Pollen limitation in two native plant species, *Samolus repens* (Primulaceae) and *Frankenia pauciflora* (Frankeniaceae), in a South Australian saltmarsh

Schlein, M.J., Whalen, M.A. and Mackay, D.A.

Flinders University, School of Biological Sciences,

GPO Box 2100, Adelaide, SA, 5001, Australia

Abstract

While many saltmarsh plants are predominantly wind-pollinated, several saltmarsh plant species depend heavily on insect pollinators for their reproduction; however, the extent to which insect-pollinated plants in salt marshes experience pollen limitation is largely unknown. This study examined the occurrence and magnitude of pollen limitation in two co-occurring native insect-pollinated plant species, *Samolus repens* (J.R.Forst. & G.Forst.) Pers. (Primulaceae) and *Frankenia pauciflora* DC. var. *gunnii* Summerh. (Frankeniaceae), in the Goolwa saltmarsh in South Australia. For several flowering seasons, *S. repens* and *F. pauciflora* were pollen-limited with significantly more fruits set and significantly more seeds per fruit when pollen addition took place. For *S. repens*, fruit set was significantly increased only once with pollen addition but the number of seeds per fruit significantly increased in three of the four pollen limitation experiments with the addition of pollen. For *F. pauciflora*, in four of the five pollen limitation experiments pollen addition significantly increased fruit set and the number of seeds per fruit. For the earliest *F. pauciflora* and *S. repens* pollen limitation experiments, conducted in drought years, consistent effects of pollen addition were not obtained, with resource (rainfall) limitation a possible cause. Responses of seed mass were inconsistent and were sometimes heavier in controls, suggesting the possibility of a trade-off between seed number and seed mass. By conducting pollen limitation

experiments over multiple flowering seasons, year-to-year variation can be examined and information that is more reliable can be gathered regarding the reproductive biology and pollen limitation of native saltmarsh species. This study concluded that pollen limitation was taking place for both species over multiple flowering seasons although at times, resource limitation was also likely to be occurring.

Introduction

Flowers of saltmarsh plant species are thought to be predominantly pollinated by wind and invertebrates (Adam 1990; Friess *et al.* 2012; Mackay and Whalen 2009; Pojar 1973) although the pollination biology of saltmarsh plants has been relatively neglected (Adam 1981; Adam 1990) with no comprehensive studies of pollen limitation in saltmarshes. Saltmarshes have low floral diversity when compared with many other ecosystems, and the number of plants with showy flowers is limited and usually confined to the upper marsh (Adam 1990), which could account for why the pollination biology of such plants is under-studied.

Pollen limitation occurs when plants do not receive an adequate quantity or quality of pollen, usually resulting in reduced fruit and/or seed set (Aizen and Harder 2007; Ashman *et al.* 2004; Caris and Smets 2004; Fagua and Gonzalez 2007; Gómez *et al.* 2010; Knight *et al.* 2006; Knight *et al.* 2005). Pollen quantity can be reduced by a decrease in the frequency of pollinator visits or conspecific pollen being delivered per visit (de Figueiredo and Sazima 2007; Fernández *et al.* 2012; Olsson and Agren 2002), whereas quality can be reduced if self, or otherwise incompatible, pollen is delivered (de Figueiredo and Sazima 2007; Fernández *et al.* 2012; Olsson and Agren 2002).

A review by Burd (1994) found high levels of pollen limitation, with 62% of species showing pollen limitation at some time or at some sites. Results often varied when pollen limitation experiments were conducted over multiple years or flowering seasons (Burd 1994). Pollen addition increased fruit set more often than seed set and when multiple studies were conducted during one flowering season, varying results were found (Burd 1994). These findings confirm the importance of conducting pollen limitation experiments over multiple flowering seasons and years to get an accurate understanding of pollen limitation.

Pollen limitation can decrease the abundances of plants in communities which has the potential to change plant composition across habitats and to cause local extinctions if pollen limitation is consistent (Aguirre and Dirzo 2008; Ashman *et al.* 2004; Fernández *et al.* 2012; Friess *et al.* 2012; Knight *et al.* 2005). Changes to interactions between plant species and their floral visitors can influence the occurrence and magnitude of pollen limitation (Alonso *et al.* 2013; Knight *et al.* 2005) as can the level of fragmentation (Gómez *et al.* 2010; Knight *et al.* 2005).

Knight *et al.* (2005) conducted a comprehensive review of pollen limitation studies and examined the role of environmental perturbations. This review presents evidence that pollinator numbers are declining in many habitats and this decline in native pollinators, and increases in non-native pollinators, is expected to increase the magnitude of pollen limitation in plants (Knight *et al.* 2005).

Gómez and Zamora (1996) studied pollen limitation in high-mountain populations of *Hormathophylla spinosa* and found that the species was not pollen limited. They concluded, however, that the lack of pollen limitation was due to its generalised pollination system that uses insects and wind to pollinate flowers, indicating that these plant species can utilise the wind to aid in pollination if insect visitors are lacking (Gómez and Zamora 1996). This generalised pollination system could be a beneficial trait in coastal saltmarshes, as the environment is harsh, on both plants and their insect pollinators.

Plant reproduction is influenced not only by pollen limitation or resource limitation but a combination of both (Fernández *et al.* 2012; Haig and Westoby 1988). A lack of resources such as water or essential nutrients can outweigh the impacts of pollen addition or pollen limitation (Cázares-Martínez *et al.* 2010; Fernández *et al.* 2012). Kalisz and Vogler (2003) indicated that even for plant species with autonomous selfing, resource limitation could still cause reductions in fruit set. Knight *et al.* (2005), as well as Harder and Aizen (2010), concluded that because of the variable nature of pollination by insects and variation in resource availability, plants will likely suffer from both resource limitation and pollen limitation at different times over their lives, a conclusion supported by Haig and Westoby (1988). Burd (1994) stated that the consequences of pollen limitation depend on the frequency of its occurrence, so if pollen limitation or resource limitation occur at certain times but not consistently, plants should not suffer any long-term consequences under natural conditions.

Aims and objectives

The aim of this study was to determine the extent of pollen limitation in populations of the native saltmarsh plant species *S. repens* and *F. pauciflora* in the Goolwa saltmarsh. This study assessed pollen limitation in fruit set and seed set, during three flowering seasons that spanned both below-average rainfall and above-average rainfall conditions; studies over such an extended timeframe are uncommon. Studies on pollination in saltmarsh communities are also uncommon, and this study examines two of the main insect-pollinated species in this saltmarsh community that are known to share pollinators (see Chapter two). This study is the first multi-year study to investigate pollen limitation in saltmarshes of South Australia, an environmentally harsh community with relatively low plant species diversity, as well as potentially low pollinator diversity.

Pollen limitation is expected to be present for both *S. repens* and *F. pauciflora* in the Goolwa saltmarsh. Although both species are visited by generalist pollinators, the harsh environmental conditions and the effects that may have on floral visitors, as well as the high density of the introduced plant species *Limonium* within the saltmarsh, may prove too competitive for the native plant species, and will potentially lead to pollen limitation. Wolowski *et al.* (2013) believed that by conducting pollen limitation experiments on multiple species in a community, a better interpretation of pollen limitation could be obtained, including the occurrence, components and mechanisms of pollen limitation, as factors such as habitat, weather and pollinator availability would be constant. If these factors were constant, any variation in pollen limitation present could be attributed to differences in plant characteristics, despite this however, few studies have examined pollen limitation in all or most plants in a community, and even less have examined plant species who share pollinators (Wolowski *et al.* 2013).

Haig and Westoby (1988) argued that levels of ovule production should evolve to reach an equilibrium with levels of pollen availability, and thus that pollen limitation should be evolutionary transient. Under this prediction, consistent pollen limitation across flowering seasons should not be expected, yet few studies have tested this prediction. Wolowski *et al.* (2013) on the other hand, predicted that pollen limitation would be consistent across flowering seasons as they expected pollinator behaviour to be constant over multiple flowering seasons; it was predicted that any variation across flowering seasons would be due to changes in resource availability and resource limitation, not pollen limitation. Another view on variation in pollen limitation is given by Knight *et al*. (2005) who believe that pollen limitation will be greater in plant species which have a higher ovule number per flower, and believe that an over production of ovules would allow plants to capitalise on times of high pollen receipt. If this were true, pollen limitation would be greater for *S. repens* than *F, pauciflora,* as ovule numbers can be much greater for flowers of *S. repens*.

Materials and methods

Study site

The Goolwa saltmarsh (-35° 31' 55.69"S, +138° 49' 36.13"E) is on the Fleurieu Peninsula of South Australia, Australia and is situated downstream of the Goolwa barrage on the Murray River and upstream of the Murray mouth on the Sir Richard Peninsula. The area used is approximately 2 km long and 100 m wide, although some areas are as small as 50 m wide, the study area borders the northern edge of the Younghusband Peninsula. Fieldwork took place over four flowering seasons, from late October 2007 to early March 2011. The Goolwa saltmarsh has only four common insect-pollinated plant species with showy flowers, the native species *S. repens* and *F. pauciflora* and introduced *Limonium* species, predominately *Limonium binervosum* (G.E.Sm.) C.E. Salmon with patches of *Limonium hyblaeum* agg. (see Chapter one).

Study species

Samolus repens

Samolus repens (J.R.Forst. & G.Forst) Pers is a native, small perennial herbaceous species (Saintilan 2009a; Toelken 1986) that has traditionally been included in the family Primulaceae (Stevens 2014; Toelken 1986). *Samolus repens* occurs commonly along the southern coast of Australia in saltmarsh and cliff communities (Saintilan 2009b). The flowers of *S. repens* are actinomorphic with a tubular corolla, pink to white in colour, with a persistent calyx (Toelken 1986) and with numerous ovules (Stahl 2004). Breeding system studies (Chapter five), indicate that *S. repen*s is facultatively xenogamous and protandrous.

Frankenia pauciflora

Frankenia pauciflora DC. var. *gunnii* Summerh., also known as common sea-heath, is a native, halophytic small shrub, commonly found in saltmarshes and on coastal cliffs in southern Australia (Barnsley 1982). It is a member of the plant family Frankeniaceae (Whalen 1986) and is henceforth referred to as *F. pauciflora*. *Frankenia pauciflora* grows as a small shrub and is usually under 30 cm tall. It has small, white to pink, actinomorphic flowers around 1 cm in diameter with a persistent tubular calyx (Whalen 1986) and is facultatively xenogamous (Mackay and Whalen (2009). Ovule numbers for *F. pauciflora* var *gunnii* range from 7 to 18 (Whalen 1986).

Seasonal variation

Studies were conducted over multiple flowering seasons to allow for year-to-year variation. Nine pollen limitation experiments were conducted during this study at Goolwa from the end of 2007 to the beginning of 2011, covering flowering seasons with varying climatic conditions. The Goolwa saltmarsh has a Mediterranean climate with the majority of rainfall at Goolwa falling over winter (Fig. 1.9). Initial pollen limitation experiments were conducted during the Millennium Drought, during the flowering seasons of 2007/2008 (400 mm annual rainfall) and 2008/2009 (365 mm annual rainfall), with later experiments undertaken during periods of average rainfall, 2009/2010 (475 mm annual rainfall) and above-average rainfall, 2010/2011, (594 mm annual rainfall), discussed in more detail in Chapter one.

Methods

In each experiment, twenty pairs each of healthy *S. repens* and *F. pauciflora* plants were selected across the saltmarsh with at least 10 and 50 open flowers on each plant respectively. On each *S. repens* plant, three flowers were tagged as controls, for which no experimental procedure was conducted. Three flowers were tagged with additional pollen added to each flower. Pollen was added directly from the anthers of at least three different donor flowers from a minimum of 10 metres away by collecting pollen from anthers with a paintbrush and then brushing the stigmas of the recipient flowers. Pollen deposition was then visually confirmed. Another three flowers were tagged as procedural controls where flowers were manipulated as for the pollen addition treatment (by brushing the flower with a fine-haired paintbrush) but no pollen was transferred. Procedural controls were not included for the *S. repens* experiment in 2008/2009 or the *F. pauciflora* experiments in 2009/2010 because of an inadequate number of open flowers. Techniques were practiced prior to the commencement of the experiment. To address the possibility of potential resource re-allocation within plants to which pollen was added, controls were used on both the treated plant and a neighbouring plant that did not receive additional pollen. On the other plant of each pair, three flowers were tagged as ‘other-plant’ controls to test for possible resource allocation shifts, as was done by

Fernández *et al.* (2012) and Gómez (2010). Thus, in total, for *S. repens*, twelve flowers were used per plant pair. The same design was used for the pairs of *F. pauciflora* plants, but because a greater number of flowers were open at any one time, ten flowers were used for each treatment, instead of three, with forty flowers used per plant pair.

Following the experimental treatments, flowers were left to set seed and then fruits were collected. Fruit set and the number of seeds per fruit, hereafter referred to as seed set, were calculated. Fruits with signs of insect damage were removed and were excluded from analyses of pollen limitation. Examining flower and fruit predation was not initially part of the study and it is unknown at what stage flowers and fruits were preyed upon.

Four pollen limitation experiments were conducted for *S. repens* from 2008/2009 to 2010/2011 at the Goolwa saltmarsh. The experiments during the 2008/2009, 2009/2010 and 2010/2011 flowering seasons were conducted in November, after flowering had commenced, but before the flowering peak for the flowering season. During the flowering season of 2010/2011, *S. repens* showed a reduction in floral display in January, as it had in previous flowering seasons; however, flowering increased again in late February/ early March, so another pollen limitation experiment was initiated in late February, to determine if pollen limitation was occurring during the extended flowering period. Note that, no pollen limitation experiment for *S. repens* was conducted during the 2007/2008 flowering season, unlike for *F. pauciflora,* as *S. repens* was only added to the study during 2008.

Five pollen limitation experiments were conducted for *F. pauciflora* from 2007/2008 to 2010/2011. The experiment during the 2007/2008 flowering season was conducted in December, after flowering had increased, but before the flowering peak for that flowering season. Two experiments were conducted for *F. pauciflora* during 2009/2010 and 2010/2011, in December and then again in February, before and after the flowering peak. These extra experiments were conducted to determine within-season variation in pollen limitation. Note that no pollen limitation experiment was conducted for *F. pauciflora* during the 2008/2009 flowering season.

Data analysis

Data analysis was conducted using IBM SPSS 20. Chi-Square tests were used to test for differences in fruit set among the treatments. Fruit set was determined by assessing the proportion of flowers that developed into a fruit, with a fruit being defined as having one or more seeds. A general linear mixed model was used to test for differences in seed set among the treatments. The number of seeds in each fruit was determined, referred to as seed set. A mixed model analysis was also used to determine if seed mass varied among treatments. In the mixed model analysis, the pollen addition treatment was treated as a fixed effect and the effect of plant pair was treated as random. Pre-planned comparisons were conducted between each of the control groups and the pollen-addition treatment group. Remnant flowers/ fruits with signs of floral or fruit predation were removed from the analysis.

Results

Samolus repens

Fruit set

Of the four pollen limitation experiments conducted for *S. repens*, only one experiment showed a statistically significant increase in fruit set following pollen addition. The experiment conducted late in the flowering season of 2010/2011 showed a significant increase in fruit set when additional pollen was added, relative to the other three treatments ($X^2 = 11.095$, df = 3, $P = 0.011$) (Fig. 3.1 and Table 3.1).

Seed set

Three of the four pollen limitation experiments conducted for *S. repens* showed significantly increased seed set in the pollen addition treatments compared with the other treatments (Fig. 3.2 and Table 3.2). Only in the pollen limitation experiment conducted during the 2008/2009 flowering season did the pollen addition fail to significantly increase seed set over the different-plant control; rather this control showed a significantly greater seed set than fruits of the pollen addition treatment. For the experiments conducted in 2009/2010, and both experiments in 2010/2011, fruits from all of the other treatments set significantly fewer seeds than those in the pollen addition treatment.

Seed masses

Seed masses were determined for the first two *S. repens* pollen limitation experiments (Table 3.3). A total of 2,708 *S. repens* seeds was weighed for the 2008/2009 flowering season, and 1,822 *S. repens* seeds were weighed for the 2009/2010 flowering season. Seeds from the pollen addition treatments were not consistently lighter or heavier than seeds from the control treatments with variation among the years. The only significant differences were in 2009/2010 with the difference between mean seed mass of the pollen addition treatment and the procedural control = -0.02 mg, t = -5.347, $P = < 0.001$ and the difference between mean seed mass of the pollen addition treatment and different plant control = -0.01 mg, t = -3.535, $P = <0.001$.

Floral and fruit predation

The number of flowers/ fruits collected at the end of the experimental study period that were discarded from the analysis because of floral or fruit predation varied greatly across the different flowering seasons and experiments (Table 3.4). The lowest percentage of insect-damaged flowers/ fruits was 19% during the 2009/2010 pollen limitation experiment but then increased to the highest percentage of insect damage, at 54%, during the early 2010/2011 flowering season.

Frankenia pauciflora

Fruit set

For all but one of the five pollen limitation experiments conducted for *F. pauciflora* (2007/2008), fruit set increased significantly following pollen addition (Fig. 3.3 and Table 3.5).

Seed set

Four of the five pollen limitation experiments conducted for *F. pauciflora*, showed a significant increase ($P < 0.05$) in seed set after pollen addition when compared to the same plant and ‘other-plant’ controls (Fig. 3.4 and Table 3.6). In the 2007/2008 experiment, fruits of the procedural control and ‘other-plant’ controls both set significantly more seed than the fruits of the pollen addition treatment.

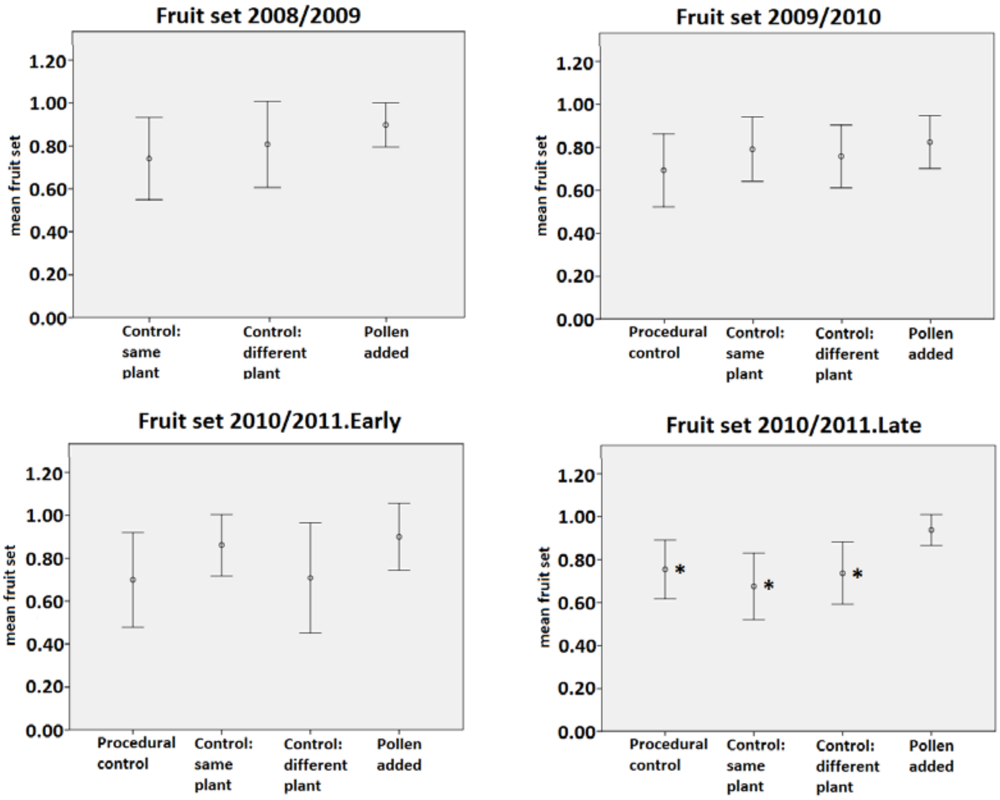

Figure 3.1 Mean fruit set for the different experimental treatments for the four *Samolus repens* pollen limitation experiments at the Goolwa saltmarsh from 2008/2009 to 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant differences with the pollen-addition treatment. Number of flowers included in analyses by year: 2008/2009 n = 103; 2009/2010 n = 146; 2010/2011 early n = 110; 2010/2011 late n = 184.

Table 3.1 Chi-square test results for the fruit set of *Samolus repens* flowers among treatments, conducted at the Goolwa saltmarsh from 2008/2009 to 2010/2011. E and L indicate an early experiment and late experiment respectively.

	X^2	df	P value	Sample size
2008.2009	1.774	2	0.412	103
2009.201	5.763	3	0.124	195
2010.2011.E	5.359	3	0.147	110
2010.2011.L	11.095	3	0.011	184

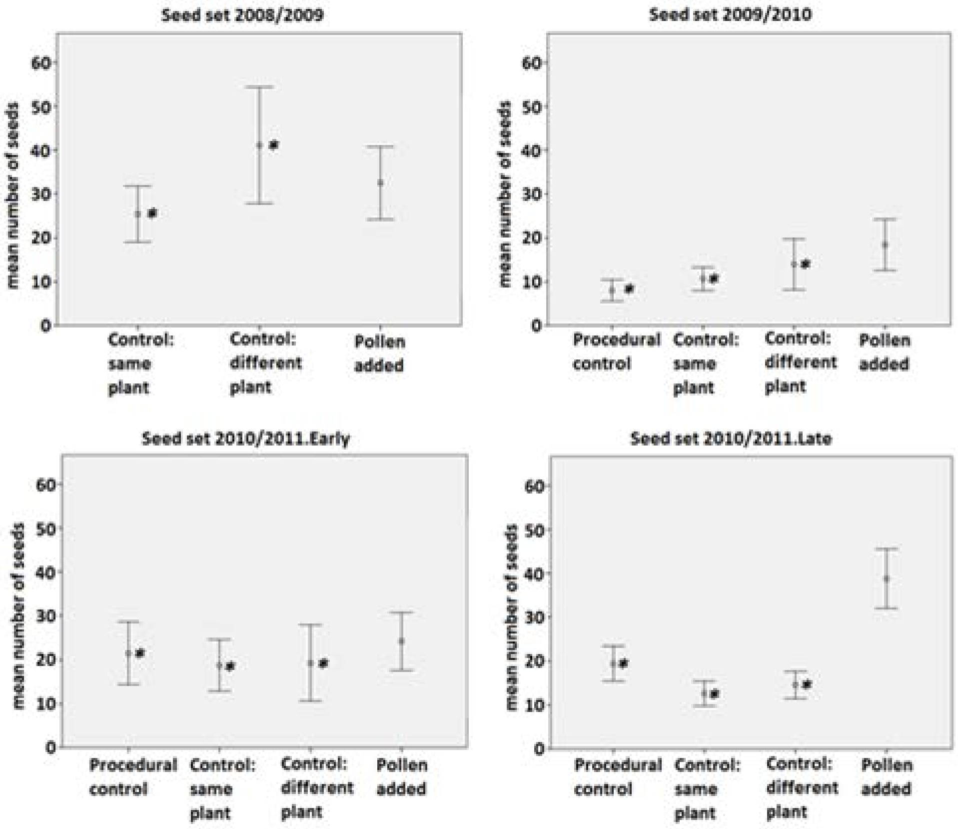

Figure 3.2 Number of mature seeds per fruit (seed set) for the different experimental treatments for the four *Samolus repens* pollen limitation experiments at the Goolwa saltmarsh from 2008/2009 to 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant difference with the pollen-addition treatment. Number represents mean seed number per fruit. Number of fruits included in analyses by year: 2008/2009 n = 84; 2009/2010 n = 115; 2010/2011 early n = 89; 2010/2011 late n = 141.

Table 3.2 Effect of pollen addition on seed set for *Samolus repens* for the four pollen limitation experiments conducted at the Goolwa saltmarsh from 2008/2009 to 2010/2011. E and L indicate an early experiment and late experiment respectively. NA represents treatment not included in the study. P-values represent significance of contrast with pollen-addition treatment.

	Procedural Control				95% confidence interval	
	Coefficient	Std.Error	t	P value	Lower	Upper
2008.2009	NA	NA	NA	NA	NA	NA
2009.2010	-0.849	0.073	-11.576	< 0.001	-0.994	-0.704
2010.2011.E	-0.232	0.064	-3.644	< 0.001	-0.359	-0.106
2010.2011.L	-0.776	0.047	-16.623	< 0.001	-0.868	-0.683
	Control (Same Plant)					
2008.2009	-0.292	0.051	-5.715	< 0.001	-0.394	-0.191
2009.2010	-0.552	0.061	-8.998	< 0.001	-0.673	-0.431
2010.2011.E	-0.177	0.064	-2.756	< 0.001	-0.304	-0.050
2010.2011.L	-1.167	0.061	-19.175	< 0.001	-1.287	-1.046
	Control (Different Plant)					
2008.2009	0.185	0.050	3.716	< 0.001	0.086	0.285
2009.2010	-0.292	0.057	-5.092	< 0.001	-0.406	-0.179
2010.2011.E	-0.468	0.074	-6.360	< 0.001	-0.614	-0.322
2010.2011.L	-0.979	0.051	-19.130	< 0.001	-1.080	-0.878

Table 3.3 Statistics for seed mass for *Samolus repens* in 2008/2009 and 2009/2010. Asterisks denote significant differences with the pollen-addition treatment.

2008.2009			95% Confidence Interval		
Seed mass	Mean	Std. Error	lower bound	upper bound	Number of seeds weighed per treatment
Control Same Plant	0.052 mg	0.003	0.046	0.058	1170
Control Different Plant	0.046 mg	0.003	0.040	0.053	862
Pollen Added	0.046 mg	0.003	0.041	0.052	676
2009.2010					
Procedural control	0.073 mg*	0.003	0.067	0.079	249
Control Same Plant	0.050 mg	0.003	0.044	0.055	416
Control Different Plant	0.066 mg*	0.003	0.061	0.071	419
Pollen Added	0.055 mg	0.003	0.049	0.060	738

Table 3.4 The percentage of non-fruits (remnant flowers with no seed present), fruits (with one or more seed present) and insect damaged remnant flowers/ fruits at the Goolwa saltmarsh by plant species and flowering season with number in parentheses (n). E and L indicate an early experiment and late experiment respectively.

Plant species	Flowering season	% **Non-Fruit** (remnant flower, no seed)	**% Fruit** (with one or more seed present)	**% Floral or fruit remnant flowers** (insect damaged flower/ fruit)
Frankenia pauciflora	2007/2008	56 (n = 225)	24 (n = 95)	20 (n = 80)
	2009/2010.E	41 (n = 124)	53 (n = 159)	6 (n = 17)
	2009/2010.L	43 (n = 171)	47 (n = 187)	10 (n = 40)
	2010/2011.E	30 (n = 237)	22 (n = 174)	49 (n = 389)
	2010/2011.L	41 (n = 324)	50 (n = 401)	9 (n = 75)
Samolus repens	2008/2009	12 (n = 19)	54 (n = 84)	34 (n = 52)
	2009/2010	17 (n = 31)	64 (n = 115)	19 (n = 34)
	2010/2011.E	9 (n = 21)	37 (n = 89)	54 (n = 130)
	2010/2011.L	18 (n = 43)	59 (n = 141)	23 (n = 56)

Seed masses

Seed masses were determined for the first three *F. pauciflora* pollen limitation experiments (Table 3.7). A total of 483 *F. pauciflora* seeds were weighed for the 2007/2008 flowering season, 1,068 for the early 2009/2010 pollen limitation experiment and 816 for the late 2009/2010 pollen limitation experiment. Seed masses were determined for the first three *F. pauciflora* pollen limitation experiments and seeds from the pollen addition treatments were not significantly heavier than those in the other treatments with one exception, 2007/2008, where the difference between mean seed mass of the pollen addition treatment and different plant control = 0.02 mg, t = 5.140, $P = < 0.001$. In the other three comparisons where significant results were obtained, the mean pollen-addition seed masses were significantly less than one or more of the controls. In 2007/2008 the difference between mean seed mass of the pollen addition treatment and procedural control = -.01 mg, t = -3.226, $P = < 0.001$; in 2007/2008 the difference between mean seed mass of the pollen addition treatment and same plant control = -.03 mg, t = -9.179, $P = < 0.001$ and in 2009/2010 early, the difference between mean seed mass of the pollen addition treatment and different plant control = -.02 mg, t = -2.017, $P = 0.045$ (Table 3.7).

Floral and fruit predation

For *F. pauciflora*, as with *S. repens*, considerable variation in floral and fruit predation was present among the different years of the pollen limitation experiments (Table 3.4). The flowers/fruits with signs of floral and fruit predation varied from 6% during the early 2009/2010 pollen limitation experiment to 49% in the early pollen limitation experiment of 2010/2011. The pattern shown during the period of the early pollen limitation experiment of 2010/2011 is similar to that for

S. repens, with the highest level of insect damage coinciding with increased rainfall at Goolwa during the winter of 2010/2011.

Association between rainfall and fruit set and seed set

Associations between the amount of rainfall leading up to each of the pollen limitation experiments and the subsequent fruit set and seed set were examined. The rainfall for the eight months leading up to the time of the experiment was used (Fig. 3.5 and 3.6).

Discussion

This multi-year study found that both *S. repens* and *F. pauciflora* are pollen-limited, at least at some times, in the Goolwa saltmarsh in both fruit set and seed set. The study species included in this study are the dominant two of only a few insect-pollinated plant species in this saltmarsh community with showy flowers, with pollinator observations revealing that they share pollinators (Chapter two). Wolowski *et al.* (2013) state that conducting community pollen limitation studies, like this study, allows for better interpretation of results than if just one species was examined but very few studies have examined pollen limitation in all, or at least most, of the plants in a community or guild.

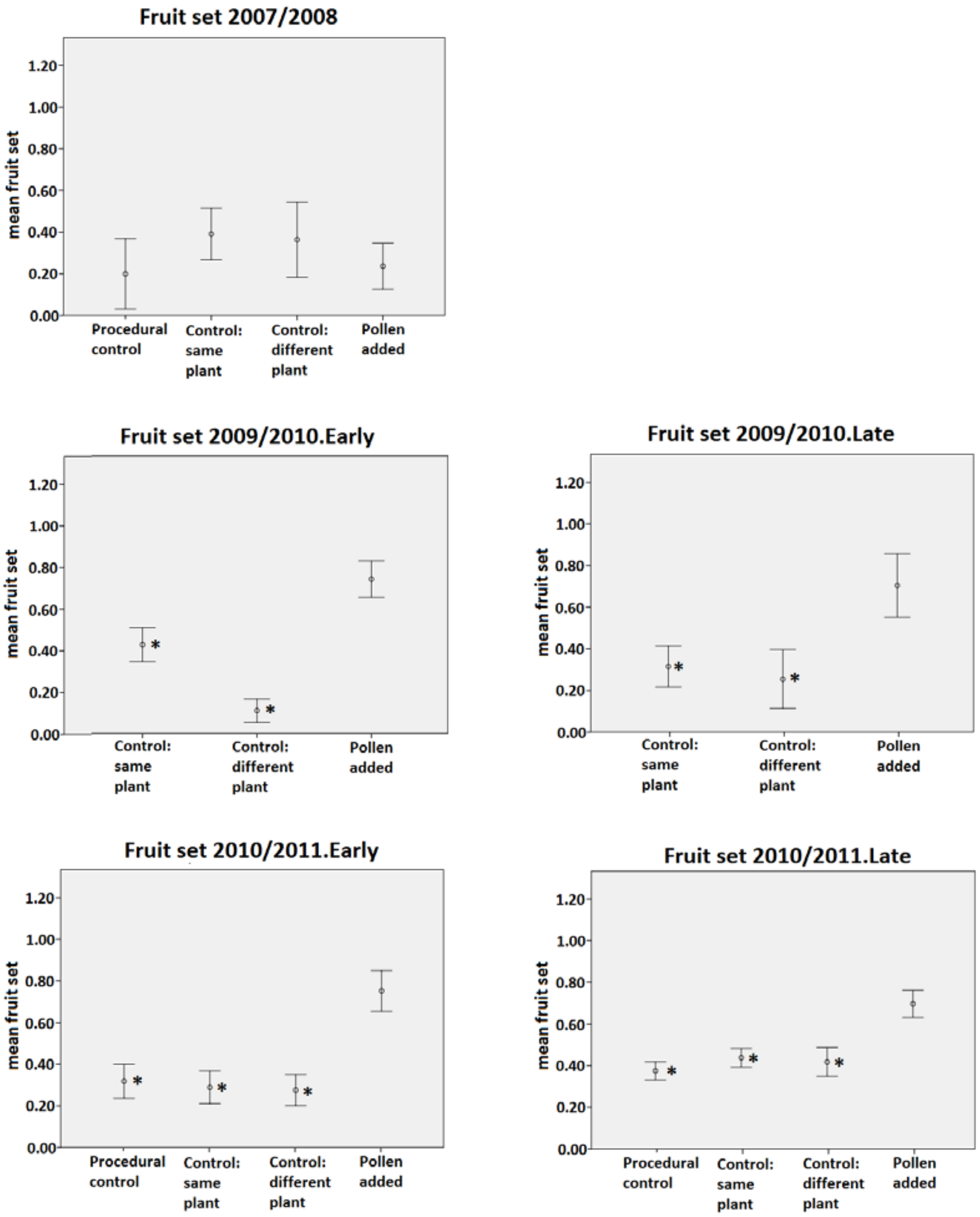

Figure 3.3 Mean fruit set for the different experimental treatments for the five *Frankenia pauciflora* pollen limitation experiments at the Goolwa saltmarsh from 2007/2008 to 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant differences with the pollen-addition treatment. Number of flowers included in analyses by year: 2007/2008 n = 320; 2009/2010 early n = 283; 2009/2010 late n = 358; 2010/2011 early n = 411; 2010/2011 late n = 725.

Table 3.5 Chi-square test comparisons of the fruit set of *Frankenia pauciflora* flowers among treatments, conducted at the Goolwa saltmarsh from 2007/2008 to 2010/2011. E and L indicate an early experiment and late experiment respectively.

	X^2	df	P value	Sample size
2007.2008	10.357	3	0.016	320
2009.2010.E	51.449	2	< 0.001	283
2009.2010.L	29.225	2	< 0.001	278
2010.2011.E	69.163	3	< 0.001	411
2010.2011.L	42.383	3	< 0.001	625

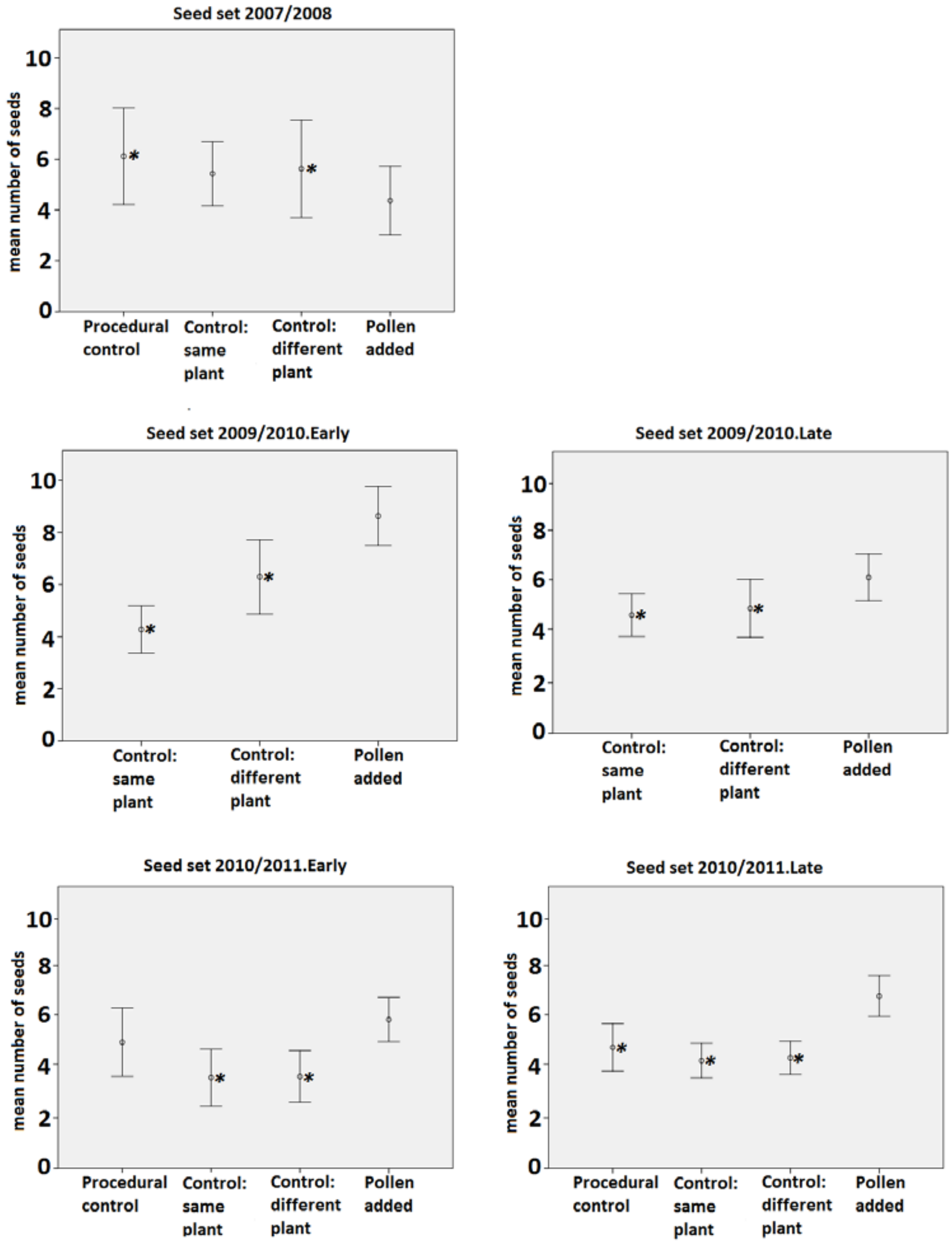

Figure 3.4 Number of mature seeds per fruit (seed set) for the different experimental treatments for the five *Frankenia pauciflora* pollen limitation experiments at the Goolwa saltmarsh from 2007/2008 to 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant differences with the pollen-addition treatment. Number represents mean seed number per fruit. Number of flowers included in analyses by year: 2007/2008 n = 95; 2009/2010 early n = 159; 2009/2010 late n = 187; 2010/2011 early n = 174; 2010/2011 late n = 401.

Table 3.6 Effect of pollen addition on seed set for *Frankenia pauciflora* for the five pollen limitation experiments conducted at the Goolwa saltmarsh from 2007/2008 to 2010/2011. E and L indicate an early experiment and late experiment respectively. NA represents treatment not included in study. P-values represent significance of contrast with pollen-addition treatment.

	Procedural Control				95% confidence interval	
	Coefficient	Std.Error	t	P value	Lower	Upper
2007.2008	0.342	0.159	2.149	0.034	0.026	0.659
2009.2010.E	NA	NA	NA	NA	NA	NA
2009.2010.L	NA	NA	NA	NA	NA	NA
2010.2011.E	-0.154	0.092	-1.674	0.096	-0.335	0.028
2010.2011.L	-0.358	0.069	-5.176	< 0.001	-0.494	-0.222
Control (Same Plant)						
2007.2008	0.188	0.134	1.406	0.163	-0.078	0.454
2009.2010.E	-0.703	0.076	-9.217	< 0.001	-0.854	-0.552
2009.2010.L	-0.286	0.085	-3.361	< 0.001	-0.455	-0.118
2010.2011.E	-0.534	0.114	-4.699	< 0.001	-0.758	-0.309
2010.2011.L	-0.498	0.072	-6.929	< 0.001	-0.640	0.357
Control (Different Plant)						
2007.2008	0.423	0.148	2.849	0.005	0.128	0.718
2009.2010.E	-0.341	0.089	-3.847	< 0.001	-0.516	-0.166
2009.2010.L	-0.200	0.089	-2.247	0.026	-0.376	-0.024
2010.2011.E	-0.476	0.108	-4.400	< 0.001	-0.690	-0.263
2010.2011.L	-0.427	0.071	-5.998	< 0.001	-0.567	-0.287

Table 3.7 Statistics for seed mass for *Frankenia pauciflora* in 2007/2008 and 2009/2010. Asterisks denote significant differences with the pollen-addition treatment. E and L indicate an early experiment and late experiment respectively.

2007.2008			95% Confidence Interval		
Seed mass	Mean	Std. Error	lower bound	upper bound	Number of seeds weighed per treatment
Procedural control	0.108 mg*	0.006	0.094	0.121	105
Control Same Plant	0.127 mg*	0.006	0.114	0.140	163
Control Different Plant	0.074 mg*	0.006	0.060	0.087	134
Pollen Added	0.094 mg	0.006	0.080	0.107	81
2009.2010.E.					
Control Same Plant	0.053 mg	0.010	0.032	0.074	230
Control Different Plant	0.059 mg*	0.010	0.038	0.081	157
Pollen Added	0.043 mg	0.009	0.023	0.063	681
2009.2010.L					
Control Same Plant	0.086 mg	0.006	0.074	0.097	201
Control Different Plant	0.097 mg	0.006	0.084	0.109	185
Pollen Added	0.084 mg	0.005	0.075	0.094	430

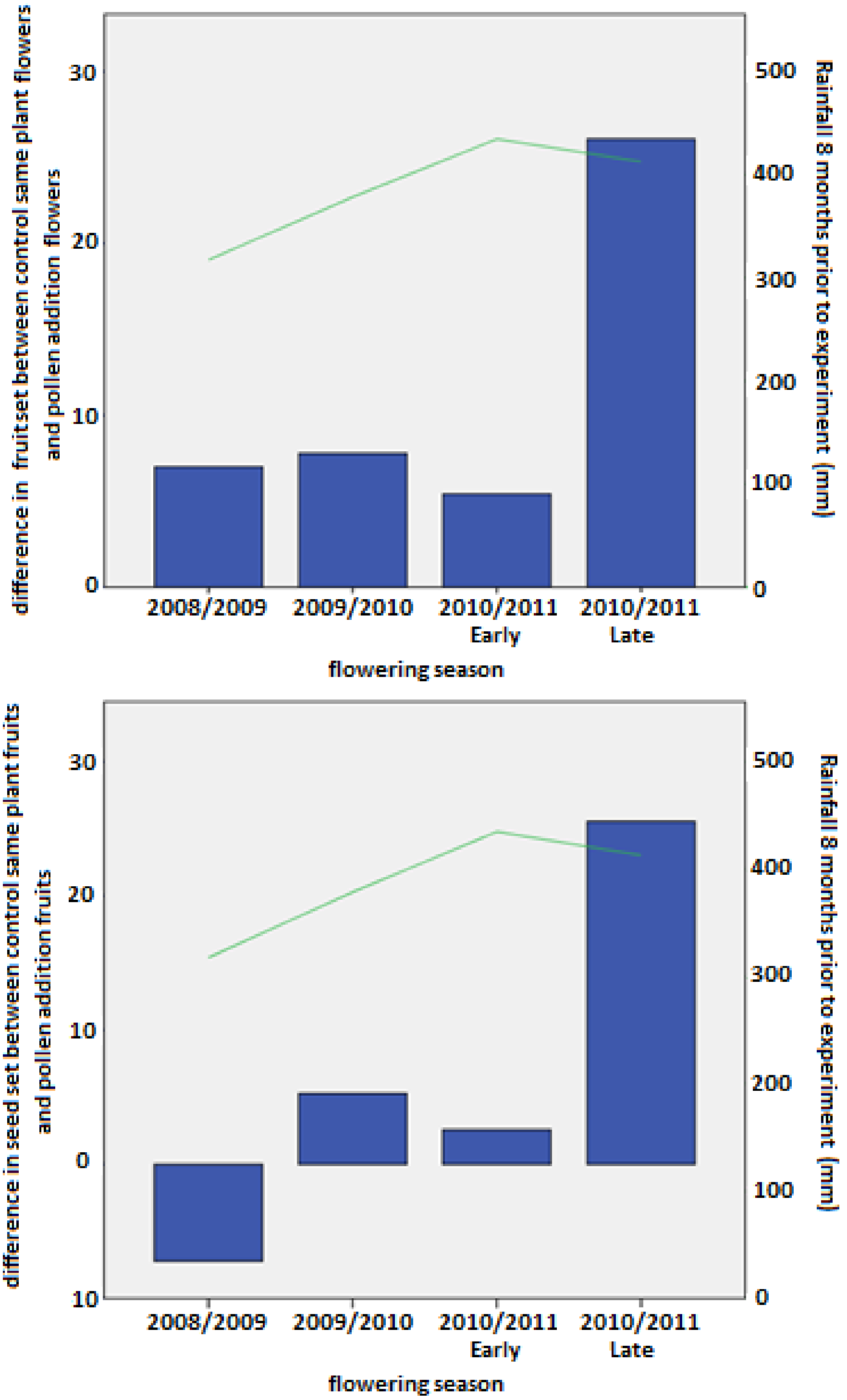

Figure 3.6 Difference in fruit set and seed set between same-plant control flowers and pollen-addition flowers for *Samolus repens* during pollen limitation experiments at the Goolwa saltmarsh from 2008/2009 to 2010/2011.

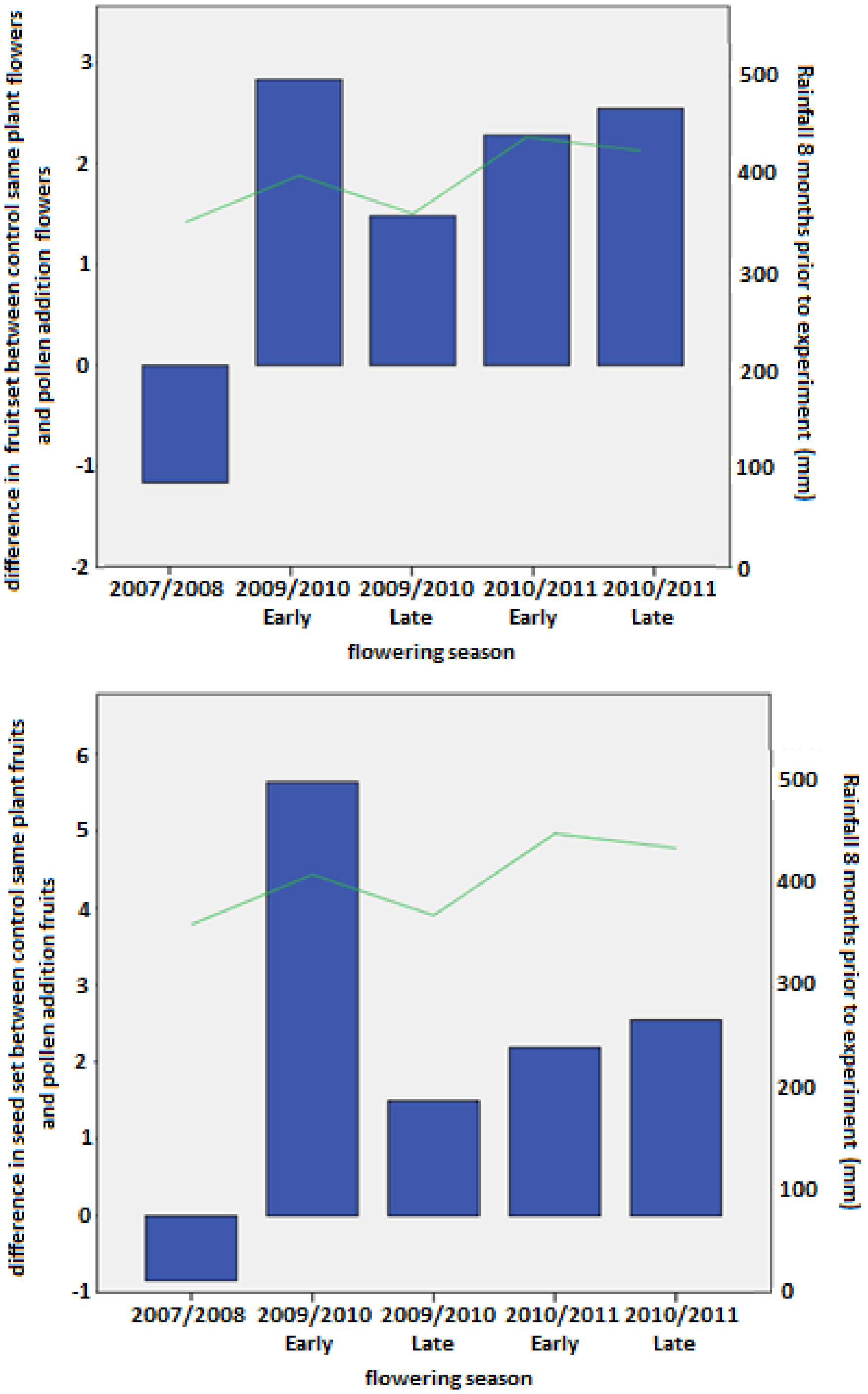

Figure 3.7 Difference in fruit set and seed set between same-plant control flowers and pollen-addition flowers for *Frankenia pauciflora* during pollen limitation experiments at the Goolwa saltmarsh from 2007/2008 to 2010/2011.

Pollen limitation in *Samolus repens* and *Frankenia pauciflora*

Of the four pollen limitation experiments conducted on *S. repens*, only one showed a significant effect on fruit set, whilst three of the four experiments showed a significant effect on seed set. The greatest effect of pollen limitation on *S. repens* was observed in the late experiment of 2010/2011, when *S. repens* was flowering beyond its typical flowering period (Fig. 3.1 and 3.2). *Samolus repens* plants growing in the later experiment may have experienced competition for floral visitors or conspecific pollen deposition because of the presence of co-occurring flowers of *F. pauciflora* and *Limonium* species. *Samolus repens* begins flowering earlier in the year than *F. pauciflora* and *Limonium* (Chapter two), and this staggered flowering may improve reproductive success for *S. repens* via reduced competition for floral visitors. For *F. pauciflora*, four of the five experiments showed a significant effect of pollen limitation for fruit set and/or seed set.

Due to differences in ovule number, it would be expected that *S. repens* would show a stronger response in seed set than *F. pauciflora*, which was the case in this study. In the later experiment of 2010/2011 both species had a statistically significant effect of pollen-addition, fruits from the pollen-addition treatment for *S. repens* had a mean seed number of 37, compared to 17, 12 and 14 for the other treatments, in contrast, fruits of the pollen-addition treatment for *F. pauciflora* had a mean seed number of six, compared to 5, 4 and 4. This would confirm the idea of Knight *et al*. (2016) who stated that by plant species over producing ovules, they can take advantage of good conditions and high pollen receipt, and in turn, have the potential to show greater pollen limitation than species with fewer ovules.

The number of flowers per plant could also explain the differences between *S. repens* and *F. pauciflora* with regard to fruit set. As discussed in Chapter two, plants of *S. repens* tend to have less than 10 open flowers per plant at any given time, whilst plants of *F. pauciflora* can have more than 60, sometimes even more than 100. Having more flowers, each with less ovules could allow for resource allocation and trade-offs across the flowering season and across years (Knight *et al.* 2006; Mackay and Whalen 2009).

For both study species, pollen limitation was not observed in the earliest experiments i.e. during 2008/2009 for *S. repens* and in 2007/2008 for *F. pauciflora*, possibly due to the prevailing drought conditions at the Goolwa saltmarsh which may have resulted in resource limitation, so regardless of how much pollen was received, environmental factors and stresses, such as reduced water availability and increased soil salinity, may have outweighed any potential benefits of pollen receipt. When rainfall returned to average levels in 2009/2010 and above average during the 2010/2011 flowering season, pollen limitation was observed. Straka and Starzomski (2015) examined the occurrence of pollen limitation in seven common alpine species in the Coast Range Mountains of Canada and found that, as in this study, variation among species in the extent of pollen limitation. They established that several factors influenced whether pollen limitation was present or not, such as temperature, abundance of pollinators and seed predation (Straka and Starzomski 2015). They also concluded that pollen limitation was not always present due to variations, across time and space, in other limiting factors to reproduction, such as resource limitation (Straka and Starzomski 2015). These results were similar to those of this study, with different species pollen limited at different times, potentially due to differences in susceptibility to resource limitation. Wagner *et al.* (2016) looked at pollen limitation in an alpine-nival ecosystem in the European Central Alps. As with the saltmarsh examined in this study, the alpine habitat studied by Wagner *et al. (*2016) represents an ecosystem with very low plant species diversity and extremely harsh environmental conditions. Plant species growing in alpine environments tend to have extended floral longevities, to compensate for variable and harsh weather conditions and variable pollinator visitation rates; however, Wagner *et al*. (2016) found that plants could not take advantage of additional pollen due to resource limitation. The findings of Straka and Starzomski (2015) and Wagner *et al*. (2016) are consistent with the findings of this study, in that the effects of pollen limitation may be mediated by levels of available resources or the presence of environmental stresses.

Pollen limitation experiments in both below-average and above-average rainfall years were included in this study. The tail end of the Millennium Drought (Department of Sustainability, Environment, Water, Population and Communities 2011), with below-average rainfall and above-average temperatures occurred in the earlier years of the study from 2007/2008 to 2009/2010 (McKeon 2006). Above-average rainfalls were recorded in the later years of the study and during the flowering season of 2010/2011, floodwaters from Victoria made their way downstream to Goolwa, as did increased flows from the above-average rainfall, that fell across the River Murray catchments (Department for Water 2010; Department for Water 2011b). Increased flows inundated the saltmarsh and would, together with the above-average rainfall, have very likely decreased salinity and increased soil moisture content, flushing the saltmarsh. These rainfall and flow patterns coincide with periods of increased flowering, seen in the flowering phenologies of *S. repens* and *F. pauciflora* (Chapter two). Many studies have confirmed that plant growth in saltmarshes is not only water limited but also nutrient-limited, particularly with regard to nitrogen (Fox *et al.* 2012; Kiehl *et al.* 1997; Rietsma *et al.* 2011). High water flows down the River Murray brought with them nutrients from upstream. The Department for Water (2011a) issued a River Murray flow advice update warning of the increased organic content in the water, due to high water levels inundating floodplains that had not been inundated for years. This would have improved the health of the plants and soil in the saltmarsh, potentially alleviating resource limitation.

Fernández *et al.* (2012) studied pollen limitation across different populations of the plant species *Erysimum popovii* and found that most of the populations were pollen-limited. Annual rainfall was positively associated with pollen limitation across populations, with plants subject to resource limitation unable to utilise the additional pollen. Some plant species in a community can be pollen-limited whereas others are not, and a plant species may be pollen-limited in some areas and not in others. This variation could be caused by differing environmental requirements with some species being more susceptible to resource limitation than others, which may be the case for *S. repens* and *F. pauciflora* plants in the Goolwa saltmarsh. A possible correlation was shown between fruit set and seed set and the amount of rainfall received in the months leading up to the time that the experiments were conducted (Fig. 3.5 and 3.6). Year-to-year variation in the difference in fruit set and seed set between the pollen addition treatment and the same plant control treatment was observed in this study for both *S. repens* and *F. pauciflora* (Fig. 3.5 and 3.6).

The earliest experiment for *S. repens* showed unexpected variation in seed set, with flowers from the control same plant setting significantly fewer seeds per fruit than the pollen-added treatment and flowers from the ‘other-plant’ control setting significantly more seeds than the pollen-added treatment. This pattern was only observed in one season, possibly because of the reduced rainfall, when compared to the later flowering seasons. It may be that within-species variation is more evident under stressful environmental conditions, like those present in the Goolwa saltmarsh during 2008/2009. Personal observations in the field suggest that *S. repens* prefers to grow in wetter areas of the saltmarsh, usually in low-lying areas. Some members of the family Primulaceae, and most species included in the family Samolaceae (the family in which the genus *Samolus* is sometimes placed), prefer areas with high soil moisture, e.g. near lakes, rivers or saltmarshes (Jones *et al.* 2012; Stahl 2004). It is possible that *S. repens* requires a higher soil moisture content to maintain a healthy condition and to set fruit than *F. pauciflora*, and could therefore be more susceptible to resource limitation when there is reduced rainfall and water availability. This is important as changes brought on by climate-change could increase temperatures, elevate sea levels and reduce rainfall, which could have considerable repercussions for *S. repens*.

Cázares-Martinez *et al.* (2010) examined pollen limitation in two co-occurring plant species, as this study did. They found that pollen limitation occurred in one of the species, *Atriplex canescens*, but not in the other, *Atriplex acanthocarpa*. Pollen addition had a significant effect on fruit production and germination rates for *A. canescens*, but not for *A. acanthocarpa* (Cázares-Martínez *et al.* 2010). They concluded that resource limitation was taking place through a lack of water and that some plant species are more susceptible than others (Cázares-Martínez *et al.* 2010), as has been suggested here for *S. repens*. Copland and Whelan (1989) examined pollen limitation in four co-occurring *Banksia* species, and found variation across species and time also. In a study on pollen limitation in the alpine species *Inula royleana*, Shabir *et al.* (2015) found that pollen limitation was present, likely as a result of harsh and variable weather conditions and the effect that these conditions have on insect visitation. Unlike previous studies mentioned, this study found that resource limitation did not seem to be affecting reproductive success of *Inula royleana*, and instead, they found that fragmentation of species within plant communities was contributing to pollen limitation (Shabir *et al.* 2015). Many species do not occur as continuous patches, because of competition for space or through different environmental requirements, and the occurrence of unsuitable areas, which can influence insect foraging behaviour and floral visitation patterns (Shabir *et al*. 2015). This finding is relevant with regard to *S. repens*, which is thought to require a higher soil moisture content than *F. pauciflora* in the Goolwa saltmarsh, which may reduce suitable areas in which it can grow. Typically, reproductive success decreases with reduced density and increased isolation (Shabir *et al*. 2015) which could prove important to *S. repens*, especially as it is known to be self-incompatible, if it tends to be restricted to moister patches within the saltmarsh.

Previous studies examining pollen limitation, as well as reviews by authors such as Burd (1994), Knight *et al.* (2005) and Ashman *et al.* (2004), had hypothesised that pollen limitation would be greater in magnitude and more common in plant species that were self-incompatible. Breeding system studies conducted as part of this broader study (Chapter five), found that *S. repens* is largely dependent on outcrossed pollen for fertilisation, which is also true for *F. pauciflora* (Mackay and Whalen 2009).

Mackay and Whalen (2009) also examined pollen limitation of *F. pauciflora* in the Goolwa saltmarsh during the 1990’s. They found that *F. pauciflora* was pollen-limited in terms of fruit set and seed set, with flowers with additional pollen added setting significantly more fruits and seeds than the unmanipulated control flowers (Mackay and Whalen 2009). Thus, *Frankenia pauciflora* has exhibited evidence of pollen limitation over an extended timeframe of 20 years. The results for *F. pauciflora* differ to those for *S. repens*, as pollen limitation was found consistently in both fruit set and seed set for four out of five experiments, whereas *S. repens* only had one flowering season with pollen-limited fruit set and seed set. This study combined with the earlier study by Mackay and Whalen (2009) is the only one to our knowledge, to evaluate pollen limitation over such an extended period of time. If these trends of pollen limitation and resource limitation continue long-term, changes to plant composition within the community may take place, potentially reducing plant numbers of *S. repens* and *F. pauciflora*, and reducing the stability of their populations.

Seed masses

Seed mass was not significantly heavier for ‘pollen addition’ seeds of both *S. repens* and *F. pauciflora* nor were seeds significantly heavier in later experiments than in earlier ones. Mackay and Whalen (2009) also examined the effect of pollen addition on seed mass in *F. pauciflora*, and found no significant effect. Wolowski *et al.* (2013) examined pollen limitation in a tropical montane rain forest and found no significant effect of pollen addition on seed mass. Hegland and Totland (2008) found a negative relationship between pollen addition and seed mass, as did a study by Muñoz and Arroyo (2006), possibly because of a trade-off between seed number and seed mass, with more seeds per fruit with a lighter mass than when there are more seeds per fruit, with a greater mass. Kindlmann and Jersáková (2006) suggest that if there is a large investment in reproduction one year, they may be an affect in subsequent years, due to depleted stores. Ideally, pollen limitation experiments would be conducted on the same plants over multiple flowering seasons to see if this is the case.

Floral and fruit predation

Floral and fruit predation increased to approximately 50% of flowers examined for both *F. pauciflora* and *S. repens* during the early 2010/2011 experiment (Table 3.4). Whilst plants may be able to increase fruit set and seed set with favourable weather conditions and increased resource availability, the number of floral and fruit predators may also increase, as was apparent in the early pollen limitation experiments in 2010/2011. Increased resource availability may no longer be beneficial to plant species after a certain point, if the levels of floral and fruit predation become so great that any benefits are counteracted. The increase in floral and fruit predation of *S. repens* and *F. pauciflora* flowers in 2010/2011 coincides with above-average rainfall during 2010. Flower and fruit predation can significantly affect plant numbers and plant recruitment, especially in plant species with a limited seed bank (Kolb *et al.* 2007; Maron and Crone 2006). The variation among years in the floral and fruit predation of *S. repens* and *F. pauciflora* observed in this study is similar to that found in other studies such as those of Fröborg and Eriksson (2003), Kelly and Dyer (2002), Ehrlen (1996) and Szentesi and Jermy (1995). The effects and magnitude of floral and fruit predation are poorly understood in saltmarsh communities and certainly warrant further study, especially considering the importance of reproductive success to the long term survival of plant species.

Conclusion

This study not only included the two main native insect-pollinated plant species with showy flowers in the Goolwa saltmarsh community but it also assessed, for two co-occurring plant species which share pollinators, pollen limitation of both fruit set and seed set over multiple flowering seasons, including years of quite variable rainfall. This study highlights seasonal variation in a plant community and reinforces the importance of conducting studies over multiple seasons to determine if pollen limitation and/or resource limitation is taking place. Numerous studies have found within-population differences in pollen limitation, so further work could be conducted, examining differences between the upper, mid or lower marsh with regard to the occurrence of pollen limitation for *S. repens* and *F. pauciflora*. This study shows that *S. repens* and *F. pauciflora* in the Goolwa saltmarsh are pollen-limited across different flowering seasons and may also be reproductively limited by rainfall in some seasons, although this was not tested experimentally. When combining the results of this study with those of Mackay and Whalen (2009), this study provides novel evidence of a long-term trend of pollen limitation in this saltmarsh habitat.

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4. The effect of plant density on the facilitative and competitive effects of introduced *Limonium* species in two South Australian saltmarshes

Schlein, M.J., Mackay, D.A. and Whalen, M.A.

Flinders University, School of Biological Sciences,

GPO Box 2100, Adelaide, SA, 5001, Australia

Abstract

Plant density can have a significant effect on whether or not an introduced plant species will have competitive or facilitative effects on the pollination biology of the native plant species with which it co-occurs. Both natural and manipulated density studies examined if different densities of plants of the introduced genus *Limonium* Mill. have different effects on the fruit and seed set of plants of the native species *Frankenia pauciflora* DC. var. *gunnii* Summerh. in saltmarshes in South Australia. Seasonal variation across flowering seasons was observed, with effects ranging from facilitative to competitive. During the first year of the natural density study at the Goolwa saltmarsh, evidence of facilitation was observed, with the presence of *Limonium binervosum* (G.E.Sm.) C.E.Salmon associated with an increase in fruit set of flowers of *F. pauciflora*. In the later years of the natural density study, high densities of *L. binervosum* were associated with *F. pauciflora* setting fewer fruits and having a lower seed number per fruit, but when at a 50/50 mix (an equal floral display), the presence of *L. binervosum* had no significant effect. In the manipulated density study, at Torrens Island, the density of plants of the *Limonium hyblaeum* agg. had no effect on the fruit set of *F. pauciflora*, but did have an effect on the number of seeds per fruit at high densities. The study highlights the importance of conducting such experiments over multiple flowering seasons to determine variation amongst seasons and whether resource limitation may also be affecting fruit set and seed set. These results suggest that if the densities of plants of the introduced *Limonium* were to be reduced, its competitive effects on the native plant species

F. pauciflora may be replaced by facilitative effects which may lead to enhanced fruit or seed set.

Introduction

As introduced plant species increase in number and range (Brown *et al.* 2002), it is becoming increasingly important for ecologists to understand the potential impacts of introduced plant species on native plant communities and their existing relationships. Understanding these impacts can help to maximise conservation efforts and improve the efficiency of money spent on control and management (Byers *et al.* 2002; Harvey *et al.* 2014; Taylor and Hastings 2004).

Introduced plant species can have a range of effects on the pollination and reproductive biology of native plant species (Mitchell *et al.* 2009; Moragues and Traveset 2005; Muñoz and Cavieres 2008; Woods *et al.* 2012) and the density of an introduced plant species can strongly affect a native plant community (Feldman *et al.* 2004; Ghazoul 2006; Grabas and Laverty 1999; Muñoz and Cavieres 2008). Introduced plant species can compete with native plant species for resources such as water, nutrients and space as well as pollinator availability; however, they can also have facilitative effects on native plant species (e.g. Herron-Sweet *et al.* 2016; Moragues and Traveset 2005; Morales and Traveset 2009). The effects of introduced plant species on native plant species can change over time through the seasonal variation in the availability of resources (Moragues and Traveset 2005), which is why it is important to study their effects over multiple flowering seasons (Morales and Traveset 2009), as was done in this study.

Introduced plant species can reduce the reproductive success of native plant species by reducing visitation rates to native plant species. For example, they can attract floral visitors away from flowers of the native plant species through greater floral displays or through the presentation of greater rewards such as pollen and nectar (Brown *et al.* 2002; Chittka and Schurkens 2001; Johnson *et al.* 2012; Liao *et al.* 2011). Many studies have found the higher the density, the stronger the competitive effect on native plant species (Muñoz and Cavieres (2008), Kitamoto *et al.* (2008), Ghazoul (2006), Stout and Morales (2009)). However, introduced plant species can have facilitative effects on native plant species by increasing reproductive success (Ghazoul 2006). Introduced plant species can attract insect visitors to native plant species by increasing the floral display in an area, improving the presentation of nectar rewards available to visitors, or by increasing the insect populations that a community can support (Brown *et al.* 2002; Chittka and Schurkens 2001; Grindeland *et al.* 2005).

In some cases, the density of an introduced plant species can influence whether an introduced species exhibits competitive or facilitative effects on native plant species (Herron-Sweet *et al.* 2016; Mitchell *et al.* 2009; Morales and Traveset 2009; Rathcke 1983). These effects can be competitive, facilitative or in fact neutral and can vary given the density of the plant species involved and can vary across co-occurring plant species. The effect of density on the reproductive output of *Impatiens capensis* and other co-occurring species was examined by Grabas and Laverty in 1999 and they concluded that at high densities, the introduced *Lythrum salicaria* reduced fruit set of *I. capensis* by 30%. Many studies have found that at high densities, introduced plant species will have a negative effect on native plant species, however at a certain point, when density is reduced, facilitation can take place. Muñoz and Cavieres (2008) studied the effects of flower density on alpine species in Chile and found that high densities of introduced plant species could reduce the visitation rates of pollinators to native plant species, while the introduced plant species when present at lower densities could attract pollinators to flowers of the native plant species and increase pollinator visitation rates. Both of the native plant species in this study suffered from competition with the introduced species at high density, whilst at low density, one species showed no effect and the other showed an effect of facilitation (Muñoz and Cavieres 2008). It was hypothesised by Ghazoul, in a 2006 study of facilitation and co-occurring species, that multiple-species floral displays would create facilitation for floral visitors within a plant community. Ghazoul found that the density and composition of floral display influenced the presence of facilitation within the plant community and that as density continued to increase, insects began to disproportionately visit the species at the higher density, to the disadvantage of other species (Ghazoul 2006). Thus, there is potential for these effects to change over time if density or abundances change and although facilitation may be present at one point, it does not necessarily mean it will continue (Feldman *et al.* 2004). For example, in the study by Moragues and Traveset (2005) on the effect of the introduced *Carpobrotus* species, the effects of density ranged from facilitative to competitive for different species, with some species even showing no effect; however, these results were not consistent over time.

When assessing the effect of density on native plant species, it is important to assess reproductive output, not just visitation rates and heterospecific or conspecific pollen deposition, as changes to these interactions do not always equate to changes in reproductive output. Dietzsch *et al.* (2011) examined interactions between the introduced plant species *Rhododendron ponticum* and native plant species *Digitalis purpurea*. It was found that although increasing densities of the introduced plant species were linked to a decrease in visitation rates and a decrease in conspecific pollen deposition for the native *D. purpurea*, this did not lead to a reduced reproductive output (Dietzsch *et al*. 2011). The native *D. purpurea* is known to be self-compatible, and it was concluded, that by being self-compatible, plant species can overcome reductions in visitation and reductions in the quality or quantity of pollen deposition, and therefore will not show signs of competition through reproductive output (Dietzsch *et al*. 2011). Studies like this highlight the importance of breeding system studies, such as those discussed in Chapter five. *Frankenia pauciflora* is known to be facultatively xenogamous (Mackay and Whalen 2009) and so is reliant on outcross pollen to set seed, which makes it more susceptible to competition and pollen limitation (Dietzsch *et al.* 2011).

This study aimed to determine if introduced plants of the genus *Limonium* P Miller had a competitive, neutral or facilitative effect on the reproductive biology of the native South Australian saltmarsh species, *F. pauciflora* DC. var. *gunnii* Summerh. and whether or not the density of the introduced plants affected the outcomes of these interactions. It was expected that the introduced *Limonium* would have a competitive effect at high density and a facilitative effect at low density. Because the native *F. pauciflora* is dependent on insect visitors as pollinators (Mackay and Whalen 2009), any change in insect visitation could potentially have major impacts on its reproductive success. Fruit and seed set were examined at Goolwa for *F. pauciflora* plants, whilst pollinator observations along with fruit and seed set were examined at Torrens Island. Experiments were conducted over multiple years, and it was expected therefore that the effects of co-occurring plant species would vary in response to climatic conditions.

Materials and methods

Study sites

Goolwa saltmarsh

The natural density experiments were conducted at the Goolwa saltmarsh, (-35° 31' 55.69"S, +138° 49' 36.13"E) on the Fleurieu Peninsula of South Australia in an area approximately 2 km long and 50-100 m wide. The saltmarsh is situated 1.6 km downstream of the Goolwa Barrage on the Murray River, and 6 km upstream of the Murray Mouth (see Chapter one).

Torrens Island saltmarsh

The manipulated density experiments were conducted at the Torrens Island saltmarsh, (-34° 47' 46.90"S, +138° 31' 23.13"E) on the west side of Torrens Island, near Adelaide, South Australia. The experiments were conducted in an area approximately 1 km by 1 km.

Study species

Frankenia pauciflora

Frankenia pauciflora DC. var. *gunnii* Summerh., (Frankeniaceae), hereafter referred to as *F. pauciflora,* is a small native shrub occurring in saltmarshes and on cliffs along the coast of South Australia and in inland saline areas (Barnsley 1982; Mackay and Whalen 2009; Whalen 1986). *Frankenia pauciflora* has small, tubular, white to pale pink, actinomorphic flowers that are insect-pollinated (Whalen 1986). *Frankenia pauciflora* produces flowers year round at the study sites, but flowering markedly increases from October to March.

Limonium

Limonium P. Miller is an introduced genus in South Australia in the family Plumbaginaceae (Kubitzki 1993; Stevens 2014) although some sources such as Toelken (Toelken 1986a; Toelken 2007) place the genus in the family Limoniaceae. The genus *Limonium* naturally occurs in saltmarshes in Europe and the Mediterranean, often co-occurring with *Frankenia* species, and has spread quite extensively across the South Australian coast. *Limonium* has also been introduced into other saltmarshes across the world, including San Francisco Bay (Archbald and Boyer 2009). Plants of *Limonium* in South Australian saltmarshes are typically small with rosettes of leaves with long flowering stems raised above the leaves (Toelken 1986b). *Limonium binervosum* (G.E.Sm.) C.E.Salmon has purple flowers and is the most common *Limonium* species found at Goolwa but is not found at Torrens Island.

The *Limonium* found at Torrens Island, as well as occurring in lesser numbers at Goolwa, is referred to here as the *Limonium hyblaeum* /*Limonium companyonis* complex or *L. hyblaeum.* aggregate (*L. hyblaeum* agg.). This naming of the complex is because of similarities between *L. companyonis* (Gren. & Billot) Kuntze and *L. hyblaeum* Brullo and issues in distinguishing them in the study sites in South Australia (see Chapter one for details). For example, given patterns of variation in and among plants in some of the key characters differentiating *L. companyonis* (Gren. & Billot) Kuntze and *L. hyblaeum* in the Flora of South Australia (Toelken 2007).

Seasonal variation

The Millennium drought took place from 2000 to 2010, across multiple years of this study (see Chapter one for details). During the drought, Adelaide had reduced rainfall and elevated temperatures, after March 2010 however, large areas of Australia experienced above-average rainfall caused by an extremely strong La Niña event in the Pacific Ocean (Department of Sustainability, Environment, Water, Population and Communities 2011).

Natural density observations

Natural density observations were conducted at the Goolwa saltmarsh during the flowering seasons of 2008/2009, 2009/2010 and 2010/2011. Plants were observed occurring under three density conditions or categories, based upon comparing the floral display of *F. pauciflora* with *L. binervosum*. The first density category included single plants of *F. pauciflora* growing alone, without the presence of neighbouring *L. binervosum* in a 1 m x 1 m plot, referred to henceforth as *F. pauciflora* alone. Secondly, a *F. pauciflora* plant with neighbouring *L. binervosum* creating an approximately equal floral display in the 1 m x 1 m plot, was referred to as 50/50 mix or medium density, and lastly, a *F. pauciflora* plant with a floral display considerably outnumbered by *L. binervosum* flowers in the 1 m x 1 m plot, was referred to as *L. binervosum* outnumbering *F. pauciflora*, or high density.

Ten 1 m x 1 m plots representing each condition were haphazardly selected across the Goolwa saltmarsh and ten flowers were tagged on each *F. pauciflora* plant. Fruits were left to mature and the fruit set, the number of flowers that develop a fruit containing one or more seeds, and seed set, the number of seeds per fruit, were then determined. Fruits that showed signs of insect damage were removed from subsequent analyses.

Manipulated density experiments

A manipulated density experiment was conducted at the Torrens Island saltmarsh over the flowering season of 2010/2011. Fifty *F. pauciflora* plants were selected across the Torrens Island saltmarsh; the plants were all healthy and flowering and were approximately 50 cm in diameter. Plants were randomly assigned to one of three treatments: *F. pauciflora* growing alone, referred to as *F. pauciflora* alone; *F. pauciflora* with one *L. hyblaeum* agg. plant or *F. pauciflora* with four *L. hyblaeum* agg. plants. For all the *F. pauciflora* plants selected, all vegetation was removed from around the *F. pauciflora plant* within a radius of one metre and one, four or no *L. hyblaeum* agg. plants were transplanted around the *F. pauciflora* plant depending on the treatment. The transplanted *L. hyblaeum* agg. plants were all flowering when transplanted, and had at least 15 open flowers as well as numerous flower buds still to open. The transplanted *L. hyblaeum* agg. plants were regularly watered and treated with Seasol, a commercial soil conditioner. If a *L. hyblaeum* agg. transplant died, it was replaced each morning with fresh flowering branches from another *L. hyblaeum* agg. plant while the experiment was being conducted. On each of the *F. pauciflora* plants, ten branches were tagged that had buds and no open flower, so that any fruit taken from those branches would have been produced under the experimental conditions. Fruits were left to mature and the fruit set, the number of flowers that develop a fruit containing one or more seeds, and seed set, the number of seeds per fruit, were then determined. High levels of fruit and seed predation took place at both Goolwa and Torrens Island reducing the numbers of fruits that could be analysed, leading to unequal sample sizes in the density treatments.

Two hundred 15-minute pollinator observation periods were also conducted at the Torrens Island saltmarsh on the *F. pauciflora* plants in the different treatments (40 observation periods for *F. pauciflora* alone, 80 for the 1 *L. hyblaeum* agg. plant density and 80 for the 4 *L. hyblaeum* agg. density). Insect visitors were also recorded if they visited flowers of the partnered *L. hyblaeum* agg. plant(s). Observers sat at least one metre from the plant being watched and allowed five minutes for settling before a 15-minute observation period was conducted. Insect visitors that came into contact with either *F. pauciflora* or *L. hyblaeum* agg. reproductive parts were counted as floral visitors and considered possible pollinators. Their identity was recorded as well as the number of flowers visited for each species, the time spent at each flower, the plant species they came from and where they went afterwards (if possible), along with the time of the day, weather conditions such as temperature and cloud cover and any other noticeable behaviours.

Data analysis

SPSS 20 was used for all analyses. A two-way analysis with a Chi-square test was used for estimating any differences between treatments in fruit set, and a general linear mixed model with a Poisson error distribution was used to examine differences in seed set. Fruit and seed set for *F. pauciflora* plants in the different density treatments were compared to the fruit set and seed set of *F. pauciflora* growing alone, which was considered the baseline for comparison. Any flowers/ fruits that showed signs of floral or fruit predation were excluded from analyses. Analyses such as chi-square tests and Independent-samples median tests were used to determine the effects of treatment on pollinator behaviour. A series of nonparametric tests were conducted to examine the effect of heterospecific densities on visitation to *F. pauciflora* and the amount of time spent at each flower.

Results

Natural density observations at Goolwa saltmarsh

Fruit set

Fruit set, or the proportion of flowers that developed into fruits containing one or more seeds, was determined for the plants growing at three different densities over the three years of the study at Goolwa (Fig. 4.1). In the 2008/2009 flowering season, the plants in the *F. pauciflora alone* treatment set significantly fewer fruits (χ^2 = 8.062, df = 2, P = 0.018) than did plants growing under the two other density conditions. In the 2009/2010 flowering season; however, *F. pauciflora* plants growing alongside high densities of *L. binervosum* set significantly fewer fruits (χ^2 = 20.024, df = 2, $P < 0.001$) than did plants of *F. pauciflora* growing alone. The *F. pauciflora* plants growing in a 50/50 mix with *L. binervosum* also set fewer fruits than *F. pauciflora* plants growing alone, although the difference was non-significant. In the 2010/2011 flowering season, no significant differences in fruit sets of *F. pauciflora* plants growing under the three different density conditions were observed (χ^2 = 2.178, df = 2, P = 0.337). The number of flowers/ fruits removed from analyses due to floral or fruit predation is shown in Table 4.1.

Seed set

Seed set, or the number of seeds per fruit, was determined for the plants growing at the three different densities over the three years of the study at Goolwa (Fig. 4.2). In the 2008/2009 flowering season no significant effect *of L. binervosum* floral density on the mean number of seeds per fruit for *Frankenia pauciflora* plants was observed ($F_{2,77} = 0.246$, $P = 0.783$). In both the 2009/2010 and 2010/2011 flowering seasons a significant difference was observed ($F_{2,146} = 8.590$, $P = < 0.001$ and $F_{2,103} = 4.551$, $P = 0.013$ respectively) with the *F. pauciflora* plants growing alone setting significantly more seed than plants growing with a high density of *L. binervosum*. No significant difference was observed between the “50/50 mix” and *F. pauciflora* alone conditions.

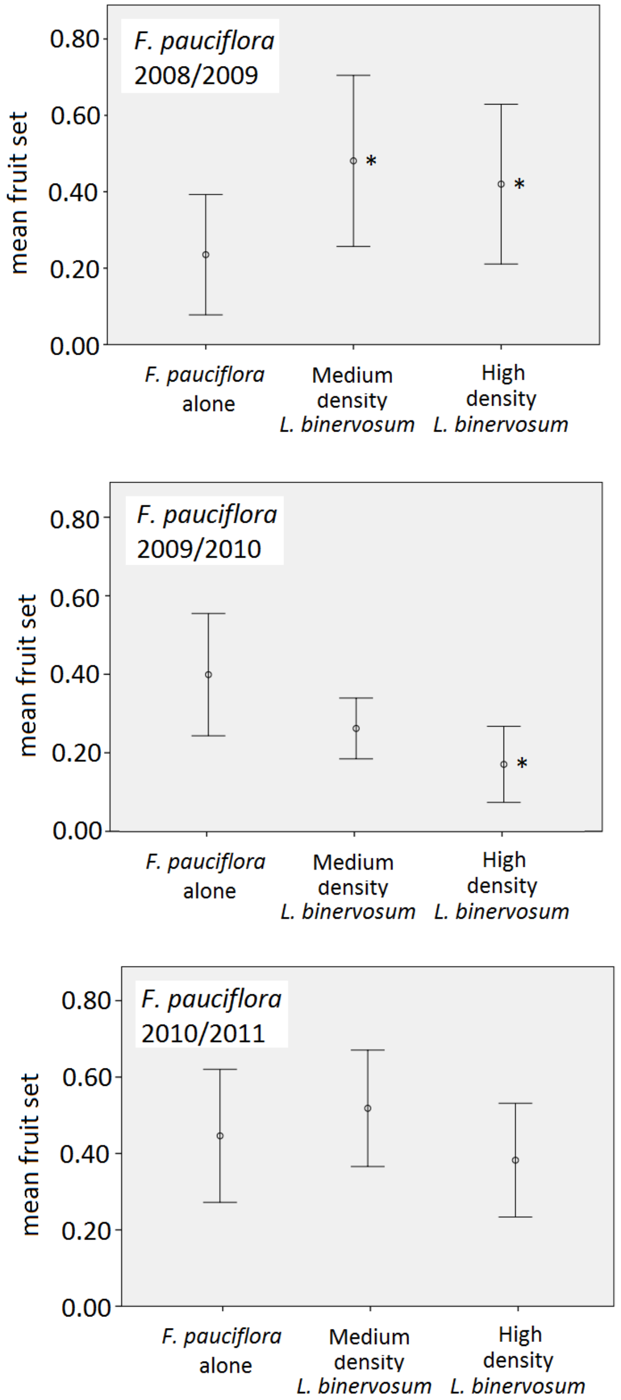

Figure 4.1 Mean proportional fruit set by *Frankenia pauciflora* plants in the different density conditions for the three natural density experiments at the Goolwa saltmarsh 2008/2009 to 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant comparisons with the *Frankenia pauciflora* alone treatment. Numbers of experimental flowers used in analysis (n) = 200 in 2008/2009, 541 in 2009/2010 and 344 in 2010/2011 (see Table 4.1 for detailed sample sizes).

Table 4.1 Number of *Frankenia pauciflora* flowers included in analysis, from the total number of flowers included in the density studies, for Goolwa and Torrens Island, after insect-damaged flowers/ fruits removed.

	Number of fruits	Number of flowers included in analyses	Number of fruits	Number of flowers included in analyses	Number of fruits	Number of flowers included in analyses
Natural density observations	*F. pauciflora* alone		Medium density *L. binervosum*		High density *L. binervosum*	
2008/2009	16	62	35	71	29	67
2009/2010	70	181	32	180	47	180
2010/2011	38	88	39	179	29	77
Manipulated density experiment	*F. pauciflora* alone		*F. pauciflora* with 1 *L. hyblaeum agg.*		*F. pauciflora* with 4 *L. hyblaeum agg.*	
2010/2011	54	157	38	151	27	101

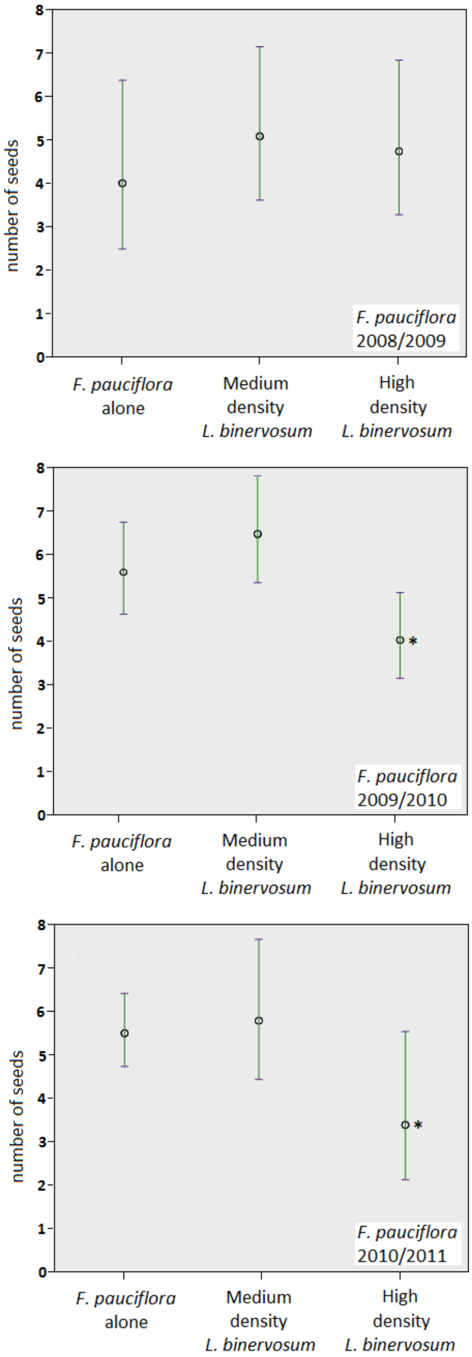

Figure 4.2 Mean number of mature seeds per fruit for *Frankenia pauciflora* plants in the different density conditions in three natural density studies at the Goolwa saltmarsh 2008/2009 to 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant comparisons with the *Frankenia pauciflora* alone treatment. Numbers of experimental fruits used in analysis (n) = 80 in 2008/2009, 149 in 2009/2010 and 106 in 2010/2011 (see Table 4.1 for detailed sample sizes).

Manipulated density experiments at Torrens Island saltmarsh

Fruit set

Fruit set was determined for the plants growing under the different *L. hyblaeum* agg. floral densities at the Torrens Island saltmarsh and no significant effect of *L. hyblaeum* agg. floral density was present on the mean proportion of *F. pauciflora* flowers that set fruit ($\chi^2 = 3.541$, df = 2, $P = 0.170$, Fig. 4.3). The number of flowers/ fruits removed from analyses due to floral or fruit predation is shown in Table 4.1.

Seed set

Seed set was determined for the plants growing at the different densities of *L. hyblaeum* agg. at the Torrens Island saltmarsh and a statistically significant effect of *L. hyblaeum* agg. floral density was present during the study ($F_{2,380} = 6.120$, $P = 0.002$). *Frankenia pauciflora* growing with 4 *L. hyblaeum* agg. plants set significantly fewer seeds than *F. pauciflora* growing alone, with no significant effect of 1 *L. hyblaeum* agg. plant (Fig. 4.4).

Visitation rates

The number of *F. pauciflora* flowers visited at Torrens Island did not differ significantly among different densities of *L. hyblaeum* agg. plants (Independent-samples median test, $P = 0.562$, Fig. 4.5). The mean time spent at different *F. pauciflora* flowers at also did not differ significantly between different densities of *L. hyblaeum* agg. plants (Independent-samples median test, $P = 0.562$, Fig. 4.6).

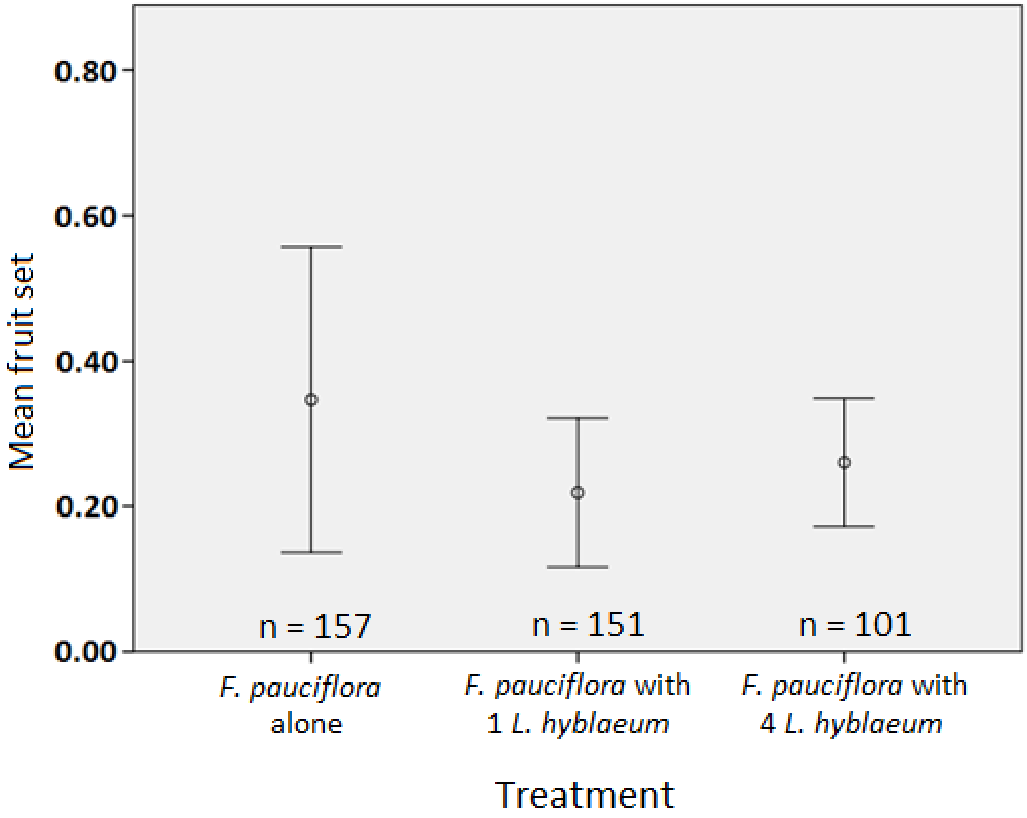

Figure 4.3 Mean fruit set for *Frankenia pauciflora* in the different experimental treatments in the manipulated density study at the Torrens Island saltmarsh 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote significant comparisons with *Frankenia pauciflora* alone treatment. Sample sizes (n) refer to numbers of experimental flowers included in analysis (see Table 4.1 for detailed sample sizes).

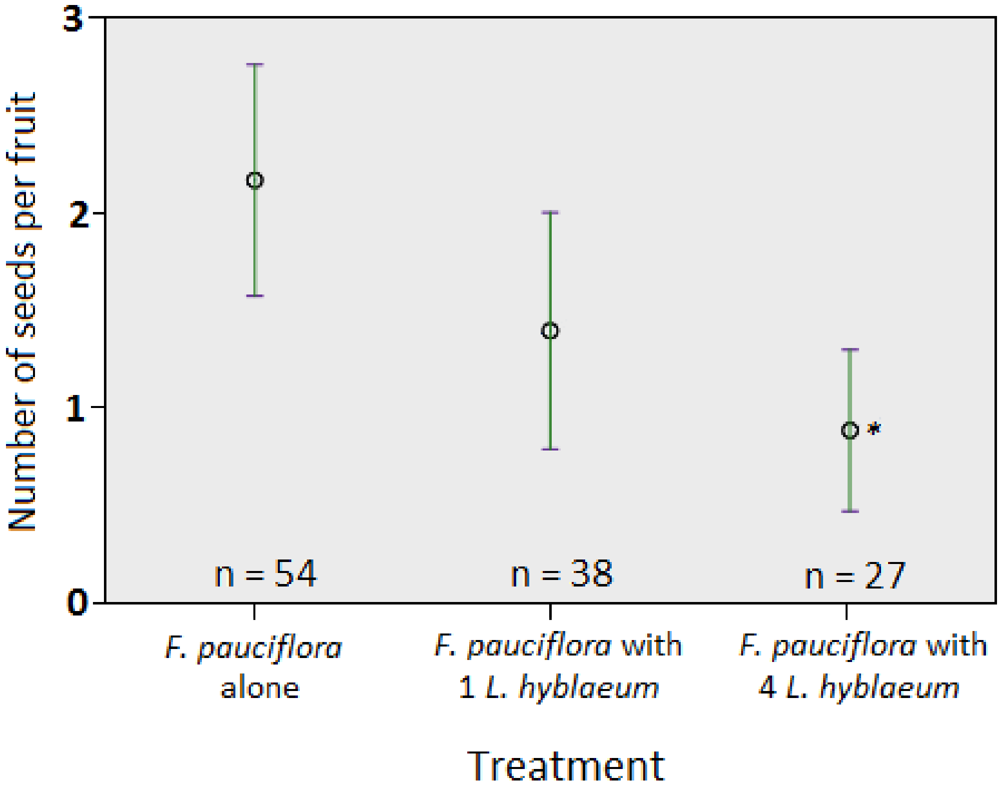

Figure 4.4 Mean number of mature seeds per fruit for *Frankenia pauciflora* by the different experimental treatments in the manipulated density study at the Torrens Island saltmarsh 2010/2011. Error bars represent 95% confidence intervals. Asterisks denote statistically significant comparisons with the *Frankenia pauciflora* alone treatment. Sample sizes (n) refer to numbers of experimental fruits included in analysis (see Table 4.1 for detailed sample sizes).

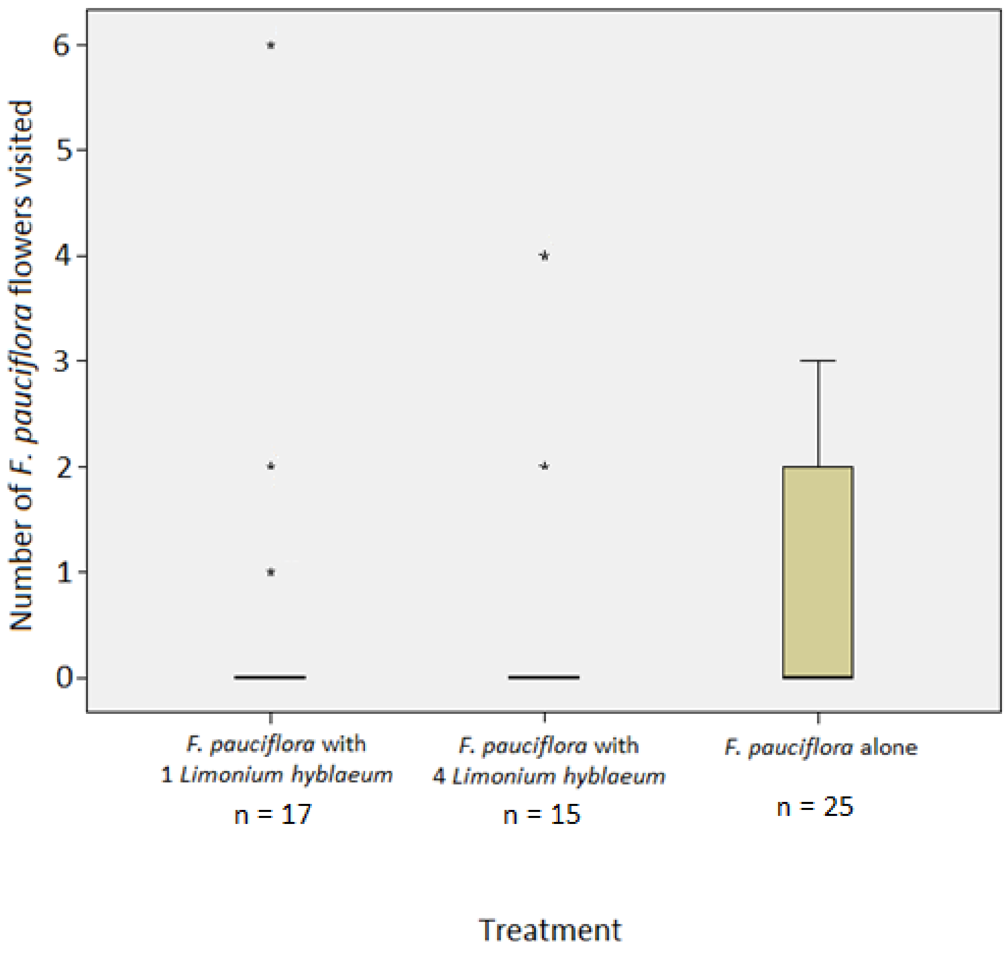

Figure 4.5 Number of *Frankenia pauciflora* flowers visited per 15-minute observation period at different *Limonium hyblaeum* agg. densities during the manipulated density study at Torrens Island 2010/2011. Asterisks and circles denotes outliers. n = the number of 15-minute observation periods with one or more *F. pauciflora* flower visited.

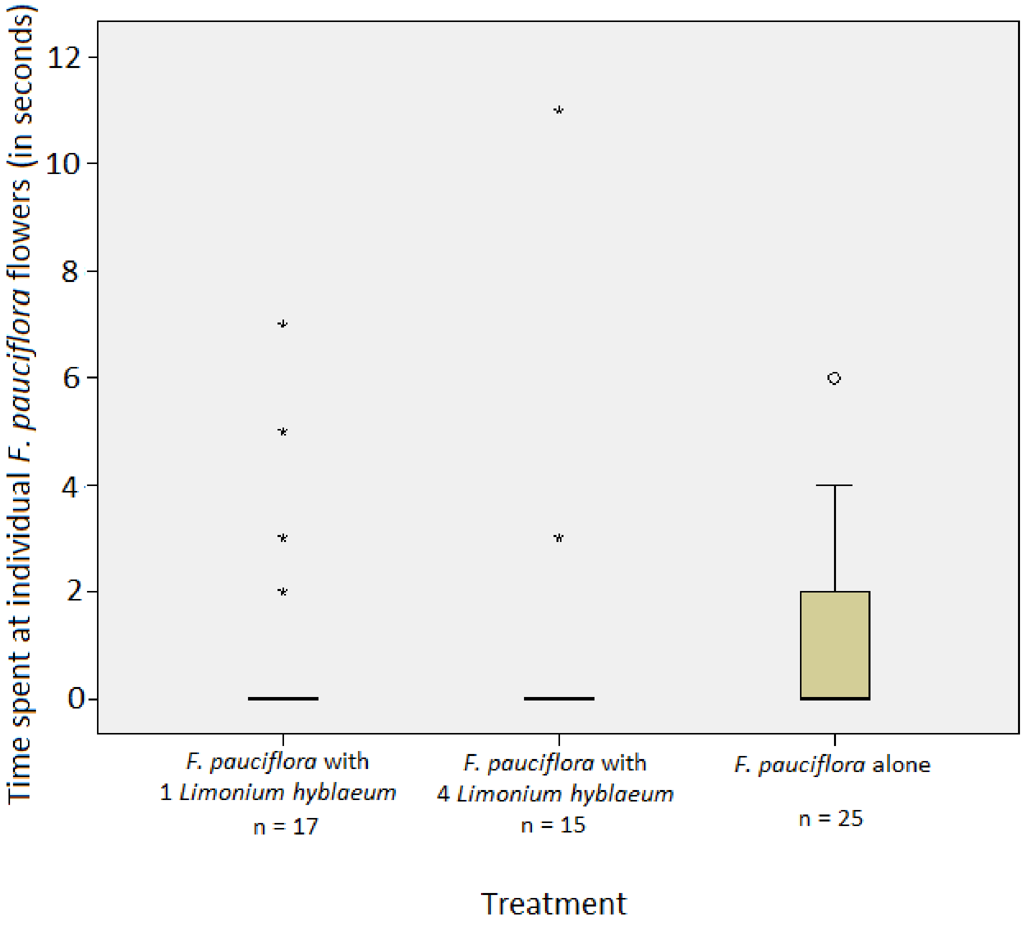

Figure 4.6 Time (in seconds) spent at individual *Frankenia pauciflora* flowers at different *Limonium hyblaeum* agg. densities during the manipulated density study at Torrens Island 2010/2011. Asterisks and circles denotes outliers. n = the number of 15-minute observation periods with one or more *F. pauciflora* flower visited and the time spent on individual flowers.

***Frankenia pauciflora* floral visitors**

During the observation periods conducted at Torrens Island, 142 insect floral visitors were observed, with 35 of these insects visiting flowers of *F. pauciflora*. Members of the Syrphidae were the most common floral visitors to flowers of *F. pauciflora* accounting for 46% of all floral visitors (Fig. 4.7). Sixteen individuals from the Syrphidae family were recorded, spending a total of 535 seconds visiting 110 *F. pauciflora* flowers (mean = 2.37 secs ± SD 2.00). Members of the Bombyllidae represented 26% of all insect visits, Apidae (*Apis mellifera*) 11%, Lycaenidae 9%, Sciomyzidae 6% and Muscidae represented 3%.

***Limonium hyblaeum* agg. floral visitors**

During the observation periods, 107 insects were recorded visiting flowers of *L. hyblaeum* agg. Members of the family Apidae (*Apis mellifera*) were the most common floral visitors to *L. hyblaeum* agg. flowers accounting for 61% of all visits (Fig. 4.7), with 65 individuals observed visiting 1,493 individual *L. hyblaeum* agg. flowers (mean = 4.46 secs ± SD 2.78). Butterflies of the family Lycaenidae made up 18% of all insect visits to flowers of the *L. hyblaeum* agg., Syrphidae 10%, Bombyllidae 9%, and Schiomyzidae and Doliochopodidae each represented 1% of all visits.

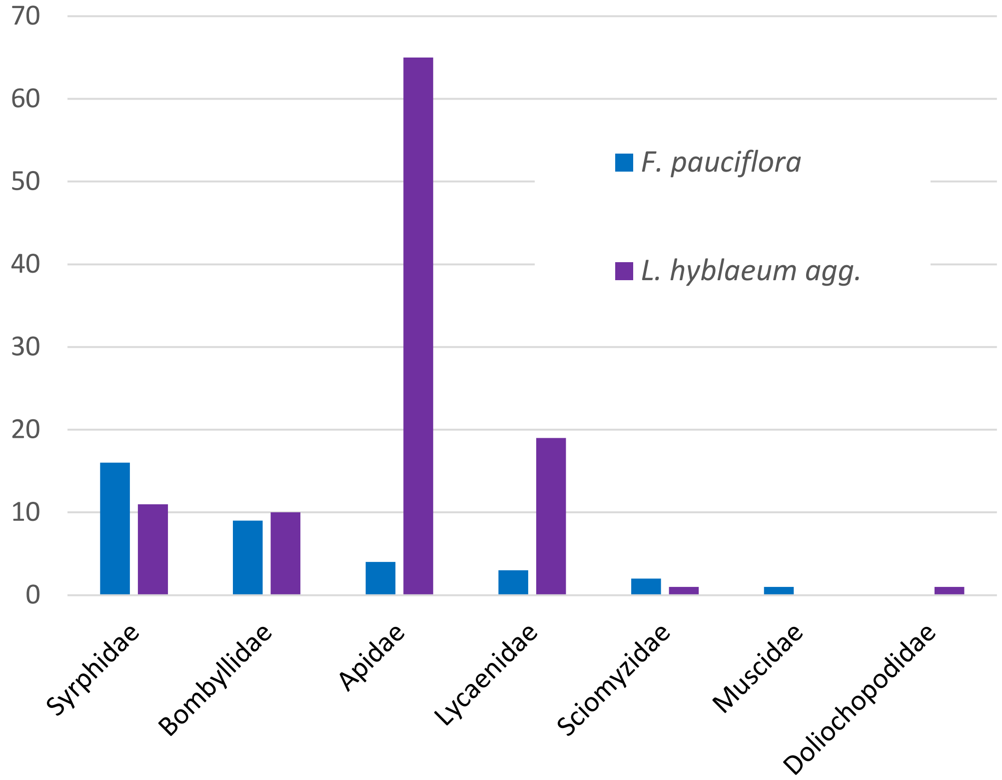

Figure 4.7 The number of floral visitors of different insect families to *Frankenia pauciflora* and *Limonium hyblaeum* agg. observed during 15-minute observation periods at the Torrens Island saltmarsh during the manipulated density study 2010/2011.

Frankenia pauciflora and *L. hyblaeum* agg. flowers show a considerable sharing of potential pollinators with the three most common visitors to flowers of *F. pauciflora* at the Torrens Island saltmarsh, syrphids, bombyliids and *Apis mellifera* which account for 81% of floral visitors to *F. pauciflora*, also accounting for 79% of floral visits to flowers of *L. hyblaeum* agg. (Fig. 4.7). Syrphids made up 46% of all insect visits to *F. pauciflora* flowers, but only 10% of insect visits to *L. hyblaeum* agg. flowers, although the number of visitors was quite similar, with sixteen individual syrphids visiting flowers of *F. pauciflora* and eleven syrphids visiting flowers of *L. hyblaeum* agg. This pattern may suggest that although many more *L. hyblaeum* agg. flowers are available, syrphids seem to prefer *F. pauciflora* flowers at the Torrens Island saltmarsh, a trend also shown in the Goolwa saltmarsh. Visitation in relation to availability is discussed in Chapter two. The introduced honeybee *A. mellifera* may prefer flowers of the introduced *L. hyblaeum* agg. whilst the native pollinators prefer the flowers of native plant species, a similar trend seen at the Port Elliott cliffs, discussed in Chapter two also.

Discussion

This is the first study to examine the impact of introduced plant species on the reproductive biology of native plants in South Australian saltmarshes, over multiple years and at multiple sites. It was found that the presence of introduced *Limonium* plants at high densities significantly reduced the reproductive success of *F. pauciflora*, for both fruit and/or seed set, at least at certain times. This effect was observed both in natural density observations at Goolwa and in the manipulated density experiments at Torrens Island. The effects of medium densities of *Limonium* plants on the reproductive success of *F. pauciflora* plants at Goolwa were not significant, except in the drought year 2008/2009 when both medium and high densities facilitated increased fruit set for flowers of *F. pauciflora*. Similarly, in the manipulated density experiment on Torrens Island the presence of 1 *L. hyblaeum* plant had no significant effects on the reproductive success of *F. pauciflora* plants. *Limonium* plants at these two sites appear to have integrated into the existing pollination network and any future management of the introduced plant species must take this integration into account. *Limonium* species have a large potential for spread, due to their rapid growth rates and ability to produce large numbers of seeds (Adair 2012), so it is vital that land managers have an understanding of their breeding system, reproductive biology and interactions with native plant species.

Herron-Sweet *et al*. (2016) examined the effects of variation in density of the introduced *Centaura stoebe* in western Montana and found results consistent with this study, in that the interactions between native and introduced plant species and their pollinators was dependent on the density of the introduced *C. stoebe*. They concluded that at high densities *C. stoebe* had a competitive effect on native plant species, whilst at low densities, the effect of *C. stoebe* was facilitative (Herron-Sweet *et al*. 2016). Ghazoul (2006) found similar results in his study on the facilitation and competition caused by plant species co-occurring with plants of *Raphanus raphanistrum* in the UK. The facilitative effect observed was dependent on the density of the other plant species, and when the density increased beyond a certain point, the effect of co-occurring species became a competitive effect (Ghazoul 2006). Ghazoul (2006) discussed the model proposed by Rathcke (1983) where facilitation is expected to occur up until a density threshold, when facilitation will turn to competition. Thijs *et al.* (2012) studied the effect of the introduced *Impatiens glandulifera* on two native species, *Lythrum salicaria* and *Alisma plantago-aquatica*, and one naturalized species, *Oenothera biennis,* in a freshwater marsh in Belgium. The three species showed different interactions with the introduced *I. glandulifera*, with one of the native species, *L. salicaria*, suffering from competitive effects whilst the other two species, *A. plantago-aquatica* and *O. biennis*, showed no effect. It was determined that by not sharing pollinators with the introduced plant species, competitive effects could be avoided (Thijs *et al.* 2012). Findings like this show the importance of investigating multiple factors, such as density, pollinator sharing, fruit and seed set, and pollen limitation, to obtain a clear understanding of the interactions taking place between species, which this study was able to do (see Chapters two, three and five).

For *F. pauciflora*, it appears that the sharing of pollinators with the introduced *L. binervosum* increases the likelihood of a competitive effect taking place at high densities of the latter species. *Limonium binervosum* appears to be male sterile at Goolwa, so effects of varying densities of *L. binervosum* on the pollination of *F. pauciflora* will most likely be due to an increase or decrease in conspecific visitations to the latter species, rather than due to effects such as stigma clogging by heterospecific pollen. Interestingly, although plants of the *L. hyblaeum* agg. present at Torrens Island do produce pollen, both sites had similar patterns with respect to the effects of *Limonium* densities in the 2010/2011 flowering season on fruit set and seed set. Surprisingly, variation in *Limonium* density did not significantly affect insect visitation rates to *F. pauciflora* flowers at Torrens Island. It may be that *F. pauciflora* pollen is being lost through visits to *Limonium* flowers, or that insect visitors to *F. pauciflora* flowers are not efficient pollinators. This finding definitely warrants further investigation in the future.

Conservation and management implications

The main visitor to flowers of *L. hyblaeum* agg. plants at Torrens Island is the introduced honeybee *Apis mellifera* (Fig. 4.7), suggesting that plants of the introduced *L. hyblaeum* agg. may not be primarily interfering with the pollination of plants of *F. pauciflora* by co-opting the services of native pollinators. Moragues and Traveset (2005) examined the effects of introduced *Carpobrotus* species on native plant pollination and found that effects were facilitative for some native plants species, competitive for other native plant species and neutral for other plant species, although effects varied over time. Understanding these impacts can help to maximise conservation efforts and improve the efficiency of money spent on control and management (Byers *et al.* 2002; Harvey *et al.* 2014; Herron-Sweet *et al.* 2016; Taylor and Hastings 2004). Given the findings of this, and similar studies, it is important to evaluate the role of introduced plant species on a local level and ideally over multiple seasons and years (Herron-Sweet *et al.* 2016). The findings of this study have confirmed those of several other studies: that variation in the density of introduced plant species can shift the effect on the native plants from facilitative to competitive, and so when conservation and management strategies are developed, it is important to know the ways in which varying densities of introduced plant species may alter the availability of resources within the plant communities and pollination networks (Herron-Sweet *et al.* 2016). With regard to *Limonium*, the management of plants to avoid dense stands in the Goolwa and Torrens Island saltmarshes could prove not only the best option ecologically but also economically for the pollination of native plant species. Future work could further examine at what density threshold facilitation becomes competition, for a more accurate understanding of the effects of *Limonium* density.

An additional concern to consider when planning the management of introduced plant species such as *Limonium*, is a suggested trend in the loss of pollinators globally (Goulsen *et al.* 2015; Potts *et al.* 2010; Stout and Morales 2009). Pollinators play a critical role in ecosystem functioning, especially when considering that at least 80% of terrestrial plant species rely on animals, usually insects, as pollinators (Herron-Sweet *et al.* 2016; Ollerton *et al.* 2011). Introduced plant species may be able to help buffer impacts of pollinator loss on native plant communities and could provide resources to pollinators that are not available via native plant species (Potts *et al.* 2010). By limiting *Limonium* populations to occur at low to medium densities, the competitive effects on native plant species may be avoided whilst still providing facilitative effects.

Seasonal variation and weather

At Goolwa, the effects of medium densities of *Limonium* plants on the reproductive success of *F. pauciflora* plants were not statistically significant, except in 2008/2009 when both medium and high densities facilitated increased fruit set for flowers of *F. pauciflora*. The flowering season of 2008/2009 saw drought conditions at the Goolwa saltmarsh; 2009/2010 had average rainfall at Goolwa, and the 2010/2011 flowering season coincided with above average rainfall at both Goolwa and Torrens Island. From the 1st of March 2010 to the 28th of February 2011, Goolwa and Torrens Island had very much above average rainfall (i.e. when rainfall was in the highest 10% of historical totals since 1900).

The varying results in the natural density study at Goolwa may be due to the effects of seasonal variation and resource limitation, such as a lack of water availability and increased soil salinity levels, implying that seasonal variation is an important factor to consider when examining the effects of introduced plant species.

When examining the relationship between natural densities of *L. binervosum* and the fruit set of *F. pauciflora* at Goolwa, evidence suggests a facilitative effect of both medium and high densities of *L. binervosum* on *F. pauciflora* during the 2008/2009 flowering season, a competitive effect of high densities of *L. binervosum* in 2009/2010 and no significant effect in 2010/2011. Facilitation may be more likely when environmental conditions are harsh, as was the case in 2008/2009, and competition may be more likely under more favourable conditions. Possibly because of resource limitations, plants of *F. pauciflora* had a reduced floral display during the 2008/2009 flowering season (see Chapter two). The presence of *Limonium* flowers at that time may therefore have increased the overall floral display in patches where *F. pauciflora* occurred, resulting in more insect visitors attracted to *F. pauciflora* flowers. Once both resource availability and floral display had improved, as was the case in the 2010/2011 flowering season, the effect of *Limonium* on fruit set *F. pauciflora* may no longer have been significant.

With regard to seed set, no significant association between *Limonium* floral density and *F. pauciflora* seed set was observed in 2008/2009 at Goolwa; however, a negative association was found in 2009/2010 and 2010/2011 with high *L. binervosum* densities, and no significant association with medium *L. binervosum* densities. These associations are consistent with the hypothesis that when environmental conditions are harsh, as in 2008/2009, any effect of the *Limonium* is likely to be neutral or facilitative, but when environmental conditions improve, perhaps through increased water availability, the presence of *Limonium* at a high density may cause a competitive effect on *F. pauciflora* seed set.

Conclusion

This study is novel in examining the impact of an introduced plant species with regard to density and its interactions with native, insect-pollinated plant species, at multiple sites in a saltmarsh habitat over multiple years. The study found that the presence of *Limonium* at medium densities can have a facilitative or neutral effect on the native plant species *F. pauciflora*, whilst at high densities have a competitive effect. This suggests that to maximise conservation of native saltmarsh plants, land managers could focus on reducing *Limonium* density to low or medium densities, without the necessity of complete eradication. The results of this study also suggest that facilitation may be more likely under unfavourable environmental conditions and competition more likely under favourable conditions. These results highlight the importance of conducting such studies over multiple flowering seasons, to identify any variation across seasons. Further studies could examine the interaction between the introduced *Limonium*, the native *F. pauciflora* and the other main native insect-pollinated plant at Goolwa and Torrens Island, *Samolus repens*.

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5. Reproductive biology of the native saltmarsh plant species *Samolus repens* (Primulaceae) and the introduced *Limonium hyblaeum* agg. and *Limonium binervosum* (Plumbaginaceae) in a South Australian saltmarsh

Schlein, M.J., Whalen, M.A. and Mackay, D.A.

Flinders University, School of Biological Sciences,

GPO Box 2100, Adelaide, SA, 5001, Australia

Abstract

Examining plant breeding systems is not only important for understanding how plant species reproduce but also to help understand the possible impacts of introduced plant species. This study examined the breeding systems of the native saltmarsh plant species *Samolus repens* (J.R.Forst. & G.Forst.) Pers. as well as of the introduced *Limonium binervosum* (G.E.Sm.) C.E.Salmon and *Limonium hyblaeum* Brullo agg., all co-occurring in South Australian saltmarshes. Natural levels of fruit set and seed set were also recorded for *S. repens* and the two *Limonium* species at the Goolwa saltmarsh in South Australia. Glasshouse experiments revealed that *S. repens* is dependent on insect visitors to serve as pollinators, being unable to set fruit without pollen being deposited on the stigma. Plants of *L. hyblaeum* agg. and *L. binervosum* were both capable of reproducing via apomixis. *Limonium binervosum* appears to be male sterile at the Goolwa saltmarsh, with pollen absent from flowers examined from the population. Natural fruit set was high for plants of the *L. hyblaeum* agg. and *L. binervosum* at the Goolwa saltmarsh, suggesting that the further spread of *Limonium* across the South Australian coast is not likely to be limited by reproduction. These results also suggest that *S. repens* would be negatively affected by a reduction in insect visitation rates, whilst similar reductions would not likely affect the introduced

Limonium populations to the same extent, thus potentially leading to further competition in natural communities.

Introduction

Very little is known about pollination and seed dispersal in saltmarsh plants, especially in saltmarshes in south-east Australia (Adam 1990), and especially those depending on insect pollination. With increasing numbers of introduced plant species in saltmarshes, such as *L. binervosum* (G.E.Sm.) C.E.Salmon and *L. hyblaeum* agg. Brullo, the relationships among native and introduced species in terms of pollination and seed dispersal are dynamic and complicated. Introduced plant species can have competitive, facilitative or neutral effects on native plant species, and understanding their reproductive biology can help to determine what impacts they may have on the native plant species (Keane and Crawley 2002; Rathcke 2003) enabling ecologists to better predict and manage the potential impacts of introduced plant species on native plant species.

Previous work in this study (Chapter two) has shown that *Samolus repens* (J.R.Forst. & G.Forst.) Pers., *L. binervosum* and *L. hyblaeum* agg. all share a common flowering period and share many insect visitors. Breeding systems are an important component of the biology of species which are likely to affect the outcomes of interactions such as competition for pollinators. For example, Baker (1966) proposed that plant species that are capable of autonomous self-pollination are more likely to succeed in expanding their ranges and becoming naturalised than plant species that are self-incompatible (Baker 1966; 1974). Other authors have suggested that an ability to reproduce asexually can help species to expand their populations and ranges (Pannell and Barrett 1998; Perglová *et al*. 2006; Rambuda and Johnson 2004; Randall 2007; Rodger and Johnson 2013).

The genus *Limonium* has apomictic and self-compatible members. Apomixis (treated here as synonymous with agamospermy) in flowering plants is asexual reproduction via seed (Koltunow and Grossniklaus 2003; Ortiz *et al.* 2013; Rathcke 2003). Apomixis may result in seed production, but comes at a potential cost, as all of the seeds produced will be clones of the parent plant (Castro *et al.* 2008; Hörandl and Hojsgaard 2012). Some apomictic plant species also seem to maintain their ability to reproduce via sexual reproduction (Koltunow and Grossniklaus 2003). Apomixis is thought to be naturally occurring in more than 400 plant species from 35 families worldwide (Estrada-Luna *et al.* 2002; Koltunow and Grossniklaus 2003) with many members of the genus *Limonium* P Miller known to be apomictic (Cowan *et al.* 1998; Palacios *et al.* 2000; Róis *et al.* 2013). Apomictic species are known to create monomorphic populations consisting entirely of self-incompatible plants (Cowan *et al.* 1998; Rodrigo *et al*. 2012).

Rambuda and Johnson (2004) examined the occurrence of autonomous self-pollination in introduced plant species in South Africa, all 17 plant species examined were either self-compatible or apomictic, and autonomous self-pollination was possible in 72% of the species examined. Rambuda and Johnson (2004) concluded that the type of breeding system present in introduced plant species can have important consequences for invasion ecology. Hao *et al.* (2011) examined the occurrence of autonomous self-pollination in 12 invasive species of the family Asteraceae, finding that 67% of plant species examined were self-compatible and 83% were capable of autonomous self-pollination (Hao *et al*. 2011), indicating that these reproductive strategies may assist introduced plant species with range expansion. These results are consistent with Baker's 1955 law and re-affirm the importance of breeding system studies for introduced plant species (Hao *et al.* 2011) as did the results of the previously mentioned study.

Aims of study

The aim of this study is to gain a better understanding of the breeding systems of one of the main native insect-pollinated plant species at Goolwa, *S. repens* and of the introduced plants *L. hyblaeum* agg. and *L. binervosum*. By increasing the understanding of breeding systems of these species in South Australia, better management decisions can be made with regard to the impact and management of introduced plant species in South Australian saltmarshes. Breeding system studies have already been conducted on the other main native insect-pollinated plant species at Goolwa, *Frankenia pauciflora* DC var. *gunnii* Summerh., by Mackay and Whalen (2009) and so this study focused on *S. repens* and on the *Limonium* species, which have been less studied in South Australia.

It is predicted that plants of the native species *S. repens* will have a breeding system which makes these plants more reliant on insect pollination than plants of the introduced *Limonium* species.

Materials and methods

Study species

Samolus repens

Samolus repens (J.R.Forst. & G.Forst.) Pers. (Primulaceae), or creeping brookweed, is a small, halophytic herb native to South Australia, found in moist, saline areas such as saltmarshes, coastal cliffs and mangroves (Stahl 2004; Toelken

1986b; Toelken 2007b). Although often considered a member of the Primulaceae (Stevens 2014; Toelken 2007b) it is sometimes included in its own family, the Samolaceae (Stahl 2004). *Samolus repens* has white to pink actinomorphic flowers, with a tubular corolla, roughly 1 cm in diameter, and numerous ovules (Toelken 1986b, Stahl 2004). It was noted through floral longevity studies that *S. repens* flowers are unable to self-pollinate because of herkogamy (the spatial separation of sexual organs) and protandry (where pollen is released prior to the stigma becoming receptive) (pers. obs.).

Limonium

The genus *Limonium* P. Miller is introduced in Australia and has been considered to be a member of the family Plumbaginaceae although sometimes included in the family Limoniaceae where the latter is distinguished from the Plumbaginaceae (Kubitzki 1993; Toelken 1986a; Toelken 2007a). The genus has become common along the coast of southern Australia, where it grows with *F. pauciflora* DC. var. *gunnii* and *S. repens*. The genus *Limonium* is native to Europe and the Mediterranean and *Limonium* species can be found growing naturally alongside *Frankenia* L. and *Samolus* L. species in these areas (Boira 1995; Chapman 1974). *Limonium* species have spread along the South Australian coast as well as in other parts of Australia and globally, such as the spread of *Limonium ramosissimum* in marshes of San Francisco (Archbald and Boyer 2009). Members of the genus *Limonium* can produce sexual and asexual seeds (Róis *et al.* 2012), and many species of *Limonium* are triploid or tetraploid and capable of agamospermy (Cowan *et al.* 1998; Palop-Esteban *et al.* 2011). *Limonium* species are known to grow at a rapid rate, *Limonium hyblaeum* was recorded spreading 0.36 m over a 3 month period at Port Fairy, Victoria (Rodrigo *et al.* 2012) with *L. ramosisimum* reported to spread 0.7 m within two months in the San Francisco Bay area (Archbald and Boyer 2009). *Limonium* species can also reproduce vegetatively through horizontal rhizomes, which can not only travel under existing plants but also can help to create a blanket of plants (Rodrigo *et al*. 2012).

Limonium binervosum

Limonium binervosum (G.E.Sm.) C.E. Salmon (Plumbaginaceae) is an introduced species of *Limonium* found at Goolwa, originally from the Atlantic coasts of Europe (Ingrouille and Stace 1986). It has purple flowers in an inflorescence protruding from a rosette of leaves (Toelken 1986a). Members of the *L. binervosum* aggregate, also referred to as microspecies (Ingrouille and Stace 1986), are agamospermous, with some male-sterile populations (Baker 1948; Cowan *et al.* 1998; Ingrouille and Stace 1986). Some taxa lack pollen altogether and can have entire colonies lacking pollen (Cowan *et al.* 1998; Ingrouille and Stace 1986). Pollen examinations were conducted at the Goolwa study site, with 500 *L. binervosum* flowers and buds randomly collected from 100 different plants. No flower or bud was observed to have pollen, suggesting that it is highly likely that the population of *L. binervosum* at Goolwa is male-sterile.

***Limonium hyblaeum* agg.**

In addition to *L. binervosum* at Goolwa, a different *Limonium* was found less commonly at Goolwa and also at Torrens Island and Port Elliott. The latter *Limonium* is referred to as the *Limonium hyblaeum*/ *Limonium companyonis* complex or *L. hyblaeum* aggregate in this study because of the similarities between *L. companyonis* (Gren. & Billot) Kuntze and *L. hyblaeum* Brullo, and issues in distinguishing them in the South Australian study sites. They have pink/ purple flowers in an inflorescence projecting above a dense rosette of leaves. The *L.*

hyblaeum agg. plants used in the breeding system studies all had a midvein that did not extend to the tip of the calyx lobe, typical of *L. hyblaeum*, and all plants had pollen and obovate leaves.

Limonium hyblaeum, *L. companyonis* and an unknown *Limonium* species (possibly *L. binervosum*), are recognised as red alert priority weeds in the Limestone Coast and Coorong region (Caton *et al.* 2011). These *Limonium* species have the potential to cause serious damage across their invasive ranges and it is hoped that this study can help scientists to understand their reproductive biology, and hence their potential spread.

Methods

Breeding system experiments

Hand pollination trials were conducted, in the glasshouse facilities at Flinders University, to quantify levels of self-compatibility in *S. repens* as well as in plants of the introduced *L. binervosum* and *L. hyblaeum* agg. The extent of self-compatibility can be examined by comparing the number of seeds per fruit, or seed set, of cross-pollinated and self-pollinated flowers (Dafni *et al.* 2005). Many studies have used this method, including those of Dicenta *et al*. (2002), Zorn-Arnold and Howe (2007) and Agren (1996).

This study examined whether or not flowers of the three study taxa can be fertilised with pollen from the same plant (geitonogamy), pollen from a different plant of the same species (xenogamy), and whether or not plants were capable of autonomous self-pollination (autogamy) or apomixis (agamospermy). To test for geitonogamy, newly opened flowers were bagged, with hand-made, fine mesh bags and pollinated with pollen from different flowers on the same plant. To test for xenogamy, flowers were bagged and pollinated with cross-pollen from flowers of the same species from a different plant. Pollen was transferred directly from the anthers of at least three flowers, from either the same plant or different plants depending on treatment. Some flowers were bagged and emasculated (anthers removed) to test for apomixis/agamospermy and other flowers were bagged and left unmanipulated to test for autonomous self-pollination/autogamy. Flowers were bagged to ensure that no additional pollen was able to contact the treated flowers, either by wind or insect. All plants were kept in a glasshouse with an evaporative cooler, with a maximum temperature of 35 ^{o} C with daily watering; a slow-release fertilizer was used, as was a soil conditioner. Fruit set is calculated as the proportion of flowers that develop into fruits containing one or more seeds. The parameter of seed set refers here to the number of seeds per fruit, with fruits having one or more seeds present. No sign of insect damage through floral or fruit predation was recorded in the glasshouse.

For the breeding system studies of *S. repens*, 199 flowers were used from 15 different plants (34 flowers in the geitonogamy treatment, 44 in the xenogamy treatment, 68 in the autogamy treatment and 53 in the agamospermy treatment), For *L. binervosum*, 342 flowers were used from 28 plants (113 flowers in the autogamy treatment and 229 flowers in the agamospermy treatment) and for *Limonium hyblaeum* agg. 864 flowers from 33 plants were used in the breeding system experiments (158 flowers in the geitonogamy treatment, 184 in the xenogamy treatment, 406 in the autogamy treatment and 116 flowers in the agamospermy treatment). All breeding system experiments were conducted in the glasshouse facilities at Flinders University, Adelaide, South Australia, during the summers of 2010/2011 and 2011/2012.

Natural fruit and seed set

Natural fruit set and seed set were compared for plants of *S. repens,* the *L. hyblaeum* agg. and *L. binervosum* in the glasshouse, with those occurring under natural conditions in the Goolwa saltmarsh during the 2010/2011 flowering season. Twenty healthy plants of *S. repens* were selected from across the Goolwa saltmarsh and on each plant 20 flowers were tagged over the flowering season. When fruits had matured, fruit set and seed set were recorded, as well as the number of fruits that had been damaged by floral or fruit predation, subsequently removed from analysis. This same method was used for 20 *L. hyblaeum* agg. plants and 20 *L. binervosum* plants.

Data analysis

Contingency-table Chi-square tests were used to compare fruit set among breeding system treatments. Levels of seed set for *S. repens* were analysed using a generalised linear mixed-effect model. SPSS 20 was used for all analyses.

Results

Samolus repens

Glasshouse breeding systems study

A significant effect of treatment on fruit set was found for *S. repens* (X^2 = 99.857, df = 3, $P < 0.001$) during the glasshouse experiment. No flower from the autogamy or agamospermy treatments set fruit, whilst 24% of the geitonogamy treatment set fruit and 76% of the flowers in the xenogamy treatment set fruit (Comparison of geitonogamy and xenogamy treatments: X^2 = 14.62, df = 1, $P = 0.002$) (Table 5.1).

Seed set (seed number per fruit) was examined for *S. repens,* the autogamy and agamospermy treatments were not included in the analysis as they set no fruit. A statistically significant effect of treatment was found for the seed set of *S. repens* (F = 27.551, df = 1,76, < 0.001) with fruits in the xenogamy treatment setting significantly more seeds than fruits in the geitonogamy treatment (Table 5.2).

Both fruit set and seed set were significantly higher for flowers in the xenogamy treatment than for the geitonogamy treatment. At no time was pollen present on stigmas from anthers of the same flower, as reproductive parts were always spatially separated, through herkogamy and protandry (pers. obs.).

Natural fruit set and seed set in the Goolwa saltmarsh

Of the *S. repens* flowers growing under natural conditions in the Goolwa saltmarsh during the flowering period of 2010/2011, 56% (n = 45/80) set fruit (Table 5.1), leaving 16% (n = 13/80) of flowers with no obvious floral or fruit predation but not developing into fruits and 28% (n = 22/80) of fruits showing signs of floral or fruit predation. Natural seed set was also examined (Table 5.2) with 78% of unpredated flowers setting fruit (n = 45/58) with a mean number of seeds per fruit of 16.11.

***Limonium hyblaeum* agg.**

Glasshouse breeding systems study

Comparisons of fruit set among treatments were made with no significant differences among treatments for *L. hyblaeum* agg. experiments in both 2010/2011 (X^2 = 1.164, df = 3, P = 0.762) and 2011/2012 (X^2 = 2.080, df = 3, P = 0.556). Treatments included tests for geitonogamy, xenogamy, autogamy and agamospermy (Table 5.3). *Limonium* species only set one seed per fruit, so seed set was not examined.

Table 5.1 Fruit set, calculated as the percentage of flowers, that develop into fruits containing one or more seeds, for *Samolus repens* flowers in the glasshouse under different treatments and in the Goolwa saltmarsh. * indicates a statistically significant difference between the geitonogamy treatment and xenogamy treatment. *n* indicates the number of flowers which set fruit out of the total number of flowers included in each treatment.

Fruit set by treatment in glasshouse:	Percentage fruit set
Autogamy	0% (*n* = 0/68)
Agamospermy	0% (*n* = 0/53)
Geitonogamy	24% (*n* = 10/34)
Xenogamy	76% (*n* = 31/44) *
Natural fruit set: Goolwa (excluding insect predated flowers/ fruits)	78% (*n* = 45/58)

Table 5.2 Seed set, as the number of seeds per fruit, for *Samolus repens* flowers in the glasshouse by treatment and in the Goolwa saltmarsh. Note: no flower from either the autogamy or agamospermy treatments set fruit so no seed was present for these treatments. Note that the range indicates minimum and maximum number of seeds per fruit.

Treatment in glasshouse	Mean number of seeds per fruit	Std. error of mean	median	mode	Range
Geitonogamy	7.90	2.173	5.00	3	2 to 21
Xenogamy	34.42	5.869	26.00	9	1 to 145
Natural seed set: Goolwa	16.11	1.854	11	4	1 to 48

Table 5.3 Fruit set, calculated as the percentage of flowers that developed into fruits with at least one seed, for *Limonium hyblaeum* agg. flowers in the glasshouse by treatment and in the Goolwa saltmarsh. *n* indicates the number of flowers which set fruit out of the total number of flowers included in each treatment.

	2010/2011	2011/2012	Combined
All glasshouse treatments combined	84%	98%	*n* = 793/864
Autogamy	84%	100%	*n* = 375/406
Agamospermy	83%	97%	*n* = 107/116
Geitonogamy	87%	100%	*n* = 145/158
Xenogamy	81%	100%	*n* = 166/184
Natural seed set: Goolwa	54%	Not recorded	N/A

Natural fruit set in the Goolwa saltmarsh

Fruit set, by plant, for plants of the *L. hyblaeum* agg. growing under natural conditions in the Goolwa saltmarsh ranged from 20% to 80% (Table 5.3). By comparison fruit set, by plant, for unmanipulated *L. hyblaeum* agg. flowers growing in the glasshouse in the autogamy treatment during the 2010/2011 flowering season ranged from 60% to 100%.

Under natural conditions in the Goolwa saltmarsh, 54% of flowers set fruit (Table 5.3) and no fruit from plants of the *L. hyblaeum* agg. collected from the Goolwa saltmarsh had signs of floral or fruit predation. By comparison in the glasshouse, 84% of unmanipulated flowers set fruit during 2010/2011, and during the 2011/2012 glasshouse study, 100% of unmanipulated flowers set fruit.

Limonium binervosum

Glasshouse breeding systems study

A significant difference was observed between treatments for *L. binervosum* in 2010/2011 ($X^2 = 8.403$, df = 1, $P = 0.004$) (Table 5.4). In the agamospermy treatment, 85% of flowers set fruit compared with 72% for the autogamy treatment. *Limonium* species only set one seed per fruit, so seed set was not examined separately.

Natural fruit set in the Goolwa saltmarsh

A natural fruit set study was also conducted at Goolwa for *L. binervosum* to determine levels of fruit set under natural conditions (Table 5.4). The natural fruit set of *L. binervosum* flowers during the 2010/2011 flowering season was 80%. No fruit of *L. binervosum* collected from the natural fruit set study had signs of floral or fruit predation. By comparison 72% and 100% respectively of unmanipulated flowers studied during the 2010/2011 and 2011/2012 flowering season in the glasshouse set fruit.

Fruit set, by plant, for *L. binervosum* growing under natural conditions in the Goolwa saltmarsh also ranged from 50% to 100%. In comparison fruit set, by plant, for unmanipulated *L. binervosum* flowers growing in the glasshouse in the autogamy treatment during the 2010/2011 flowering season ranged from 50% to 100%.

Discussion

Samolus repens

The breeding systems studies for *S. repens* indicated that flowers are unable to self-pollinate, most likely because of herkogamy (the spatial separation of sexual organs) and protandry (where pollen is released prior to the stigma becoming receptive) (pers. obs.), and although flowers can show some fruit set and seed set through geitonogamy, they show a markedly higher fruit set and seed set through xenogamy. These results suggest a level of self-incompatibility for *S. repens*. Starr (1993) found a similar trend; *S. repens* flowers to which self-pollen was added set considerably fewer seeds than flowers that had been cross-pollinated. Natural seed set in the Goolwa saltmarsh, presumably from insect (and possibly wind) pollination, was considerably higher than seed set of fruits in the geitonogamy treatment and not as high as seed set of fruits in the xenogamy treatment in glasshouse experiments. It could be that the growth habit of *S. repens*, whereby several plants grow intertwined with one another, may allow for a mixture of pollen from both the same plant and different plants to be deposited onto stigmas by insect visitors.

Table 5.4 Fruit set, calculated as the percentage of flowers that develop into fruits with a seed, for *Limonium binervosum* flowers in the glasshouse by treatment and in the Goolwa saltmarsh. Note: as *Limonium binervosum* is male sterile, no pollen was available to use in geitonogamy or xenogamy treatments. *n* indicates the number of flowers which set fruit out of the total number of flowers included in each treatment.

	2010/2011	2011/2012	Combined
All glasshouse treatments combined	81%	100%	*n* = 279/342
Autogamy	72%	100%	*n* = 83/113
Agamospermy	85%	100%	*n* = 196/229
Natural seed set: Goolwa	80%	Not recorded	N/A

Numerous studies, such as those by Gutierrez *et al.* (2006) and Hirayama *et al*. (2005), have found an increased fruit set and seed set when cross pollen was added as opposed to self-pollen. Vaughton *et al*. (2010) found that the seed set of individuals of *Cyrtanthus breviflorus* hand pollinated with self-pollen, was significantly reduced by 80% and 67% when compared to individuals hand pollinated with cross-pollen and open pollinated flowers, respectively (Vaughton *et al.* 2010). It was thought that these differences in seed set were due to late-acting self-incompatibility.

In *S. repens*, the significant reduction in fruit set and seed set of self-pollinated flowers relative to cross-pollinated flowers, would be consistent with a self-incompatibility system (Hirayama *et al.* 2005), but could also reflect inbreeding depression. The low numbers of fruit and seed set through self-pollen addition may be due to a partial breakdown in a self-incompatibility system, as reported for *Inula royleana* by Shabir *et al*. (2015).

None of the *S. repens* flowers left unmanipulated in the glasshouse (autogamy treatment) set fruit. For flowers growing under natural conditions in the Goolwa saltmarsh, seed set seemed relatively low for many of the fruits, with a mean of 16.11, but with a maximum number of seeds per fruit reaching 51, suggesting that seed set could be increased given more adequate pollen loads. Pollinator observations, discussed in Chapter two, revealed that *S. repens* shared nearly all of its pollinators with the other plant species included in the study. In addition, none of the three main insect floral visitors preferentially visited *S. repens* flowers and increased floral densities of both conspecific and heterospecific flowers negatively affected per-flower visitation rates to *S. repens*.

When pollen limitation experiments were conducted for *S. repens* (Chapter three), pollen addition treatments increased mean seed set to 37.09 seeds per fruit during the 2010/2011 late experiment. These experiments showed that pollen limitation was taking place over a number of flowering seasons, possibly due to insufficient visitation, which has been shown to reduce fruit and seed set. The fact that fruit and seed set in *S. repens* were considerably reduced for flowers that had been self-pollinated compared to those that had been cross-pollinated in glasshouse trials makes the occurrence of pollen limitation more concerning. For species which are self-compatible, even when visitation and pollen receipt are reduced, the ability to self-pollinate can alleviate these changes (Dietzsch *et al.* 2011). For *S. repens,* the further spread of *Limonium* plants is likely to lead to competition for insect visitors, and hence increased levels of pollen limitation may occur which would likely reduce the reproductive success of this species which exhibits reduced fruit and seed set from self-pollination relative to cross pollination.

Limonium hyblaeum agg. and *Limonium binervosum*

The results of this study suggest that both *L. hyblaeum* agg. and *L. binervosum* are capable of apomixis (agamospermy) in the South Australian populations studied, not a surprising result given that apomixes is common within the genus. Lledo *et al*. (2005) believed that species of *Limonium* are commonly apomictic, which could have proved important in the expansion of *Limonium* throughout its range. Lledo *et al*. (2015) also indicated that reproductive strategies such as agamospermy, present across the genus *Limonium,* can make it difficult to identify species, a theory also expressed by Cowan *et al*. (1998).

Previous studies examining the presence of pollen in plants of *Limonium* species have concluded that plants in many populations of agamospermous *Limonium* have no mature or viable pollen; however, some populations of the species *L. binervosum* group may still have normal seed production (Cowan *et al.* 1998). An earlier study by Ingrouille and Stace (1985) found that *L. binervosum* in the British Isles was obligately agamospermous. This lack of mature/ viable pollen is unusual in other species of *Limonium* outside of the *L. binervosum* aggregate. This makes for an interesting interaction between *L. binervosum*, native plant species and insect visitors present at the Goolwa saltmarsh, as no *L. binervosum* pollen is likely to be added to the network.

Rodrigo *et al*. (2012) investigated whether apomixis was present in *L. hyblaeum* and found that in their study site at Port Fairy, Victoria, in the south east of Australia; *L. hyblaeum* had one flower type, with self-incompatible pollen. They also found that even when flowers were bagged to prevent cross-fertilization, seeds were still produced and they concluded on the basis of this, and on the monomorphic and self-incompatible flower type, that this *L. hyblaeum* population in Victoria was apomictic (Rodrigo *et al.* 2012).

When examining natural fruit set for plants from the *L. hyblaeum* agg. and *L. binervosum*, similar results were observed for both the natural fruit set at Goolwa saltmarsh and fruit set in glasshouse experiments for *L. binervosum;* however, for the *L. hyblaeum* agg. fruit set was quite reduced in the Goolwa saltmarsh when compared to fruit set in the glasshouse. This difference may be due to variation across the Goolwa saltmarsh in water availability having a greater effect on plants of the *L. hyblaeum* agg. than on plants of *L. binervosum*, or may simply be due to within-species variation. Even if fruit set is reduced in the Goolwa saltmarsh, given that these species are able to produce seed via apomixis, it would potentially only take one seed to germinate in a new range for the species to expand its range further (Rodrigo *et al.* 2012).

None of the flowers/ fruits from the natural fruit set studies for *Limonium* showed signs of floral or fruit predation, although *Samolus repens* had 28% of its flowers damaged by insects in the same year (2010/2011). This finding is consistent with the enemy release hypothesis, which states that introduced species can gain a competitive advantage in new ranges because they are released from natural predators (Kirichenko *et al.* 2013; Lawton *et al.* 1986; Wolfe 2002; Yang *et al.* 2014). In Europe, *Limonium* suffers from attack by aphids, species of weevils are confined to *Limonium,* and *Limonium* leaves are often eaten by moth larvae (Packham and Willis 1997). Khan *et al*. (2012) found that plants of *Limonium barceloi* were attacked by two seed predators, a moth species and an ant species, and this predation affected both fruit and seed numbers. The enemy release hypothesis predicts that the insect herbivores that would naturally have consumed *Limonium* flowers and/ or seeds may not be present in the Goolwa saltmarsh, and that none of the native insect species that are present utilise this novel resource. Wolfe (2002) studied rates of predation in *Silene latifolia* in both its native range and introduced range. In terms of flower herbivory and fruit predation, individual plants were 17 times more likely to be attacked in their native ranges than in their invaded ranges (Wolfe 2002). The relatedness of the introduced plant species to native species, the geographic distance between ranges and the amount of time since introduction into the new range can all influence the strength of the enemy release hypothesis (Yang *et al.* 2014). At the Goolwa saltmarsh, the introduced *Limonium* is not closely related to any of the native plant species, with significant distances, across continents between the old and new range. A significant time has elapsed, since the introduction of *Limonium* in the south-east of Adelaide, for local insects and herbivores to discover the new resource (AVH 2014). These three ecological factors suggest the applicability of the enemy release hypothesis with regard to *Limonium* in South Australia. An important feature of many successfully introduced plant species is their ability to produce large numbers of small seeds (Ghazoul 2002; Rejmánek and Richardson 1996; Rodrigo *et al.* 2012), and in the case of the introduced *Limonium*, additionally these taxa are able to produce seeds via apomixis, regardless of pollinator visitation. Archbald and Boyer (2009) suggested that *L. ramosissimum* could produce around 10,000 seeds per m^2 of soil. Apomixis and the potential large number of small seeds produced, combined with an apparent lack of floral/seed predators, means that *Limonium* is likely to pose a significant threat to the local plant communities that they invade in South Australia as suggested for *Limonium* in other regions (Adair 2012).

These *Limonium* species have the potential to cause serious damage across their invasive ranges and this study can help scientists to understand their reproductive biology, and hence their potential spread. Management should focus on *Limonium* density in established populations (see Chapter four for details), reducing density to low or medium density, at most, and removal of new populations. Hand removal of plants, especially young plants, is relatively easy (pers. obs.; Rodrigo *et al*. 2012) and should be the focus for new populations, where the *Limonium* population can be removed entirely, before seed production can aid in range expansion.

Conclusion

The results of this study are very likely to be of practical conservation significance, since saltmarsh habitats are likely to be substantially altered in the future as a result of coastal development, soil salinisation, introduced plant species and climatic warming. The understanding of breeding systems and floral biologies that was gained in this study should enhance our ability to predict what changes may take place, what species will be threatened by such changes and what species are most likely to persist. *Samolus repens* was found to set no seed in the glasshouse autogamy treatment and to set much reduced fruit and seed set for self-pollinated flowers relative to cross-pollinated flowers, and so is heavily dependent on insect visitors for pollination. Also, at the Goolwa saltmarsh, where S. repens co-occurs with the introduced *Limonium* species, *S. repens* had a high natural fruit set, similar to that of the xenogamy treatment in the glasshouse, but with apparently low levels of seed set relative to this xenogamy treatment. Thus, the breeding system of *S. repens* has the potential to influence population numbers of this species and should be taken into consideration for future management decisions. The observation that plants of the *L. hyblaeum* agg. and *L. binervosum* both appear to be capable of reproducing via apomixis, at least in parts of their introduced ranges in South Australian saltmarshes, suggests large potential for further spread as insect visitors may not be required for reproduction. Of additional concern, is that fact that no *Limonium* flowers included in the study showed signs of floral or fruit predation, which is another factor which favours the expansion of this introduced genus. These introduced *Limonium* species should be recognised as a major threat to saltmarshes and other coastal communities across southern Australia, and immediate action should be taken to prevent further spread and invasion.

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6. General discussion

Saltmarshes are known to be extremely important ecosystems for many reasons, yet have remained understudied and poorly understood (Adam 1990; Adam 2009a; Foster *et al.* 2013), especially with regard to the reproductive biology of plant species and the interactions between plant species and their insect pollinators (Mackay and Whalen 2009). Although saltmarshes in southern Australia have greater plant diversity than more northerly saltmarshes (Adam 2009a; Saintilan and Rogers 2013), they are still quite species-poor in comparison to other plant communities. This may have contributed to the lack of research on saltmarshes, along with their apparent unattractiveness as study sites and the lack of land occupied by saltmarshes both locally and globally (Adam 1990; Chapman 1974).

The study aimed to provide insight into and understanding of the impact that the introduced plant genus *Limonium* P Miller is having on the ecology and reproductive biology of the two common, co-occurring, insect pollinated plant species at the Goolwa saltmarsh and Port Elliott cliffs, *Frankenia pauciflora* DC. var. *gunnii* Summerh. (Frankeniaceae) and *Samolus repens* (J.R.Forst. & G.Forst.) Pers. (Primulaceae). To optimise conservation efforts in saltmarshes, and maintain best practices in management, there must be an understanding of the biology and ecology of the plant species present (Sommerville *et al.* 2012). This study has added to this knowledge, not only for the two native species *F. pauciflora* and *S. repens*, but also for the introduced genus *Limonium*.

Overview

When looking at the results of the different experiments conducted over the course of this study and other studies examining the impacts of *Limonium*, it can be concluded that *Limonium* has the potential to seriously alter the composition and diversity of saltmarshes that it may invade. Adair (2012) labelled *Limonium hyblaeum* as a catastrophic invader, and described it as a major threat to the valuable and unique halophytic communities which it invades, sentiments which this study has confirmed for *L. binervosum* and *L. hyblaeum* agg. at the Goolwa saltmarsh and Port Elliot cliffs.

Limonium is now known to share a flowering period with *F. pauciflora* and *S. repens* and was shown to share many insect floral visitors with these co-occurring native plant species. Pollen limitation was found to be present over multiple flowering seasons for the two native plant species, and density studies conducted at two sites over multiple years concluded that at high densities, *Limonium* was exhibiting competitive effects on *F. pauciflora*. When breeding systems were examined, both *Limonium* species examined were found to be capable of producing apomictic seeds, whilst *S. repens* was found to be self-incompatible (previous breeding system studies on *F. pauciflora* have shown a similar pattern in this species).

The integration of *Limonium* into the saltmarshes studied however, seems to have affected *F. pauciflora* and *S. repens* differently, despite the fact that the two native species share many similarities, with *S. repens* being more adversely affected than *F. pauciflora* with regard to attracting insect visitors. Both *F. pauciflora* and *Limonium* were found to have competitive interactions with *S. repens*, with syrphids and lycaenid butterflies actively preferring flowers of *F. pauciflora* whilst no common floral visiting insects preferred flowers of *S. repens* over those of the other two taxa.

Samolus repens does not seem to be more attractive to floral visitors than *F. pauciflora* and plants tend to favour areas with high soil moisture. Breeding system studies indicated that flowers of *S. repens* are unable to self-pollinate because of herkogamy and protandry, and although they can exhibit low levels of fruit set and seed set through geitonogamy, have a significantly increased fruit set and seed set through xenogamy. Thus, although *S. repens* is dependent on insect visitors to serve as pollinators, it is not preferentially selected by any of the three main insect visitors within the Goolwa saltmarsh when growing together with flowers of *F. pauciflora* or *Limonium*.

Floral densities, of both native and introduced plant species, have the potential to influence the interactions between flowers and their visitors (Essenberg 2013; Feldman *et al.* 2004; Grabas and Laverty 1999; Kitamoto *et al.* 2008; Muñoz and Cavieres 2008). Studies by Grabas and Laverty (1999), Flanagan *et al.* (2010) and a meta-analysis on the impacts of introduced plant species by Morales and Traveset (2009) confirm that the competitive effects of introduced plant species may increase with their density. These findings, as well as those in this study, suggest that when introduced plant species occur at high densities, competition is more likely to take place than facilitation. This is an important result, especially when trying to maximise conservation efforts. If land managers can reduce densities of introduced species, then not only will they dramatically reduce the competitive effects of the introduced plant species, but they can also allow any facilitative effects to continue taking place. This will then capitalise on species diversity, and still allow for the novel interactions and functions which have developed since the introduction of new species.

Both *F. pauciflora* and *S. repens* are pollen limited at times and are likely to be resource limited at other times. Over several flowering seasons, *S. repens* and *F. pauciflora* were pollen limited in both fruit set and seed set. The earlier experiments for both species failed to show a significant effect of pollen addition, likely due to the presence of resource limitation, as a result of the Millennium Drought, so regardless of how much pollen was received, environmental factors such as a lack of water and increased soil salinity, may have outweighed any potential benefits of pollen receipt. When rainfall returned to average levels in 2009/2010 and even above average levels during the 2010/2011 flowering season, pollen limitation was observed for both species in both fruit and seed set.

Differences between outcomes for fruit set and seed set through the addition of pollen certainly indicated that pollen limitation affects both components of reproduction in *S. repens* and *F. pauciflora.* Burd (1994) found that fruit set typically showed a greater response to pollen limitation than seed set. The optimal allocation of floral resources among seeds and fruits can depend on the resources available to individual flowers or inflorescences (Wesselingh 2007) and may also be influenced by levels and patterns of fruit and seed predation.

When the mean fruit set and seed set of *F. pauciflora* and *S. repens* were examined with regard to the rainfall in the period leading up to the experiments, results suggested that rainfall may have an effect on fruit set and seed set, supporting the idea that the lack of rainfall in the early pollen limitation experiments was responsible for resource limitation, an idea consistent with findings from studies by Fernandez (2012) and Petanidou *et al*. (1998). If predictions of climate change are correct, and the altered hydrology of the River Murray remains, the likelihood of resource limitation from low levels of rainfall is likely to increase.

What are the implications for introduced species?

Limonium has integrated into the saltmarsh and cliff environments included in this study, and whilst it can provide facilitation for native pollinators, at high densities it can reduce the reproductive output of native species. Native insect species have altered their behaviour to include flowers of *Limonium* species in their foraging for pollen and nectar and in some circumstances, the introduced *Limonium* could prove beneficial to the native insect community. Interestingly, insect herbivores have apparently not included *Limonium* fruits or seeds into their diets, with no signs of fruit or seed predation within the study sites.

It is important to remember though, that whilst a reduction in floral visitors to the two native species as a result of competition with *Limonium* may lead to a reduction in their reproductive output, the *Limonium* species included in this study were capable of producing apomictic seed, which limits or removes their dependence on floral visitors for reproduction. The results of these breeding system studies and those of Rodrigo *et al.* (2012) in Victoria certainly help to explain how *Limonium* species are such successful invaders across the southeast of Australia, and suggest the potential for their further range expansion. *Limonium* plants produce hundreds of flowers per plant, per season, and their ability to achieve maximal levels of seed set through apomixis poses a threat to native plant communities in southern

Australian saltmarshes, especially since a single seed can potentially create an entirely new population (Rodrigo *et al.* 2012).

One of the goals of conservation biology and invasion biology is to determine characteristics which make introduced plant species successful when expanding into new ranges (Daehler 2003; Platt and Jeschke 2014; Richardson and Pyšek 2006; van Kleunen and Richardson 2007). Pyšek *et al.* (2011) concluded that if an introduced plant species self-pollinates, it will not only be quicker at expanding its range, but it will also naturalise faster than introduced plant species that are reliant on insect pollinators. Pyšek *et al.* (2011) recognized that the most successful introduced plant species tended to have the same pollination modes as the native species, allowing the introduced plant species to enter already existing pollinator networks, and utilise the same insect visitors (Pyšek *et al.* 2011). This was found to be the case with the introduced *Limonium* taxa in this study, although it is now known that they do not depend on insect visitors, as they can reproduce through apomixis. Regardless of visitation rates to flowers of the *L. hyblaeum* agg. and *L. binervosum*, fruit set can reach 100%. Not only do these *Limonium* taxa have the ability to attract pollinators from the native plants, but they are also likely to have 100% fruit set, potentially flooding the communities they inhabit with large quantities of seeds. To further determine the impact of introduced species of *Limonium* on invaded communities in South Australia, seed bank and demographic studies would be useful in determining whether such high levels of fruit set result in high rates of seed germination and seedling survival. Boorman (1968) commented on the very low recruitment of saltmarsh plant species and Daehler (2003) warned that introduced plant species can use their high seed production to aid in their establishment.

It is interesting to note that none of the flowers from the natural fruit set studies for *Limonium* showed signs of seed or fruit predation, although 28% of flowers of *S. repens* examined that same year, were predated by insects. The lack of floral/fruit predation in *Limonium* is consistent with the enemy release hypothesis, which states that introduced plant species can gain a competitive advantage in their new ranges because their natural predators are not present (Kirichenko *et al.* 2013; Lawton *et al.* 1986; Platt and Jeschke 2014; Wolfe 2002; Yang *et al.* 2014).

Novel ecosystems

Novel ecosystems, also known as emerging ecosystems, occur when new interactions take place between flora and fauna, that had not existed together prior to human interference (Hobbs *et al.* 2006; Lindenmayer *et al.* 2008). A growing consensus amongst conservation biologists is that rather than try and return these ecosystems back to a natural or previous state, we should simply try to maximise genetic and species diversity and maintain the presence of favourable species (Seastedt *et al.* 2008). One of the main arguments in favour of the novel ecosystem approach is that there is no true consensus on what is considered a ‘natural’ environment (Hobbs *et al.* 2009). Another issue with current conservation efforts is the very high level of integration that takes place when introduced species enter new environments. It is often extremely difficult for conservationists and land managers to effectively remove introduced species and research has now shown that in certain situations, introduced species are actually having a facilitative effect in the communities that they are invading (Hobbs *et al.* 2009), a finding consistent with the results of this study. An example of a novel ecosystem with introduced species facilitating native species can be found in California, with native butterflies utilizing introduced plant species for their ovipositing and feeding (Graves and

Shapiro 2003). Graves and Shapiro (2003) found that 34% of the butterfly species surveyed were recorded either ovipositing on introduced plant species, using the flowers of introduced plant species for nectar or had larvae feeding on the vegetation of introduced plant species. It would appear that introduced plant species have integrated into this community and are providing additional resources to butterfly species (Graves and Shapiro 2003). Studies of the impacts of introduced plant species on the communities that they have invaded can help to determine whether or not facilitative or competitive effects are exerted on the native species and hence whether the novel ecosystem approach may be useful in the management and planning of conservation in that community.

Examination of the interactions between the introduced plant genus *Limonium* and the native insect-pollinated *F. pauciflora* and *S. repens* and their pollinator networks in this study has shown how introduced plant species can become integrated into local communities. In the studied saltmarshes, *Limonium* was found to facilitate the reproduction of one of these native plant species, as long as its populations remain at low densities. Thus, removal of the introduced plant species may only be necessary if densities become too high, causing a shift from facilitation to competition, and this must be taken into consideration when preparing management strategies. Adam (2009b) calls into question the removal of introduced plant species in saltmarshes without first assessing if they are actually causing harm to the community and whether or not their removal and control will actually be successful, concluding that whilst new infestations and new species introductions should be prevented and controlled, existing introductions should be managed cautiously and conservation efforts may need to be revisited.

The removal of introduced plant species is discussed in the Best Practice Guidelines for Coastal Saltmarsh, published in 2008 by the Department of Environment and Climate Change NSW. These guidelines discuss the removal of the introduced plant *Juncus acutus*; however, nowhere do they discuss any possible resource or connectivity lost through the removal of *J. acutus*. The guidelines recommend that the bulk of the weeds be removed section by section; however, this does not take into account the possibility that *J. acutus* may have integrated with the surrounding flora and fauna, and that other species may now be utilising this new resource. Given the growing number and range of introduced species and the limited funds available for their management, it is imperative that these relationships are assessed to maximise conservation efforts.

This context-dependent view of management corresponds with the novel ecosystem approach where a shift is made in conservation efforts. Novel ecosystems are likely to become more and more common not only as human interference causes introduced flora and fauna to move into new and expanded ranges, but as the climate changes due to an accelerated greenhouse effect (Hobbs *et al*. 2009). A shift from the “native vs. introduced” debate to a novel ecosystem approach could prove beneficial both environmentally, by maintaining species diversity and ecosystem functioning, and economically, as funds for conservation could be spent in the most efficient manner possible, creating the most environmentally sound management practices.

Implications of altered hydrology

The hydrology of the River Murray and its tributaries has been significantly altered by substantial reductions in flows (now around half of naturally occurring levels) as well as reductions in flow variability, i.e. year-round constant water releases and flows and reductions in flood events and volumes (Gawne *et al.* 2011; Gippel and Blackham 2002). Before the construction of weirs and barrages and the removal of water for human consumption (including agricultural uses), peak flows typically took place during spring before reducing in volume to lower flows during late summer/ early autumn, but due to the capture of water for use during the drier months, there is now minimal variation across the months (Murray-Darling Freshwater Research Centre 2013), low to medium scale flooding has been eliminated completely and the River Murray mouth is now closed 40% of the time, as opposed to historical occurrences of 1% of the time (Department of the Environment, Water, Heritage and the Arts 2010; Gawne *et al*. 2011; Gippel and Blackham 2002). An assessment was done on the condition of the Macquarie Marshes, a site on the lower Macquarie River, which feeds into the Darling River. The assessment found that the greatest threat to the marshes was the altered hydrology of the river, mainly the changes to flow patterns and water extraction, which have influenced the magnitude and composition of the marshes, which has then gone on to affect the breeding patterns of birds and fish that utilise the area (Gawne *et al.* 2011). An assessment was also completed on the Coorong and Murray Mouth, a RAMSAR wetland of significance (Department of the Environment, Water, Heritage and the Arts 2010), an area including the Goolwa saltmarsh. The main threats to the area were reduced flows and the change in occurrence and magnitude of flood events, a problem intensified by drought conditions (Gawne *et al*. 2011). Reduced flows have caused increased salinity levels, both in water and soil (Gawne *et al*. 2011). There have been drastically reduced river flows into the Coorong and Lower Lakes and the lack of flushing of the Murray Mouth has also contributed to increased salinity levels, with salinity levels so great that severe and significant effects have been suffered physically and ecologically, with reductions in species diversity across the area common (Gawne *et al.* 2011; Department for Environment and Heritage 2010).

In recent years, infrastructure throughout the Murray-Darling catchment has been improved to reduce water loss through evaporation in irrigation systems through a series of pipeline installations and projects have been undertaken to re-allocate water for bioremediation (Department for Environment and Heritage 2010; Swirepik *et al.* 2015); however, although steps have been taken to improve the health and functioning of the River Murray and its flows, without long-term increases in water being released through the mouth, the system can only be improved so far (Murray-Darling Basin Authority 2011). The increased flows after the Millennium Drought broke, along with the arrival of the flood waters from upstream, showed the effect that flushing and increased flows can have on the health of the environment along the path of the River Murray. Increased, un-regulated flows will allow for wetlands and floodplains to be inundated, vegetation and wetland health will improve and ecological systems and processes can be maintained (Murray-Darling Basin Authority 2016). Since the end of the Millennium Drought, there have been improved flows through the River Murray, which have re-opened the River Murray mouth, fish and bird populations have increased and salinity levels across the lower River Murray and lakes have decreased (Murray-Darling Basin Authority 2016).

However the long-term impacts of climate change, such as a decrease in annual rainfall, decrease in freshwater resources and an increase in drought frequency

(Bureau of Meteorology and Commonwealth Scientific and Industrial Research Organisation 2014; Neave *et al.* 2015), could prove an insurmountable challenge, making the future of the River Murray and its associated ecosystems and habitats uncertain. The challenge will be to manage and maintain environmental processes, ecosystem functioning and biodiversity with a sound scientific basis, whilst continuing the extraction of high water volumes for human use. The variation in the outcomes of the pollen limitation experiments between the 2007/2008 Millennium Drought and subsequently when rainfall and river flows had increased indicates that flow and salinity levels can have a marked influence on the reproductive biology of saltmarsh plants and their pollination ecology.

Comparison between Goolwa and Port Elliott

Although the Goolwa saltmarsh and Port Elliott cliffs differ in habitat and ecology, they both have harsh environmental conditions and share plant species. The most striking difference between the plant communities occurring in the Goolwa saltmarsh and the Port Elliott cliffs is the size and growth habit of the two native species, *F. pauciflora* and *S. repens*, probably because of the harsher environmental conditions present at the cliff community at Port Elliott. The phenology studies also revealed that *F. pauciflora* had between 30 and 60 open flowers per plant at the peak of flowering at Goolwa; however, at Port Elliott, *F. pauciflora* peaked at less than five open flowers per plant across the different flowering seasons. Plants of *F. pauciflora* were smaller at the Port Elliott cliffs, and most were found growing close to the ground, instead of growing as upright bushes and shrubs as at Goolwa (Fig. 1.10). *Samolus repens* had fewer flowers per plant than *F. pauciflora* and showed similar flowering peaks at both Goolwa and Port Elliott, with only one to five open flowers per plant; however, plants tended to be larger at Goolwa (Fig. 1.12). This difference in plant size is possibly due to *S. repens* tending to favour areas with higher soil moisture than *F. pauciflora*, so whilst *F. pauciflora* may be more restricted by wind speeds and exposure at Port Elliott, *S. repens* can only grow in wetter areas at either site. Interestingly, *Limonium* showed similar flower numbers at both sites, seemingly unaffected by the extreme conditions at the Port Elliott cliffs.

All three of the most common insect visitors, syrphids (Syrphidae), *Z. labradus* (Lycaenidae) and *A. mellifera* (Apidae), were present at both sites; however, the reduced floral resources at Port Elliott may not be able to maintain the same insect communities, as the floral resources at Goolwa, as there was less diversity in insect visitors. A striking difference between the main pollen carriers at Goolwa and Port Elliott was that only 31% of all insects caught at Goolwa carried pollen whilst at Port Elliott, 67% of all insects caught carried pollen. The insects carrying the most pollen at Goolwa were hymenopterans from the families Bombyliidae, Pompilidae and Stratiomyidae whilst at Port Elliott, *A. mellifera* (Apidae) and *Z. labradus* (Lycaenidae) carried the most pollen. It may be that because of the harsher and windier environmental conditions at Port Elliott, insects reduce the time they spend in flight and spend more time on flowers and thus collect more pollen than they do at Goolwa.

Future directions

Saltmarshes are generally understudied, and the reproductive biology of saltmarsh plants and their pollination networks are particularly neglected. Further work should be conducted on the reproductive biology of saltmarsh plants in other saltmarshes across South Australia and Victoria. Seed bank and demographic studies are also lacking for saltmarsh communities, and now with the knowledge that the introduced *Limonium* species can reproduce apomicticly at Goolwa, Torrens Island and Port Elliott, it would be useful to learn about how the seed set of the different plant species in these communities is reflected in the composition of the soil seed banks and in the diversity and abundance of germinating plants.

Concerning the introduced genus *Limonium*, monitoring should be started to quantify the current range and to identify further range expansions and invasions. Research should be conducted on the identity of the different *Limonium* taxa that are present across south-east Australia, focussing on their reproductive biologies, especially for members of the *L. hyblaeum* aggregate.

Further monitoring and research on the effects of the altered hydrology along the River Murray on the Goolwa saltmarsh and its vegetation need to be conducted. This study found that flushing had a positive effect on the saltmarsh, and this needs to be further examined for the long-term health and management of the saltmarsh.

Climate change is also likely to be responsible for future changes in vegetation composition and growth of saltmarshes, (Prahalad *et al.* 2011). Some work has been done looking at the effects and potential effects of climate change on the ability of saltmarshes to act as carbon pools (Kirwan and Mudd 2012), yet little work has been done on the possible effects on the composition of plant communities (Prahalad *et al.* 2011) or the potential effects of climate change on the breeding systems, reproductive and pollination biologies of saltmarsh native plant species. These interactions between plants, pollinators and environmental variables require further study to help mitigate the effects of climate change. Studies looking at the fauna of saltmarshes, especially invertebrate communities, are rare, and the invertebrate fauna present in saltmarshes is largely unknown (Adam 1995). Adam (1995) urged that further research was necessary not only on saltmarsh ecology in general, but on the plant-pollinator interactions in saltmarshes.

Conclusion

This study is novel in examining, over multiple seasons and at multiple sites, the reproductive biology and success of native and introduced insect-pollinated saltmarsh plants in South Australian saltmarshes. The introduced genus *Limonium* has become integrated into the plant communities of all three of the study sites, and whilst at high density is proving competitive, has been found to be have facilitative effects on native plant species at low and medium densities. It may therefore be considered to play a role in the ecosystem functioning at these sites, with management efforts needing to reflect this fact.

This study has greatly added to our knowledge of the reproduction and pollination of saltmarsh plant species in South Australia and of the impact of the introduced *Limonium* on the reproductive biologies of the native *F. pauciflora* and *S. repens.* The study also suggested possible effects of variation in rainfall and river flows arising from the Millennium Drought on the reproductive biologies of these species. These findings should assist conservation efforts by providing crucial information about the breeding systems of the introduced *L. binervosum* and *L. hyblaeum* agg, and by revealing the integration of these introduced species into the Goolwa and Torrens Island saltmarshes, and Port Elliott cliffs, highlighting the importance that plant and floral densities have on these interactions.

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