

# Living on the edge: ecological and evolutionary mechanisms of range expansion in invasive species

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## **Declaration**

This work has not previously been submitted for a degree or diploma in any other university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself. All people that have contributed to this work have been given credit. No ethics approval was required for this work to be undertaken.

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*“If it’s worth caring about, no matter how impossible you think it is, you take the shot.”*

— Clint Barton (Hawkeye), Marvel Comics

## **Table of contents**

Acknowledgements	<b>iv</b>
General abstract	<b>vi</b>
CHAPTER 1: Introduction	
Background	<b>10</b>
Thesis scope and structure	<b>17</b>
References	<b>20</b>
CHAPTER 2: Does enemy damage vary across the range of exotic plant species? Evidence from two coastal dune plant species in eastern Australia	
Abstract	<b>27</b>
Introduction	<b>28</b>
Materials and methods	<b>31</b>
Results	<b>35</b>
Discussion	<b>40</b>
References	<b>44</b>
Appendices	<b>47</b>
CHAPTER 3: Mixed evidence for shifts to faster carbon capture strategies towards range edges of two coastal invasive plants in eastern Australia	
Abstract	<b>51</b>
Introduction	<b>53</b>
Materials and methods	<b>56</b>
Results	<b>61</b>
Discussion	<b>69</b>
References	<b>75</b>
Appendices	<b>79</b>

**CHAPTER 4: Have your cake and eat it too: greater dispersal ability and faster germination towards range edges of an invasive plant species in eastern Australia**

Abstract	<b>81</b>
Introduction	<b>82</b>
Materials and methods	<b>85</b>
Results	<b>89</b>
Discussion	<b>96</b>
References	<b>101</b>
Appendices	<b>105</b>

**CHAPTER 5: It doesn't take two to tango: increased capacity for self-fertilisation towards range edges of two coastal invasive plant species in eastern Australia**

Abstract	<b>107</b>
Introduction	<b>108</b>
Materials and methods	<b>111</b>
Results	<b>117</b>
Discussion	<b>121</b>
References	<b>126</b>
Appendices	<b>131</b>

**CHAPTER 6: Discussion**

General summary	<b>136</b>
Are dune systems good models for species' range expansions?	<b>141</b>
Range shifts and climate change: what can and can't we learn from invasions?	<b>143</b>
Concluding remarks	<b>145</b>
References	<b>147</b>

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## General abstract

Despite ongoing research into traits of successful invasive species, few studies have examined how selection on such traits change during the course of an invasion. This is despite increasing recognition that populations in the invaded range are generally not at equilibrium and many invasive species are still undergoing range expansion. As a species expands in its range from its range core where it was first introduced, populations at the edge of the range may experience different ecological (e.g. fewer enemies) and evolutionary conditions (i.e. selection for increased dispersal, growth and reproduction). Changes in these conditions towards range edges may facilitate continual range expansion and examining how such selective pressures vary across the range of invasive species may increase our understanding of factors determining species' ranges. This thesis explores the ecological and evolutionary mechanisms of range expansion in exotic invasive species using two coastal exotic dune plants (*Gladiolus gueinzii* Kunze (Iridaceae) and *Hydrocotyle bonariensis* Lam. (Apiaceae)) occurring along eastern Australia. In particular, it examines variation in enemy attack across ranges (Chapter 2) as well as selection for increased growth (Chapter 3), dispersal (Chapter 4) and reproduction (Chapter 5) towards range edges to facilitate further range expansion. Both *H. bonariensis* and *G. gueinzii* did not show consistent responses in terms of enemy release and trait shifts towards range edges. *Hydrocotyle bonariensis* experienced increased enemy attack and growth towards range edges however there were no such differences towards range edges for *G. gueinzii*. These inconsistent responses highlight the complexity of understanding factors influencing range expansion within and across species and provide a rich avenue for continued research.



# **1 | Introduction**

I, Samiya Tabassum, conducted the literature review and wrote this chapter.  
Michelle Leishman and Anthony Manea provided comments.

## Background

An invasive non-native species can be defined as a species that has been introduced (deliberately or otherwise) to an area outside its native range and has formed self-sustaining populations and spread from its area of initial introduction (Richardson et al. 2000; Blackwell et al. 2011). Increasing globalisation and trade have led to an exponential increase in the number of species introductions into new regions (Hulme 2009). Invasive species have widely been reported to have detrimental impacts on their recipient communities, with many posing a significant threat to biodiversity and ecosystem functioning (Clavero & García 2005; Molnar et al. 2008; Hedja et al. 2009). Due to the devastating impacts of invasive species, a key research direction in invasion biology has been to find ways to prevent invasive species becoming a threat (Kolar & Lodge 2001). In 1974, Herbert Baker published a seminal paper describing several characteristics pertaining to the ‘ideal’ invasive species. Traits characteristic of such species include having mixed reproductive strategies, short time to sexual maturity and high phenotypic plasticity (Baker 1974). Although much work has been dedicated to ascertaining a broad suite of traits to predict when a species may become invasive, a general consensus is still lacking (Sakai et al. 2001; Moles et al. 2008).

One reason for this lack of consensus may be that ecological and evolutionary processes acting on species may change as the invasion proceeds (Dietz & Edwards 2006). Most studies in invasion biology assume that ecological conditions in the invaded range are relatively benign beyond those encountered upon first introduction (Phillips et al. 2010, but see Dlugosch et al. 2015). When a species is first introduced into a novel environment, it may experience an initial rapid increase in population size due to factors such as release from natural enemies, availability of niche space and human facilitation (Dietz & Edwards

2006). As the abundance of the introduced species increases, populations at the edge of the range may experience selection for continual spread due to fitness advantages associated with a lower density of conspecifics (Phillips et al. 2010), increased availability of resources beyond the range (Carol et al. 2009; Brown et al. 2013; Houston et al. 2013) and avoidance of kin competition (Kubisch et al. 2013; Van Petegem et al. 2018). As a species continues to expand its range, populations at the edge of the range may experience different ecological and evolutionary conditions compared to populations near the initial point of introduction. For example, towards expanding range edges, traits that enhance colonisation ability such as those associated with increased dispersal (Phillips et al. 2006; Burton et al. 2010), growth (Siemann & Rogers 2001; Burton et al. 2010; Kilkenny & Galloway 2013) and interspecific competitive ability (Lankau et al. 2009) should be selected for to facilitate further range expansion. In contrast, populations nearer to the initial point of introduction are older and more likely to experience greater intraspecific competition compared to range edge populations (Evans et al. 2013). Thus traits enhancing intraspecific competitive ability are expected to be favoured as populations become older and heavily colonised (Huang & Peng 2016).

Few studies have investigated how ecological and evolutionary processes vary across the invaded range of introduced species (Gaston 2009; Lankau et al. 2009; Kilkenny & Galloway 2013). This is an aspect of invasion biology that has received relatively little attention until recently. In fact, a recent horizon scan examining current and future issues in weed management identified the role of evolution within the invaded range as a priority research question (Neve et al. 2018). Findings of such research have important implications for not only understanding range expansion of invasive species, but also for understanding range

contractions of endangered species and range shifts due to climate change (Hargreaves & Eckert 2014; Chuang & Peterson 2016). As little is known about how factors influencing invasive species change over the course of an invasion, gaining a better understanding of the ecological and evolutionary processes governing range expansion may shed light on how species are able to rapidly expand their range.

Release from natural enemies is often cited as a key ecological factor driving the success of invasive species in novel environments. The enemy release hypothesis posits that when a species is introduced into a novel environment it leaves behind many of its co-evolved enemies (parasites, herbivores, pathogens, etc.) in its native range (Keane & Crawley 2002; Torchin et al. 2003). However, with time introduced species do eventually accumulate a suite of local enemies in their new ranges (Torchin et al. 2003; Castells et al. 2013; Harvey et al. 2013). A potential factor affecting the acquisition of enemies is associated with the location of populations within the introduced range. A species' abundance is generally greater at the centre of its range and decreases towards the range edges, either as a result of declining habitat quality (abundant centre model, Angert & Schemske 2005; Vaupel & Matthies 2012, but see Saragin & Gaines 2002a, b; Samis & Eckert 2007) or as a product of range expansion as species gradually disperse from their point of introduction/establishment (Fagan & Bishop 2000; Tsai & Manos 2010; Kelehear et al. 2012). This greater abundance of a species at its range centre may facilitate a greater accumulation of enemies compared to at its range edges (Alexander et al. 2007; Kambo & Kotanen 2014). Lower conspecific density in range edge populations potentially reduces apparency of species, thereby decreasing enemy accumulation at range edges (Sletvold &

Grindeland 2008; Vaupel & Matthies 2012; Castagneyrol et al. 2013), potentially allowing invasive species to once again escape from their enemies.

In addition to ecological conditions varying across the invaded range of species, selection for particular traits may also vary in the same manner. Arguably one of the most well studied trait types in the introduced range of species is dispersal traits. The process of range expansion itself leads to the accumulation of the furthest dispersing phenotypes at the range edge each generation, a process termed spatial sorting (Phillips et al. 2010; Shine et al. 2011). These highly dispersive range edge populations will tend to reproduce with each other ('Olympic Village effect', Phillips et al. 2010) and if dispersal ability is heritable then this trait will be passed down to future offspring (Phillips et al. 2010). In addition, individuals at the range edge may experience higher fitness due to lower conspecific density. The combined effect of these two processes will lead to the continual evolution of increased dispersal ability at the range edge (Phillips et al. 2010; Alex Perkins et al. 2013). The cane toad invasion across northern Australia has become an excellent study system with which to investigate changes in dispersal ability across the course of an invasion. Cane toads at the vanguard of the invasion have been found to have longer legs (Phillips et al. 2006), faster locomotion (Phillips et al. 2006), greater activity (Alford et al. 2009; Lindström et al. 2013; Pizzatto et al. 2017), increased path straightness (Lindström et al. 2013; Pizzatto et al. 2017) and bolder personalities (Gruber et al. 2017a, b) compared to older populations near the initial point of introduction. In addition to cane toads, increased dispersal ability towards range edges has been found in a variety of range expanding plants (Cwynar & MacDonald 1987; Huang et al. 2015, but see Bartle et al. 2013), insects (Hill et al. 1999; Hughes et al. 2003; Hassell et al. 2009; Laparie et al. 2013), birds (Duckworth 2008; Berthouly-Salazar et

al. 2012) and fish (Myles-Gonzalez et al. 2015, but see Lopez et al. 2012), emphasising dispersal as a fundamental trait for population spread.

Following dispersal to a site, additional life-history traits may be under selection to promote range expansion. During range expansion, while population density is low at the range edge, traits pertaining to faster growth resulting in shorter time to reproduction are likely to be under strong selection as individuals that can complete the most number of generations in a given amount of time will be selected for (Phillips 2009; Phillips et al. 2010). Faster growing individuals are more likely to be sexually mature at any one time and thus benefit from the arrival of new mates, allowing for faster reproduction and continual expansion of the range front (Chuang & Peterson 2016). Faster growth rates have been found in range edge populations of a variety of invasive taxa (fish (Bøhn et al. 2004; Carol et al. 2009; Feiner et al. 2012), amphibians (Phillips 2009; Brown et al. 2013) and plants (Siemann & Rogers 2001; Siemann et al. 2006; Kambo & Kotanen 2014)), suggesting that faster growth may be a major contributor to facilitating species' range expansion.

Another life-history trait that is expected be under strong selection towards range edges is reproduction. As populations expand in their range, increased allocation to reproductive biomass is expected to occur as a means of significantly increasing colonisation opportunities, hence facilitating further range expansion. Increased allocation to reproductive biomass in frontal populations has been found in a variety of range expanding invasive taxa (urchins (Lester et al. 2007; Ling et al. 2008), gobies (Gutowsky & Fox 2012; Houston et al. 2013; Masson et al. 2016), fish (Lopez et al. 2012) and plants (Kambo & Kotanen 2014)). However, within a given reproductive event, organisms can produce a small

number of large offspring or a large number of small offspring due to resource allocation trade-offs (Westoby et al. 1992). Although offspring with greater maternal provisioning have been shown to be more competitive (Jakobsson & Eriksson 2000, 2003), lower conspecific density towards range edges of invasive taxa is expected to drive selection for increased reproductive rate at the expense of competitive ability (Burton et al. 2010; Alex Perkins et al. 2013).

In terms of reproduction, another trait associated with successful invasions in novel environments (particularly with respect to invasive plants) is the ability to produce offspring through self-fertilisation (Petanidou et al. 2012; but see Atlan et al. 2015). Plants that are able to self-pollinate and self-fertilise are likely to be successful invaders because reproduction is not constrained by reliance on mates and/or pollinators (Baker 1974; Pannell et al. 2015). Increased capacity for uniparental reproduction is beneficial for range edge populations of expanding species where individuals may occur at lower densities (experiencing mate limitation) and also be less apparent to pollinators (Pannell & Barrett 1998; Pannell et al. 2015). Many studies have found increased capacity for self-fertilisation in peripheral populations of native species where local extinction and continual re-colonisation may be occurring (e.g. Busch 2005; Herlihy & Eckert 2005; Darling et al. 2008; Griffin & Willi 2014). However, few studies have examined whether this is a mechanism facilitating range expansion in invasive plants (Colautti et al. 2010).

Despite strong selection for range edge populations to continually evolve faster dispersing and colonising phenotypes, direct trade-offs between key traits may hamper such progress.

For example, trade-offs between dispersal and reproduction, two key traits important for facilitating range expansion, have been found in edge populations of range expanding taxa (insects (Hughes et al. 2003; Simmons & Thomas 2004), arachnids (Van Petegem et al. 2016), fish (Lopez et al. 2012) and amphibians (Hudson et al. 2015)). This phenomenon is not solely confined to biological range expansions but can also be seen in cancer biology – metastatic cancer cells are highly dispersive because they lack contact inhibition for locomotion (Abercrombie 1979) however, this key trait impedes their ability to proliferate (Biddle et al. 2011). Alternatively, traits pertaining to faster range expansion may instead trade-off with traits that increase fitness in high density populations (e.g. competitive ability and enemy defence) such as those found at the range core (Phillips et al. 2010). For example, earlier sexual maturation in range edge populations of an invasive fish species in Europe has been shown to lead to shorter life span (Amundsen et al. 2012) and body size (Bøhn et al. 2004). Furthermore, larger body size and more frequent movements associated with highly dispersive range edge populations of invasive cane toads in Australia has been found to increase susceptibility to pathogens (Brown et al. 2004). Thus, understanding more about the nature of trade-offs in range edge populations may help elucidate potential limits for range expansion.

Identifying the ecological and evolutionary processes that shape species' distributions is a fundamental goal in ecology (Hargreaves & Eckert 2014). Invasive species provide a natural experiment with which to understand such processes as many species have not yet reached equilibrium within their invaded landscapes and are still experiencing range expansion. This thesis examines how invasive species are able to rapidly expand in their range once introduced by exploring the ecological and evolutionary mechanisms underlying such



processes. As the effects of climate change and anthropogenic disturbance intensify, both invasive and native species will likely experience accelerated shifts in their range. Therefore, understanding the mechanisms underpinning species' range distributions may help to formulate more effective conservation and management practices in the face of global change.

### **Thesis scope and structure**

This thesis examines variation in ecological and evolutionary pressures across the range of two exotic invasive coastal dune plant species occurring along the east coast of Australia (*Gladiolus gueinzii* Kunze (Iridaceae) and *Hydrocotyle bonariensis* Lam. (Apiaceae)).

*Gladiolus gueinzii* is a perennial dune plant native to dune systems in South Africa. The first record of *G. gueinzii* in Australia was from the port of Stockton, New South Wales in 1950, suggesting that the species was accidentally introduced through ballast water (Heyligers 1999). This species is solely confined to beach dunes and has become naturalised nearly 800 km along the east coast of Australia, from South West Rocks, New South Wales to Mallacoota, Victoria. *Gladiolus gueinzii* is facultatively sexual, reproducing clonally by cormels (round, buoyant, underground growths) and sexually through self-compatible flowers (Heyligers 1999; Manning et al. 2011). Flowering occurs between October to December, with plants producing a single flower stem containing 1-6 pink flowers that open sequentially (Heyligers 1999). Fruits ripen through the austral summer (December – February), with each fruit producing up to 40 winged seeds (samaras) (Heyligers 1999). This species typically occurs in dense populations close to the high-tide mark, with individuals occurring 20-30 cm apart (Manning et al. 2011).

*Hydrocotyle bonariensis* is a perennial plant native to Africa, North and South America and has become widely naturalised on coastal dunes along eastern Australia. The first record of *H. bonariensis* in Australia was made in 1893 near Botany Bay, New South Wales. Although *H. bonariensis* is primarily found on dune systems, it occasionally occurs along rivers and lagoon outlets (Heyligers 1998). Its distribution in Australia is along the east coast from southern Queensland to Victoria (approximately 1200 km). *Hydrocotyle bonariensis* was accidentally introduced to Australia, possibly through ballast water (Heyligers 2008; Murray & Phillips 2012). It is facultatively sexual, reproducing clonally using rhizomes (modified underground stems) and sexually using flowers. *Hydrocotyle bonariensis* produces compound inflorescences containing hundreds of cream coloured flowers throughout the year, with each flower potentially producing one indehiscent schizocarp containing two seeds (Evans 1992). Roots, leaves and inflorescences arise from nodes along the rhizome which is generally buried in the sand at a depth of 2-5 cm (Knight & Miller 2004). The extensive rhizome systems of *H. bonariensis* can be comprised of over a thousand individual ramets that span many square meters of dune systems (Evans 1991).

Coastal invasive species represent an ideal system with which to study questions pertaining to species' ranges as their range is essentially linear with capacity for spread being on two range edges only (Sagarin & Gaines 2002b; Samis & Eckert 2007), making it possible to sample across their entire range. Across the four data chapters (Chapters 2-5), the following questions were addressed: (i) Does amount of enemy damage (from herbivores and pathogens) decrease towards range edges? (ii) Do range edge populations have faster growth strategies? (iii) Is there a trade-off between dispersal and colonisation ability towards range edges? and (iv) Are there changes in reproductive strategies towards range

edges? Although each data chapter has been formatted for submission to different journals, they have been re-formatted in a consistent style for this thesis. As each data chapter was prepared as a stand-alone publication, there is unavoidable repetition with regards to the Introductions and Methods sections.

In this thesis, Chapter 2 describes a field study which quantified the level of enemy damage across the range of *G. gueinzii* and *H. bonariensis* and related this to distance from range core as well as to leaf-level traits and climatic variables. This chapter has been published as a highlighted student research paper in *Oecologia*. Chapter 3 describes a glasshouse experiment which tests whether range edge populations have shifted to faster growth strategies by examining shifts in pairwise leaf trait relationships between range core and range edge populations of *G. gueinzii* and *H. bonariensis*. This chapter has been submitted to *Diversity and Distributions*. Chapter 4 describes another glasshouse experiment that investigates the potential trade-off between dispersal and colonisation ability, two traits central to the range expansion process, towards range edges of *G. gueinzii*. This chapter has been published in *Biological Invasions*. The final data chapter, Chapter 5, examines changes in reproductive strategies across the range of *G. gueinzii* and *H. bonariensis*, namely whether range edge populations invest more resources into reproduction, produce more propagules and have greater capacity for self-fertilisation compared to populations near the range core. This chapter is currently in review at *Biological Invasions*. Finally, Chapter 6 is a general discussion which integrates the findings of these data chapters and contextualises the thesis within the broader research on species' range limits.

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## **2 | Does enemy damage vary across the range of exotic plant species? Evidence from two coastal dune plant species in eastern Australia**

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## Abstract

Release from natural enemies is often cited as a key mechanism for understanding the success of invasive plant species in novel environments. However, with time invasive species will accumulate native enemies in their invaded range, with factors such as spread distance from site of introduction, climate and leaf-level traits potentially affecting enemy acquisition rates. However, the influence of such factors is difficult to assess without examining enemy attack across the entire species' range. We tested the significance of factors associated with range expansion (distance from source population and maximum population density), climatic variables (annual temperature and rainfall) and leaf-level traits (specific leaf area (SLA) and foliar nitrogen concentration) in explaining variation in enemy damage across multiple populations of two coastal invasive plants (*Gladiolus gueinzii* Kunze and *Hydrocotyle bonariensis* Lam.) along their entire introduced distribution in eastern Australia. We found that for *H. bonariensis*, amount of foliar damage increased with distance from source population. In contrast, for *G. gueinzii*, probability and amount of foliar damage decreased with decreasing temperature and increasing rainfall, respectively. Our results show that patterns of enemy attack across species' ranges are complex and cannot be generalised between species or even range edges.

**Key words:** Enemy release, plant invasions, population density, leaf traits, range limits

## Introduction

Why particular species thrive when introduced to novel environments is a long standing question in invasion biology (Kolar & Lodge 2001). One of the most popular hypotheses explaining the success of invasive species is the enemy release hypothesis which posits that when a species is introduced into a novel environment it leaves behind many of its co-evolved enemies (parasites, herbivores, pathogens, etc.) in its native range (Keane & Crawley 2002; Torchin et al. 2003). This reduction in enemies has often been cited as a reason for the increased biomass (Blossey & Nötzold 1995; Siemann & Rogers 2001; Jacobs et al. 2004), reproductive investment (Stastny et al. 2005) and survival (Siemann et al. 2006) of invasive species in their introduced compared to their native range.

However, with time invasive species do eventually accumulate a suite of local enemies in their introduced ranges (Torchin et al. 2003; Castells et al. 2013; Harvey et al. 2013). A potential factor affecting the acquisition of enemies is associated with the location of populations within the introduced range. A species' abundance is generally greater at the centre of its range and decreases towards the range edges, either as a result of declining habitat quality (abundant centre model, Angert & Schemske 2005; Vaupel & Matthies 2012) or as a product of range expansion as species gradually disperse from their point of establishment/introduction (Fagan & Bishop 2000; Tsai & Manos 2010; Kelehear et al. 2012). This greater abundance of species at their range centre may facilitate a greater accumulation of enemies compared to at their range edges (Alexander et al. 2007; Kambo & Kotanen 2014). Lower conspecific density in range edge populations potentially reduces apparency of species, thereby decreasing enemy accumulation at range edges (Sletvold & Grindeland 2008; Vaupel & Matthies 2012; Castagneyrol et al. 2013).

In addition to population attributes associated with the range expansion process, variation in enemy damage may be influenced by environmental factors such as temperature and rainfall as well as leaf-level traits that may vary across species' ranges. For example, the latitudinal herbivory hypothesis posits that interactions between plants and herbivores should be weaker with increasing latitude (Moles et al. 2011), potentially due to decreased abundance of insects at higher latitudes (Pennings & Silliman 2005). Therefore, a latitudinal gradient in herbivory may instead favour the expansion of invasive species into colder climates where enemy pressure is decreased (assuming that the climatic conditions can support the plant species' establishment, growth and reproduction). Specific leaf area (SLA, amount of structural investment in a leaf per unit of light capture area) and leaf nitrogen concentration, are traits that have been shown to influence levels of enemy attack. Leaves with higher SLA (i.e. thinner, less waxy leaves) and leaf nitrogen concentration have been found to have greater enemy damage (Pérez-Harguindeguy et al. 2003; Vergeer & Kunin 2011). Nonetheless, studies investigating variation in enemy damage across invasive species' ranges are distinctly lacking. Understanding differences in enemy damage along a species' range may shed light on how invasive species are able to expand their range, even after the novelty of enemy release has passed.

Evidence for variation in enemy damage towards range edges is equivocal, with studies finding decreased (Siemann et al. 2006; Castilla et al. 2013; Kambo & Kotanen 2014; Gruntman et al. 2016), increased (Castells et al. 2013; Harvey et al. 2013; Nunes et al. 2016) or no difference (Garcia et al. 2000; Jump & Woodward 2003; Wan & Bonser 2016) in enemy damage towards range edges. Other studies have found equivocal relationships

between amount of enemy damage and latitude (and hence underlying climatic variables, Anstett et al. 2016) (i.e. García et al. 2000; Vaupel & Matthies 2012; Nunes et al. 2016). A potential reason for the inconsistency in results may be that most of these studies only sampled from one range edge and/or only compared populations from just the range centre and range edge, potentially obscuring our ability to find consistent patterns (Sagarin & Gaines 2002a; Sexton et al. 2009; Vaupel & Matthies 2012; Nunes et al. 2016). As range edges often correspond with changes in latitude, disentangling the influence of spread distance from climatic variables that correspond with latitude would require sampling across the entire species' range (Kilkenny & Galloway 2013).

In this study, we investigated variation in population density, enemy damage and leaf-level traits across the entire distribution of two coastal exotic invasive species growing along the east coast of Australia. Coastal species represent an ideal system to study such questions as their range is essentially linear with capacity for spread being on two range edges only (Sagarin & Gaines 2002b; Samis & Eckert 2007). In eastern Australia where this study was conducted, these northern and southern range edges are associated with variation in latitude and consequently climate. We aimed to investigate the influence of factors associated with range expansion (e.g. population density and distance from source population) and variation in climatic variables and leaf-level traits in explaining differences in enemy damage across species' ranges. We predicted the following potential relationships for our two study species: (1) amount of enemy damage would decrease with increasing distance from source population (i.e. would be less towards range edges); (2) amount of enemy damage would increase with higher values for leaf-level traits such as specific leaf area (SLA) and nitrogen concentration; and (3) amount of enemy damage would increase

with increasing temperature, potentially as a result of increased enemy abundance in warmer climates.

## Materials and methods

### STUDY SPECIES

*Gladiolus gueinzii* Kunze (Iridaceae) is a perennial dune plant native to South Africa. The first record of *G. gueinzii* in Australia was from the port of Stockton, New South Wales in 1950, suggesting that the species was accidentally introduced through ballast water (Heyligers 1999). *G. gueinzii* is solely confined to beach dunes and has a distribution of nearly 800 km along the east coast of Australia, from South West Rocks, New South Wales to Mallacoota, Victoria. *G. gueinzii* is facultatively sexual, reproducing clonally by cormels and sexually through flowers. Flowering occurs between October to December and the fruits ripen through the summer months (December to February), with each fruit producing up to 40 winged seeds (samaras) (Heyligers 1999).

*Hydrocotyle bonariensis* Lam. (Apiaceae) is a perennial plant native to Africa, North and South America. The first record of *H. bonariensis* in Australia was made in 1893 near Botany Bay, New South Wales. Although *H. bonariensis* is primarily found on dune systems, it occasionally occurs along rivers and lagoon outlets (Heyligers 1998). Its distribution in Australia is along the east coast from southern Queensland to Victoria (approximately 1200 km). *Hydrocotyle bonariensis* was accidentally introduced to Australia, possibly through ballast water (Heyligers 2008; Murray & Phillips 2012). *Hydrocotyle bonariensis* is facultatively sexual, reproducing clonally using rhizomes and sexually using flowers. A clone of *H. bonariensis* produces new ramets through vegetative growth and branching at nodes

(Knight & Miller 2004). *Hydrocotyle bonariensis* produces inflorescences throughout the year, with each flower potentially producing one schizocarp containing two seeds (Evans 1992).

## FIELD SURVEY

The distribution of *G. gueinzii* and *H. bonariensis* along the east coast of Australia was determined by examining occurrence records from Australia's Virtual Herbarium (AVH). From November to December 2015 we conducted an extensive field survey across the recorded ranges of both species (and 100 km beyond) and found 23 populations of *G. gueinzii* and 28 populations of *H. bonariensis* along the east coast of Australia (Appendix S1). Each population was separated by at least 1 km. Two 20 m x 20 m quadrats were established within the densest area of each population to assess maximum population density (Jump & Woodward 2003). Abundance of *G. gueinzii* was assessed by counting the number of clumps of plants within each quadrat as it was difficult to distinguish individual plants. Percentage cover was used as a measure of population density for *H. bonariensis* due to its extensive clonal growth. If both species occurred at the same site then separate quadrats were conducted to avoid pseudo-replication.

For each population of *G. gueinzii* and *H. bonariensis*, we randomly collected fully deployed leaves to assess enemy damage. A maximum of 50 leaves were collected for each population of *H. bonariensis* and *G. gueinzii*. Fewer leaves were collected from *G. gueinzii* due to the presence of fewer plants at each site compared to *H. bonariensis* (Appendix S1). For each leaf, percentage damage by enemies (i.e. herbivores and pathogens) including leaf chew, skeletonising, gall damage, mould, discolourisation and necrosis was visually



estimated (Leishman et al. 2014). The same person (S. Tabassum) conducted the leaf damage surveys to ensure consistency of results.

#### LEAF-LEVEL TRAITS AND CLIMATIC VARIABLES

To assess the relationship between leaf quality and the amount of enemy damage, we measured specific leaf area (SLA) and leaf nitrogen concentration. SLA (measured as leaf area per unit dry mass) is a measure of the amount of structural investment in a leaf per unit of light capturing area. To assess SLA we randomly collected healthy, fully deployed and sun exposed leaves for each population of *G. gueinzii* and *H. bonariensis*. A maximum of 15 leaves were collected for each species from each population of *H. bonariensis* and *G. gueinzii*. Fewer leaves were collected from *G. gueinzii* due to the presence of fewer plants at each site compared to *H. bonariensis* (Appendix S1). Due to the clonal nature of both study species, all leaves were collected across multiple plants and pooled across plants for each population. Leaves were scanned using a flatbed scanner to determine area before being brought back to Macquarie University and dried in an oven at 70°C for 2 days and weighed to the nearest 0.01g. Dried leaves were ground to a fine powder and analysed for percentage nitrogen concentration by the dry combustion method using a LECO CHN-900 analyser (St. Joseph, USA) at the Plant Growth Facility at Macquarie University.

Climatic variables for each site (mean annual rainfall (mm) and mean annual maximum temperature (°C)) were obtained from the Australian Bureau of Meteorology website ([www.bom.gov.au](http://www.bom.gov.au)). For each site, data was obtained from the nearest weather station. The average straight line distance between each site and its nearest weather station was 7.4 km (range 0.46 km – 58.0 km).

To assess the spread distance of each population from the site of introduction, we designated the first herbarium record for each species from the AVH as the source population (Stockton (32° 55' 00"S, 151° 46' 00"E) for *G. gueinzii* and Lady Robinson's Beach (33° 58' 00"S, 151° 09' 00"E) for *H. bonariensis*) and calculated the straight line distance to each population.

## STATISTICAL ANALYSIS

We conducted linear regressions to test for associations between amount of enemy damage and spread distance, maximum population density, leaf-level traits and climatic variables for both species independently. Due to the high number of zeros in our enemy damage data set that produced non-normally distributed data even when transformed, we examined proportional data for the presence of enemy damage for all leaves sampled within a population and our explanatory variables using a logistic multiple regression with a logit link function and binomial error distribution.

Linear multiple regression models were then used on the leaves that showed any sign of enemy damage (enemy damage > 0%) to assess the effects of distance and maximum population density (and their interaction), leaf-level traits and climatic variables on the amount of enemy damage. The models were based on population means. Predictor variables and two-way interactions were included in the models based on the Akaike information criterion (AIC) using stepwise forward selection. For each model, the significance of each explanatory variable was assessed by comparing the model to that with the target explanatory variable and any associated interaction removed using likelihood

ratio tests. When more than one variable was present in the model, variance inflation factors (VIF) were calculated for each variable in each model to test for multicollinearity. Low VIF values ( $<3$ ) suggest that multicollinearity was not a major issue in the final models. Variables were log, square root or logit transformed to fulfil assumptions of normality and homogeneity of variances where applicable. All analysis was conducted using R version 3.2.4 (R Development Core Team 2016).

## Results

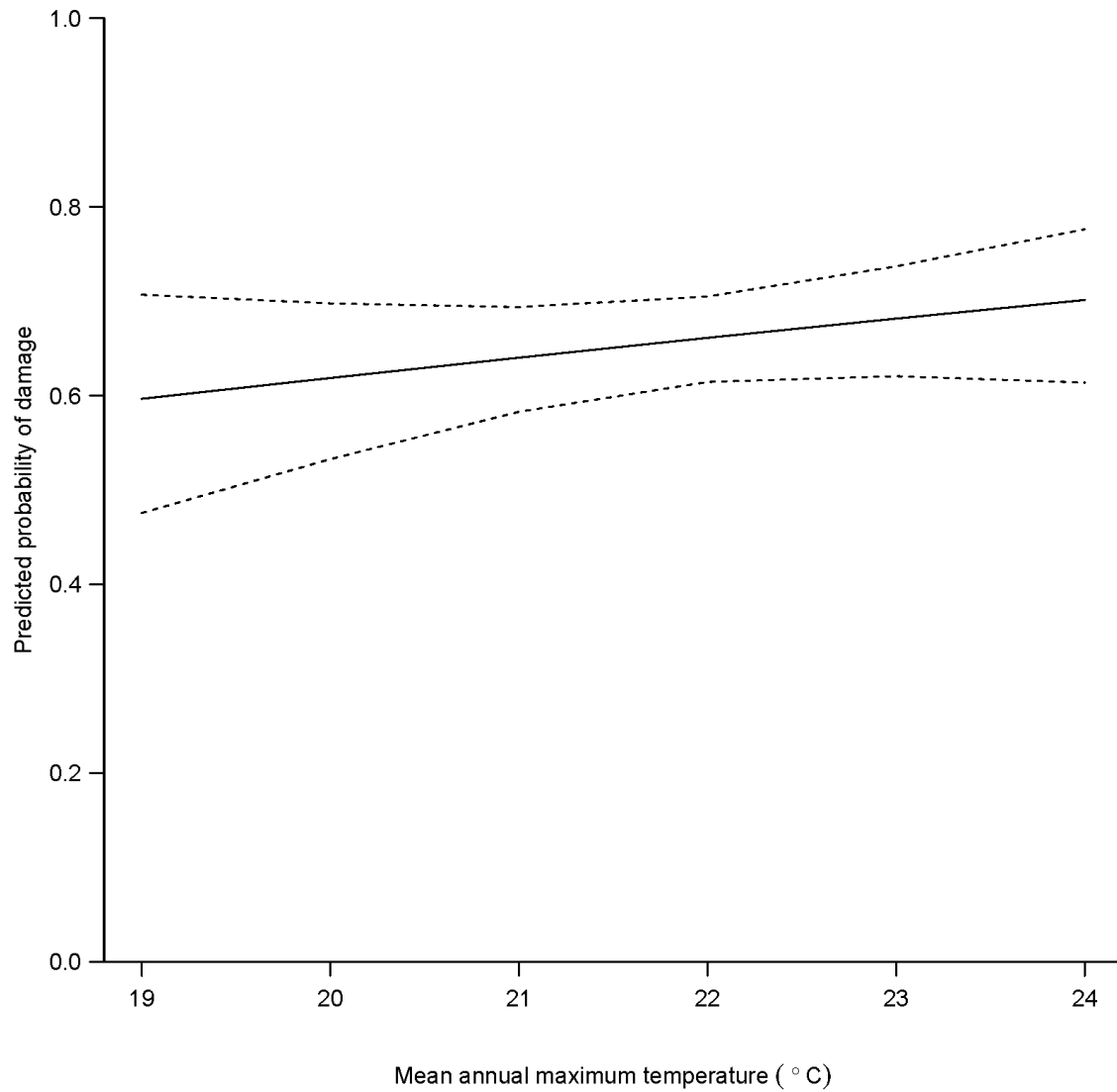
### SPREAD DISTANCE AND PROBABILITY OF ENEMY DAMAGE

In terms of total range size, *G. gueinzii* had spread a total of 794 km from its source population, with a 240 km spread north and a 553 km spread south. *H. bonariensis* had a much larger range size totalling 1155 km, with a 677 km spread north and a 478 km spread south from its introduction site. Of 1390 leaves from 28 populations for *H. bonariensis*, 38% showed no signs of enemy damage. Of 422 leaves from 23 populations of *G. gueinzii*, 34% showed no signs of enemy damage. For the probability of enemy damage, addition of any predictor variables did not improve the model fit for *H. bonariensis* compared to an intercept-only model, based on AIC values (p value for intercept  $< 0.0001$ , Appendix S2). For *G. gueinzii*, the best model based on AIC values included mean annual maximum temperature (p value for mean annual maximum temperature = 0.0038, Appendix S2). Probability of enemy damage weakly but significantly increased with increasing mean annual maximum temperature (odds ratio = 1.097,  $p = 0.003$ , Fig. 1).

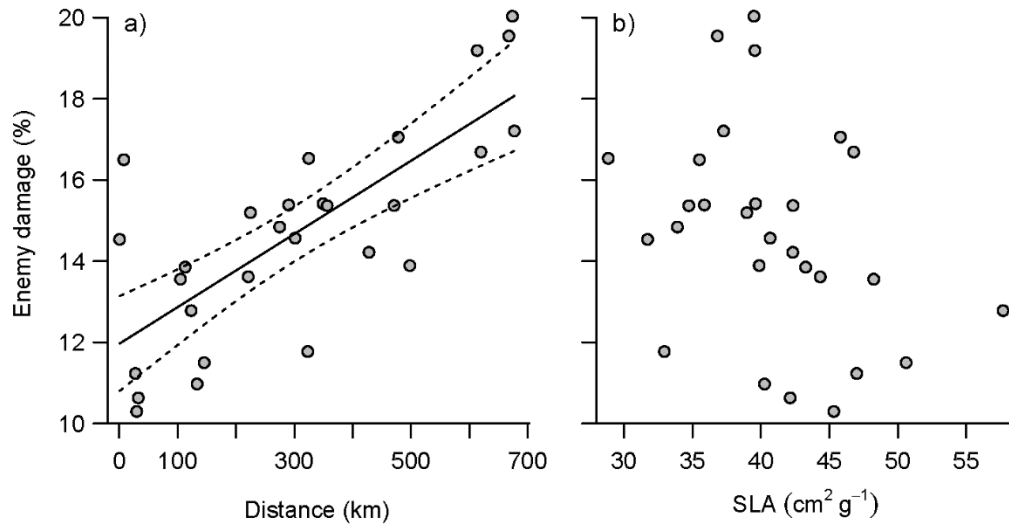
## AMOUNT OF ENEMY DAMAGE

For *H. bonariensis*, when only the leaves with enemy damage were analysed, the best model based on AIC values included distance ( $p < 0.0001$ , Appendix S2) and SLA ( $p = 0.072$ , Appendix S2). Amount of enemy damage significantly increased with increasing distance from source population (Fig. 2a). There was a non-significant trend for decreasing enemy damage with increasing SLA (Fig. 2b).

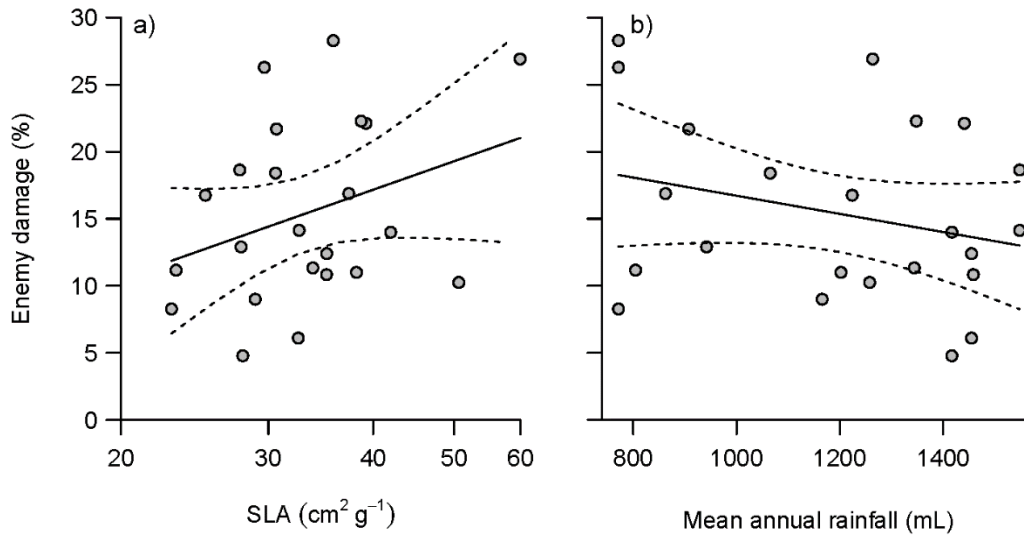
For *G. gueinzii*, when only the leaves with enemy damage were analysed, the best model based on AIC values included SLA ( $p = 0.034$ , Appendix S2) and mean annual rainfall ( $p = 0.02$ , Appendix S2). Amount of enemy damage was found to significantly increase with increasing SLA (Fig. 3a) and significantly decrease with increasing mean annual rainfall (Fig. 3b). Percent enemy damage varied more for *G. gueinzii* compared to *H. bonariensis*, potentially due to smaller sample sizes at each population (Appendix S1).



**Fig. 1** Logistic regression showing the predicted probability of enemy damage as a function of mean annual maximum temperature. Dotted lines show 95% confidence limits for each fitted value.



**Fig. 2** Linear regressions showing the relationship between a) distance from source population ( $y = 0.009x + 11.97$ ,  $R^2_{\text{adj}} = 0.56$ ,  $p < 0.0001$ ) b) specific leaf area (SLA;  $y = -0.15x + 20.81$ ,  $R^2_{\text{adj}} = 0.093$ ,  $p = 0.072$ ) on amount of enemy damage for *H. bonariensis*. Each point represents the average value for a single site. Dotted lines show 95% confidence limits of significant regressions.



**Fig. 3** Linear regressions showing the relationship between a) specific leaf area (SLA;  $y = 22.00x - 18.08$ ,  $R^2_{adj} = 0.065$ ,  $p = 0.034$ ) b) mean annual rainfall ( $y = -0.007x + 23.49$ ,  $R^2_{adj} = 0.032$ ,  $p = 0.020$ ) on amount of enemy damage for *G. gueinzii*. Each point represents the average value for a single site. Dotted lines show 95% confidence limits of significant regressions. Note that a back transformed common log x axis is shown for figure a).

## Discussion

Enemy damage can have a significant impact on the successful spread of invasive species in novel environments. However, factors affecting the amount of enemy damage across invasive species' ranges have rarely been studied. Our study of enemy damage across the range of two exotic invasive coastal plants revealed that factors influencing the amount of enemy damage were not consistent across species. For *H. bonariensis*, factors associated with the range expansion process (i.e. spread distance) explained much of the variation in enemy damage across the range whilst for *G. gueinzii*, factors associated with climate and leaf quality were significantly associated with enemy damage. These results highlight the complexity of understanding factors affecting enemy damage across invasive species away from their point of introduction in a novel location.

We predicted that enemy damage would increase with increasing values of leaf-level traits such as SLA and leaf nitrogen concentration and increase with increasing values of climatic variables such as temperature. Our results for *G. gueinzii* support our predictions. The amount of enemy damage significantly increased with increasing specific leaf area (SLA). Leaves with higher SLA have also been found to sustain higher levels of enemy damage in previous studies (Pérez-Harguindeguy et al. 2003; Vergeer & Kunin 2011). Climatic variables also influenced levels of enemy damage in *G. gueinzii*, with the probability of enemy damage significantly increasing with increasing temperature and the amount of enemy damage significantly decreasing with increasing rainfall. These relationships suggest that *G. gueinzii* may be experiencing reduced enemy attack at its southern range edge as the species expands to colder environments, potentially as a result of a reduction in herbivore abundance with colder climates (Lee & Kotanen 2015).



We also hypothesised that amount of enemy damage may decrease with increasing distance from the source, potentially as a result of decreasing conspecific density (Sletvold & Grindeland 2008; Vaupel & Matthies 2012; Castagneyrol et al. 2013). However, contrary to expectations, we found an increase in amount of enemy damage with increasing distance from source population for *H. bonariensis*. This finding is consistent with some previous studies that have also found increased enemy damage towards range edges of invasive species (Harvey et al. 2013; Nunes et al. 2016). This result may be due to potential changes in enemy guilds across species' ranges. Different enemy species vary in the relative damage they inflict due to differences in their feeding behaviour and can therefore have an impact on results based on visual assessments of enemy damage. Harvey et al. (2013) found invertebrate abundance and diversity both decreased towards edge populations of the invasive *Senecio madagascariensis* in Australia. However, edge populations suffered greater amounts of leaf damage which they believe may have been due to the greater abundance of generalist defoliators at range edges that cause more conspicuous damage compared to greater abundances of sap sucking insects at the range core which cause less obvious damage. In the field, both *G. gueinzii* and *H. bonariensis* were observed to be growing with a mixture of *Cakile edentula* Scop., *C. maritima* Scop. and *Spinifex sericeus* R.Br. across their entire ranges, suggesting that changes in enemy guilds due to changes in vegetation composition across their ranges may not be likely. However, we did not quantify the different types of enemy damage nor did we study enemy abundance and diversity and therefore cannot reliably comment on their influence on our results.

Biotic interactions such as attack by herbivores and pathogens can play a key role in determining range sizes of invasive species, with changes in enemy damage along species' ranges contributing to range expansion or the formation of range boundaries (Fagan & Bishop 2000; Sexton et al. 2009; Katz & Ibáñez 2016). Increased enemy damage towards range edges of *H. bonariensis* may be contributing to the formation of range limits by affecting performance (Hochberg & Ives 1999; Briers 2003; Alexander et al. 2007) and decreased enemy damage towards the southern range edge of *G. gueinzii* may be facilitating range expansion towards colder climates (Lee & Kotanen 2015; Wan & Bonser 2016). However, quantifying the amount of enemy damage is not necessarily a good indicator of plant performance as populations may vary in their ability to tolerate damage and thus not experience reductions in fitness (Mitchell et al. 2006; Anstett et al. 2016). For example, Katz & Ibáñez (2016) found greater enemy damage towards range edges of three invasive species but found that this increased damage did not translate to effects on survival. Furthermore, although there were significant relationships between amount of enemy damage and numerous predictor variables, the difference in damage was ~5-10% for both species and may not be significant enough to elicit fitness differences. Future studies would need to quantify the effect of enemy damage on plant performance to elucidate the influence of enemies in controlling range limits.

In conclusion, we found that factors affecting the acquisition of enemies in the invaded range of species are complex and vary across species and even range edges. We found that for *H. bonariensis*, distance from source population was positively related to amount of enemy damage, while for *G. gueinzii*, temperature and SLA positively related to the probability and amount of enemy damage, respectively. These contrasting relationships

could only have been deduced by quantifying foliar damage across the entire range of the two species and highlights the importance of adopting such a method when studying factors relating to species' ranges. Nonetheless, variation in enemy damage within species' ranges can have profound effects on the geographic distribution of species in novel environments and future studies examining identity of enemies and tolerance to enemy attack within species' ranges may help to better understand the role of enemies in shaping species' range boundaries.

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**Appendix S1.** Sites sampled for *Hydrocotyle bonariensis* and *Gladiolus gueinzii* from north to south showing the straight line distance from their first herbarium record. For the percent enemy damage and specific leaf area (SLA) data, values outside the parentheses represent the number of leaves assessed for each population while values inside the parentheses represent means and standard errors, respectively.

Species	Site	Latitude (S)	Longitude (E)	Distance (km)	% enemy damage	SLA
<i>H. bonariensis</i>	Kingscliff	28°14'25"	153°34'06"	677	50(17.2±1.3)	15(37.3±2.1)
	Casuarina	28°16'32"	153°34'50"	673.7	50(20.2±1.6)	15(39.5±3.3)
	Cabarita Beach	28°19'55"	153°34'19"	667.5	50(19.5±1.2)	15(36.8±1.9)
	Lennox Head	28°47'55"	153°35'43"	619.7	40(16.7±1.2)	12(46.8±6.5)
	Ballina	28°51'37"	153°35'46"	613.4	50(19.2±1.2)	15(39.6±3.9)
	Wooli	29°51'58"	153°16'02"	497.8	50(13.9±1.0)	15(39.9±7.8)
	Woolgoolga	30°06'20"	153°11'59"	470.8	50(15.4±0.9)	15(42.3±2.9)
	Mylestom	30°28'08"	153°02'45"	427.9	50(14.2±0.8)	15(42.3±2.8)
	Richardson's Crossing	31°09'32"	152°58'57"	356.3	50(15.4±1.1)	15(34.7±1.7)
	Port Macquarie	31°28'39"	152°55'51"	323	50(11.8±0.8)	15(32.9±1.9)
	Dunbogan	31°39'12"	152°49'50"	301.4	50(14.6±1.5)	15(40.6±1.5)
	Crowdy Head	31°52'09"	152°42'23"	274.8	50(14.8±0.7)	15(33.9±1.4)
	Blueys Beach	32°21'13"	152°32'06"	220.9	50(13.6±1.2)	15(44.3±2.3)
	Warriewood	33°41'50"	151°18'37"	32.42	50(10.6±0.7)	15(42.1±2.3)
	Collaroy	33°43'46"	151°18'02"	29.82	50(10.3±0.6)	15(45.3±9.0)
	Dee Why	33°45'06"	151°17'50"	27.5	50(11.2±0.7)	15(47.0±0.6)
	La Perouse	33°59'07"	151°13'53"	7.78	50(16.5±1.3)	15(35.5±1.9)
	Brighton le Sands	33°58'13"	151°09'09"	0.46	50(14.5±1.0)	15(31.7±2.2)
	Shoalhaven Heads	34°51'01"	150°45'01"	104.9	50(13.5±0.8)	15(48.2±3.6)
	Culburra	34°55'54"	150°46'20"	112.8	50(13.8±0.7)	15(43.3±4.3)
	Callala	35°00'36"	150°41'57"	123.2	50(12.8±0.6)	15(57.7±4.6)

<i>G. gueinzii</i>	Hyams	35°06'18"	150°41'36"	133.3	50(11.0±1.3)	15(40.3±1.4)
	Swanhaven	35°11'18"	150°34'58"	145.4	50(11.5±0.5)	15(50.6±3.4)
	Tomakin	35°49'45"	150°11'42"	224.7	50(15.2±1.0)	15(39.0±2.1)
	North Tura Beach	36°25'21"	150°04'13"	290.2	50(15.4±0.9)	15(35.9±1.6)
	Tathra Beach	36°43'32"	149°58'53"	324.6	50(16.5±0.9)	15(28.8±2.1)
	Pambula	36°56'25"	149°54'32"	349.2	50(15.4±1.0)	15(39.6±2.8)
	East Cape Beach	37°47'55"	148°44'36"	478	50(17.1±1.3)	15(45.8±2.2)
	Hat Head	31°02'50"	153°02'39"	240.3	23(16.7±4.6)	10(25.2±1.4)
	Richardson's Crossing	31°09'32"	152°58'57"	226.6	14(10.8±4.8)	9(35.2±1.5)
	Point Plomer	31°24'31"	152°54'51"	199.5	22(4.8±1.6)	10(28.0±1.4)
	Port Macquarie	31°29'03"	152°55'24"	192.9	20(14±4.2)	9(42.0±1.6)
	Bonny Hills	31°35'07"	152°50'21"	179.1	18(18.6±5.5)	10(27.7±1.4)
	Dunbogan	31°39'08"	152°49'48"	172.5	19(14.1±4.3)	10(32.6±1.5)
	Crowdy Head	31°52'09"	152°42'24"	146.1	18(11.3±3.7)	11(33.9±1.5)
	Boomerang Beach	32°20'07"	152°32'47"	97.53	17(6.1±2.2)	11(32.6±1.5)
	Blueys Beach	32°21'13"	152°32'06"	95.37	19(12.4±4.0)	10(35.2±1.5)
	Hawks Nest	32°40'34"	152°11'09"	47.44	21(22.1±5.0)	8(39.2±1.6)
	Nobbys Beach	32°55'22"	151°47'34"	2.53	11(22.3±7.1)	7(38.7±1.6)
	Dudley Beach	32°58'19"	151°43'51"	6.997	17(9±3.1)	10(28.9±1.5)
	Swansea Heads	33°05'47"	151°39'30"	22.39	25(18.5±3.5)	10(30.6±1.5)
	Culburra	34°55'54"	150°46'20"	242.1	16(11±5)	10(38.2±1.6)
	Currarong	35°00'52"	150°48'50"	249.3	18(10.2±3.1)	8(50.6±1.7)
	Swanhaven	35°11'19"	150°34'58"	275.2	16(26.9±5.6)	7(59.9±1.7)
	Tomakin	35°49'46"	150°11'40"	354.6	18(11.2±4.9)	11(23.3±1.4)
	Bermagui	36°25'21"	150°04'13"	419.5	18(21.7±5.5)	10(30.7±1.5)
	Tathra Beach	36°43'29"	149°58'50"	453.7	19(16.9±5.0)	8(37.4±1.6)
	North Tura Beach	36°49'42"	149°56'08"	465.9	19(26.3±5.8)	8(29.7±1.5)
	Merimbula	36°53'49"	149°54'57"	473.6	18(8.3±2.7)	12(23.0±1.4)
	Pambula	36°56'26"	149°54'32"	478.4	17(28.3±6.3)	10(35.9±1.5)
	Mallacoota	37°34'05"	149°45'42"	548.3	19(12.9±4.8)	12(27.8±1.4)



**Appendix S2.** Key features of the intercepts and predictor variables for each (a) logistic regression (b) linear regression analysis. Stepwise forward selection was used to select predictors for each model. Only the subset of predictors that were present in the models with the lowest AIC values are shown. Significant p values are highlighted in bold.

(a) Logistic regression

Species	Variable	Estimate	Standard error	z/t value	P value	AIC
<i>H. bonariensis</i>	Intercept	0.49	0.05	8.92	<b>&lt;0.0001</b>	214.72
<i>G. gueinzii</i>	Intercept	-2.17	1.80	-1.20	0.23	119.03
	Max temp	0.27	0.09	2.89	<b>0.0038</b>	-13.54

(b) Linear regression

Species	Variable	Estimate	Standard error	z/t value	P value	AIC
<i>H. bonariensis</i>	Intercept	15.98	2.30	6.95	<b>&lt;0.0001</b>	54.87
	Distance	0.01	0.001	5.71	<b>&lt;0.0001</b>	-21.77
	SLA	-0.09	0.05	-1.80	0.072	-23.18
<i>G. gueinzii</i>	Intercept	-19.87	19.57	-1.01	0.32	88.38
	Log SLA	31.42	13.56	2.32	<b>0.034</b>	-2.61
	Rainfall	-0.01	0.005	-2.12	<b>0.02</b>	-3.29

### **3 | Mixed evidence for shifts to faster carbon capture strategies towards range edges of two coastal invasive plants in eastern Australia**

This chapter has been submitted to *Diversity and Distributions*.

My contribution to the research and paper: Concept – 90%; Data collection – 100%; Analysis – 100%; Writing – 90%

## Abstract

Understanding shifts in traits across the course of an invasion can significantly increase our understanding of mechanisms underpinning range expansion. For example, shifts to traits associated with faster growth may be advantageous in range edge populations of invasive species to decrease generation time and thus promote rapid range expansion. We tested whether populations at the expanding range edges of two coastal plant species invasive in eastern Australia (*Gladiolus gueinzii* Kunze and *Hydrocotyle bonariensis* Lam.) possessed different carbon capture strategies compared with range core populations where they were first introduced. We collected seed and vegetative material from a total of 30 populations of *G. gueinzii* and *H. bonariensis* across their range and grew them in the glasshouse to measure leaf-level traits (specific leaf area (SLA), assimilation rate ( $A_{\text{mass}}$ ), foliar nitrogen ( $N_{\text{mass}}$ ) and foliar phosphorus ( $P_{\text{mass}}$ )). Investigating relationships between these leaf-level traits can reveal information about growth strategies, with higher values for these traits often conferring faster growth. Pairwise leaf trait relationships between SLA,  $A_{\text{mass}}$ ,  $N_{\text{mass}}$  and  $P_{\text{mass}}$  were investigated for range edge and range core populations using standardised major axis (SMA) regression. Across species, SMA slopes for range core and range edge populations for all pairwise comparisons did not differ significantly from each other, suggesting that both species have similar carbon capture strategies across their range. However, at a species level, *H. bonariensis* displayed significant shifts in trait values along a common axis for many pairwise comparisons. Range edge populations were found to have higher values for  $N_{\text{mass}}$ ,  $A_{\text{mass}}$  and SLA compared to range core populations, suggesting that range edge populations are positioned further along the leaf economics spectrum towards faster growth strategies. In contrast, for *G. gueinzii*, leaf traits were positioned along a common slope with no difference in the relative positions of range core and range edge

populations. Our results suggest that there is selection for faster carbon capture strategies at range edges for some non-native introduced species undergoing range expansion and this may be a contributing factor in explaining rapid range advance.

**Key words:** assimilation rate, carbon capture strategy, leaf economics spectrum, leaf nitrogen, leaf phosphorus, plant invasions, specific leaf area

## Introduction

Invasive species constitute one of the major threats to biodiversity worldwide (Clavera & García-Berthou 2005; Molnar et al. 2008; Hedja et al. 2009). As such there has been much effort dedicated to understanding what constitutes a successful invasive species (van Kleunen et al. 2010) yet relatively less on understanding the processes underlying successful range expansion of a species introduced into a new region. As a species expands its range from the initial point of introduction, selection may favour a different suite of traits across the range, depending on the location of the population. For example, traits that confer greater colonisation potential such as increased dispersal ability (Phillips et al. 2006; Berthouly-Salazar et al. 2012; Huang et al. 2015) and reproductive output (Lopez et al. 2012; Masson et al. 2016) may be favoured towards the range edge where conspecific density may be low as a means of facilitating further range expansion. In contrast, traits conferring greater competitive ability (Evans et al. 2013; Huang & Peng 2016) may be selected for in longer established populations where conspecific density may be high as a mechanism for population persistence. However to date little is known about the dynamics of species' invasions, particularly how selection for traits changes during the course of an invasion (Evans et al. 2013). Understanding more about such processes may lead to novel solutions for mitigating the spread of invasive species.

During range expansion, as the abundance of introduced species increases, populations at the edge of the range may experience selection for continual spread due to fitness advantages associated with a lower density of conspecifics (Phillips et al. 2010), increased availability of resources beyond the range (Carol et al. 2009; Brown et al. 2013; Houston et al. 2013) and avoidance of kin competition (Kubisch et al. 2013). One way to facilitate such

spread is through increased selection for faster growth strategies towards range edges.

Faster growing individuals are more likely to be sexually mature and benefit from the arrival of new mates, thus allowing for faster reproduction and continual expansion of the range front (Chuang & Peterson 2016). Faster growth strategies have been found in range edge populations of a variety of invasive taxa (fish (Bøhn et al. 2004; Carol et al. 2009; Feiner et al. 2012), amphibians (Phillips 2009; Brown et al. 2013) and plants (Siemann & Rogers 2001; Siemann et al. 2006; Kambo & Kotanen 2014)), suggesting that faster growth may be a major contributor to facilitating species' range expansion.

For plants, growth strategies have been found to be intimately tied to the leaf economics spectrum, which describes ecological strategies of plants in relation to carbon fixation and fundamental trade-offs between a number of key plant traits (Wright et al. 2004). Species with low structural investment per unit leaf area (high specific leaf area, SLA) tend to have short leaf lifespans but experience a fast growth return on their investment (i.e. they have high leaf nutrient concentrations, assimilation and respiration rates). On the other hand, species with high structural investment per unit leaf area (low SLA) tend to produce well-defended leaves with long leaf life spans at the expense of fast growth returns (i.e. they have low leaf nutrient concentrations, assimilation and respiration rates) (Wright et al. 2004). Numerous studies have found that these relationships are consistent across habitats and growth forms, suggesting tight trade-offs between traits (Reich et al. 1997, Reich et al. 1999; Wright et al. 2004).

Examining pairwise relationships between these ecologically important traits can reveal important information on the carbon capture strategies of plants. For example, species with

higher foliar phosphorus content or assimilation rate for a given amount of foliar nitrogen content will experience greater carbon returns for a given investment in leaf tissue. However, only a few studies have examined pairwise leaf trait relationships for invasive species. Gulías et al. (2003) and Leishman et al. (2007, 2010) compared leaf trait relationships between invasive and native species and found that they both have the same carbon capture strategy; however, invasive species were situated at the higher end of the leaf economics spectrum representing faster growth strategies. However, to our knowledge, no studies have examined leaf trait relationships among populations across the invaded range of species. Selection for faster growth strategies towards range edges of invasive plants should favour populations that have inherently higher values for leaf level traits and/or can achieve a greater gain for a given investment in leaf tissue. Understanding fundamental differences in carbon capture strategies across populations can provide greater insights into the range expansion process.

In this study we explored pairwise leaf trait relationships between range core populations of coastal invasive plants where they were first introduced, to range edge populations at the invasion front, to examine whether range edge populations have shifted towards faster growth strategies. Coastal species represent an ideal system to study such questions as their range is essentially linear (restricted to the coast) with capacity for spread being on two range edges only (Sagarin & Gaines 2002; Samis & Eckert 2007). Across all comparisons we hypothesised that pairwise combinations of leaf traits (SLA, assimilation rate ( $A_{\text{mass}}$ ), foliar nitrogen ( $N_{\text{mass}}$ ) and foliar phosphorus ( $P_{\text{mass}}$ )) would scale positively with one another across all populations for each species. Across population location we tested the following alternative hypotheses between pairwise trait combinations: (i) slopes for range edge

populations will be higher in elevation compared to core populations, suggesting greater carbon gains for a given investment in leaf tissue for range edge populations; (ii) slopes for range edge populations will be shifted further along a common slope compared to core populations, suggesting inherently higher values for leaf level traits and thus greater carbon gains for range edge populations; (iii) slopes for range core and edge populations will not differ in elevation or shift along a common slope, suggesting similar carbon gains; and (iv) slopes will differ between range core and edge populations, suggesting different carbon fixation strategies across the invaded range.

## Materials and methods

### STUDY SPECIES

*Gladiolus gueinzii* Kunze (Iridaceae) is a perennial dune plant native to South Africa. The first record of *G. gueinzii* in Australia was from the port of Stockton, New South Wales in 1950, suggesting that the species was accidentally introduced through ballast water (Heyligers 1999). *Gladiolus gueinzii* is solely confined to beach dunes and has a distribution of nearly 800 km along the east coast of Australia, from South West Rocks, New South Wales to Mallacoota, Victoria. *Gladiolus gueinzii* is facultatively sexual, reproducing clonally by cormels (round, buoyant, underground growths) and sexually through self-compatible flowers (Heyligers 1999; Manning et al. 2011). Flowering occurs between October to December, with plants producing a single flower stem containing 1-6 pink flowers that open sequentially (Heyligers 1999). Fruits ripen through the austral summer (December – February), with each fruit producing up to 40 winged seeds (samaras) (Heyligers 1999). This species typically occurs in dense populations close to the high-tide mark, with individuals occurring 20-30 cm apart (Manning et al. 2011).



*Hydrocotyle bonariensis* Lam. (Araliaceae) is a perennial plant native to Africa, North and South America. The first record of *H. bonariensis* in Australia was made in 1893 near Botany Bay, New South Wales. Although *H. bonariensis* is primarily found on dune systems, it occasionally occurs along rivers and lagoon outlets (Heyligers 1998). Its distribution in Australia is along the east coast from Kingscliff in southern Queensland to East Cape Beach in northern Victoria (approximately 1200 km). *Hydrocotyle bonariensis* was accidentally introduced to Australia, possibly through ballast water (Heyligers 2008; Murray & Phillips 2012). This species is facultatively sexual, reproducing clonally using rhizomes (modified underground stems) and sexually using flowers. *Hydrocotyle bonariensis* produces compound inflorescences containing hundreds of cream coloured flowers throughout the year, with each flower potentially producing one indehiscent schizocarp containing two seeds (Evans 1992). Roots, leaves and inflorescences arise from nodes along the rhizome which is generally buried in the sand at a depth of 2-5 cm (Knight & Miller 2004). The extensive rhizome systems of *H. bonariensis* can be comprised of over a thousand individual ramets that span many square meters of dune systems (Evans 1991).

#### FIELD COLLECTION

The distribution of *G. gueinzii* and *H. bonariensis* along the east coast of Australia was determined by examining occurrence records from the Australian Virtual Herbarium (AVH). From November to December 2015 we conducted an extensive field survey across the recorded ranges of both species (and 100 km beyond) and located 15 populations of *G. gueinzii* and 15 populations of *H. bonariensis* (Appendix S1). For each species, five populations were located from the range centre (designated as within 100 km from site of

first herbarium record, (Stockton (32° 55' 00"S, 151° 46' 00"E) for *G. gueinzii* and Lady Robinson's Beach (33° 58' 00"S, 151° 09' 00"E) for *H. bonariensis*), five populations were located from the northern edge and five populations were located from the southern edge.

Mature seeds were collected from the *G. gueinzii* populations while rhizomes were collected from *H. bonariensis* populations as many of the populations were not seeding at the time of collection. This material was then used to grow plants in the Plant Growth Facility glasshouses at Macquarie University. For each *H. bonariensis* population, two or three 10 cm long rhizomes were lightly buried in shallow trays (28 cm x 34 cm) filled with 100% washed beach sand sourced from a commercial supplier (Australian Native Landscapes). Due to space constraints, each population was confined to one tray. *Gladiolus gueinzii* seeds were lightly scarified before being set to germinate on moist filter paper in petri dishes. Seeds were kept moist using 1% bleach solution to reduce the chance of mould. Petri dishes were placed in a temperature controlled growth cabinet set at 20°C with a 12 hour photo-period. For each population, 10 randomly selected germinated seeds were carefully transplanted into pots (diameter 17 cm, depth 17 cm) containing 100% washed beach sand. *Hydrocotyle bonariensis* and *G. gueinzii* plants were placed in two separate glasshouses set to  $25 \pm 3^{\circ}\text{C}$  during the day and  $18 \pm 3^{\circ}\text{C}$  during the night. Temperature was monitored continuously using a Multigrow Controller System (Autogrow Systems, Auckland, New Zealand). Plants were mist watered twice daily for two minutes with additional watering being provided on hot days. Pots/trays were randomly assigned a new position in the glasshouse every two weeks to minimise any glasshouse microclimate effect. After two weeks of growth, plants were given a low concentration (0.15 g dissolved in 125 mL of water) of liquid fertiliser (Aquasol™; Hortico, Arthur Yates & Company, Homebush, NSW,

Australia, 23 N:3.95 P:14 K). This was repeated every two weeks to prevent nutrient depletion.

#### TRAIT MEASUREMENT

Plants were allowed to grow under standard glasshouse conditions for 15 months to overcome any maternal effects due to environmental conditions in the field (see Weiner et al. 1997; van Kleunan & Fisher 2003). After 15 months, growth related traits (SLA,  $A_{\text{mass}}$ ,  $N_{\text{mass}}$  and  $P_{\text{mass}}$ ) were measured for both species. Due to the clonal nature of our study species, trait measurements were pooled across plants at the population level. For each population 15-30 healthy, sun exposed and fully developed leaves were collected to measure SLA. This was done by measuring the area of each leaf (including petiole) using a LI-COR LI-3100C area meter (Lincoln, Nebraska, USA) and then oven-drying them at 60°C for 72 hours before weighing them. SLA was then calculated as the leaf area divided by dry mass.

For each population of *G. gueinzii* and *H. bonariensis*, assimilation rate was measured on 10 healthy, sun exposed and fully developed leaves using a LI-COR LI-6400 portable photosynthesis machine (Lincoln, Nebraska, USA). Reference CO<sub>2</sub> was set at 400 ppm, relative humidity between 25-45%, block temperature at 22°C and photosynthetically active radiation at 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to mimic glasshouse conditions. Measurements were taken after two to three minutes when assimilation rates in the chamber stabilised. Three measurements for each leaf were taken with leaves selected randomly across populations for both species. These leaves were then collected, measured to determine area, oven-dried and weighed as described above for SLA. Maximum assimilation rate was then calculated on a mass basis by multiplying assimilation output by the SLA of the leaf. If leaves did not fill

the entire IRGA chamber, measurements were adjusted based on leaf area. All assimilation measurements were conducted on cloudless days.

For each population of *G. gueinzii* and *H. bonariensis*, 5-10 leaves were collected to measure foliar nitrogen and phosphorus content. For each population, four replicates were measured for both nitrogen and phosphorus. Leaves were dried at 60°C for 3 days then ground to a fine powder. Foliar nitrogen content was obtained by the dry combustion method using a LECO CHN-900 analyser at the Plant Growth Facility at Macquarie University, NSW, Australia. Foliar phosphorus content was obtained using inductively coupled plasma-atomic emission spectrometry (ICP-AES) by digesting samples in a 5:1 mixture of nitric and perchloric acid at the School of Agricultural and Food Sciences, University of Queensland, Australia.

## STATISTICAL ANALYSIS

Measurements were averaged at each population for each species. As we were interested in the scaling relationship between range core and range edge populations, we used standardised major axis (SMA) regressions to investigate relationships between pairwise comparisons for each of our leaf traits. First, we examined the correlation coefficients of edge and core populations for each of our pairwise comparisons to justify the use of SMA regression. If correlation coefficients were significant, then for each pairwise comparison SMA slopes were fitted separately for range core and range edge populations and tested for homogeneity. If slopes were found to be homogenous then a common slope was estimated. Shifts along a common slope and/or elevation of SMA slopes were then tested using the WALD test. All data were  $\log_{10}$  transformed prior to analysis and all analyses were

performed using R version 3.2.3 (R Development Core Team 2015) using the package *smatr* v. 3 (Warton et al. 2012).

## Results

All traits were positively correlated, however many of the pairwise relationships for range core populations were not significant, with correlation coefficients as low as 0.15 (Table 1).

Across both species, four out of the 12 comparisons for core populations were not significant at the  $p < 0.1$  level (Table 1).

For the pairwise trait comparisons for which correlation coefficients were significant for both range core and range edge populations (eight out of the 12 comparisons), SMA regression analysis found homogenous slopes for all comparisons (Table 2). Common slopes were then fitted and tested for shifts in elevation and shifts along the common slope. For *H. bonariensis*, pairwise trait relationships could be compared for  $P_{\text{mass}}$  vs.  $N_{\text{mass}}$ , SLA vs.  $N_{\text{mass}}$ ,  $A_{\text{mass}}$  vs. SLA and  $A_{\text{mass}}$  vs.  $N_{\text{mass}}$  (Table 1). The SMA slope for  $P_{\text{mass}}$  and  $N_{\text{mass}}$  differed in elevation, with range core populations having higher  $P_{\text{mass}}$  for a given  $N_{\text{mass}}$  compared with range edge populations (Table 2; Fig. 1a). Comparisons for  $A_{\text{mass}}$  vs. SLA, SLA vs.  $N_{\text{mass}}$ , and  $A_{\text{mass}}$  vs.  $N_{\text{mass}}$  showed significant shifts along the common fitted slope, with range edge populations having higher values for these leaf level traits compared to range core populations (Table 2; Fig. 1b, c, d). For these significant regressions, when edge populations were separated into northern and southern edges, both northern and southern edges differed significantly from core populations but not each other for all comparisons (Table 3).

For *G. gueinzii*, pairwise trait relationships could be compared for  $P_{\text{mass}}$  vs.  $N_{\text{mass}}$ ,  $A_{\text{mass}}$  vs.  $P_{\text{mass}}$ , SLA vs.  $N_{\text{mass}}$  and  $A_{\text{mass}}$  vs.  $N_{\text{mass}}$  (Table 1). However there were no significant shifts along a common slope and no shifts in elevation for these pairwise comparisons (Table 2; Fig. 2a, b, c, d).

**Table 1.** Correlation coefficients for pairwise trait comparisons between range core and range edge populations of *Gladiolus gueinzii* and *Hydrocotyle bonariensis*. Significant p values ( $p < 0.1$ ) are highlighted in bold.

Species	Trait pair	Area	R <sup>2</sup>	P value
<i>H. bonariensis</i>	$P_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	0.78	<b>0.04</b>
		Edge	0.79	<b>0.0005</b>
	SLA vs. $P_{\text{mass}}$	Core	0.25	0.39
		Edge	0.71	<b>0.002</b>
	SLA vs. $N_{\text{mass}}$	Core	0.71	<b>0.07</b>
		Edge	0.81	<b>0.0004</b>
	$A_{\text{mass}}$ vs. SLA	Core	0.71	<b>0.071</b>
		Edge	0.92	<b>&lt;0.0001</b>
	$A_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	0.63	<b>0.097</b>
		Edge	0.85	<b>&lt;0.0001</b>
	$A_{\text{mass}}$ vs. $P_{\text{mass}}$	Core	0.27	0.37
		Edge	0.87	<b>&lt;0.0001</b>
<i>G. gueinzii</i>	$P_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	0.71	<b>0.07</b>
		Edge	0.81	<b>0.0004</b>
	SLA vs. $P_{\text{mass}}$	Core	0.37	0.28
		Edge	0.85	<b>0.0001</b>
	SLA vs. $N_{\text{mass}}$	Core	0.65	<b>0.083</b>
		Edge	0.85	<b>0.0001</b>
	$A_{\text{mass}}$ vs. SLA	Core	0.15	0.52
		Edge	0.76	<b>0.001</b>
	$A_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	0.64	<b>0.089</b>
		Edge	0.86	<b>0.0001</b>
	$A_{\text{mass}}$ vs. $P_{\text{mass}}$	Core	0.88	<b>0.017</b>
		Edge	0.90	<b>&lt;0.0001</b>

**Table 2.** Results of the standardised major axis regression analysis for all pairwise combinations of traits for range core and range edge populations of *H. bonariensis* and *G. gueinzii*. NAs indicate the presence of non-significant correlations for edge and/or core populations (Table 1) to conduct SMA regression. Significant p values ( $p < 0.1$ ) for slope homogeneity, shift in elevation and shift along a common slope are highlighted in bold.

Species	Trait pair	Area	Common slope	Intercept	Slope homogeneity (P)	Shift in elevation (P)	Shift along slope (P)
<i>H. bonariensis</i>	$P_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	1.99	-1.31	0.51	<b>0.04</b>	0.15
		Edge		-1.38			
	SLA vs. $P_{\text{mass}}$	Core	0.41	2.30	NA	NA	NA
		Edge		2.34			
	SLA vs. $N_{\text{mass}}$	Core	0.80	1.77	0.15	0.54	<b>0.04</b>
		Edge		1.78			
	$A_{\text{mass}}$ vs. SLA	Core	1.80	-0.47	0.17	0.91	<b>0.02</b>
		Edge		-0.47			
	$A_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	1.36	2.73	0.90	0.22	<b>0.01</b>
		Edge		2.76			
	$A_{\text{mass}}$ vs. $P_{\text{mass}}$	Core	0.72	3.65	NA	NA	NA
		Edge		3.73			
<i>G. gueinzii</i>	$P_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	2.78	-1.23	0.13	0.71	0.53
		Edge		-1.25			

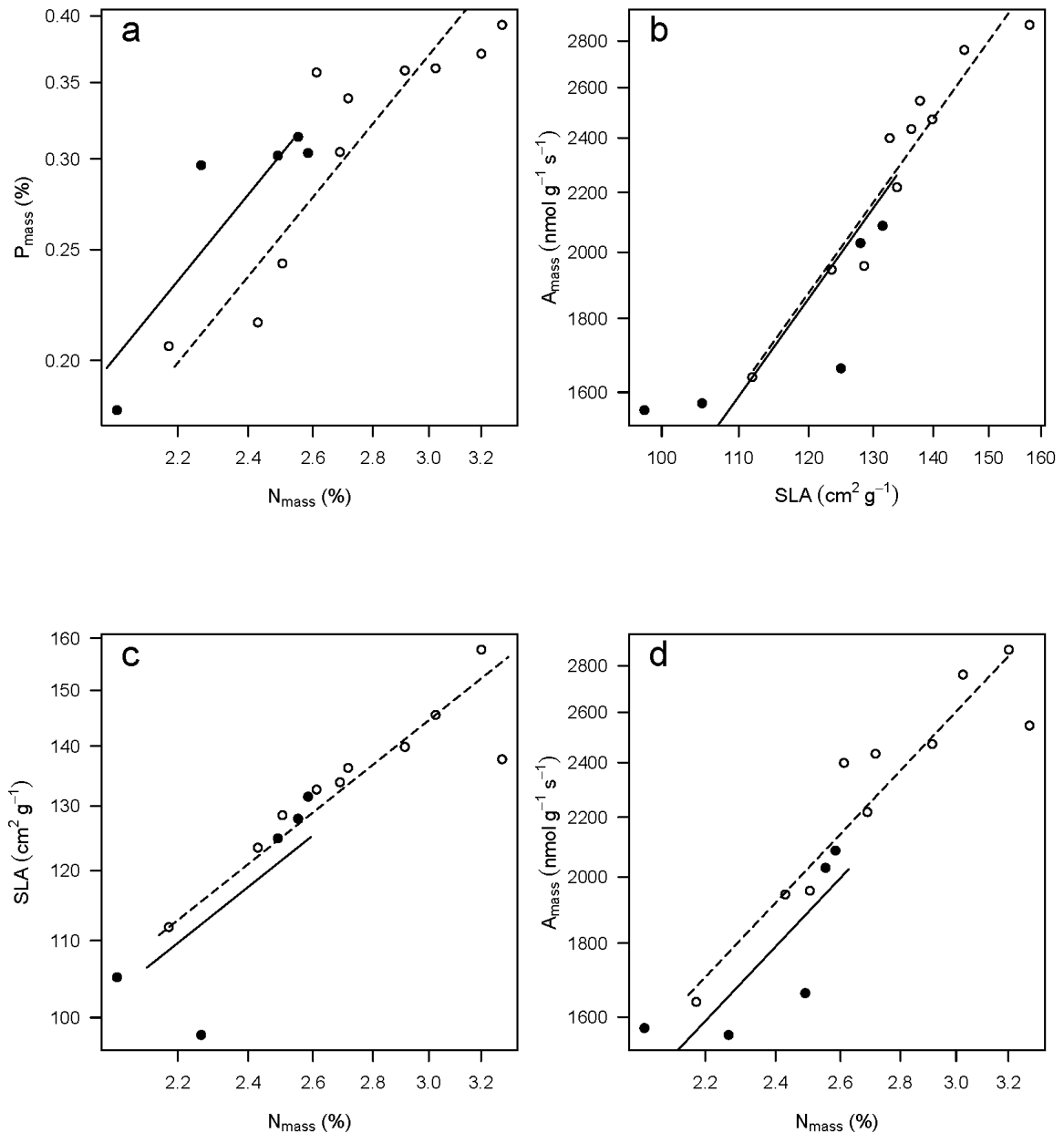


SLA vs. $P_{\text{mass}}$	Core	0.09	1.81	NA	NA	NA
	Edge		1.82			
SLA vs. $N_{\text{mass}}$	Core	0.26	1.69	0.47	0.51	0.79
	Edge		1.70			
$A_{\text{mass}}$ vs. SLA	Core	2.55	-1.51	NA	NA	NA
	Edge		-1.52			
$A_{\text{mass}}$ vs. $N_{\text{mass}}$	Core	0.66	2.83	0.75	0.83	0.61
	Edge		2.82			
$A_{\text{mass}}$ vs. $P_{\text{mass}}$	Core	0.24	3.12	0.08	0.82	0.48
	Edge		3.12			

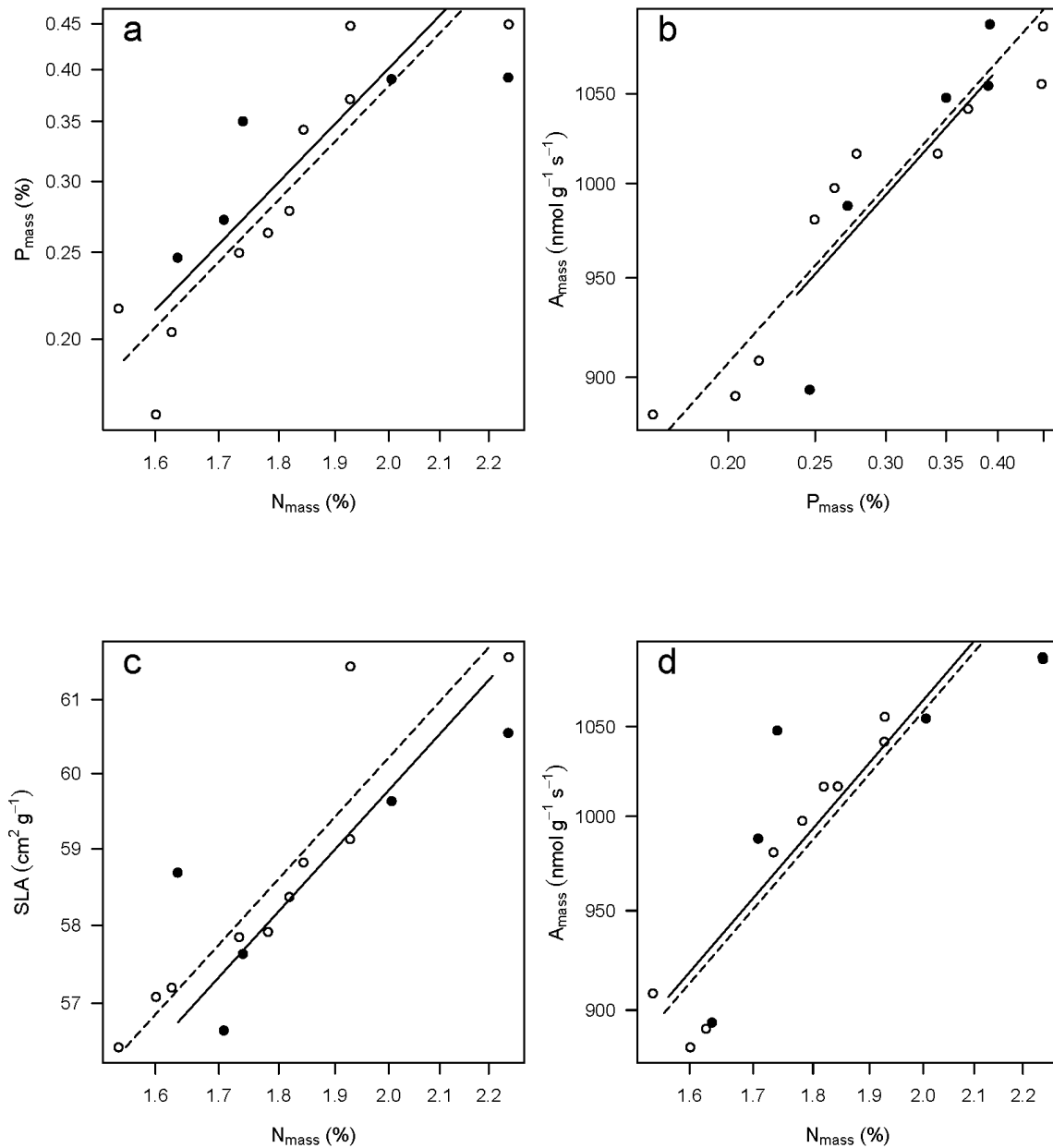
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**Table 3.** Results of multiple comparisons between northern edge, southern edge and core populations of *H. bonariensis* for which there were significant shifts in elevation or along the common slope when edge populations were grouped (Table 2). Significant p values ( $p < 0.1$ ) are highlighted in bold.

Trait pair	Comparison	WALD statistic	P value
$P_{\text{mass}}$ vs. $N_{\text{mass}}$	core vs. north	3.59	<b>0.06</b>
	core vs. south	2.43	0.12
	north vs. south	0.03	0.86
$SLA$ vs. $N_{\text{mass}}$	core vs. north	2.94	<b>0.09</b>
	core vs. south	2.92	<b>0.09</b>
	north vs. south	0.19	0.66
$A_{\text{mass}}$ vs. $SLA$	core vs. north	3.61	<b>0.06</b>
	core vs. south	3.97	<b>0.05</b>
	north vs. south	0.21	0.65
$A_{\text{mass}}$ vs. $N_{\text{mass}}$	core vs. north	3.60	<b>0.06</b>
	core vs. south	4.21	<b>0.04</b>
	north vs. south	0.11	0.74



**Fig. 1** Standardised major axis regressions for the significant relationships between (a)  $P_{\text{mass}}$  and  $N_{\text{mass}}$  (b)  $A_{\text{mass}}$  and SLA (c) SLA and  $N_{\text{mass}}$  and (d)  $A_{\text{mass}}$  and  $N_{\text{mass}}$  for core and edge populations of *Hydrocotyle bonariensis*. Core populations are denoted by closed circles and a continuous line while edge populations are denoted by open circles and a broken line. Note that back transformed  $\log_{10}$  are shown for all graphs.



**Fig. 2** Standardised major axis regressions for the significant relationships between (a)  $P_{\text{mass}}$  and  $N_{\text{mass}}$  (b)  $A_{\text{mass}}$  and  $P_{\text{mass}}$  (c) SLA and  $N_{\text{mass}}$  and (d)  $A_{\text{mass}}$  and  $N_{\text{mass}}$  for core and edge populations of *Gladiolus gueinzii*. Core populations are denoted by closed circles and a continuous line while edge populations are denoted by open circles and a broken line. Note that back transformed log<sub>10</sub> axes are shown for all graphs.

## Discussion

In terms of our original hypotheses we found that pairwise comparisons of  $P_{\text{mass}}$ ,  $N_{\text{mass}}$ ,  $A_{\text{mass}}$  and SLA scaled positively with one another for both *H. bonariensis* and *G. gueinzii* across their range. This is consistent with previous studies that have compared pairwise leaf trait relationships for invasive species within different environments (Leishman et al. 2007, 2010). In addition, we found no difference in the slopes for pairwise comparisons of traits between range core and range edge populations, suggesting that both species possess fundamentally similar carbon capture strategies across their range.

Overall, we found mixed evidence to suggest that range edge populations may be shifting to a faster growth strategy to facilitate further range expansion. For *H. bonariensis*, significant shifts along SMA slopes between range core and range edge populations were found for SLA vs.  $N_{\text{mass}}$ ,  $A_{\text{mass}}$  vs. SLA and  $A_{\text{mass}}$  vs.  $N_{\text{mass}}$  relationships. For each of these comparisons, range edge populations possessed higher values for SLA,  $N_{\text{mass}}$  and  $A_{\text{mass}}$  compared to range core populations. Higher values for these leaf level traits position range edge populations of *H. bonariensis* at the upper end of the leaf economics spectrum, towards faster growth returns.

As range edges often correspond with changes in latitude, both climatic variables and the process of range expansion may select for similar range edge phenotypes (Evans et al. 2013; Therry et al. 2014b; Van Petegem et al. 2016). For example, species undergoing poleward expansion may experience selection to undergo faster growth in order to hasten development in response to shorter growing seasons, a process known as counter-gradient variation (Conover & Schultz 1995; Sanford et al. 2006). We found no significant shifts in

elevation or shifts along the common slope when range edge populations were separated into northern and southern range edges. This suggests that these shifts in growth strategies are not influenced by differences in climatic conditions between the edges but instead may be a product of the range expansion process. Although some studies have controlled for the influence of counter-gradient variation when understanding the mechanisms behind range expansion (e.g. Therry et al. 2014a, b; Van Petegem et al. 2016), others have not (e.g. Evans et al. 2013; Kilkenny & Galloway 2013; Dangremond & Feller 2016; Macel et al. 2017), highlighting the need for more studies such as ours to disentangle the underlying processes influencing range expansion.

The evolution of increasingly faster growth strategies in frontal populations during the course of an invasion may be impeded by trade-offs with other important life history traits. For example, earlier sexual maturation in edge populations of range expanding taxa have been shown to lead to shorter life span (Hanski et al. 2006; Amundsen et al. 2012) and shorter body length (Bøhn et al. 2004). Similarly Brown et al. (2007) found that larger cane toads at the invasion front experienced reduced immune defence. For plants, there exists a trade-off between growth and defence traits which may lead to individuals with faster growth strategies being more vulnerable to enemy attack (Blossey & Notzold 1995). For example, leaves with higher SLA and foliar nitrogen content have been found to sustain greater enemy damage (Pérez-Harguindeguy et al. 2003; Vergeer & Kunin 2011). This may be the case for *H. bonariensis*, which not only had higher growth trait values in their range edge populations but also higher incidences of enemy damage in those field populations (Tabassum & Leishman 2018a). Thus increased capacity for range expansion due to shifts to

faster growth strategies at the range edge may be hampered by increasing vulnerability to enemy attack.

Contrary to *H. bonariensis*, there were no significant shifts along the common slope or shifts in slope elevation found for pairwise leaf trait relationships for *G. gueinzii*. A potential explanation for this discrepancy may be that *H. bonariensis* and *G. gueinzii* differ in time since introduction. *Hydrocotyle bonariensis* was first recorded on the east coast of Australia in 1893 while *G. gueinzii* appeared almost 60 years later in 1950. Therefore, differences between species may simply reflect the fact that *H. bonariensis* has had a longer residence time and thus greater opportunity to undergo change (Moran & Alexander 2014).

Previous studies have also found no shifts in growth related traits between range edge and core populations of invasive species (e.g. Alexander et al. 2007; Masson et al. 2016), suggesting that this is not a common syndrome for range expanding species. Rather, selection for alternative traits such as increased dispersal may be more important in some species for range expansion. For example, *G. gueinzii* is capable of long distance dispersal by water through the use of cormels, which are buoyant asexual growths produced by adult plants. Cormels are capable of dispersing over many kilometres and have been speculated to have contributed to the early range advance of this species (Heyligers 1999). Additionally, *G. gueinzii* produces copious amounts of wind dispersed seeds through sexual reproduction (Heyligers 1999). It is possible that selection for increased dispersal ability of winged seeds (Tabassum & Leishman 2017b) or increased allocation to the production of cormels may be more influential for range expansion in *G. gueinzii* compared with selection for faster growth strategies. In contrast *H. bonariensis* does not produce modified seeds for dispersal

and therefore faster carbon gains at the range edge may contribute more to range advance in this species.

It is worth noting that the plants used in this experiment were derived from material collected in the field (stolons for *H. bonariensis* and seeds for *G. gueinzii*) and thus our leaf trait data may reflect the influence of maternal environment to some extent. Previous studies have observed effects of maternal environment on seed size and subsequently growth traits associated with establishment (e.g. Platenkamp & Shaw 1993; González-Rodríguez et al. 2011) and also biomass of clonal offspring (González et al. 2016). However Weiner et al. (1997) showed that although maternal effects influenced growth at the early seedling stage in *Centaurea maculosa*, these effects dissipated after eight weeks of growth. Similarly van Kleunen and Fisher (2003) found no significant maternal effects on the growth of stolons of *Ranunculus reptans* from high and low density experiments after being grown in identical conditions for 63 days. Ideally, studies on selection would be conducted on plants from the  $F_1$  generation (and beyond) to eliminate the influence of plasticity and/or maternal effects. Although we endeavoured to minimise the influences of maternal effects in this experiment by allowing plants to grow in controlled conditions in the glasshouse for 15 months, the influence of maternal effects cannot be entirely ruled out. Understanding more about the influence of plasticity, maternal effects and genetic differences can increase our understanding of the drivers of range expansion (Gruber et al. 2017). Future studies examining traits from the  $F_1$  generation will help to untangle the influence of these different drivers.



As well as increasing our understanding of biological invasions, this study has important implications for understanding native species' responses to climate change. As species shift their ranges to track suitable climates, populations at the leading edge and trailing end of the expansion front should experience similar selection pressures for range expanding populations. Numerous studies have found support for shifts towards not only faster growth traits in edge populations tracking climate change (Sanford et al. 2006; Therry et al. 2014b) but also shifts in other important life history traits such as dispersal (Cwynar & MacDonald 1987; Hughes et al. 2003; Simmons & Thomas 2004; Hanski et al. 2006; Therry et al. 2014c) and reproduction (Ling et al. 2008). However, unlike biological invasions, range shifts due to climate change are limited by a slowly shifting cloud of suitable climatic conditions, which imposes strong selection against phenotypes which may over-disperse ahead of this cloud or under-disperse and fall behind (Boeye et al. 2013). Given that the movement of climatically suitable space generally proceeds slower than the rate of biological invasions, it is likely that the intensity of selection for expanding phenotypes at range edges may be stronger for invasive species than native species tracking climate change (Chuang & Peterson 2016).

In general, we found that range edge and range core populations of our invasive study species have similar carbon capture strategies as they are positioned along a common axis describing pairwise leaf trait relationships. We found evidence suggesting that range edge populations of *H. bonariensis* lie at the higher end of this spectrum, potentially shifting towards a faster growth strategy. However, for *G. gueinzii* we found that range edge and range core populations were similarly positioned along a common axis describing carbon capture strategies, suggesting similar capacity for carbon gains across the range for this

species. Thus differences in carbon capture strategies across species' ranges may be a contributing factor in explaining the rapid range expansion of a select group of non-native introduced species. Additional life-history traits such as dispersal and reproductive investment may be more influential in explaining rapid range advances. Nonetheless, increasing our knowledge of variation in traits affecting adult life stages may provide a more complete picture of range expansion dynamics.

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**Appendix S1.** Sites sampled for *Hydrocotyle bonariensis* and *Gladiolus gueinzii*. Core sites

were sites located within 100 km from the site of the first herbarium record. Edge sites were the furthest populations found across the ranges of *H. bonariensis* and *G. gueinzii*.

Species	Site	Area	Latitude (S)	Longitude (E)
<i>H. bonariensis</i>	Kingscliff	Edge	28°14'25"	153°34'06"
	Casuarina	Edge	28°16'32"	153°34'50"
	Cabarita Beach	Edge	28°19'55"	153°34'19"
	Lennox Head	Edge	28°47'55"	153°35'43"
	Ballina	Edge	28°51'37"	153°35'46"
	Warriewood	Core	33°41'50"	151°18'37"
	Collaroy	Core	33°43'46"	151°18'02"
	Dee Why	Core	33°45'06"	151°17'50"
	La Perouse	Core	33°59'07"	151°13'53"
	Brighton le Sands	Core	33°58'13"	151°09'09"
	Tomakin	Edge	35°49'45"	150°11'42"
	North Tura Beach	Edge	36°25'21"	150°04'13"
	Tathra Beach	Edge	36°43'32"	149°58'53"
	Pambula	Edge	36°56'25"	149°54'32"
	East Cape Beach	Edge	37°47'55"	148°44'36"
<i>G. gueinzii</i>	Hat Head	Edge	31°02'50"	153°02'39"
	Richardson's Crossing	Edge	31°09'32"	152°58'57"
	Point Plomer	Edge	31°24'31"	152°54'51"
	Port Macquarie	Edge	31°29'03"	152°55'24"
	Dunbogan	Edge	31°39'08"	152°49'48"
	Blueys Beach	Core	32°21'13"	152°32'06"
	Hawks Nest	Core	32°40'34"	152°11'09"
	Nobbys Beach	Core	32°55'22"	151°47'34"
	Dudley Beach	Core	32°58'19"	151°43'51"
	Swansea Heads	Core	33°05'47"	151°39'30"
	North Tura Beach	Edge	36°49'42"	149°56'08"
	Merimbula	Edge	36°53'49"	149°54'57"
	Pambula	Edge	36°56'26"	149°54'32"
	Mallacoota	Edge	37°34'05"	149°45'42"
	Secret Beach	Edge	37°36'31"	149°43'14"

## **4 | Have your cake and eat it too: greater dispersal ability and faster germination towards range edges of an invasive plant species in eastern Australia**

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My contribution to the research and paper: Concept – 90%; Data collection – 100%; Analysis – 100%; Writing – 90%



**Abstract**

The process of range expansion often selects for traits that maximise invasion success at range edges. For example, during range expansion, individuals with greater dispersal and colonisation ability will be selected for towards range edges. For wind dispersed plants, however, there exists a fundamental trade-off between dispersal and colonisation ability (germination success and growth) that is mediated by seed size; smaller seeds often have greater dispersal ability but poorer colonisation ability. We investigated the nature of the dispersal/colonisation trade-off by comparing dispersal ability (wing loading ratio: seed mass/wing area), germination success and growth related traits across multiple populations of a coastal exotic invasive plant species (*Gladiolus gueinzii* Kunze) along its entire introduced distribution in eastern Australia. We found that *G. gueinzii* had significantly greater dispersal ability towards its range edges which was mediated by a decrease in seed mass. However, this was not associated with a decrease in probability of germination or growth after 3 months. In fact, seeds from range edge populations had significantly faster germination times. Our results suggest that a shift towards greater dispersal ability does not have an associated negative effect on the colonisation ability of *G. gueinzii* and may be a key factor in promoting further range expansion of this exotic invasive species.

**Key words:** dispersal, colonisation, trade-offs, range expansion, introduced range, invasive species

## Introduction

Invasive species constitute a major threat to biodiversity and ecosystem function worldwide (Clavero & García 2005; Molnar et al. 2008; Hedja et al. 2009). As a result, there has been a strong research focus on determining which traits contribute to a species' 'invasive' ability (e.g. van Kleunen et al. 2010; Davidson et al. 2011). The majority of empirical studies investigating trait shifts during range expansion have focused on comparing invasive populations from their native and invaded ranges (home and away studies), on the basis that novel ecological and evolutionary conditions may promote invasiveness (Lankau et al. 2009; Kilkenny & Galloway 2013). A key assumption of this view is that species remain static and that evolutionary processes in the introduced range are relatively unimportant (Phillips et al. 2010, but see Dlugosch et al. 2015). However, as species expand in their range, the process of range expansion itself may be selecting for traits that aid in persistence at the range edge (i.e. traits that enhance dispersal, reproduction and/or competitive ability), hence facilitating further range expansion (Burton et al. 2010; Evans et al. 2013; Kilkenny & Galloway 2013).

One such trait that may be under selection towards range edges is dispersal ability (Travis & Dytham 2002). This is because the process of range expansion essentially sorts individuals through space according to dispersal ability. This process, known as spatial sorting, leads to individuals with the greatest dispersal ability being most prevalent at the range edges of expanding fronts (Phillips et al. 2010; Shine et al. 2011). Spatial selection, coupled with the notion that individuals at the invasion front often experience greater absolute fitness due to lower conspecific density, leads to the evolution of increased dispersal ability at the range edge with each successive generation (Phillips et al. 2010; Alex Perkins et al. 2013). The

propensity for greater dispersal at range edges has been demonstrated with greater leg length (Phillips et al. 2006) and increased path straightness (Brown et al. 2014) in cane toads, larger wings in crickets (Simmons & Thomas 2004), and decreased seed mass in plants (Huang et al. 2015).

Another key trait that may evolve upwards at range edges is colonisation ability, which includes growth and establishment. For plants, greater establishment at the range edge may be promoted by faster germination times and greater probability of germination, as a means of establishing peripheral populations while intraspecific competition is relatively low (Bartle et al. 2013). Similarly, faster growth rates are often associated with earlier sexual maturation, shorter generation time and greater biomass gains, which may be advantageous for range edge populations in promoting population growth and persistence (Kilkenny & Galloway 2013; Chuang & Peterson 2015). Indeed, greater percent germination (Bartle et al. 2013, but see Sugiyama 2003) and faster growth rates (Bøhn et al. 2004, Phillips 2009; Kilkenny & Galloway 2013) have been found in edge populations of many invasive species. Together, selection for enhanced dispersal and colonisation ability at leading edges of an invasion may greatly accelerate rates of range expansion.

However, for wind dispersed plants, there exists a fundamental trade-off between dispersal and colonisation ability that is mediated by seed mass. Wind dispersed (anemochorous) diaspores consist of a seed (achene) attached to a specialised dispersal structure (i.e. parachute-like pappus, papery wings, etc.). The architecture of wind dispersed diaspores is a compromise between dispersal and colonisation ability, with larger achenes having greater competitive ability and probability of germination but poorer dispersal ability (Strykstra et

al. 1998; Meyer & Carlson 2001; Cappuccino et al. 2002; Gravuer et al. 2003; Tabassum & Bonser 2017, but see Soons & Heil 2002; Skarpaas et al. 2011). Thus, opposing selection pressures on seed morphology may constrain evolution in edge populations of wind dispersed range-expanding plants.

Many empirical studies exist that explore trade-offs due to selection for particularly advantageous phenotypes in range edge populations. For example, selection for greater dispersal ability in cane toads in range edge populations has been associated with increasing incidences of spinal arthritis (Brown et al. 2007). Similarly, selection for faster sexual maturation at the range front has been found to be associated with reduced life expectancy for the Granville fritillary butterfly (Hanski et al. 2006) and vendace fish (Amundsen et al. 2012). However, trade-offs associated with traits that may be under concurrent selection at range edges are somewhat less well explored. A classic example is the trade-off between dispersal and reproduction at range edges in many insects (Hughes et al. 2003; Simmons & Thomas 2004; but see Hanski et al. 2006), where selection on one trait is constrained by the other by means of resource limitations (Zera & Denno 1997). For plants, a similar trade-off between dispersal and colonisation ability exists and is mediated by seed mass, however, this trade-off has never been explored across the range of wind dispersed invasive species. Understanding more about this fundamental trade-off is essential for elucidating potential evolutionary limits to range advance.

In this study, we explored the dispersal/colonisation ability trade-off by examining dispersal ability, germination success (probability and time of first germination) and seedling growth across the entire distribution of a coastal exotic invasive species growing along the east

coast of Australia. Coastal species represent an ideal system to study such questions as their range is essentially linear with the capacity for spread being on two range edges only (Sagarin & Gaines 2002; Samis & Eckert 2007). We hypothesised that either one of these two scenarios would occur towards range edges: (1) dispersal ability would increase and this would be associated with decreased germination success (probability and time of first germination) and growth, or, (2) dispersal ability would decrease and this would be associated with increased germination success (probability and time of first germination) and growth.

## Materials and methods

### STUDY SPECIES

*Gladiolus gueinzii* Kunze (Iridaceae) is a perennial dune plant native to South Africa. The first record of *G. gueinzii* in Australia was from the port of Stockton, New South Wales in 1950, suggesting that the species was accidentally introduced through ballast water (Heyligers 1999). *Gladiolus gueinzii* is solely confined to beach dunes and has a distribution of nearly 800 km along the east coast of Australia, from South West Rocks, New South Wales to Mallacoota, Victoria. *Gladiolus gueinzii* is facultatively sexual, reproducing clonally by corms and sexually through flowers which are self-compatible (Heyligers 1999; Manning et al. 2011). Flowering occurs between October to December and the fruits ripen through the summer months (December – February), with each fruit producing up to 40 winged seeds (samaras) (Heyligers 1999).

## FIELD DIASPORE COLLECTION

The distribution of *G. gueinzii* along the east coast of Australia was determined by examining occurrence records on Australia's Virtual Herbarium (AVH). From November to December 2015 we conducted an extensive field survey across the recorded range of *G. gueinzii* (and beyond) and found 23 populations that were seeding at the time (Fig. 1, Appendix S1). Each population was separated by at least 1 km. From each *G. gueinzii* population, we collected mature diaspores from multiple plants. Diaspores were loosely stored in envelopes until measurement.

## MEASUREMENT OF DIASPORE TRAITS

Due to the clonal nature of *G. gueinzii*, diaspores were pooled for each population as individuals could not be reliably identified. The dispersal ability of 30 randomly selected diaspores from each population of *G. gueinzii* was measured. Wing area for each diaspore was calculated by scanning each diaspore using a flatbed scanner and calculating the area of the wing using the software program ImageJ (W. Rasband, USA). The weight of each diaspore was determined by removing the wing and weighing the seed to the nearest 0.0001g using an analytical electronic balance (Mettler Toledo, Switzerland). Dispersal ability of *G. gueinzii* diaspores was assessed by calculating wing loading (seed mass/wing area). Wing loading has been established as a reliable indicator for dispersal ability of rotating samaras, such as those of *G. gueinzii*, with lower wing loading values indicating greater dispersal ability (Green 1980; Minami & Azuma 2003). We also assessed variation in the components of wing loading (seed mass and wing area) between populations to understand which component is the primary driver for differences in wing loading.

## SEED GERMINATION TRIAL

Seeds used to measure dispersal traits were subsequently used in the germination trial. Seeds were lightly scarified before being set to germinate on moist filter paper in petri dishes. Three petri dishes were used for each population, with ten seeds in each petri dish. The position of each seed was carefully tracked to investigate how seed traits affected germination. Seeds were kept moist using 1% bleach solution to reduce the chance of mould. Petri dishes were placed in a temperature controlled growth cabinet set at 20°C with a 12 hour photo-period. Petri dishes were randomly placed within shelves in the growth cabinet and relocated every two days to minimise any microclimatic effects that may have been present. Germination of seeds (emergence of radicle) was assessed at the same time every day for 3 weeks, after which the rate of germination had remained constant.

## GROWTH MEASUREMENTS

For each population, 10 randomly selected germinated seeds were used in a subsequent experiment to measure growth. Seedlings were carefully transplanted into pots (diameter 17 cm, depth 17 cm) containing 100% washed beach sand sourced from a commercial supplier (Australian Native Landscapes). The glasshouse temperature was set to  $25 \pm 3^\circ\text{C}$  during the day and  $18 \pm 3^\circ\text{C}$  during the night. Temperature was monitored continuously using a Multigrow Controller System (Autogrow Systems, Auckland, New Zealand). Seedlings were mist watered twice daily for two minutes. If mortality occurred, seedlings were replaced up to one week after the commencement of the experiment. After two weeks of growth, seedlings were given a low concentration (1200 p.p.m.) of liquid fertiliser (Aquasol, Hortico Nurseries, 23 N:3.95 P:14 K). This was repeated every two weeks to prevent complete nutrient depletion. Pots were also randomly assigned a new position in the

glasshouse every two weeks to minimise any glasshouse microclimate effect. After 3 months of growth, total leaf number, total leaf length and plant height were measured. Leaf length was measured as the length from the base of the leaf blade to the tip. Height was measured as the length from the point of attachment to the seed (indicated by a leaf scar) to the tip of the highest point of foliage. As the seedlings were planned to be used for a future experiment and due to seed limitations for some populations, we decided not to conduct destructive biomass harvesting.

## STATISTICAL ANALYSIS

To assess the spread distance of each population from site of introduction, we designated the first herbarium record for *G. gueinzii* from the AVH (Stockton, 32° 55' 00"S 151° 46' 00"E) as the source population and calculated the straight line distance to each population.

We conducted linear regressions to test for associations between wing loading, seed mass, wing area and time to first germination with distance from source population. Data was pooled across all sites to investigate any trade-offs between seed mass, wing loading and germination/growth traits. For analyses with count data (i.e. number of days to first germination, number of leaves), generalised linear models with Poisson distribution and log link functions were used. We examined the probability of germination with distance, seed mass and wing loading using logistic regression with a logit link function and binomial distribution. Significance of each variable was tested using likelihood ratio tests. Variables were log transformed to fulfil assumptions of statistical tests where applicable. All analyses were conducted using R version 3.2.4 (R Development Core Team 2016).



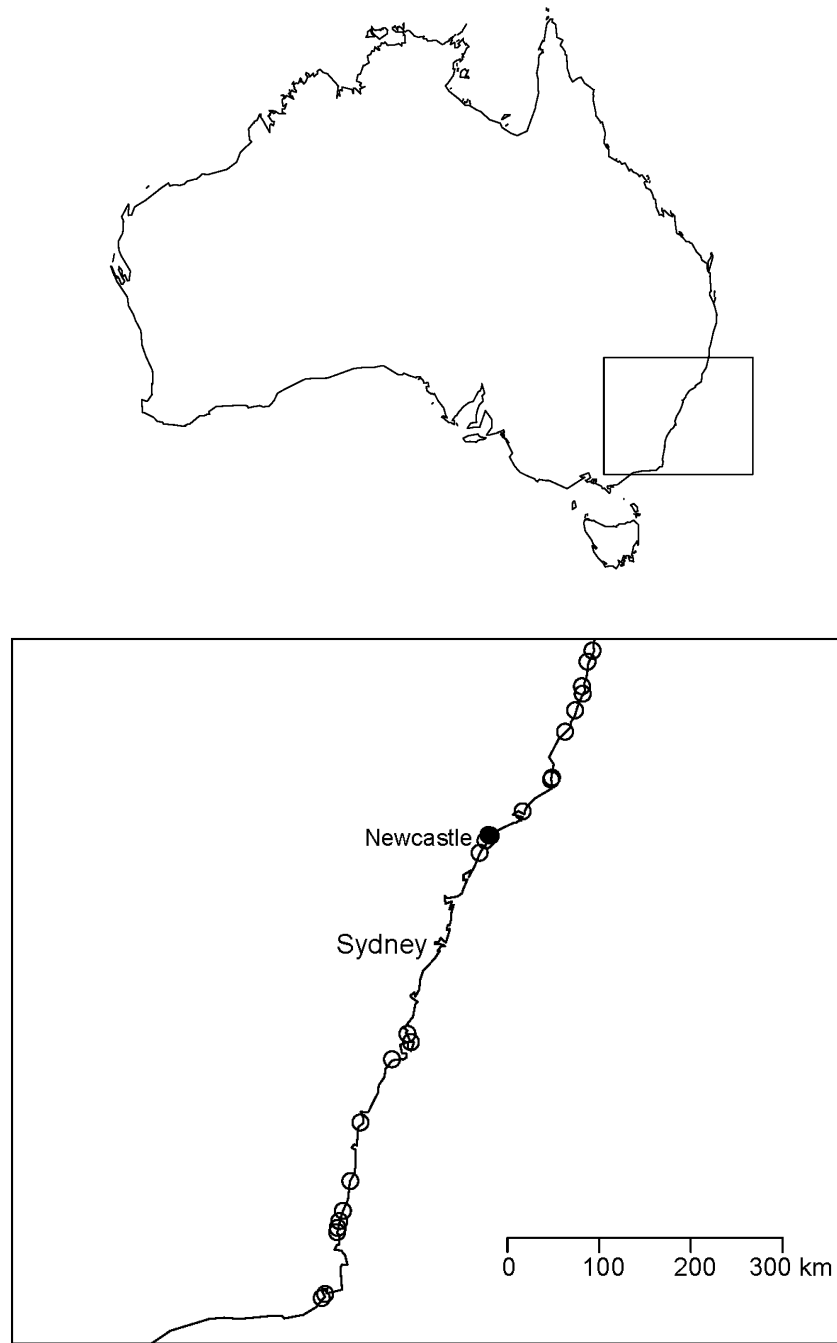
## Results

For population level data, we found a significant negative relationship between wing loading and distance from source population ( $F_{1,21} = 4.99$ ,  $p = 0.025$ ,  $R^2 = 0.19$ , Fig. 2a), suggesting that higher dispersal propensity was favoured towards the range edge. Examining the components of wing loading separately revealed a significant negative relationship between seed mass and distance from source population ( $F_{1,21} = 4.37$ ,  $p = 0.037$ ,  $R^2 = 0.17$ , Fig. 2b). However, there was no significant relationship between wing area and distance from source population ( $F_{1,21} = 0.65$ ,  $p = 0.42$ ,  $R^2 = 0.030$ , Fig. 2c).

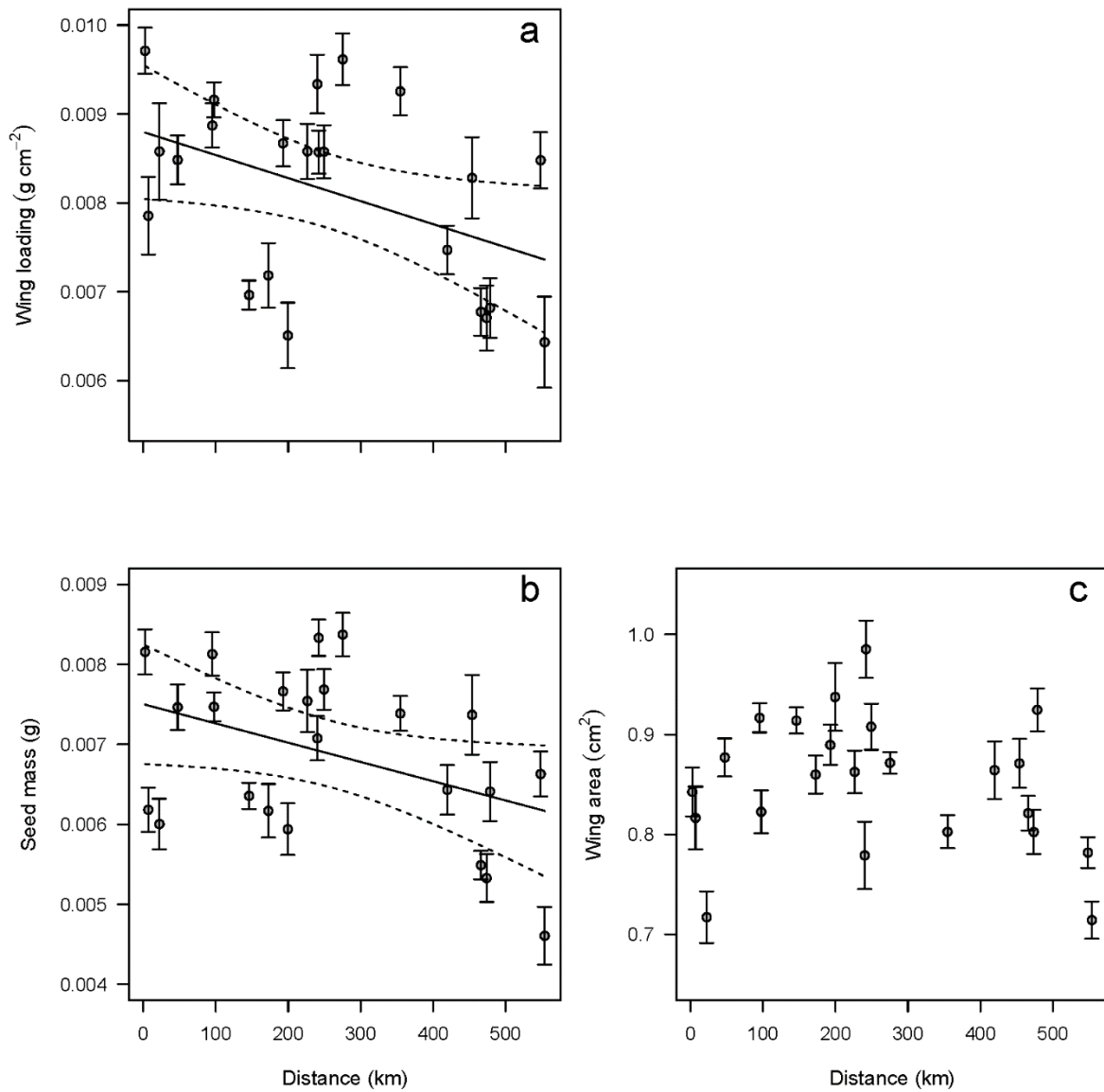
After 21 days, we observed 97% germination across all populations of *G. guenzii*. Time to first germination decreased with increasing distance from source population (d.f. = 1,21, coefficient = -0.001,  $p = 0.032$ , Fig. 3a). However, there was no relationship between probability of germination after 21 days and distance (coefficient = -0.0007,  $p = 0.23$ ). In terms of growth related traits, there was no significant relationship between number of leaves (d.f. = 1,21,  $p = 0.17$ ,  $R^2 = 0.083$ , Fig. 3c), plant height (d.f. = 1,21,  $p = 0.24$ ,  $R^2 = 0.062$ , Fig. 3b) and total leaf length (d.f. = 1,21,  $p = 0.053$ ,  $R^2 = 0.15$ , Fig. 3d) with distance from source population.

When data were pooled across all populations, days to germinate increased with seed mass (d.f. = 1,667, coefficient = 74.47,  $p < 0.0001$ , Fig. 4a), indicating that lighter seeds were also quicker to germinate. However, there was no significant relationship between probability of germination after 21 days and seed mass (coefficient = -29.80,  $p = 0.80$ ) or wing loading (coefficient = -82.10,  $p = 0.44$ ). There were significant positive relationships between plant height and total leaf length and seed mass (height:  $F_{1,228} = 7.11$ ,  $p = 0.008$ ,  $R^2 = 0.030$ , Fig.

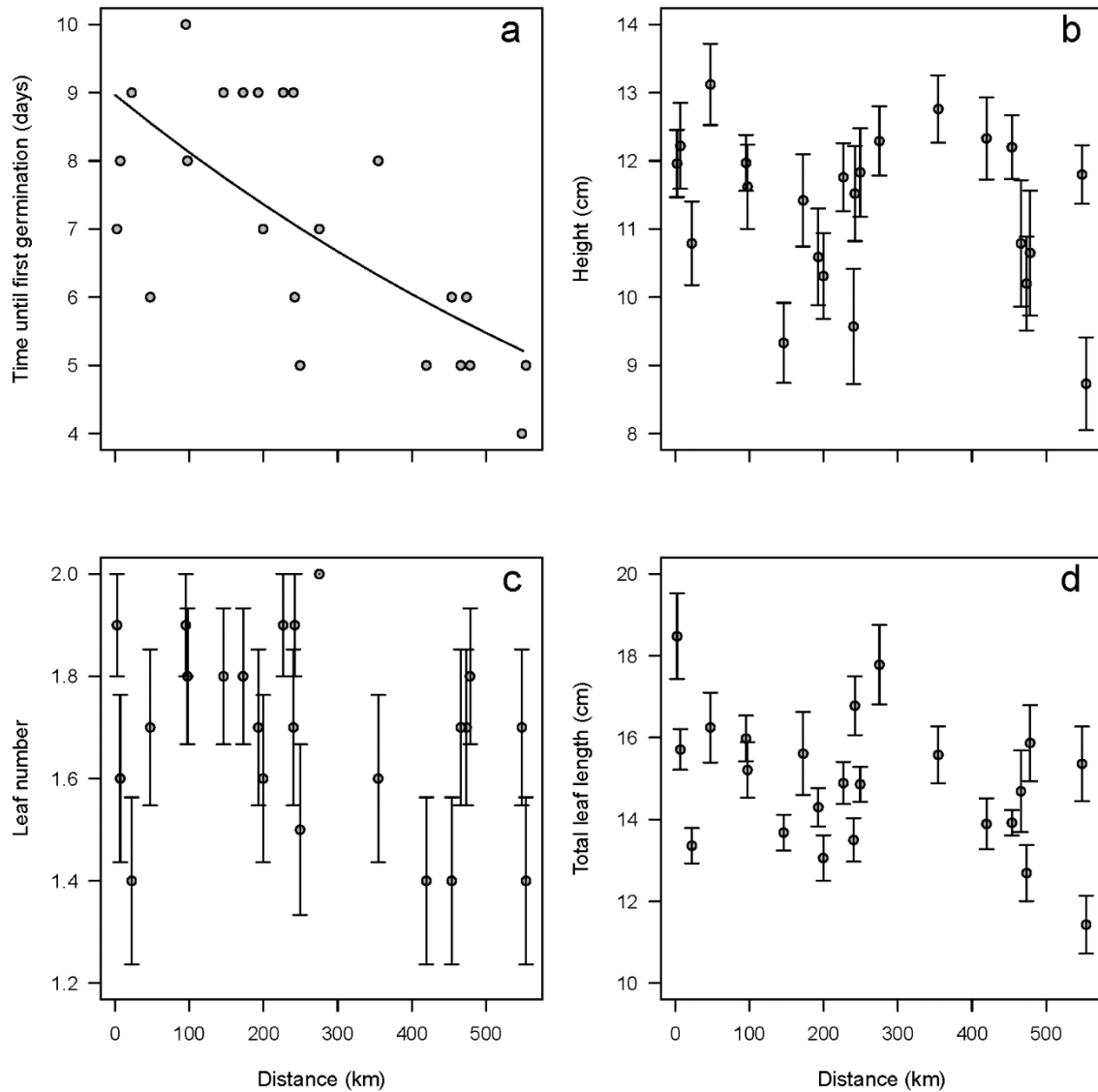
4b; leaf length:  $F_{1,228} = 15.47$ ,  $p < 0.0001$ ,  $R^2 = 0.064$ , Fig. 4c), indicating that heavier seeds produced larger seedlings. Days to germinate increased with increasing wing loading (d.f. = 1, 668, coefficient = 55.01,  $p < 0.0001$ , Fig. 5a), indicating that diaspores with higher dispersal ability were also quicker to germinate. Plant height and total leaf length scaled positively with wing loading (height:  $F_{1,228} = 5.10$ ,  $p = 0.024$ ,  $R^2 = 0.022$ , Fig. 5b; leaf length:  $F_{1,228} = 7.83$ ,  $p = 0.005$ ,  $R^2 = 0.033$ , Fig. 5c) indicating that diaspores with high dispersal ability produce smaller seedlings.



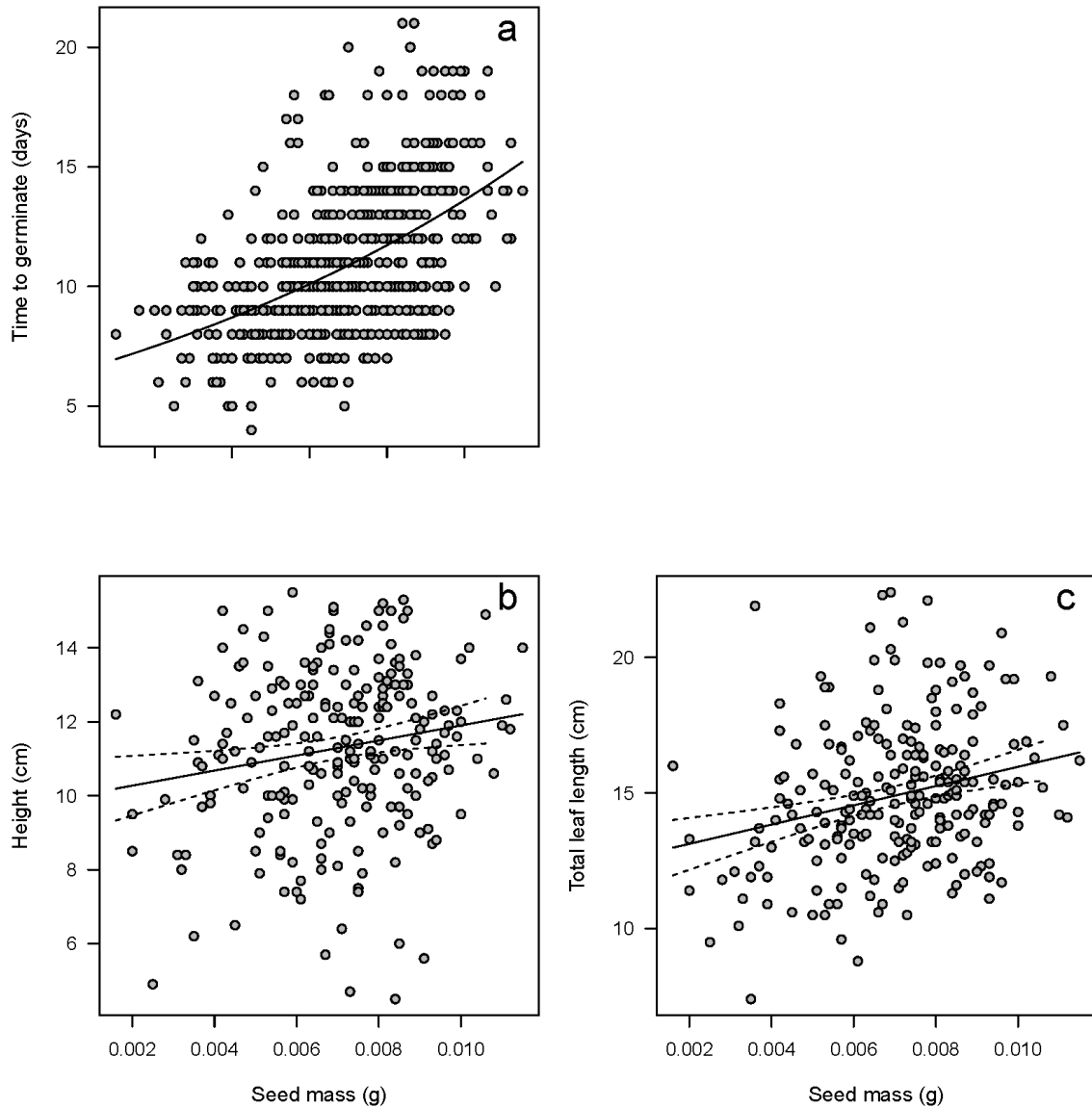
**Fig. 1** Map of the populations of *Gladiolus gueinzii* from which seeds were collected in this study. Open circles represent sample sites and the closed circle represents the assumed introduction point based on herbarium records.



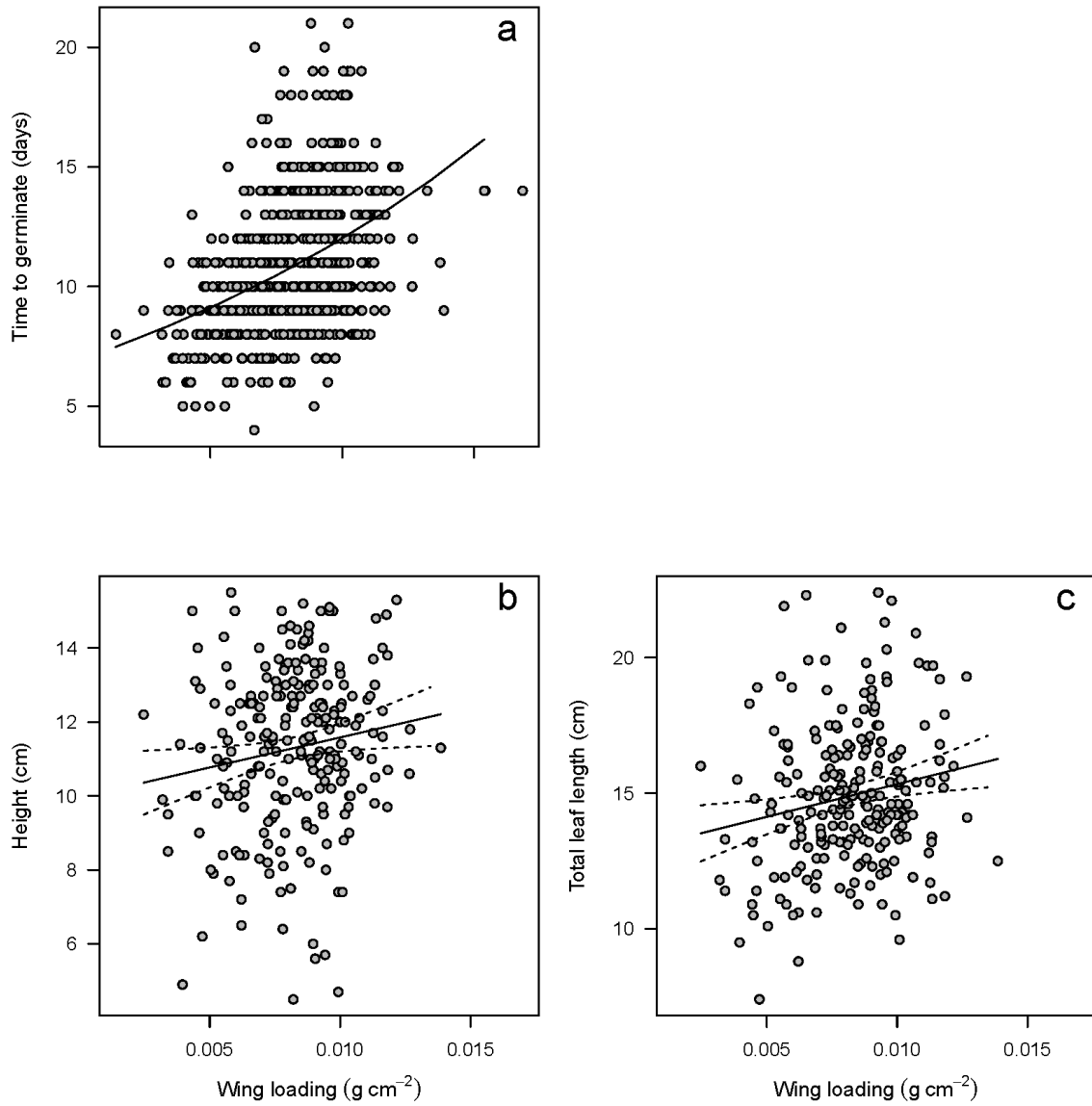
**Fig. 2** Linear regressions showing the relationships between (a) wing loading ( $y = -0.0000026x + 0.0088$ ,  $R^2 = 0.19$ ,  $p = 0.025$ ) (b) seed mass ( $y = -0.0000024x + 0.0075$ ,  $R^2 = 0.17$ ,  $p = 0.037$ ) (c) wing area ( $y = -0.000066x + 0.087$ ,  $R^2 = 0.030$ ,  $p = 0.42$ ) and distance from source population for *G. gueinzii*. Each point represents the average value for a single population. Error bars represent standard errors. Dotted lines show 95% confidence limits of significant regressions.



**Fig. 3** Regressions showing the relationships between (a) time until first germination ( $\log(y) = -0.0010x + 2.19$ ,  $p = 0.032$ ) (b) height ( $y = -0.0016x + 11.70$ ,  $R^2 = 0.062$ ,  $p = 0.24$ ) (c) leaf number ( $y = -0.00029x + 1.77$ ,  $R^2 = 0.083$ ,  $p = 0.17$ ) (d) total leaf length ( $y = -0.0036x + 15.84$ ,  $R^2 = 0.15$ ,  $p = 0.053$ ) and distance from source population for *G. gueinzii*. Figure (a) represents a Poisson regression while figures (b), (c) and (d) represent linear regressions. For figures (b), (c) and (d) each point represents the average value for a single population. Error bars represent standard errors. Note that for figure (c) there is one population with no error bars as each replicate for this population had the same number of leaves. Regression lines are only shown for significant relationships.



**Fig. 4** Regressions showing the relationships between (a) time to germinate ( $\log(y) = 74.47x + 1.87$ ,  $p < 0.0001$ ) (b) height ( $y = 203.45x + 9.86$ ,  $R^2 = 0.03$ ,  $p = 0.008$ ) (c) total leaf length ( $y = 355.25x + 12.41$ ,  $R^2 = 0.06$ ,  $p < 0.0001$ ) and seed mass for *G. gueinzii*. Figure (a) represents a Poisson regression while figures (b) and (c) represent linear regressions.



**Fig. 5** Regressions showing the relationships between (a) time to germinate ( $\log(y) = 55.01x + 1.93$ ,  $p < 0.0001$ ) (b) height ( $y = 162.22x + 9.96$ ,  $R^2 = 0.02$ ,  $p = 0.024$ ) (c) total leaf length ( $y = 240.78x + 12.92$ ,  $R^2 = 0.03$ ,  $p = 0.005$ ) and wing loading for *G. gueinzii*. Figure (a) represents a Poisson regression while figures (b) and (c) represent linear regressions.

## Discussion

Trade-offs associated with traits under selection at species' range edges have seldom been explored and represent a major gap in our understanding of evolutionary constraints on range expansion. In this study we explored the potential trade-off between dispersal and colonisation ability towards range edges of *G. gueinzii*, an exotic invasive plant occurring along eastern Australia.

We found an increase in dispersal ability (mediated by a decrease in seed mass) towards the range edges of *G. gueinzii*, which is consistent with several other studies on different taxa (e.g. Cwynar & MacDonald 1987; Phillips et al. 2006; Darling et al. 2008; Berthouly-Salazar et al. 2012). However, contrary to our expectations, we did not observe a reduction in colonisation ability towards range edges. That is, seeds from range edge populations of *G. gueinzii* did not experience a reduction in growth or probability of germination. This is despite there being a significant positive relationship between wing loading and growth related traits when data were pooled (diaspores with higher dispersal ability produced shorter seedlings with shorter leaves). This disconnect between theorised and observed relationships in terms of seed dispersal/colonisation traits has previously been observed. Investigating the relationship between dispersal ability, germination and growth, Skarpaas et al. (2011) found no relationship between dispersal ability and germination or growth despite observing smaller seeds to have higher dispersal ability and lower germination and growth. They attribute some of this disconnect to additional factors that may explain much of the variation in dispersal ability other than seed mass such as release height. The non-significant relationships between growth traits and distance from the source population that we observed may be due to additional factors other than seed mass affecting plant growth.



Furthermore, because we measured growth-related traits after three months of growth we may have missed any effects of seed size that may have manifested in the early or even later stages of life. For example, larger seed sizes towards core populations may have resulted in faster initial growth rates or produced more competitive individuals as the plants matured compared to range edge populations. However, because we only measured growth-related traits at one time point we cannot reliably comment on this effect.

Despite there being no reduction in probability of germination towards range edges, seeds from range edge populations were observed to have faster germination times compared to seeds from populations near the range core. Faster germination may be a beneficial life history strategy as it may aid in competition avoidance while interspecific vegetation cover is low, and is a trait that is synonymous with invasion success (Bartle et al. 2013; Gioria & Pyšek 2016). As *G. gueinzii* is a pioneer dune species (Heyligers 1999), faster germination time would be more beneficial for colonisation compared to larger seedling size.

Nevertheless, the combination of increased dispersal ability and faster germination towards range edge populations of *G. gueinzii* may be suggestive of a shift towards a faster spread strategy, potentially accelerating the rate of range expansion.

An alternative explanation for the reduction in seed mass towards range edges of *G. gueinzii*, irrespective of selection on dispersal ability, is a reduction of habitat quality towards range edges. Transplant experiments have revealed fitness declines towards and beyond species range edges (e.g. Levin & Clay 1984; Angert & Schemske 2005; Geber & Eckhart 2005, but see Samis & Eckert 2009; Katz & Ibáñez 2016), suggesting that deteriorating environmental conditions may be an explanation for the existence of species'

range boundaries (Geber 2008). If range edge populations of *G. gueinzii* experience more stressful conditions compared to range core populations, then reduced seed mass in range edge populations may instead reflect quality of the maternal environment (Wulff 1986). Another possible explanation for decreased seed mass towards range edges may be the existence of a seed size/seed number trade-off across the species' range. Seed size/seed number trade-offs, where plants either produce a large amount of small seeds or a small amount of large seeds for a given investment in reproduction, have been observed across many species (i.e. Werner & Platt 1976; Jakobsson & Eriksson 2000, 2003; Leishman et al. 2000; Leishman 2001). Across an invasive species' range, selection for a greater number of recruitment opportunities towards range edges may favour individuals who produce a large number of small seeds. Although reductions in seed size (Sugiyama 2003; Huang et al. 2015) and increased seed number (Alexander et al. 2007) have been found towards range edges of invasive species, no studies have examined this trade-off across species' ranges. Both maternal effects and seed size/seed number trade-offs may explain the observed variation in seed size across the range of *G. gueinzii* and controlled glasshouse studies are needed to investigate the relative contribution of each of these factors.

A significant caveat of laboratory studies is that they often fail to accurately reflect conditions in the field (Gioria & Pyšek 2016). Dispersal ability, germination success and growth have been found to be significantly influenced by various biotic and abiotic conditions that may have differed between our sampled populations. For example, factors such as wind velocity, release height and height of surrounding vegetation may significantly affect dispersal ability in the field (Gavuer et al. 2003; Skarpaas et al. 2011). Likewise, germination is significantly influenced by abiotic conditions such as temperature and light

availability (Bellairs & Bell 1990; Benech-Arnold et al. 2000; Milberg et al. 2000) and growth may be affected by associations with soil microbes (Andonian et al. 2011). Thus, the conditions presented in the laboratory may have influenced differences in dispersal ability, germination success and growth between populations and inferences to patterns in the field should be made with caution. Nonetheless, laboratory studies are necessary to test underlying mechanisms that may be driving patterns of dispersal, germination and growth in the field (Gravuer et al. 2003; Tabassum & Bonser 2017).

The process of range expansion imposes strong selection pressures to maximise traits associated with dispersal and colonisation at the range edge. However, the presence of a trade-off between these two fundamental life history traits may set limits to the range expansion process. Our study revealed that dispersal ability increased towards range edges of *G. gueinzii*, however, this was not associated with a decrease in probability of germination or growth after 3 months. In fact, seeds from range edge populations had faster germination than seeds nearer to the site of first introduction. Greater dispersal ability and faster germination at range edges potentially represents a shift towards a faster spread strategy and may contribute to accelerated range advance. Thus, understanding the nature of trade-offs in range edge populations can provide valuable insights into the evolutionary processes underpinning species' range shifts. Future studies investigating the role of maternal effects and existence of a seed size/seed number trade-off at the range edge will provide further insights into the dynamic nature of species' range expansion.

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**Appendix S1.** Sites sampled for *Gladiolus gueinzii* from north to south showing the straight line distance from its first herbarium record (Stockton 32° 55' 00"S 151° 46' 00"E).

Site	Latitude (S)	Longitude (E)	Distance (km)
Hat Head	31°02'50"	153°02'39"	240.3
Richardson's Crossing	31°09'32"	152°58'57"	226.6
Point Plomer	31°24'31"	152°54'51"	199.5
Port Macquarie	31°29'03"	152°55'24"	192.9
Dunbogan	31°39'08"	152°49'48"	172.5
Crowdy Head	31°52'09"	152°42'24"	146.1
Boomerang Beach	32°20'07"	152°32'47"	97.53
Blueys Beach	32°21'13"	152°32'06"	95.37
Hawks Nest	32°40'34"	152°11'09"	47.44
Nobbys Beach	32°55'22"	151°47'34"	2.53
Dudley Beach	32°58'19"	151°43'51"	6.997
Swansea Heads	33°05'47"	151°39'30"	22.39
Culburra	34°55'54"	150°46'20"	242.1
Currarong	35°00'52"	150°48'50"	249.3
Swanhaven	35°11'19"	150°34'58"	275.2
Tomakin	35°49'46"	150°11'40"	354.6
Bermagui	36°25'21"	150°04'13"	419.5
Tathra Beach	36°43'29"	149°58'50"	453.7
North Tura Beach	36°49'42"	149°56'08"	465.9
Merimbula	36°53'49"	149°54'57"	473.6
Pambula	36°56'26"	149°54'32"	478.4
Mallacoota	37°34'05"	149°45'42"	548.3
Secret Beach	37°36'31"	149°43'14"	553.8

## **5 | It doesn't take two to tango: increased capacity for self-fertilisation towards range edges of two coastal invasive plant species in eastern Australia**

This chapter is currently in review at *Biological Invasions*.

My contribution to the research and paper: Concept – 90%; Data collection – 85%; Analysis – 100%; Writing – 90%

## Abstract

Understanding how selection pressures change during the course of an invasion is a key question in invasion biology. Shifts to greater reproductive success and output are expected to occur towards range edges of expanding invasive species as a means of increasing colonisation opportunities and accelerating further spread. In a glasshouse experiment, we investigated shifts in reproductive traits (allocation to reproduction, seed number vs. seed size, capacity for self-fertilisation) across multiple populations spanning the entire range of two coastal exotic invasive plant species (*Gladiolus gueinzii* Kunze and *Hydrocotyle bonariensis* Lam.) in eastern Australia. Although there was no significant increase in allocation to reproduction towards range edges or changes in seed provisioning, range edge populations displayed an increased capacity for self-fertilisation in the absence of pollinators for both species. For *H. bonariensis* this entailed an increase in the probability of fruit production towards range edges while for *G. gueinzii* it was an increase in the probability of forming developed seeds towards range edges. Greater capacity for self-fertilisation may facilitate further range expansion as it alleviates any reliance on external factors for pollination at the range edge. Our results suggest that increased capacity for self-fertilisation towards range edges may be a key factor in promoting range expansion in some invasive species.

**Key words:** autonomous self-fertilisation, invasive species, introduced range, range expansion, reproductive traits

## Introduction

Invasive species have been implicated in a number of adverse environmental outcomes such as loss of biodiversity and ecosystem function (Clavero & García 2005; Molnar et al. 2008; Hedja et al. 2009). As such, much research has been dedicated to studying the key life history traits of successful invasive species in order to best understand what makes a species a successful invader. Studies comparing trait differences between invasive species from their native range compared to their introduced range have found that introduced populations of invasive species have a suite of different traits including greater biomass (Siemann & Rogers 2001; Caño et al. 2008), fecundity (Caño et al. 2008; Correia et al. 2016), and plasticity (Davidson et al. 2011). These “home and away” studies are predicated on the assumption that evolutionary processes in the introduced range are relatively unimportant beyond those encountered upon first introduction (Phillips et al. 2010, but see Dlugosch et al. 2015). However, invasive species rarely remain static in their introduced range, with many still undergoing range expansion and continually experiencing novel environments and selection pressures towards range edges. As such, many of our existing ideas regarding successful invasions can be applied within the invaded range of species. As species expand in their range, the process of range expansion itself may select for advantageous “invasive” traits in range edge populations to facilitate further range expansion (Phillips et al. 2010).

Increased allocation to reproductive structures and increased fecundity have commonly been associated with the success of invasive species when introduced to novel environments (e.g. Caño et al. 2008; Correia et al. 2016). As populations expand, these traits are likely to be under strong selection towards range edges as a means of significantly increasing colonisation opportunities, hence facilitating further range expansion. Increased

allocation to reproductive biomass in frontal populations has been found in a variety of range expanding invasive taxa (e.g. Lester et al. 2007; Ling et al. 2008; Gutowsky & Fox 2012; Lopez et al. 2012; Houston et al. 2013; Kambo & Kotanen 2014; Masson et al. 2016). However, within a given reproductive event, organisms can produce a small number of large offspring or a large number of small offspring due to resource allocation trade-offs (Westoby et al. 1992). Although offspring with greater maternal provisioning have been shown to be more competitive (Jakobsson & Eriksson 2000, 2003), lower conspecific density towards range edges of invasive taxa is expected to drive selection for increased reproductive rate at the expense of competitive ability (Burton et al. 2010; Alex Perkins et al. 2013). In terms of reproduction in plants, producing smaller seeds will result in a greater number of offspring for a given reproductive event and as such small seed size has been widely regarded as a key element in ecological strategies that rely on frequent colonisation events (Henery & Westoby 2001) such as at invasion fronts. Changes in seed number (Alexander et al. 2007) and seed provisioning (Huang et al. 2015, Tabassum & Leishman 2018, but see Kambo & Kotanen 2014) have been reported towards range edges of many invasive plants, however a shift towards a greater number of smaller seeds towards range edges has not been explored.

Another trait associated with successful invasions in novel environments (particularly with respect to invasive plants) is the ability to produce offspring through self-fertilisation (Petanidou et al. 2012; but see Atlan et al. 2015). Plants that are able to self-pollinate and self-fertilise are likely to be successful invaders because reproduction is not constrained by external factors such as mate and/or pollinator limitation (Baker 1974; Pannell et al. 2015). Numerous studies have found significantly higher numbers of self-compatible invasive

plants than self-incompatible ones in given regional floras, many of which have also been found to autonomously self-fertilise (self-fertilise without pollination) (Rambuda & Johnson 2004; van Kleunen & Johnson 2007; van Kleunen et al. 2008; Hao et al. 2011, but see Sutherland 2004). Increased capacity for uniparental reproduction is not only beneficial for initial establishment but also at range edges of expanding species where individuals may occur at lower densities (experiencing mate limitation) and also be less apparent to pollinators (Pannell & Barrett 1998; Pannell et al. 2015). Many studies have found increased capacity for self-fertilisation in peripheral populations of native species where local extinction and continual re-colonisation may be occurring (e.g. Busch 2005; Herlihy & Eckert 2005; Darling et al. 2008; Griffin & Willi 2014). However, few studies have examined whether this is a mechanism facilitating range expansion in invasive plants (Colautti et al. 2010).

Despite ongoing research into understanding traits of successful invasive species, we still know relatively little about how selection for traits alters during the course of an invasion (Gaston 2009; Lankau et al. 2009; Kilkenny & Galloway 2012). Reproductive traits such as increased fecundity and increased capacity to self-fertilise are likely to be under strong selection towards range edges, however few studies have examined these relationships. Additionally, most of these studies have sampled from a single range edge and/or only compared populations from the range centre and range edge, potentially obscuring our ability to find consistent patterns (Sagarin & Gaines 2002b; Sexton et al. 2009; Vaupel & Matthies 2012; Nunes et al. 2016).

In this study, we explored shifts in key reproductive traits across the entire distribution of two coastal exotic invasive species growing along the east coast of Australia. Coastal species represent an ideal system to study such questions as their range is essentially linear with the capacity for spread being on two range edges only (Sagarin & Gaines 2002a, b; Samis & Eckert 2007). We hypothesised that range edge populations would: (i) allocate a greater proportion of biomass to reproduction; (ii) display a greater capacity for autonomous self-fertilisation (ability to self-fertilise without pollination); and (iii) produce a greater number of smaller seeds, compared to populations from where they were first introduced (range core populations).

## Materials and methods

### STUDY SPECIES

*Hydrocotyle bonariensis* Lam. (Araliaceae) is a perennial plant native to Africa, North and South America and has become widely naturalised on coastal dunes along eastern Australia. The first record of *H. bonariensis* in Australia was made in 1893 near Botany Bay, New South Wales. Although *H. bonariensis* is primarily found on dune systems, it occasionally occurs along rivers and lagoon outlets (Heyligers 1998). Its distribution in Australia is along the east coast from southern Queensland to Victoria (approximately 1200 km). *Hydrocotyle bonariensis* was accidentally introduced to Australia, possibly through ballast water (Heyligers 2008; Murray & Phillips 2012). It is facultatively sexual, reproducing clonally using rhizomes (modified underground stems) and sexually using flowers. *Hydrocotyle bonariensis* produces compound inflorescences containing hundreds of cream coloured flowers throughout the year, with each flower potentially producing one indehiscent schizocarp containing two seeds (Evans 1992). Roots, leaves and inflorescences arise from nodes along

the rhizome which is generally buried in the sand at a depth of 2-5 cm (Knight & Miller 2004). The extensive rhizome systems of *H. bonariensis* can be comprised of over a thousand individual ramets that span many square meters of dune systems (Evans 1991).

*Gladiolus gueinzii* Kunze (Iridaceae) is a perennial dune plant native to dune systems in South Africa. The first record of *G. gueinzii* in Australia was from the port of Stockton, New South Wales in 1950, suggesting that the species was accidentally introduced through ballast water (Heyligers 1999). This species is solely confined to beach dunes and has become naturalised nearly 800 km along the east coast of Australia, from South West Rocks, New South Wales to Mallacoota, Victoria. *Gladiolus gueinzii* is facultatively sexual, reproducing clonally by cormels (round, buoyant, underground growths) and sexually through self-compatible flowers (Heyligers 1999; Manning et al. 2011). Flowering occurs between October to December, with plants producing a single flower stem containing 1-6 pink flowers that open sequentially (Heyligers 1999). Fruits ripen through the austral summer (December – February), with each fruit producing up to 40 winged seeds (samaras) (Heyligers 1999). This species typically occurs in dense populations close to the high-tide mark, with individuals occurring 20-30 cm apart (Manning et al. 2011).

#### FIELD COLLECTION

The distribution of *H. bonariensis* and *G. gueinzii* along the east coast of Australia was determined based on occurrence records from Australia's Virtual Herbarium (AVH). From November to December 2015 populations of *H. bonariensis* and *G. gueinzii* from their entire range along the east coast of Australia were sampled. We travelled 100 km further than the last recorded occurrence of both species on AVH at each range edge to ensure accurate



sampling. For *G. gueinzii* we collected seeds from 23 populations (Appendix S1). From each population we collected approximately 100 seeds from up to ten individuals that were at least 5 m apart. As *H. bonariensis* was not seeding throughout its range during fieldwork, we collected 3-4 5 cm long rhizomes from 24 populations (Appendix S1).

## PLANT GROWTH

*Hydrocotyle bonariensis* rhizomes were wrapped in moist paper towel and transported back to Macquarie University, North Ryde, New South Wales. Rhizomes were lightly buried in shallow trays (20 cm x 25 cm x 5 cm) using 100% washed beach sand sourced from a commercial supplier (Australian Native Landscapes, Sydney, New South Wales). Due to space constraints in the glasshouse, 2-3 rhizomes from each population were grown together in one tray. For *G. gueinzii*, 20 randomly selected seeds from each population were lightly scarified before being set to germinate on moist filter paper in petri dishes. Seeds were kept moist using 1% bleach solution to reduce the chance of mould. Petri dishes were placed in a temperature controlled growth cabinet set at 20°C with a 12 hour photo-period until germination (approximately 1-2 weeks). Ten randomly selected germinated seedlings from each population were then transplanted into separate pots (diameter 17 cm, depth 17 cm) containing 100% washed beach sand sourced from a commercial supplier (Australian Native Landscapes, Sydney, New South Wales). Glasshouse temperatures were set to 25 ± 3°C during the day and 18 ± 3°C during the night. Temperature was monitored continuously using a Multigrow Controller System (Autogrow Systems, Auckland, New Zealand). Plants were mist watered twice daily for two minutes with additional watering being provided on hot days. After two weeks of growth, plants were provided with a low concentration (0.15g dissolved in 125 mL of water) of liquid fertiliser (Aquasol, Hortico Nurseries, 23 N:3.95 P:14

K). This was repeated every two weeks to prevent nutrient depletion. To help control an outbreak of leaf scale on *H. bonariensis*, plants were sprayed with a non-hazardous insecticide (PestOil™, Arthur Yates & Company, Homebush, Australia) every week.

## REPRODUCTIVE TRAITS

After approximately one year (*H. bonariensis*) and 1.5 years (*G. gueinzii*) of growth, plants began to flower. For each population, up to 20 inflorescences were randomly selected to assess fertilisation and seed traits. More inflorescences were sampled for *G. gueinzii* due to the significantly lower number of flowers per inflorescence compared to *H. bonariensis*. The number of flowers were counted on each inflorescence unaided (*G. gueinzii*) or with a 10x magnifying hand lens (*H. bonariensis*) to determine autonomous self-fertilisation ability. Flower counting occurred from November 2016 to January 2017 (*H. bonariensis*) and July 2017 to December 2017 (*G. gueinzii*), with flowers counted randomly across populations. As flowers on inflorescences opened sequentially, for each inflorescence, flowers and developing flower buds were counted when 75% of the flowers on the inflorescence were open. Counted inflorescences were tagged and subsequently bagged with mesh cloth to prevent the loss of developing fruit and seeds.

After approximately 1-3 months the bagged inflorescences developed fruit and began to wither or dehisce. At this stage fruit were harvested and allowed to air dry in their mesh bags in the lab for two weeks. We then counted the number of fruit produced per inflorescence for each species. Additionally for *G. gueinzii* we counted the number of filled and aborted seeds within each seed pod on each inflorescence. This could not be performed for *H. bonariensis* as the fruit were indehiscent and therefore did not open to release their

seeds, although each fruit typically contains two seeds (Evans 1992). Once the seeds were counted they were then weighed to the nearest 0.0001g using an analytical electronic balance (Mettler Toledo, Switzerland).

Once flowering and seed set had completely finished, all *G. gueinzii* plants were destructively harvested to examine biomass allocation to sexual and asexual reproduction. For *H. bonariensis*, ongoing problems with leaf scale infestation and the frequent trimming of vegetative growth that was required to try and control this infestation throughout the experiment meant that we were unable to adequately measure biomass allocation for this species. For *G. gueinzii*, biomass was separated into vegetative (leaves and roots), clonal (cormels) and sexual (flower stalks, flowers, seed pods and seeds) components, dried at 70°C for 48 hours and weighed. As we required the seeds for future experiments, we left them to air dry to avoid heat damaging them. Sexual reproductive effort (SRE) and clonal reproductive effort (CRE) were then calculated as:

$$\text{SRE} = S/(C+V+S) \quad \text{CRE} = C/(S+V+C)$$

where S is the total dry mass of sexual components, C is the total dry mass of clonal components and V is the total dry mass of vegetative components.

## STATISTICAL ANALYSIS

To assess the spread distance of each population from the site of introduction, we designated the first herbarium record for each species (Stockton (32° 55' 00"S, 151° 46' 00"E) for *G. gueinzii* and Lady Robinson's Beach (33° 58' 00"S, 151° 09' 00"E) for *H.*

*bonariensis*) as the source population and calculated the straight line distance to each population.

Due to the clonal nature of both of our study species, values for all reproductive traits were averaged for each population. To investigate differences in reproductive effort across the range of *G. gueinzii*, we used linear regressions to examine the relationships between SRE and CRE with distance from source population and total biomass (as reproductive allocation has been shown to correlate strongly with plant size e.g. Samson & Werk 1986). Plants that did not flower during the entirety of the experiment were removed from the analysis (this led to 18 replicates being removed). To assess autonomous self-fertilisation ability, we examined the probability of flowers developing into fruit for each inflorescence across the range of our study species using logistic regression with a logit link function and binomial distribution. Additionally for *G. gueinzii*, we assessed the probability of forming filled seeds by comparing the number of filled and aborted seeds in each inflorescence using the same analysis. Finally, we conducted linear regressions to assess the relationships between average seed number and seed size per inflorescence with distance from source population for each species. For *H. bonariensis*, populations that produced seed on less than two inflorescences (Callala, Dunbogan, Kingscliffe and Port Macquarie) were excluded from analysis due to inability to obtain average values. For *G. gueinzii*, total biomass was included as an additional explanatory variable due to the influence of plant size on seed partitioning (e.g. Jakobsson & Eriksson 2000). Significance of variables in each model was tested using likelihood ratio tests. Variables were log transformed to fulfil assumptions of statistical tests where applicable. All analyses were conducted using R version 3.2.4 (R Development Core Team 2016).

## Results

### REPRODUCTIVE EFFORT

For both *G. gueinzii* and *H. bonariensis*, there was no significant relationship between average number of flowers per inflorescence and distance from source population (*G. gueinzii*:  $F_{1,21} = 0.26$ ,  $p = 0.61$ ,  $R^2 = 0.012$ ; *H. bonariensis*:  $F_{1,22} = 0.35$ ,  $p = 0.55$ ,  $R^2 = 0.016$ ). Additionally, for *G. gueinzii* there was no relationship between average number of flowers per plant and distance from source population ( $F_{1,21} = 1.52$ ,  $p = 0.22$ ,  $R^2 = 0.067$ ). For *G. gueinzii*, there was no significant relationship between clonal reproductive effort ( $F_{1,21} = 0.004$ ,  $p = 0.40$ ,  $R^2_{\text{adj}} = -0.05$ , Fig. 1a) or sexual reproductive effort ( $F_{1,21} = 0.83$ ,  $p = 0.43$ ,  $R^2_{\text{adj}} = -0.008$ , Fig. 1c) with distance from source population. There was, however, a significant positive relationship between clonal reproductive effort ( $F_{1,21} = 5.71$ ,  $p = 0.012$ ,  $R^2_{\text{adj}} = 0.18$ , Fig. 1b) and total biomass but this was not found for sexual reproductive effort ( $F_{1,21} = 0.22$ ,  $p = 0.84$ ,  $R^2_{\text{adj}} = -0.04$ , Fig. 1d).

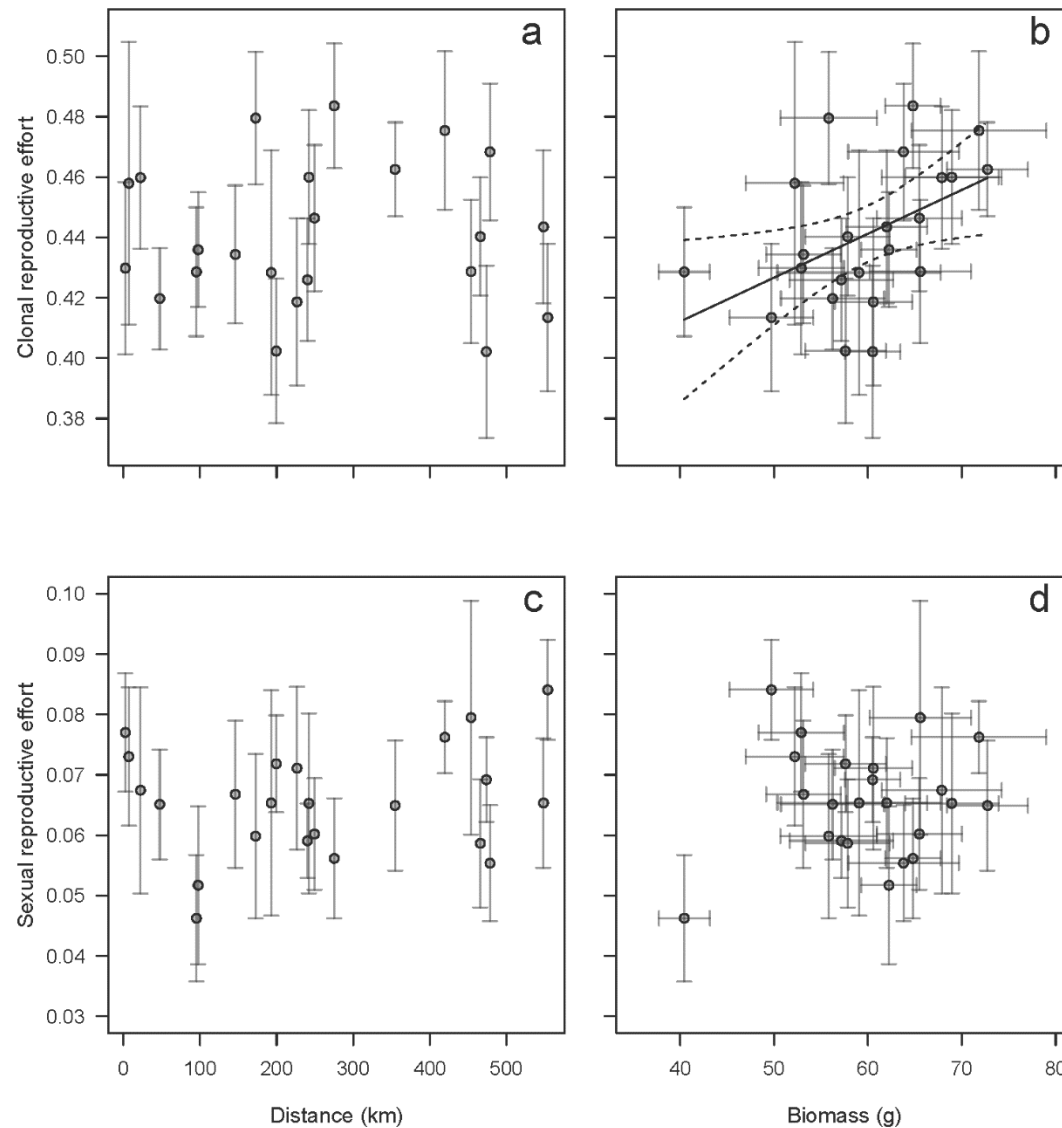
### AUTONOMOUS SELF-FERTILISATION

For *G. gueinzii*, all populations produced fruit through autonomous self-fertilisation (fertilisation in the absence of pollinators) while for *H. bonariensis* one population (Port Macquarie) did not autonomously self-fertilise. Within populations, the degree of fruit production through autonomous self-fertilisation was higher for *G. gueinzii* (60-100%) compared to *H. bonariensis* (13-100%). There was a significant increase in the probability of producing fruit through autonomous self-fertilisation with increasing distance from source population for *H. bonariensis* (odds ratio = 1.0005, d.f. = 1,24,  $p < 0.0001$ , Fig. 2b) but not for *G. gueinzii* (odds ratio = 1.0004, d.f. = 1,22,  $p = 0.19$ , Fig. 2a). For *G. gueinzii* there was a

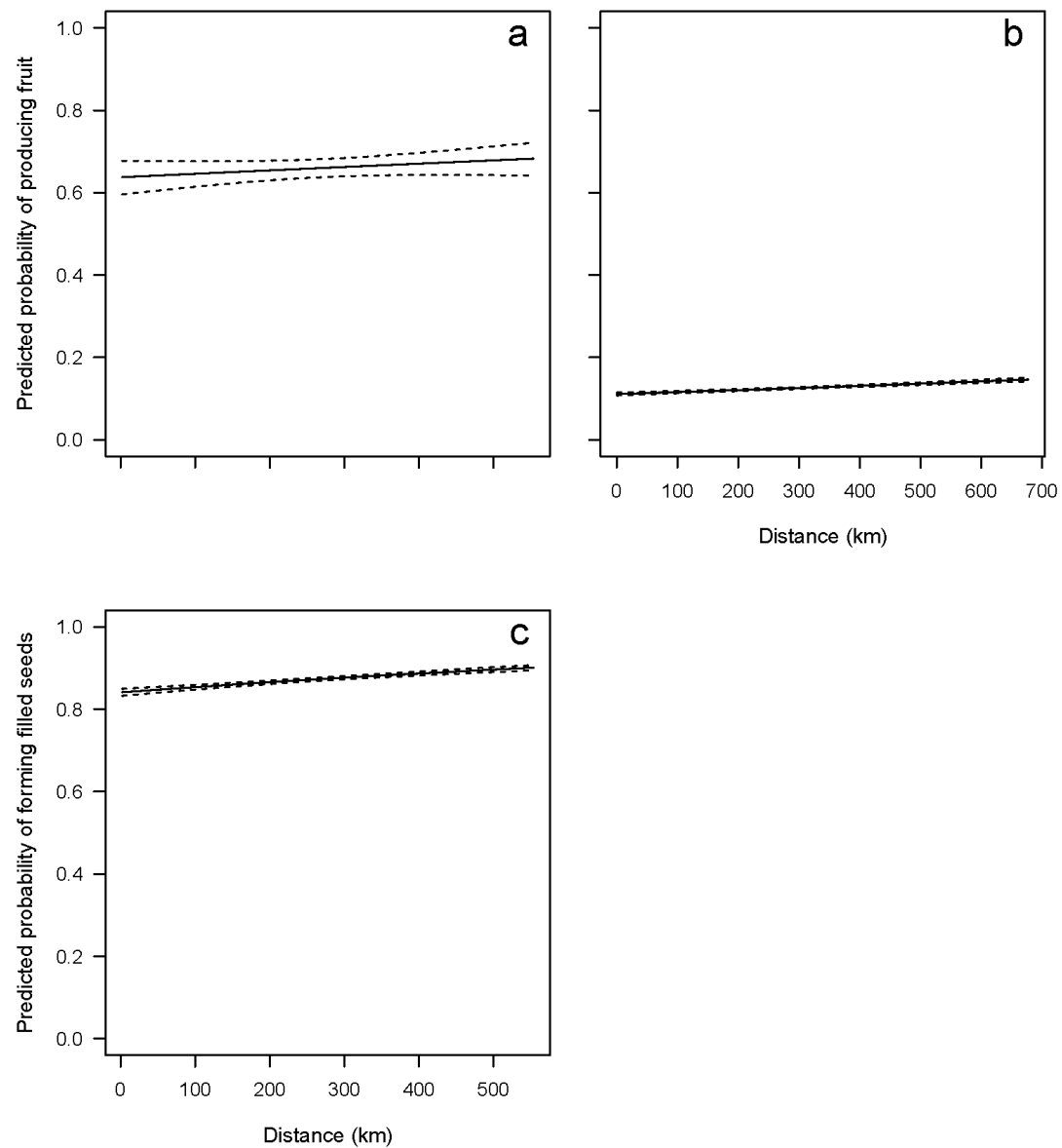
significant increase in the probability of producing fully formed seeds with increasing distance from source population (odds ratio = 1.001, d.f. = 1,22,  $p < 0.0001$ , Fig. 2c).

#### SEED SIZE/SEED NUMBER

Among populations, seed number varied more than seed size for both *G. gueinzii* (CV seed number = 23%, CV seed mass = 9%) and *H. bonariensis* (CV seed number = 128%, CV seed size = 24%). For *G. gueinzii* and *H. bonariensis*, there was no significant relationship between total seed mass per inflorescence and distance from source population (*G. gueinzii*:  $F_{1,21} = 1.76$ ,  $p = 0.30$ ,  $R^2_{\text{adj}} = 0.03$ , Appendix S2; *H. bonariensis*:  $F_{1,18} = 0.0073$ ,  $p = 0.93$ ,  $R^2 = 0.0004$ , Appendix S3), indicating that there was no increase in biomass investment in seed production towards range edges. For *G. gueinzii* and *H. bonariensis*, there were no significant relationships found between average individual seed mass per inflorescence and distance from source population (*G. gueinzii*:  $F_{1,21} = 1.05$ ,  $p = 0.25$ ,  $R^2_{\text{adj}} = 0.002$ , Appendix S2; *H. bonariensis*:  $F_{1,18} = 0.0024$ ,  $p = 0.96$ ,  $R^2 = 0.0001$ , Appendix S3). At the whole plant level there was also no significant relationship between total seed mass per plant and distance from source population for *G. gueinzii* ( $F_{1,21} = 3.84$ ,  $p = 0.24$ ,  $R^2_{\text{adj}} = 0.11$ ). There was no significant relationship found between number of seeds per inflorescence with distance from source population for either species (*G. gueinzii*:  $F_{1,21} = 3.45$ ,  $p = 0.090$ ,  $R^2_{\text{adj}} = 0.10$ , Appendix S2; *H. bonariensis*:  $F_{1,18} = 0.0007$ ,  $p = 0.98$ ,  $R^2 < 0.0001$ , Appendix S3). For *G. gueinzii*, there was no significant effect of total biomass on total seed mass ( $F_{1,21} = 1.47$ ,  $p = 0.37$ ,  $R^2_{\text{adj}} = 0.021$ ), average seed size ( $F_{1,21} = 0.11$ ,  $p = 0.53$ ,  $R^2_{\text{adj}} = -0.04$ ) or total seed number ( $F_{1,21} = 1.10$ ,  $p = 0.11$ ,  $R^2_{\text{adj}} = 0.004$ ). There was however a significant effect of total biomass on total seed mass at the whole plant level ( $F_{1,21} = 21.1$ ,  $p < 0.0001$ ,  $R^2_{\text{adj}} = 0.48$ ), with larger plants producing a greater volume of seeds.



**Fig. 1** Linear regressions for the relationships between (a) clonal reproductive effort and distance (b) clonal reproductive effort and biomass (c) sexual reproductive effort and distance and (d) sexual reproductive effort and biomass for *Gladiolus gueinzii*. Each point represents the average value for a single population. Error bars represent standard errors. Dotted lines show 95% confidence limits of significant regressions.



**Fig. 2** Logistic regressions showing the predicted probabilities of (a) producing fruit for *Gladiolus gueinzii*, (b) producing fruit for *Hydrocotyle bonariensis* and (c) forming filled seeds for *Gladiolus gueinzii*. Dotted lines show 95% confidence limits for each fitted value.



## Discussion

This study examined whether there were changes in reproductive strategies across the course of an invasion, namely whether populations at the expanding range edge allocated more resources to reproduction, produced a greater number of small seeds and/or had an increased capacity for autonomous self-fertilisation compared to longer established populations. We found evidence for increased capacity to autonomously self-fertilise towards range edges, however range edge populations did not invest more resources into reproduction nor were there any changes in the seed size/seed number relationship across the invaded range. To our knowledge, this is the first study to extensively examine changes in reproductive strategies across the invaded range of species.

Both *H. bonariensis* and *G. gueinzii* displayed increased capacity for autonomous self-fertilisation towards range edges. We found an increase in the probability of fruit production towards range edges for *H. bonariensis* and an increase in the number of developed seeds towards range edges for *G. gueinzii*, indicating that range edge populations are more reproductively assured compared to longer-established populations closer to the core of the range. Previous studies have found an increase in the number of developed seeds in invasive populations of species (Correia et al. 2015, 2016) and towards range edges within the invaded range of species (Nunes et al. 2016). Despite there being a significant increase in autonomous self-fertilisation towards range edges, the increase was relatively small (10% increase for *H. bonariensis*, 5% increase for *G. gueinzii*). However, a complete shift to autonomous self-fertilisation towards range edges would not be beneficial as recombination of alleles may be required as range edge populations expand into novel and potentially adverse environments (Pannell et al. 2015). Nonetheless, any increase in

capacity for autonomous self-fertilisation may facilitate further range expansion as it alleviates reliance on pollinators and/or mates for reproduction as low density populations at the range edge may be mate limited and less apparent to pollinators (Moeller 2006, Moeller et al. 2012).

An alternative explanation for increase in capacity for autonomous self-fertilisation towards range edges of invasive species may be selection to reduce gene swamping from more dense central populations (Pannell et al. 2015). Higher conspecific density and thus reproductive output from central populations may lead to a net flow of genes from central to edge populations (Kirkpatrick & Barton 1997), leading to an influx of maladapted genes that hinder adaptation at range margins and restrict range expansion (Sexton et al. 2009). Theoretical (García-Ramos & Kirkpatrick 1997; Kirkpatrick & Barton 1997) and empirical (Wang et al. 2016, but see Jump et al. 2003) studies have found a net flow of genes from central to peripheral populations, impeding adaptation in peripheral populations. Thus autonomous self-fertilisation in range edge populations may instead be a means of reducing gene swamping and retaining beneficial allele combinations.

For *G. gueinzii*, we found no increase in reproductive effort (either sexual or clonal) towards range edges. As conspecific density is relatively low in an expanding range front, increased reproductive effort is expected to be selected for in edge populations (Burton et al. 2010). Studies examining allocation to reproduction towards range edges are equivocal, with studies finding increased (e.g. Jump & Woodward 2003; Yakimowski & Eckert 2007; Masson et al. 2016) and decreased (e.g. Brandner et al. 2013; Courant et al. 2017) allocation to reproduction towards range edges. This may in part be due to selection for concurrent traits

in range edge populations that may directly trade-off with reproduction (Courant et al. 2017). For example, highly dispersive range edge populations of speckled wood butterflies had reduced allocation to reproduction compared to conspecifics at the range core (Hughes et al. 2003). Hudson et al. (2015) also found a decrease in the probability of female cane toads laying eggs from highly dispersive range edge populations compared to at the range core. However, as plants are sessile, reproduction and dispersal are not mutually exclusive, thus any trade-off with reproduction at the range edge may exist with another unmeasured trait.

We found no relationship between seed number and seed size with distance from source population, with neither species producing a greater number of small seeds towards range edges. A greater number of smaller seeds at the range edge would greatly increase colonisation opportunities and facilitate further range expansion. A previous study on *G. gueinzii* found a significant decrease in seed size towards range edges from field collected seeds (Tabassum & Leishman 2018). Considering this trend was not mirrored when plants were grown in controlled conditions in the glasshouse suggests that smaller seed sizes towards range edges may not have been due to a seed size/number trade-off but rather a reflection of the quality of the maternal environment (Wulff 1986). Furthermore, although strong negative relationships between seed number and seed size have been found between species (e.g. Jakobsson & Eriksson 2000; Henery & Westoby 2001; Leishman 2001), many studies examining this relationship within species have found no relationship (e.g. Shaal 1980; Wulff 1986; Michaels et al. 1988). This is because seed size is a highly conserved trait within species compared to between species (Westoby et al. 1996, Weiner et al. 1997). Instead, individual plants are more likely to express reproductive differences through

variation in seed number rather than individual seed size (Weiner et al. 1997; Leishman et al. 2000). In support of this we did observe greater variation in seed number compared to seed size between populations for both *G. gueinzii* and *H. bonariensis* however this was not significantly related to spread distance.

An important caveat of glasshouse studies is that they may not be directly related to advantages in the field (Gioria & Pyšek 2016). Although we found an increase in the propensity for autonomous self-fertilisation towards range edges in our experiment, it is not entirely clear whether this leads to fitness benefits in range edge populations of our study species in the field. Investigating the advantageous of autonomous self-fertilisation in natural populations of our study species would therefore be a useful next step.

Furthermore, this study only examined the propensity for populations to produce seeds using 'self' pollen and did not investigate how 'self' vs. 'non-self' pollen affected fruit/seed set across the range of our study species. Low density range edge populations have been observed to shift towards a greater propensity for self-pollination to provide reproductive assurance (Moeller & Geber 2005). Therefore another interesting future direction would be to compare fruit/seed set of selfed and outcrossed individuals to investigate whether range edge populations have shifted away from an outcrossing system.

Overall, this study revealed some differences between reproductive traits between range edge and range core populations of *G. gueinzii* and *H. bonariensis*. In general, although range edge populations did not invest more resources into reproduction or alter seed number or partitioning, edge populations were more reproductively assured due to greater capacity for autonomous self-fertilisation. Increased capacity for autonomous self-

fertilisation in range edge populations may help to facilitate further range expansion as it alleviates constraints imposed on reproduction due to reliance on external factors such as mate and/or pollinator availability. Understanding how selection varies in range edge populations has important implications for not only expansion of invasive species but also range contractions of endangered species and range shifts due to climate change (Hargreaves & Eckert 2014). Because of this, studies such as our own will become increasingly valuable for understanding species' range shifts in the face of future global change.

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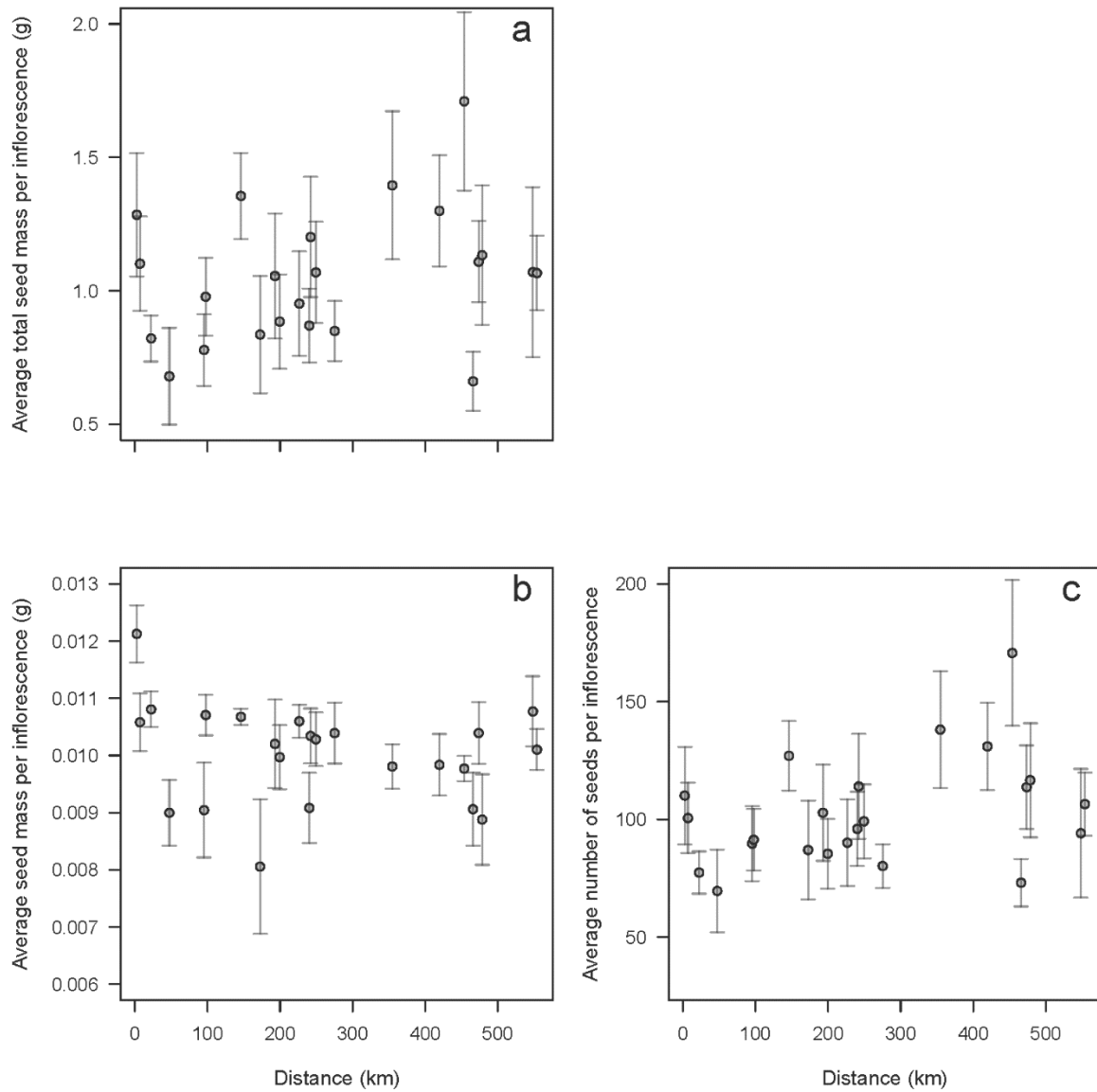
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**Appendix S1.** Sites sampled for *Hydrocotyle bonariensis* and *Gladiolus gueinzii* from north to south showing the straight line distance from their first herbarium record (Stockton (32° 55' 00"S, 151° 46' 00"E) for *G. gueinzii* and Lady Robinson's Beach (33° 58' 00" S, 151° 09' 00" E) for *H. bonariensis*).

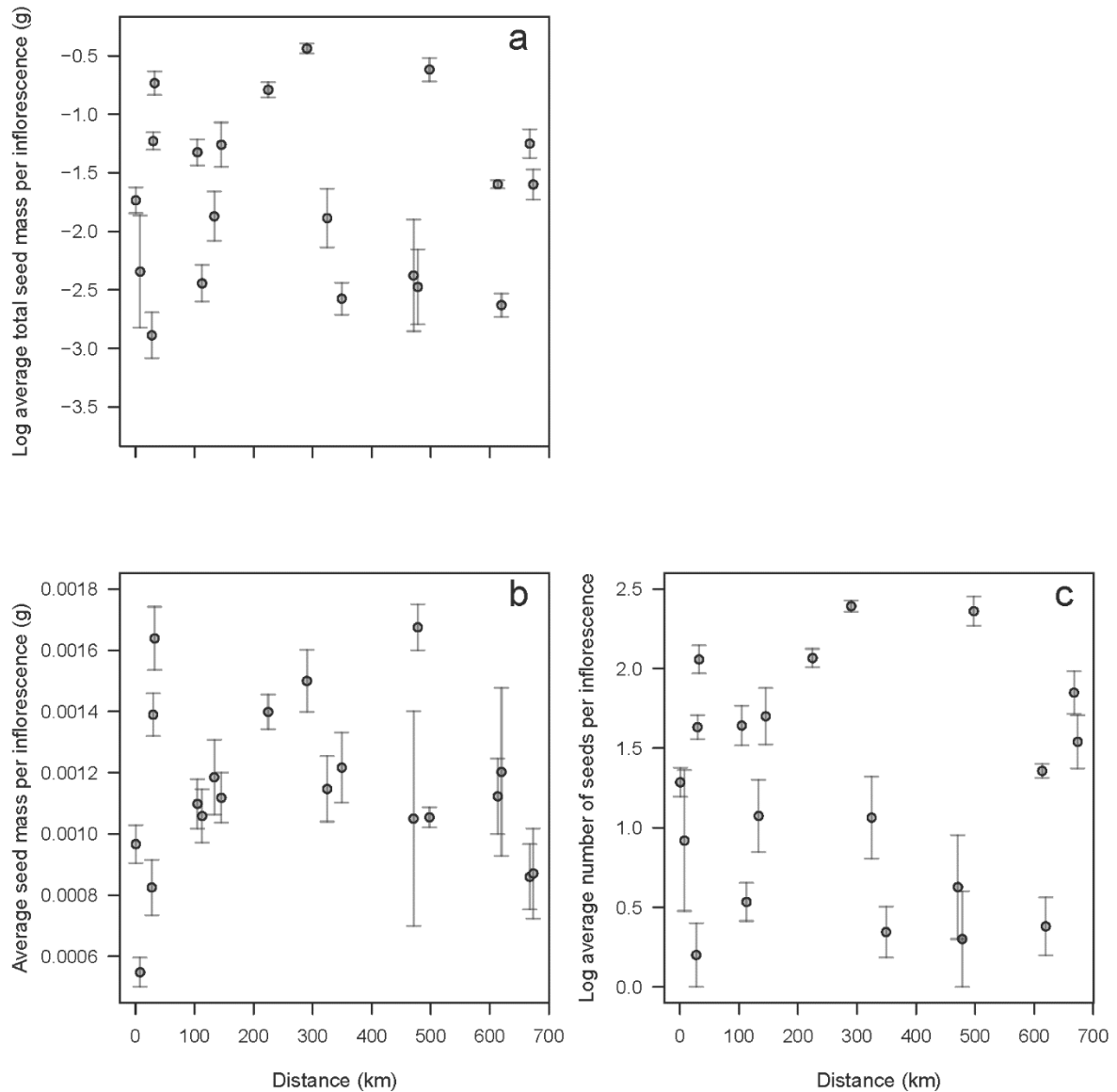
Species	Site	Latitude (S)	Longitude (E)	Distance (km)
<i>H. bonariensis</i>	Kingscliff	28°14'25"	153°34'06"	677
	Casuarina	28°16'32"	153°34'50"	673.7
	Cabarita Beach	28°19'55"	153°34'19"	667.5
	Lennox Head	28°47'55"	153°35'43"	619.7
	Ballina	28°51'37"	153°35'46"	613.4
	Wooli	29°51'58"	153°16'02"	497.8
	Woolgoolga	30°06'20"	153°11'59"	470.8
	Port Macquarie	31°28'39"	152°55'51"	323
	Dunbogan	31°39'12"	152°49'50"	301.4
	Warriewood	33°41'50"	151°18'37"	32.42
	Collaroy	33°43'46"	151°18'02"	29.82
	Dee Why	33°45'06"	151°17'50"	27.5
	La Perouse	33°59'07"	151°13'53"	7.78
	Brighton le Sands	33°58'13"	151°09'09"	0.46
	Shoalhaven Heads	34°51'01"	150°45'01"	104.9
	Culburra	34°55'54"	150°46'20"	112.8
	Callala	35°00'36"	150°41'57"	123.2
	Hyams	35°06'18"	150°41'36"	133.3
	Swanhaven	35°11'18"	150°34'58"	145.4
	Tomakin	35°49'45"	150°11'42"	224.7
	North Tura Beach	36°25'21"	150°04'13"	290.2
	Tathra Beach	36°43'32"	149°58'53"	324.6
	Pambula	36°56'25"	149°54'32"	349.2
	East Cape Beach	37°47'55"	148°44'36"	478
<i>G. gueinzii</i>	Hat Head	31°02'50"	153°02'39"	240.3
	Richardson's Crossing	31°09'32"	152°58'57"	226.6
	Point Plomer	31°24'31"	152°54'51"	199.5
	Port Macquarie	31°29'03"	152°55'24"	192.9
	Dunbogan	31°39'08"	152°49'48"	172.5
	Crowdy Head	31°52'09"	152°42'24"	146.1
	Boomerang Beach	32°20'07"	152°32'47"	97.53
	Blueys Beach	32°21'13"	152°32'06"	95.37
	Hawks Nest	32°40'34"	152°11'09"	47.44
	Nobbys Beach	32°55'22"	151°47'34"	2.53
	Dudley Beach	32°58'19"	151°43'51"	6.997
	Swansea Heads	33°05'47"	151°39'30"	22.39

Culburra	34°55'54''	150°46'20''	242.1
Currarong	35°00'52''	150°48'50''	249.3
Swanhaven	35°11'19''	150°34'58''	275.2
Tomakin	35°49'46''	150°11'40''	354.6
Bermagui	36°25'21''	150°04'13''	419.5
Tathra Beach	36°43'29''	149°58'50''	453.7
North Tura Beach	36°49'42''	149°56'08''	465.9
Merimbula	36°53'49''	149°54'57''	473.6
Pambula	36°56'26''	149°54'32''	478.4
Mallacoota	37°34'05''	149°45'42''	548.3
Secret Beach	37°36'31''	149°43'14''	553.8

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**Appendix S2.** Linear regressions showing the relationships between (a) average total seed mass per inflorescence (b) average individual seed mass per inflorescence and (c) average seed number per inflorescence and distance from source population for *Gladiolus gueinzii*. Each point represents the average value for a single population. Error bars represent standard errors.



**Appendix S3.** Linear regressions showing the relationships between (a) log average total seed mass per inflorescence (b) average individual seed mass per inflorescence and (c) log average seed number per inflorescence and distance from source population for *Hydrocotyle bonariensis*. Each point represents the average value for a single population. Error bars represent standard errors.

## 6 | Discussion

I, Samiya Tabassum, conducted the literature review and wrote this chapter.  
My supervisor, Michelle Leishman, provided comments.

## General summary

Biological invasions present an ever increasing threat to biodiversity worldwide yet little is still known about how some species are able to rapidly increase in their range when introduced into novel environments. Understanding more about how the ecological and evolutionary pressures change during the course of an invasion may provide insights into the success of invasive species (Lankau et al. 2009). This thesis examined the ecological and evolutionary mechanisms of range expansion in invasive species. In particular, it examined the role of ecological phenomena such as enemy release and selective pressures such as increased growth rates, dispersal ability and reproductive output towards range edges as a means of facilitating further range expansion. Two exotic invasive coastal dune plants (*Hydrocotyle bonariensis* Lam. and *Gladiolus gueinzii* Kunze) were chosen as study species because their relative confinement along dune systems enabled sampling across their entire range in order to answer questions pertaining to entire species' ranges. The aim of this final chapter is to synthesis the key findings of this thesis and to consolidate them within the broader research on species' range expansions.

The success of many invasive species has been attributed to the enemy release hypothesis which posits that when a species is introduced into a novel environment it leaves behind many of its co-evolved enemies (parasites, herbivores, pathogens, etc.) that may have functioned to control its abundance in its native range (Keane & Crawley 2002; Torchin et al. 2003). However, with time, invasive species do eventually accumulate a suite of local enemies in their introduced ranges (Torchin et al. 2003; Castells et al. 2013; Harvey et al. 2013). Chapter 2 tested the idea that populations towards range edges would be subject to less enemy pressure than at the range core as local enemies have had less time to



accumulate and conspecific density is lower. Even though there was a significant decrease in conspecific density towards range edges for *H. bonariensis*, there was an increase in enemy damage towards range edges. Although this was a surprising result, the results of Chapter 3 helped to understand these findings in light of trade-offs between traits. Chapter 3 looked at shifts towards faster growth strategies at range edges by examining pairwise leaf trait relationships. Fast growth is likely to be selected for towards range edges in order to reduce time to sexual maturity, thus allowing for faster time to reproduction and continual expansion of the range front (Chuang & Peterson 2016). Range edge populations of *H. bonariensis* were found to possess higher values for specific leaf area (SLA), leaf nitrogen content ( $N_{\text{mass}}$ ) and assimilation rate ( $A_{\text{mass}}$ ), resulting in a faster growth strategy. However, leaves with higher SLA and foliar nitrogen content are generally considered to be less well-defended and have been found to sustain greater enemy damage (Pérez-Harguindeguy et al. 2003; Vergeer & Kunin 2011). Thus, the benefit of faster growth strategies towards range edges to facilitate further range expansion may be lost due to increased vulnerability to enemy attack.

Understanding the nature of trade-offs in range edge populations can provide valuable insights into the evolutionary processes underpinning species' range shifts. In addition to the trade-off between growth and defence, additional trade-offs with regards to range edge phenotypes may also constrain range expansion. One such trade-off, especially for invasive plants, is that between dispersal and colonisation ability (germination success and growth) which was explored in Chapter 4. For wind dispersed plants this trade-off is mediated by seed size; smaller seeds often have greater dispersal ability but poorer colonisation ability (Strykstra et al. 1998; Meyer & Carlson 2001; Cappuccino et al. 2002; Gravuer et al. 2003;

Tabassum & Bonser 2017, but see Soons & Heil 2002; Skarpaas et al. 2011). Range edge populations of *G. gueinzii* were found to have significantly greater dispersal ability, which was mediated by a decrease in seed mass, compared to populations nearer to the range core. However, this was not associated with a decrease in growth or probability of germination. In fact, seeds from range edge populations had significantly faster germination times. The lack of a trade-off between dispersal and colonisation ability towards range edges of *G. gueinzii* may be suggestive of a shift towards a faster spread strategy. As *G. gueinzii* is a pioneer dune species (Heyligers 1999), fast seedling growth rate and germination time are likely to be important traits for facilitating further spread. However, an alternative explanation for the reduction in seed mass towards range edges of *G. gueinzii*, irrespective of selection on dispersal ability, is a reduction of habitat quality towards range edges that may have affected seed size.

These alternative explanations were explored in Chapter 5 along with a suite of shifts in reproductive strategies expected to occur in range edge populations of expanding species. Chapter 5 examined whether range edge populations allocated more resources to reproduction, produced a greater number of smaller seeds and/or displayed a greater capacity for autonomous self-fertilisation (fertilisation in the absence of pollinators) compared to range core populations. As populations expand, traits pertaining to increased reproductive output are likely to be under strong selection towards range edges as a means of significantly increasing colonisation opportunities, hence facilitating further range expansion. Despite there being no significant increase in reproductive allocation towards range edges, both *H. bonariensis* and *G. gueinzii* displayed a greater capacity for self-fertilisation towards range edges. An increase in the capacity for autonomous self-

fertilisation may facilitate further range expansion as it alleviates reliance on pollinators for reproduction as low density populations at the range edge may be less apparent to pollinators (Moeller 2006; Moeller et al. 2012). Chapter 5 also examined whether there were shifts in seed size which would provide evidence for selection for increased dispersal ability (mediated through decreased seed mass) towards range edges of *G. gueinzii* observed in Chapter 4. *Gladiolus gueinzii* plants grown in controlled conditions in the glasshouse did not produce smaller seeds towards range edges which suggests that the pattern observed in field collected seeds may instead reflect plasticity in response to maternal environmental conditions (Wulff 1986).

The most pertinent finding of this thesis is that *H. bonariensis* and *G. gueinzii* did not show consistent responses across the traits and strategies investigated in the different research chapters of this thesis. For example, in Chapter 2 which examined drivers of enemy damage across species' ranges, amount of enemy damage on *H. bonariensis* was significantly related to spread distance whereas for *G. gueinzii* the most important drivers were climate and leaf-level traits. Similarly, although *H. bonariensis* displayed a shift to a faster growth strategy towards range edges (described in Chapter 3), *G. gueinzii* showed no such shifts. A potential explanation for this inconsistency may be that *H. bonariensis* and *G. gueinzii* differ in time since introduction. *Hydrocotyle bonariensis* was first recorded on the east coast of Australia in 1893 while *G. gueinzii* appeared almost 60 years later in 1950 (Heyligers 1999). Therefore, differences between species may simply reflect the fact that *H. bonariensis* has had a longer residence time and thus greater opportunity to undergo change (Moran & Alexander 2014). This may explain why there were no differences in growth strategies between range core

and range edge populations of *G. gueinzii* in Chapter 3 but contradicts the patterns of enemy damage found in Chapter 2.

The findings of this thesis show that *G. gueinzii* and *H. bonariensis* differ in how range expansion may be facilitated at their edges; *G. gueinzii* has been found to be more dispersive at the range edge while *H. bonariensis* has potentially shifted to a faster growth strategy at the range edge, emphasising the difficulty in making generalisations about a common strategy across species. In addition to differences between species it is also important to note that not all range edges are influenced by the same limiting factors (Sagarin et al. 2006; Bontrager & Angert 2016). For example, Sugiyama (2003) found faster germination and lower reproductive output only at the southern and not northern range edge of an invasive grass in Japan. Taken together, this evidence highlights the complexity of understanding factors influencing range advance within and across species and provides a rich avenue for continued research.

A noteworthy limitation of this thesis is that traits were either measured on field-collected material (Chapter 2 and 4) or on plants grown directly from material obtained from the field (Chapters 3 and 5). Ideally, studies on selection would be conducted on plants from the  $F_1$  generation and beyond to eliminate the influence of plasticity and/or maternal effects. Although care was taken to minimise the influence of maternal effects when measuring growth traits in Chapter 3 (by allowing plants to grow in controlled conditions for 15 months before trait measurement), the influence of maternal effects cannot be ruled out. The influence of maternal effects was most noticeable when the patterns of smaller seed sizes found towards range edges of field-collected seeds of *G. gueinzii* in Chapter 4 was not found

for plants grown in the glasshouse in Chapter 5. Understanding more about the influence of plasticity, maternal effects and genetic differences can increase our understanding of the drivers of range expansion (Gruber et al. 2017). Further studies comparing traits measured on field populations with those from F<sub>1</sub> generations produced in controlled conditions in the glasshouse are needed to disentangle the influence of these factors on range expansion of *H. bonariensis* and *G. gueinzii*.

### **Are dune systems good models for species' range expansion?**

This thesis examined ecological and evolutionary mechanisms of range expansion using exotic invasive dune plants as study systems. Coastal species represent an ideal system in which to study processes underlying species' range dynamics as their range is essentially linear with capacity for spread being on two range edges only (Sagarin & Gaines 2002a; Samis & Eckert 2007). Most studies examining processes affecting species' ranges have only sampled from one range edge and/or only compared populations from just the range centre and range edges, potentially obscuring our ability to find consistent patterns (Sagarin & Gaines 2002b; Sexton et al. 2009; Vaupel & Matthies 2012; Nunes et al. 2016). As range edges often correspond with changes in latitude, both climatic variables and the process of range expansion may select for similar range edge phenotypes (Evans et al. 2013; Therry et al. 2014b; Van Petegem et al. 2016). For example, species undergoing poleward expansion may experience selection to undergo faster growth in order to hasten development in response to shorter growing seasons, a process known as counter-gradient variation (Conover & Schultz 1995; Sanford et al. 2006). Although some studies have controlled for this phenomenon when understanding the mechanisms behind range expansion (e.g. Therry et al. 2014a, b; Van Petegem et al. 2016), others have not (e.g. Evans et al. 2013; Kilkenny &

Galloway 2013; Dangremond & Feller 2016; Macel et al. 2017). Coastal dune systems provide an excellent opportunity to study processes affecting range expansion independently of climatic variables as they allow for systematic sampling across the entire range of species.

Despite the benefits of using coastal dune systems to study factors influencing species' ranges, there are some features of dune systems that may not enable them to be applicable across all systems. Firstly, *H. bonariensis* and *G. gueinzii* often experience interspecific competition from species such as *Cakile edentula* Scop., *C. maritima* Scop. And *Spinifex sericeus* R.Br. along much of their range (S. Tabassum, personal observation). The influence of changing competitive environments (interspecific vs. intraspecific competition) in shaping selection for particular traits across the course of an invasion has been rarely investigated. Intraspecific competitive ability (measured as biomass accumulation) has been found to be selected against towards range edges of invasive plants in response to lower conspecific density (Huang & Peng 2016, but see Evans et al. 2013). Furthermore, using a theoretical model Burton et al. (2010) showed that during range expansion, range edge populations will invest less towards dispersal if they encounter a competing species, hence slowing range expansion. Thus the nature of competitive interactions across a species' range can have contrasting effects on selection for traits that promote range advance during the course of an invasion (i.e. faster growth and greater dispersal ability); however coastal dune systems may not provide the best opportunity to investigate these differences.

Another caveat of coastal dune systems is that they often present a relatively homogenous and uninterrupted landscape to colonise. In fact, the majority of studies investigating how

invaders spread through the environment are concerned with movement through homogenous landscapes (Phillips et al. 2010; Pachevsky & Levine 2011). However, in reality, invaders are likely to move through a matrix of suitable and unsuitable patches which may affect the rate of spread (Pachevsky & Levine 2011; Williams et al. 2016). Studies on movement through heterogeneous landscapes have shown that when faced with an inhospitable environment, the rate of spread decreases until population density is high enough to produce enough propagules in order to overcome such a gap (Pachevsky & Levine 2011; Williams & Levine 2018), leading to the accumulation of individuals with high competitive tolerance at the range edge (Williams et al. 2016). Furthermore, if highly dispersive range edge populations continually disperse into adverse environments, then dispersal at the range front may be strongly selected against (Phillips 2012; Hargreaves & Eckert 2014). Movement through heterogeneous landscapes can often produce opposite results to movement through homogenous landscapes (Williams et al. 2018). However, our understanding of these dynamics in natural systems is still lacking. In summary, although coastal dune systems provide some excellent opportunities to study processes affecting species' ranges, results from such studies are not necessarily applicable to all systems.

### **Range shifts and climate change: what can and can't we learn from invasions?**

When invasive species are first introduced to a novel environment, they are usually accidentally introduced somewhere along their tolerance range and subsequently expand their range to fill their niche limit. This is in contrast to native species which are generally at equilibrium within their range (Hargreaves et al. 2014; Lee-Yaw et al. 2016). However, as the climate becomes warmer with anthropogenic climate change, native species are no longer expected to be at equilibrium with their suitable climate envelope (Moran & Alexander

2014). Biological invasions can thus provide useful analogues with which to understand range shifts in non-equilibrium systems in response to climate change (Caplet et al. 2013; Sargent et al. 2017).

In order for species to shift their distribution to occupy new areas with suitable climates, range edge populations may need to undergo trait shifts (Griffith & Watson 2006) much like those explored in this thesis. Numerous studies have observed increased dispersal ability towards range edges of many taxa undergoing range expansion in response to climate change as well as through natural colonisation (butterflies (Hill et al. 1999; Hughes et al. 2003; Hanski et al. 2006), crickets (Thomas et al. 2001; Simmons & Thomas 2004), damselflies (Hassell et al. 2009; Therry et al. 2014a, c), birds (Duckworth & Badyaey 2007), plants (Cwynar & MacDonald 1987)). Similar shifts to faster growth (Sanford et al. 2006; Therry et al. 2014b; Macel et al. 2017), shifts in reproductive strategies (Hill et al. 1999; Ling et al. 2008; Dangremond & Feller 2016) and enemy damage (Fagan & Bishop 2000; Mendéneez et al. 2008; Macel et al. 2017) towards range edges have also been observed in native species tracking climate change.

While there are several parallels between range expansion due to the invasion process and that due to shifting climates, there are several reasons why invasions cannot be used as complete surrogates for native species' spread. Firstly, invasive species that are not at equilibrium will continue to spread into more favourable habitats until equilibrium is reached. In contrast, native species tracking climate change are likely to encounter available sites that are more fragmented within the landscape (Travis & Dytham 2002) and this may affect selection on range edge phenotypes. It has also been speculated that range shifts due



to climate change may occur slower as they are constrained by a slowly shifting cloud of suitable climatic conditions which impose strong selection against phenotypes which may over-disperse ahead of this cloud or under-disperse and fall behind (Boeye et al. 2013). Lastly, a major feature unique to range expansions caused by climate change is that co-occurring species are likely to also respond to such changes, however species may move at different rates, leading to the formation of novel ecological communities (Alexander et al. 2016). To date, little is known about how native species' ranges expand in response to climate change and our understanding of how invasive species undergo range expansion may increase our understanding of such processes to some extent (Macel et al. 2017).

### **Concluding remarks**

Biological invasions have had a significant impact on native biodiversity worldwide and understanding the success of invasive species is a leading question in invasion biology. This thesis used two exotic invasive coastal dune species from eastern Australia to examine how ecological and evolutionary pressures change during the course of an invasion, to provide novel insights into the success of invasive species. Although understanding how invasion dynamics change through time is a relatively new venture, studies of such dynamics are already accumulating from a variety of different taxa. The general picture that is emerging from such studies and those presented in this thesis is that trait shifts at expanding range edges are not consistent and are highly context specific. Thus a pertinent research direction would be to amalgamate these studies with a meta-analytical approach to understand general patterns. Furthermore, although shifts in life history strategies have been found towards range edges in this thesis and other published research, little is known about how these changes will affect interactions with species at the expanding range front (Tomuolo &

Ward 2018). Perhaps another promising next step would be to better understand species interactions at range edges and their consequences for range dynamics.

Biological invasions provide excellent natural experiments with which to study pertinent questions in ecology such as determinants of species' range limits. However, since many invasive species are still undergoing range expansion in their introduced ranges, understanding causes of range limits from biological invasions must be done with caution (Sargent et al. 2017). Nonetheless, biological invasions provide invaluable knowledge regarding species' range dynamics, which will only become more important in the face of increasing global change.

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