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**Ecological interactions of an invading insect:
the planthopper *Prokelisia marginata***

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University of Sussex
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For Indigo

My greatest challenge and my deepest joy.

“It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty; the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living.”

Sir David Attenborough

University of Sussex
Claire Harkin, Doctor of Philosophy
Ecological interactions of an invading insect:
the planthopper *Prokelisia marginata*

Summary

The planthopper *Prokelisia marginata* Van Duzee is native to the eastern coast of North America, where densities on its foodplant, the cordgrass *Spartina alterniflora*, frequently exceed several thousand per square metre. It has little impact on its host plant in its native range where both species have co-evolved, however where the plant has been introduced and has had no recent exposure to the planthopper, it has a major impact and has been trialled as a biological control agent.

P. marginata was recorded for the first time in Britain in 2008, where it feeds primarily on the widespread *S. anglica*, itself listed as one of the world's 100 worst invasive species, as well as its progenitors *S. alterniflora*, *S. maritima* and *S. x townsendii*. *P. marginata* appears to be in the successful early stages of invasion in Britain, having already spread extensively. Significantly outnumbering all other saltmarsh arthropod groups, it is benefitting from partial natural enemy escape, and a high proportion of macropterous individuals in all populations indicates strong potential for further range expansion. Utilising both glasshouse and field manipulations, I show that exposure to *P. marginata* has a significant negative impact on *S. anglica*, an interaction which has the potential to destabilise Britain's important saltmarsh habitat. I suggest that the four host species that occur in Britain represent a 'gradient' of shared co-evolutionary history with the planthopper. I show that, whilst all species are negatively impacted by *P. marginata* exposure, *S. alterniflora*, the species with which it shares the longest co-evolutionary history, is the least affected. I further show that *P. marginata* exhibits a preference for, and performs better on, *S. anglica*. As *S. anglica* is by far the most abundant of the four *Spartina* species in Britain, these results suggest *P. marginata* may be undergoing rapid evolution in its new range to take advantage of this widespread host species, thereby maximising its potential for further range expansion.

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Chapter one

General introduction

1.1 Biological invasions

Biological ‘invaders’ or ‘invasive’ species, those which establish in areas outside their native range and subsequently spread beyond the location of their introduction (*sensu* Colautti & MacIsaac 2004), are the subject of considerable political, public and academic interest (Huenneke 1988; IUCN 2000; Mack *et al.* 2000). Charles Elton’s (1958) seminal text was among the first to highlight the importance of invasions as a model for biological research. His publication outlined patterns and impacts of species introductions, giving particular consideration to the effects of invasions on isolated ecosystems and the damage invasive species can cause to both natural systems and human wellbeing. More recent work has focused largely on two main areas: attempting to determine generalities about what makes species invasive and communities invulnerable (Sakai *et al.* 2001; Hayes & Barry 2008; Bacon *et al.* 2014; Gidoin *et al.* 2015; Saul & Jeschke 2015); and a more applied approach looking at the impacts of invasive species and how these can best be avoided or minimized (Strayer *et al.* 2006; Thomsen *et al.* 2011; Vila *et al.* 2011; Ricciardi *et al.* 2013; Roy *et al.* 2014).

Invasions are certainly a widespread, some may even say ubiquitous, phenomenon. Most communities now contain at least one, often several, invading species, with invaders already representing over a fifth of many countries’ flora (Mooney & Cleland 2001). A recent report published by the Royal Botanic Gardens, Kew (2016) reports that at least 13,168 species of vascular plant are known to have become naturalised outside their native range, with 4,979 of them documented as causing harm to the environment, the economy or human health. A key factor underlying this statistic is the unprecedented role that modern human societies are playing in transporting species outside of their native ranges, either intentionally or as unwanted passengers of growing international travel (Anderson *et al.* 2015), trade (Seebens *et al.* 2015) and anthropogenic land-use change (Vitousek *et al.* 1996). Such introductions show no sign of abating, and indeed rates of invasions and species’ range changes are expected to increase even further in this era of economic and cultural globalisation (Maron & Vila 2001; Meyerson & Mooney 2007).

There can be little doubt that biological invasions are one of the most high profile global issues facing both policy makers and conservation biologists today. Identified at the turn of the millennium as one of the five primary drivers of biodiversity change for the coming century (Sala *et al.* 2000), second only to habitat destruction and degradation, they are the focus of the Invasive Species Specialist Group, a network of over 2000 experts worldwide forming part of the Species Survival Commission of the International Union for Conservation of Nature (IUCN 2000). Identification, control and eradication of 'priority' invasive species, along with effective pathway management to prevent further invasions, is identified as one of the Aichi Biodiversity Targets for 2020 in the Strategic Plan for Biodiversity 2011-2020 agreed at the Tenth Meeting of the Conference of the Parties to the Convention of Biological Diversity (CBD) in Nagoya, Japan in October 2010. Consequently, it is a requirement that similar targets are reflected in the regional and national biodiversity strategies and action plans of the 193 member nations, or 'Parties' (CBD 2010). These targets are therefore a key element of the recent EU regulation 1143/2014 on the prevention and management of the introduction and spread of invasive species which came into effect on 1 January 2015 (European Parliament and the Council of the European Union 2014), itself cautiously welcomed by the scientific community (Beninde *et al.* 2015; Genovesi *et al.* 2015).

1.2 Why are biological invasions important?

1.2.1 Ecological impacts and economic costs

The Millennium Ecosystem Assessment (2005) identifies invasive species as among the top five most severe threats to global biodiversity and ecosystem integrity, along with habitat change, climate change, over-exploitation and pollution. Both the rate and extent of biological invasions continue to escalate (McGeoch *et al.* 2010). An analysis of invading plants, mammals and invertebrates establishing populations in Europe between 1500 and 2000 shows a persistently increasing trend, with the highest rates across all taxa occurring in the latter 25 years of measurement (Hulme 2009). Invasions of new species are therefore altering the structure and composition of often long-established communities (Davis 2003), with consequent effects on ecological interactions, and even the physical and chemical structure of the invaded environments (Ehrenfeld 2010). Allison & Vitousek (2004) found that leaf litter from invasive plants in Hawaii decayed at a rate of up to fifty times that of natives, resulting in highly elevated levels of nitrogen and phosphorous in the surrounding soils. As the invaders performed better than native species under high nutrient conditions, positive feedback in the nutrient cycling process could serve to facilitate even higher levels of invasion and further soil enrichment. Impacts of invasive species can be varied and complex, acting as both direct and indirect agents of change. Impacts on populations of native species can occur through mechanisms such as hybridization (Jensen *et al.* 2005), predation (Doherty *et al.* 2015a), herbivory (Poland & McCullough 2006) and parasitism (Boettner *et al.* 2000), the introduction and spread of pathogens (Morin *et al.* 2007; Banks & Hughes 2012), the breakdown of mutualisms (Kenta *et al.* 2007; Aizen, Morales & Morales 2008; Lach 2008) and competition for a range of resources (Norbury 2001; Hulme 2007). Additionally, invasive species can have wider community and ecosystem impacts via habitat degradation, disruption of natural disturbance regimes, altered biogeochemical and hydrological processes and changes to water and soil chemistry (Lovett *et al.* 2006; Strayer *et al.* 2006; Ford & Vose 2007; Mainka & Howard 2010; Simberloff 2011; Penk *et al.* 2015).

These effects on biodiversity and ecosystem services often result in economic costs arising from factors such as prevention, eradication and management of invaders, compromised harvests, and the indirect costs associated with human and domestic animal health impacts, and the loss of ecosystem services such as clean water and pollination. The estimated annual costs attributed to invasive species worldwide amount to some US\$1.4 trillion, equivalent to 5% of the global GDP (Mainka & Howard

2010). The Delivering Alien Invasive Species Inventories for Europe (DAISIE) project (www.europe-aliens.org) has resulted in the production of the first continent-wide dataset detailing the scale and impact of over 11,000 invasive species within Europe (DAISIE 2009). Recent estimates of the costs associated with these species amounts to at least €12 billion per year (EEA 2012); this is considered to be a substantial underestimate, however, as the potential economic and ecological costs resulting from European invasions remain unknown for approximately 90% of these species (Vila *et al.* 2010). The Great Britain Non-Native Species Secretariat (2015) reports that nearly 2,000 non-native species are currently established in Britain, with a further 10-12 species becoming established each year. They estimate that 10-15% of these species cause significant adverse impacts, at a cost of at least £1.7 billion every year.

1.2.2 Extinctions, biodiversity decline and biotic homogenization

Whilst the primary driver of extinctions in most areas is habitat loss, there have been numerous well-evidenced examples of species extinctions directly attributable to invading predators and pathogens (Davis 2003). The introduction of vertebrate predators represents the primary cause of global avian extinctions (Vila *et al.* 2010), with species endemic to oceanic islands proving especially vulnerable (Blackburn *et al.* 2004; Szabo *et al.* 2012). The accidental, and now infamous, introduction of the brown tree snake, *Boiga irregularis*, to the Micronesian island of Guam in the 1950s, thought to be transported by US military cargo vessels, proved devastating to the native vertebrate fauna. Twenty-four species of bird, bat and reptile, some endemic to Guam, had become extinct by 1998. Whilst some of these losses were linked to habitat destruction, the vast majority were directly attributable to predation by the snake (Fritts & Rodda 1998). Despite numerous attempts at eradication, the severity of the snake's impact remains of high concern (Kahl *et al.* 2012). Increased military movements in the region indicate other islands in the Pacific are at high risk of future invasion, whilst credible sightings of the predator have already been confirmed in Hawaii and mainland USA (Perry & Vice 2009; Kahl *et al.* 2012).

Closer to home, the invasive American mink, *Mustela vison*, introduced to Britain in the late 1920s, has been implicated in the dramatic decline of the water vole, *Arvicola terrestris*, observed over the same timescale (Jefferies *et al.* 1989). Habitat fragmentation was found to be a key factor in the voles' decline, however mink predation doubled the probability of local population extinction when compared to that explained by habitat fragmentation alone (Rushton *et al.* 2000). Such effects are by no means limited to vertebrate predators. The New Zealand flatworm, *Arthurdendyus*

triangulates, established in Scotland, is implicated in the decline and local extirpations of earthworms of which it is an obligate predator (Boag & Yeates 2001). Wider impacts of earthworm decline may include changes to soil structure and drainage, compromising agricultural grassland production and exacerbating risks of flooding and surface water pollution (Murchie & Gordon 2013), as well as disruption to the abundance, distribution and foraging behaviour of native mammalian predators (Alford 1998). The harlequin ladybird, *Harmonia axyridis*, a predatory coccinellid native to central and eastern Asia, was widely released across Europe from the 1980s as a biological control agent of agricultural aphid and coccid pests (Brown *et al.* 2011). Subsequently undergoing a period of extremely rapid range expansion, it colonised many countries, including Britain, in which it had not been deliberately released (Brown *et al.* 2008a; Brown *et al.* 2008b), triggering concerns about its potential deleterious impact on non-target species, particularly other aphidophages (Majerus *et al.* 2006). Following the first English record of the species in 2004, the UK Ladybird Survey, a citizen science recording scheme, was initiated to monitor the spread of *H. axyridis* and record the distribution of the other ladybird species across the country (Roy *et al.* 2012). Analysis of this long-term dataset has found a sharp decline in seven of the eight native ladybird species assessed, correlated with increasing abundance of the invader (Roy & Brown 2015). This correlation is thought to be the result of a complex range of interactions, including the weaker competitive abilities of the native species, and highly efficient intra-guild predation by *H. axyridis* (Brown *et al.* 2011; Roy *et al.* 2016). Identifying the direct causes of species decline and extinction is often problematic due to the presence of numerous confounding factors and the paucity of autecological studies. However, in the USA over 40% of the species listed as threatened or endangered under the Endangered Species Act are considered to be at risk due to predation by, or competition with, one or more of the estimated 50,000 invasive species present in the country. In some regions of the world, this percentage is thought to be as high as 80% (Pimentel *et al.* 2005).

Invasions, often by generalist and already widespread species, accompanied by the decline and extirpation of local, often more specialist, species are key factors in the progression of biotic homogenization, the process by which the genetic, taxonomic or functional diversity between biotic regions is reduced over time (Olden 2006; Dar & Reshi 2014). Whilst biotic homogenization has played an important role in structuring the distribution and composition of Earth's taxa throughout history (Vermeij 1991), the anthropogenic homogenization of regions through land-use change and species translocations has dramatically increased the pace and extent of this process (Olden *et*

al. 2004). Taxonomic homogenization, represented by the widespread invasion of a comparatively small number of successful, and often generalist, 'winning species', potentially at the expense of a larger number of more specialist 'losing' species (McKinney & Lockwood 1999), is relatively easy to observe and measure (Le Viol *et al.* 2012; Ibarra & Martin 2015). The impacts of genetic and functional homogenization however can prove more challenging to quantify (Devictor *et al.* 2008).

Local adaptations of geographically isolated populations may be lost as a consequence of intraspecific hybridization. Such losses may result in reductions in individual fitness, potentially even compromising the ability of entire populations to maintain and expand their distributions (Olden *et al.* 2004; Lowe *et al.* 2015). The survival and reproduction of anadromous salmonid fish relies heavily on a range of genetically determined adaptations to local environments encountered throughout their migratory lifecycle, including the timing of spawning and the orientation behaviour of fry. Genetic homogenization caused by introductions of artificially-bred salmon across the native ranges of these species may result in outbreeding depression linked to the loss of local adaptations and the breakdown of co-adapted gene complexes (Rhymer & Simberloff 1996; Yates *et al.* 2015). Interspecific hybridization may result in the mixing of genotypes and the loss of genetic integrity between previously distinct taxa. Survival of the European wildcat, *Felis silvestris*, is threatened by hybridization with feral housecats, *Felis catus* (Daniels *et al.* 2001). It was thought that some of the most genetically isolated wildcats in Europe were to be found in remote areas of northern and western Scotland, however some 80% of individuals studied were found to display genetic and phenotypic traits associated with domestic cats (Hubbard *et al.* 1992). More recent molecular studies have confirmed genetically distinct populations of the wildcat in eastern Germany (Hertwig *et al.* 2009) and north eastern France (O'Brien *et al.* 2009). Analysis of 71 individuals from 30 captive zoo populations, however, found that 68% shared a common mtDNA haplotype with domestic cats and only two individuals could be classified as 'purebred' wildcats, leading the authors to recommend the cessation of captive breeding and reintroduction programmes (Witzenberger & Hochkirch 2014).

Functional diversity, the value and range of functional traits within and between species in a community, has been identified as a key determinant of ecosystem processes and stability (Tilman *et al.* 1997; Diaz *et al.* 2007; Farias & Jaksic 2011). The relationship between taxonomic and functional diversity is not always clear however, and the replacement of an ecological specialist by a widespread generalist invader may have

much graver consequences for regional ecosystems than the loss of a single species may be thought to imply (Flynn *et al.* 2009; Baiser & Lockwood 2011; Mori *et al.* 2015). Functional homogenization may endanger ecosystem functioning and resilience by increasing similarities between communities, thereby reducing the potential for landscape and regional buffering of environmental disturbance (Olden 2006). Species and communities respond differently to disturbance. Whilst any individual specialist species may be more negatively affected by disturbance than an equivalent generalist species, Clavel *et al.* (2011) theorise that an entire meta-community consisting of many specialist species would be relatively more stable due to greater niche partitioning and complementarity. In their study of serpentine grasslands, Hooper and Dukes (2010) found more functionally diverse communities were better able to resist biological invasions, and that native species were most successful at repelling potential invaders from their own functional group. As biological invaders contribute to the loss of rare and specialist species from local communities, the potential for further invasion, homogenization and the associated ecological impacts consequently continues to increase (Tilman *et al.* 1997; Sousa *et al.* 2011; Capinha *et al.* 2015).

1.2.3 Confounding factors

1.2.3.1 Climate change

Almost 60 years after Elton (1958) focussed attention on the importance of biological invasions, understanding of occurrence and impacts continues to be complicated by emerging, and often synergistic, anthropogenic risk factors (Doherty *et al.* 2015b). The simultaneous action of apparently separate processes, for example climate change and biological invasions, can have a greater total impact on species, communities and ecosystems than the sum of individual effects alone (Occhipinti-Ambrogi 2007; Brook, Sodhi & Bradshaw 2008; Mainka & Howard 2010; Gallardo & Aldridge 2013). Climate change adds particular complexity to the prediction and understanding of biological invasions (Bradley *et al.* 2010; Cosner 2014). Direct impacts on abiotic factors including temperature, precipitation and CO₂ levels, nitrogen deposition, disturbance regimes and the occurrence of extreme events, strongly influence the spread and abundance of species (Hellmann *et al.* 2008). Such changes may enable species to expand into regions in which they previously could not survive or reproduce (Hochkirch & Damerau 2009; Peltanova *et al.* 2012; Barbet-Massin *et al.* 2013), or may result in native species becoming increasingly poorly adapted to local environmental conditions (Walther *et al.* 2009). The picture is further complicated by the predicted impacts of changing climate on human travel and trade activities, opening up new dispersal pathways, especially at higher latitudes, and increasing the risks of habitat destruction

and land use change (Brook *et al.* 2008; Chown *et al.* 2012; Greenslade & Convey 2012; Guo *et al.* 2012; Ware *et al.* 2012; Seebens *et al.* 2015). Changes in phenology and disruption of the interactions between species and their natural enemies, competitors or mutualists, are all linked to changing climatic conditions; all serve to add complexity to predictions of species range expansion and impact (Hellmann *et al.* 2008; Cosner 2014; Leishman & Gallagher 2015). Menendez *et al.* (2008) found that the Brown Argus butterfly, *Arícia agestis*, was able to expand its distribution in Britain much more rapidly than previously thought possible due to the combined effects of increasing climatic suitability at northern latitudes and disruption of the interaction with its natural enemies. This was despite many of its parasite species already being present in the new range. In their study of two species of invasive freshwater mysid shrimp, *Hemimysis anomala* and *Mysis diluviana*, Iacarella *et al.* (2015) found that maximum predation rates of both species showed a distinct peak at temperatures that most closely matched their thermal growth optima, identifying temperature as a primary mediator of invasive species impact.

Invasive species are widely considered to have particular traits that will favour them under changing climatic conditions: superior dispersal and colonisation abilities, high growth rates and fecundity, and broad environmental tolerances (Bertelsmeier *et al.* 2013; Wolkovich & Cleland 2014). It has been suggested that the rapid growth strategy of many invasive plants may make them particularly successful under elevated CO₂ levels (Dukes *et al.* 2011; Blumenthal *et al.* 2013; Tooth & Leishman 2014). Evidence for superior responses by invasive species is equivocal however, with both meta-analytical (Sorte *et al.* 2013) and experimental approaches (Perry *et al.* 2013) finding only a weak advantage of invasive over native species, complex results dependent on other factors, or no advantage at all. Leishman and Gallagher (2015) therefore conclude that the relative responses of native and exotic plants to changing climatic conditions will not consistently favour one group over another and will instead be strongly species and context dependent. Such complexity is not restricted to plants. A study modelling future distributions of fifteen of the worst invasive ant species under a range of predicted climatic conditions found that, unexpectedly, range expansions were forecast for only five of the species under a changing climate, whereas the majority were predicted to decline (Bertelsmeier *et al.* 2015). The authors were particularly surprised by this finding as all the species studied are from the same family (Formicidae) and have similar ecological characteristics. However, as much of the predicted range expansion coincided with the location of global biodiversity hotspots (Mittermeier *et al.* 2004), the authors concluded that ant invasions are likely to remain a

major global problem. Contrary to expectation, however, no systematic synergies were found between the two potential extinction drivers of climate change and species invasions.

1.2.3.2 Invasion lag

Introduced species are broadly described as passing through four non-discrete spatio-temporal stages of invasion: transportation to a new region; initial escape or release to colonise the new habitat; establishment of a viable, self-perpetuating population, possibly including short-range dispersal into closely interacting sub-populations; and finally long-distance dispersal and spread at a regional scale (Vermeij 1996; Theoharides & Dukes 2007). Some models include a fifth stage, often termed 'impact', in which the spreading population takes on pest status (Williamson & Brown 1986; Williamson 2006). A range of biotic and abiotic factors act as filters on each phase (see Theoharides & Dukes 2007 for a discussion of suggested predominant factors at each stage), strongly constraining the successful passage between stages. Indeed, Williamson and Fitter (1996) contend that only 10% of species entering one stage will successfully pass to the next.

Detection of invading species and determination of the level and nature of their impact can be severely confounded by the occurrence of one or multiple lag phases. Lag phases can occur at any point in the invasion process and may arrest progression through the stages for decades or even centuries (Crooks 2005; Simberloff 2011). Even without a distinct lag phase, invading species frequently take considerable time to reach their fullest extent in the new range. A model using data from across five European countries estimated the time taken for invading plants to reach their maximum extent to be around 150 years (Gasso *et al.* 2010). Commonly, a lag phase may occur between establishment and spread (Theoharides & Dukes 2007; Figure 1.1) and, whilst the mechanisms behind lag phases are not fully understood, this may be related to a lack of genetic variation in the founding population restricting the speed by which it can adapt to its new environment (Strayer *et al.* 2006), or the time needed to reach a population size sufficient to enable it to spread (Sakai *et al.* 2001). Accumulation of genetic variation, including via hybridization, during the lag phase may result in the rapid generation of novel genotypes capable of flourishing under different abiotic conditions, outcompeting native species or escaping from natural enemies, leading to a sudden increase in the spread or impact of the invader (Lee 2002; Colautti & Barrett 2013). The potential for further, sometimes rapid, adaptation at these new

range limits may subsequently result in even greater population growth, spread and impact (Prentis *et al.* 2008; Figure 1.1).

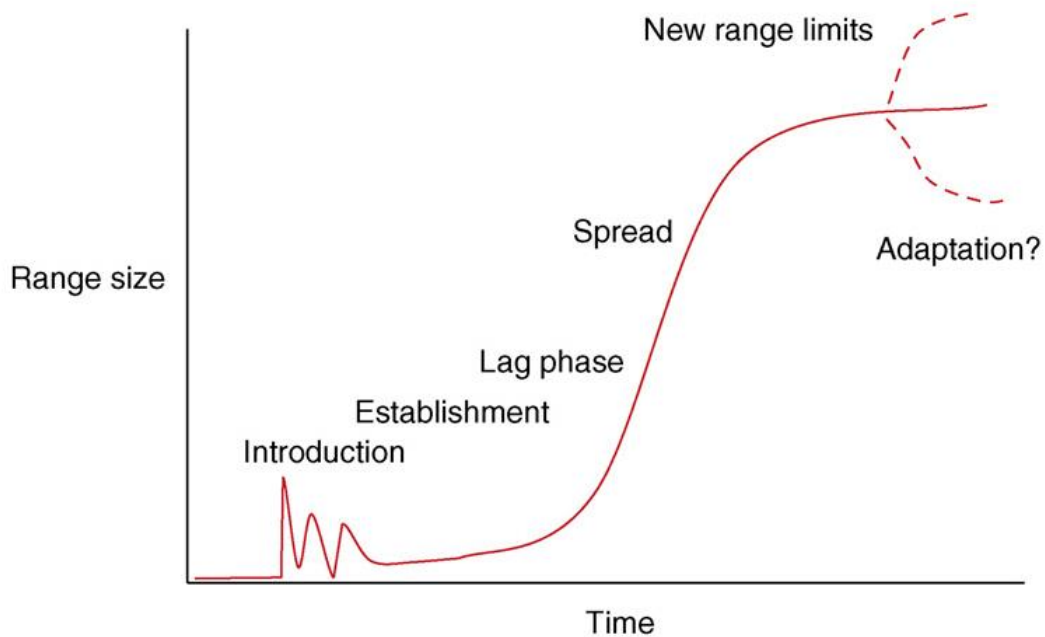


Figure 1.1 Key stages in biological invasions with regard to the change in range size over time (from Prentis *et al.* 2008).

A number of authors argue that the frequency of long lag phases before sudden changes in invader dynamics means the default assumption must be that any invading species has the potential for substantial negative effects on its recipient community, regardless of a seemingly benign coexistence at any given point in time (Crooks 2005; Simberloff 2011).

Essl *et al.* (2011) used data on ten taxonomic groups across 28 European countries to show that the number of currently established invading species are more closely linked to human trade and transport activities in the year 1900 rather than 2000, despite the majority of introductions having occurred in the latter 50 years. They suggest this lag demonstrates that any attempts to prevent future invasions will be ineffective as the past 100 years of human activity will have already set in motion a significant number of, albeit currently unrecognised, invasions: a so-called “invasion debt”. A study of native and exotic plant assemblages in Canada found numerous lines of evidence consistent with the invasion debt hypothesis, however concluded that the presence of numerous other confounding factors continued to make predictions of future invasion trends and impacts extremely difficult to quantify (Bennett *et al.* 2013).

1.2.3.3 Controversies over invasive species impacts

Despite considerable levels of international political concern, the causes, impacts and importance of biological invasions remain a hotly-debated topic. A commentary published in *Nature* in June 2011 (Davis *et al.* 2011) argued that the concepts of 'native' and 'invasive' species have become largely meaningless, and that actually other anthropogenic risk factors such as climate change and urbanization pose a far greater threat to global biodiversity. A rebuttal appearing a month later in *Science* (Lambertini *et al.* 2011), along with a number of other responses in *Nature* (Alyokhin 2011; Lockwood, Hoopes & Marchetti 2011; Simberloff *et al.* 2011), maintained that, whilst not all invasive species have so far proved damaging to the recipient communities, numerous invaders have caused significant harm to native species, ecosystem function and economic interests, and as such warrant "aggressive intervention" (Lambertini *et al.* 2011). Thomas and Palmer (2015a) used the British Countryside Survey (BCS) data, covering plant occurrence and cover at 479 British sites between 1990 and 2007 (Carey *et al.* 2008), to test whether, over time, spread of invasive plants was causing regional-scale extinctions of native species by competitive exclusion. Contrary to predictions, the authors found introduced species to be less widespread than natives, that their diversity and cover were increasing no more than native species, and that changes to the diversity of both groups were positively associated. The authors therefore suggest that both groups are likely to be responding similarly to other drivers of environmental change and that the negative effects of invading plants on British biodiversity, and perhaps that of other parts of the world, have been overstated. A prompt challenge by Hulme *et al.* (2015) was highly critical of their interpretation, outlining the inappropriateness of utilising the BCS dataset to investigate non-native species impacts in this way. Hulme *et al.* (2015) further stated that the conclusions drawn by Thomas and Palmer (2015a) indicated a lack of understanding of invasion ecology and, worse, that their "misleading analyses" could ultimately result in the mismanagement of plant invasions with a consequent detrimental impact on worldwide conservation. The debate continues (Schlaepfer *et al.* 2011; Richardson & Ricciardi 2013; Thomas 2013; Valery *et al.* 2013; Blackburn *et al.* 2014; Blondel *et al.* 2014; Simberloff & Vitule 2014; Thomas & Palmer 2015b; Buckley & Catford 2016).

1.2.4 Invasive species as model study systems

Whilst invasion biology is an area of theoretical and applied research in its own right, invasive species can act as useful models for the study of basic ecological and evolutionary processes. In such cases, invasions serve to act as ‘natural experiments’ which can be used to complement and extend the understanding gleaned from planned manipulative investigations (HilleRisLambers *et al.* 2013). This utility was recognised from the mid-1800s (Darwin 1859) and explicitly referenced as early as 1919 (Grinnell 1919). As such, it has been used to inform our understanding of many ecological and evolutionary concepts, including adaptation, speciation, life history dynamics and community assembly (Lambrinos 2004; Rice & Sax 2005; Vellend *et al.* 2007; Prentis *et al.* 2008; Rogers *et al.* 2012; Valladares *et al.* 2015). Invasions occur over such sizeable spatial and temporal scales they would be largely impossible to recreate under planned experimental conditions (Seiter *et al.* 2013; Roy *et al.* 2016). Similarly, they involve so many different taxa and environment types that a full range of biological questions can appropriately be investigated via their study (Sax *et al.* 2007). The time, place and characteristics of introductions are often well documented and consequently allow the rate of change processes to be accurately measured (Blackburn 2008). Furthermore, evolutionary and ecological processes can be studied in real time, often in the absence of confounding effects of long-term co-evolution, rather than inferred from past events (Strauss *et al.* 2006; Carroll *et al.* 2007). Notably, invasions provide the opportunity to accumulate data on the effects of occurrences such as wide-scale species additions, pathogen translocations and hybridization events that would be unethical to collect in any other circumstances, thereby contributing significantly to understanding of these and other processes (Sax *et al.* 2007; Ainouche *et al.* 2009; Popkin 2015).

Coastal marine areas are at extremely high risk of invasion, being amongst the most heavily invaded systems on Earth (Grosholz 2002). This thesis aims to examine the ecological impacts of one such invasion, that of the planthopper *Prokelisia marginata*, which has recently arrived in British saltmarshes.

1.3 Study system

1.3.1 Saltmarshes

Saltmarshes are intertidal areas of fine sediment stabilised by vegetation. Distinct from terrestrial zones, they are subject to the full range of tidal inundation from micro-tidal to macro-tidal regimes (Adam 2002). In Britain and Ireland, these tidal fluctuations typically range from 1.7m to 12.2m (Boorman 2003). Found fringing many of the world's soft coasts, saltmarshes represent a critical interface between land and sea on sheltered shores and are one of the key habitats of the European Community Directive 92/43/EEC ('Habitats Directive') (Townend *et al.* 2011). Saltmarshes provide a complex of habitats with the main vegetated surface being dissected by creeks, intertidal flats, and occasionally shell ridges (Adam 2002). British saltmarshes are categorised into four main zones, although not all may be clearly distinguishable in all marshes. Categories range from the pioneer zone bordering open mudflats, through to the lower, middle and high or upper marsh, following a broadly increasing surface elevation due to the accretion of sediments. Each zone is characterised by the extent of tidal inundation and associated plant species distribution, with a fifth zone representing the transition to non-halophytic terrestrial areas (Boorman 2003; Foster *et al.* 2013; Figure 1.2).

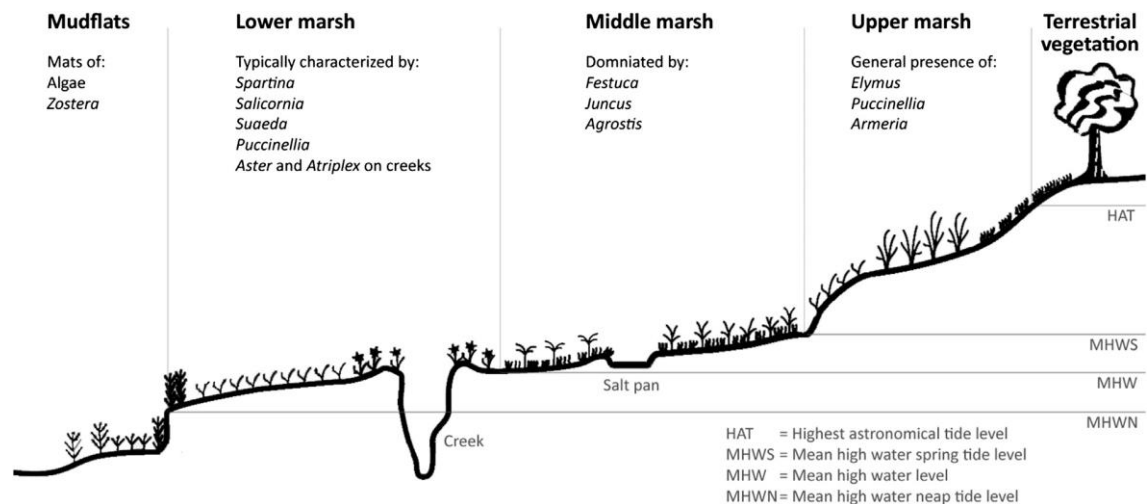


Figure 1.2 Indicative British intertidal mudflat and saltmarsh profile (from Foster *et al.* 2013).

Saltmarshes are of high conservation value, providing a unique habitat for a wide diversity of fauna and flora that are unable to survive elsewhere, as well as an important refuge for overwintering wildfowl (Boorman 2003; Finch *et al.* 2007; Townend *et al.* 2011). Rising sea levels and extreme weather events predicted under global climate change scenarios (Lowe & Meiner 2012), combined with the presence of hard

coastal defences such as seawalls preventing landward migration of saltmarshes, represent a significant threat to this important habitat and its associated species (Irmiler *et al.* 2002; Finch *et al.* 2007).

England has an estimated 32,500 hectares (ha) of saltmarsh, with approximately 6,000ha in Wales and a similar area in Scotland (Figure 1.3). A further 239ha can be found in Northern Ireland (not shown).



Figure 1.3 Distribution of British saltmarshes (from Boorman 2003).

Around 40 species of vascular plant are found exclusively in British saltmarshes, several of these being Red Data Book species (Cheffings & Farrell 2005), although individual marshes commonly only have between ten and twenty species (Boorman 2003). Terrestrial saltmarsh invertebrates are estimated to number 293 resident species, of which 148 are found only in saltmarshes (Doody 1992b). Invertebrates are distributed throughout the marsh zones (Boorman 2003), however a number of studies have found communities located around the mean high tide elevation to be particularly species poor (Irmiler *et al.* 2002; Finch *et al.* 2007).

Coastal marine areas are at extremely high risk of invasion, being amongst the most heavily invaded systems on Earth, yet ones in which species introduction is particularly difficult to predict or prevent (Grosholz 2002). Whilst terrestrial and coastal invading species display a similar mean rate of range expansion over long periods, coastal invasions exhibit much greater variation in their rate of spread, frequently demonstrating explosive and extensive range expansions over very short timescales (Grosholz 1996). Coastal invasions are driven largely by the shipping industry, particularly through hull biofouling and the exchange of ballast water. Up to 83% of the species introduced to North American coastal areas between 1981 and 2010 are thought to have arrived in this way, whilst rates of invasion are increasing exponentially (Ruiz *et al.* 2015). A study by Cohen and Carlton (1998) of the highly-invaded San Francisco Bay estuary found that as many as three or four new invading species are detected every year. The European Union is similarly affected. Numbers of non-indigenous species in the Mediterranean Sea were found to have increased by 204% between 1970 and 2013 (Galil *et al.* 2014) with shipping via the Suez Canal representing a primary vector (Galil *et al.* 2015). Minchin *et al.* (2013) report the presence of some ninety alien species in British marine and brackish environments, the majority of which were initially reported from the English Channel. Despite these concerns and the importance of the habitat type itself, there has been relatively little investigation of the occurrence and impact of invading species on saltmarshes, either globally or in Britain (Adam 2002). One notable exception to this is the *Spartina* genus, which will be discussed in the following section. This relative paucity of research notwithstanding, Boorman (2003) stated that invading animal species have had no significant impact on British saltmarshes, and that any further introductions “cannot be seen as a major threat, given the current awareness of the dangers inherent in the introduction of non-native species of plants or animals.” (Boorman 2003, p.54).

1.3.2 *Spartina* spp.

1.3.2.1 *Spartina* spp. in Britain

Spartina is a genus of perennial rhizomatous polyploid C₄ grasses in the sub-family Chloridoideae (Poaceae). The genus is comprised of around fifteen species, including a number of hybrids, all of which colonise coastal or inland saltmarshes (Ainouche *et al.* 2009). *Spartina* species are halophytic and consequently capable of osmoregulation when grown in these saline environments (Bacheller & Romeo 1992). Within their native range, species of *Spartina* fulfil an important role as primary colonists of intertidal mud flats. Able to trap and stabilise sediment efficiently, they act as powerful ecosystem engineers (Jones *et al.* 1994) of both the physical and biological environment and as key facilitators of successional saltmarsh development (Castillo *et al.* 2000; Bouma *et al.* 2010; Figure 1.4).



Figure 1.4 *Spartina anglica* in flower at Hythe *Spartina* Marsh field site, Hampshire. August 2013.

Spartina maritima (Curtis) Fernald, a hexaploid native to the UK, has a wide, although discontinuous, range along the Atlantic seaboard from South Africa through to Northern Europe (Adams *et al.* 2012). Up until the 19th century it was the only known *Spartina* species along the Atlantic European coast (Marchant & Goodman 1969). Whilst robust, sexually reproducing, populations remain in the African and south European areas of its range, north European populations of *S. maritima* have been found to display a lack of vigour, typified by a scarcity of viable seed and only marginal vegetative spread (Marchant 1967; Yannic *et al.* 2004). *S. maritima* achieved its maximal British distribution in the early 1900s, recorded from the Exe estuary to Chichester on the south coast, and from the Thames estuary to Lincolnshire on the east coast (Perring & Walters 1976). Its range has subsequently decreased dramatically, with an extensive survey undertaken by Raybould *et al.* (1991a) suggesting a rapid retreat into the centre of its distribution: the south Suffolk and Essex high saltmarshes. Indeed, a very small population identified on the Northney Saltings of Hayling Island in the same survey was found to be present at the outset of my research but was undetectable by its conclusion (pers. obs.).

In the early 19th century, *Spartina alterniflora* Loiseleur, a hexaploid native of the eastern coast of North America, was accidentally introduced to Southampton Water, Hampshire, presumed to be the result of the transport of seeds in shipping ballast (Thompson 1991). Unlike *S. maritima*, which is found as scattered individuals or small clumps in generally species-rich communities of middle and upper saltmarshes (Raybould *et al.* 1991a), *S. alterniflora* typically forms dense, monospecific stands that dominate the lower portion of the intertidal zone (Thompson 1991). Its native range extends along the eastern seaboard of North America from southern Canada to northern Florida, occurring as far south as northern Argentina, and, in the Gulf of Mexico, from Florida to southern Texas (Mobberley 1956; Adams *et al.* 2012).

Interspecific hybridization of *S. alterniflora* with *S. maritima* resulted in the sterile homoploid hybrid *Spartina x townsendii* Groves, first described in 1880 from samples collected at Hythe in Southampton Water (Groves & Groves 1880). *S. x townsendii* demonstrates vigorous growth by lateral vegetative spread (Renny-Byfield *et al.* 2010) and was deliberately and widely distributed around the world in the nineteenth century (Strong & Ayres 2013). Fertile plants were first recorded in Lymington, Hampshire in 1892 which appeared to have resulted from chromosome doubling in *S. x townsendii* (Marchant 1967; Gray *et al.* 1991). These were later described as the new fertile allododecaploid species *Spartina anglica* Hubbard (Hubbard *et al.* 1968). *S. anglica* is

extremely vigorous, spreading both vegetatively and through the setting of abundant seed (Strong & Ayres 2013). It rapidly colonised British coasts via both natural dispersal of seeds and rhizomes and by deliberate introduction for marsh reclamation (Thompson 1991), occupying approximately 10,000ha of intertidal saltmarsh by 1990 (Charman 1990). The extent of the natural distribution of the species is believed to be between Poole in Dorset and Pagham in Sussex (as well as some areas in France); all other populations are thought to have been established by deliberate introduction (Raybould *et al.* 1991b). The first record of deliberate planting of *S. anglica* is from the Beaulieu Estate in 1898 (Gray *et al.* 1991). *S. anglica* displays a large ecological amplitude along the successional marsh gradient, tolerating several hours of immersion at high tides and therefore able to occupy a previously vacant niche as a pioneer species in the low marsh zone (Ainouche *et al.* 2009). The species has a particularly robust rhizome, shoot and root system, enabling the accumulation of large volumes of tidal sediments. This modification of the physical structure of intertidal zones consequently facilitates colonisation by other saltmarsh species and alters the prevailing dynamics of the marsh. Due to its properties as an ecological engineer, *S. anglica* was deliberately introduced widely around the world (North Europe; North America; Australia; New Zealand) in the early twentieth century (Ainouche *et al.* 2009; Strong & Ayres 2013) and most recently to China in 1963 (An *et al.* 2007) for land reclamation and coastal protection. Although *S. anglica* continues to spread in many areas both in Britain and worldwide, degeneration of swards along the south coast of England was first noted in the mid-1920s. Poole Harbour had 775ha of *S. anglica* in 1924, but this had reduced to 415ha by 1980. In a process categorised as 'natural die-back', it is thought that *S. anglica* alters the sedimentary characteristics of the saltmarsh to cause waterlogged anaerobic conditions which consequently result in its own demise. The extent to which this explains the phenomenon has not been conclusively resolved however, and other factors are also likely to be involved (Gray *et al.* 1991; Lacambra *et al.* 2004; Coverdale *et al.* 2012).

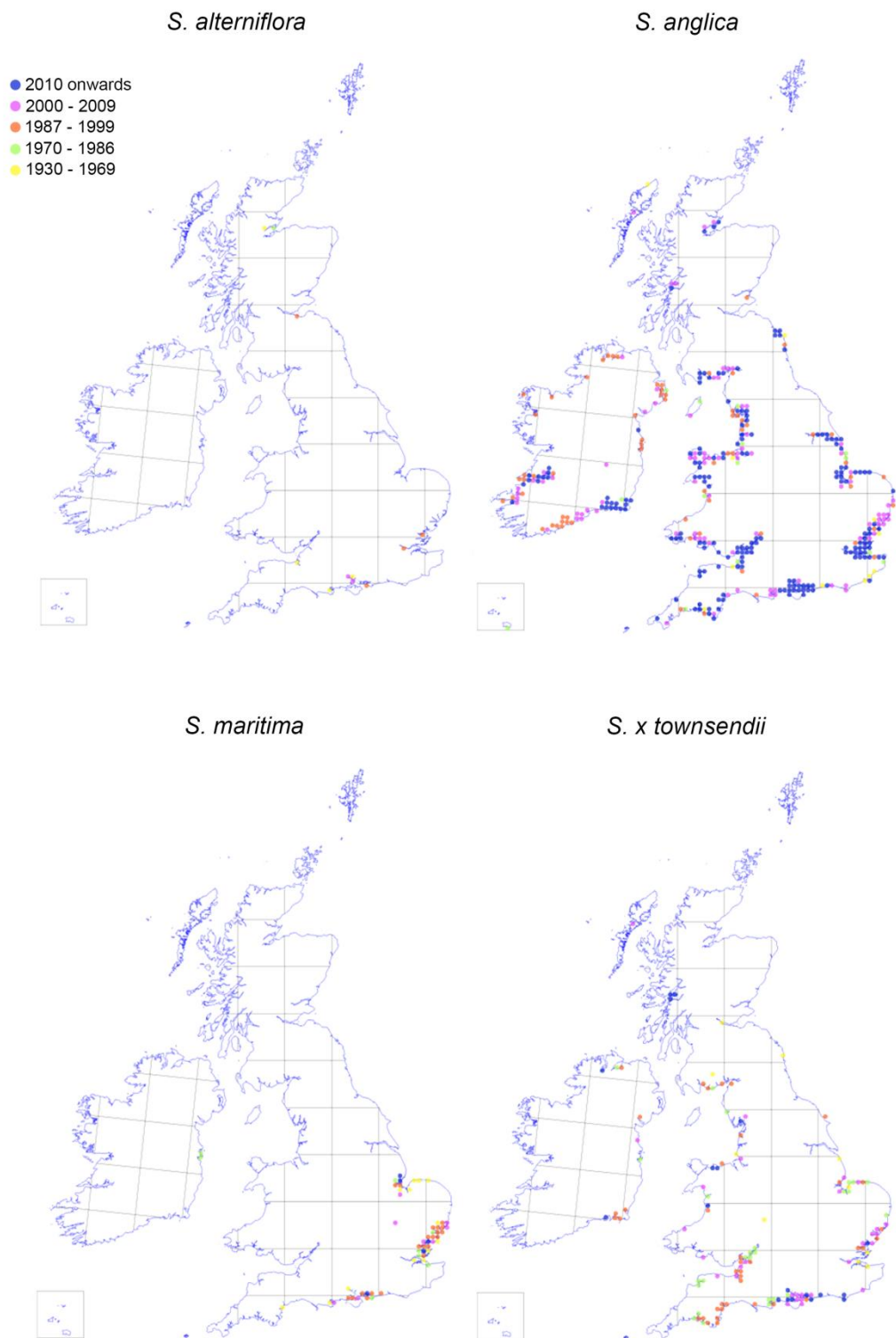


Figure 1.5 British and Irish distribution of (clockwise from top left): *S. alterniflora*; *S. anglica*; *S. x townsendii* and *S. maritima*. Maps copyright of BSBI produced using interactive data. Obtained from <http://www.brc.ac.uk/plantatlas/> (accessed 9 July 2016).

Whilst genetic variation in both *S. alterniflora* and *S. maritima* in Britain is extremely low, rendering the specifics of the speciation pathway difficult to elucidate (Gray *et al.* 1991; Ayres & Strong 2001), isozyme studies (Raybould *et al.* 1991b) and, latterly, sequencing of nuclear and chloroplast DNA (Ferris *et al.* 1997; Baumel *et al.* 2002b), have shown *S. alterniflora* to be the maternal parent of the resulting hybrid *S. x townsendii* (Figure 1.6). These studies further suggest that both the original hybridization event and the subsequent chromosome doubling to produce *S. anglica* happened as extremely rare, or possibly even unique, occurrences.

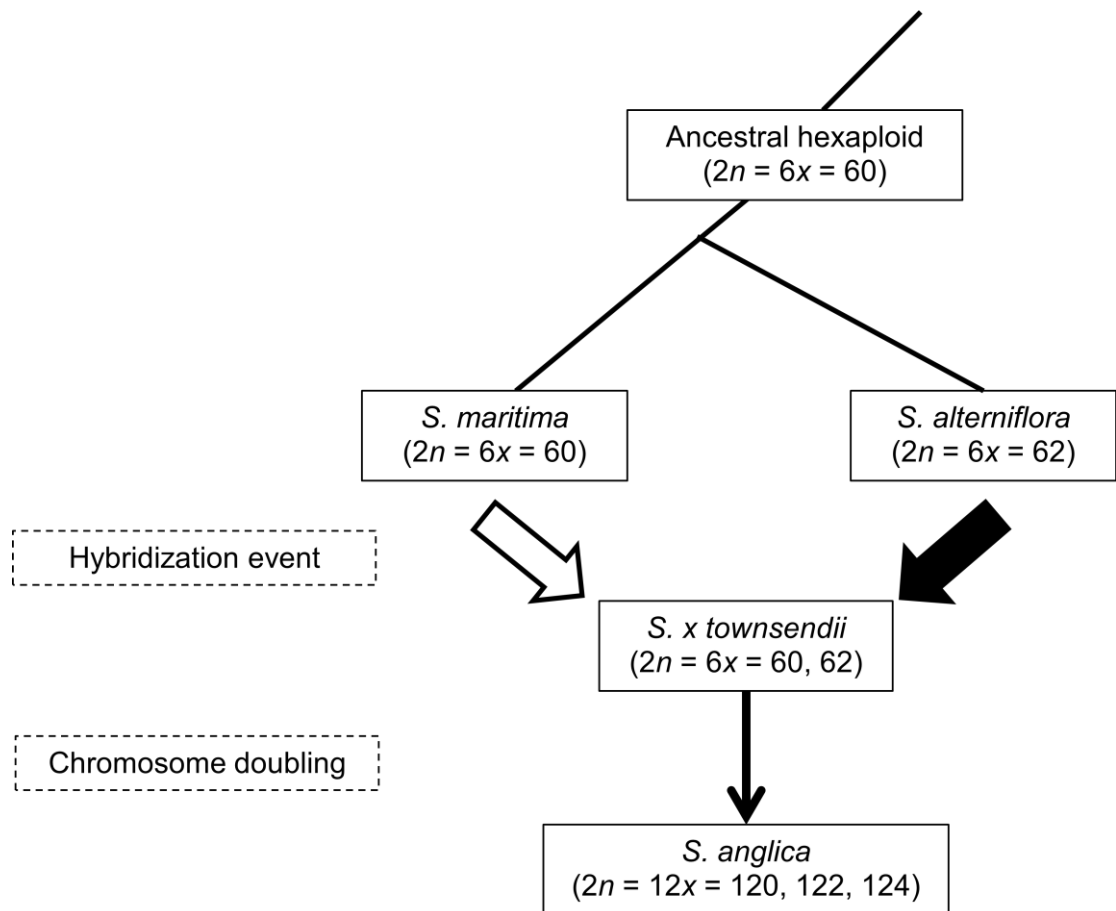


Figure 1.6 Hybridization and allopolyploid speciation of *Spartina* species in Britain following *S. alterniflora* introduction. Species boxes include details of polyploidy and chromosome number. Filled arrow represents maternal and empty arrow represents paternal genome donors in the hybridization event. Adapted from Ainouche *et al.* (2004).

1.3.2.2 *Spartina* spp. as invaders

Spartina species have been intentionally introduced to many parts of the world due to their ability to colonise open areas, stabilise eroding shorelines, reclaim land and provide defence against extreme coastal weather events (Callaway & Josselyn 1992; Wang *et al.* 2015). Many of the introductions have been considered successful in achieving these aims (Ranwell 1967; Chung 1983), however the ecological impact that

these species have in much of their invaded range now overshadows the ecosystem engineering benefits expected of their initial introductions. Potential negative impacts include competition with native flora, altered habitat for native fauna, changed sediment, nitrogen and carbon dynamics, threats to local economies, and the loss of shorebird and wading bird foraging areas (Callaway & Josselyn 1992; Neira *et al.* 2006; Wang *et al.* 2006; An *et al.* 2007; Adams *et al.* 2012; Zhou *et al.* 2015a).

The only species of *Spartina* native to the Pacific coast of North America is *S. foliosa*, however four other species have become established: *S. alterniflora*, *S. anglica*, *S. patens* and *S. densiflora*. Across its introduced range, *S. alterniflora* has been found to readily hybridise with its native congeners, threatening their genetic integrity and potentially resulting in local extirpation (Adam 2002). *S. alterniflora* was introduced to San Francisco Bay in the late 1970s (Callaway & Josselyn 1992). It grew intermixed with the native *S. foliosa* resulting in the creation of a backcrossing hybrid swarm which aggressively invaded restoration sites, native marshes and open mudflats around the Bay. Hybrids produced 400 times the pollen of the native species, whilst 90% of seed production and 80% of seedling recruitment between 1998 and 2004 was found to be of hybrid origin (Strong & Ayres 2013). The hybrid *Spartina* canopy was found to shade the substrate, reducing light for microalgae, whilst also triggering a range of physical, chemical and biological changes in the benthic system. The resulting changes significantly altered the composition of the benthic invertebrate community (Neira *et al.* 2006). The open intertidal mudflats of San Francisco Bay represent the most important foraging habitat on the Pacific coast of North America for over 900,000 migrating shorebirds. An estimated loss of between 27% and 80% of available foraging area was attributed to the spread of hybrid *Spartina* (Stralberg *et al.* 2010). Fledgling success of the rare Alameda song sparrow, *Melospiza melodia*, was reduced by 30% when nesting in hybrid *Spartina* compared with native vegetation. The invader occurred at significantly lower elevations, exposing the nests to tidal flooding (Nordby *et al.* 2009).

In Willapa Bay, Washington, the introduction and spread of *S. alterniflora* resulted in the conversion of open intertidal mudflats to dense monospecific marshes with a consequent 20% reduction in habitat for aquatic birds (Hedge *et al.* 2003). Shorebird numbers were reduced by as much as 67% during the height of the invasion (Strong & Ayres 2013). Despite Washington State Department of Agriculture establishing a wide ranging control programme in 1995, populations of *Spartina* species increased in the area by 250% between 1995 and 2000 (Hedge *et al.* 2003). Intensified efforts from 2003 onwards (at a total cost of ~\$30 million) finally resulted in the rapid decrease and

elimination of all but a few hectares of *S. alterniflora* by 2011 (Strong & Ayres 2013). During this period, bird numbers were found to greatly increase in areas in which herbicide treatment had not been used, but from which *S. alterniflora* had been removed (Patten & O'Casey 2007). Similar problems have been experienced in China (Ma *et al.* 2003; Chen *et al.* 2004; Li *et al.* 2009; Wu *et al.* 2009; Wang *et al.* 2015) where *S. alterniflora* was listed amongst the most harmful invasive alien plants in the country by the State Environmental Protection Administration in 2003 (Wang *et al.* 2006; Zuo *et al.* 2012).

S. anglica was intentionally distributed in Britain, North Europe, North America, China, Australia and New Zealand for land reclamation, marsh restoration and the stabilisation of tidal flats (Adam 2002). Over 175,000 plant fragments were transported by ship to ~130 different sites around the world from Poole Harbour between 1924 and 1936 (Hubbard 1965; Ranwell 1967) and the species now has a worldwide distribution (Ainouche *et al.* 2009). The rapid spread of the introduced populations has resulted in various attempts to control or eradicate the plant (Kriwoken & Hedge 2000; Hacker *et al.* 2001; Cottet *et al.* 2007) and it is now listed by the IUCN as one of the world's 100 worst invasive species (Lowe *et al.* 2000). Introduced to Puget Sound, Washington in 1961, *S. anglica* coverage increased from less than 3ha in the mid-1970s to affect 3,311ha across 73 sites by 2000 (Hacker *et al.* 2001). *S. anglica* was deliberately introduced to Australia in the 1930s and has subsequently invaded approximately 880ha of intertidal mudflats, saltmarshes, mangrove and seagrass habitats in Victoria and Tasmania. The effect of *S. anglica* on benthic macroinvertebrates in Australia is equivocal (Kriwoken & Hedge 2000), however Cutajar *et al.* (2012) found that invaded areas had lower diversity and 50% less species richness than both native saltmarsh and bare mudflats, and 60% lower macrofaunal abundance in comparison with native marshes. Additionally, they found that below ground biomass was 72% denser in *S. anglica* patches than native saltmarsh, which they suggested was likely to inhibit burrowing by infauna.

S. anglica is thought to threaten the populations of wildfowl and wading birds that overwinter on British and Irish estuaries due to its impacts on the abundance and composition of macroinvertebrate communities and the overgrowth of bare intertidal flats necessary for foraging (Nairn 1986). Severe reductions have been seen in populations of dunlin, *Calidris alpina*, whose numbers reduced by almost half between 1973-74 and 1985-86. Goss-Custard and Moser (1988) found that the spread of *S. anglica* was strongly correlated with reduced dunlin numbers in British estuaries. Whilst

bird numbers were not found to increase in estuaries where *S. anglica* had receded due to natural die-back, the authors suggested this may be due to the growth of thick algal mats which followed the invader's disappearance. This theory was tested by Tubbs *et al.* (1992), however, who found that the spread of *S. anglica* was not the ultimate cause of the national reduction in dunlin numbers. They instead linked the overall population decrease to hunting mortality, although allowed that *S. anglica* spread may represent the proximate cause of dunlin decline in specific estuaries.

Whilst considered a major pest in many areas around the world, the status of *Spartina* species in Britain is more complex. British populations of *S. maritima*, *S. x townsendii* and *S. alterniflora* have all undergone rapid declines, and the species are scarce in Europe (Lacambra *et al.* 2004). Both *S. maritima* and *S. alterniflora* communities are listed in Annex I of the Habitats Directive (92/43/EEC), requiring reports on the progress and outcomes of conservation measures by member states. Additionally, *S. maritima* is listed as a species "of principal importance for the purpose of conserving biodiversity" under section 41 of the Natural Environment and Rural Communities Act 2006 and a priority species under the UK Biodiversity Action Plan (Joint Nature Conservation Committee 2007). The status of *S. anglica* is particularly controversial. Its spread is still encouraged in some areas where it is viewed as an important agent for stabilising shores and facilitating coastal defence. The ecological consequences of its expansion for coastal habitats and species, however, have triggered a range of monitoring and population management interventions in a number of sensitive British sites (Lacambra *et al.* 2004).

1.3.3 *Prokelisia marginata*

1.3.3.1 *Prokelisia marginata* in its home range

Prokelisia marginata Van Duzee is one of ~2000 species of planthopper, phytophagous hemipterans, in the family Delphacidae, the most well-studied of nineteen families making up the superfamily Fulgoroidea (Denno & Perfect 1994). Planthoppers are found on every continent, with the exception of Antarctica, and in all major biomes (O'Brien & Wilson 1985). Most delphacids feed on monocots, especially grasses; frequently mono- or stenophagous, many species are ruinous pests of major agricultural crops worldwide (Benrey & Lamp 1994; Gallagher *et al.* 1994). Planthoppers cause damage directly by feeding, during which they insert their stylets into the vascular tissue of plants and ingest the sap (Cook & Denno 1994). This process often causes a characteristic yellowing of plant tissues referred to as "hopperburn" (Denno & Roderick 1990) (Figure 1.9). Planthoppers may also cause damage indirectly as vectors of a range of plant pathogens (Nault 1994). *Claviceps purpurea* is a fungal pathogen of saltmarsh grasses which causes ergot disease, preventing seed production in infected inflorescences (Jarosz & Davelos 1995). Over 70% of *S. anglica* inflorescences in Poole Harbour were found to be infected between 1985 and 1995, however this was found to have little impact on the population dynamics of the grass (Raybould *et al.* 1998) as dispersal and colonisation occur predominantly via vegetative spread (Mullins & Marks 1987). Fisher *et al.* (2005) contend that *P. marginata* is a prime candidate for transmission of *C. purpurea* spores, however molecular studies have found no empirical evidence to show that the planthopper acts as a vector of such diseases (Davis *et al.* 2002; Garcia-Rossi *et al.* 2003; Grevstad *et al.* 2003).

P. marginata are small delphacids (length of males: 2.3-4.1 mm; females 3.4-4.4 mm) with uniform colouration ranging from pale yellow to light brown, and two pairs of distinctive dark brown longitudinal lines on the frons (Wilson 1982) (Figure 1.7).



Figure 1.7 Left: lateral view of adult *P. marginata*. Right: frontal view of adult *P. marginata* frons showing distinctive longitudinal markings. Photo copyright: Charles R. Bartlett, 2008.

In common with most species of planthopper, *P. marginata* exhibits wing dimorphism, having populations composed of two wing forms (Denno *et al.* 1986) (Figure 1.8). Long-winged individuals, macropters, have both pairs of wings fully developed for flight, capable of covering distances of up to 30km (Denno *et al.* 1996). Short-winged individuals, brachypters, have abbreviated mesothoracic and vestigial metathoracic (hind) wings and consequently are capable of dispersing only over short distances (up to several metres) by walking or hopping (Denno & Grissell 1979). Wing morphology is determined by a developmental switch that responds to proximate environmental cues such as levels of crowding and host plant nutrition. The sensitivity of the switch is heritable and under polygenic control (Denno 1994). Macropters are the more adaptive form in unstable habitats where resources fluctuate (Denno & Grissell 1979), whereas brachypters have greater fecundity and are therefore the more adaptive form in stable patches when crowding is relatively low and plant nutrition, crucially the level of available nitrogen (Olmstead *et al.* 1997), is adequately high (Denno *et al.* 1980; Denno *et al.* 1989).

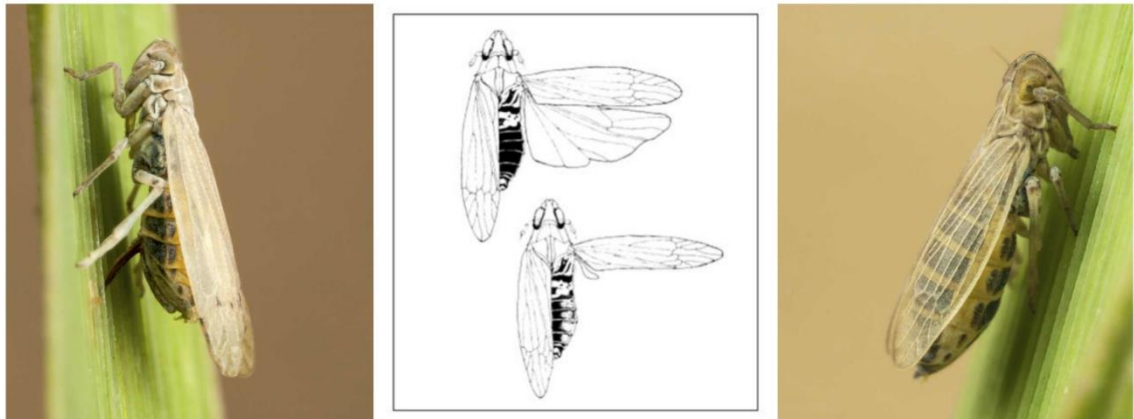


Figure 1.8 Wing forms of *P. marginata*. Left: adult macropter; Right: adult brachypter. Photo copyright: Theodoor Heijerman, 2010. Centre: diagram of wing forms from Denno *et al.* (1985).

P. marginata is native to the Gulf and Atlantic coasts of North America where it is the most abundant herbivore of *S. alterniflora*, frequently reaching densities exceeding 1000 adults/m² and 100,000 nymphs/m² (Denno *et al.* 1986; Denno *et al.* 2000). Whilst *P. marginata* eggs are laid inside the plant tissue (Figure 2.2) and therefore subject to regular tidal inundation, adults and nymphs escape rising waters by retreating up *Spartina* stems, or hopping along the surface film to the next plant if dislodged or completely inundated (Denno & Grissell 1979). Adults have been found to survive complete submersion for between four and five hours under experimental conditions (Davis & Gray 1966). *P. marginata* is also present in San Francisco Bay and nearby estuaries, where it is thought to have been introduced prior to the introduction of *S. alterniflora* (Daehler & Strong 1997b; Grevstad *et al.* 2003) and would therefore have utilised the native *S. foliosa* as its host plant. *P. marginata* is a stenophagous phloem feeder. Across its entire North American range, the species feeds only on *S. alterniflora*, *S. foliosa* and their hybrids, and the introduced *S. anglica*, avoiding all other sympatrically occurring *Spartina* and other monocot species (Grevstad *et al.* 2003).

P. marginata reproduce sexually; there have been no recorded instances of parthenogenesis. Males actively search for sexually receptive females, the two sexes communicating via acoustic signals transmitted as vibrations through the host plant or between separate plants that are in physical contact (Claridge & De Vrijer 1994). The structure of the signal has been found to be species-specific, helping to maintain reproductive isolation in sympatrically occurring *Prokelisia* species (Heady & Wilson 1990; Heady & Denno 1991). Once in close contact, males and females align in the same orientation with the male to the side and slightly to the rear of the female (Figure 1.9). Courtship proceeds with the continuing exchange of acoustic signals, wing fluttering, and the male placing one of his forelegs on top of the female's thorax before

mating begins (Heady & Denno 1991). Multiple mating is common in male delphacids, with as many as five copulations recorded in one day; however recently-mated females usually reject further copulation attempts (Drosopoulos 1985). Female *P. marginata* lay their eggs, often in small groups, either in a slit made with their ovipositor in the leaf epidermis or between the closely-spaced veins on the adaxial surface of the leaf blade (Denno & Roderick 1990). The resulting oviposition scars routinely turn brown due to invasion by a mite, *Ogmotarsionemus erepsis*. This easily visible discolouration acts as a highly reliable indicator of oviposition; presence of the mite has been found to have no apparent effect on egg development (Strong 1988). After hatching, nymphs pass through five instars before moulting into adults (Denno *et al.* 1986; Figure 1.9).



Figure 1.9 Left: *P. marginata* nymphs and adults on *S. anglica* at Hythe field site. Characteristic yellowing of plant tissue indicates damage to the plant referred to as “hopperburn”. Top right: Adult *P. marginata* emerging from nymph case. Bottom right foreground: *P. marginata* mating. Bottom right background (indicated by arrow): oviposition scars on *S. anglica* leaf surface.

Generation times are relatively short, responding to climatic conditions and the associated growing season of their host plants. In the mid-Atlantic coast range, *P. marginata* are trivoltine with generation times of approximately 40 days, the adult population peaking in May, July and September (Gratton & Denno 2003a). Adults are completely absent between December and March. Active nymphs overwinter in litter or rolled dead leaves, the presence of which is crucial for survival; winter mortality is extremely high with population densities falling by two or more orders of magnitude between autumn and spring (Denno 1976; Denno *et al.* 1996). Conversely, the sub-tropical climate of the Gulf coast allows for continuous growth of *S. alterniflora* throughout the year, resulting in five overlapping generations of *P. marginata* in which adults are always present (Denno & Grissell 1979).

P. marginata are subject to attack by a variety of natural enemies in their home range. The mirid bug *Tytthus vagus* is the primary egg predator (Gratton & Denno 2003a), whilst wolf spiders (Lycosidae) (Denno *et al.* 2003) and web-building linyphiid spiders (Gratton & Denno 2005) are the primary predators of nymphs and adults. Mymaridae (Hymenoptera) are important egg parasitoids (Stiling & Strong 1982a). Nymphs and adults are subject to parasitoid attack by Dryinidae (Hymenoptera) and Elenchidae (Strepsiptera), although rates are relatively low and vary both spatially and temporally (Stiling *et al.* 1991; Denno *et al.* 2003). Egg mortality is thought to be the key factor in planthopper population dynamics. After accounting for egg and nymph mortality factors, as little as 4% of the population may survive to adulthood. In addition to predation and parasitism, habitat disturbance (Grevstad *et al.* 2003), crowding (Denno *et al.* 1986) and poor host plant nutrition (Bowdish & Stiling 1998) also have negative fitness and performance impacts on individuals and populations (Denno & Peterson 2000).

1.3.3.2 *Prokelisia marginata* as a biological control agent

The extensive invasion of Willapa Bay by *S. alterniflora* prompted the University of Washington to research a range of methods for its control, ultimately recommending biological control as the most promising new approach (Wecker *et al.* 2000). Experimental glasshouse studies found that *S. alterniflora* clones originating from Willapa Bay, an area in which *P. marginata* was absent, were severely stressed or killed by exposure to moderate populations of the insect (Daehler & Strong 1997b). After two seasons of herbivory, plants achieved only 12% of the above-ground biomass of herbivore-free control plants, and 37% of treatment plants had died. In contrast, *S. alterniflora* clones collected from San Francisco Bay, in which the grass and

planthopper species had co-existed for at least 20 years, achieved 83% of the above-ground biomass of control plants after two seasons of herbivory and suffered no mortality. Field reductions of approximately 70% of *P. marginata* densities in San Francisco Bay did not result in greater vegetative growth, inflorescence production or lateral spread of *S. alterniflora*; plants grew vigorously whilst exposed to the highest densities of the planthopper (Daehler & Strong 1995).

In further glasshouse studies, Wu *et al.* (1999) found that clones of *S. anglica* taken from a naive population, i.e. one that had had no previous contact with *P. marginata*, introduced to Puget Sound in Washington were highly vulnerable to damage caused by *P. marginata* herbivory and oviposition. After four months of exposure to high densities of the planthopper, experimental plants had suffered 92% mortality, with surviving plants averaging only 37% of the dry weight biomass of control plants. Blossey and Notzold (1995) suggest that an evolved loss of herbivore resistance might commonly occur in introduced plants as an evolutionary trade-off for increased competitive ability (although evidence for this is mixed; see Vellend *et al.* (2007) and references therein). Wu *et al.* (1999) suggested that vulnerability to *P. marginata* seen in *S. anglica* (their study) and introduced populations of *S. alterniflora* (Daehler & Strong 1997b) is explained by these populations never having been exposed to, or having been exiled from, *Prokelisia* spp. for tens of generations, consequently having no defensive adaptations against these specialist herbivores. In contrast, *Spartina* spp. populations that have evolved with, and never been exiled from, *P. marginata* are unaffected by extremely high densities of the insect (Daehler & Strong 1995; Gustafson *et al.* 2006).

In 2000, the Washington State Department of Agriculture approved the introduction of *P. marginata* into Willapa Bay to act as a biological control agent against the invasive *S. alterniflora*: the first application of classical biological control in a marine intertidal environment. Initial results of the study were promising. After three months of exposure to *P. marginata* in field cages, *S. alterniflora* showed an average reduction in biomass of 49.6% and reduction in height of 14.7% in comparison with paired control cages. Visible damage to uncaged plants was also noted where *P. marginata* was present in high densities, and planthopper populations were found to persist beyond the first winter in the new range (Grevstad *et al.* 2003). In combination with previous studies (Daehler & Strong 1995; Daehler & Strong 1997b; Wu *et al.* 1999), these results support the hypothesis that the Willapa Bay population was unusual in its vulnerability to *P. marginata* herbivory, possibly by virtue of an evolved loss of resistance or tolerance in the ~100 years since its introduction (Grevstad *et al.* 2003). It was not

possible to gather further data however, as the State agencies involved decided to implement a significant programme of herbicide application across the entirety of Willapa Bay and Puget Sound before the study was able to reach its conclusion (F.S. Grevstad, pers. comm.). Garcia-Rossi *et al.* (2003) compared populations of *S. alterniflora* from San Francisco Bay, which had been consistently exposed to *P. marginata*, with long-exiled and naive introduced populations of *S. alterniflora* and *S. anglica*. As expected, they found both lower tolerance of and lower resistance to the planthopper amongst the exiled and naive plants. The rate of nymphal emergence was twice as high on these plants and a significantly greater proportion of second instar nymphs successfully developed to adults, whilst negative impacts on plant performance and mortality were significantly greater for this group. Resistance, tolerance and planthopper population growth were all found to vary as a function of plant-herbivore contact history. Importantly however, the authors also noted much greater variability in both tolerance and resistance between genotypes in the naive populations, with some plant genotypes experiencing more than 50% shoot mortality and other genotypes experiencing none. The authors suggest this causes substantial problems for the use of biological control against invasive *Spartina* species. Introducing *P. marginata* to the system could result in plants re-evolving resistance or tolerance; if planthoppers differentially kill or reduce the spread of vulnerable genotypes, the frequency of tolerant genotypes could rapidly increase throughout the population (Garcia-Rossi *et al.* 2003).

1.3.3.3 *Prokelisia marginata*: an invading insect?

The first (unpublished) European record of *P. marginata* was from the Algarve in Portugal in 1994, with later recordings from Spain in 2008 and France in 2009 (Mifsud *et al.* 2010). The first published record was of a well-established population discovered in Slovenia in 2004 where it was collected from *S. maritima* (Seljak 2004), with high abundances subsequently reported in both Belgium and the Netherlands where it was present on either *S. anglica* or *S. x townsendii* (de Blauwe 2011).

Whilst it is possible that it was present in extremely low densities at an earlier date, *P. marginata* probably arrived in Britain in the last ten to fifteen years. A comprehensive invertebrate survey of the Hythe marshes in 2000 (Kirby 2000) found no evidence of the planthopper, whilst the first recorded specimen anywhere in Britain was collected in the same location in 2008 (Wilson & Muhlerthaler 2009). It is possible that *P. marginata* reached Britain by natural dispersal from continental European populations, however there is no evidence to support this. Alternatively, it may have arrived as eggs in plant

material carried in ships' ballast, which is perhaps more likely given that the site of its discovery is the same as that of introduced *S. alterniflora*. *P. marginata* has spread rapidly since its introduction to Britain. It has been recorded in all surveyed *Spartina* sites from the Humber estuary through East Anglia and south east England to South Wales, as far as the Gower Peninsular (Figure 1.10). No surveys have yet been conducted beyond this range, although it is possible that its distribution has extended further north (A.J.A. Stewart, unpublished data; Appendix A).

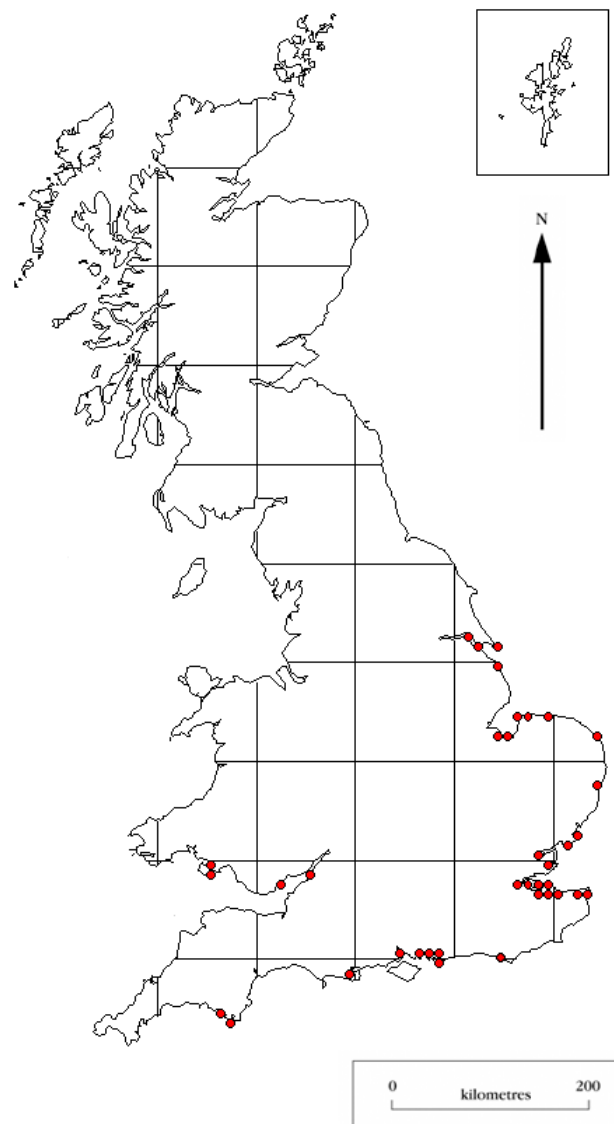


Figure 1.10 Distribution of *P. marginata* in Britain. Map created by A.J.A Stewart from unpublished data (Appendix A) using DMAP software written by A. Morton, Aberystwyth, UK; adapted by W. Harkin.

1.4 Thesis aims and structure

The aim of this thesis is to elucidate the ecological interactions of *Prokelisia marginata* with its new environment in Britain, specifically with its host plants and the wider arthropod community of the saltmarsh, and to assess its position as an invading insect. In Chapter 2, I begin by examining the demography of *P. marginata* along with the composition of the arthropod community in its immediate habitat, including the levels to which *P. marginata* may be subject to natural enemy attack. In Chapters 3 to 5, I then employ a combination of glasshouse and field manipulations to explore the relationship between *P. marginata* and its host plants in greater depth. In Chapter 3, I investigate the impact of *P. marginata* exposure on its main host plant in Britain, *S. anglica*. In Chapter 4, I expand this investigation to include the other three British host species: *S. alterniflora*, *S. maritima* and *S. x townsendii*, and aim to determine whether the level of any identified impact varies by species. This comparison is of particular interest in light of the evolutionary background of the four plant species and what might be characterised as a 'gradient' of shared co-evolutionary history with the planthopper. To my knowledge, no studies have ever been conducted on the interaction between *P. marginata* and either *S. x townsendii* or *S. maritima*. In Chapter 5, I develop these ideas to explore whether *P. marginata* makes a preferential choice between the four *Spartina* species, and whether different host species have a differential impact on *P. marginata* performance and outcomes. Finally, in Chapter 6, I conclude with a discussion of the results presented in the previous chapters, my thoughts on the current and potential future impact of *P. marginata* as an invading species in Britain, and suggest future areas of research.

Chapter two

***Prokelisia marginata* demography, community composition and natural enemies**

2.1 Introduction

Biological invasions represent the outcome of a complex interaction between propagule pressure (Colautti *et al.* 2006; Brockerhoff *et al.* 2013; Fensham *et al.* 2013), the physical and biological characteristics of the recipient ecosystem, and the biotic characteristics of the invading species (Catford *et al.* 2009; Mata *et al.* 2013). Much research has focussed on establishing patterns of generality in these characteristics in order to predict which species may become invaders in a new range (van Kleunen *et al.* 2010; Blackburn *et al.* 2015; van Kleunen *et al.* 2015). These generalities have in some cases been used to construct risk assessments acting as ‘early warning indicators’ to help identify and prevent emerging invasion risks (Ruesink *et al.* 1995; Roy *et al.* 2014; Singh *et al.* 2015). Whilst the numerous complexities of these interactions have resulted in exceptions being found to counter every ecological ‘rule of thumb’ elucidated by this research, a number of characteristics are common to a broad range of successful invasions (Hayes & Barry 2008).

Characteristics common to successful invaders across a range of taxonomic groups include *r*-selected traits such as short generation times, high growth rates and high fecundity (Pianka 1970; Amundsen *et al.* 2012). When coupled with strong adaptations for dispersal, such rapidly-growing populations are likely to expand their range (Sakai *et al.* 2001). Indeed, Philips *et al.* (2010) argue that evolutionary pressures act to further increase dispersal capabilities and reproductive rates at the edge of expanding population ranges (see also Thomas *et al.* 2001; Perkins *et al.* 2013). They argue that expanding populations are assorted by dispersal ability with individuals having the strongest adaptations, be they behavioural or morphological, dispersing the furthest and consequently mating with those whose adaptations have also enabled them to reach the outer population edge. Phenotypic plasticity has been frequently proposed as an important determinant of invasion success, allowing colonisers to adapt more readily to a range of potentially differing environmental conditions encountered in their new location (Gray 1986; Richards *et al.* 2006; Colautti & Barrett 2013; Turner *et al.* 2015). Greater empirical investigation is needed, however, to determine the extent to

which such plasticity contributes to successful establishment beyond the initial colonisation stage (Sakai *et al.* 2001; Palacio-Lopez & Gianoli 2011).

Characteristics of the invading species alone have proved insufficient to determine invasion success. Competitive abilities that make a species successful in one habitat do not necessarily make it successful in invading another; the interactions between the invader and the biotic and abiotic properties of the recipient community are of equal importance (Catford *et al.* 2009; Pysek *et al.* 2015). In their review of studies aiming to predict invasion success, Hayes and Barry (2008) found that only a match between the climate and habitat of the native and invaded range was a consistent indicator of successful establishment across a range of taxa including birds, insects, plants, mammals and amphibians (see also Casado *et al.* 2015). Research conducted both over paleobiological timescales and within island ecosystems, has suggested that less speciose communities are likely to be more at risk of invasion, and to being fundamentally changed by these invasions (Lodge 1993; Alpert *et al.* 2000). This is particularly the case where competition has been less intense over evolutionary time compared to more biologically 'sophisticated' communities that exhibit a greater complexity of trophic and competitive interactions (Vermeij 1991). Simberloff and von Holle (1999) introduced the concept of 'invasional meltdown' whereby the establishment of one non-native species would facilitate the arrival of another, with their compound effects on the recipient community being greater than either would be expected to produce alone. Some years later, Simberloff (2006) suggested that the metaphor had perhaps been stretched too far in the public imagination beyond its biological meaning, but asserted numerous cases where at least one invading 'partner' had been aided by the presence of another (see also Engelkes & Mills 2013). Recent investigation of the impacts of two invading rodents on the population of wood mice and pygmy shrews in Ireland found support for the invasional meltdown model; the presence of one invader facilitated the expansion of the other, with concurrent detrimental and compound effects on the native species (Montgomery *et al.* 2012).

An additional hypothesis which garners much support amongst invasion research, in particular when considering the early stages of invasion (Heger & Jeschke 2014), is the Enemy Release Hypothesis (ERH) or natural enemy escape (Elton 1958; Torchin *et al.* 2001; but see also Colautti *et al.* 2004; Roy *et al.* 2011). Under this hypothesis invaders are predicted to gain considerable advantage over native species as: i) the specialist predators, parasitoids and pathogens of the introduced species will be absent from the new range; ii) host switching by the specialist enemies of native congeners will

be rare; and iii) generalist natural enemies will vary in their ability to attack the invader, resulting in a greater overall impact on native competitors (Keane & Crawley 2002; Shea & Chesson 2002; Colautti *et al.* 2004). Conversely, predation is one of the factors found to prevent long-term invasion success (Sakai *et al.* 2001; Zenni & Nunez 2013). Deliberate introduction of the cinnabar moth, *Tyria jacobaeae*, as a biological control agent of ragwort, *Senecio jacobaea*, failed in both Australia and New Zealand due to predation by birds and a variety of generalist invertebrate predators (Goeden & Louda 1976). In a review of the population ecology of invading insects, Lawton and Brown (1986) cite further examples of naturally invading populations of the sphingid moth, *Erinnyis ello*, and the thistle gallfly, *Urophora cardui*, being rapidly exterminated by polyphagous parasitoid wasps. Parasites and parasitoids are of particular interest when considering natural enemy escape as their development within or attached to the host organism implies some level of co-evolution (Quicke 1997), and they can have very strong regulatory effects on host population dynamics (Hudson *et al.* 1998; Hatcher *et al.* 2006; Roy *et al.* 2008). Further, Phillips *et al.* (2010) argue that, not only is the founder effect of initial introduction likely to release invaders from the majority of their parasites and parasitoids, but as populations expand in their new range, even the few enemies that may have made the initial translocation with their hosts may be lost from the expanding population edge. They suggest this may be due to repeated founder effects, the need for multiple host species or minimum host density. However, such enemy release may be short lived (Schultheis *et al.* 2015). In their study of invasive round goby, *Neogobius menalostomus*, in the Great Lakes St Lawrence Basin, Gendron *et al.* (2012) found that abundance and species richness of associated parasites had doubled in the 15 years following their introduction, with the number of parasite species per host reaching levels typical of native fish. A minimum residence period of at least twenty years was suggested by Grabenweger *et al.* (2010) as being necessary for the adaptation of local parasitoids to the invasive horse chestnut leaf miner *Cameraria ohridella*, whereas invasive cane toads, *Bufo marinus*, in newly invaded areas of tropical Australia have been found to evade their parasitic lungworm *Rhabdias pseudosphaerocephala* for only 1-3 years (Phillips *et al.* 2010) following colonisation.

Studies of *Prokelisia marginata* in their home range show they exhibit a variety of characteristics which may confer the potential to be successful invaders. As with the majority of insects, *P. marginata* life history is characterised by *r*-selected traits. Generation times are relatively short, responding to climatic conditions and the associated growing season of their host plants. In the mid-Atlantic coast range, *P.*

marginata are trivoltine with generation times of approximately 40 days (Gratton & Denno 2003a), whereas the sub-tropical climate of the Gulf coast allows for continuous growth of its host plant, *Spartina alterniflora*, throughout the year, resulting in five overlapping generations of *P. marginata* (Denno & Grissell 1979). Population growth is rapid with natural densities reaching up to 1000 adults and 100,000 nymphs per m², even in the shorter Atlantic coast season (Denno *et al.* 2000). Like many planthoppers, *P. marginata* exhibit phenotypic plasticity in their wing morphology, with both brachypterous and macropterous forms present in the same population (Denno *et al.* 1986). Wing morphology is determined by a developmental switch that responds to proximate environmental cues such as levels of crowding and host plant nutrition. The sensitivity of the switch is heritable and under polygenic control (Denno 1994). Such plasticity enables *P. marginata* to maximally exploit the environmental conditions in which they find themselves. Brachypters live longer and brachypterous females breed earlier than their macropterous counterparts (Heady & Denno 1991). As *P. marginata* are iteroparous, there is a resulting positive correlation between this enhanced longevity and lifetime realised fecundity, with brachypterous females leaving more offspring which grow to a larger size before winter. Brachypters are therefore the more adaptive form, particularly for females, where habitats are stable, crowding is low and host plant nutrition is high (Denno *et al.* 1989). Conversely, macropters are the more adaptive form in unstable habitats where resources fluctuate (Denno & Grissell 1979). Macropters are capable of long-distance migration by flight of up to 30km (Denno *et al.* 1996), thereby providing *P. marginata* with the strong dispersal capabilities essential both for insects exploiting transient habitats (Langellotto & Denno 2001) and for the expansion of invading populations.

The aim of the work reported in this chapter is to survey the populations of *P. marginata* previously identified at selected sites along the south coast of England and the communities in which they have established, examining these findings in light of the biological characteristics identified as indicative of prospective invasion success. It is hypothesised that:

- i) *P. marginata* are bivoltine in Britain and have the potential to reach population densities similar to those seen in their native range;
- ii) Consequently, *P. marginata* will dominate the relatively species-poor saltmarsh invertebrate community;
- iii) *P. marginata* populations will display a high proportion of macroptery, consistent with elevated levels of dispersal expected on an invasion front; and
- iv) *P. marginata* will have experienced some element of natural enemy escape, particularly in relation to specialist enemies such as parasitoids.

2.2 Materials and methods

2.2.1 Field sites

The Solent extends from Hurst Spit in Hampshire to Pagham Harbour in West Sussex, including the north shore of the Isle of Wight. Although subject to heavy industrial and recreational use, over 80% of its coastline is designated for its nature conservation interest (Foster *et al.* 2014). Field work was undertaken at two principal field sites along the Solent coast: Pagham Harbour and Hythe *Spartina* Marsh, along with limited sampling at three other locations (Fig 2.1).

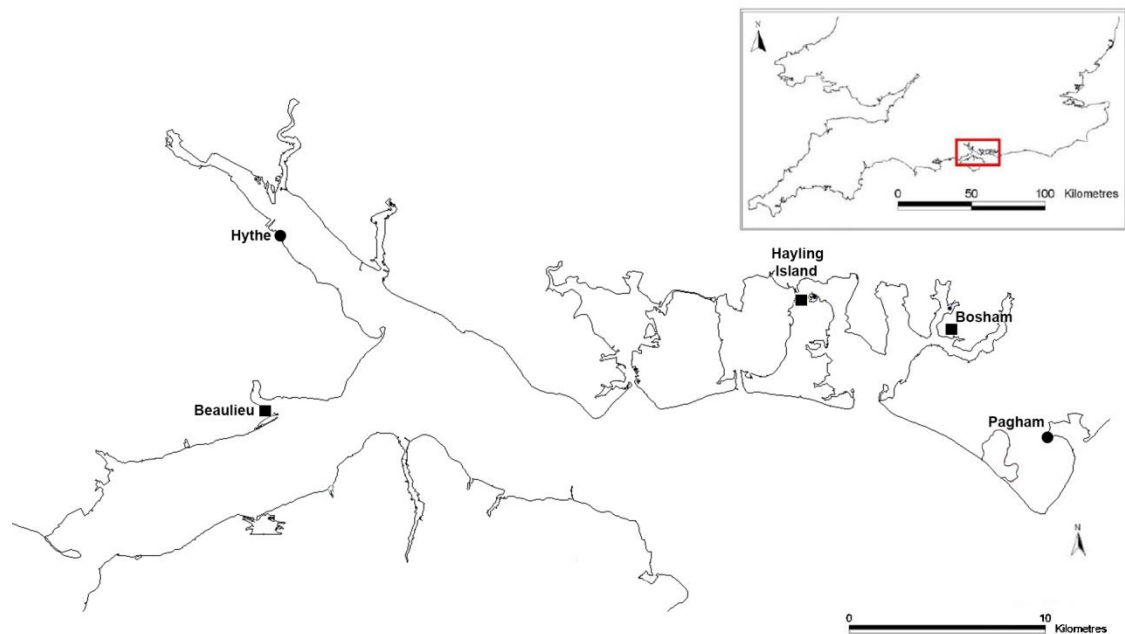


Figure 2.1 Location of principal field research sites (circles) and secondary sampling sites (squares). Adapted from Foster *et al.* (2014) by W. Harkin.

2.2.1.1 Pagham Harbour

Pagham Harbour Local Nature Reserve (LNR) is an area of almost 600ha on the south east of the Manhood Peninsula in West Sussex. The research site (National Grid reference SZ 862972) is located within a central area of some 156ha of saltmarsh surrounded by a complex of other habitats including open water, reed swamp, shingle and tidal mudflats (West Sussex County Council 2007) as well as a small amount of residential housing on the periphery. The lower marsh is entirely dominated by *S. anglica*, with small patches of *Salicornia* spp. and *Atriplex portulacoides* also present in the upper marsh where sampling took place. Pagham Harbour is designated a Special Protection Area (SPA) under the European Community Directive 79/409/EEC ('Birds Directive'), a Special Area of Conservation (SAC) and part of the Natura 2000 suite of protected sites established across the European Union under the European

Community Directive 92/43/EEC ('Habitats Directive'). Additionally it is classified as a European Marine Site, included in the list of Wetlands of International Importance under the 1976 Ramsar Convention, and a national Site of Special Scientific Interest (SSSI) (West Sussex County Council 2007). It was designated as one of the 27 new English Marine Conservation Zones on 21 November 2013 (DEFRA 2013).

2.2.1.2 Hythe *Spartina* Marsh

Hythe *Spartina* Marsh LNR is a 10 ha site within the 683ha Hythe to Calshot Marshes SSSI (Natural England 1994). The research site (National Grid reference SU 432073) is a narrow stretch of saltmarsh divided by large creeks, with a shell-shingle fringe bordered by Southampton Water on one side and a road on the other. The site forms part of the Solent and Southampton Water SPA, holds SAC designation, and is listed under the 1976 Ramsar Convention (Fowler 2003). Hythe is considered to be the site of origin for both *Spartina x townsendii* and *Spartina anglica* (Raybould *et al.* 1991b) and supports nationally and internationally important populations of migratory and over-wintering waders and wildfowl (Natural England 1994). *S. alterniflora* is still present on the site in a monospecific stand of approximately 125m², with the remainder of the marsh populated by *S. x townsendii* and *S. anglica* (Renny-Byfield *et al.* 2010). However, *S. anglica* has exhibited extensive die-back within its monospecific swards, and the marsh is now characterised by a mixed matrix, largely dominated by *Atriplex portulacoides* and *Puccinellia maritima* with sparse patches of *Salicornia* spp. and *Aster tripolium*.

2.2.1.3 Secondary sampling sites

Beaulieu River is a 233ha area within the 1188ha North Solent SSSI, which is also designated as a Ramsar site, SPA and SAC. The SSSI comprises an extensive diversity of habitats, ranging from coastal mudflats and saltmarshes, through a range of grass- and heathlands, to ancient semi-natural woodlands. The river's estuary is fringed by a 132ha saltmarsh largely dominated in the lower marsh sampling area (National Grid reference SZ 425976) by *S. anglica*, but interspersed with isolated stands of *S. x townsendii*. *S. anglica* loses its dominance in the higher marsh area, forming more of a matrix with *Atriplex portulacoides* and *Limonium vulgare* among other species (Natural England 1991).

Bosham channel is a narrow inlet of around 28.5km², bordered by Bosham village. It falls within the 74km² Chichester Harbour Area of Outstanding Natural Beauty (AONB), the larger 3695ha Chichester Harbour SSSI and the Chichester and Langstone

Harbours SPA, SAC and Ramsar sites (Natural England 2010). The channel is partially fringed with an approximately 100m wide saltmarsh, widening at the channel mouth. The saltmarsh is heavily dissected by creeks and gullies with large areas of mudflat apparent at low tide. The sampling area (National Grid reference SU 800019) is dominated by *S. anglica* with extensive areas of bare substrate.

Hayling Island also falls within the Chichester Harbour SSSI and the Chichester and Langstone Harbours SPA, SAC and Ramsar designations. The sampling site within the Northney foreshore area (National Grid reference SU 724042) is a narrow strip of saltmarsh confined by the main road onto the island and the sea wall. The saltmarsh is dominated by *S. anglica* with sparse patches of *S. maritima* present in the higher marsh. This represents one of the last remaining populations of *S. maritima* along the southern coast, however Natural England have identified the area as being adversely affected by sea level rise and 'coastal squeeze' which is resulting in declining habitat and loss of the saltmarsh (Natural England 2010).

2.2.2 *Prokelisia marginata* demography and invertebrate community sampling

Ten field visits to both principal sites were made between October 2011 and October 2012. During each visit, four replicate samples each consisting of ten 10-second 'sucks' from the vegetation were taken with a Vortis suction sampler. The sampler has an 18cm diameter intake aperture and is driven by a McCulloch GBV 325 two-stroke engine. The total surface area sampled was therefore 1m² per site per visit. During sampling the equipment was lowered over the vegetation to within a centimetre of the ground to avoid surface water. Samples were bagged and labelled in the field and placed in a freezer at -20°C on return to the laboratory. They were subsequently examined at 10x magnification using a Meiji EMZ binocular microscope and the following details of *P. marginata* demography were recorded: number of nymphs; number of adults; sex of adults; wing morph of adults. Additionally, samples were examined for the presence of other invertebrates. Individuals were sorted to ordinal level and the majority (with the exception of Diptera, parasitoid Hymenoptera and Lepidopteran larvae) were identified to family level and then as morphospecies (Oliver & Beattie 1996). Hemiptera were further identified to species level. Numbers of individuals in each group were counted and recorded.

In August 2013, one further sampling visit was made to Pagham, Hythe and each of the secondary sampling sites described in section 2.2.1.3. During each visit, eight repeats of ten 10-second suction samples were taken with the Vortis suction sampler to

obtain an equivalent sample are of 2m² per site. Samples were bagged and labelled in the field and placed in a freezer on return to the laboratory. They were subsequently examined with community composition details being recorded as previously described.

2.2.3 *Prokelisia marginata* natural enemies

Community sampling included identifying the presence and abundance of spiders, wolf spiders in particular having been identified as a primary natural enemy of both nymph- and adult-stage *P. marginata* in the United States (Denno *et al.* 2002). Native populations are also subject to attack by parasitoids, including Dryinidae, Pipunculidae and Strepsiptera (Stiling & Strong 1982b). *P. marginata* collected in the suction samples were individually examined for the presence of parasitoids. Signs of parasitism include the presence of a strepsipteran cephalothorax appearing from the abdomen, usually between segments 7 and 8, or a dryinid sac between segments 5 and 6 (see Stiling & Strong 1982b for images). Abdomens were not dissected for the presence of Pipunculidae, but no external indications of such parasitism (swollen abdomen) were recorded.

During the field visits described in section 2.2.2, seven *S. anglica* tillers from each site were cut at ground level, bagged, labelled and returned to the laboratory. Each leaf was examined under x10 magnification for evidence of *P. marginata* oviposition and details were recorded, including the number and position of eggs and the presence / absence of egg parasitoids. *Spartina* leaves were numbered from one at the base of the plant upwards towards the apex (following Stiling & Strong 1982a). *P. marginata* insert their eggs into incisions made between adaxial leaf blade ridges, thereby slightly distorting the linearity of the ridges; this provides a useful indicator of egg presence. Leaves can be carefully dissected using a pin to expose the eggs (Figure 2.2). Healthy eggs exhibit a pale yellow colouration, often with a red eyespot in the latter stages of development, whereas parasitized eggs initially appear milky-white or transparent, later becoming orange in colour (Stiling & Strong 1982b). It was not possible to undertake an egg collection in April 2012 as there was no living *Spartina* standing crop at either location.

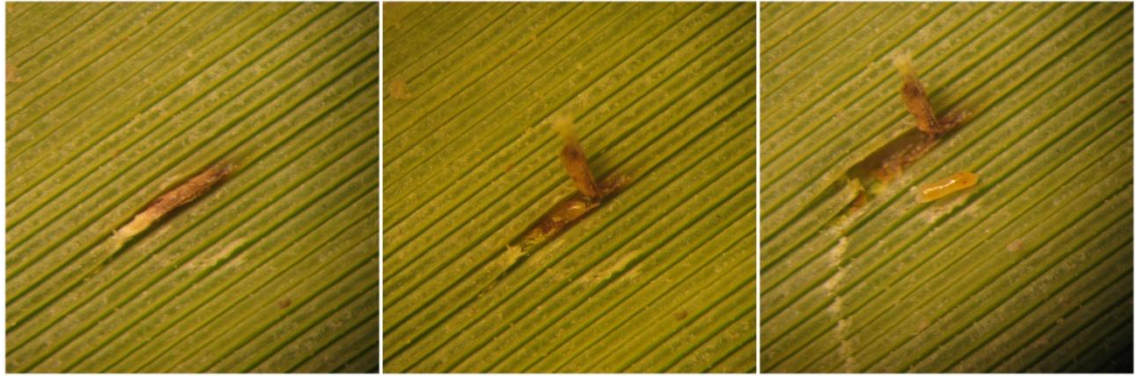


Figure 2.2 Left: *P. marginata* oviposition scar on adaxial surface of *S. anglica*. Centre and Right: exposed healthy egg of *P. marginata*. Photo credit: A.J.A Stewart.

2.2.4 Statistical analysis

Analysis of community composition was performed with the statistical software R version 3.1.3 (R Core Team 2015) using the *nlme* (Pinheiro *et al.* 2012) package. Assumptions about the distribution and variance of the response data (see Quinn & Keough 2002) were tested prior to analysis. Shapiro Wilk tests were used to test for normality of residuals; Fligner-Killeen tests were used to test for constancy of variance, as recommended by Crawley (2013). Detailed analyses were performed on all predator / parasitoid groups, and on any other groups in which at least 200 individuals were collected.

Linear mixed effects models were used to identify any correlation between the population density of *P. marginata* and that of other arthropod groups. The mean number of *P. marginata* per 0.25m² per site visit was the explanatory variable and mean numbers per 0.25m² per site visit of Diptera, Hemiptera (excluding *P. marginata*), Coleoptera, Collembola, Gastropoda, Hymenoptera and Araneae were individually fitted as response variables. In each case tests showed the data to be non-normal, however log(x+1) transformations were successful in achieving normality. Site was fitted as a random factor to account for any issues of pseudoreplication. Each model was compared to a null model containing only the random factor to determine whether removal of *P. marginata* numbers as an explanatory variable led to a significant ($p < 0.05$) increase in deviance. This was assessed by comparing the model with and without this term using log-likelihood ratio tests. The resulting minimum adequate model describes that which best fits the data, produces the least unexplained variation (the minimum residual deviance) and where all parameters in the model are significant. Significance levels are reported for the addition of non-significant terms and removal of significant terms from the minimum adequate model.

2.3 Results

2.3.1 *Prokelisia marginata* demography

Abundance of *P. marginata* at the Hythe field site declined gradually over the autumn / winter period from a high starting point, then exhibited two main peaks during the following year: the first (for adults) in April with the second considerably larger peak in August, when densities reached 1252 adults per m² (Figure 2.3).

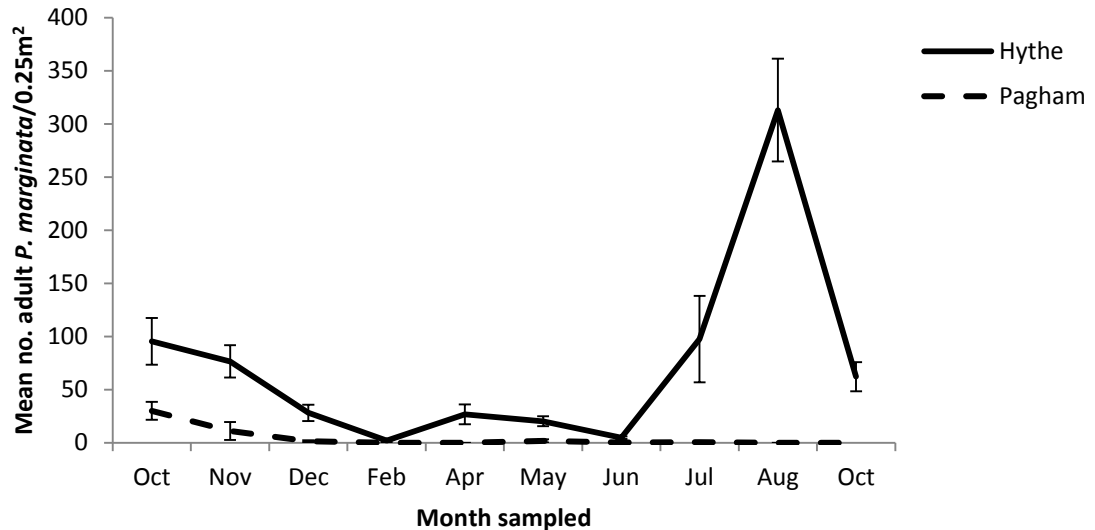


Figure 2.3 Mean number of adult *P. marginata* per 0.25m² sample area. Samples obtained between October 2011 and October 2012 at Hythe and Pagham field sites. Means \pm 1 S.E.M.

This pattern was replicated approximately a month later for eggs (Figure 2.4) and a further two months later for nymphs (Figure 2.5) suggesting *P. marginata* are bivoltine at this location. The spring / early summer generation was much smaller than the second generation in the autumn, probably due to high mortality over the previous winter.

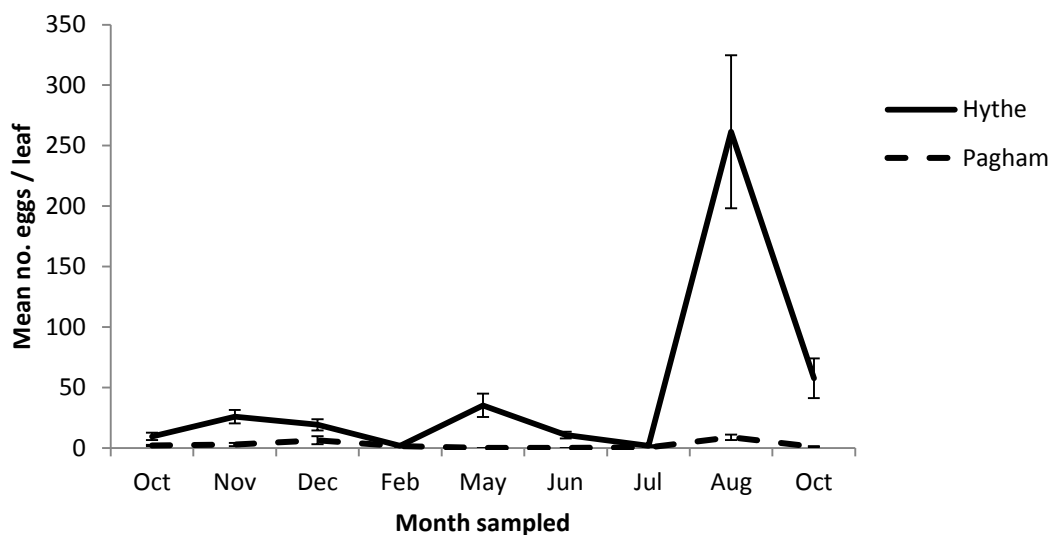


Figure 2.4 Mean number of *P. marginata* eggs per leaf sampled. Samples obtained between October 2011 and October 2012 at Hythe and Pagham field sites. Means \pm 1 S.E.M. It was not possible to undertake an egg collection in April 2012 as there was no living *Spartina* standing crop at either location.

Nymphal abundance at the Hythe site varied widely, from 26/m² in May to 25,030/m² in August.

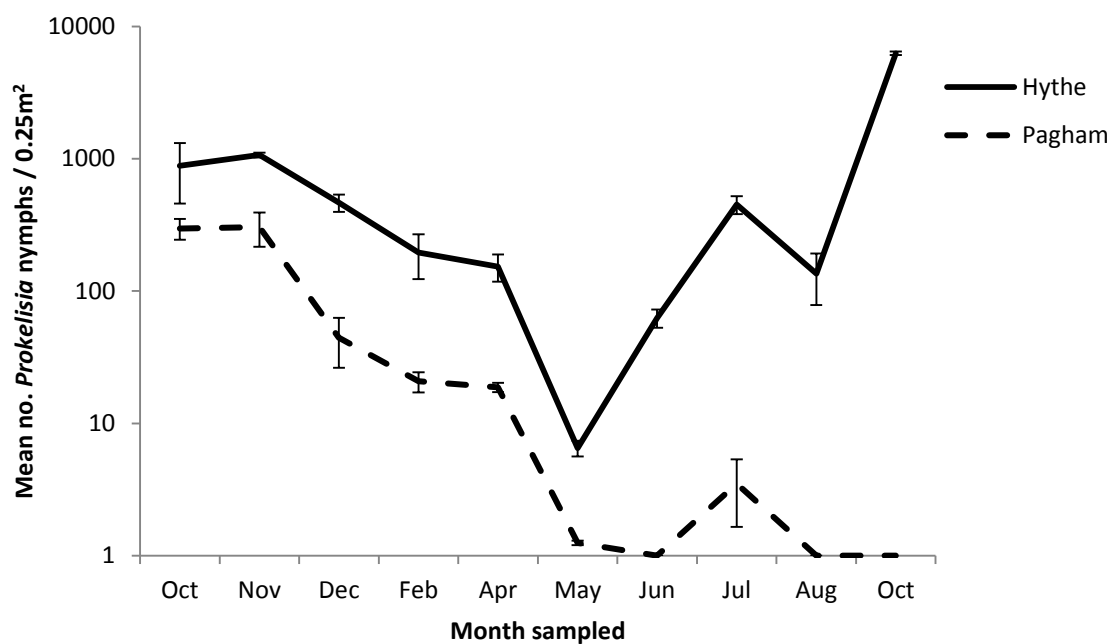


Figure 2.5 Mean number of *P. marginata* nymphs per 0.25m² sample area. Samples obtained between October 2011 and October 2012 at Hythe and Pagham field sites. Means \pm 1 S.E.M. Abundance of nymphs is presented on a log scale due to the high range of densities at the Hythe site.

Throughout the sampling period, numbers of all life-history stages at Pagham were found to be substantially lower than at Hythe, with the Pagham population becoming almost undetectable by the end of the sampling period.

Analysis of egg distribution suggests that *P. marginata* oviposits preferentially some distance above the base of the plant (Figure 2.6). The mean number of eggs per centimetre of leaf length was normally distributed in relation to leaf number. This distribution peaked at leaf number 5 leaf at the Hythe site (3.68 ± 1.56) and at leaf number 7 at Pagham, although at a much lower density (0.64 ± 0.29).

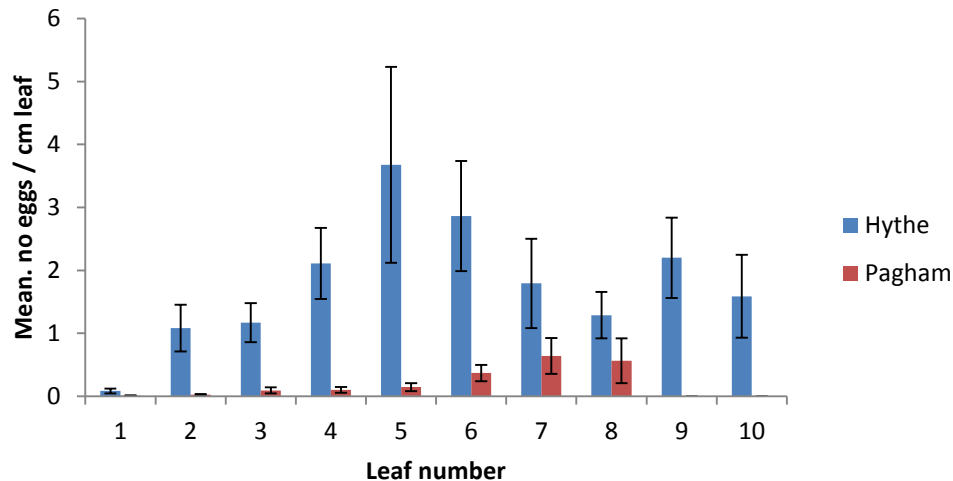


Figure 2.6 Mean number of *P. marginata* eggs per centimetre of *Spartina* leaf length showing the distribution of eggs within individual plants. Leaves are numbered from one at the base of the plant upwards towards the apex. Samples obtained between October 2011 and October 2012 at Hythe and Pagham field sites. Means \pm 1 S.E.M

Egg distribution must be viewed in light of the size variance between individual plants (Figure 2.7). All plants had at least 3 leaves; 51% of plants at Pagham had at least 5 leaves; 51% of plants at Hythe had at least 6 leaves. Few plants at either site had more than 6 leaves. Whilst egg density was high in leaf number 9 where present, only 11% plants at Hythe had at least 9 leaves. No plants at Pagham had more than 8 leaves.

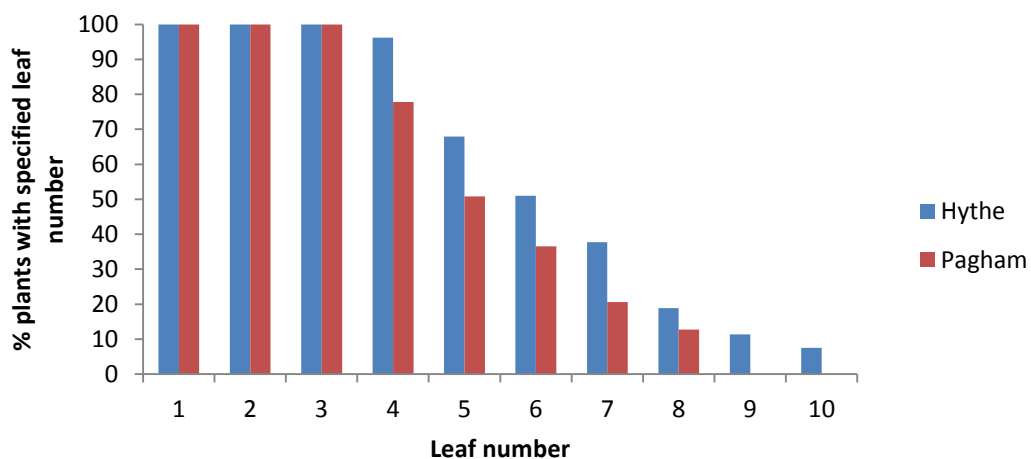


Figure 2.7 The percentage of *Spartina* plants bearing at least the specified number of leaves. Samples obtained between October 2011 and October 2012 at Hythe and Pagham field sites.

Sex and wing morph ratios were examined in the adult *P. marginata* population at Hythe. A comparable analysis was not possible at Pagham because overall numbers were too low. The percentage of females in the Hythe population declined considerably between October and December 2011 from a mean of 49% to 28% (Figure 2.8), coinciding with the overall population decrease at the end of the previous breeding season. Chi squared tests revealed the ratio of males to females differed significantly from 1:1 in November ($\chi^2 = 39.54$, $p < 0.001$) and December 2011 ($\chi^2 = 28.75$, $p < 0.001$). The mean percentage of females showed two peaks over the course of the year, in February (58%) and June (64%), both coinciding with the lowest overall numbers of adults recorded during the sampling period (8/m² and 19/m² respectively; Figure 2.3). The February ratio of males to females was not significantly different from 1:1 ($\chi^2 = 0.5$, $p = 0.480$), however the June ratio had a significantly higher proportion of females ($\chi^2 = 3.86$, $p = 0.05$). A second significant decline in the mean percentage of females followed the June peak, again coinciding with an increase in the adult population (Figure 2.3; Figure 2.8). In both August ($\chi^2 = 25.88$, $p < 0.001$) and October 2012 ($\chi^2 = 4.37$, $p = 0.037$) the ratio of males to females differed significantly from a 1:1 relationship. There was no statistically significant relationship between population density and the proportion of females in the population ($t = -0.85$, $p = 0.423$).

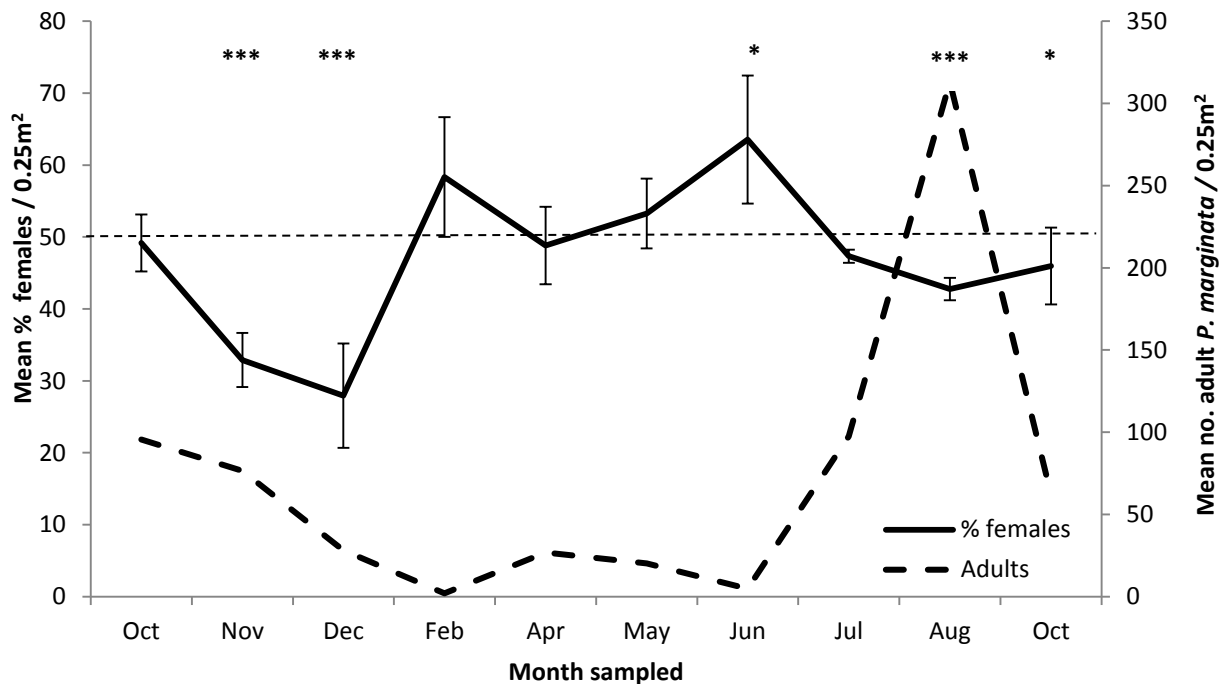


Figure 2.8 Mean number of adults per 0.25m² sample area and the mean percentage of *P. marginata* adults at Hythe field site that were female per 0.25m² sample area. Samples obtained between October 2011 and October 2012. Thin dashed line indicates 50%. Means for % female ± 1 S.E.M. Stars indicate months where the ratio of males to females differs significantly from 1:1. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

The percentage of adult *P. marginata* that were macropters in the Hythe population dropped dramatically in April (Figure 2.9) following the population decline recorded in the preceding months (Figure 2.3). The second, and greatest, population peak seen in July – August was reflected in the highest proportion of macroptery in the July – October sampling period (Figure 2.9). There was, however, no statistically significant relationship between population density and the mean percentage of male ($F=0.67$, $p=0.436$) or female ($F=1.37$, $p=0.276$) macropters, or of macroptery in the population as a whole ($t=1.04$, $p=0.328$).

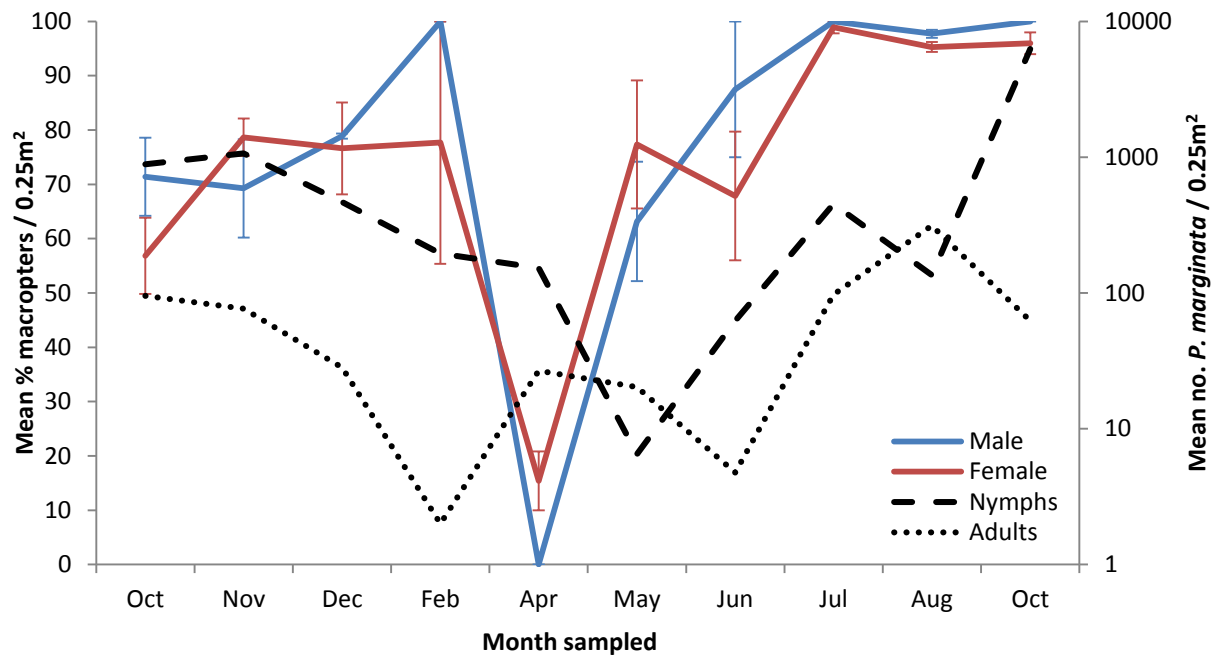


Figure 2.9 Mean number of *P. marginata* adults and nymphs per 0.25m² sample area (log scale), and the mean percentage of male and female adult *P. marginata* at Hythe field site that were macropters per 0.25m² sample area. Samples obtained between October 2011 and October 2012. Means for % macropters \pm 1 S.E.M.

2.3.2 Invertebrate community composition

Numbers of individuals within all other invertebrate groups were found to be lower than *P. marginata* by at least one, and more frequently two, orders of magnitude. Collembola formed the second most numerous group. Across all groups, with the exception of Gastropoda, numbers at Hythe were higher by approximately one order of magnitude than those at Pagham.

P. marginata was the only member of the family Delphacidae present at any of the sites, however two leafhopper species (family Cicadellidae) were found in very small numbers. *Aphrodes aestuarina* was present at Hythe and *Conosanus obsoletus* at Pagham with, respectively, 24 and 63 individuals recorded over the entire sampling period. The two most numerous hemipterans were from the suborder Heteroptera. *Ischnodemus sabuleti* was the most abundant with 652 individuals recorded at Hythe and 9 at Pagham over the entire sampling period. *Parapiesma quadratum* was also relatively numerous with 252 individuals at Hythe and 6 at Pagham.

The majority of Coleoptera were from the family Carabidae, however isolated individuals of the Curculionidae, Coccinellidae and Staphylinidae were recorded. Araneae numbers were dominated by the families Linyphiidae and Clubionidae, with Lycosidae also present in very low numbers. Parasitoid hymenopterans were present at the Hythe field site, but none were found at Pagham (Table 2.1).

Table 2.1 Number of individuals and trophic level of classified invertebrate groups collected at the Hythe and Pagham field sites between October 2011 and October 2012.

Order	Sub-order	Family	Genus / Species	Trophic level	Hythe	Pagham
Amphipoda	Gammaridea	Talitridae		Detritivore	368	409
Araneae		Clubionidae	<i>Clubiona</i>	Predator	55	
		Linyphiidae		Predator	186	14
		Lycosidae		Predator	12	2
Coleoptera		Carabidae	<i>Paradromius linearis</i>	Predator	17	
			Other	Predator	9	3
		Coccinellidae	<i>Harmonia axyridis</i> <i>Coccinella septempunctata</i>	Predator	1	
		Curculionidae		Herbivore	5	
		Staphylinidae	<i>Tachyporus</i>	Predator	2	1
Collembola	Arthropleona	Entomobryoidea		Detritivore	7709	292
Diptera	Brachycera	Dolichopodidae		Predator	234	3
		Scathophagidae		Predator	2	
		Syrphidae		Nectarivore	1	
		Unclassified			354	54
	Nematocera	Chironomidae		Scavenger	121	2
		Tipulidae			153	1
Gastropoda	Hypsogastropoda	Hydrobiidae	<i>Hydrobia ulvae</i>	Herbivore	855	2021
Hemiptera	Heteroptera	Lygaeidae	<i>Ischnodemus sabuleti</i>	Herbivore	652	9
		Miridae		Herbivore	9	
		Nabidae	<i>Nabis ferus</i>	Predator	1	
		Piesmidae	<i>Parapiesma quadratum</i>	Herbivore	252	6
		Salididae	<i>Saldula</i>	Predator	1	
	Auchenorrhyncha	Cicadellidae	<i>Aphrodes aestuarina</i> <i>Conosanus obsoletus</i>	Herbivore	24	63
		Delphacidae	<i>Prokelisia marginata</i>	Herbivore	49899	3675
	Sternorrhyncha	Psyllidae		Herbivore	3	1
Hymenoptera	Parasitica			Parasitoid	50	
Ixodida		Ixodidae		Ectoparasite	95	
Lepidoptera				Herbivore	85	
Psocoptera				Scavenger	17	
Trombidiformes		Tetranychidae		Herbivore	55	54

No significant correlation was found across the five sample sites, or within the Hythe or Pagham sites individually, between the density of *P. marginata* and that of Diptera, Collembola or Gastropoda species (Table 2.2; Figure 2.10).

Table 2.2 Relationship between densities of *P. marginata* and selected invertebrate groups across all five sampling sites and within Hythe and Pagham sites individually. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

	d.f.	L	<i>p</i>	
i)				
Araneae				
All sites	17	9.83	0.002	**
Hythe only	9	14.43	<0.001	***
Pagham only	7	1.50	0.22	
ii)				
Coleoptera				
All sites	17	0.81	0.368	
Hythe only	9	0.63	0.427	
Pagham only	7	0.24	0.627	
iii)				
Collembola				
All sites	17	1.81	0.179	
Hythe only	9	0.64	0.424	
Pagham only	7	0.28	0.595	
iv)				
Diptera				
All sites	17	0.97	0.326	
Hythe only	9	0.89	0.345	
Pagham only	7	0.31	0.578	
v)				
Gastropoda				
All sites	17	0.26	0.608	
Hythe only	9	0.23	0.638	
Pagham only	7	0.04	0.838	
vi)				
Hemiptera				
All sites	17	0.62	0.432	
Hythe only	9	0.52	0.470	
Pagham only	7	5.63	0.018	*
vii)				
Parasitoid Hymenoptera				
All sites	17	0.11	0.736	
Hythe only	9	1.67	0.196	
Pagham only	n/a	n/a	n/a	

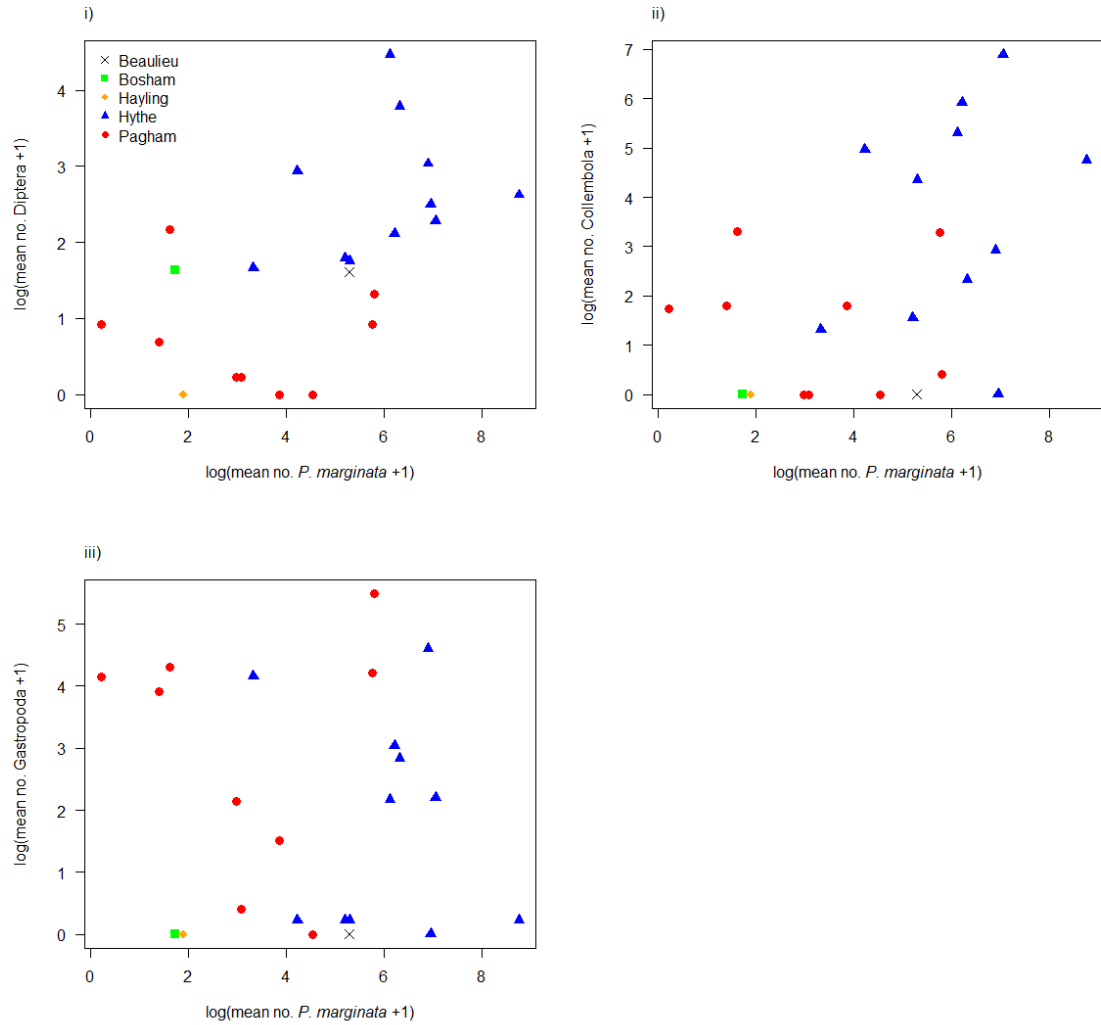


Figure 2.10 Mean number of *P. marginata* compared to i) Diptera; ii) Collembola and; iii) Gastropoda per 0.25m² per site visit at main (Hythe and Pagham) and secondary sampling sites. Samples collected between October 2011 and October 2012 for main sites only, and in August 2013 for all sites. Figure shows $\log(x+1)$ transformed data.

There was a significant negative correlation between the density of *P. marginata* and that of other Hemiptera at the Pagham field site. The same relationship was not evident at Hythe however, or across the combined five-site sample (Table 2.2; Figure 2.11).

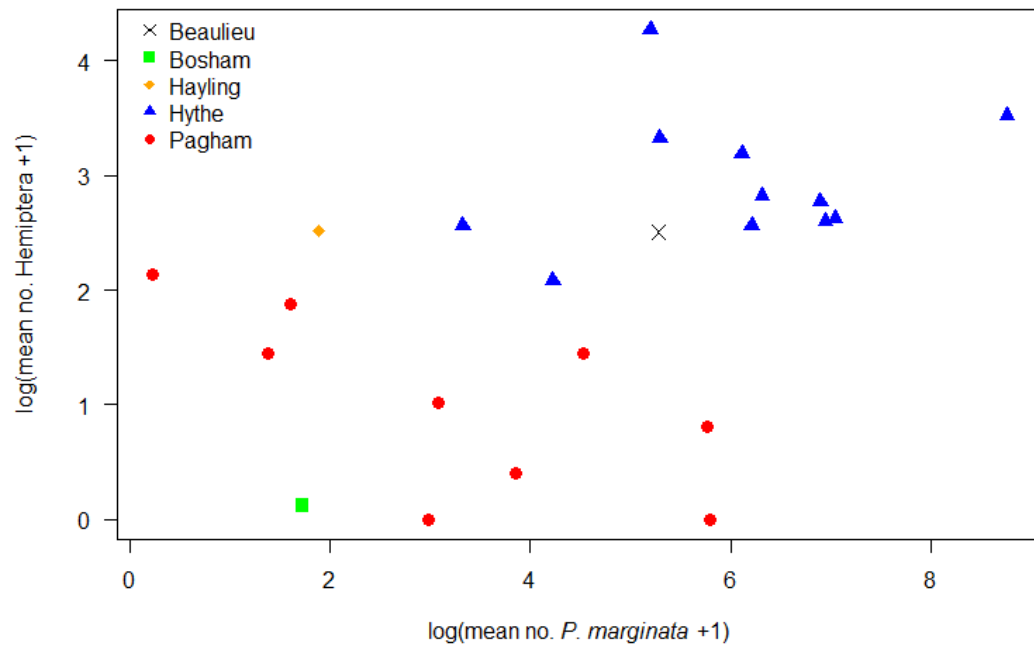


Figure 2.11 Mean number of *P. marginata* and other Hemiptera per 0.25m² per site visit at main (Hythe and Pagham) and secondary sampling sites. Sampling dates as described in Figure 2.10. Figure shows log(x+1) transformed data.

2.3.3 Potential natural enemies of *Prokelisia marginata*

A significant positive correlation was found between the numbers of *P. marginata* and the density of spiders across all sites and for the Hythe field site treated individually, although not for Pagham (Table 2.2; Figure 2.12; Figure 2.13). There were no significant relationships between the density of spiders and that of other potential prey groups: Hemiptera excluding *P. marginata* (all sites, $L=1.74$, $p=0.187$); Collembola (all sites, $L=2.78$, $p=0.096$); Coleoptera (all sites, $L=0.01$, $p=0.920$); Diptera (all sites, $L=2.74$, $p=0.098$).

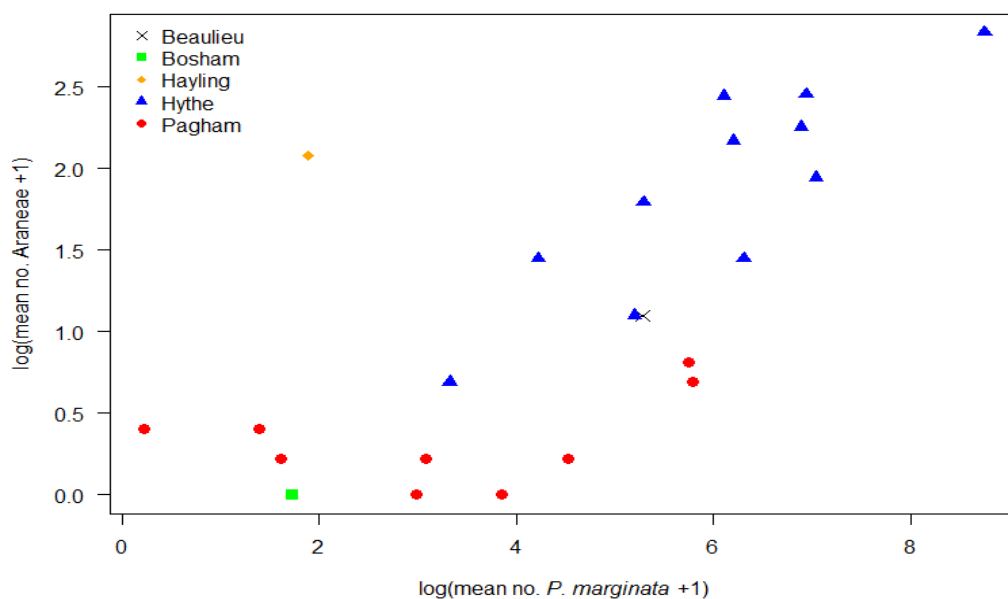


Figure 2.12 Mean number of *P. marginata* and Araneae per 0.25m² per site visit at main (Hythe and Pagham) and secondary sampling sites. Sampling dates as described in Figure 2.10. Figure shows log(x+1) transformed data.

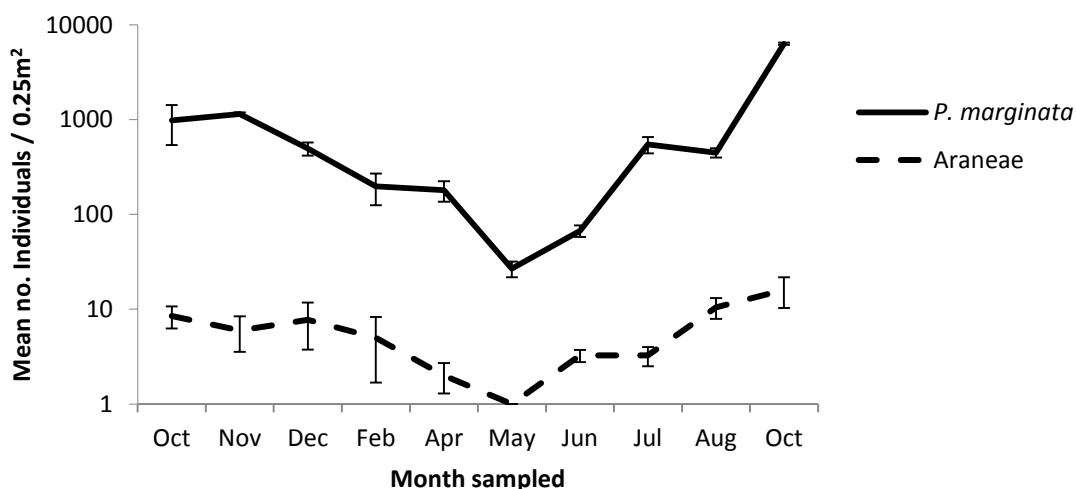


Figure 2.13 Mean number of *P. marginata* (adults and nymphs) and Araneae at the Hythe field site per 0.25m² sample area. Samples obtained between October 2011 and October 2012. Means \pm 1 S.E.M. Note the log scale.

There was no significant relationship between the density of *P. marginata* and that of predatory Coleoptera (Table 2.2; Figure 2.14). A significant positive correlation was however found between densities of Coleoptera and that of all other Hemiptera across all five sample sites ($L=7.01$, $p=0.008$) and for the Hythe site individually ($L=5.96$, $p=0.015$) although not for Pagham ($L=0.25$, $p=0.620$). No significant relationship was found between Coleoptera and any other group.

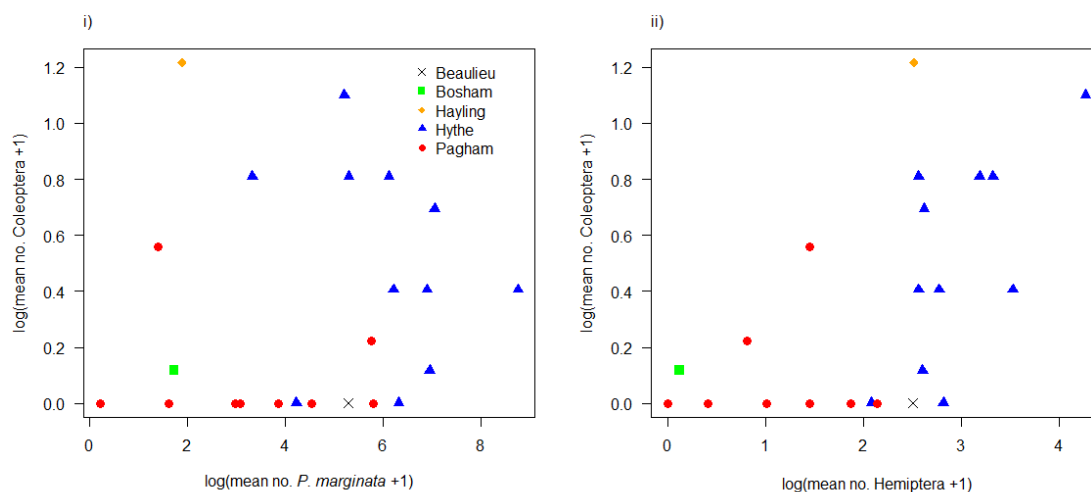


Figure 2.14 Mean number of Coleoptera compared to i) *P. marginata* and ii) all other Hemiptera per 0.25m² per site visit at main (Hythe and Pagham) and secondary sampling sites. Sampling dates as described in Figure 2.10. Figure shows log(x+1) transformed data.

No significant relationship was found across the five sample sites, or within the Hythe site individually, between the density of parasitoid Hymenoptera and that of *P. marginata* (all sites: $L=0.11$, $p=0.736$), or with any other groups. No parasitoid Hymenoptera were found at the Pagham site (Table 2.1; Table 2.2; Figure 2.15).

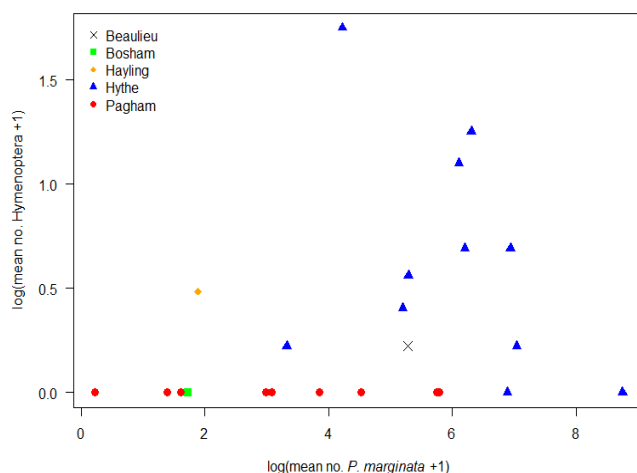


Figure 2.15 Mean number of *P. marginata* and parasitoid Hymenoptera per 0.25m² per site visit at main (Hythe and Pagham) and secondary sampling sites. Sampling dates as described in Figure 2.10. Figure shows log(x+1) transformed data.

All *P. marginata* eggs, nymphs and adults collected from the main Hythe and Pagham field sites over the course of 13 months, and additionally from the three secondary sites on one occasion, were examined for the presence of parasitoids. In total, 16,681 eggs, 45,435 nymphs and 9797 adults were examined (Table 2.3).

Table 2.3 The number of individual *P. marginata* eggs, nymphs and adults collected at main (Hythe and Pagham) and secondary sampling sites. Samples collected between October 2011 and October 2012 for main sites only, and in August 2013 for all sites.

	Life stage		
	Egg	Nymph	Adult
Site	No. individuals	No. individuals	No. individuals
Beaulieu	2376	315	1261
Bosham	0	10	27
Hayling Island	213	7	38
Hythe	13190	42098	7801
Pagham	902	3005	670
Total	16681	45435	9797

No evidence of parasitism was found across any of the sampled sites or life stages.

2.4 Discussion

2.4.1 *Prokelisia marginata* demography

In planthoppers, the number of generations per year is generally determined by the length of their host plant growing season, itself largely determined by latitude. The native Atlantic coast *P. marginata* population (39°N) produces three generations per year, whilst data collected for this study show the Hythe (51°N) *P. marginata* population to be bivoltine (Figure 2.3; Figure 2.5). This is consistent with populations of other planthopper species found at higher latitudes across Europe and North America (Denno & Roderick 1990). Despite having fewer generations, population densities in the British range are substantial. *P. marginata* have been recorded in their home range as reaching densities of up to 1000 adults and 100,000 nymphs per m² (Denno *et al.* 2000). Similar densities have been found at the Hythe field site, with 1252 adults/m² collected in August 2012 (Figure 2.3) and over 25,000 nymphs/m² two months later (Figure 2.5). This level of population establishment and growth is particularly noteworthy as it is likely to have been achieved in less than a decade (Kirby 2000; Wilson & Muhlerthaler 2009). It is clear, however, that populations fluctuate dramatically within and among seasons in the same site, and spatially between sites; Pagham population density was substantially below that of the Hythe population, becoming almost undetectable by the end of the sampling period (Figure 2.3; Figure 2.4; Figure 2.5). Such fluctuations are consistent with those found in the native range, where populations can show exponential growth during the host plant growing season, particularly in local patches where aggregations of brachypterous adults occur. Numerous other factors, including host plant nutrition, dispersal, and the effects of natural enemies, competitors and physical stresses, influence both spatial and temporal variation in population growth and density (Denno & Roderick 1990).

Determination of adult wing form has been shown to be density-dependent in native populations of female *P. marginata*, particularly those inhabiting temporary habitats, with increased crowding resulting in a greater percentage of macroptery. Males have been found to exhibit no such relationship; levels of macroptery can remain high even when individuals are raised in isolation (Denno *et al.* 1985). Whilst macropters of both sexes display reduced longevity compared to their brachypterous counterparts, the lifetime reproductive cost of flight capability appears to be less for males, perhaps due to their smaller gamete size. Additionally, flight capability is adaptive for males in very low density populations typical of those colonising new habitats. Males employ a “fly and call” strategy when looking for mates, flying among plants in search of stationary females (Denno 1994). The proportion of macroptery varies significantly between

populations however. In North America, Atlantic coast habitats are temporary, making seasonal dispersal essential for survival; here, over 80% of the *P. marginata* population are macropters (Denno *et al.* 1989). Conversely, vegetation along the Gulf Coast persists all year round, with levels of macroptery rarely exceeding 10% (Denno *et al.* 1980). Whilst wing form is dependent on proximate environmental cues acting upon a developmental switch, the sensitivity of that switch has high heritability, particularly for males (0.42) (Denno 1994). Selection for increased or decreased macroptery should therefore result in rapid change (Roff 1990) and may, at least temporarily, be constrained by founder effects when invading a new range (Hochkirch & Damerau 2009). Levels of macroptery were generally high within the Hythe population, with both male and female levels exceeding 90% in the latter portion of the sampling period. No statistically significant relationship was found between population density and levels of macroptery, nor were there any significant differences between the sexes (Figure 2.9). A seemingly anomalous and substantial drop in the proportion of macroptery was seen in April 2012, with 100% of males and 85% of females in the samples being brachypterous. No definitive explanation was found in the data for this, although the overall population density was at its lowest two months preceding this event. It has additionally been found that macroptery can be suppressed by fertilizing host plants (Denno *et al.* 1985; Denno *et al.* 1986), perhaps offering clues to some other environmental explanation that would warrant further investigation. As expected from native population dynamics, an exponential growth in overall population numbers immediately followed this event.

The data presented here show that, both within their home range and in the emerging British population, *P. marginata* exhibit a number of characteristics consistent with predictors of invasion success: relatively short generation times; high fecundity with the potential for exponential population growth; strong mechanisms for dispersal; and phenotypic plasticity previously shown to be responsive to environmental cues (Denno *et al.* 1985).

2.4.1 Community composition

P. marginata's recipient British community also displays characteristics indicative of their potential invasion success. A match between home and invasive range habitat, as illustrated when comparing North American to British saltmarshes, is cited as one of the only consistent predictors of successful invasions (Hayes & Barry 2008). Whilst providing extremely important habitats for a range of terrestrial invertebrate fauna, a high proportion of which are found in no other habitat (Boorman 2003), British

saltmarshes host a relatively unsaturated invertebrate community (Ford et al. 2013). Sampling at both primary sites revealed low species diversity and density across all major guilds, suggestive of low competitive pressure. As is the case in North American *Spartina* marshes (Vince *et al.* 1981; Stiling & Bowdish 2000), sample data show that *P. marginata* dominates the invertebrate community in this new range, outnumbering all other groups by at least one, and more frequently two, orders of magnitude (Table 2.1). Competition specifically within the Hemiptera warrants closer investigation. A significant negative correlation was found between the density of *P. marginata* and that of other Hemiptera at the Pagham field site (Table 2.1; Figure 2.11). This is potentially indicative of interspecific competition, although the same relationship was not evident at Hythe or across the five sample sites combined. Further examination of individual species numbers reveals that the most numerous Hemipteran, other than *P. marginata*, at the Hythe field site is *Ischnodemus sabuleti*, constituting 69% of the 'other Hemiptera' population. *Ischnodemus* is a seed feeder, and hence not in direct competition with the sap-sucking *P. marginata* for food resources. *Parapiesma quadratum* and *Aphrodes aestuarina*, the two sap-sucking species occupying the same feeding guild as *P. marginata* and therefore likely to be direct competitors, make up only 29% of the 'other Hemiptera' population at this site. In contrast, *Parapiesma quadratum* and *Conosanus obsoletus*, the two sap-feeding species found at Pagham, represent some 88% of the 'other Hemiptera' population. This dichotomy of feeding guild occupation lends some support to the suggestion of interspecific competition as an explanatory mechanism for the negative correlation between *P. marginata* and other Hemiptera seen at Pagham but not across the other sites. However, overall numbers of Hemiptera species at Pagham are relatively low and further investigation in the form of experimental manipulations of community composition would be required before any firmer conclusions could be drawn.

Coastal habitats have been identified as amongst the most heavily invaded ecosystems in the world and ones in which the theoretical potential for invasional meltdown is high, but for which empirical investigation is lacking (Grosholz 2002). Available evidence suggests that delphacid planthoppers do not easily adapt to novel host species. Whilst the possibility for exploitation of closely-related plant varieties exists, for example the brown planthopper, *Nilaparvata lugens*, has successfully adapted to new 'resistant' rice cultivars, switching to entirely new host species is considered to be much less likely (Denno & Roderick 1990). Across their native range, *P. marginata* breed only on *Spartina alterniflora*, *S. foliosa* and their hybrids, and the introduced *S. anglica*, whilst avoiding sympatrically occurring populations of *S. patens*,

S. bakeri and *S. cynosuroides* (Wu *et al.* 1999). *P. marginata* has additionally been recorded in Slovenia where it has successfully colonised populations of *S. maritima* (Seljak 2004). This is consistent with the molecular phylogeny published by Blaumel *et al.* (2002b), showing that *S. maritima* is in the same clade as *S. alterniflora* (as are *S. x townsendii* and *S. anglica* being progeny of the two species), whereas the unexploited *S. patens*, *S. bakeri* and *S. cynosuroides* are more distantly related. This narrow host range was confirmed by Grevstad *et al.* (2003) who undertook no-choice tests on 23 potential North American host species, finding that *P. marginata* was able to complete its full life cycle only on the same three previously recorded hosts: *S. alterniflora*, *S. anglica* and *S. foliosa*.

Britain's invasion by *S. alterniflora* and the subsequent hybridisation and dispersal events ultimately resulting in the widespread colonisation of *S. anglica* throughout British saltmarshes is therefore a critical factor in facilitating the establishment and spread of *P. marginata*. *P. marginata*'s successful colonisation, as evidenced by the data reported in this chapter, would have been impossible prior to the plant's introduction due to the extreme scarcity of *S. maritima* (Raybould *et al.* 1991a) and the complete absence of any other suitable hosts.

2.4.3 Natural enemy escape

Spiders (order Araneae), particularly mobile hunting wolf spiders of the family Lycosidae (Denno *et al.* 2002) and the dominant marsh spiders, web-building linyphiids (Gratton & Denno 2005), have been identified as the primary natural enemies of both adult and nymphal *P. marginata* in their home range. *P. marginata* have been shown to be extremely susceptible to capture by mobile predators when compared to other co-occurring prey species such as leafhoppers and mirid bugs due to their ineffective behavioural response to predator presence (simply moving down the stem in full view rather than jumping away or moving out of sight) (Dobel & Denno 1994). Vince *et al.* (1981) additionally found *P. marginata* to be the primary prey of web-building members of the Linyphiidae due to their small body size relative to other potential prey species and to their typical adoption of low vertical positions within the *Spartina* canopy.

Some predator groups, including spiders, have been shown to respond numerically to an increase in the population size of their prey by increasing reproduction and / or by aggregating in areas of high prey density (Riechert & Lockley 1984). Positive correlations between predator and prey density are often used as evidence for such a response, although care must be taken in inferring any directly causal relationship as

aggregations could be attributable to some other environmental factor independent of prey density (Murdoch *et al.* 1985). Studies have demonstrated both aggregative and reproductive numerical responses by spiders to planthopper density. The wolf spider *Pardosa littoralis* was found to make a significant and positive numerical response to a short term manipulation of densities of *P. marginata* in experimental saltmarsh plots (Dobel & Denno 1994), whilst in another study a positive correlation was found between spiders and *P. marginata* in samples taken from 12 unmanipulated field sites across the Pacific coast of North America (F.S. Grevstad, pers. comm.). This study shows a significant positive correlation between spiders and *P. marginata* across all five study sites, and for the Hythe site individually (Table 2.2; Figure 2.12; Figure 2.13). No such relationship exists between spiders and any other prey group, suggesting the substantial *P. marginata* population may be sufficiently abundant to elicit a numerical response whilst other groups are too sparsely populated to do so. No correlation was found between densities of spiders and *P. marginata* at the Pagham field site. This may be due to the significantly lower densities of *P. marginata* at this site, in which case experimental manipulations would be useful in exploring the relationship further. The suction sampling method utilised in this research has been noted as a “highly effective” approach for the sampling of ground active arthropods, and as being particularly useful for long-term arthropod monitoring (Zou *et al.* 2012). The samples obtained can therefore be considered representative of the wider natural enemy population.

Although generalist predators such as spiders are expected to respond to the presence of a novel food resource more rapidly than specialist predators and parasitoids, it is also possible that the lack of correlation at Pagham is due to a lag in predator response. Vince *et al.* (1981) argued that spiders prevented a long term increase of *P. marginata* abundance in fertilised plots of *Spartina* by responding numerically to the initial population increase, resulting in an inverse correlation between spider and planthopper densities. This pattern was not consistently seen across all experimental plots until several years following commencement of fertilisation treatment however, suggesting a delayed numerical response by spiders in some plots. Longer term sampling at both primary sites is needed to establish whether the current lack of correlation between spider and *P. marginata* populations at Pagham can be explained by a similar numerical delay, and whether the currently positive relationship at Hythe will, over time, become an inverse relationship. The emergence of an inverse relationship would signify an increasingly density-dependent effect of spider predation which may ultimately check population growth of the planthopper.

In their review of studies examining the parasitoids of phytophagous insects, Cornell and Hawkins (1993) found that parasitoid attack rates on invading hosts are generally lower, that parasitoid complexes are generally less rich, and that they contain a greater proportion of generalists when compared to the host organism in their native range. They further predicted that adaptation by specialist parasitoids to novel hosts would take at least 150 years. Grabenweger *et al.* (2010) investigated the parasitoid complex of the invasive leafminer *Cameraria ohridella* across a range of European populations where the host had been resident for between one and at least twenty years. They found that in all cases, parasitism rates remain very low, but that the rate increases as a function of host residence time (among other factors). They found the first signs of basic adaptations by some generalist parasitoids in the older host populations, however these complexes remained completely lacking in specialists. They therefore concluded that a minimum period of at least twenty years was required for basic adaptation of native parasitoids to the invading host, but that adaptation by specialists would require much longer.

P. marginata nymphs and adults are subject to parasitoid attack across the USA. Parasitism by dryinids in Florida has been found to remain beneath 1% of the population, however that by Strepsiptera can reach 40%, averaging around 20%. In New Jersey, levels of parasitism by the two groups combined can reach 80% (Stiling *et al.* 1991). Stiling and Strong (1982a) found egg parasitism to be inversely density dependent which they posited was due to the disruption of parasitoid search patterns by periodic tidal inundation, although they did not test this hypothesis. Despite this potential impediment, recorded levels range from 27% to 100% with a mean of ~40% parasitism reasonably to be expected (Stiling & Strong 1982a). Over 55,000 individual *P. marginata* and almost 17,000 eggs were examined in the course of this study; none were parasitized.

This study shows *P. marginata* to be in the successful early stages of invasion in Britain. The Hythe population has persisted for at least seven years since the first official record (Wilson & Muhlerthaler 2009), and possibly for longer (see section 1.3.3.3). As predicted, population density and growth rates equivalent to those seen in the native range have been recorded. High levels of macroptery and the extensive spread of its host plant (Boorman 2003; Figure 1.5) indicate a strong probability of further dispersal to other saltmarsh sites. Sampling of the entire British and Irish saltmarsh habitat would be informative to determine the full extent of *P. marginata*'s current distribution and, with repeated sampling, to document the rate at which any

further dispersal is achieved. *P. marginata* currently appears to be benefitting from natural enemy escape. Whilst data are suggestive of a numerical response by generalist Araneae predators, the positive nature of this correlation indicates that predation is not sufficient to regulate population growth at present. Additionally, *P. marginata* appear to be completely free from parasitoid attack. Records document a considerable rate and diversity of parasitoids already adapted to native European planthopper species (Stiling 1994) which may ultimately switch to exploit this substantial new resource. Repeated sampling and manipulative experiments are needed to examine the adaptive potential of both predator and parasitoid species to respond to the presence of *P. marginata* in this new range, and to determine whether these interactions will contribute to population regulation over the longer term.

Chapter three

The impact of *Prokelisia marginata* on *Spartina anglica*

3.1 Introduction

3.1.1 British saltmarshes: importance and extent

Covering an area of around 44,000ha, approximately 80% of Britain's saltmarshes have been notified under one or more national or international conservation designations, including Annex I of the European Community Directive 92/43/EEC ('Habitats Directive') (Paramor & Hughes 2004). Although widely distributed (Figure 1.3), they represent a relatively scarce habitat type by comparison with other protected and rare habitats (Adnitt *et al.* 2007). Despite this relative scarcity, saltmarshes are considered to be highly valuable, not only in terms of their national and international conservation importance, particularly for wildfowl and wading birds (Norris 2000; Atkinson *et al.* 2001) and as nursery grounds for economically important fisheries (Green *et al.* 2009; Foster *et al.* 2013), but also in the provision of crucial ecosystem services (Adnitt *et al.* 2007; Gardiner 2015).

Increasing flood risk, linked to a combination of relative sea level rise and increased incidence of extreme weather events, is of high and growing concern for many inhabitants of low-lying coastal areas (Nicholls *et al.* 2007). An estimated 2.5 million people and £150 billion of assets are currently at risk from coastal flooding in the UK (Haigh *et al.* 2015). Much of the coast of south-east England is low-lying and protected by seawalls (King & Lester 1995; Paramor & Hughes 2004), an intervention which is both economically and environmentally costly to maintain, and which may prove increasingly ineffective in the face of continuing global climate change (French 2001; Goda 2010). Estuarine and coastal wetlands, and in particular saltmarshes (Moller *et al.* 1999; Moller 2006), play a significant role in alleviating flood risk to coastal communities, both by attenuating wave energy and in reducing coastline erosion (Cooper 2005; Austen *et al.* 2011). This natural buffering function considerably reduces the height, and associated cost, requirements of seawall construction (Brampton 1992; Moller & Spencer 2002). Costs for building a seawall in the absence of any mitigating habitat are estimated to reach £5400 per metre (Hudson *et al.* 2015), an expense which can be reduced by as much as 90% in the presence of saltmarsh (Paramor & Hughes 2004).

In addition to their conservation importance and provision of coastal defences, saltmarshes play a vital role in the delivery of many other ecosystem services. These include the processing of nutrients, water and soil; the provision of food, fuel and biochemical resources; and the regulation of disease, pollution and climate (Angus *et al.* 2011; Foster *et al.* 2013). Rates of carbon sequestration in saltmarshes are very high (Cannell *et al.* 1999), with the potential for long-term storage in accreting systems (Shepherd *et al.* 2007). Physical, biological and chemical processes within saltmarshes act to remove nutrients from sea, river and ground waters, and from agricultural run-off, thereby reducing diffuse pollution to the marine environment (Andrews *et al.* 2006; Andrews *et al.* 2008). The high rate of sediment accumulation in many saltmarshes also contributes to the immobilisation and storage of heavy metals and pollutants. Andrews *et al.* (2008) recorded the storage of 171 tonnes of pollutants, including lead and arsenic, in a 54ha area of saltmarsh in the Humber Estuary. Whilst methods of estimating the economic value of such ecosystem services are subject to debate, and frequently limited by the validity of the assumptions on which they are based (Pascual *et al.* 2010; Morris & Camino 2011), the value of saltmarshes to the 2,000km of UK coastline they protect (Doody 1992a) must be considerable.

Major saltmarsh losses occurred prior to the 1980s due to widespread and large-scale reclamation of land for development and agriculture (Morris *et al.* 2004). Whilst this process had been ongoing for hundreds, if not thousands, of years (Doody 2004), increased activity during the 20th century resulted in significant losses: 3,000ha of saltmarsh in the Wash alone (Doody 2008). Currently, major losses to saltmarsh extent continue to occur in south and south-east England (Cooper *et al.* 2001; Angus *et al.* 2011; Foster *et al.* 2013), due predominantly to erosion and 'coastal squeeze', the process whereby intertidal habitat is lost to rising sea levels whilst prevented from realigning landwards due to the presence of hard sea defences (Pethick 2001). Across the Solent, c. 670ha of saltmarsh was lost to erosion between 1971 and 2001, some 40% of that present at the earlier date. Forward extrapolation of past changes predicts a further loss of c. 870ha over the coming century: 78% of the remaining habitat (Foster *et al.* 2014). There have been some gains in saltmarsh extent, notably on the larger west coast marshes (Dargie 2000), however saltmarsh losses continue to significantly exceed gains (Rupp-Armstrong & Nicholls 2007; Foster *et al.* 2014) with ongoing net losses forecast to be as high as 2% per year (Angus *et al.* 2011).

3.1.2 *Spartina anglica*: friend or foe?

Perhaps the most significant change to British saltmarshes over the past century has been the evolution, spread and, in some areas, subsequent die back of *S. anglica* (Lacambra *et al.* 2004). In many areas of the world, the grass is considered to be a noxious pest, one of the “100 worst invasive species” (Lowe *et al.* 2000). The situation in Britain, however, is rather more complicated (Doody 2008), not least because *S. anglica* evolved here and could therefore be considered a native species which has simply colonised a vacant niche immediately seaward of other communities (Gray *et al.* 1991; Raybould *et al.* 1991b; Preston *et al.* 2002; Lacambra *et al.* 2004). It plays an important role in stabilising mudflats and protecting the coast, the function for which it was so widely distributed, and in contributing a considerable amount of organic material to the estuarine ecosystem (Doody 1990).

S. anglica is believed to dominate almost a quarter of Britain’s saltmarsh area, notably on the south and east coasts. Whilst its extent is now declining in these areas, it continues to spread on the west, north-west and north-east coasts, with around twenty new sites and a 40% increase in area reported between 1965 and 1997 (Gray *et al.* 1997). The species is generally perceived to be of detrimental conservation impact in the sites where it has successfully out-competed the native *S. maritima*, or other intertidal species including *Zostera* and *Salicornia* spp. (Lacambra *et al.* 2004). However, it has been suggested that the widespread loss of *Zostera marina* in the 1920s and 1930s was in fact due to a ‘wasting disease’, and that *S. anglica* merely took advantage of its demise rather than caused it (Davison & Hughes 1998). *S. anglica* is often reported as aggressively outcompeting ‘native’ species and preventing natural succession to other communities (Adam 2002). Other studies, however, suggest that *S. anglica* may actually play an important role in facilitating the establishment of other perennial saltmarsh species by providing protection against tidal currents, increasing surface elevation via the accretion of sediments, and improving upper sediment layers through increased litter accumulation (Gray *et al.* 1991). Whilst the species often plays a determining role in colonising bare mudflats, studies in the Dee Estuary show pioneer communities to be co-dominated by *Salicornia* spp. (Huckle *et al.* 2004) and further suggest *S. anglica* may act as a pioneer species for the successional formation of Atlantic salt meadow, the community for which the area is designated under the European Habitats Directive (Dargie 2000). The upper limit of *S. anglica* is likely to be determined by its competitive interaction with more landward species including *Puccinellia maritima* and *Atriplex portulacoides*. Here conditions

increasingly favour the competitor species as mudflat levels rise due to sediment accretion (Gray *et al.* 1991).

Strong conservation concerns remain in many areas regarding the abilities of *S. anglica* to colonise tidal mudflats, thereby reducing the available habitat for infaunal invertebrates and the populations of wildfowl and wading birds that feed on them (Doody 1990). Indirect evidence has been given for *S. anglica*'s role in the decline of dunlin, *Calidris alpina*, at a local scale (Goss-Custard & Moser 1988), however there has been no evidence directly attributing any decrease in the numbers of other bird species to the expansion of *S. anglica* marshes (Gray *et al.* 1997; Lacambra *et al.* 2004). The lack of definitive evidence regarding the impacts of *S. anglica* in Britain at anything other than a local scale has resulted in the adoption of three divergent management strategies (Lacambra *et al.* 2004; Adnitt *et al.* 2007):

1. *S. anglica* is considered to be of significant concern and (expensive and largely unsuccessful) control measures have been applied e.g. Lindisfarne and Morecombe Bay;
2. The species is not considered a problem and is neither protected nor controlled e.g. Bridgwater Bay; and
3. The spread, including further planting, of *S. anglica* continues to be encouraged to combat coastal erosion e.g. West Sussex.

Despite its rapid spread, and concerns in many locations over its control, *S. anglica* is now experiencing extensive natural die-back in some marshes without any management intervention having taken place. The total British extent of the species is estimated to have fallen by 19% between 1967 and 1990 (Doody 2008). Particularly affecting southern England, the mechanisms behind this process are not fully understood, however it seems to be associated to some degree with badly drained anaerobic soils which may be toxic to the plant's rhizomes (Gray *et al.* 1991). An additional explanation for the reduction in *S. anglica* extent is colonisation by other species, particularly in areas of low salinity (Lacambra *et al.* 2004). This has led some authors to suggest that, in Britain at least, natural succession of *S. anglica* marshes will, over time, result in the development of habitats of high nature conservation value. However, for this to take place, *S. anglica* must be given adequate time and freedom from management interventions to develop and accrete sufficient sediment to facilitate this process (Lacambra *et al.* 2004; Doody 2008).

3.1.3 Herbivores of *S. anglica*

Very little attention has been focused on the macroinvertebrate communities of *S. anglica* marshes in Britain (Gray *et al.* 1991). Payne (1973) found only four commonly-occurring species in Poole Harbour, three of which appeared to feed on *S. anglica*, although none exclusively. Jackson *et al.* (1985) conducted a two year study of the invertebrate population of the *S. anglica* marsh in Seafeld Bay, Suffolk. During this time, they noted fifteen benthic species and a further thirteen inhabiting the canopy, only six of which occurred regularly. The most abundant of the canopy-dwellers, the sap-sucking froghopper *Philaenus spumarius* (Aphrophoridae), was the only significant consumer of live *S. anglica* material. The authors concluded that this species would have no discernible impact on *S. anglica* however, as its annual consumption amounted to less than 0.3% of the total above-ground net primary production of the grass. The most abundant infaunal invertebrate identified in the Jackson *et al.* (1985) study was the polychaete, *Nereis diversicolor*. Primarily a scavenger and detritivore, the ragworm has been implicated in the erosion of south-east coast saltmarshes due to the combined effects of bioturbation and herbivory of *Salicornia* spp. seeds and seedlings (Hughes & Paramor 2004; Paramor & Hughes 2004). These conclusions were strenuously contested by Morris *et al.* (2004) however, and the only interaction noted between *N. diversicolor* and *S. anglica* is via the detrital food chain rather than consumption of live material (Jackson *et al.* 1985). Possible explanations for the lack of herbivory on *S. anglica* in its British range are thought to be the general indigestibility of C₄ plants (Caswell & Reed 1976), with *S. anglica* being one of only eight known C₄ species in Britain (Long 1983), or a degree of natural enemy escape afforded by its relatively recent origin (Gray *et al.* 1991).

The picture is very different in North America where *Spartina* marshes are heavily attacked by *P. marginata* and the mirid bug *Trigonotylus uhleri*, both of which are *Spartina* specialists (Denno 1977; Strong & Stiling 1983; Stiling & Bowdish 2000). They suffer further attack from a range of less-abundant invertebrates, including: stem-boring lepidopterans, beetles and flies (Stiling & Strong 1983); herbivorous flies and acridids that feed in flower heads and seeds (Bertness *et al.* 1987); leaf-mining flies (Stiling *et al.* 1984); midges feeding inside inflorescence stems (Gagne 1981); scale insects (Boyer & Zedler 1996); and leaf-grazing snails (Silliman & Zieman 2001). *T. uhleri* has been recorded at densities of up to ten individuals per mature culm of *S. alterniflora* (Daehler & Strong 1995), whereas *P. marginata* densities routinely reach several thousand individuals per square metre (Denno *et al.* 1986; Denno *et al.* 2000). Despite their high densities, *P. marginata* have been found to have no significant adverse effect

on *S. alterniflora* performance and survival where both species have co-evolved (Gustafson *et al.* 2006; Roberts & Pullin 2008), or in invaded ranges where both have coexisted for a substantial period (Daehler & Strong 1995). However, both *S. alterniflora* and *S. anglica* populations that had experienced a lengthy period of exile from, or had never been in contact with, *P. marginata* suffered significant deleterious impacts when exposed to the herbivore, both in terms of growth and survival (Daehler & Strong 1997b; Wu *et al.* 1999; Garcia-Rossi *et al.* 2003). Naive *S. anglica* plants were found to suffer 92% mortality after four months of exposure to high densities of *P. marginata* in glasshouse trials, with surviving plants averaging only 37% of the biomass of control plants over the same period (Wu *et al.* 1999). Field trials of *P. marginata* as a biological control agent of invasive *S. alterniflora* also showed early promise, with an almost 50% reduction seen in the biomass of exposed field cages in comparison to paired controls (Grevstad *et al.* 2003, and see 1.3.3.2 for further discussion).

British populations of *S. anglica* had no exposure to *P. marginata* prior to its arrival here, probably sometime between 2000 and 2008 (Kirby 2000; Wilson & Muhlerthaler 2009). A test of *S. anglica*'s susceptibility to the herbivore would shed light on the consistency with which increased vulnerability to *P. marginata* is found in naive and exiled *Spartina* populations, the first time this has been looked at outside of North America. Importantly, it would also give an early indication of what potential impacts *P. marginata*'s continuing invasion may have on Britain's important and vulnerable saltmarsh habitat.

The aim of the work reported in this chapter is to investigate the impact of *P. marginata* exposure on British populations of *S. anglica*, the dominant structuring species in a quarter of Britain's lower saltmarsh communities. To pursue this aim, I also assess the extent to which results from glasshouse experiments can be considered reliable indicators of impacts expected under field conditions. It is hypothesised that:

- i) *S. anglica* performance will be negatively affected by exposure to *P. marginata* feeding and oviposition;
- ii) The strength of this impact will be more severe at greater *P. marginata* densities, although the relationship may be non-linear; and
- iii) Negative impacts on *S. anglica* performance evident under glasshouse conditions will be replicated in experimental manipulations of field populations.

3.2 Materials and methods

3.2.1 Establishing a *Spartina anglica* culture

Spartina anglica root material was manually extracted from the Pagham field site (Grid ref: SZ 862972) in April 2012 and returned to the University of Sussex. Mud was removed from the roots by washing in fresh water and all above-ground biomass was discarded. Sections of rhizome approximately 12cm long and containing at least one node were carefully separated and planted in 10cm diameter pots containing horticultural grade silver sand. Pots were arranged in trays (14 pots per tray) and watered *ad libitum* with fresh water. This method follows Daehler and Strong (1994) in which the congeneric *S. alterniflora* was shown to grow 'vigorously' in fully fresh water. Pots were kept continually wet but not inundated, following Denno *et al.* (2000). One litre of 100% Hoagland nutrient solution (Hoagland & Arnon 1950) was added to each tray fortnightly. Trays were kept in a glasshouse in which the temperature was maintained within the range 15-30°C with supplementary lighting (100W Supanova LED grow lights, 8:2 light ratio comprising 660nm Red and 430nm Blue) on an 18:6 hour light:dark regime. After approximately one month, all plants showing signs of growth were re-potted in 15cm diameter pots. Conditions were maintained as before.

3.2.2 Calculating *Spartina anglica* biomass reference measurements

A method was required to compare the effect of different herbivory levels on plant biomass as a measure of plant performance. It is impossible, however, to directly measure plant biomass non-destructively (Evans 1972). Gonzalez Trilla *et al.* (2013) have shown that the strength of the allometric relationship between *Spartina* spp. biomass, leaf length and leaf area is sufficiently great to enable non-destructive monitoring and assessment of plant growth and spread. Stem length and biomass has also been found to be strongly correlated in the congeneric *S. alterniflora* (Nixon & Oviatt 1973). Accordingly, twenty *S. anglica* plants were removed from the culture and 150 leaves were measured to the nearest millimetre from leaf axil to tip. Each leaf was separately bagged, labelled and dried for 72 hours at 70°C in a Gallenkamp OV-420 drying oven. Dried leaves were weighed to an accuracy of 0.1mg using a Precisa 125A balance. The linear regression of leaf biomass on leaf length was used to estimate leaf biomass non-destructively in all subsequent experiments.

3.2.3 Establishing a *Prokelisia marginata* culture

S. anglica and *S. alterniflora* plants showing brown markings indicative of *P. marginata* oviposition (Strong 1988) were dug up and removed from the Hythe field site (Grid ref: SU 432 073) in November 2011 and returned to the University of Sussex. Individual

plants were planted in 15cm diameter pots containing horticultural grade silver sand. Pots were clustered in six trays with a combined area of 2m². The substrate in the pots was kept continually wet, but not inundated, by maintaining a 2-3cm depth of fresh water in the trays. One litre of 100% Hoagland nutrient solution was added to each tray fortnightly. Trays were kept in a glasshouse in which conditions were maintained as described in 3.2.1 with the exception of the supplementary lighting which was provided by 400W high pressure sodium lamps. Eggs that had been laid in the plants hatched within approximately one week, initiating a breeding culture of *P. marginata*. The culture was regularly monitored with the aim of keeping the impact of potential predators, notably spiders, to a minimum. Additional plants were added to the culture as required over time to maintain a consistent supply of host plant biomass.

3.2.4 *Prokelisia marginata* impact on *Spartina anglica* under glasshouse conditions

Forty-five potted *S. anglica* plants from the culture described in 3.2.1 were placed in individual trays. Individual plants were randomly assigned to three treatment levels (control, low planthopper density and high planthopper density) and then labelled. Five plants of each treatment group were randomly assigned to each of three experimental blocks, and to a random position within that block. Blocking was used to stagger the start date of each experimental unit to allow for the generation of sufficient numbers of *P. marginata* nymphs within the stock culture. Each experimental treatment was therefore replicated fifteen times. Starting metrics were recorded for each plant: number of leaves; number of tillers; overall height of plant and the length of each leaf. Starting biomass was inferred from leaf length using the equation described in 3.2.2 and 3.3.1.

Plants allocated to the low density treatment were inoculated with 15 second-to-third instar *P. marginata* nymphs from the culture described in 3.2.3; plants allocated to the high density treatment were inoculated with 30 nymphs. Plants allocated to the control group did not receive any nymphs. Transparent cylindrical cages were constructed from 175µm PET polyester film. Cages were 13cm in diameter and 50cm tall with a 5x7cm² nylon gauze-covered ventilation window positioned 18cm from the base of the cage and a gauze lid. Each plant was enclosed by a cage, the base of which was embedded approximately one centimetre below the surface of the sand. Plants were arranged in their designated position within the 3x5 grid configuration of the assigned experimental block (Figure 3.1) and watered *ad libitum* with fresh water; 100ml 100%

Hoagland nutrient solution was added to each tray fortnightly. The experiment ran for eight weeks with glasshouse conditions maintained as described in 3.2.1.

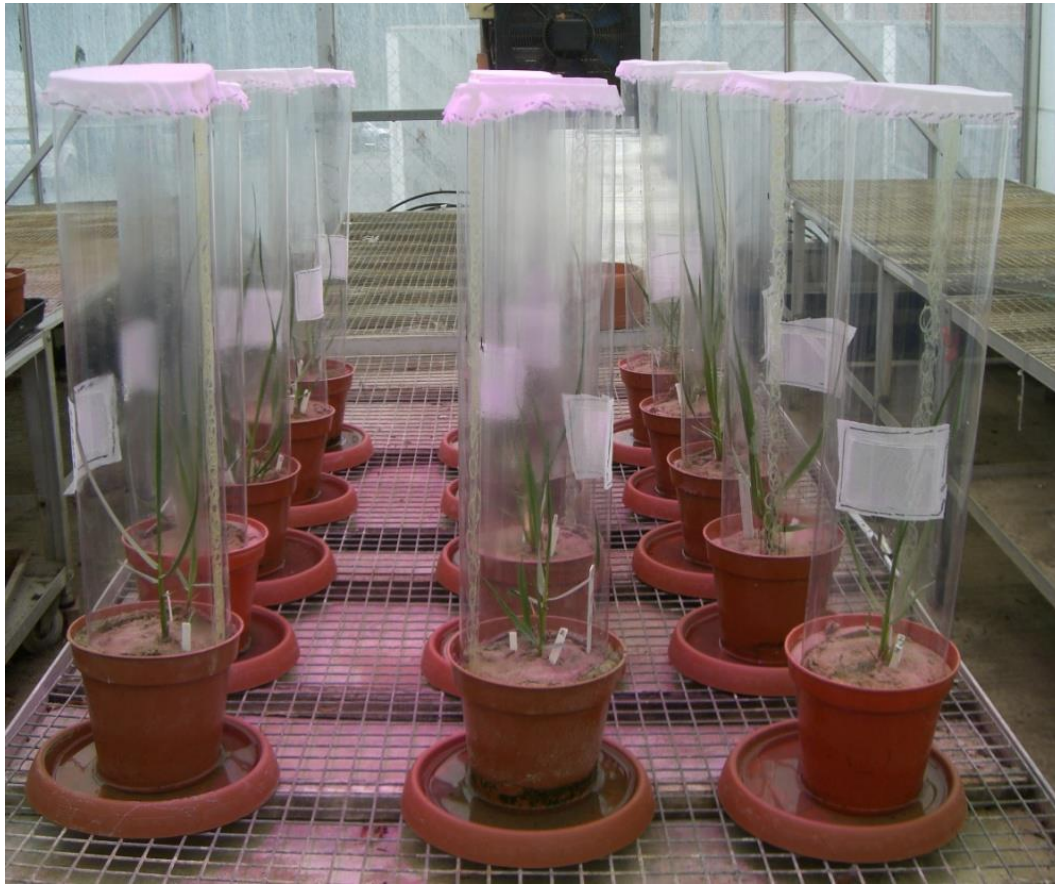


Figure 3.1 Experimental block in 3x5-pot grid

At the end of the experimental period, each replicate was placed inside a 1m³ Perspex box with a small access door. The cage was lifted off the pot and all *P. marginata* adults and nymphs were individually removed using an aspirator and counted. Overall plant height, total length of all leaves, number of leaves and number of tillers were recorded. Each plant was removed from its pot, all sand was carefully washed from the roots, and plants were then divided into above- and below-ground material. Each component was then weighed to an accuracy of 0.01g using a Precisa 125A balance, dried for 72 hours at 70°C in a Gallenkamp OV-420 drying oven and finally re-weighed to establish both fresh and dry biomass measurements.

3.2.5 *Prokelisia marginata* impact on *Spartina anglica* under field conditions

Field cages were constructed from inverted white opaque polypropylene buckets. Each cage was constructed by removing the base of the bucket and covering the resulting opening with nylon gauze, secured using Bostik Hot Melt adhesive. Six holes were drilled in the lip of each bucket through which 12cm steel tent pegs were inserted to secure the cage to the substrate when *in situ*. Cages were 42cm tall and 31.5cm in diameter, enclosing a surface area of 780cm² (Figure 3.2).



Figure 3.2 Field cage *in situ* at Pagham field site

In June 2013, twenty cages were distributed within an area of dense *S. anglica* at the Pagham field site. Cages were positioned to ensure, as far as was possible, consistent levels of plant coverage (i.e. avoiding patches of bare substrate) and elevation (i.e. avoiding creeks and gullies). The area was subject to a moderate level of tidal inundation, with water reaching two thirds of the height of the cages during high tides. However, it was sufficiently far from the lower marsh edge to be protected from any wave action which might dislodge the cages. Pagham was selected as the experimental site because natural background densities of *P. marginata* had been found to be extremely low (section 2.3.1). Cages were randomly assigned to either *Prokelisia* or control (no *Prokelisia*) treatments and then labelled (Figure 3.3). Four

individual *S. anglica* stems in each cage were loosely tagged with PVC tape and designated as ‘focal’ plants. The total leaf length for each focal plant was measured to the nearest millimetre from leaf axil to tip using a ruler. Starting biomass was subsequently inferred from leaf length using the equation described in 3.2.2 and 3.3.1. Cages designated as *Prokelisia* treatment were also fitted with a platform on which to place the insects, comprised of a circular piece of filter paper loosely fixed around a central stem. This was to ensure that *P. marginata* subsequently introduced into the cage would not fall onto the mud substrate and thus have a better chance of surviving the transplant. Neither the PVC tape nor the filter paper were fixed so tightly as to inhibit plant growth.



Figure 3.3 Experimental field cages at Pagham field site, June 2013.

P. marginata were collected on the following day from the Hythe field site (Grid ref: SU 432073) using the Vortis suction sampler described in 2.2.2. These were transported inside large polythene bags to the Pagham site, where groups of 200 adults were aspirated into individual sample pots and carefully transferred to the filter paper platform in each *Prokelisia* treatment cage. The number of *P. marginata* utilised in the experiment (equivalent to 256 individuals per 1m²) was chosen to reflect that of the high density treatment described in 3.2.4 (224 / m²) and of mean natural densities found at the Hythe field site in June the previous year (268 / m²; section 2.3.1). The experiment was left to run for 58 days.

At the end of the experimental period cages were removed, focal plants were cut at ground level, bagged and labelled. Leaf length of each focal plant was measured from leaf axil to tip using a ruler. Plants were individually bagged and dried for 72 hours at 70°C in a Gallenkamp OV-420 drying oven. The remaining standing crop in each cage was cut at ground level, bagged, labelled and removed for drying. Once dried, plants were weighed to an accuracy of 0.01g using a Precisa 125A balance to determine final biomass. No attempt was made to retain or count any *P. marginata* individuals remaining in the cages.

3.2.6 Statistical analysis

Statistical analysis was performed in R version 3.1.3 (R Core Team 2015) using the *nlme* (Pinheiro *et al.* 2012), *lme4* (Bates *et al.* 2012) and *multcomp* (Hothorn *et al.* 2008) packages. Assumptions about the distribution and variance of the response data (see Quinn & Keough 2002) were tested prior to analysis. Shapiro Wilk tests were used to test for normality of residuals; Fligner-Killeen tests were used to test for equal variance, as recommended by Crawley (2013). In cases where these assumptions were not met for continuous variables, square root transformations were successful in normalising the data.

For the glasshouse experiment, appropriate models were determined by the nature of the response variables. For continuous response variables, differences in performance between treatment groups were analysed using a linear mixed-effects model (LMM) with plant change as the response variable, treatment as the explanatory variable and block as a random factor. Plant change was analysed using relative growth rates, RGR (i.e. growth during the experimental period divided by the starting measurement), rather than absolute value in order to standardise for unavoidable variation in plant size between replicates at the beginning of the experiment. Root:shoot ratios were calculated at the individual plant level. Relative water content was calculated as a percentage of dry biomass. Count data were analysed using generalised linear mixed-effect models (GLMMs) with a Poisson error structure, log link function and the Laplace approximation technique (Bolker *et al.* 2009). As with the LMM structure, plant change was used as the response variable, treatment as the explanatory variable and block as a random factor. A test of Pearson's residuals showed no evidence of over-dispersion.

Each analysis began by fitting the explanatory variable (treatment) as a fixed factor and block as a random factor in a maximal model. This was compared to a null model containing only the random factor to determine whether removal of treatment as an

explanatory variable led to a significant ($p < 0.05$) increase in deviance. This was assessed by comparing the model with and without the term in question using log-likelihood ratio tests for LMMs and χ^2 values for GLMMs. The resulting minimum adequate model is the one that best fits the data, produces the least unexplained variation (the minimum residual deviance) and where all parameters in the model are significant (Crawley 2013). Significance levels are reported for the addition of non-significant terms and removal of significant terms from the minimum adequate model. Plots of model residuals against fitted values were visually inspected for normal distribution, homogeneity of variance and the presence of influential outliers following procedures outlined in Crawley (2013). Results showing significant treatment effects were further investigated using a Tukey HSD (Honest Significant Differences) *post-hoc* test to identify differences between treatment means. This test corrects for family-wise error rates when making multiple comparisons, adjusting the resulting p-value accordingly (Crawley 2013).

For the field experiment, all response variables were continuous, normally distributed and with constant variance. The effect of treatment was analysed using ANOVA with, for focal plants, relative growth rates (RGR) as the response variable and treatment as the explanatory variable. For whole cage standing crop, the final biomass was used as the response variable. Plots of model residuals against fitted values were visually inspected for normal distribution, homogeneity of variance and the presence of influential outliers as before.

3.3 Results

3.3.1 Estimating *Spartina anglica* biomass from leaf dimensions

A linear regression applied to the data described in 3.2.2 revealed a strong correlation between leaf length and biomass ($n=150$, $r^2=0.9637$, $p<0.001$):

$$\text{biomass (g)} = 0.0009736 * \text{leaf length (mm)}$$

This equation was used in subsequent analyses to estimate the starting biomass of experimental plants with known leaf length measurements.

3.3.2 Effect of *Prokelisia marginata* colonisation on *Spartina anglica* under glasshouse conditions

At the end of the experimental period, visual inspection suggested that exposure to *P. marginata* had a detrimental effect on *S. anglica* performance. Overall plant height, the number and length of leaves, as well as general plant 'robustness', all appeared to have suffered in plants exposed to *P. marginata* colonisation (Figure 3.4).

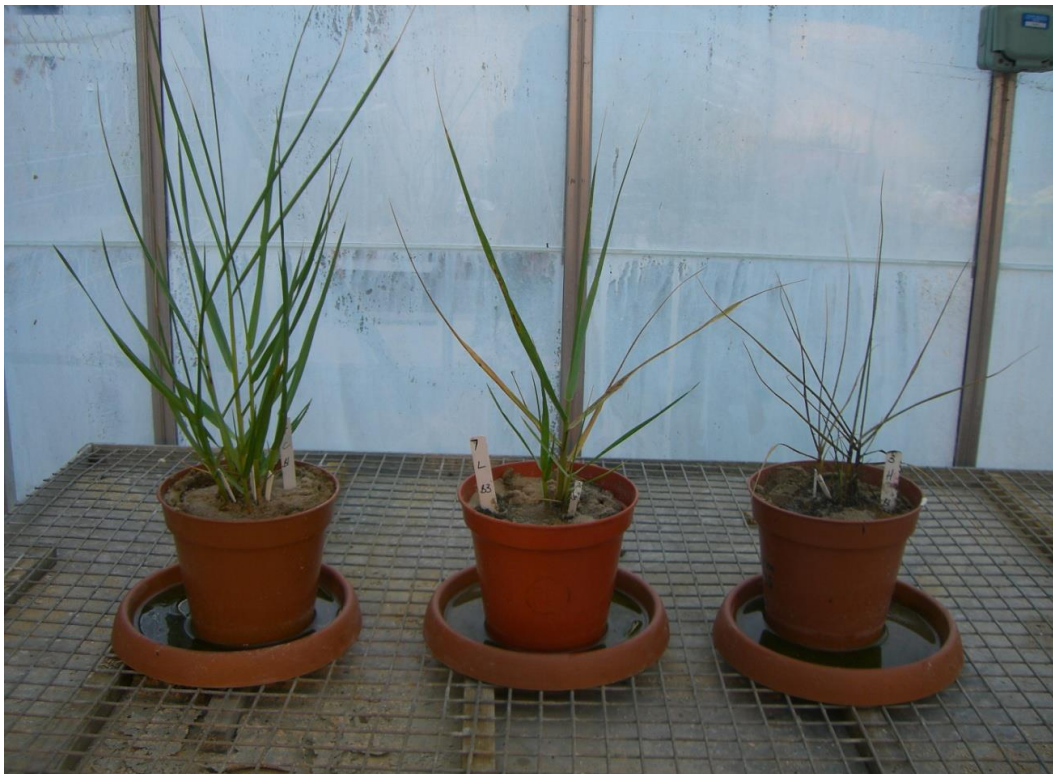


Figure 3.4 A selection of *S. anglica* plants at the end of the experimental period illustrating the negative effects of *P. marginata* exposure on plant growth. Treatments (from left to right): Control (no. *P. marginata*); Low density (15 *P. marginata* individuals added); High density (30 *P. marginata* individuals added).

Detailed analysis of the data confirmed that exposure to *P. marginata* had strong negative effects on a range of *S. anglica* performance metrics (Table 3.1). Plant height, leaf length and biomass RGR response variables were found to be highly correlated; consequently only one metric (biomass RGR) is reported.

Table 3.1 Effect of *P. marginata* on *S. anglica* performance. Individual treatment levels refer to low *P. marginata* density (15 individuals added) and high *P. marginata* density (30 individuals added) treatments. Whole model analyses refer to comparisons with control (no *P. marginata* added) replicates. Post-hoc test refers to Tukey HSD using 95% CI. Test statistics are log-likelihood (L) ratio for LMMs and χ^2 for GLMMs. Relative metrics indicate ratios of post- to pre-experiment measurements. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

	d.f.	Control mean	Treatment mean	L ratio	χ^2	p
i)						
Biomass relative gain						
Treatment (whole model)	40	2.423	1.697	15.582	<0.001	***
Low density (<i>post-hoc</i>)			2.035		0.271	
High density (<i>post-hoc</i>)			1.360		<0.001	***
ii)						
No. new tillers gained						
Treatment (whole model)	40	2.067	1.167		6.653	0.036 *
Low density (<i>post-hoc</i>)			1.400		0.394	
High density (<i>post-hoc</i>)			0.933		0.037	*
iii)						
No. new leaves gained						
Treatment (whole model)	40	15.867	11.500		15.089	<0.001 ***
Low density (<i>post-hoc</i>)			12.067		0.015	*
High density (<i>post-hoc</i>)			10.933		<0.001	***
iv)						
Relative water content						
Treatment (whole model)	40	2.171	1.679	17.561	<0.001	***
Low density (<i>post-hoc</i>)			1.686		<0.001	***
High density (<i>post-hoc</i>)			1.672		<0.001	***
v)						
Final root biomass (g)						
Treatment (whole model)	40	8.419	6.850	6.419	0.040	*
Low density (<i>post-hoc</i>)			8.138		0.988	
High density (<i>post-hoc</i>)			5.561		0.042	*
vi)						
Root:shoot ratio						
Treatment (whole model)	40	1.441	1.537	3.385	0.184	
Low density (<i>post-hoc</i>)			1.738		0.363	
High density (<i>post-hoc</i>)			1.336		0.916	

The relative above-ground biomass growth rate and the number of new tillers gained were significantly negatively impacted at high (but not at low) *P. marginata* density (Figure 3.5; Table 3.1).

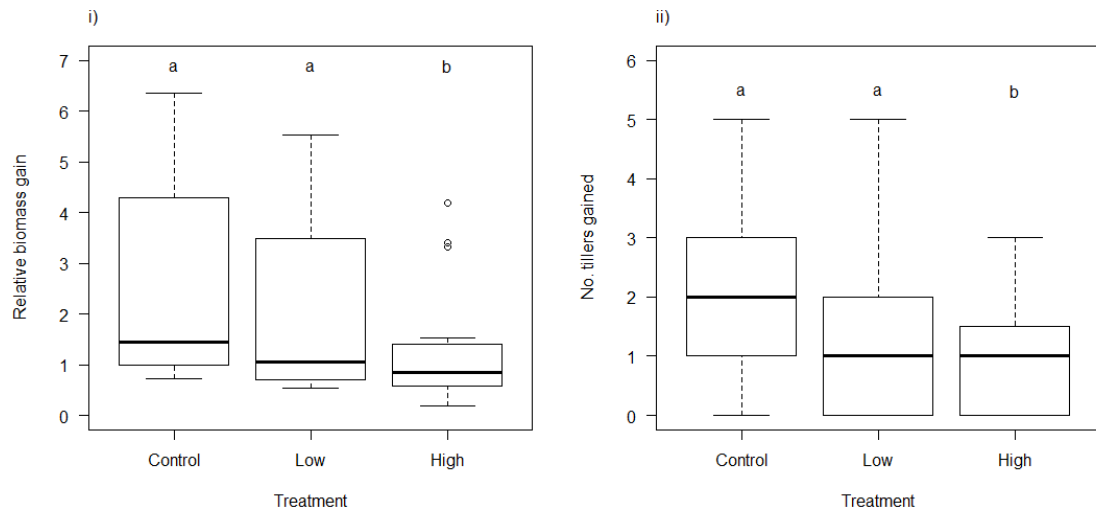


Figure 3.5 Effect of *P. marginata* on *S. anglica* performance measured as i) above-ground biomass relative growth rate; and ii) number of new tillers gained. Treatment refers to control (no *P. marginata*), low *P. marginata* density (15 individuals added) and high *P. marginata* density (30 individuals added) treatments. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD *post-hoc* test, using 95% CI). Figures show untransformed data. Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present. Relative metrics indicate ratios of post- to pre-experiment measurements.

The mean above-ground biomass relative growth rate (RGR) of high density treatment plants was 56% of that of control plants.

The number of new leaves gained was negatively affected in both the low and high *P. marginata* density treatments. Low density treatment plants on average gained 76% and high density treatment plants 69% of the number of leaves gained by control plants. The relative water content of shoot material was similarly impacted by both levels of treatment with mean levels for both groups representing approximately three quarters of those found in the control group (Figure 3.6; Table 3.1).

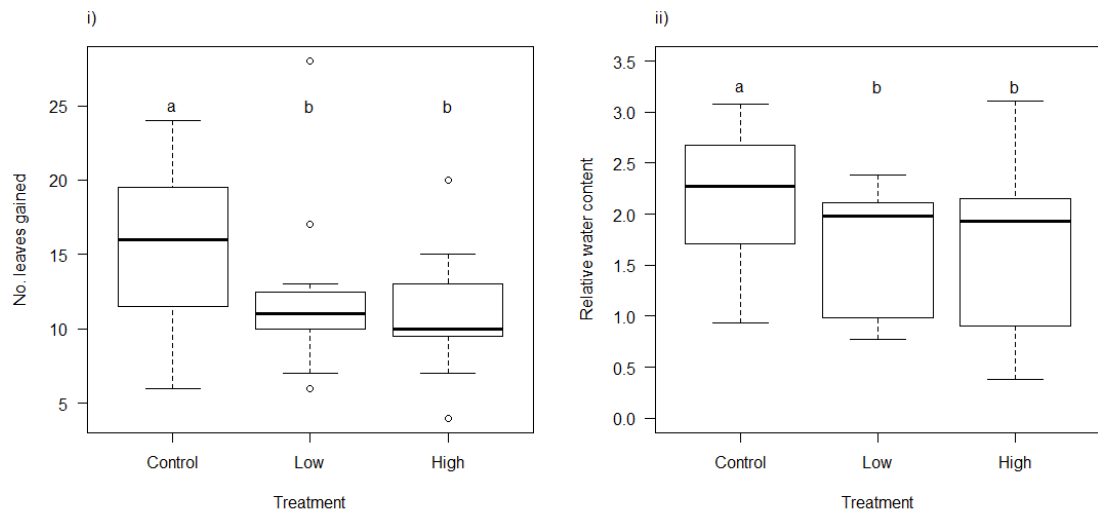


Figure 3.6 Effect of *P. marginata* on *S. anglica* performance measured as i) number of new leaves gained; and ii) shoot relative water content in control (no *P. marginata*), low *P. marginata* density (15 individuals added) and high *P. marginata* density (30 individuals added) treatments. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD *post-hoc* test, using 95% CI). Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present.

Inspection of root material suggested a negative impact of *P. marginata* exposure similar to that observed for shoot material (Figure 3.7).

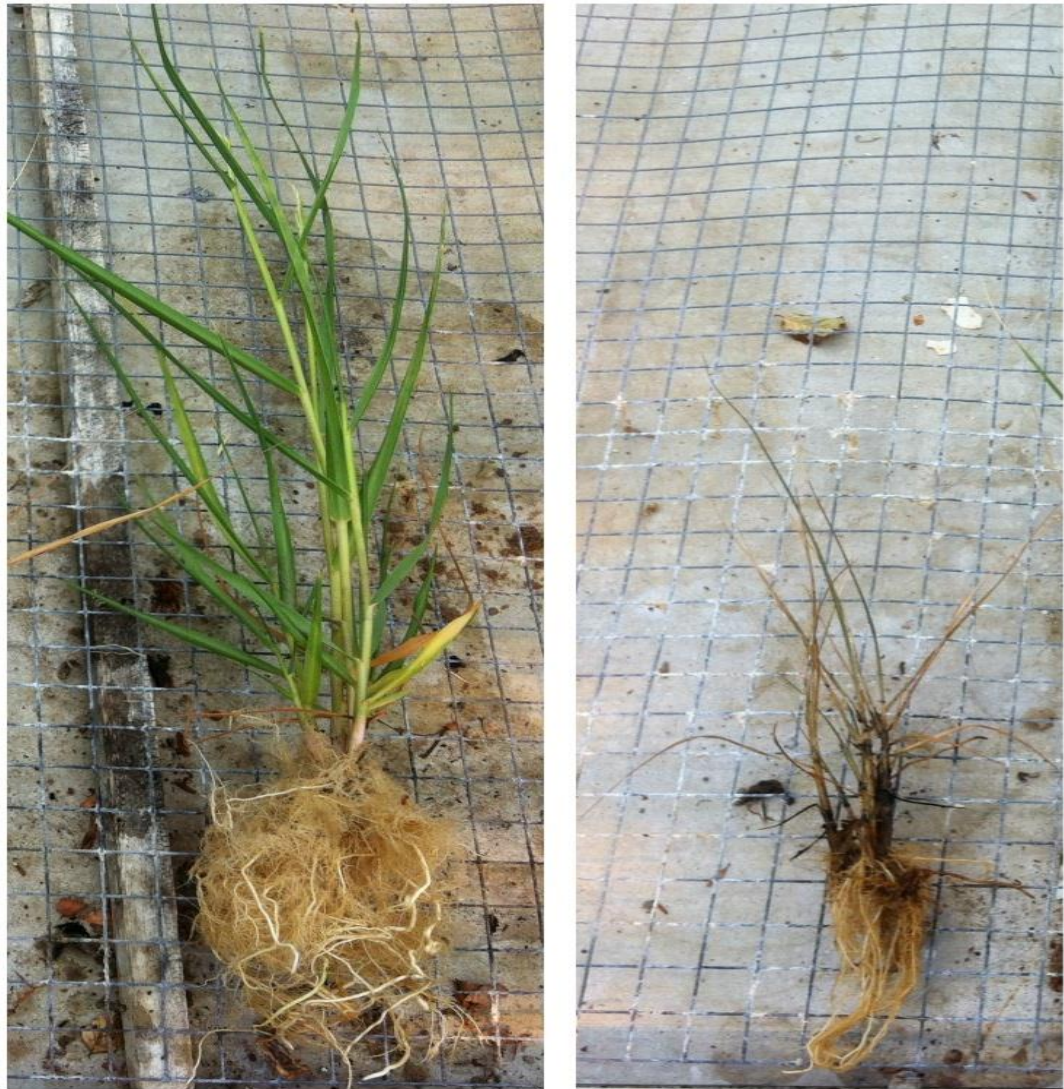


Figure 3.7 A selection of *S. anglica* plants at the end of the experimental period illustrating the negative effects of *P. marginata* exposure on both above and below ground plant growth. Left: Control (no. *P. marginata*); Right: High density treatment (30 individuals added).

Root biomass was negatively affected at high (but not low) *P. marginata* density treatments, with plants in the high density treatment group having on average 66% of the biomass of control plants. No significant effect of either treatment level was found on the root:shoot ratio of plants (Table 3.1; Figure 3.8).

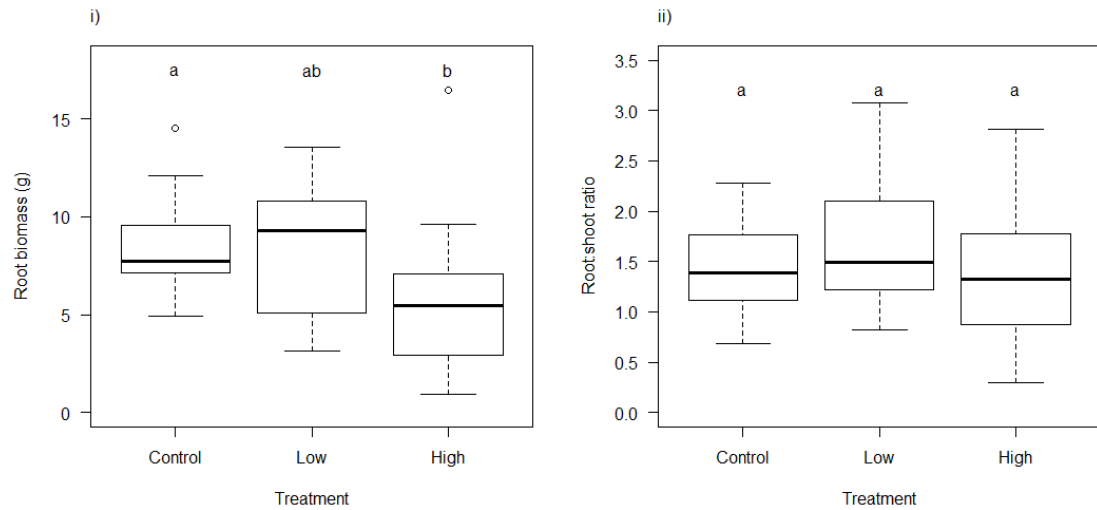


Figure 3.8 Effect of *P. marginata* on *S. anglica* performance measured as i) final root biomass and ii) root:shoot ratio in control (no *P. marginata*), low *P. marginata* density (15 individuals added) and high *P. marginata* density (30 individuals added) treatments. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD *post-hoc* test, using 95% CI). Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent the full range of the data.

3.3.3 Effect of *Prokelisia marginata* exposure on *Spartina anglica* under field conditions

Exposure to *P. marginata* under semi-natural field conditions was found to have a negative impact on plant performance metrics (Table 3.2).

Table 3.2 Effect of *P. marginata* on *S. anglica* performance measures in field conditions. Treatments are control (no *P. marginata*) and *Prokelisia* (200 individuals added). Relative metrics indicate ratios of post- to pre-experiment measurements. Significance levels indicated by: * ≤ 0.05

	d.f.	Control mean	Treatment mean	F	<i>p</i>	
i)						
Relative leaf biomass gain						
Treatment	1	2.65	2.51	0.29	0.599	
Residuals	18					
ii)						
Relative leaf length gain						
Treatment	1	1.14	0.84	4.88	0.040	*
Residuals	18					
iii)						
Final whole cage biomass (g)						
Treatment	1	83.69	60.42	5.99	0.025	*
Residuals	18					

A significant negative treatment effect was found on the leaf length RGR of focal plants, which produced a mean growth rate of 74% of that of the control plants. Treatment also had a significant negative effect on final whole cage biomass, with cages exposed to *P. marginata* having 72% of the mean biomass of control cages. The mean relative biomass gain of focal plants exposed to *P. marginata* was lower than that for control plants, however the difference was not statistically significant (Figure 3.9; Table 3.2).

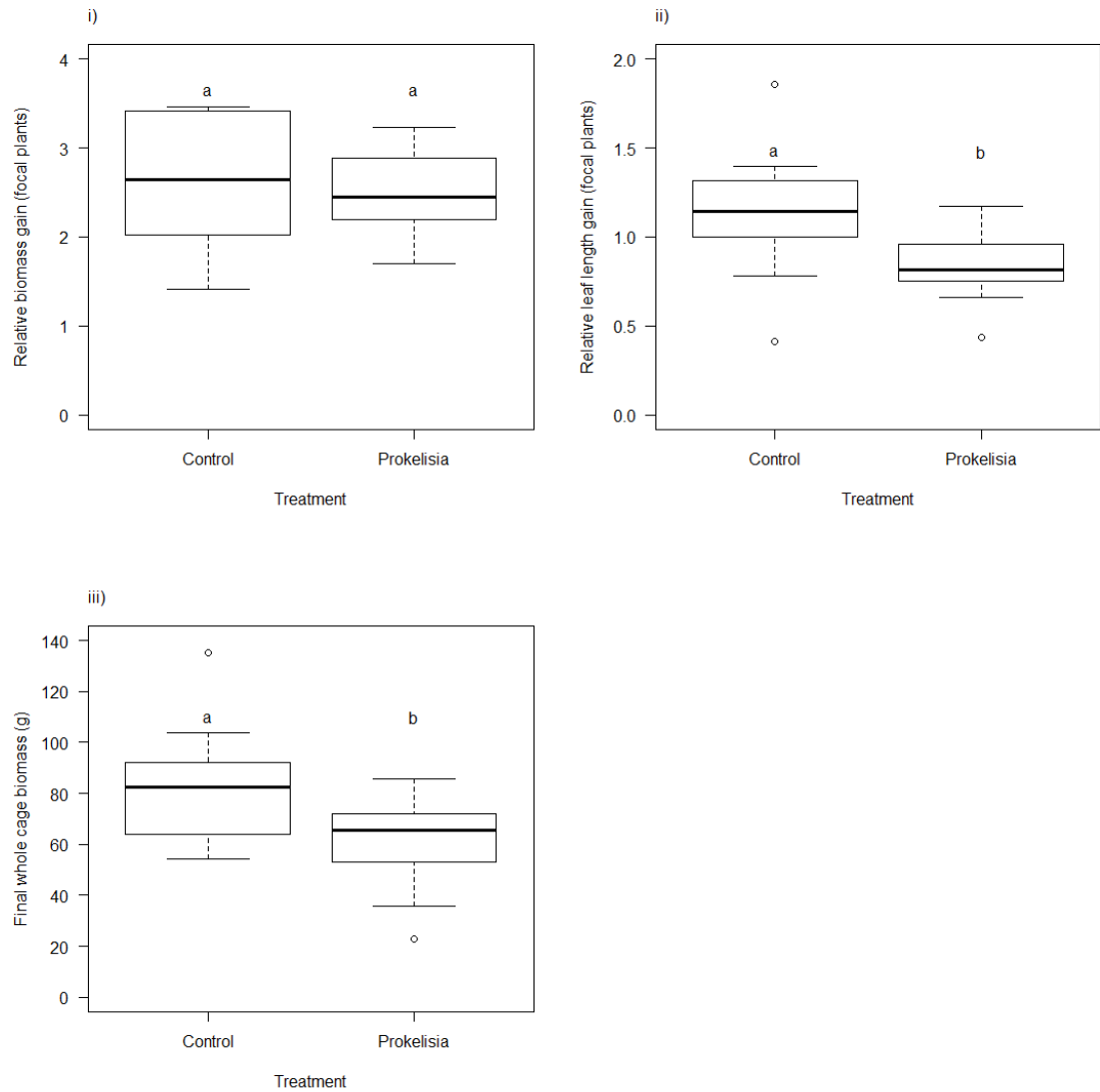


Figure 3.9 Effect of *P. marginata* on *S. anglica* performance measured as i) relative leaf biomass gain of focal plants; ii) relative leaf length gain of focal plants; and iii) final biomass of entire cage in control (no *P. marginata*) and *Prokelisia* (200 individuals added) treatments. Treatments sharing lower case letters are not significantly different from each other. Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present. Relative metrics indicate ratios of post- to pre-experiment measurements.

3.4 Discussion

The measurement of plant functional traits, any morphological, physiological or phenological feature quantifiable at the individual level, provides a reliable indication of a herbivore's impact upon its host (Strong *et al.* 1984). Reflecting the ability of a plant to grow, reproduce or survive, such traits allow for indirect measurement of a plant's fitness at any given point in time (Violle *et al.* 2007). With the exception of root:shoot ratio, exposure to *P. marginata* herbivory and oviposition had a significant negative impact on all of the *S. anglica* functional traits examined under glasshouse conditions. As predicted, impacts were more severe in high density treatments, with only the number of new leaves and the relative above-ground water content being significantly affected at low density treatment levels. It is relevant to note, however, that crowding levels, even in the high density treatment, were lower than the average natural field densities recorded at Hythe (section 2.3.1 and 3.2.5). Plants subject to high density treatments had greatly reduced height and root biomass, indicative of reduced competitive ability, whilst the reduced number and total length of leaves is indicative of reduced photosynthetic potential (Strong *et al.* 1984; Olmstead *et al.* 1997). A reduction of relative water content, found in plants subject to both treatment levels, has the potential to restrict transpiration, restrict nutrient uptake, reduce efficiency of photosynthesis and ultimately limit productivity (Zhang *et al.* 2012).

Wherever possible, it is prudent to test whether vulnerabilities identified under glasshouse conditions are replicated in the field (Fletcher *et al.* 1990; Malmberg *et al.* 2005). Numerous confounding factors, including a range of environmental conditions and the presence of natural enemies can result in a significant difference in outcomes (Wu *et al.* 1999). Field manipulations were thought to be of particular importance in judging the applicability of the glasshouse experiments described in this thesis as experimental plants were all grown in fresh, rather than salt, water. This approach was adopted for a number of reasons. Firstly, the great majority of published glasshouse experiments examining this system were conducted with fresh water (Denno *et al.* 1985; Denno *et al.* 1986; Thompson *et al.* 1991; Daehler & Strong 1995; Olmstead *et al.* 1997; Denno *et al.* 2000; Stiling & Bowdish 2000). In the limited number of instances where saltwater was used, it was at salinities significantly lower than experienced in natural conditions (Wu *et al.* 1999; Garcia-Rossi *et al.* 2003). Secondly, the use of salt water in glasshouse conditions can be heavily compromised by the effects of evapotranspiration, resulting in pore water in the pot having much greater salinity than that which was applied. It is possible to account for this by utilising multiple replicates representing a gradient of salinities and mitigating treatments. Practical limitations

prevented the adoption of this approach, however, as the number of possible replicates was restricted by the length of *S. anglica* growing season (which was limited even under glasshouse conditions), and difficulties in synchronising the timing between obtaining sufficient numbers of *P. marginata* at the appropriate life stage and plants of the optimum size for experimentation. Finally, whilst *Spartina* species are salt-tolerant, affording them a competitive advantage over non-halophytic species in brackish and saline environments, individual plants actually perform better under low- and no-salt conditions (Linthurst & Seneca 1981; Courtney *et al.* 2016). Vasquez *et al.* (2006) found that *S. alterniflora* achieved greater shoot height, more new shoots, and greater above- and below-ground biomass when grown in fresh water in comparison to plants exposed to an assay of saline conditions. The use of fresh water therefore represents the adoption of a conservative approach when assessing the impacts of *P. marginata* exposure on plant performance. *P. marginata* host plant preferences (as inferred from field densities) are not significantly affected by altered salinities under manipulated natural conditions (Bowdish & Stiling 1998). The impact of their exposure on the host, however, could be more severe under saline conditions which confer a level of stress on the plant (Daehler & Strong 1995). Whilst the lower biomass gain seen in exposed focal plants under semi-natural field conditions was not significantly different from controls (Table 3.2; Figure 3.9), both the height of focal plants and the biomass gain of entire cages exposed to *P. marginata* were significantly impacted. It is reasonable to conclude, therefore, that results from glasshouse experiments are representative of impacts under field conditions.

P. marginata are stenophagous; able to successfully complete their lifecycle on only a small number of *Spartina* species (Grevstad *et al.* 2003). As all other *Spartina* species are rare and extremely localised in the UK (Lacambra *et al.* 2004; Figure 1.5), the interaction between *P. marginata* and *S. anglica*, its primary British host and the main mechanism enabling its further establishment and spread, is of particular interest. This study is the first time the interaction between *P. marginata* and *S. anglica* has been examined outside North America where the herbivore showed early promise as a potential biological control agent of the grass. The findings reported in this chapter lend weight to the conclusions of North American studies that exiled and naive *Spartina* populations are vulnerable to attack by *P. marginata* in a way that co-evolved and non-exiled plants are not (Daehler & Strong 1995; Wu *et al.* 1999; Grevstad *et al.* 2003). This should be of interest to environmental managers in countries such as China where *S. alterniflora* is listed amongst the most harmful invasive alien plants in the country (Wang *et al.* 2006; Zuo *et al.* 2012), and where biological control attempts utilising the

superior competitive abilities of other exotic plants have shown early promise (Zhou *et al.* 2015b).

It appears likely that British populations of *S. anglica* have so far benefitted from a degree of natural enemy escape (Gray *et al.* 1991). In high contrast to North American *Spartina* marshes which hold a diverse assemblage of host-specific free-living herbivores (Gratton & Denno 2005), including the highly abundant *Spartina* specialists *P. marginata* and *T. uhleri*, there are no specialist *Spartina* herbivores native to any part of Europe (Garcia-Rossi *et al.* 2003). Although pressure from larger grazers such as *Littoraria* spp. snails and the purple marsh crab, *Sesarma reticulatum*, has been shown to act synergistically with other drivers to cause die-back in some western Atlantic saltmarshes (Silliman *et al.* 2005; Jefferies *et al.* 2006; Alberti *et al.* 2008; Holdredge *et al.* 2009; Coverdale *et al.* 2012), Daehler and Strong (1995) found that the top-down effects of insect herbivores on *S. alterniflora* in San Francisco Bay were minimal. They suggested the effects of herbivory may be low due to a lack of other stressors impacting the plant which occurs in large, monospecific stands and is subject to little or no interspecific competition (Strong & Ayres 2013). These dense, monospecific stands are less apparent in Britain, however, where *S. anglica* marshes frequently present as more of a mixed matrix with other halophytes (Gray *et al.* 1991; Dargie 2000; Boorman 2003; Huckle *et al.* 2004; and see section 2.2.1). British *Spartina* may consequently be subject to greater levels of competition-induced stress, already noted as a potential contributing factor to the die-back recorded over recent decades (Lacambra *et al.* 2004), and thus be inherently more vulnerable to the deleterious impacts of a novel and abundant insect herbivore.

P. marginata is currently in the very early stages of invasion in the UK (section 2.4.1; 2.4.3). It has, however, already spread rapidly from its probable site of initial colonisation (section 1.3.3.3) and reached densities shown in this chapter to have significant deleterious impacts on *S. anglica*, both under glasshouse and semi-natural field conditions. Invasion lag and other complexities of the invasion process mean that the full effects of an invader may not be seen for a considerable time after its arrival (Strayer *et al.* 2006). Further investigation is needed to assess whether continued *P. marginata* population growth and spread may act additively, or even synergistically, with other biotic and abiotic factors to restrain or inhibit growth in British *Spartina* spp., or increase the rate of die-back. A reduction in plant vigour, such as that seen in *S. anglica* die-back in southern England, may have a deleterious impact on the efficiency of wave and current dissipation across affected saltmarshes (Adnitt *et al.* 2007),

consequently reducing the extent to which they act to prevent coastal flooding. Whilst the position of *S. anglica* in Britain remains complex, and in some cases contentious, early indications suggest that *P. marginata* has the potential to contribute to a significant change in the composition of British saltmarsh over the coming decades. Further research is needed to categorise the nature and extent of these potential impacts on this important and already heavily challenged habitat.

Chapter four

The impact of *Prokelisia marginata* on four *Spartina* species

4.1 Introduction

4.1.1 Tolerance to herbivory

Insect herbivory can have significant detrimental impacts on plant growth, reproduction and survivorship, ultimately affecting plant fitness (Shen & Bach 1997) and exerting a major influence in both ecological and evolutionary timescales (Coley *et al.* 1985; Fornoni *et al.* 2004). Selection by herbivores has been shown to result in the evolution of a wide diversity of defensive plant traits (Didiano *et al.* 2014), which are widely categorised as relating to either tolerance or resistance (Nunez-Farfan *et al.* 2007). Resistance traits are morphological (e.g. thorns, trichomes) or chemical (e.g. tannins, glucosides) constitutive or induced traits that deter and reduce herbivore damage (Nunez-Farfan *et al.* 2007; Didiano *et al.* 2014). Tolerance of herbivory has been defined as the capacity of plants to reduce the negative impacts of herbivore-inflicted damage on their fitness (Stowe *et al.* 2000), with individual plant species differing markedly in their ability to survive and reproduce after herbivore attack (Strauss & Agrawal 1999). Compensatory growth by plants to replace damaged leaves, shoots and roots represents the most commonly studied plastic tolerance response (McNaughton 1983; Maschinski & Whitham 1989; Lehtila & Syrjanen 1995; Lennartsson *et al.* 1997), however tolerance to insect herbivory is not limited solely to induced responses. In its wider definition, tolerance is expected to be related to a plant's phenotypic expression of ecologically relevant traits, expressed both before and after damage (Strauss & Agrawal 1999). Such traits include: plant architecture and resource allocation patterns, including the number and distribution of leaves, branches and tillers, and the ratio of root to shoot biomass; the rate of photosynthetic activity; and phenological patterns (Hendrix 1979; Briske *et al.* 1996; Marquis 1996; Stowe *et al.* 2000; Tiffin 2000). Studies utilising quantitative genetics have shown that plant tolerance is comprised of heritable traits, subject to natural selection, and therefore likely to evolve as an adaptive defence against herbivore pressure (Stowe *et al.* 2000; Fornoni *et al.* 2003). However, the evolution of tolerance is restricted by a range of factors including phylogenetic history and genetic constraints, the existence, magnitude and type of tradeoffs with other traits affecting fitness, and context-dependent interactions between plant genotype and the physical and biotic environment (Nunez-Farfan *et al.* 2007; Agrawal & Fishbein 2008; Fornoni 2011). Tolerance can only evolve

by natural selection if there is heritable variation that affects plant fitness. Additionally, if allocation costs of maintaining tolerance mechanisms are sufficiently great in environments where herbivores are scarce or have little impact, non-tolerant genotypes could be favoured (Van Der Meijden *et al.* 1988; Rosenthal & Kotanen 1994; Strauss & Agrawal 1999).

4.1.2 Post-introduction evolution in plants

Little is known about the factors that contribute to geographic variation in tolerance and how this affects co-evolutionary dynamics between plants and their herbivores (Baucom & Mauricio 2008; Agrawal 2011; Fornoni 2011). A popular explanation for the success of invasive plants is that they are introduced to their new range without the full suite of natural enemies, particularly specialists, with which they have co-evolved (Maron & Vila 2001; Keane & Crawley 2002), thereby benefitting from natural enemy escape. With fewer specialist enemies, the Evolution of Increased Competitive Ability (EICA) hypothesis predicts that invasive plants may reallocate resources from defence to growth, reproduction, or both, consequently out-competing native species (Blossey & Notzold 1995). Examples of reduced defence (Maron *et al.* 2004; Wolfe *et al.* 2004), increased growth or fecundity (Flory *et al.* 2011), and for both elements of the EICA hypothesis (Zou *et al.* 2008; Feng *et al.* 2009) have been evidenced for some invasive species. Other studies, however, have found either no evidence to support the hypothesis, with species faring equally well in both their native and invasive range, or opposing results where individuals from invasive populations are more highly defended than their native-range counterparts (Willis *et al.* 1999; Leger & Forister 2005; Muller & Martens 2005; Cano *et al.* 2009; Parker *et al.* 2013). Tolerance remains a relatively under-studied component of the EICA hypothesis (Muller-Scharer *et al.* 2004; Liao *et al.* 2014), with the majority of studies focussed on resistance, as indicated by the presence and concentration of toxic defensive chemicals, and the hypothesised trade-off between tolerance and resistance, which suggests that highly resistant plants are expected to demonstrate low levels of tolerance, and vice versa (Bossdorf *et al.* 2004; Muller-Scharer *et al.* 2004; but see also Wilsey & Polley 2006; Ashton & Lerdau 2008; Li *et al.* 2012).

Fitness benefits accrued via enemy release and associated reallocation of resources away from defence may, however, be temporary, as native herbivores tend to accumulate on novel species over time, and may even prefer them (Strong 1974; Strong *et al.* 1977; Andow & Imura 1994; Morrison & Hay 2011). Co-evolved natural enemies may themselves arrive in the new range, either through unintended invasion

processes, or through deliberate introduction for the purposes of biological control. The character and intensity of the re-association between plant and herbivore can differ from that found in the native range (Agrawal 2011), and plants may undergo rapid evolutionary changes to defend against these new or re-established interactions (Thompson 1998; Zangerl & Berenbaum 2005; Prentis *et al.* 2008; Zangerl *et al.* 2008; Pearse *et al.* 2013). Siemann and Rogers (2001; 2003) found evidence for the post-introduction evolution of reduced defence and increased growth in populations of the Chinese tallow tree, *Sapium sebiferum*, introduced to North America in the previous 100 to 233 years. Further investigation using a combination of common garden experiments and bioassays along a “chronosequence of introduction time” (Siemann, Rogers & Dewalt 2006), reported that newly-established populations had the greatest growth and lowest defence in contrast with trees in the native range, thereby contributing to early success of the invader, but that accumulation of insect herbivores had reduced this benefit, and realigned the direction of resource allocation, over time. Similarly, Fukano and Yahara (2012) found populations of common ragweed, *Ambrosia artemisiifolia*, naturalised in Japan displayed greater growth rate and reduced defence in comparison to their native-range counterparts. In areas where the specialist herbivore *Ophraella communa* had also recently invaded, however, plants demonstrated the ability to rapidly re-evolve defence capacity after renewed exposure to the native enemy. Additionally, Lu and Ding (2012) found that plants originating from populations with historical exposure to herbivory had an increased capacity for compensatory growth, compared to conspecifics with no such history, when subjected to herbivore attack in their new range .

4.1.3 Vulnerability of invasive *Spartina* spp. to *Prokelisia marginata*

Qing *et al.* (2012) found that *S. alterniflora* plants originating from invasive populations directed a greater proportion of leaf nitrogen to photosynthetic processes (a proxy for competitive ability) when compared to plants originating from native populations. The authors suggest that these findings indicate *Spartina* spp. may benefit from rapid evolutionary changes in their introduced range when released from the selection pressures of specialist natural enemies, contributing to invasion success. Despite reaching extremely high population densities (Denno *et al.* 1986; Denno *et al.* 2000), *P. marginata* herbivory has been shown to have no significant impact on otherwise unmanipulated *Spartina* spp. populations where both species are native, or where they have co-existed for a substantial period of time (Daehler & Strong 1995; Gustafson *et al.* 2006; Bertness *et al.* 2008). However, a number of studies suggest that tolerance is lost (or has not been gained) in long-exiled (or naive) populations. In both field and

glasshouse studies of such populations, exposure to field-equivalent densities of *P. marginata* has been shown to significantly increase *Spartina* spp. mortality and reduce biomass, growth rates, fertility, plant height, leaf length and the number of new leaves and tillers in comparison to herbivore-free controls (Daehler & Strong 1997b; Wu *et al.* 1999; Garcia-Rossi *et al.* 2003; Grevstad *et al.* 2003; and see section 1.3.3.2 for further discussion). Garcia-Rossi *et al.* (2003) found genetic variation in vulnerability to *P. marginata* to be much greater in long-exiled populations of *S. alterniflora* in comparison to a population that had never been separated from it, suggesting that this had arisen as a result of relaxed selection in the enemy-free invaded range. The authors further suggest that uniform tolerance to the herbivore could be rapidly re-evolved as vulnerable individuals are killed or controlled while tolerant genotypes persist and spread throughout the population.

The genetic origins of the four *Spartina* species present in Britain (see section 1.3.2.1 for a full discussion) could be considered to represent a “gradient” of shared co-evolutionary history with *P. marginata*. British populations of *S. alterniflora* are presumed to have been established from seeds transported in shipping ballast from the eastern coast of North America (Thompson 1991) where *P. marginata* is present in high densities (Denno *et al.* 1986). The founding population can therefore be considered to have shared a long, unbroken, co-evolutionary history with *P. marginata* prior to its arrival in Britain. Initial high levels of tolerance to the herbivore suggested by previous studies (Daehler & Strong 1995; Gustafson *et al.* 2006) are reasonably to be expected for the founding population, although this may subsequently have been eroded due to lack of selection pressure in the c.120 years since its arrival. Conversely, *S. maritima* had no exposure to *P. marginata* prior to the planthoppers’ arrival, presumed to be sometime between 2000 and 2008 (Kirby 2000; Wilson & Muhlerthaler 2009) and there are no known specialist *Spartina* herbivores native to Britain (Payne 1973; Jackson *et al.* 1985). *S. maritima* is therefore expected to display a lack of tolerance to *P. marginata* consistent with that reported for naive congeners (Wu *et al.* 1999). Whilst having no direct prior exposure to *P. marginata*, British populations of both *S. x townsendii* and *S. anglica* may be considered to have intermediate levels of co-evolutionary history with the herbivore in terms of the genetic material contributed by the respective parental species (Raybould *et al.* 1991b; Figure 1.6). A test of the relative vulnerability of all four British *Spartina* spp. to *P. marginata* would build on the work reported in Chapter 3 to shed further light on the consistency with which increased vulnerability to *P. marginata* is found in naive and exiled *Spartina* populations. It would additionally contribute to understanding of the degree to which

Spartina vulnerability may be linked to the level of genetic co-evolutionary history with its specialist herbivore and the extent to which tolerance may be lost, evolved, or re-evolved in such populations.

The aim of the work reported in this chapter is to investigate the tolerance of the four species of *Spartina* present in Britain to herbivory by *P. marginata*. I aim to assess whether exposure to *P. marginata* has a deleterious impact on all four species of *Spartina* and, if so, to determine if there is any link between the level of impact and the extent of the co-evolutionary history between the grass species and the planthopper. It is hypothesised that:

- i) Exposure to *P. marginata* feeding and oviposition will have a deleterious impact on all four species of *Spartina*;
- ii) The level of impact will be least severe for *S. alterniflora*, the species with which *P. marginata* shares the longest co-evolutionary history;
- iii) The level of impact will be most severe for *S. maritima*, the species which is presumed to have evolved in the absence of any specialist herbivores;
- iv) The level of impact for *S. anglica* and *S. x townsendii* will be intermediate between that for *S. alterniflora* and *S. maritima*; and
- v) The level of impact will be the same for the two intermediate species given that *S. anglica* originated from a chromosomal doubling of *S. x townsendii* without the introduction of any additional genetic material.

4.2 Materials and methods

4.2.1 Establishing *Spartina* spp. cultures

Spartina spp. source populations were identified at the following sites along the south coast of England (Figure 4.1; see Chapter 2 for description of sites):

S. alterniflora – Hythe *Spartina* Marsh (Grid ref: SU 433070);

S. anglica – Pagham Harbour (Grid ref: SZ 862972);

S. maritima – Hayling Island (Grid ref: SU 724042); and

S. x townsendii – Beaulieu Estate (Grid ref: SZ 425976).

Collection sites were determined by the population distribution of each species, *S. anglica* being the only species with wide distribution throughout the study area.

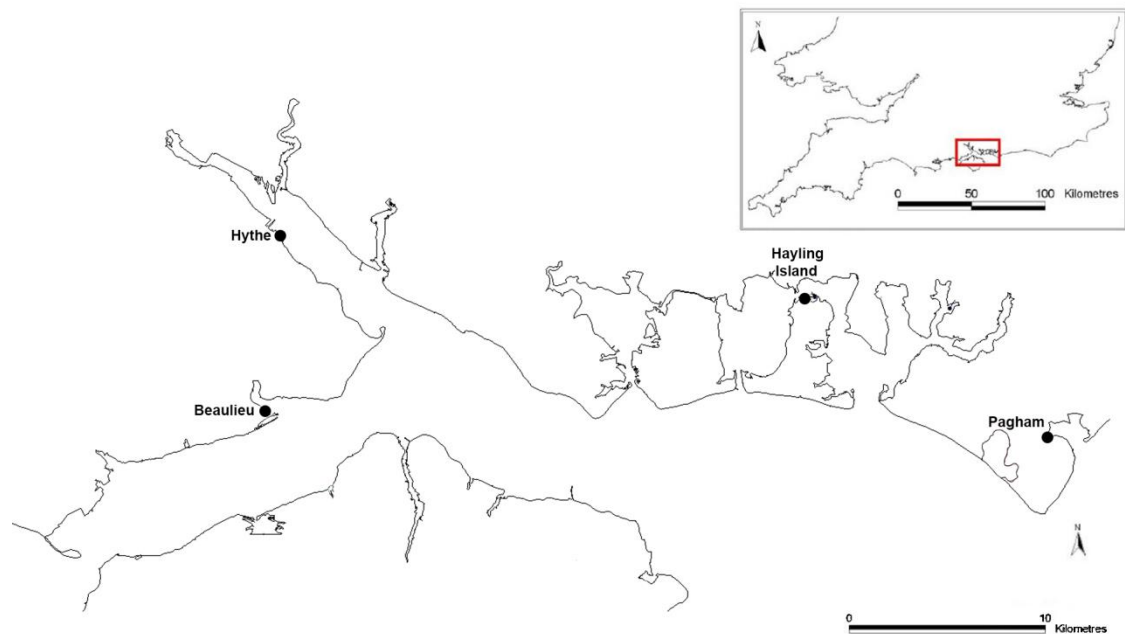


Figure 4.1 Location of field sites from which *Spartina* spp. stock material was collected. Adapted from Foster *et al.* (2014) by W. Harkin.

Root material was manually extracted for each species and returned to the University of Sussex. Mud was removed from the roots by washing in fresh water, and all above-ground biomass was discarded. Sections of rhizome approximately 12cm long and containing at least one node were carefully separated and planted in 10cm diameter pots containing horticultural grade silver sand. Pots were arranged in trays (14 pots per tray) and watered *ad libitum* with fresh water. This method follows Daehler and Strong (1994) in which *S. alterniflora* was shown to grow ‘vigorously’ in fully fresh water. Pots were kept continually wet but not inundated, following Denno *et al.* (2000). One litre of 100% Hoagland nutrient solution (Hoagland & Arnon 1950) was added to each tray

fortnightly. Trays were kept in a glasshouse in which the temperature was maintained within the range 15-30°C with supplementary lighting (100W Supanova LED grow lights, 8:2 light ratio comprising 660nm Red and 430nm Blue) on an 18:6hr light:dark regime. After approximately one month, all plants showing signs of growth were re-potted in 15cm diameter pots. Conditions were maintained as before. Cultures were supplemented with further field collections as required. *S. maritima* was found to be absent from Hayling Island by 2014; a new collection site was identified at Wallasea Island in Essex (Grid ref: TQ 925946) and material extracted from here was added to the culture.

4.2.2 Calculating *Spartina* spp. biomass reference measurements

A method was required to assess the effect of herbivory on plant biomass as a measure of plant performance. It is impossible, however, to directly measure plant biomass non-destructively (Evans 1972). In order to compare biomass change during the course of the experiment, reference plants were therefore used to determine a correlation between biomass and leaf length as a reliable but non-destructive measure (Nixon & Oviatt 1973; Gonzalez Trilla *et al.* 2013). Fifteen plants were removed from each species culture (with the exception of *S. anglica*) and a minimum of 75 leaves were measured from leaf axil to tip using a ruler. Each leaf was separately bagged, labelled and dried for 72 hours at 70°C in a Gallenkamp OV-420 drying oven. Leaves were then weighed to an accuracy of 0.1mg using a Precisa 125A balance. *S. anglica* reference measurements had previously been established as described in 3.2.2. The linear regression of leaf biomass on leaf length was used to estimate leaf biomass non-destructively in all subsequent experiments.

4.2.3 *Prokelisia marginata* impact on four *Spartina* spp.

Twenty potted plants of each species were placed in individual trays. Equal numbers of individual plants from each species were randomly assigned to either *Prokelisia* or control (no *Prokelisia*) treatments and then labelled. Plants were randomly assigned to a position within the experimental arena which extended along one side of the glasshouse in a 3x20-pot grid. Starting metrics were recorded for each plant: number of leaves; number of tillers; overall height of plant and length of each leaf. Starting leaf biomass was inferred from leaf length using the equations described in 4.3.1.

Plants allocated to the *Prokelisia* treatment were inoculated with 30 second-to-third instar *P. marginata* nymphs from the culture described in 3.2.3. Plants allocated to the control group did not receive any nymphs. Transparent cylindrical cages were

constructed from 175µm PET polyester film. Cages were 13cm in diameter and 50cm tall with a 5x7cm² nylon gauze-covered ventilation window positioned 18cm from the base of the cage and a gauze lid. Each plant was enclosed by a cage, the base of which was embedded approximately one centimetre below the surface of the sand. Plants were arranged in their designated position within the glasshouse (Figure 4.2) and watered *ad libitum* with fresh water; 100ml 100% Hoagland nutrient solution was added to each tray fortnightly. The experiment ran for eight weeks with glasshouse conditions maintained as described in 4.2.1.

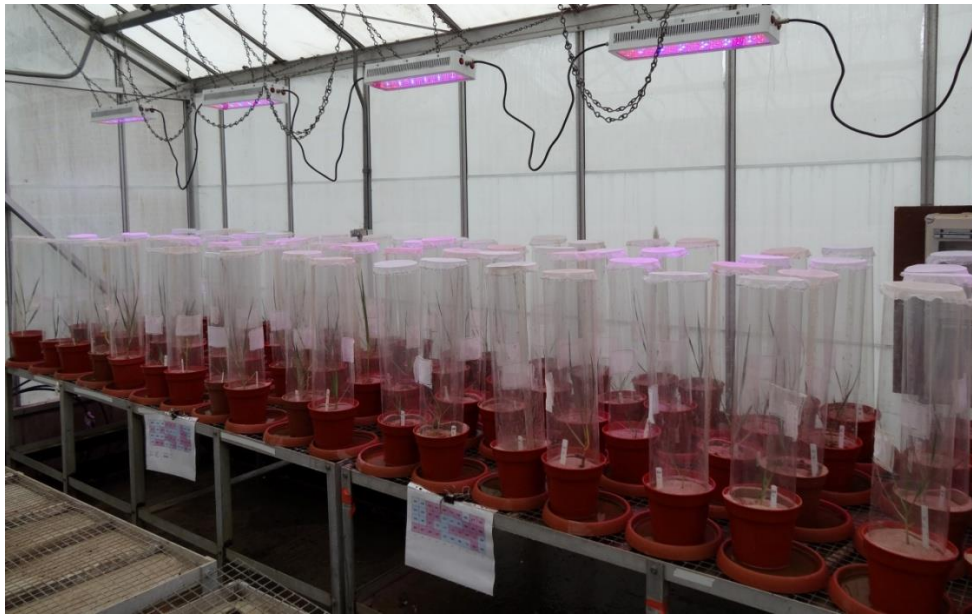


Figure 4.2 Experimental set up

At the end of the experimental period, each replicate was placed inside a 1m³ Perspex box with a small access door. The cage was lifted off the pot and all *P. marginata* adults and nymphs were individually removed using an aspirator and counted. Overall plant height, total length of all leaves, number of leaves and number of tillers were recorded. Each plant was removed from its pot, all sand was carefully washed from the roots, and plants were then divided into above- and below-ground material. Each component was then weighed to an accuracy of 0.01g using a Precisa 125A balance, dried for 72 hours at 70°C in a Gallenkamp OV-420 drying oven and finally re-weighed to establish both fresh and dry biomass measurements.

4.2.4 Statistical analysis

Statistical analysis was performed in R version 3.1.3 (R Core Team 2015) using the *sciplot* (Morales & R Development Core Team 2012) package. Assumptions about the distribution and variance of the response data (see Quinn & Keough 2002) were tested

prior to analysis. Shapiro Wilk tests were used to test for normality of residuals; Fligner-Killeen tests were used to test for constancy of variance, as recommended by Crawley (2013). In cases where these assumptions were not met for continuous variables, square root transformations (height and length measures), and log transformations (root:shoot ratio and root biomass measures) were successful in normalising the data. Appropriate models were determined by the nature of the response variables. Where these were continuous, ANOVA was initially used with plant change as the response variable and treatment, species and their interaction as explanatory variables. Plant change was analysed using relative growth measures (i.e. growth during the experimental period divided by the starting measurement) rather than absolute value in order to standardise for unavoidable variation between replicates at the beginning of the experiment. Root:shoot ratios and root biomass were calculated at the individual plant level using final measures only as starting measurements could not be obtained non-destructively. Relative water content was calculated as a percentage of dry biomass.

Count data were analysed using generalized linear models (GLMs) with a Poisson error structure and log link function. Plant change was used as the response variable, treatment, species and their interaction as explanatory variables. In all cases, analyses compared models with and without treatment as an explanatory variable to determine whether its removal led to a significant ($p < 0.05$) increase in deviance. For GLMs this was assessed using χ^2 tests. Significance levels are reported for the removal of significant terms from the minimum adequate model. Plots of model residuals against fitted values were visually inspected for normal distribution, homogeneity of variance and the presence of influential outliers following procedures outlined in Crawley (2013). In cases where the interaction between species and treatment was found to be significant, interaction plots were used to visualise the level of effect.

4.3 Results

4.3.1 Calculation of *Spartina* spp. biomass reference measurements

Individual linear regressions applied to the data described in 4.2.2 revealed a strong correlation between leaf length and biomass for each species:

S. alterniflora: biomass (g) = $0.0008737 \times \text{leaf length (mm)}$
(n=121, $r^2=0.932$, $p<0.001$).

S. maritima: biomass (g) = $0.0002432 \times \text{leaf length (mm)}$
(n=76, $r^2=0.892$, $p<0.001$).

S. x townsendii: biomass (g) = $0.0006128 \times \text{leaf length (mm)}$
(n=90, $r^2=0.9685$, $p<0.001$).

S. anglica reference measurements had previously been established in 3.3.1 as:
biomass (g) = $0.0009736 \times \text{leaf length (mm)}$
(n=150, $r^2=0.9637$, $p<0.001$).

These equations were applied in subsequent analyses to estimate the starting biomass of experimental plants with known leaf length measurements.

4.3.2 *Prokelisia marginata* impact on four *Spartina* spp.

Two-way ANOVAs revealed significant interactions between exposure to *P. marginata* and *Spartina* species for a range of plant performance metrics. Interactions are said to exist when the response to one factor depends upon the level of another factor (Dodge 2008). Negative impacts of these interactions on plant performance were seen for height, overall leaf length and above ground biomass relative growth rates, along with relative above ground water content (Table 4.1; Figure 4.3).

Table 4.1 Effect of *P. marginata* exposure, *Spartina* species and their interactions on plant performance measured as: i) height; ii) total leaf length; and iii) shoot biomass relative growth rates; and iv) relative shoot water content. Relative metrics indicate ratios of post- to pre-experiment measurements. Control refers to no *P. marginata*; Treatment refers to 30 *P. marginata* individuals added. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

	d.f.	Control mean (C)	Treatment mean (T)	T as % of C	F	p	
i)							
Relative height gain							
Treatment	1				58.74	<0.001	***
Species	3				30.33	<0.001	***
<i>S. alterniflora</i>		0.46	0.33	72.5			
<i>S. anglica</i>		0.29	0.06	20.2			
<i>S. maritima</i>		0.94	0.48	50.4			
<i>S. x townsendii</i>		0.59	0.14	23.4			
Treatment x Species	3				3.08	0.033	*
ii)							
Relative leaf length gain							
Treatment	1				100.88	<0.001	***
Species	3				12.21	<0.001	***
<i>S. alterniflora</i>		1.69	1.08	63.8			
<i>S. anglica</i>		1.16	0.60	51.2			
<i>S. maritima</i>		2.59	0.91	35.0			
<i>S. x townsendii</i>		1.96	0.89	45.3			
Treatment x Species	3				4.31	0.007	**
iii)							
Relative biomass gain							
Treatment	1				40.17	<0.001	***
Species	3				98.22	<0.001	***
<i>S. alterniflora</i>		1.05	0.60	57.0			
<i>S. anglica</i>		0.74	0.26	34.5			
<i>S. maritima</i>		5.30	2.71	51.2			
<i>S. x townsendii</i>		1.19	0.50	41.7			
Treatment x Species	3				9.58	<0.001	***
iv)							
Relative water content							
Treatment	1				303.24	<0.001	***
Species	3				5.96	0.001	***
<i>S. alterniflora</i>		1.83	1.02	55.8			
<i>S. anglica</i>		1.63	0.89	54.6			
<i>S. maritima</i>		1.84	0.56	30.4			
<i>S. x townsendii</i>		1.61	0.59	36.2			
Treatment x Species	3				4.85	0.004	**
Residuals (all models)	72						

A significant interaction effect means that it is not possible to conduct any post-hoc tests to make further statistical comparisons between variables (Crawley 2013). Interaction plots can be used, however, to visualise the impact of interactions between independent variables on resulting performance metrics. Interactions are interpreted from differences in the gradient of plotted trace lines i.e. if lines are not parallel there is an effect of the interaction. Visual inspection of interaction plots for the four functional traits to which this applies indicates that the impact of the interaction between *P. marginata* exposure and plant species was least for *S. alterniflora* (i.e. the trace line had the shallowest gradient). In contrast, the impact on each metric was notably greater for *S. x townsendii* and *S. anglica* (i.e. the trace shows a steeper gradient) and for *S. maritima*. The relative severity of impact, however, varied between *S. maritima*, *S. x townsendii* and *S. anglica* according to functional trait. For some performance metrics (leaf length RGR and relative shoot water content) the treatment effect on *S. maritima* is the strongest of all the species, whereas for other metrics (relative height and biomass gain) it is weaker only than that for *S. alterniflora* (Figure 4.3). Comparisons of mean outcomes for control and treatment groups in each metric are consistent with this result (Table 4.1).

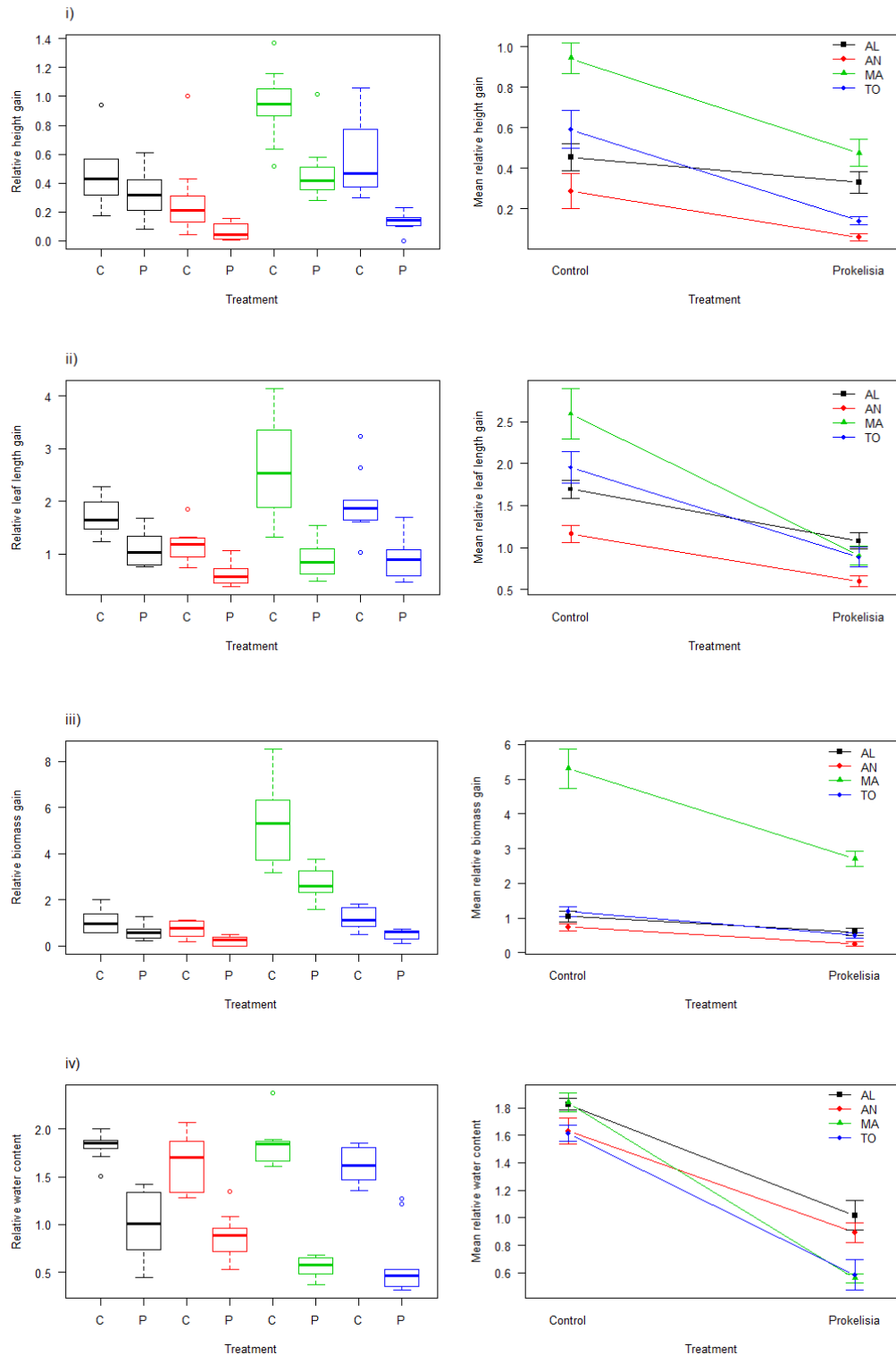


Figure 4.3 Effect of *P. marginata* exposure on *S. alterniflora* (AL), *S. anglica* (AN), *S. maritima* (MA) and *S. x townsendii* (TO) performance measured as i) height; ii) total leaf length; and iii) biomass relative growth rates; and iv) relative shoot water content. Treatment refers to C - control (no *P. marginata*) and P - *Prokelisia* (30 individuals added) treatments. Boxes (left hand column) show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present. Interaction plots (right hand column) show means ± 1 S.D. Plots show untransformed data. Relative metrics indicate ratios of post- to pre-experiment measurements.

A significant overall negative treatment effect was found on the number of new leaves and the number of new tillers gained, the root:shoot ratio and the mean root biomass of experimental plants. However, the interaction between treatment and species was not significant.

The individual effect of species was significant for root:shoot ratio and mean root biomass, but not for leaf or tiller gain (Table 4.2; Figure 4.4). This contrast is unsurprising as the first two measures reflect absolute differences between species, whereas the final two are reflective of performance differences throughout the duration of the experiment relative to the starting position.

Whilst the interaction between species and treatment was not found to be significant for these four measures, *S. alterniflora* treatment plants continued to display the highest mean performance level of any species in comparison to its control group (Table 4.2; Figure 4.4). Post-hoc tests examining root:shoot ratio and mean root biomass found no significant difference between *S. alterniflora* and *S. anglica*, or between *S. maritima* and *S. x townsendii*, but significant differences between the two groupings ($p < 0.001$). The lack of a significant interaction, however, indicates that this result is reflective of absolute differences between the species rather than differential responses to *P. marginata* herbivory.

Table 4.2 Effect of *P. marginata* treatment, *Spartina* species and their interaction on plant performance measured as i) new leaf gain; ii) new tiller gain; iii) root:shoot ratio; and iv) mean root biomass. Control refers to no *P. marginata*; Treatment refers to 30 *P. marginata* individuals added. Test statistics are F values for two-way ANOVAs and χ^2 for GLMs. Significance levels indicated by: *** ≤ 0.001

	d.f.	Control mean (C)	Treatment mean (T)	T as % of C	χ^2	F	p
i)							
No. new leaves gained							
Treatment	1				78.37		<0.001 ***
Species	3				6.27		0.099
<i>S. alterniflora</i>		8.20	4.50	54.9			
<i>S. anglica</i>		7.50	2.70	36.0			
<i>S. maritima</i>		7.90	2.70	34.2			
<i>S. x townsendii</i>		9.30	4.10	44.1			
Treatment x Species	3				3.44		0.329
ii)							
No. new tillers gained							
Treatment	1				33.83		<0.001 ***
Species	3				7.63		0.054
<i>S. alterniflora</i>		2.30	0.80	34.8			
<i>S. anglica</i>		2.40	0.40	16.7			
<i>S. maritima</i>		1.30	0.40	30.8			
<i>S. x townsendii</i>		1.30	0.30	23.1			
Treatment x Species	3				1.35		0.717
iii)							
Root:Shoot ratio							
Treatment	1					43.68	<0.001 ***
Species	3					17.76	<0.001 ***
<i>S. alterniflora</i>		2.84	1.87	65.8			
<i>S. anglica</i>		2.76	1.77	64.0			
<i>S. maritima</i>		1.68	0.89	52.8			
<i>S. x townsendii</i>		1.53	0.85	55.6			
Treatment x Species	3					0.41	0.744
iv)							
Mean root biomass (g)							
Treatment	1					68.61	<0.001 ***
Species	3					61.12	<0.001 ***
<i>S. alterniflora</i>		2.99	1.97	65.9			
<i>S. anglica</i>		3.04	1.30	42.6			
<i>S. maritima</i>		0.87	0.32	36.9			
<i>S. x townsendii</i>		0.84	0.39	46.7			
Treatment x Species	3					1.13	0.342
Residuals (all models)	72						

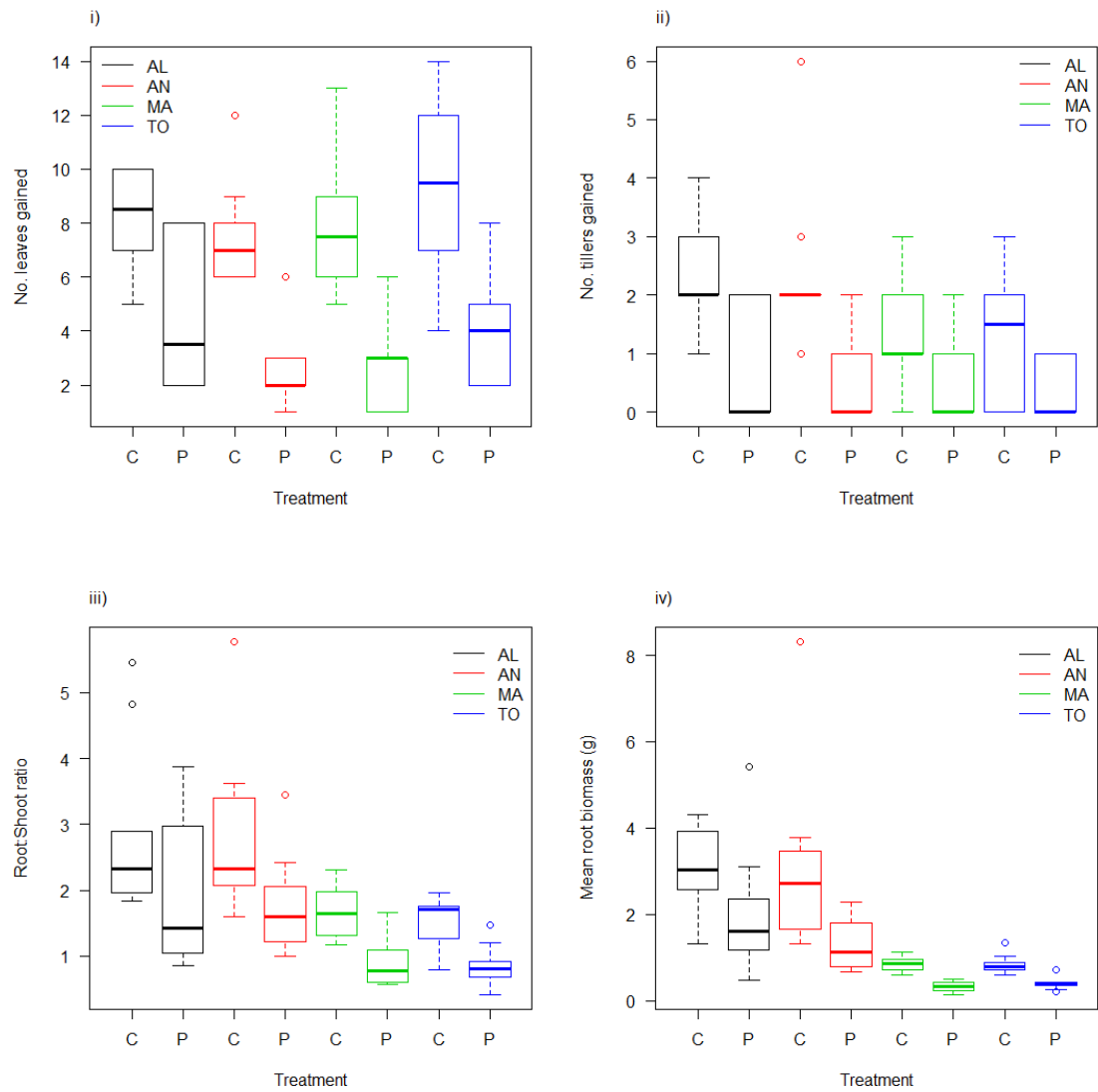


Figure 4.4 Effect of *P. marginata* on *S. alterniflora* (AL), *S. anglica* (AN), *S. maritima* (MA) and *S. x townsendii* (TO) performance measured as i) number of new leaves and ii) number of new tillers gained, iii) root:shoot ratio and iv) mean root biomass. Treatment refers to C - control (no *P. marginata*) and P - *Prokelisia* (30 starting individuals) treatments. Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present.

4.4 Discussion

Previous studies have examined the impact of *P. marginata* exposure only on *S. alterniflora*, its native host, and *S. anglica* populations that were introduced to Puget Sound, Washington, in 1961. Long-separated (*S. alterniflora*) and naive (*S. anglica*) populations have been found to suffer significant detrimental impact when exposed to the herbivore (Daehler & Strong 1997b; Wu *et al.* 1999; Garcia-Rossi *et al.* 2003; Grevstad *et al.* 2003). The work reported in this chapter extends this investigation to include two further species of *Spartina*, *S. x townsendii* and *S. maritima*, for the first time. As predicted, exposure to *P. marginata* herbivory and oviposition had a significant negative impact on all four species of *Spartina* for all performance metrics.

Interactions are said to exist when a change in the level of one factor (i.e. exposure to *P. marginata*) has different effects on the response variable, depending on the value of the other factor (i.e. the different species of *Spartina*) (Dodge 2008). The impact of the interaction between plant species and planthopper exposure was found to be significant for four of the traits measured: relative height, leaf length and biomass growth rates, and relative water content. These traits are reflective of plants' compensatory growth and photosynthetic potential (Strong *et al.* 1984; Zhang *et al.* 2012); the significant interaction is therefore indicative of differential tolerances of the four species to *P. marginata* exposure (Maschinski & Whitham 1989).

The gradients of trace lines on the interaction plots for these four metrics show the impact of *P. marginata* exposure to be the least severe for *S. alterniflora*. This is consistent with the stated hypothesis that the plant species sharing the longest co-evolutionary history with the planthopper would display the greatest level of tolerance to it. In contrast to non-exiled populations which are not significantly affected by *P. marginata* herbivory (Daehler & Strong 1995; Gustafson *et al.* 2006), however, the species did suffer significant negative effects of exposure. These findings go some way to supporting the conclusions drawn by other studies (Wu *et al.* 1999; Qing *et al.* 2012) that defence against *P. marginata* is ecologically costly to maintain in the absence of selection pressure by the herbivore, and that resources may be directed to other fitness-related traits, consistent with the EICA hypothesis (Blossey & Notzold 1995). Daehler and Strong (1997b) found significantly reduced tolerance of *S. alterniflora* after ~100 years of separation from *P. marginata*. British populations of the grass are likely to have been separated from the herbivore for approximately twice that period (Thompson 1991), yet *S. alterniflora* remained the least severely impacted of all four species examined. It is possible that the post-introduction evolutionary response of

British *S. alterniflora* to enemy escape has not been as rapid, or as strong, as that of North American exiles, with a considerable level of tolerance still maintained in the population. Wu *et al.* (1999) hypothesised that the selective forces which may result in an evolutionary trade-off of defensive traits in favour of interspecific competitive ability were likely to be of minimal importance to *Spartina* spp. which, in North America, live primarily in monospecific stands. This argument is less persuasive for British populations of the grass, however, which more frequently occur as one element of a multi-species matrix with other halophytes (Gray *et al.* 1991; Dargie 2000; Boorman 2003; Huckle *et al.* 2004; and see section 2.2.1). An alternative explanation for the results presented in this chapter is that British populations of *S. alterniflora* are already displaying a rapid re-evolution of defensive traits in response to selection pressures imposed by the recent arrival of *P. marginata* (Kirby 2000; Wilson & Muhlerthaler 2009). If so, this would add support to concerns raised by Garcia-Rossi *et al.* (2003) regarding the potentially “self-defeating” use of the planthopper as a biological control agent of the grass. Evolved loss of susceptibility to chemical pesticides is a well-studied issue of growing concern to applied ecology (Gould 1991; Harker 2013); conversely, acquired resistance and tolerance as a result of selection by herbivorous biological control agents is reported to be an exceedingly rare event (Holt & Hochberg 1997). The very few documented cases where this has occurred (Burdon *et al.* 1981), however, have led some authors to suggest that such evolved defences represent clear and emerging threats to the ongoing success of biological control programmes (Goldson *et al.* 2014). Whilst deliberate use of *P. marginata* as a biological control agent is not currently a consideration in Britain, further studies of the possible mechanisms influencing the results for *S. alterniflora* reported in this chapter are warranted as a topic of interest to the fields of evolutionary ecology, invasion biology and biological control (Holt & Hochberg 1997; Garcia-Rossi *et al.* 2003; Muller-Scharer *et al.* 2004; Goldson *et al.* 2014).

It was hypothesised that the negative effect of *P. marginata* exposure would be the same for *S. x townsendii* and *S. anglica* as the latter arose from a chromosome doubling of the former, consequently sharing the same level of “genetic co-evolutionary history” with the planthopper. The results do not provide compelling support for this argument. Two possible mechanisms may offer some explanation for this disparity. Firstly, it is possible that divergent evolutionary changes have occurred between the two species, either due to selective adaptation, or random evolutionary processes such as genetic drift (Schluter 2001). *S. anglica* was formed as a separate species c.120 years ago (Marchant 1967; Gray *et al.* 1991), with contemporary studies showing that

significant adaptive evolution can occur in a range of species within twenty or fewer generations (Muller-Scharer *et al.* 2004; Prentis *et al.* 2008). There is very little inter-individual genetic variation in *S. anglica* populations (Ayres & Strong 2001; Baumel *et al.* 2002b), consistent with a severe genetic bottleneck due to the rare, or even unique, occurrence which resulted in the origin of the species (Baumel *et al.* 2001), and very little structural change has been observed in the genomes of either *S. x townsendii* or *S. anglica* (Baumel *et al.* 2002a). However, considerable epigenetic alteration (Salmon *et al.* 2005) as well as changes to the transcriptome (Chelaifa *et al.* 2010) arose from both the hybridization event that led to the formation of *S. x townsendii*, and in the subsequent chromosome doubling which resulted in *S. anglica*. These epigenetic and regulatory changes are thought to account for the high levels of phenotypic plasticity documented for *S. anglica* (Renny-Byfield *et al.* 2010), and may similarly offer an explanation for the differential tolerance response to *P. marginata* evidenced for the two species in this chapter.

An alternative explanation for these results could be the difference in ploidy levels between *S. anglica* (allododecaploid) and *S. x townsendii* (hexaploid) (Ainouche *et al.* 2004). Polyploids occur with greater frequency amongst invasive plants than among angiosperms in general (Pandit *et al.* 2006; Prentis *et al.* 2008); for species that have multiple ploidy levels in the native range, invasive populations are frequently composed entirely of individuals with the higher ploidy level (Hollingsworth & Bailey 2000). Reasons for this are currently unresolved; however it is possible that there may be fitness differences between populations with different ploidy levels in some species complexes (Flegrova & Krahulec 1999; Soltis & Soltis 2000; Prentis *et al.* 2008). Ploidy level has also been shown to have a significant impact on plant-animal interactions (Thompson *et al.* 2004; Halverson *et al.* 2008; Hull-Sanders *et al.* 2009), with herbivores found to exert differential damage and selection pressure on conspecifics with different ploidy levels (Munzbergova 2006). Approximately 70% of flowering plants are thought to be descended from polyploid ancestors (Masterson 1994), however the effects of polyploidy on plant-animal interactions remains relatively unexplored (Thompson *et al.* 2004; Munzbergova 2006). The recent association of *P. marginata* with the multi-ploidy *Spartina* species complex in Britain offers a valuable opportunity for further research in this area with relevance to both evolutionary ecology and an understanding of how these interactions may influence the outcome of invasion dynamics.

It was predicted that *S. maritima* would suffer the greatest detrimental impact of exposure to *P. marginata* herbivory and oviposition as it shares no co-evolutionary history with the planthopper, nor with any other specialist herbivore (Payne 1973; Jackson *et al.* 1985). Whilst *S. maritima* did suffer a significant negative impact of exposure in all performance metrics, the relative impact in comparison to the other *Spartina* species is less clear. For some performance metrics (leaf length RGR and relative shoot water content) the treatment effect on *S. maritima* is the strongest of all the species, whereas for other metrics (relative height and biomass gain) it is weaker only than that for *S. alterniflora*. It is interesting to note that *S. maritima* plants in the control group performed substantially better than the control group for any other species, displaying the greatest relative height, leaf length and biomass mean growth rates, and the highest relative shoot water content. In its current British distribution, *S. maritima* is extremely localised, sparsely populated and routinely out-competed by *S. anglica* (Lacambra *et al.* 2004); in this experiment, each plant was placed individually in its own pot and hence freed from competition. In future experiments, it would be interesting to examine how *S. maritima* fares in the presence of both *S. anglica* and *P. marginata*, examining the relative impacts of competition and herbivory and the interaction between the two. Even in the absence of other biotic stressors, *P. marginata* was found to have a significant negative impact on the performance of all *Spartina* species across all functional traits. *S. maritima* is listed as a species “of principal importance for the purpose of conserving biodiversity” under section 41 of the Natural Environment and Rural Communities Act 2006 and a priority species under the UK Biodiversity Action Plan (Joint Nature Conservation Committee 2007). Dramatic declines in its range have previously been documented (Raybould *et al.* 1991a), with a further local extirpation at Hayling Island potentially occurring during the course of this research. *P. marginata* is currently in the very early stages of invasion in the UK (section 2.4.1; 2.4.3). The results presented in this chapter, however, suggest that its continued population growth and spread may pose a very real threat to the ongoing survival of *S. maritima* across its remaining British distribution.

The expression, and adaptive potential, of a species’ functional traits is constrained by its phylogenetic history, however it has proved difficult to quantify the strength of such constraints, and the extent to which they inform observed differences, when conducting interspecific comparative analyses (Cheverud *et al.* 1985; Agrawal & Fishbein 2008). More recent statistical approaches allow for inclusion of phylogenetic distance as a factor within analytical models (Beckmann *et al.* 2015; Paradis 2016). It would be useful to examine the extent to which phylogenetic distance may inform the differential

response of the four *Spartina* species to exposure to *P. marginata* in any future analyses.

Chapter five

***Prokelisia marginata* choice between *Spartina* species and associated impacts on *P. marginata* performance**

5.1 Introduction

5.1.1 Host plant specialisation

5.1.1.1 Host specificity in phytophagous insects

Approximately 90% of all phytophagous insects exhibit highly specialised diet preferences, feeding on a single plant species or a small number of closely-related hosts (Futuyma & Gould 1979; Strong *et al.* 1984; Bernays & Graham 1988), although distribution of diet breadth varies geographically, with a higher frequency of specialists in the tropics (Forister *et al.* 2015). It has been suggested that such specialisation may be explained by two, mutually non-exclusive, mechanisms: firstly, that adaptations to overcome the wide range of constitutive and inducible host plant defences (Nunez-Farfan *et al.* 2007) are too costly for generalist herbivores to maintain (Rausher 1992); or secondly, that variable fitness outcomes achieved on different host species results in selection for behavioural genotypes that restrict feeding to the best available hosts (Prokopy *et al.* 1988; Rausher 2001; Agosta 2006). Selection for such genotypes consequently acts to increase selection for physiological adaptation to the specific host (Fry 1996; Reznick & Ghalambor 2001). Even if they are able to feed on them, specialist herbivores are expected to display reduced fitness on non-host plants (Rausher 2001), further strengthening the specialist host-herbivore association (Carroll *et al.* 1997).

5.1.1.2 Host specificity in planthoppers

Host plant specialisation is widespread in the Delphacidae. Polyphagy is reported for a small proportion of delphacids (Prestidge & McNeill 1983), in some cases having resulted from the range expansion of introduced host species (Metcalf 1969), however mono- or stenophagy occurs with much greater frequency (Sogawa 1982; Denno & Roderick 1990). Plant defences are thought to play a less substantial role in shaping the interactions between host plants and planthoppers in comparison with insects from other feeding guilds, as phloem-feeders are believed to avoid many toxic allelochemicals (Sogawa 1982; Denno & Roderick 1990; but see also Denno *et al.* 2000; Long *et al.* 2011). Conversely, host plant nutrition, and in particular, available nitrogen, is understood to play a significant role in the shaping of planthopper-host

plant relationships, life history patterns, population dynamics and community structure (Denno 1983; Denno 1985; Denno *et al.* 1985; Denno & Roderick 1990; Denno & Peterson 2000; Denno *et al.* 2002). Empirical evidence suggests that adaptation to novel host species may be heavily constrained in some planthopper species. Selection experiments in *Ribautodelphax* spp. point to the existence of a strong genetic correlation between oviposition preference and nymphal performance for this complex (den Bieman 1987), expected to render host shifting more difficult and less likely to occur (Thompson 1988). Notable exceptions do exist. The brown planthopper, *Nilaparvata lugens*, is one of the most virulent pests of rice crops (Sogawa 1982; Gallagher *et al.* 1994) and has demonstrated the ability to adapt to novel “resistant” rice cultivars (or “varieties”) within as little as ten generations (Claridge & den Hollander 1982; Pathak & Heinrichs 1982). Studies, however, suggest this virulence is under polygenic control (den Hollander & Pathak 1981; Claridge *et al.* 1982), whilst for many other planthopper species a gene-for-gene relationship is thought to exist between host resistance and planthopper virulence (den Bieman 1987). Denno and Roderick (1990) therefore argue that, whilst the potential for rapid adaption to host varieties exists amongst the Delphacidae, the step to include a novel species in the diet is much more difficult and less likely to occur.

5.1.1.3 Host specificity in *Prokelisia marginata*

P. marginata is stenophagous. Across its entire North American range, it has been reported to feed only on *S. alterniflora*, *S. foliosa* and their hybrids, and the introduced *S. anglica*, whilst avoiding the sympatrically-occurring *S. patens*, *S. cynosuroides* and *S. bakeri* (Denno *et al.* 1996). Grevstad *et al.* (2003) examined the host-switching potential of *P. marginata* prior to a trial of its use as a biological control agent of *S. alterniflora*. No-choice host specificity tests for oviposition, nymph and adult survival were conducted on more than twenty plant species, including *Spartina* spp., closely- and distantly-related monocotyledons and one dicotyledonous species. *P. marginata* consistently achieved the greatest survival rates on *S. alterniflora*, and was unable to complete a full life cycle on any species other than the three previously recorded *Spartina* spp. hosts. The authors concluded that the likelihood of *P. marginata* switching to any other untested plant species in the new range was extremely remote (Grevstad *et al.* 2003). Across its introduced continental European range, *P. marginata* has been recorded primarily on *S. anglica* or *S. x townsendii* (de Blauwe 2011), with a Slovenian population recorded on *S. maritima* (Seljak 2004). Prior to the work presented in this thesis, *S. anglica* was the only recorded host for *P. marginata* in its British range (Ouvrard & Soulier-Perkins 2012; Badmin 2013).

Molecular studies of nuclear and chloroplast DNA sequences of the *Spartina* genus have identified the evolution of two distinct clades (Ainouche *et al.* 2004). Clade II has diverged from a common tetraploid ancestor and includes *S. patens*, *S. cynosuroides* and *S. bakeri*, all of which *P. marginata* avoids under natural field conditions (Denno *et al.* 1996), as well as *S. gracilus* and *S. pectinata* (Baumel *et al.* 2002b). *P. marginata* was unable to complete a full life cycle on either of the two latter species in no-choice host specificity tests (Grevstad *et al.* 2003). Clade I has diverged from a common hexaploid ancestor and includes *S. alterniflora*, *S. foliosa*, *S. maritima* and their hybrid descendents, including *S. x townsendii* and *S. anglica*. Within Clade I, *S. alterniflora* and *S. foliosa* have been shown to be closely-related sister species, differing by only four nucleotide changes in the 1981 base pairs that have been sequenced (Baumel *et al.* 2002b). This minimal genetic distance, combined with the propensity for the species to readily hybridise and for the resulting offspring to backcross with the parents in both directions following *S. alterniflora*'s recent introduction to *S. foliosa*'s natural Pacific coast range (Daehler & Strong 1997a), has raised questions regarding the taxonomic distinction between the two species (Baumel *et al.* 2002b). Conversely, despite occupying the same clade, *S. maritima* and *S. alterniflora* contain two well-differentiated and divergent genomes (Baumel *et al.* 2001). In addition to differences in their coding sequence, the two species have been shown to diverge substantially at the transcriptome level. These regulatory changes are consistent with morphological and ecological differences between the species, with the majority of differentially expressed genes being over-expressed in *S. alterniflora*, and down-regulated in *S. maritima* (Chelaifa *et al.* 2010). This extended genetic and transcriptomic differentiation between *S. alterniflora* and *S. maritima* adds weight to the suggestion raised in 4.2.3 of a "gradient" of shared co-evolutionary relationships between *P. marginata* and the four *Spartina* species present in Britain. *S. alterniflora* is *P. marginata*'s native-range host, the species with which it has shared the longest co-evolutionary history, and on which it has been shown to achieve the greatest rates of survival (Denno *et al.* 1986; Grevstad *et al.* 2003). *S. maritima* is the most genetically distant from the native host species (Baumel *et al.* 2002b; Chelaifa *et al.* 2010), with *S. x townsendii* and *S. anglica* occupying an intermediate position in terms of the genetic material contributed by the respective parental species (Raybould *et al.* 1991b; Figure 1.6). *P. marginata* has shown some ability to host shift, evidenced by the adoption of *S. maritima* as its host plant in Slovenia (Seljak 2004; Ouvrard & Soulier-Perkins 2012). This ability appears to be somewhat constrained however, as it has been unable to successfully utilise any hosts outside of Clade I (Denno *et al.* 1996; Baumel *et al.* 2002b; Grevstad *et al.* 2003).

It is possible therefore, that detrimental performance impacts may be realised on *S. maritima*, the most genetically distinct species within the clade.

5.1.3 Morphology and fitness trade-offs in *Prokelisia marginata*: host plant influence

5.1.3.1 Wing dimorphism

In common with the majority of delphacid species, *P. marginata* exhibits wing dimorphism. Flight-adapted, long-winged macropters, and non-flying brachypters with abbreviated mesothoracic and vestigial metathoracic wings (Figure 1.8), are both present in the majority of populations (Denno *et al.* 1986; Denno *et al.* 1996), although the proportion of each can vary significantly by season, location and between the sexes (McCoy & Rey 1981; Denno & Roderick 1990; and see Chapter 2). Levels of brachyptery in *P. marginata* populations along the Gulf Coast of North America rarely fall below 90%, whereas brachypters only constitute around 20% of North Atlantic coast populations (Denno *et al.* 1980; Denno *et al.* 1989). Wing morphology is determined by a developmental switch that responds to proximate environmental cues such as host plant nutrition, temperature, photoperiod and the extent of habitat stability, with population density being the most influential factor (Denno *et al.* 1985). The sensitivity of the developmental switch is under polygenic control and has been shown to have high heritability, particularly for males (Denno 1994). However, the threshold density that triggers the production of macropters can differ markedly between sexes and populations (Denno & Grissell 1979; Denno *et al.* 1994). Whilst determination of adult wing form is strongly density-dependent in females, with increased crowding resulting in a greater proportion of macropters, macroptery levels in males can remain high even when individuals are raised in isolation (Denno *et al.* 1985; Denno *et al.* 1991).

The existence of wing dimorphism within populations suggests that there are fitness costs associated with the ability to fly (Roff 1984). Macropterous individuals are able to escape the fitness-reducing effects of poor-quality hosts, over-crowding and deteriorating habitats (Denno 1976), abilities which are essential in heterogeneous and transient environments (Novotny 1994; Zera & Denno 1997; Langelotto & Denno 2001). However, the investment of resources necessary to meet the physiological demands of such dispersal capability is associated with significant reproductive costs (Denno *et al.* 1989; Denno & Roderick 1990). Macropterous females experience a reproductive delay in comparison to their brachypterous counterparts, associated with protracted oogenesis and deferred weight gain, sexual maturity and mate receptivity

(Heady & Denno 1991). Brachypters live longer than macropters which, combined with the earlier age of first reproduction, results in greater lifetime realised fecundity. In stable habitats, brachypterous females leave more offspring which grow to a larger size before winter (Denno 1994). Although male macropters also display reduced longevity in comparison to brachypters, the lifetime reproductive cost of flight capability appears to be less for males than for females, perhaps due to their smaller gamete size (Denno *et al.* 1985).

5.1.3.2 Interaction between host plant quality and population density

In addition to the impact on *P. marginata* wing morphology, the interaction between crowding and host plant nutrition has a substantial influence on other important life history traits. In experimental manipulations of host plant nutrition, *P. marginata* females were able to select the most nitrogen-rich hosts on which to feed and oviposit (Denno 1985; Denno *et al.* 1986); a preference which was reflected by macropterous adults of both sexes in field-scale studies (Denno 1983). Nymphs raised on more nutritious host plants eclose as significantly larger adults, which in females is positively correlated with daily fecundity (Denno & McCloud 1985). In contrast, high population density has been shown to reduce survivorship, decrease body size and delay nymphal development, consequently increasing the age of first reproduction and reducing lifetime realised fecundity (Denno & McCloud 1985; Denno *et al.* 1986). Prolonged development time further increases the risk of attack from nymphal parasitoids, mortality from which can reach 80% in the native range (Stiling & Strong 1982a; Denno 1983). Nymphal emergence, development and survivorship are negatively impacted by poor quality or unsuitable host plants (Garcia-Rossi *et al.* 2003; Grevstad *et al.* 2003), whilst conversely, highly nutritious hosts have been shown both to moderate the fitness-reducing impacts of crowding and to suppress the associated trigger for macroptery (Denno *et al.* 1986). The sensitivity of *P. marginata*'s response to the interaction between crowding and host plant suitability therefore suggests that expression of the planthopper's life history traits, and associated performance outcomes, may differ depending on which host plant species is being exploited, and that the level of crowding that triggers macroptery, at least in females, may be similarly influenced.

The aim of the work reported in this chapter is to investigate whether *P. marginata* makes a preferential choice between the four potential *Spartina* host species present in Britain. I further aim to assess whether different host species have a differential impact on *P. marginata* performance and outcomes and, if so, to determine if there is any link between these impacts and the extent of the co-evolutionary relationship between the grass species and the planthopper. It is hypothesised that:

- i) *P. marginata* will make a preferential choice of oviposition host between the four *Spartina* species, displaying a preference for *S. alterniflora*, the species with which it shares the longest co-evolutionary history and on which it achieved the greatest rate of survival in previous no-choice host specificity tests (Grevstad *et al.* 2003);
- ii) Host plant species will have a significant impact on *P. marginata* development times, proportion of individuals that are macropterous, body size and population size;
- iii) Increased crowding levels will have a negative impact on *P. marginata* body size and will result in an increased proportion of females that are macropterous. Impacts will vary significantly according to host plant species;
- iv) *P. marginata* will perform better when raised on *S. alterniflora*, will suffer the greatest negative impact when raised on *S. maritima*, the species which is most distantly related to its native host, and will achieve intermediate performance outcomes when raised on *S. anglica* and *S. x townsendii*.

5.2 Materials and methods

5.2.1 *Prokelisia marginata* oviposition choice in *Spartina* spp. field assay

It was not possible to sample discrete populations of *P. marginata* on all four *Spartina* species under natural field conditions due to the extremely sparse and isolated nature of the non-*S. anglica* plant populations. *P. marginata* populations would consequently have been too low to produce meaningful comparisons. Comparison of oviposition choice in a field assay under semi-natural conditions was therefore conducted. Ten potted plants, each with at least five leaves, of each of the four *Spartina* species were removed from the cultures described in 4.2.1 and transported to the Hythe field site (Grid ref: SU 433070). Plants were randomly assigned to one of ten groups, each group containing one plant of each species. Groups were labelled and haphazardly distributed in an area of established saltmarsh measuring 20m x 30m, with a minimum of 1.5m between each group. Each group of pots was buried so that the tops were level with the surrounding substrate (Figure 5.1).



Figure 5.1 Experimental replicate at Hythe field site.

Pots were left *in situ* for 24 days, at which point they were extracted from the field site and returned to the University of Sussex. Leaves number three and four (counting upwards from the base of each plant) were removed and measured to the nearest millimetre. Leaves were examined at 10x magnification using a Meiji EMZ binocular

microscope and numbers of *P. marginata* eggs in each leaf were counted. To control for potential differences in plant size, egg density was calculated as the number of eggs per centimetre of leaf length.

5.2.2 *Prokelisia marginata* oviposition choice between *Spartina* spp. under glasshouse conditions

Ten plants of each *Spartina* species were removed from the cultures described in 4.2.1 and re-potted in 7cm diameter pots. Plants were randomly assigned to one of ten groups, each group containing one plant of each species, and then labelled. Transparent cylindrical cages were constructed from 175µm PET polyester film. Cylinders were 23cm in diameter and 60cm tall with an 18cm² nylon gauze-covered ventilation window positioned 14cm from the base. On the opposite side of the tube, access was provided by a 9.5cm diameter hole positioned 18cm from the base, attached to a nylon gauze sleeve and secured with a crocodile clip. A 10cm deep plastic base was inserted into the cylinder, which was then sealed with the addition of a 2.5cm deep plastic lid (Figure 5.2).

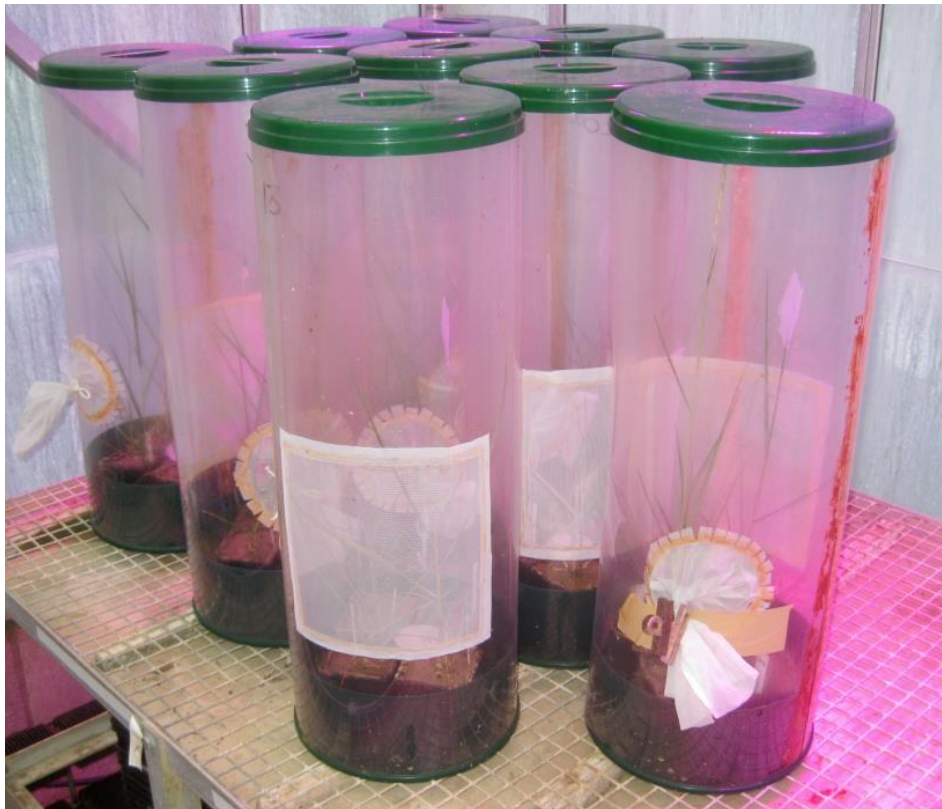


Figure 5.2 Experimental cages containing one plant of each *Spartina* species.

Each group of plants was placed inside a cage, the base of which was half filled with fresh water; 200ml 100% Hoagland solution was added. A 55mm diameter circle of filter paper was placed at the centre of the group at substrate level, positioned to be equidistant between the four plants (Figure 5.3).

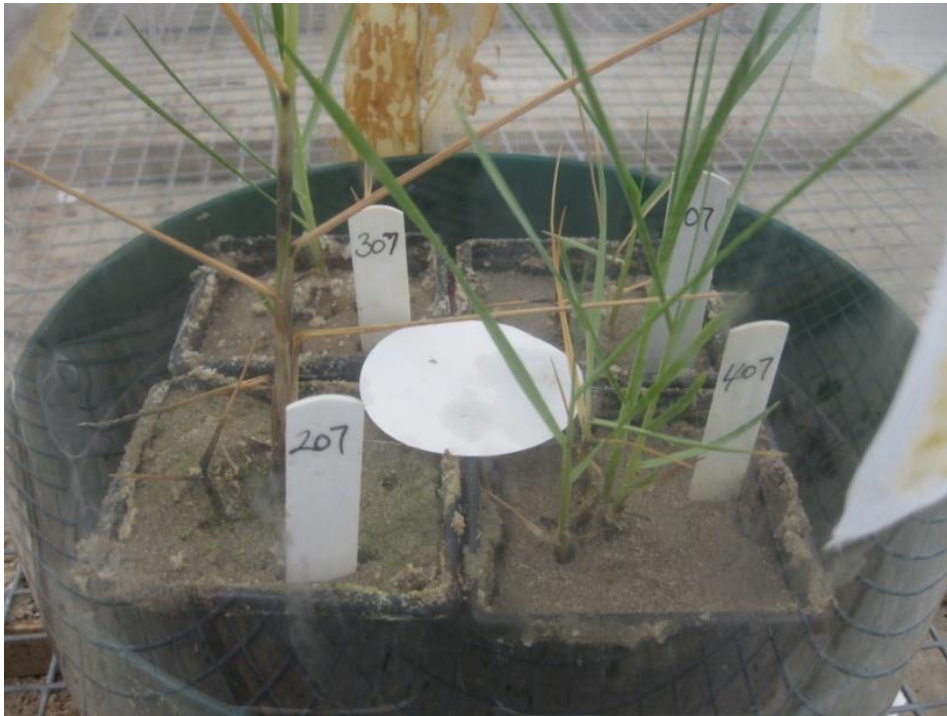


Figure 5.3 Experimental replicate showing one plant of each *Spartina* species caged with filter paper onto which *P. marginata* were introduced.

For each replicate, eight female and four male adult *P. marginata* were aspirated from the breeding culture described in 3.2.3 and placed onto the filter paper. A cylinder cage was quickly placed over the plants as soon as the planthoppers had been introduced onto the filter paper. Caged replicates were arranged in the glasshouse, allocated to a randomly assigned position within a 2x5 grid. Cages were checked twice weekly and watered if required using the access sleeve at the side of the cage. The experiment ran for 14 days; glasshouse temperature was maintained within the range 15-30°C with supplementary lighting (100W Supanova LED grow lights, 8:2 light ratio comprising 660nm Red and 430nm Blue) on an 18:6hr light:dark regime.

At the end of the experimental period, cages were removed and plants were cut at the base. No attempt was made to retrieve or count the number of surviving planthoppers. Leaves were individually removed and their length measured to the nearest millimetre. Leaves were examined at 10x magnification using a Meiji EMZ binocular microscope and numbers of *P. marginata* eggs in each leaf were counted. To control for potential

differences in plant size, egg densities were calculated as the number of eggs per centimetre of leaf length.

5.2.3 Impact of *Spartina* spp. on *Prokelisia marginata* development times and outcomes

Twelve plants of each of the four *Spartina* species were removed from the cultures described in 4.2.1 and placed in individual trays. Six plants of each species were randomly assigned to each of two experimental blocks and to a random position within that block. Each block was arranged in a 3x8 grid. Plants were inoculated with 2 male and 2 female adult *P. marginata* from the stock culture described in 3.2.3. Blocking was used to stagger the start date of each experimental unit to allow time for sufficient numbers of *P. marginata* adults to become available in the culture. Transparent cages were constructed from 175µm PET polyester film. Cages were 13cm in diameter and 50cm tall with a 5 x 7cm nylon gauze-covered ventilation window positioned 18cm from the base of the cage and a gauze lid. Each plant was covered by a cage, the base of which was embedded approximately one centimetre below the surface of the sand. Plants were arranged according to their designated position within the glasshouse and watered *ad libitum* with fresh water; 100ml 100% Hoagland nutrient solution was added to each tray fortnightly. Cages were monitored three times per week until egg hatch and twice weekly thereafter to determine the date of first hatching, the date of first adult emergence and the date by which all adults had emerged. The experiment ran for 64 days; glasshouse conditions were maintained as described in 5.2.2.

At the end of the experimental period, each replicate was placed inside a 1m³ Perspex box with a small access door. The cage was lifted and a plastic bag was placed over the top of the plant. The plant was cut at the base and secured, along with its associated *P. marginata*, inside the bag. Any remaining *P. marginata* either inside the cage or the box were aspirated and added to the bag. Bags were labelled and placed in the freezer for 24 hours. After freezing, plants and associated *P. marginata* were carefully separated and allowed to thaw. Plants were placed within individually labelled paper bags and dried for 72 hours at 70°C in a Gallenkamp OV-420 drying oven. Dry biomass was weighed to an accuracy of 0.01g using a Precisa 125A balance. *P. marginata* were examined at 10x magnification using a Meiji EMZ binocular microscope to determine the sex and wing morph of each individual. No eggs hatched from two of the *S. maritima* plants and there was no adult emergence on four of the *S. alterniflora* plants; affected replicates were excluded from relevant analyses.

5.2.4 Impact of *Spartina* spp. and crowding densities on *Prokelisia marginata* size and wing form

Fifteen plants of each of the four *Spartina* species were removed from the cultures described in 4.2.1 and placed in individual trays. Five plants of each species were randomly assigned to each of three crowding treatment levels: Low, Medium and High, and to a random position within the glasshouse. Plants assigned to the Low density treatment were inoculated with ten first instar *P. marginata* nymphs taken from the culture described in 3.2.3. Plants assigned to the Medium density treatment were inoculated with thirty nymphs; plants assigned to the High density treatment were inoculated with fifty nymphs. Plants were individually caged, randomly arranged within a 3x20 grid, and maintained as described in 5.2.3. The experiment ran for 64 days; glasshouse conditions were maintained as described in 5.2.2. At the end of the experimental period, cages were harvested and data collected as described in 5.2.3 with the addition of body length data (frons to tip of abdomen) which was measured using a graticule eyepiece. One of the *S. maritima* plants was found to have died during the experimental period and was consequently excluded from subsequent analyses.

5.2.5 Statistical analysis

Statistical analysis was performed with R version 3.1.3 (R Core Team 2015) using the *nlme* (Pinheiro *et al.* 2012), *lme4* (Bates *et al.* 2012), *effects* (Fox 2003) and *multcomp* (Hothorn *et al.* 2008) packages. Assumptions about the distribution and variance of the response data (see Quinn & Keough 2002) were tested prior to analysis. Shapiro Wilk tests were used to test for normality of residuals; Fligner-Killeen tests were used to test for equal variance, as recommended by Crawley (2013). *P. marginata* load was calculated for each replicate as the number of harvested individuals per gram of *Spartina* dry mass. This allows for the standardization of crowding levels between differently sized plants in subsequent analyses, following a similar approach adopted by Denno *et al.* (1986).

Appropriate models were determined by the nature of the response variables. In cases where distribution and variance assumptions were not met for continuous variables, transformations were successful in normalising the data. For oviposition choice experiments, differences between host plant selection were analysed using a linear mixed-effects model (LMM) with the number of eggs per cm of leaf length as the response variable, host plant species as the explanatory variable and group as a random factor. For the experiment described in 5.2.3, the number of days from the start

of the experiment to first egg hatch and to first adult emergence were analysed using generalised linear models (GLMMs) with number of days as the dependent variable, plant species as the fixed explanatory variable and individual plant identifier nested within block as the random factor. Initial model fitting in both cases used a Poisson distribution however, in the case of days to egg hatch, Pearson's residuals showed this to be over-dispersed, so the model was refitted using a negative binomial distribution (Ver Hoef & Boveng 2007; Crawley 2013). Sex and wing morph proportion data were analysed using a GLMM. Sex or wing morph was the response variable, plant species was the fixed explanatory variable, with individual plant reference nested within block as the random factor. Data were analysed using a binomial distribution and logit link function. A test of Pearson's residuals showed no evidence of over-dispersion. Random factors were used to account for pseudoreplication and for any variation due to blocking and differential levels of crowding (as expressed by *P. marginata* load) between individual plants.

For the experiment described in 5.2.4, body length data were analysed with a negative binomial GLMM using body length as the response variable, plant species and crowding treatment and the interaction between them as the fixed explanatory variables, and individual plant reference as the random factor to account for pseudoreplication. Wing morph data were analysed using a GLMM. Wing morph was the response variable, plant species and crowding treatment and the interaction between them were the fixed explanatory variables and individual plant reference was the random factor. Interactions were included in order to test whether the impact of crowding varies according to host species. Data were analysed using a binomial distribution and logit link function. Pearson's residuals showed no evidence of over-dispersion. In both cases, male and female data were analysed separately as the *a priori* expectation is that the sexes exhibit different physiological responses to crowding levels (Denno *et al.* 1985).

Each analysis began by fitting all relevant explanatory variables, interactions and random factors in a maximal model. Model simplification then proceeded by a backwards deletion of non-significant terms until further removals led to a significant ($p < 0.05$) increase in deviance. This was assessed by comparing the model with and without the term in question using log-likelihood ratio tests for LMMs and χ^2 values for GLMMs. The resulting minimum adequate model is the one that best fits the data, produces the least unexplained variation (the minimum residual deviance) and where all parameters in the model are significant (Crawley 2013). Significance levels are

reported for the addition of non-significant terms and removal of significant terms from the minimum adequate model. Plots of model residuals against fitted values were visually inspected for normal distribution, homogeneity of variance and the presence of influential outliers following procedures outlined in Crawley (2013). Results showing significant treatment effects were further investigated using a Tukey HSD (Honest Significant Differences) *post-hoc* test to identify differences between treatment means. This test corrects for family-wise error rates when making multiple comparisons, adjusting the resulting p-value accordingly (Crawley 2013).

5.3 Results

5.3.1 *Prokelisia marginata* oviposition choice between *Spartina* spp.

Oviposition varied significantly among host plant species in both the field and greenhouse experiments (Figure 5.4; Table 5.1). In the field, significantly fewer eggs were laid on *S. maritima* than on *S. alterniflora*; there were no significant differences between any other species comparisons. In the glasshouse experiment, *P. marginata* were found to significantly favour *S. anglica* over all other species.

Table 5.1 Effect of host plant species on the number of *P. marginata* eggs laid per centimetre of leaf in i) field and ii) glasshouse experiments and mean values for each host species ± 1 S.E.M. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

	d.f.	Mean (\pm S.E.M.)	L	<i>p</i>	
i) Field					
Mean no. eggs / cm leaf					
Species	6		7.87	0.049	*
<i>S. alterniflora</i>		0.55 (0.19)			
<i>S. anglica</i>		0.32 (0.15)			
<i>S. maritima</i>		0.14 (0.05)			
<i>S. x townsendii</i>		0.30 (0.21)			
ii) Glasshouse					
Mean no. eggs / cm leaf					
Species	6		26.85	<0.001	***
<i>S. alterniflora</i>		0.16 (0.10)			
<i>S. anglica</i>		1.40 (0.30)			
<i>S. maritima</i>		0.29 (0.12)			
<i>S. x townsendii</i>		0.24 (0.08)			

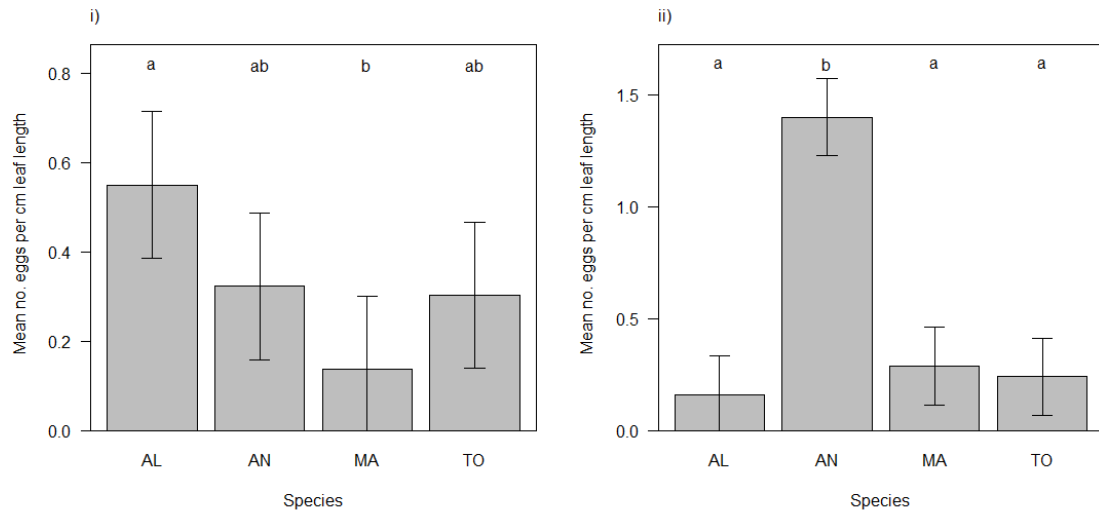


Figure 5.4 Mean number of *P. marginata* eggs laid per cm of *Spartina* spp. leaf length in i) field and ii) glasshouse experiments. Species refers to: *S. alterniflora* (AL), *S. anglica* (AN), *S. maritima* (MA) and *S. x townsendii* (TO). Treatments sharing lower case letters are not significantly different from each other (Tukey HSD post-hoc test, using 95% CI). Error bars show means ± 1 S.E.M.

5.3.2 Impact of *Spartina* spp. on *Prokelisia marginata* development times and outcomes

Levels of macroptery were high across all host plant species, consistent with those recorded in field populations (Figure 2.9), ranging from 85% for adults reared on *S. alterniflora* and *S. anglica* to 96% for those reared on *S. maritima*. The effect of host plant species was not, however, found to be significant (Figure 5.5; Table 5.2). The sex ratio of adult *P. marginata* did not significantly differ between host plant species ($\chi^2=2.99$, $p=0.394$) (Figure 5.5).

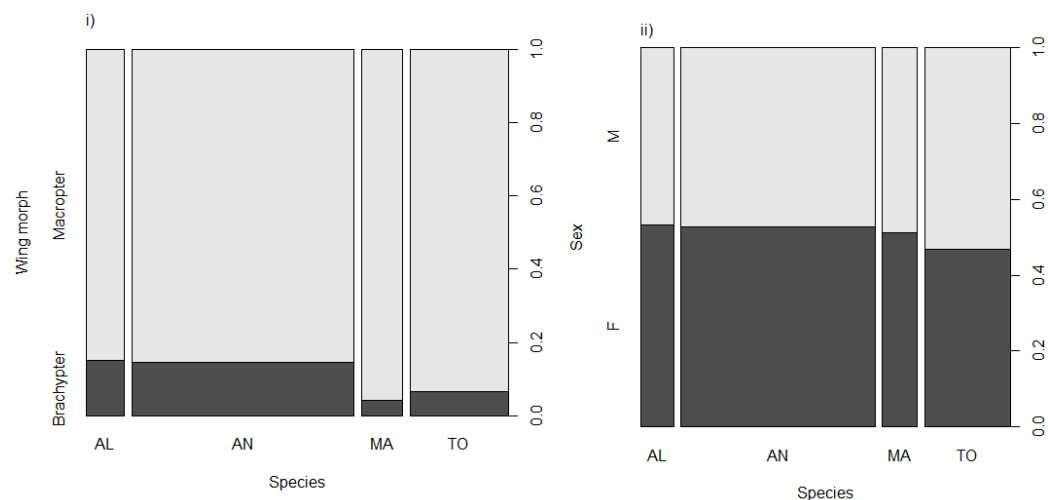


Figure 5.5 Proportion of i) wing morph and ii) sex ratios of adult *P. marginata* reared from four species of *Spartina* under glasshouse conditions. Width of bars reflects the relative number of data points in each category. Species as per Figure 5.4.

Plant species was found to have a significant effect on the final number of *P. marginata* per plant, with the mean number of individuals on *S. anglica* significantly greater than for any other species. The mean number of *P. marginata* per gram of dry *Spartina* biomass (*P. marginata* load) was also much greater on *S. anglica*, however the difference between host plant species was not found to be statistically significant (Figure 5.6; Table 5.2).

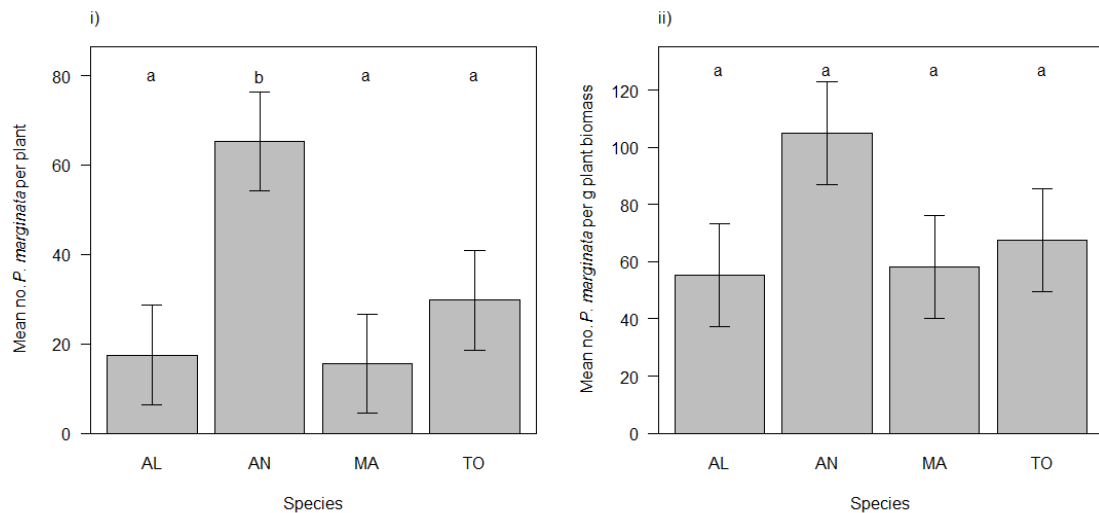


Figure 5.6 Mean number of *P. marginata* i) per plant and ii) per gram of *Spartina* dry biomass reared from four species of *Spartina* under glasshouse conditions. Species as per Figure 5.4. Error bars show means ± 1 S.E.M. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD post-hoc test, using 95% CI). Figure shows untransformed data.

Table 5.2 Effect of host plant species on i) % macroptery; ii) mean number of *P. marginata* per host plant; iii) mean number of *P. marginata* per gram of host plant biomass; iv) days to first egg hatch; and v) days to first adult emergence. Mean values are given for each host species ± 1 S.E.M. Test statistics are log likelihood (L) ratio for linear models and χ^2 for GLMMs. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

	d.f.	Mean (\pm S.E.M.)	L	χ^2	p
i) % macropter	3			2.28	0.516
Species					
<i>S. alterniflora</i>		85.05			
<i>S. anglica</i>		85.35			
<i>S. maritima</i>		95.73			
<i>S. x townsendii</i>		93.57			
ii) Mean no. <i>P. marginata</i> / plant	7		15.58		0.001 ***
Species					
<i>S. alterniflora</i>		12.33 (3.39)			
<i>S. anglica</i>		65.33 (17.29)			
<i>S. maritima</i>		13.00 (3.77)			
<i>S. x townsendii</i>		29.75 (7.44)			
iii) Mean no. <i>P. marginata</i> / g plant	7		5.26		0.153
Species					
<i>S. alterniflora</i>		39.47 (9.62)			
<i>S. anglica</i>		105.16 (24.04)			
<i>S. maritima</i>		48.53 (16.65)			
<i>S. x townsendii</i>		67.57 (11.03)			
iv) Days to first egg hatch	7			1.37	0.714
Species					
<i>S. alterniflora</i>		16.83 (1.40)			
<i>S. anglica</i>		15.00 (0.30)			
<i>S. maritima</i>		15.90 (0.77)			
<i>S. x townsendii</i>		16.33 (1.04)			
v) Days to first adult emergence	7			8.29	0.040 *
Species					
<i>S. alterniflora</i>		51.25 (3.53)			
<i>S. anglica</i>		47.58 (3.10)			
<i>S. maritima</i>		53.10 (1.93)			
<i>S. x townsendii</i>		50.25 (2.82)			

The mean number of days from the start of the experiment until first adult emergence was lowest for *P. marginata* reared on *S. anglica*. There was a significant effect of host plant species, with days to first adult emergence being significantly lower for individuals reared on *S. anglica* than for those on *S. alterniflora* or *S. maritima*. The mean number of days from the start of the experiment to first egg hatch was also lowest for eggs laid on *S. anglica*, although the difference between species was not significant (Figure 5.7; Table 5.2).

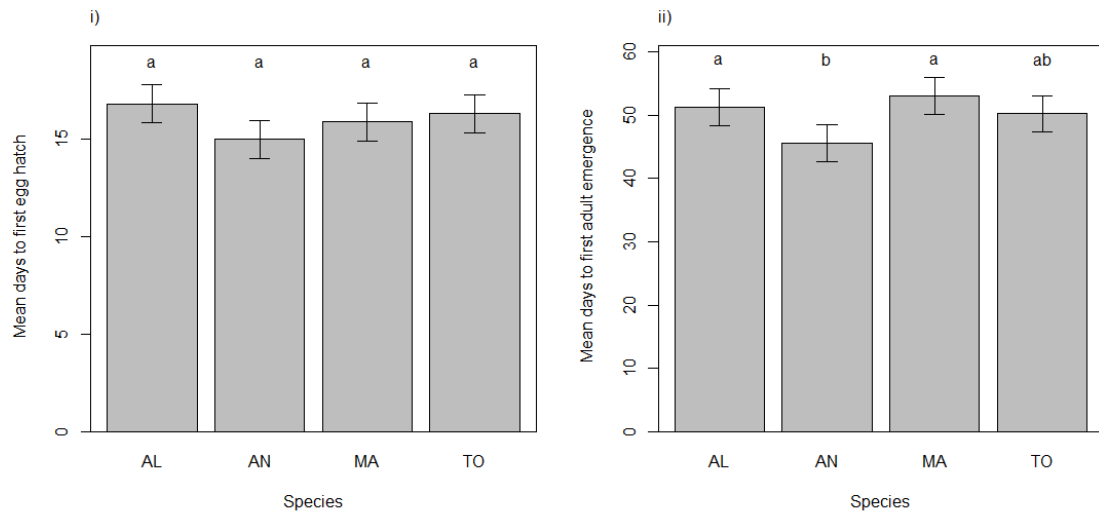


Figure 5.7 Mean number of days from the start of the experiment until i) first egg hatch and ii) first adult emergence. Species as per Figure 5.4. Error bars show means \pm 1 S.E.M. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD post-hoc test, using 95% CI).

5.3.3 Impact of *Spartina* spp. and crowding densities on *Prokelisia marginata* body length and wing form

Both host plant species and levels of crowding were found to have a significant impact on the body size of female *P. marginata*. The interaction between host plant species and crowding level was not significant, however post-hoc tests show that individuals reared on *S. alterniflora* and *S. anglica* were significantly larger (Figure 5.8; Table 5.3).

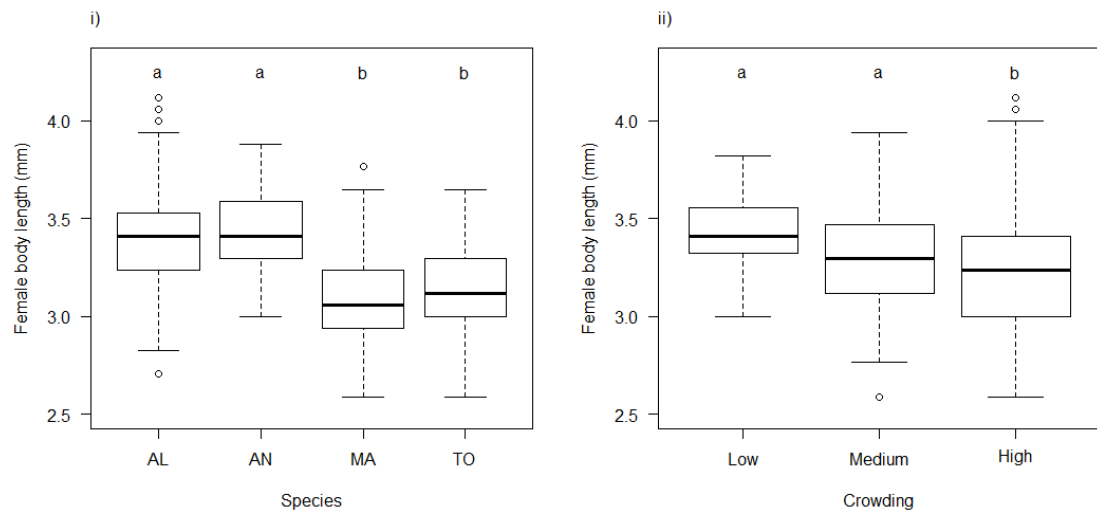


Figure 5.8 Effect of i) host plant species and ii) levels of crowding on body length of female *P. marginata*. Species as per Figure 5.4. Crowding refers to: Low (10 individuals added); Medium (30 individuals added); High (50 individuals added). Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD post-hoc test, using 95% CI).

Host plant species had a significant impact on the body size of male *P. marginata*. Individuals reared on *S. alterniflora* and *S. anglica* were larger than those reared on *S. maritima* and *S. x townsendii*, although the difference between individuals reared on *S. anglica* and *S. x townsendii* was not significant. The effect of crowding levels on male body size was not significant (Figure 5.9; Table 5.3).

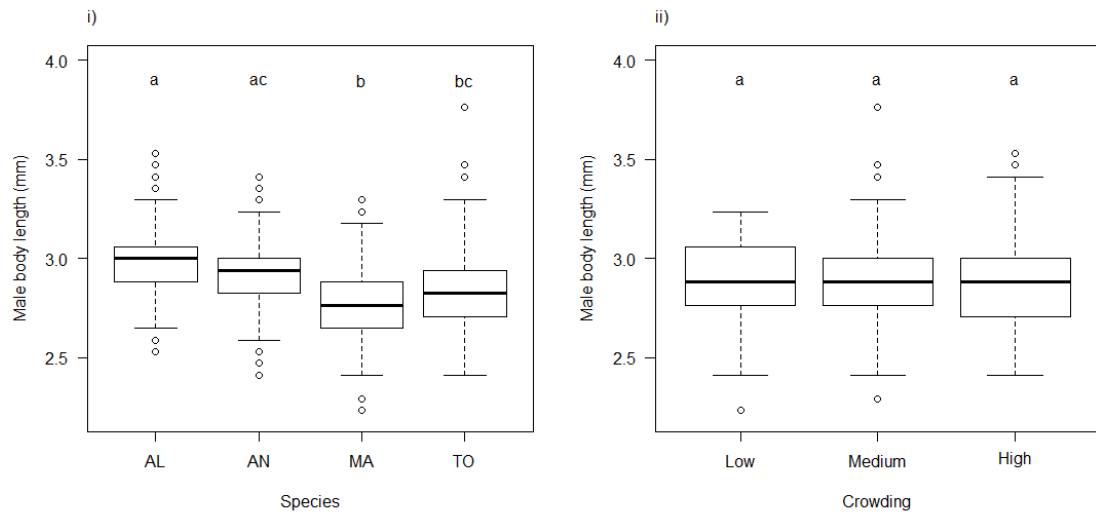


Figure 5.9 Effect of i) host plant species and ii) levels of crowding on body length of male *P. marginata*. Species as per Figure 5.4. Crowding as per Figure 5.8. Boxes show the interquartile range, the enclosed horizontal line representing the median. The tails of the vertical dashed lines represent approximately 2 standard deviations around the interquartile range in the presence of outliers (circles), or the full extent of the data where outliers are not present. Treatments sharing lower case letters are not significantly different from each other (Tukey HSD post-hoc test, using 95% CI).

Table 5.3 Effect of host plant species and crowding treatment on body length of i) female and ii) male *P. marginata*. Crowding refers to: Low (10 individuals added); Medium (30 individuals added); High (50 individuals added). Mean values are given for each host species and crowding treatment ± 1 S.E.M. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

		d.f.	Mean (\pm S.E.M.)	χ^2	p	
i)						
Female body length (mm)						
	Species	8		30.43	<0.001	***
	<i>S. alterniflora</i>		3.38 (0.02)			
	<i>S. anglica</i>		3.44 (0.01)			
	<i>S. maritima</i>		3.11 (0.02)			
	<i>S. x townsendii</i>		3.14 (0.02)			
	Crowding	8		12.07	0.002	**
	Low		3.43 (0.02)			
	Medium		3.30 (0.02)			
	High		3.24 (0.01)			
	Species x Crowding	14		9.60	0.143	
ii)						
Male body length (mm)						
	Species	8		19.60	<0.001	***
	<i>S. alterniflora</i>		2.99 (0.01)			
	<i>S. anglica</i>		2.91 (0.01)			
	<i>S. maritima</i>		2.77 (0.01)			
	<i>S. x townsendii</i>		2.84 (0.02)			
	Crowding	8		0.83	0.659	
	Low		2.89 (0.03)			
	Medium		2.90 (0.01)			
	High		2.87 (0.01)			
	Species x Crowding	14		6.13	0.409	

Host plant species had no effect on wing morph in female *P. marginata*; all groups displayed a high percentage of macroptery consistent with that seen in field populations (Figure 2.9). Crowding treatment had a significant effect on female wing morph; levels of macroptery were significantly lower in Low crowding treatments than those in Medium (Tukey HSD: $z=3.95$; $p<0.001$) and High (Tukey HSD: $z=5.47$; $p<0.001$) treatments. The difference between Medium and High crowding treatments was not significant (Tukey HSD: $z=1.69$; $p=0.203$) (Figure 5.10; Table 5.4).

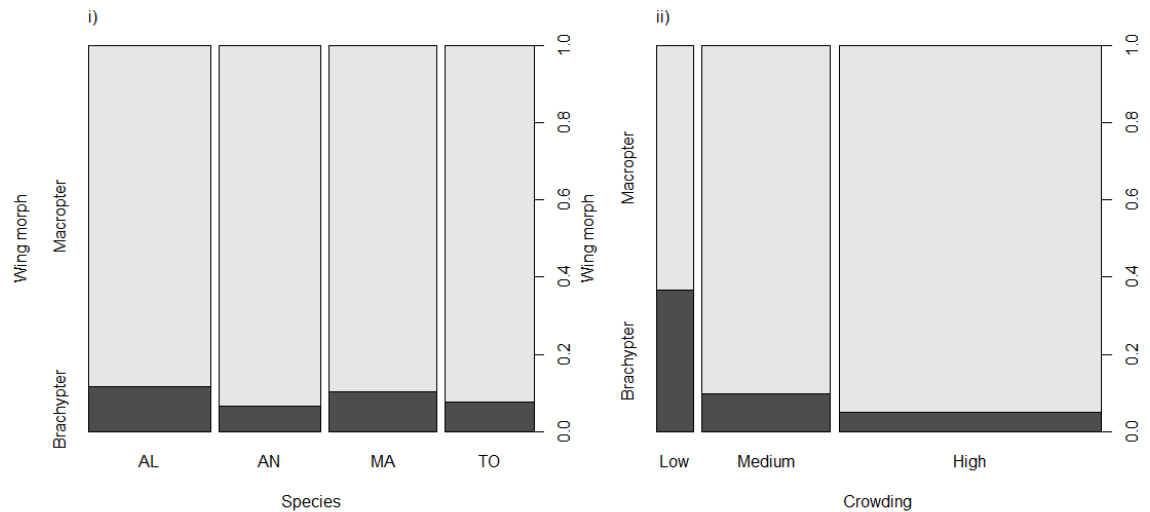


Figure 5.10 Effect of i) host plant species and ii) levels of crowding on proportion of brachypter and macropter wing morphs of female *P. marginata*. Width of bars reflects the relative number of data points in each category. Species as per Figure 5.4. Crowding as per Figure 5.8.

Host plant species had no effect on wing morph in male *P. marginata*; all groups displayed a very high percentage of macroptery which, in line with *a priori* expectation (Denno *et al.* 1985), was greater than that for females in all cases. Crowding treatment had a significant effect on male wing morph; levels of macroptery were significantly lower in Low crowding treatments than those in High crowding treatments (Tukey HSD: $z=3.23$; $p=0.004$). Differences between Low and Medium (Tukey HSD: $z=2.26$; $p=0.060$) and Medium and High ($z=1.10$; $p=0.520$) crowding treatments however were not significant (Figure 5.11; Table 5.4).

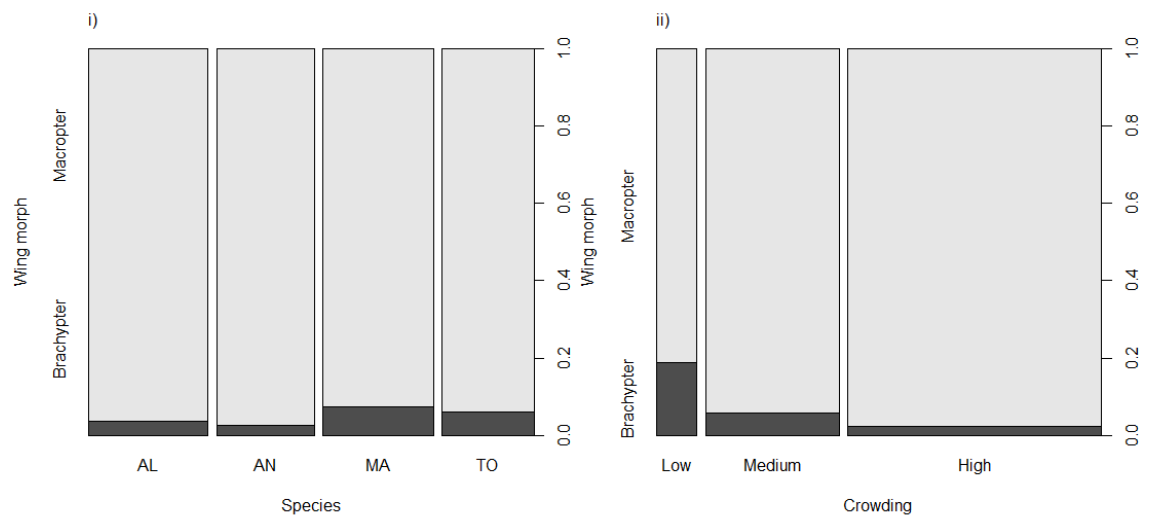


Figure 5.11 Effect of i) host plant species and ii) levels of crowding on proportion of brachypter and macropter wing morphs of male *P. marginata*. Width of bars reflects the relative number of data points in each category. Species as per Figure 5.4. Crowding as per Figure 5.8.

Table 5.4 Effect of host plant species and crowding treatment on wing morph of i) female and ii) male *P. marginata*. Crowding refers to: Low (10 individuals added); Medium (30 individuals added); High (50 individuals added). Percentage of macroptery is given for each host species and crowding treatment ± 1 S.E.M. Significance levels indicated by: * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001

		d.f.	%	χ^2	<i>p</i>	
i)						
Females % macropter						
	Species	7		3.65	0.301	
	<i>S. alterniflora</i>		88.44			
	<i>S. anglica</i>		93.37			
	<i>S. maritima</i>		89.83			
	<i>S. x townsendii</i>		92.41			
	Crowding	4		26.07	<0.001	***
	Low		63.33			
	Medium		90.29			
	High		95.01			
	Species x Crowding	13		3.90	0.691	
ii)						
Males % macropter						
	Species	7		2.73	0.436	
	<i>S. alterniflora</i>		96.34			
	<i>S. anglica</i>		97.45			
	<i>S. maritima</i>		92.66			
	<i>S. x townsendii</i>		93.88			
	Crowding	4		9.94	0.007	**
	Low		81.25			
	Medium		94.29			
	High		97.74			
	Species x Crowding	13		2.25	0.896	

5.4 Discussion

P. marginata is able to preferentially select between potential host plant species, utilising a small number of closely-related *Spartina* species whilst avoiding sympatrically occurring congeners (Denno *et al.* 1996), and between nutritionally differentiated hosts of the same species (Denno *et al.* 1980; Denno 1985). Plant species was found to have a significant effect on oviposition host selection in this study; however results differed between field and glasshouse manipulations. As predicted, field populations showed a significant preference for *S. alterniflora* over *S. maritima*, although there were no other significant pairwise differences. Under controlled glasshouse conditions, however, *P. marginata* unexpectedly showed a significant preference for *S. anglica* over all other species (Figure 5.4; Table 5.1).

A priori expectations were that *P. marginata* would achieve the greatest performance outcomes on *S. alterniflora*, the host to which it is expected to be most readily adapted by virtue of their shared co-evolutionary history, and on which it has previously been shown to achieve the greatest survivorship in no-choice host specificity tests (Denno *et al.* 1986; Grevstad *et al.* 2003). In contrast, performance was predicted to suffer on *S. maritima*, the species most distantly related to its native host (Baumel *et al.* 2002b; Chelaifa *et al.* 2010). Empirical evidence gathered from no-choice host tests offered weak support for this prediction (Figure 5.8; Figure 5.9); however, overall performance outcomes were unexpectedly greatest on *S. anglica*, the host species most favoured by ovipositing females under controlled glasshouse conditions. Eggs laid on *S. anglica* hatched more quickly than those laid on any other species (although differences were not statistically significant), and nymphal development was significantly faster for individuals raised on *S. anglica* than for those raised on *S. alterniflora* and *S. maritima* (Figure 5.7; Table 5.2). Fitness benefits are expected to be realised as a consequence of faster development times: individuals more rapidly escape the comparatively vulnerable egg and nymph stages and the elevated mortality risks with which they are associated (Stiling & Strong 1982a; Denno 1983), whilst potentially increasing their lifetime realised fecundity as a consequence of achieving first reproduction at an earlier age (Denno 1985; Denno *et al.* 1986). Access to host plant nutrition is one of the key factors underlying intraspecific phenotypic differences in insect body size (Chown & Gaston 2010). *P. marginata* eclose as larger adults when raised on nutritionally superior hosts (Denno *et al.* 1986) and there is a positive correlation between body size and the average daily fecundity of females (Denno & McCloud 1985; Denno 1994). In the experiments reported here, females were significantly larger when raised on *S. alterniflora* and *S. anglica*, although host plant species did not appear to mitigate the

negative effects of crowding on body size (Figure 5.8; Table 5.3), nor to differentially impact the level of crowding at which macroptery was triggered (Figure 5.10; Table 5.4). Greater fecundity and survivorship was realised on *S. anglica* however, with significantly larger final populations achieved per host plant (Figure 5.6; Table 5.2).

A distinction is frequently drawn between evolutionary and ecological timescales (Thompson 1998). Rapid evolutionary adaptation to novel environments has, however, been evidenced for a wide range of invading species (Hendry *et al.* 2000; Huey *et al.* 2000; Dlugosch & Parker 2008; Prentis *et al.* 2008; Buswell *et al.* 2011), with genetic studies demonstrating that ecologically significant adaptation can occur within twenty or fewer generations (Stockwell *et al.* 2003; Muller-Scharer *et al.* 2004; Carroll *et al.* 2007; Hendry *et al.* 2007). Indeed, contemporary evolution is expected to have the greatest impact when it acts on traits that most strongly influence ecological interactions (Thompson 1998; Yoshida *et al.* 2003; Hairston *et al.* 2005; Carroll *et al.* 2007), often most clearly evidenced by host-natural enemy associations (Singer *et al.* 1993; Fussmann *et al.* 2007; Hendry *et al.* 2007). Australian soapberry bugs, *Leptocoris tagalicus*, have evolved genetically based changes in mouthpart morphology which increase their feeding efficiency on the introduced balloon vine *Cardiospermum grandiflorum*. These changes were realised in less than forty years and have resulted in almost doubling the rate at which the bugs are able to attack the vine's seeds (Carroll *et al.* 2005). Thomas *et al.* (2001) studied the host plant choices of the brown argus butterfly, *Aricia agestis*, which has rapidly expanded both its habitat and geographical range across south and central England in response to climate warming. They found that females from the most recently established populations chose to lay eggs on the more widespread and available host plant species during range expansion, rather than *Helianthemum chamaecistus*, the ancestral host species utilised by long-established, non-expanding populations. Subsequent breeding experiments provided evidence of genetic adaptation in behavioural phenotypes governing host choice, which was thought to have occurred in less than twenty years. The authors suggest that such adaptation has served to facilitate significant range expansion that otherwise would have been impossible due to the fragmented distribution of the butterfly's ancestral host.

Post-invasion evolution by invading species appears to be common (Mooney & Cleland 2001; Reznick & Ghalambor 2001; Vellend *et al.* 2007; Dlugosch & Parker 2008), and has the potential to amplify ecological impacts by substantially increasing species' local population size or range (Strayer *et al.* 2006; Phillips *et al.* 2010). The frequent

occurrence of a lag between the arrival of an invader and its population growth or spread (Theoharides & Dukes 2007; Figure 1.1; and see section 1.2.3.2) may initially mask the nature and extent of its ultimate impact on the recipient community (Crooks 2005). Rapid adaptation both during and after lag phases, however, can result in sudden and unexpected changes in invader dynamics which may substantially intensify the consequences of its arrival (Stockwell *et al.* 2003; Prentis *et al.* 2008; Burton *et al.* 2010). Data presented in this chapter provide evidence of *P. marginata* achieving relatively small, but significant, improvements in performance outcomes, including greater overall population growth (Figure 5.6; Table 5.2), when utilising *S. anglica* as its host plant. Further investigation is needed to determine the mechanisms underlying these unexpected outcomes. It is possible, however, that *P. marginata* has undergone, or is in the process of, rapid adaptation to maximise its performance on *S. anglica* in the 10 - 15 years since its arrival in Britain (Kirby 2000; Wilson & Muhlerthaler 2009). *S. anglica* is the most abundant and widespread of potential host species for *P. marginata* in Britain (Lacambra *et al.* 2004). If such adaptation is occurring, this may offer some explanation for the rapid spread of the planthopper documented to date (section 1.3.3.3; Figure 1.10; Appendix A), and suggests that further population growth and expansion is to be expected. These results add weight to the classification of *P. marginata* as an invading insect (Chapter 2), further highlighting its potential to play a significant role in shaping the future of Britain's saltmarsh communities.

Chapter six

General discussion

6.1 Research purpose

The classification of invasion ecology as a distinct research discipline continues to attract some level of controversy, largely focussed on the relative impact of invasive species in comparison with other conservation threats (Blackburn *et al.* 2014; Jeschke *et al.* 2014; section 1.2.3.3). Such debates notwithstanding, invasive species have been identified as one of the five greatest threats to global biodiversity and ecosystem integrity, second only to habitat destruction and degradation (Sala *et al.* 2000; Millennium Ecosystem Assessment 2005). An analysis of the IUCN Red List database (IUCN 2003) found that, of the 680 animal species listed as extinct, the underlying causes were known for 170 cases. Invasive species were identified as a contributing factor in 54% of these 170 extinctions, and the sole listed cause in 20% of cases (Clavero & Garcia-Berthou 2005). Over 10,000 species are known to have been introduced to Europe (EEA 2012), with almost 2,000 of these established in Britain (Great Britain Non-native Species Secretariat 2015). Both the rate and extent of biological invasions continue to escalate (Hulme 2009; McGeoch *et al.* 2010). Between ten and twelve new species are becoming established in Britain each year, with consequent impacts on the structure and composition of ecological communities and physical environments. The direct and indirect costs of these impacts to the British economy are estimated to amount to £1.7 billion p.a. (Great Britain Non-native Species Secretariat 2015). Across Europe this figure is estimated to be at least €12 billion each year (EEA 2012). Biological invasions are widely understood to be one of the most important drivers of global change in natural ecosystems (Valladares *et al.* 2015). However, despite broad international political (CBD 2010) and scientific (Simberloff *et al.* 2011) consensus that invasive species are likely to play a significant role in driving further biodiversity loss and extinctions, a recent review concluded that the topic remains “vastly understudied” (Roberts *et al.* 2013).

Coastal marine areas are amongst the most heavily invaded systems on Earth, yet ones in which species introduction is especially difficult to predict or prevent (Grosholz 2002). Saltmarshes represent a key component of coastal marine systems. Despite their relative scarcity (Adnitt *et al.* 2007), they are highly valued for their importance to conservation (Norris 2000; Foster *et al.* 2013), role in coastal defence (Cooper *et al.*

2001; Nicholls *et al.* 2007; Austen *et al.* 2011) and provision of other critical ecosystem services (Angus *et al.* 2011). At least ninety alien species have been recorded in British marine and brackish environments (Minchin *et al.* 2013), yet there has been relatively little investigation of the occurrence and impact of invading species on saltmarshes, either globally or in Britain (Adam 2002).

Prokelisia marginata probably first arrived in British saltmarshes at some point within the last ten to fifteen years (Kirby 2000; Wilson & Muhlerthaler 2009) and has spread extensively in the short time since its arrival (Figure 1.10; Appendix A). Studies conducted in its native North American range suggest it has the potential both to reach population densities that would overwhelmingly dominate the relatively species-poor native British saltmarsh invertebrate community (Denno & Peterson 2000), and to have a major negative impact on its *Spartina* spp. host plants where they have been long-exiled from, or never exposed to, the herbivore (Daehler & Strong 1997b; Wu *et al.* 1999; Garcia-Rossi *et al.* 2003; Grevstad *et al.* 2003). The purpose of the research reported in this thesis is therefore to study the ecological interactions of *P. marginata* with its new environment in Britain, specifically with its host plants (Chapter 3; Chapter 4; Chapter 5) and the wider arthropod community of the saltmarsh (Chapter 2). Further, it aims to assess the herbivore's position as an invading insect (Chapter 2; Chapter 5) and its potential impact on Britain's valuable and vulnerable saltmarsh habitat (Chapter 3; Chapter 4). The work reported here adds to the existing body of research investigating the consistency with which increased vulnerability to *P. marginata* is found in naive and exiled *Spartina* populations, and represents the first time that these interactions have been studied outside of North America. Interactions between *P. marginata* and *S. x townsendii* and *S. maritima* are also examined here for the first time, which, in concert with *S. alterniflora* and *S. anglica*, addresses questions regarding the degree to which *Spartina* vulnerability may be linked to the level of genetic co-evolutionary history with its specialist herbivore.

6.2 Summary of key findings

6.2.1 *Prokelisia marginata*: early indications of invasion success

The data presented in this thesis show British populations of *P. marginata* to be in the successful early stages of invasion. The population at Hythe is well-established, having persisted for at least seven years since the first official record (Wilson & Muhlerthaler 2009), achieving population densities equivalent to those recorded in its native range (Denno *et al.* 2000; Chapter 2). Surveys show evidence of extensive population spread in the relatively short period of time since its arrival (Stewart, unpublished data; Figure 1.10; Appendix A).

Characteristics common to successful invaders across a broad range of taxonomic groups include *r*-selected traits such as high fecundity, high growth rates and short generation times (Pianka 1970; Amundsen *et al.* 2012). Significant range expansion is expected when such rapidly-growing populations additionally display strong adaptations for dispersal (Sakai *et al.* 2001). The work presented in this thesis shows *P. marginata* to be bivoltine in its British range and to be capable of exponential population growth (Chapter 2; Figure 2.3). Phenotypic plasticity has been frequently proposed as an important determinant of invasion success, enabling colonisers to adapt more readily to a range of potentially differing environmental conditions encountered in their new location (Gray 1986; Richards *et al.* 2006; Colautti & Barrett 2013; Turner *et al.* 2015). *P. marginata* displays phenotypic plasticity, its wing morphology determined by a developmental switch that responds to proximate environmental cues such as levels of crowding and host plant nutrition (Denno 1994). Extremely high, and consistent, levels of macroptery found in surveyed populations (Chapter 2), coupled with the extensive spread of its preferred host plant (Boorman 2003; Figure 1.5; Chapter 5), indicate a strong probability of further dispersal.

Interaction with natural enemies is one of the major factors believed to regulate the population growth and dispersal rate of invasive species (Shea & Chesson 2002). The Enemy Release Hypothesis (ERH) predicts that invaders may gain considerable advantage over native species by escaping their natural enemies, particularly specialist predators, parasitoids and pathogens, when establishing within a new range (Torchin *et al.* 2001; Keane & Crawley 2002). Empirical evidence supporting the ERH is equivocal (Colautti *et al.* 2004; Roy *et al.* 2011), and any associated benefits may be short lived (Phillips *et al.* 2010; Gendron *et al.* 2012; Schultheis *et al.* 2015), however enemy escape has been identified as playing a significant role in the success of a number of species invasions (Torchin *et al.* 2001; Torchin & Mitchell 2004; Harvey *et*

al. 2012). The work presented in this thesis (Chapter 2) indicates *P. marginata* is currently benefitting from natural enemy escape. Whilst data are suggestive of a numerical response by generalist Araneae predators, the positive nature of this correlation indicates that predation is not sufficient to regulate population growth at present (Vince *et al.* 1981). *P. marginata* eggs, nymphs and adults are all subject to parasitoid attack across its native North American range (Stiling & Strong 1982a; Stiling & Strong 1982b; Stiling *et al.* 1991), whilst a considerable diversity of parasitoids are adapted to native European planthopper species (Stiling 1994). In Britain, however, *P. marginata* was found to be completely free from parasitoid attack across all sites and life stages (Chapter 2).

6.2.2 Potential impacts on Britain's saltmarsh communities

Saltmarsh is a relatively scarce, yet highly valued habitat type, providing a wide range of important conservation and ecosystem services (Norris 2000; Atkinson *et al.* 2001; Adnitt *et al.* 2007; Green *et al.* 2009; Foster *et al.* 2013; Gardiner 2015; Chapter 1; Chapter 3). Major losses in saltmarsh extent were recorded throughout the 20th century, with significant further losses predicted, particularly in the south and south-east of England (Angus *et al.* 2011; Foster *et al.* 2013; Foster *et al.* 2014). *S. anglica* is believed to dominate almost a quarter of Britain's saltmarsh area, notably on the south and east coasts (Gray *et al.* 1997). Whilst the species is generally perceived to be of detrimental conservation impact (Lacambra *et al.* 2004), and is listed as one of the world's "100 worst invasive species" (Lowe *et al.* 2000), empirical evidence for its impact specifically on Britain's saltmarsh communities is somewhat equivocal (Doody 1990; Gray *et al.* 1991; Gray *et al.* 1997; Davison & Hughes 1998; Huckle *et al.* 2004; Doody 2008). This lack of definitive evidence regarding its impact in Britain has resulted in the adoption of divergent strategies for its management, including the encouragement of further planting along the south coast in an effort to combat coastal erosion (Lacambra *et al.* 2004; Adnitt *et al.* 2007).

The work presented in this thesis represents the first time that the interaction between *P. marginata* and *S. anglica* has been examined outside North America, where the herbivore showed early promise as a potential biological control agent of the grass (Wu *et al.* 1999). The data reported here show *P. marginata* to have reached natural field densities (Chapter 2) which result in a significant detrimental impact on all *S. anglica* performance metrics, under both glasshouse and semi-natural field conditions (Chapter 3; Chapter 4). These findings lend weight to the conclusions drawn from North American studies that exiled and naive *Spartina* populations are vulnerable to attack by

P. marginata in a way that co-evolved and non-exiled plants are not (Daehler & Strong 1995; Wu *et al.* 1999; Grevstad *et al.* 2003). This interaction is of particular interest as, in addition to its role in coastal stabilisation (Doody 1990), *S. anglica* represents the primary host for *P. marginata* in Britain, and the main mechanism enabling the invading herbivore's further establishment and spread.

The data reported in Chapter 4 show *P. marginata* to have a significant negative impact on the performance of all *Spartina* species present in Britain across all functional traits. *S. maritima*, the native British species, is extremely localised, sparsely populated and listed as a species "of principal importance for the purpose of conserving biodiversity" under section 41 of the Natural Environment and Rural Communities Act 2006. It is additionally a priority species under the UK Biodiversity Action Plan (Joint Nature Conservation Committee 2007). Dramatic declines of the species have previously been reported (Raybould *et al.* 1991a), with a further local extirpation at Hayling Island, the site of one of a handful of remaining documented populations (Lacambra *et al.* 2004; Figure 1.5), possibly occurring during the course of this research (Chapter 1; pers. obs.). The work presented in this thesis suggests that the expected population growth and spread of *P. marginata* (Chapter 2; Chapter 5) may pose a very real and immediate threat to the ongoing survival of *S. maritima* across its remaining British distribution (Chapter 4).

A high proportion of the terrestrial invertebrate fauna found in Britain's saltmarshes are endemic to the habitat type (Boorman 2003). The invertebrate community is, however, relatively unsaturated in terms of both species diversity and population density, particularly at the mean high tide elevation (Irmiler *et al.* 2002; Finch *et al.* 2007; Ford *et al.* 2013; Chapter 2). The work presented in this thesis shows that *P. marginata* dominates its recipient invertebrate community, outnumbering all other groups by at least one, and more frequently two, orders of magnitude. This has the potential to dramatically alter community structure, offering both a potential resource to predators and parasitoids which may be able to adapt to exploit it, and a potential competitive threat to other herbivores, particularly those occupying the same feeding guild (Chapter 2).

6.2.3 Rapid evolution in invading species

Post-invasion adaptive evolution by invading species appears to be common (Mooney & Cleland 2001; Reznick & Ghalambor 2001; Vellend *et al.* 2007; Dlugosch & Parker 2008; Prentis *et al.* 2008; Buswell *et al.* 2011). Studies have shown that ecologically significant adaptation can occur within twenty or fewer generations (Stockwell *et al.* 2003; Muller-Scharer *et al.* 2004; Carroll *et al.* 2007), and that such adaptation has the potential to amplify ecological impacts by substantially increasing species' local population size or range (Strayer *et al.* 2006; Phillips *et al.* 2010). The Evolution of Increased Competitive Ability (EICA) hypothesis, associated with the ERH (section 2.1; 4.1.2; 6.2.1), predicts that invasive plants may reallocate resources from defence to growth, reproduction or both when introduced to a new range in the absence of their native natural enemies (Blossey & Notzold 1995; Keane & Crawley 2002). Whilst empirical evidence regarding the EICA is equivocal (Maron *et al.* 2004; Cano *et al.* 2009; Feng *et al.* 2009; Flory *et al.* 2011; Parker *et al.* 2013; and see section 4.2.2 for further discussion), studies suggest that long-exiled populations of *S. alterniflora* may lose tolerance to *P. marginata* herbivory (Daehler & Strong 1997b; Grevstad *et al.* 2003). Further, Qing *et al.* (2012) have shown that plants originating from invasive populations may increase their competitive abilities, and consequent invasive success, by diverting a greater proportion of leaf nitrogen to photosynthetic processes in the absence of native herbivores. The work presented in this thesis shows that, in contrast to non-exiled populations which show no adverse impacts of *P. marginata* herbivory (Daehler & Strong 1995; Gustafson *et al.* 2006), British populations of *S. alterniflora* are negatively impacted across all functional traits by exposure to the planthopper (Chapter 4). However, despite British populations of the grass having been separated from the herbivore for approximately twice the period of time in which exiled North American populations were shown to have lost their tolerance (Thompson 1991; Daehler & Strong 1995), *S. alterniflora* was the least severely impacted of all four *Spartina* species examined for this thesis. It is possible that the post-introduction evolutionary response of British *S. alterniflora* to enemy escape has not been as rapid, or as strong, as that of North American exiles, with a considerable level of tolerance still maintained in the population. Alternatively, it may be that British populations are already displaying a rapid re-evolution of defensive traits in response to selection pressures imposed by the recent arrival of *P. marginata* (Chapter 4).

P. marginata is stenophagous (Denno *et al.* 1996) with very limited ability to host switch within a strictly defined subset of species within the *Spartina* genus (i.e. Clade I; see Baumel *et al.* 2002b; Grevstad *et al.* 2003). *P. marginata* shares the longest co-

evolutionary history with *S. alterniflora*, its native-range host, and has been shown to achieve the greatest rates of survival on this species (Denno *et al.* 1986; Grevstad *et al.* 2003). The work presented in this thesis, however, shows British populations of *P. marginata* to favour *S. anglica* and to achieve relatively small, but significant, improvements in performance outcomes when utilising this host in comparison to populations raised on the other available host species, including *S. alterniflora* (Chapter 5). This suggests that *P. marginata* may have undergone, or is in the process of, rapid adaptation to maximise its performance on *S. anglica*, the most abundant and widespread of its potential host species in Britain, in the 10 – 15 years since its arrival. If such adaptation is occurring, this may offer some explanation for the rapid spread of the planthopper documented to date (Figure 1.10; Appendix A), and adds weight to other evidence presented in this thesis (Chapter 2) suggesting that further population growth and expansion is to be expected.

6.3 Future research recommendations

6.3.1 Population spread and community interactions

P. marginata has achieved rapid population growth (Chapter 2) and spread (Figure 1.10; Appendix A) in the relatively short time since its arrival in Britain (Kirby 2000; Wilson & Muhlerthaler 2009), with data presented in this thesis (Chapter 2; Chapter 5) suggesting it has the potential for even further dispersal and establishment. Sampling of the whole geographical range of British saltmarsh habitat would be informative to determine the full extent of *P. marginata*'s current distribution and, with repeated sampling, to document the rate at which any further dispersal is achieved.

It has been noted (Chapter 2; section 6.2.1) that *P. marginata* appears to be benefitting from natural enemy escape in its new range. Whilst data are suggestive of a numerical response by generalist Araneae predators (Chapter 2), the positive nature of this correlation indicates that predation is not sufficient to regulate population growth at present (Vince *et al.* 1981). To date, there have been no recorded observations of *P. marginata* predation by birds, despite the herbivore representing a primary food source for insect-feeding birds in its native *S. alterniflora* saltmarsh habitat (Payne 1973). Additionally, British populations of *P. marginata* appear to be completely free from parasitoid attack. Records document a considerable rate and diversity of parasitoids already adapted to native European planthopper species (Stiling 1994) which may ultimately switch to exploit this substantial new resource. Repeated sampling and manipulative experiments are needed to examine the adaptive potential of both predator and parasitoid species to respond to the presence of *P. marginata* in this new range (see Berthon (2015) for a review of the various mechanistic and trait-based approaches by which adaptation of native species to invaders may be investigated), and to determine whether these interactions will contribute to population regulation over the longer term.

A review of pair-wise interactions between phytophagous insect species found interspecific competition to occur in 76% of the 193 cases examined. In many instances, this competition was indirect, involving delayed plant-mediated competition in which feeding by one species induced either nutritional or allelochemical changes in the host plant that negatively affected the performance of another species feeding later in the season (Denno *et al.* 1995). Poor host plant nutrition, particularly with respect to the levels of available nitrogen, can have significant adverse impacts on the performance and fitness of planthoppers and other sap feeding insects (Denno & Roderick 1990; Cook & Denno 1994; Denno *et al.* 2002). Olmstead *et al.* (1997) found

that feeding by the planthopper *Prokelisia dolus* significantly reduced the quality of *S. alterniflora* host plants by limiting plant growth and decreasing the levels of a wide range of amino acids. The authors posited that this may act as a mechanistic basis for mediating competitive interactions between *P. dolus* and the congeneric *P. marginata*. This hypothesis was explicitly tested by Denno *et al.* (2000) who found a significant detrimental impact on survival and performance of one species following feeding by its congener earlier in the season, even though no significant fitness reductions were evident during contemporaneous interactions. The impact was asymmetrical, with *P. marginata* suffering a greater negative impact following feeding by *P. dolus*, than was seen in the opposite scenario.

Analysis of the data reported in Chapter 2 revealed a significant negative correlation between the density of *P. marginata* and other Hemiptera at the Pagham field site. This is potentially indicative of interspecific competition, although species diversity and overall population numbers of all groups were low and it was not possible to draw any firm conclusions (see section 2.4.1 for a fuller discussion). *P. marginata* is the only member of the *Prokelisia* genus currently present in Britain; however it is possible that interactions between the invader and other sap feeding invertebrates may be mediated by indirect effects similar to those evidenced between congeners in North American studies (Olmstead *et al.* 1997; Denno *et al.* 2000). The Hemiptera population at the Pagham field site, at which a negative correlation was found with densities of *P. marginata*, is predominantly composed of sap feeding species (section 2.4.1). Further investigation in the form of experimental manipulations of community composition would be useful to shed more light on this potential interaction. In their meta-analysis of the relative competitive strengths of native versus exotic herbivores, Radville *et al.* (2014) suggest that plant-insect co-evolutionary history may be an important predictive factor for the outcome of interspecific competition between phytophagous insects, and ultimately for the impacts of invading species. The gradient of co-evolutionary history suggested in this thesis between *P. marginata* and the four *Spartina* species present in Britain (Chapter 4) would make a useful and tractable system for further exploration of this hypothesis.

6.3.2 Impacts on saltmarsh vegetation and structure

S. anglica is believed to dominate around a quarter of Britain's saltmarsh area, particularly in the south and south-east (Gray *et al.* 1997). Here, despite general concerns regarding its potential adverse conservation impact (Lacambra *et al.* 2004), it plays an important role in stabilising mudflats and protecting the coast, the function for

which it has been so widely distributed (Doody 1990; Doody 2008). In addition to its role in coastal stabilisation, and in contrast to studies suggesting *S. anglica* is an aggressive and destructive competitor of 'native' species (Adam 2002), some authors have identified the grass as an important pioneer species in the development of habitats with high nature conservation value, including Atlantic salt meadow (Dargie 2000; Doody 2008). Indeed, Doody (2008) suggests that, given sufficient time for natural processes to take effect, invading *S. anglica* will become benignly integrated into the 'natural' saltmarsh habitat.

Despite its initial rapid spread, *S. anglica* is now experiencing extensive natural die-back in some marshes, particularly in southern England. The total British extent of the species is thought to have declined by 19% between 1967 and 1990 (Doody 2008), although the specifics of the mechanisms behind the process are not fully understood (Gray *et al.* 1991). Pressure from larger grazers, such as crabs and snails, has been shown to act synergistically with other drivers to cause die-back in some western Atlantic saltmarshes (Silliman *et al.* 2005; Jefferies *et al.* 2006; Alberti *et al.* 2008; Holdredge *et al.* 2009; Coverdale *et al.* 2012). However, Daehler and Strong (1995) found that the top-down effects of insect herbivores on *Spartina* marshes in San Francisco Bay were minimal, suggesting the lack of interspecific competition implied by the occurrence of the grass in large, monospecific stands meant that any effects of herbivory were easily overcome in the absence of other significant stressors. In British saltmarshes, *S. anglica* commonly grows as part of a mixed community with other halophyte species (Gray *et al.* 1991; Dargie 2000; Boorman 2003; Huckle *et al.* 2004) and may consequently be subject to greater levels of competition-induced stress, a factor noted as potentially contributing to the die-back recorded over recent decades (Lacambra *et al.* 2004). British populations may therefore be inherently more vulnerable than their North American counterparts to the adverse effects of a novel and abundant insect herbivore. The work presented in Chapters 3 and 4 indicates that *P. marginata* is capable of exerting a significant deleterious impact on British populations of *S. anglica* at naturally-occurring field densities (Chapter 2). Further investigation is therefore needed to determine whether the predicted ongoing population growth and spread of *P. marginata* (Chapter 2; Chapter 5) has the potential to act additively, or synergistically, with other factors to increase the rate or impact of die-back. Such an outcome may not yet be apparent due to invasion lag (section 1.2.3.2; Figure 1.1), in which the full effects of an invader may not be seen for an extended period following its arrival (Strayer *et al.* 2006), but has the potential to contribute to a significant change in the composition and character of British saltmarshes. An increase in *S. anglica* die-

back may have a deleterious impact on the efficiency of wave and current dissipation across affected saltmarshes (Adnitt *et al.* 2007), consequently reducing the extent to which they act to prevent coastal flooding. It may also reduce the ultimate conservation value of saltmarshes if the reduction in plant vigour results in insufficient accretion of sediments to facilitate the establishment of successional communities (Doody 2008). Further research is needed to categorise the nature and extent of these potential impacts on this important and already heavily challenged habitat.

The work presented in this thesis suggests the predicted population growth and spread of *P. marginata* (Chapter 2; Chapter 5) may pose a significant threat to the ongoing survival of *S. maritima* across its remaining British distribution (section 1.3.2; Chapter 4; section 6.2.2). Ongoing field observations are recommended to monitor the extent of *P. marginata* population establishment and growth in the remaining *S. maritima* marshes, and to identify any further declines of the grass. The data presented in Chapter 4 show *P. marginata* to have a significant deleterious impact on the performance of *S. maritima* across all measured functional traits. It was however predicted that *S. maritima* would suffer the greatest detrimental impact of exposure to *P. marginata* herbivory in comparison with the other *Spartina* species as it shares no co-evolutionary history with the planthopper, nor with any other known specialist herbivore (Payne 1973; Jackson *et al.* 1985). This was not consistently found to be the case. In its current British distribution (Figure 1.5), *S. maritima* is extremely localised, sparsely populated and routinely outcompeted by *S. anglica* (Lacambra *et al.* 2004). For the experiment reported in Chapter 4, each plant was placed individually in its own pot and hence freed from competition. Further manipulative experiments are needed to determine how *S. maritima* fares in the presence of both *S. anglica* and *P. marginata*, examining the relative impacts of competition and herbivory and how the interaction between the two may act as synergistic drivers of the native species' further decline.

6.3.3 Impacts of polyploidy on plant-herbivore interactions

Polyploidy is a widespread phenomenon amongst plant species, with an estimated 70% of angiosperms thought to have polyploid ancestry (Masterson 1994). Newly-formed polyploids often exhibit rapid range expansion (Hull-Sanders *et al.* 2009; Treier *et al.* 2009) and polyploidy has recently been proposed as an important factor determining invasiveness in plants (te Beest *et al.* 2012). In a review of 281 species of danthonioid grasses (Poaceae), Linder and Barker (2014) found successful long-distance dispersal to be significantly more frequent in polyploid, compared to diploid, clades. Polyploids occur with greater frequency amongst invasive plant species than in

angiosperms in general (Pandit *et al.* 2006; Prentis *et al.* 2008); for species that have multiple ploidy levels in the native range, invasive populations are frequently composed entirely of individuals with the higher ploidy level (Hollingsworth & Bailey 2000; te Beest *et al.* 2012). In their review of a global dataset of 890 angiosperm species across 62 genera, Pandit *et al.* (2014) found invasiveness to be positively related to higher ploidy levels, whilst in an earlier paper, invasiveness was found to be 12% more likely as ploidy ratio doubles, and 20% more likely for polyploids than diploids (Pandit *et al.* 2011). Similarly, the same analysis found polyploid species to be 14% less likely than diploids to be endangered. Reasons for these findings are as yet unresolved; however, it is possible that there may be competitive or fitness differences between populations with different ploidy levels in some species complexes, or that polyploids display greater ecological tolerance, consequently rendering them more successful early colonisers (Flegrova & Krahulec 1999; Soltis & Soltis 2000; Prentis *et al.* 2008).

The genetic structure of plant populations is known to exert a strong influence on the evolutionary ecology of phytophagous insect communities (Mopper 1996; Hochwender & Fritz 2004; Perez-Lopez *et al.* 2016). Very few studies, however, have examined the role of polyploidy in shaping plant-insect interactions, with virtually nothing known about its effects prior to the last decade (Thompson *et al.* 2004; Munzbergova 2006; Hull-Sanders *et al.* 2009). A few recent studies have, however, found that polyploidy may be an important factor in determining the structure and diversity of associated insect herbivore communities and the character of their co-evolution with their host plants. Thompson *et al.* (2004) showed that specialist moths of the genus *Greya* differentially attack diploid and tetraploid individuals of the saxifrage *Heuchera grossulariifolia*. Notably however, the preferred ploidy level varied between moth species. *G. politella* attacks both diploid and tetraploid populations, however where both are sympatric, the moth attacks a higher percentage of tetraploids. Conversely, *G. piperalla* displays a significant preference for diploids. Similarly, Halverson *et al.* (2008) found differential attack rates of five gall-making insect herbivores on diploid, tetraploid and hexaploid populations of the goldenrod *Solidago altissima*. All five insect species responded significantly to ploidy level, but for four of the species the most preferred ploidy level differed by site. It appears that herbivores may therefore exert differential selection on plants of different ploidy levels, but that these effects may be subject to influence from other biotic or abiotic factors. Munzbergova (2006) found hexaploid individuals of the perennial herb *Aster amellus* to host significantly greater populations of the monophagous seed-feeding moth *Coleophora obscenella* than their diploid counterparts, and to suffer a correspondingly higher rate of seed damage, although

these effects were mediated to some degree by interactions with plant population size. Reciprocal effects also appear to be evident. It is believed that polyploidy can change the quality and quantity of plant secondary metabolites which may result in changes to the interaction between plants and their herbivores, with consequent impacts on herbivore success (te Beest *et al.* 2012). Hull-Sanders *et al.* (2009) examined the response of a generalist (*Spodoptera exigua*) and specialist (*Trirhabda virgata*) herbivore to diploid, tetraploid and hexaploid individuals of the goldenrod *Solidago gigantea*. They found no significant differences in the response of the specialist herbivore, but the generalist suffered negative survival and performance outcomes on both the diploid and hexaploid plants in comparison to tetraploid hosts.

The relatively small amount of research so far conducted suggests that the effects of polyploidy on plant-herbivore interactions are complex and that they have the potential to exert a significant impact on the structure and composition of terrestrial communities. The multi-ploidy *Spartina* species complex present in Britain represents an excellent model for the study of the impact of ploidy levels on the invasiveness of plant species: the allododecaploid *S. anglica* is of recent origin, the hexaploid progenitors are all still extant with overlapping distributions (Figure 1.5), and likely timings of speciation events are widely agreed upon based on historical records (Gray *et al.* 1991). The recent association of *P. marginata* with these species further adds to the research value of this complex, providing a tractable model for the study of the impact of ploidy levels on the evolutionary ecology of plant-insect interactions, and of how these interactions may influence the outcome of invasion dynamics.

6.3.4 Climate change

The prediction, management and understanding of biological invasions is greatly complicated by the influence of climate change, due to its impacts on both the abiotic environment and on the nature and occurrence of species interactions (Bradley *et al.* 2010; Bellard *et al.* 2013; Sorte *et al.* 2013; Cosner 2014; Ibanez *et al.* 2014; Ju *et al.* 2015; Stewart *et al.* 2015; and see 1.2.3.1). Climate change, and its interaction with other global change factors, is a vast and complex research area, however there are a number of elements which are of direct relevance to the study of *P. marginata* and the interaction with its host plant in its invading range.

Warming temperatures, and to some extent, elevated atmospheric CO₂ levels, have the potential to significantly impact the distribution, performance and competitive relationships of the *Spartina* species in Britain, which represent four of only eight

known C₄ species in the country (Gray & Mogg 2001). C₄ photosynthesis is most commonly found in semi-arid tropical and subtropical species, being only partially adapted to cooler climates. The length of the growing season for C₄ species is substantially curtailed in cooler climates as active growth is only possible once mean air temperatures reach 9°C (Long 1983); the optimum temperature range for C₄ photosynthesis falls between 30 – 47°C (Gray *et al.* 1991). In contrast, the majority of North European saltmarsh plants, notably including *S. anglica*'s main competitor in the high marsh elevation, *Puccinellia maritima*, utilise the C₃ pathway in which active growth becomes possible at 5°C and photosynthesis is optimised between 15 – 25°C (Long 1983; Loebl *et al.* 2006). Long *et al.* (1990) utilised a combination of field and controlled environment studies, along with a dynamic model, to predict the effects of a range of climate-related variables on the biomass and productivity of *S. anglica*. Results suggest that a 2°C rise in mean temperatures would more than double the resulting canopy biomass, whilst a mean rise of 3°C would enable the plant to significantly extend its growing season, with spring growth beginning up to 50 days earlier (Long *et al.* 1990; Gray *et al.* 1991). Whilst the outcome of competitive interactions with *P. maritima* remain difficult to predict, in part due to the C₃ competitor's greater ability to capitalise on elevated levels of CO₂ (Gray & Mogg 2001), these findings suggest that a warming climate would enable *S. anglica* to extend its distribution further northwards and to extend the length of its growing season in its current range (Gray *et al.* 1991; Gray & Mogg 2001; Loebl *et al.* 2006).

Such changes have the potential to significantly impact the population establishment and continued spread of *P. marginata* in Britain. Whilst macropterous individuals are capable of dispersing over large distances (Denno *et al.* 1996), the planthopper's distribution is limited entirely to that of its host plant, being unable to successfully complete its life cycle on any host outside a narrow subset of *Spartina* species (Grevstad *et al.* 2003). Northward spread of *S. anglica* distribution could therefore facilitate similar population expansion of the herbivore. *P. marginata* generation times are determined by climatic conditions and the associated growing season of their host plant (Gratton & Denno 2003b). Data presented in Chapter 2 show *P. marginata* to be bivoltine in Britain. However, it is trivoltine in the mid-Atlantic coast range (Gratton & Denno 2003b) and has five overlapping generations in the sub-tropical climate of the Gulf coast, facilitated by the year-round growth of *S. alterniflora* at this latitude (Denno & Grissell 1979). An earlier start to the *S. anglica* growing season in Britain conferred by climate warming could result in an increase in *P. marginata* voltinism, with consequent impacts on population growth and the rate at which potential adaptations

such as those discussed in 6.2.3 may accumulate throughout the population. Although highly complex, any future research seeking to investigate the potential impact of *P. marginata* invasion on Britain's saltmarsh communities would benefit from consideration of these and other global change-related factors where possible.

In conclusion, the work presented in this thesis shows *P. marginata* to be in the successful early stages of invasion in Britain. Data suggest that further population expansion and spread is to be expected, and that it has the potential to significantly impact Britain's important and vulnerable saltmarsh communities. The work presented in this thesis also highlights the value of *P. marginata*, in association with its host plant species complex, as a model system for further research pertinent to the dynamics of invasion biology and to the evolutionary ecology of plant-insect interactions.

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Appendix A

Prokelisia marginata British distribution records

Adapted from a list compiled by A.J.A. Stewart from unpublished data.

Date	Site name	Grid reference	Collector
05/08/2008	Fawley	SU473034	M.R. Wilson
05/08/2008	Hythe	SU435075	M.R. Wilson
12/08/2008	Poole	SY9888	M.R. Wilson
10/09/2008	Bosham Ferry	SU801020	A.J.A. Stewart
04/06/2009	Bosham Ferry	SU801020	A.J.A. Stewart
04/06/2009	Bosham Harbour	SU806037	A.J.A. Stewart
18/06/2009	Hythe	SU431072	A.J.A. Stewart
18/06/2009	Ashlet Creek, Fawley	SU469032	A.J.A. Stewart
18/06/2009	Hayling Billy	SU715025	A.J.A. Stewart
19/07/2009	Bosham Ferry	SU801020	A.J.A. Stewart
01/08/2009	Blythburgh	TM454754	A.J.A. Stewart
13/08/2009	S Swale Res nr Faversham	TR003655	J.S. Badmin
13/08/2009	S Swale Res nr Faversham	TQ992657	J.S. Badmin
13/08/2009	S Swale Res nr Faversham	TQ986656	J.S. Badmin
13/08/2009	S Swale Res nr Faversham	TQ975655	J.S. Badmin
14/08/2009	Conyer Creek	TQ961648	J.S. Badmin
14/08/2009	Conyer Creek	TQ958652	J.S. Badmin
14/08/2009	Conyer Creek	TQ965656	J.S. Badmin
14/08/2009	Oare Creek Faversham	TR008631	J.S. Badmin
14/08/2009	Oare Creek Faversham	TR013634	J.S. Badmin
15/08/2009	Isle of Grain	TQ6774	J.S. Badmin
15/08/2009	Isle of Grain	TQ6874	J.S. Badmin
15/08/2009	Isle of Grain	TQ694748	J.S. Badmin
15/08/2009	Isle of Grain	TQ698751	J.S. Badmin
15/08/2009	Isle of Grain	TQ703754	J.S. Badmin
15/08/2009	Medway Country Park, Gillingham	TQ807685	J.S. Badmin
15/08/2009	Funton Creek	TQ876681	J.S. Badmin
15/08/2009	Funton Creek	TQ883683	J.S. Badmin
15/08/2009	Funton Creek	TQ888684	J.S. Badmin
15/08/2009	Sheppey Bridge,mainland	TQ913691	J.S. Badmin
15/08/2009	Sheppey Bridge,mainland	TQ914689	J.S. Badmin
15/08/2009	Sheppey, near Kingsferry Bridge	TQ916694	J.S. Badmin
15/08/2009	Sheppey, near Kingsferry Bridge	TQ908702	J.S. Badmin
18/08/2009	Pagham Harbour	SZ878975	A.J.A. Stewart
19/08/2009	Aust	ST562891	A.J.A. Stewart
19/08/2009	Peterstone-Wentlooge	ST270798	A.J.A. Stewart
19/08/2009	Faversham Creek	TR018641	J.S. Badmin
19/08/2009	Faversham Creek	TR018638	J.S. Badmin
19/08/2009	Faversham Creek	TR024636	J.S. Badmin
19/08/2009	Faversham Creek	TR032631	J.S. Badmin
19/08/2009	Faversham Creek	TR031626	J.S. Badmin
19/08/2009	Faversham Creek	TR027625	J.S. Badmin
20/08/2009	Faversham Creek	TR022622	J.S. Badmin
09/09/2009	Pegwell Bay	TR353642	J.S. Badmin
09/09/2009	Pegwell Bay	TR345636	J.S. Badmin

09/09/2009	Minnis Bay	TR253694	J.S. Badmin
20/09/2009	Aveton Gifford	SX683468	J.S. Badmin
26/09/2009	Batson, Salcombe	SX738397	J.S. Badmin
29/09/2009	Lutton Marsh, The Wash	TF484287	A.J.A. Stewart
13/10/2009	Rushenden Sheppey	TQ902713	J.S. Badmin
13/10/2009	Rushenden Sheppey	TQ898714	J.S. Badmin
13/10/2009	Shellness Sheppey	TR053681	J.S. Badmin
13/10/2009	Shellness Sheppey	TR053678	J.S. Badmin
13/10/2009	Shellness Sheppey	TR049678	J.S. Badmin
13/10/2009	Harty Ferry Sheppey	TR016658	J.S. Badmin
13/10/2009	Harty Ferry Sheppey	TR021658	J.S. Badmin
21/07/2010	Cuckmere Haven	TQ517013	C. Harkin
03/10/2010	Bedlams Bottom	TQ8868	E.G. Philp
07/10/2011	Hythe	SU432073	C. Harkin
07/10/2011	Pagham Harbour	SZ862972	C. Harkin
09/08/2011	Colne Point	TM107123	A.J.A. Stewart
09/08/2011	Crabknowe Spit	TM240285	A.J.A. Stewart
12/08/2011	Hunstanton	TF693439	A.J.A. Stewart
13/08/2011	Titchwell	TF748450	A.J.A. Stewart
13/08/2011	Holkham Dunes	TF902460	A.J.A. Stewart
14/08/2011	Winterton Dunes	TG495205	A.J.A. Stewart
31/08/2011	Middle Stoke, Grain	TQ833752	J.S. Badmin
21/09/2011	Aveton Gifford	SX683468	J.S. Badmin
23/08/2013	Oxwich	SS509872	A.J.A. Stewart
23/08/2013	Crofty	SS532957	A.J.A. Stewart
27/08/2013	Beaulieu River	SZ425976	C. Harkin
28/08/2013	Bosham Channel	SU800019	C. Harkin
29/08/2013	Hayling Island	SU724042	C. Harkin
01/09/2013	Nene Outfall	TF506265	P.R. Kirby
17/07/2014	Grain Power Station (Site C: NE corner)	TQ8975	T.M. Bantock
22/07/2014	Portsmouth	SU676003	I.R. Thirlwell
25/07/2014	Rimac	TF471920	A.J.A. Stewart
02/09/2014	Wallasea Island	TQ925946	A.J.A. Stewart
15/06/2015	Northey Island (B)	TL875057	T.M. Bantock
15/06/2015	Northey Island (A)	TL878061	T.M. Bantock
08/08/2015	Stone Creek (West)	TA2319	W.R. Dolling
11/08/2015	Paull Strays	TA1725	W.R. Dolling
02/09/2015	Kilnsea Spurn Bight	TA410156	W.R. Dolling
