Phenotypic plasticity and population differentiation in *Suaeda maritima* on a salt marsh

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Abstract

Suaeda maritima (L) Dumort is a polymorphic annual species of the family Chenopodiaceae that in the UK occurs exclusively in coastal salt marshes. The main aim of this study has been to examine the phenotypic variations within and between its populations in the heterogeneous microenvironments of a salt marsh.

Detailed field characterizations of the growth, seed production and seed heteromorphism of four *Suaeda maritima* populations at Stiffkey salt marsh were conducted over three consecutive years, revealing considerable consistent phenotypic variation between populations on the high marsh, high-marsh creek bank, upper low marsh, and low marsh.

Field environmental heterogeneity was assessed by taking measurements of sediment salinity, water content, organic content, redox potential, elevation in the tidal frame and annual number of tidal inundations. They demonstrated that different *Suaeda maritima* populations do indeed experience divergences between their environments that could both affect the phenotypic responses of developing plants and constitute selection pressures for the evolution of genetically differentiated populations.

Experiments involving seedling reciprocal transplantation in the field and seedling transplantation to uniform laboratory conditions revealed significant differences among populations in terms of survival, growth and fecundity parameters.

Detailed experiments examining the effect of salinity, temperature, light and storage conditions on seed germination and dormancy also revealed seed dimorphism and significant variation in the germination behaviour among populations.

Suaeda maritima populations exhibited substantial evidence of genetic differentiation (reflecting the complex heterogeneity in their natural microhabitats) and, therefore, it can be suggested that this differentiation reflects adaptive ability to colonize wider range of habitats along the environmental gradients.

Key words: *Suaeda maritima*, Salt marsh, Microhabitat, Population differentiation, Phenotypic plasticity, Fecundity, Seed heteromorphism, Dormancy, Germination, Survival, Reciprocal transplantation, Spatial heterogeneity

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Introduction

1.1 Salt marsh habitats

Coastal habitats represent important ecosystems in terms of biodiversity; they can be broadly classified into four distinct types: salt marshes and mangroves, sand dunes, vegetated shingle and rocky cliffs. Of these, salt marshes are particularly important habitats, in terms of their ecosystem value, because of the unique communities of specialised plants that occur there together with the organisms that depend on them. Although considered as ecosystems in their own right, coastal salt marshes function as part of other coastal or estuarine ecosystems and, largely free of human interference, are rich in rare plants and invertebrates (Hughes and Paramor 2004); they have higher concentrations of plant and animal life than other coastal habitats. Although salt marshes are generally located in coastal areas, they also exist as inland salt marshes in appropriate geological and climatological settings around the globe but the latter have entirely different characteristics to the former and will not be considered in this study (Packham and Willis 1997).

The plants that grow on coastal marshes must be tolerant to the varying saline conditions, and have the ability to colonise areas characterized by regular inundation, shifting sedimentation patterns and the hydraulic power of waves. The sites best suited to the development of coastal marshes are those with shallow gradients, affording appreciable but not excessive sedimentation, and mud flats with raised areas and drainage channels (Little 2000). Such sites are typically found behind sandbars and spits, and in estuaries. Once pioneer species, usually annual angiosperms such as *Suaeda maritima*, have colonized and stabilized the rich sediments, other species may then become established on the newly stabilized soils. These secondary species, often perennials including broad leaved plants such as *Limonium vulgare* and grasses such as *Puccinella maritima*, typically favour the upper reaches of the marsh, where flooding is less frequent. Once established, a salt marsh replenishes itself by growing vertically, countering the effects of tidal erosion and the flow of sediment-rich water away from the marsh through the drainage channels (Little 2000). Over time, a salt marsh may develop a series of zones, typically described as the high marsh, the middle marsh and the low marsh; this latter also has a distinctive seaward or pioneer edge. These zones are drained through a complex arrangement of creeks that facilitate gene transfer across the marsh, and a final feature is that of ancient shingle banks cutting across the marsh that may inhibit gene transfer (Adam 2002).

1.1.2 Key characteristics of salt marshes

Salt marshes have three main characteristics: varying levels of salinity (salt stress), the inundation regime (periodic waterlogging) and the discrete spatial distribution of species (zonation); the last of these is dependent on the first two (Pennings and Bertness 2001). A further feature of coastal salt marshes, from the perspective of the ecologists, is that temporally they are highly stable environments. This is because the key driver in their development and sustainability is the frequency and duration of the tidal inundation regime; (Odum 1988) described this stability as pulse-stabilised, although variations may be evident over longer timescales, as accretion changes the profile of the marsh and therefore the inundation regime. Because of this stability, seasonal trends in salinity, water potential and osmolarity are all predictable. On the other hand, they exhibit a degree of spatial variability, determined by accretion rates, as well as by erosion rates at the seaward edge and along the drainage channels, which in turn are related to gradient.

Notwithstanding this spatial variability, their temporal stability make coastal salt marshes, on the annual scale, excellent field case studies for the investigation of life-histories, allowing researchers to identify any genetic differentiation resulting from localised selective forces. This may be conducted in the knowledge that salt marshes have only one key element of periodic change, the circadian inundation cycle, modulated by the equinox-solstice cycle and the lunar cycle (Pennings and Bertness 2001).

1.2.1.1 Salinity and waterlogging in salt marshes

Salinity levels vary across a salt marsh, reflecting the gradient and elevation. The lower part of the marsh is frequently flushed through, which limits any build-up of salt. On the other hand, the upper marsh is covered completely only by spring tides and it might not be covered at all in the cycles immediately before and after the solstice for about 8 weeks, thus, in particularly summer, salinity may rise to levels three or four times that of sea water (Adam 1981, Davy and Smith 1985).

Tidal inundation results in flooding and thence to waterlogging. The higher reaches of a marsh suffer less frequent inundation and therefore, with less waterlogging and a deeper, more quickly falling water table, these higher parts have higher redox potentials (Pennings and Callaway, 1992). However, in the creek banks, the soils have much greater drainage abilities and accordingly have higher redox potentials and lower sulphide levels than the soils of the low marsh but not of the high marsh (Adam 2002).

1.2.1.2 Zonation in salt marshes

The discrete distributions of plant species across salt marshes has been noted for nearly a century; particular zones within a marsh are often dominated by usually a few particular plant species, which are found in less abundance elsewhere in the marsh (Pennings and Bertness 2001). These zones form identifiable patterns apparently linked to the gradient of tidal inundation to which the marsh is subjected (Vince and Allison 1984). This spatial variation in plant distribution, or zonation, is a key characteristic of salt marshes, although the drivers of this process are not yet fully understood. Although much research has focused on local variations in topographical or environmental gradients extending from land to sea and, and while they are major influences, spatial zonation is now considered to be not entirely dependent on them (Pennings et al. 2005). Relatively little research has been undertaken on the other, more biologically centred factors, such as a plant's tolerance to varying environmental conditions (Vince and Allison 1984). These may also be important causal mechanisms in zonation, but determining them requires that they can be demonstrated experimentally.

The vegetation that is subjected to this zonation is comprised mainly of grasses, sedges, rushes and other herbaceous angiosperms, and the manner in which they respond may be due to the prevailing environmental characteristics, but they may also be due to the nature of the evolutionary adaptation of each plant type, in terms of their tolerance to varying levels of salinity. Salt-tolerant (or halophytic) species are adapted to regular seawater immersion; they are relatively few in number. As a result, salt marshes tend to be species-poor, with approximately 40 species of higher plants in British marshes. Of these, on the seaward edge (the pioneer zone of low marsh), glassworts (*Salicornia spp*.) and *Suaeda maritima* are often dominant as they are one of the few species that can tolerate twice-daily inundations while further landwards, plant species that can tolerate less frequent rates of inundation are found (Best et al. 2007). The most abundant perennial halophytic species that are typical of British salt marsh communities, and of particular zones within them include *Armeria maritima*, *Atriplex portulacoides*, *Limonium vulgare*, *Puccinellia maritima*, *Spergularia media* and *Triglochin maritimum* (Packham and Willis 1997).

1.1.3 British salt marshes

British salt marshes tend to develop on mineral-rich sands and silts, rather than peat. These minerogenic marshes are common to Europe but, in the USA, salt marshes tend to be organogenic, i.e. growing on a bed of organic matter derived from plant litter and peat. (Allen and Pye 1992) classify British salt marshes into five types: open coast, which are exposed to wave action and are therefore relatively uncommon; back-barrier, which develop in the shelter of spits etc.; estuarine-fringing, generally on Britain's larger rivers; embayment, which exploit the calm of large bays (e.g. The Wash); and loch or fjord-head, which develop on rocky coasts.

The vegetative communities that make up the salt marshes of Great Britain are fairly representative of those found in Western Europe in general. Here, approximately one fifth of the total area of British salt marshes is found on the south-eastern coast (Boorman 1992). In this region, the salt marshes of the Norfolk coast are particularly important areas owing to their age (c. 6000 years) (Mossman et al. 2012a), distinctive communities of vegetation and the range of invertebrate and bird life that inhabit them. Their value has been reflected in the significant body of ecological research conducted on these marshes over a period spanning almost one hundred years.

All salt marshes, whether minerogenic or organogenic, are subject to changes in the level of sea or land, and to the rates of sedimentization within the marsh (accretion rates). Thus, salt marshes are ecosystems that combine a wide variety of factors in their generation and maintenance: sediment supply, tidal regime, wind-wave action/climate, and movement of relative sea level (Allen and Pye 1992). This picture is further complicated by the presence of creeks and channels, which can divide a salt marsh into zones of differing plant species distribution and abundance.

1.2 Halophytes

Halophytes represent salt-tolerant species that thrive in the inhospitable habitats of inland and coastal salt marshes, dunes, beaches, deserts and salt flats. They are adapted to survive under extreme conditions, represented by temperature, (freezing to very hot) salinity (hypo- to hyper-saline) and moisture (drought to water-logging) (Flowers et al. 1986, W.H.O 1990). They must also cope with localised environmental variability; populations in these habitats may suffer changing conditions from year to year and there may be variations on the micro-spatial scale as well. For halophytes to thrive under such challenging regimes, they must not only be able to exploit any prevailing conditions but also have a strategy for adapting to sudden changes; flexibility in terms of seed germination and seedling growth is key to surviving in such unpredictable habitats (Glenn et al. 1999) .

Reflecting such high levels of habitat heterogeneity, halophytes exhibit variations in form as ephemerals, shrubs and trees. Both annuals and perennials are present in temperate climes but in the subtropics, only perennials are generally abundant. These two plant types reflect varying habitat regimes and evolutionary processes; in annuals there is only one reproductive event in a lifetime, and this necessitates the ability to produce seeds with dormancy characteristics; when the seeds reach maturity, the environmental conditions may not be favourable for seedling growth and so germination is postponed until conditions are again more favourable. Perennials can reproduce by seeds on multiple occasions, which means that producing genetic variations is not so paramount as it is with annuals; they may reproduce clonally (through ramets), seeking to reproduce only the most successful genotype. However, as a long-term strategy, they may need to introduce new genetic individuals as and when conditions allow (Khan 2003).

Halophytes have evolved to cope with a wide variety of climatic conditions, all around the world, and they are found at varying altitudes, although in greatest numbers at sea level. (Khan et al. 2006a). The number of halophytic species is an area of some debate, and will remain so while there are no highly specific definitions for levels of salinity or for the mechanisms employed in salt tolerance. Thus, (Lieth *et al.* 2008) claimed there to be 1,560 terrestrial species, whereas (Khan et al. 2006b) put this at almost 2,600. However, (Khan et al. 2006c) stated that the number could be as high as 6,000 for terrestrial halophytes (including tidal zones). Almost all halophytes are angiosperms (flowering plants); however, there are some algae and even some fungi but very few halophytic mosses, liverworts or gymnosperms. Additionally, at least two fern families have halophytic genera (Pteridaceae and Ophioglossaceae). (Flowers et al. 1986) found that about one third of all the angiosperm families contain halophytic genera, indeed it has been argued that glycophytes are plants that, through evolutionary processes, have lost salt-tolerance.

It is now accepted that there are approximately 20 significant families containing some 500 halophytic genera. The most widespread and well-studied family is one of the Dicotyledoneae: Chenopodiaceae, which, according to (Flowers et al. 1986) includes 44 halophytic genera with 312 halophytic species; these 44 genera represent 44% of the family. Of the Monocotyledoneae, the most significant is Poaceae, which has 45 halophytic genera which represent only 7% of the family. Perhaps the most important genera within the research community are *Atriplex*, *Salicornia* and *Suaeda*; this last one is to play a central part in this research (Flowers et al. 1986).

1.2.1 Phenotypic variation

Halophytic species growing in the heterogeneous salt marsh environment respond to abiotic and biotic stress factors through various mechanisms. There is strong evidence among salt marsh plants of the existence of phenotypic plasticity, which reflects the ability of an organism to produce different physiological and morphological phenotypes in response to changing environmental conditions (Wu et al. 2004, Valladares et al. 2007). Most plants are highly plastic within certain parameters, and this plasticity is manifested in varying growth rates, sizes at maturity, reproductive characteristics and rates, and tissue chemistry. Furthermore, phenotypic plasticity, once established in a genotype, may be temporary, being exploited in a fixed manner on a seasonal scale or in a lifecycle stage, or it may be dynamic, reflecting, for example, the changing levels of photosynthetic activity in response to daylight (Callaway et al. 2003).

Another important mechanism in response to the heterogeneity of salt marshes is the presence of genetic differentiation within halophyte species. Indeed, there is evidence that much of the morphological variation evident within halophytes is genetically based, and that this genetic differentiation is the driver in maintaining the variation in phenotypes, both within and between populations (Jefferies 1977, Davy and Smith 1985, Davy et al. 1990, Howard and Rafferty 2006, Baythavong and Stanton 2010a).

A further phenomenon in the physiological and morphological variation of halophytes is the mixed strategy of phenotypic plasticity and genetic differentiation in order to tolerate environmental heterogeneity (Jefferies and Rudmik 1991). Thus, notwithstanding whether a particular species adopts phenotypic plasticity or genetic differentiation, both strategies provide a possible mechanism that could determine the plant’s ability to adapt to environmental heterogeneity (Richards et al. 2005).

1.2.2 Seed production and heteromorphism

Heteromorphism in seeds is the ability of the plant to produce different seed forms during the lifecycle, and this adaptation is found, in particular, in Asteraceae, Chenopodiaceae and Poaceae (Imbert 2002). Heteromorphism is reflected in seed size, colour and shape, dispersal, dormancy and germination characteristics, and seed bank and seedling growth strategies; it far from common but over 200 species have now been identified around the world as having heteromorphic traits in seed production (Mandák and Pysek 2005, Lu et al. 2010). These traits in terms of seed heteromorphism have been found in both annuals and perennials from the sub-arctic to the tropics.

Dimorphism (two types) and polymorphism (multiple types) in seed production represent strategies on the part of a species to tolerate and exploit environmental variations in their habitat; a phenomenon typical of saline habitats (Khan and Ungar 1986). Accordingly, certain halophytes exhibit dimorphism (or even polymorphism), notably *Arthrocnemum, Atriplex, Chenopodium, Cakile, Salicornia, Salsola, Spergularia, Suaeda* and *Trianthema* (Ungar 1977, Redondo-GÃ³mez et al. 2008, Redondo-Gómez et al. 2008, Wang et al. 2008). However, whether these morphs in halophytes are the result of phenotypic plasticity or genetic variation is not well understood; this will be addressed in this thesis.

1.2.3 Seed germination

The germination of halophytic seeds has been widely studied but there is still much to learn (Khan 1999); of the 2,400 or so species that have been investigated (Lieth *et al.* 2008), the germination characteristics have been studied in depth for only a few hundred species. Nevertheless, it is known that a key factor in halophytic seed germination is level of salinity, although temperature, light, life form, habitat, water etc. are also important drivers (Khan and Ungar 1997). Ecologists and geneticists would be greatly interested to know whether there are any patterns or dependencies among these factors, and so this study also endeavours to identify any such germination characteristics.

The germination of halophytes is inhibited by salinity through four main mechanisms: i) salinities above a certain species-specific threshold totally halt the germination process, ii) high salinities below this threshold merely delay the germination process, iii) the combination of high temperatures and salinity levels render the seed non-viable, and iv) the penetration of salt through the testa creates a chemical imbalance that interrupts the initiation of germination (Läuchli and Grattan 2007). However, halophytes vary in their responses to these stresses (Khan et al. 2001), i.e. halophytes have adopted a range of seed strategies and mechanisms to tolerate salt stress through the various stages of their lifecycle. These may be grouped into three: i) tolerating high levels of salinity whilst being stored in the soil (i.e. a seed bank strategy), ii) germinating successfully at high salinity levels, and iii) completing the lifecycle at high salinity levels.

As with the vast majority of angiosperms, light is a critical determinant for successful germination, particularly for the timing of its initiation, and phytochromic responses represent a key factor in dormancy, which in turn represents an evolutionary strategy for delaying germination in order to protect seedlings from unfavourable conditions. The response to light during seed germination varies from no germination (Khan and Gulzar 2003a, Ahmed and Khan 2010) to substantial reduction (Khan and Gulzar 2003a) to a certain level of reduction, and finally to little effect (Li *et al.* 2005). Generally, halophytic seeds only successfully germinate when a particular combination of day length, temperature and salinity interact in an optimal manner.

Of particular interest in the current study are the latter two; these have been found to significantly affect the germination of halophytic seeds. However, the effects vary widely; in some species, high temperatures inhibit seed germination and interfere with the plant’s ability to tolerate salt (Khan et al. 2006a) but in others, lower temperatures actually assist in germination (Tobe et al. 2000). Temperature is thus a key driver, and in certain species, germination is initiated under optimal temperatures, even at high levels of salinity. To further complicate the picture, the seeds of some species benefit from certain variations in soil and air temperature. This mechanism, it is argued, assists in colonizing areas where there are gaps in the vegetation, and may be seen as a strategy to exploit variations in environmental conditions in depth of burial or in the inundation regime; such temperature variation may be a competitive strategy associated with dormancy processes (Brändel 2006).

As salt is generally an inhibitor of germination, seeds may only germinate when sufficient rain has fallen to dilute the salinity level of the soil (Li *et al.* 2005). On the other hand, some halophytic species actually require a period of hyper-saline conditions before germination can be successfully initiated; in such species, high salinity levels do not compromise seed viability and they may remain dormant for extended periods until the salinity levels fall below a certain threshold (Khan et al. 2002, Song et al. 2006).

1.2.4 Chenopodiaceae

The largest halophytic family is Chenopodiaceae; it contains a great many species and genera, but the exact figures are still a matter of debate. According to (Khan *et al*. 2006c), this family contains 382 species and 63 genera. However, some researchers argue that certain species of Chenopodiaceae belong to Amaranthaceae and consequently conclude that the family has over 1,000 species. This has also resulted in some non-halophytic species being included in the family. However, species classification is beyond the remit of this research. Chenopodiaceae tend to be distributed more widely in the arid and semi-arid zones of both the northern and southern hemispheres; however, some species are also found in temperate lands, and very few are found in the tropics. They are wind-pollinated. The majority of this family can be classified as herbaceous perennials or annuals, but it also contains a few shrubs and small trees (Coile and Artaud 1997).

This family is of particular interest to researchers because of its ability to adapt to various environments; indeed, Chenopodiaceae have successfully modified many of their characteristics in order to colonize extreme environments (in terms of aridity and salinity). Their adaptive mechanisms have allowed them to increase their seed dormancy, reduce leaf area and transpiration, adopt heterocarpy, and exploit alternative photosynthetic pathways. These have enabled Chenopodiaceae to thrive in arid and semi-arid environments as other desert families do through xeromorphological adaptation (Mandak and PySek 1998).

Chenopodiaceae are invasive plants and, having high reproductive rates, are consequently good colonizers of land disturbed by human activity, by utilizing their adaptive modifications detailed above (Wang et al. 2008). A further critically important characteristic of their adaptability, for which Chenopodiaceae are particularly recognized, is seed heteromorphism; this technique is also common in both the Asteraceae and Poaceae species. The saline environment is often unpredictable, whether it is arid or marsh, and consequently, Chenopodiaceae have successfully evolved the ability to produce seeds with a wide range of characteristics. They differ in colour, shape and size, but more importantly, these visual characteristics represent varying survival, dispersal, dormancy, germination and development strategies (Wang *et al.* 2008). Accordingly, Chenopodiaceae are considered to be ecologically important pioneers, and this complex survival strategy of seed heteromorphism will be addressed at greater length later in this research.

Some species of Chenopodiaceae have been successfully developed for a variety of commercial activities; such crops now include plants grown for their sugar content and for animal feed (e.g. *Beta vulgaris* - sugar beet), as well as plants grown for industrial purposes (e.g. *Salicornia* for ash extraction in glass manufacture), *Haloxylon persicum* and *Haloxylon salicornicum*, for taking anti-desertification measures, and the valuable *Haloxylon persicum* for firewood. There are also a few examples of its exploitation for human consumption, for example, samphire (*Salicornia bigelovii*). In this context, Chenopodiaceae have the potential for further exploitation of their drought- and salt-resistant traits through genetic manipulation in order to develop other commercially viable plants for human consumption in the future (Yasseen and Abu-Al-Basal 2010).

1.2.4.1 *Suaeda*

Of the Chenopodiaceae family, one genus is of particular note: *Suaeda*. Its salt-tolerance has been widely studied and it is favoured by researchers because it contains species that are relatively easy to propagate, produce high seed yields, are diverse and relatively easy to locate in the field.

Suaeda, as a genus, is characterized by halophytic leaf-succulents and, typical of Chenopodiaceae, its species are found in both arid, saline soils and in coastal marsh lands around the world (although there are few tropical species) (Fisher *et al.* 1997). In concert with the difficulties encountered in the classification of Chenopodiaceae, the taxonomy of *Suaeda* poses problems; *Suaeda*, along with several other Chenopods, is characterized by heteromorphism. Although an important strategy for thriving in the changeable environments of salt marshes or arid saline lands (Li *et al.* 2005), dimorphism is widely evident in *Suaeda* species and all forms of polymorphism result in classification difficulties. The confusing taxonomy has also been compounded by the many and various synonyms currently in use for *Suaeda* (Fisher *et al.* 1997). Notwithstanding classification difficulties generally, the variability in the maximum levels of salt to which halophytes are tolerant has also been widely studied, and it has been reported that this variation in maximum tolerance in *Suaeda* halophytic species is between 400 and 1000 mM NaCl (Li *et al.* 2005).

Seed dimorphism is a particular characteristic of *Suaeda*, with several species producing both brown and black seeds simultaneously; as mentioned above, these varying colourations reflect, for example in *Suaeda splendens*, two different dormancy and germination strategies (Redondo-Gómez et al. 2008). A further adaptive mechanism resulting from *Suaeda*'s exposure to stressful environments is the use of C_4 photosynthetic pathways. This genus has both C_3 and C_4; the latter, within its epidermis, has water-storage tissues located between two layers of chlorenchyma cell tissues (one upper and one lower) (Fisher *et al.* 1997).

1.2.4.2 *Suaeda maritima*

Of the *Suaeda* genus, the current research is focused particularly on *Suaeda maritima* (L.) DUMORT, also known as Seablite, which is an annual obligate halophyte that has the distinctive feature in that it thrives in both coastal and inland salt marshes. It is a model plant and its salt-tolerance has been extensively investigated (Flowers et al. 1977, Yeo 1981, Greenway and Munns 1983, Hajibagheri et al. 1985, Clipson 1987). It is found throughout Europe, on most sea coasts and many inland saline marshes. It is also widespread across Russia, East Asia and South India, and has recently been recorded in the Americas and Australia (Polić *et al.* 2009). However, in the UK, it is found only in coastal salt marshes (Yeo and Flowers 1977).

This research is focused on the analysis of the phenotypic variations within and between local populations of the salt tolerant *Suaeda maritima* at Stiffkey salt marsh. *Suaeda maritima*, which is common and widespread in UK salt marshes, produces seeds in abundance, and so is a suitable choice for this research. In British salt marshes, the seeds of *Suaeda maritima* germinate in late March to April, and the plant's growing season is from April to October. Thus, its dormant period lasts from October to March, i.e. about five months (Wetson *et al*. 2008). Also, it is a fast-growing plant, which facilitates its study under laboratory conditions, and there is an extensive body of knowledge available, affording the opportunity to compare and complement this research with others.

The *Suaeda maritima* species description in relation to the current study sites has been mentioned briefly by (Smith 1985). In the high marsh, the common form of *Suaeda maritima* resembles the description by (Clapham et al. 1962) of *Suaeda maritima* var. *flexilis*. On much higher parts of the high marsh close to the creeks, a distinctive prostrate form of *Suaeda maritima* occurs which resembles the description by (Clapham et al. 1962) of *Suaeda maritima* var. *macrocarpa*. On the edges of these creeks there is an abundant form of *Suaeda maritima* where its growth is much more vigorous than in the general parts of the high marsh. Both of the high marsh forms of *Suaeda maritima* occur on the top of low marsh site. On the low marsh the *Suaeda maritima* is distinctive from the high marsh forms, resembling the description by (Clapham et al. 1962) of *Suaeda maritima* var. *maritima*. The description of *Suaeda maritima* populations addressed by (Clapham et al. 1962) is mentioned in Chapter 3 besides other descriptions for this species.

It has been well documented that the *Suaeda maritima* plant is an extreme halophyte that thrives in 100-350 mM (Flowers *et al*. 1977) (Yeo and Flowers 1980). Of particular interest with respect to its potential for exploitation, *Suaeda maritima* has significant salt accumulating properties, indeed, the vacuole can tolerate 500-600 mM (Maathuis *et al*. 1992), allowing its cultivation in highly saline lands with the intention, after repeated cropping, of extracting salts from the soil (Ravindran *et al.* 2007).

1.3 Effects of salinity and waterlogging on halophytes

1.3.1 Effects of salinity

Salt has consequences for the morphological, physiological and biochemical processes of plants but there are three key areas of concern: soil salinity can lower the water potential, introduce Na and Cl, both of which are toxic, and reduce the plant's ability to absorb nutrients (Flowers and Flowers 2005). These can result in stunted growth and a weakened plant, prone to injury, and ultimately in untimely death; these are similar to dehydration stress, and to a lesser extent, heat and cold stress. Thus, the consequences of salinity are various; osmotic effects and the aforementioned toxicity of Na^+ and Cl^- can result in plant injury. Chlorosis on leaf margins may be early evidence of Cl^- contamination, which may develop into scorching of the full leaf surface. Na^+ contamination may result in mottling and necrotic patches on the leaves, but excessively high levels can cause leaf abscission and dieback, resulting in the loss of leaf-bearing branches. Moreover, (Kozlowski 1997) reported that salt contamination not only suppresses shoot and leaf growth generally, but also interferes with leaf initiation. Salt accumulation can also have consequences for the reproductive cycle, including flowering, pollination, fruit growth, yield mass and seed development and viability. Poor seed viability has been found in both halophytic and glyophytic plants; salt lowers the osmotic potential of the soil solution available to seeds, and is toxic to embryos (Khan *et al*. 2006a).

1.3.2 Effects of waterlogging

Waterlogging can seriously disturb the normal physiochemical processes necessary for plants to function; there are four key difficulties for the waterlogged plant: a) reduced aerobic root respiration, which may reduce the root's ability to absorb and transport essential nutrients and to restrict the uptake of sodium chloride (of particular importance in halophytes), b) increased metabolic processes in order to maintain a vacuole osmotic potential that is higher than that of the saline soil solution, c) increased absorption of the available levels of reduced iron and manganese, and d) impaired hormonal metabolism and photosynthesis (Parent et al. 2008)..

According to (Barrett-Lennard 2003), waterlogging affects plants in three particular ways; it interferes with the plant's ability to grow through stunting root development and advancing the natural aging processes, which reduces growth at the leaf-bearing shoots and increases die-back. It also interferes with solute movement across membranes, which includes the absorption of inorganic nutrients, the regulation of cytoplasmic pH and membrane potentials, and the efflux of internal cell constituents such as k^+, Cl^-, organic and amino acids, and 'basic and acidic metabolites'. Finally, waterlogging also has implications for the ability of the leaf stomata to regulate leaf water potentials.

1.3.3 Combined effects of salinity and waterlogging

To date, most of the research conducted on halophytes has concentrated on their tolerance to high levels of salinity, although inundation has sometimes been considered. However, very little research has been conducted on the combined effects of salinity and waterlogging, even though the effects on plants of this synergism may be a key driver of zonation. (English and Colmer 2011) have observed that the zonation of halophyte communities may be influenced by the elevational gradient (and therefore inundation frequency) as well as salt concentration in the soil but it may also be the case that waterlogging is a function of gradient, and that therefore it is tolerance to waterlogging as well as salinity that determines where halophyte communities thrive.

(Song 2009) briefly addressed the combination of waterlogging and salinity, observing that waterlogging can dramatically reduce crop yields and increase mortality regardless of the levels of salinity. (Barrett-Lennard 2003) also mentioned that, although there is little hard evidence, halophytes cope with waterlogging within plant tissue through the formation of respiratory tissue (aerenchyma) and of an endodermis for regulating the uptake and transport of ions, through reducing stomatal conductance, and through efficiently removing salt from their metabolic processes.

The combination of waterlogging and salinity has received little attention on the part of researchers, and this may be a reflection of the fact that in any given location, neither aspect is constant and, accordingly, the interplay between them is highly complex. This is compounded by the fact that waterlogging is difficult to quantify, particularly in areas that are subject to unpredictable waterlogging events. The picture is complicated by the varying ability of halophytes to emit water vapour through stomata, and by their varying tolerance to waterlogging in the different life-cycle stages, such as during germination and flowering. However, in coastal areas, waterlogging may be quantified, as inundations and water-table fluctuations are regular, both spatially and temporally, and averages for the concentrations of water in soil can be calculated (Belford et al. 1980, Ball 1998, Barrett-Lennard and Malcolm 1999, Hatton et al. 2002).

1.4 Research aim and objectives

The main aim of this research is to investigate the phenotypic variations within and between the local populations of the halophyte *Suaeda maritima* at Stiffkey salt marsh, on the north Norfolk coast, and two other, different creek bank populations (from the Tollesbury and Abbot's Hall salt marshes), UK. This research is conducted to add to our understanding of the strategies they utilise in order to cope with the steep environmental gradients, and to determine whether any phenotypic variations are the result of plasticity or genetic differentiation. In order to address this aim, a number of objectives need to be met, and these are addressed in the chapters as follows:

Chapter 2: provides an overview of the physical environmental factors of the study site.

Chapter 3: characterizes the phenotypic variations within and between *Suaeda maritima* populations, in terms of shoot and root growth parameters. This will serve to deepen our understanding of how the observed inter- and intra-specific variations may act as an adaptive response to environmental heterogeneity.

Chapter 4: examines seed heteromorphism within and between *Suaeda maritima* populations, in terms of seed mass and seed morphology in order to explain how such phenomena may be related to temporally unpredictable or spatially heterogeneous environments.

Chapter 5: examines the variations in survival, morphology and fecundity of the *Suaeda maritima* populations in a reciprocal transplant experiment in the field, in order to identify the relative importance of genetic variation and phenotypic plasticity in terms of the variations observed in these natural populations.

Chapter 6: examine variations in survival, morphology and fecundity of the *Suaeda maritima* populations under artificially controlled growth chamber conditions in order to confirm whether or not the *Suaeda maritima* populations' overall performance is plastic and/or genetic.

Chapter 7: characterizes variations in seed germination behaviour of the *Suaeda maritima* populations in order to evaluate the combined effect of salinity and certain other abiotic factors (including; temperature, light and different storage conditions) on *Suaeda maritima* seed germination in such a way as to reflect the adaptation of those populations to the local environmental conditions in the field.

Chapter 8: presents a general discussion of the preceding chapters and an evaluation of their results in order to draw conclusions and suggest recommendations for future research.

Chapter 2

The study sites

2.1 Introduction

The current study of *Suaeda maritima* populations was based mainly at Stiffkey salt marsh on the north Norfolk coast of England, UK (52°57’N, 0°55’E) (Fig. 2.1). Stiffkey salt marsh consists of two distinctive marshes: low and high, which are separated by a shingle ridge that runs parallel to the coastline (Jefferies et al. 1981). There is also a distinctive creek system within the high marsh that drains into a channel, passing around the western end of the dividing ridge and thence into the sea, whereas the low marsh creeks drain directly into the sea (Noble et al. 1992). Due to the existence of the shingle ridge in the middle of the marsh, tidal water does not pass directly between the low and high areas of the marsh, but through a complex system of minor creeks connected with a primary drainage channel “Cabbage creek”, which passes around the western end of the ridge. The result is a physiographically complex site which represents a wide range of environmental heterogeneity, reflecting the overall elevational gradient from land to sea, the drainage system and local microtopography.

Suaeda maritima occurs widely in this mosaic of local environments and therefore a series of sites was selected in order to investigate its potential for local adaptation. It has long been appreciated that variation in elevation in the tidal frame is a major determinant of tidal inundation and therefore potentially of variation in sediment salinity (Jefferies, Davy & Rudmik 1979, 1981; Noble, Davy & Oliver 1992) and redox potential (Armstrong et al. 1985). More recently it has been appreciated that factors acting independently of elevation such as localised variation in topography, sediment structure and drainage conditions, through their effects on waterlogging also contribute significantly to the redox environment experienced by plants (e.g. Davy et al. 2011; Mossman *et al*. 2012). Four more or less internally homogeneous sites that supported *Suaeda maritima* populations were selected to encompass the known ranges of environmental heterogeneity at Stiffkey: high marsh creek bank, high marsh, top of the low marsh and low marsh (Fig. 2.1).

Figure 2.1 Aerial photograph of the Stiffkey salt marsh showing the location of the four sites that were used to represent the *Suaeda maritima* populations studied. Source; Google maps 2012.

The low marsh, with a hydrological regime of consistent semi-diurnal tidal inundation, is characterized by individual clumps and mounds (up to 25 cm high) of *Limonium vulgare*, *Puccinellia maritima*, *Aster tripolium* and *Atriplex portulacoides*, and of areas of *Salicornia europaea* and *Suaeda maritima*; it also has numerous shallow salt pans some of which occupied by *Spartina anglica*. The pioneer zone of the low marsh is dominated by the tetraploid *Salicornia dolichostachya*.

The bar of shingle that divides the lower from the upper marsh has upper flanks dominated variously by *Juncus maritimus*, *Plantago maritima* and *Triglochin maritima*, and by *Aster tripolium* and *Armeria maritima* immediately adjacent to the ridge (Jefferies 1977). The lower part of the seaward flank is a shingle-rich substrate, with abundant *Suaeda maritima* and *Salicornia pusilla*, that is designated ‘top of the low marsh’.

In contrast to these two low marsh zones, the upper marsh experiences inundation generally by a fortnightly spring tide regimes, although it is usually not inundated at all for two to four-week periods on either side of the solstices (Noble et al. 1992) (Davy and Smith 1985). This zone has plant communities that are relatively diverse compared to the lower marsh zone and include *Armeria maritima*, *Limonium vulgare*, *Atriplex portulacoides*, *Plantago maritima*, *Triglochin maritima* and *Salicornia ramosissima*, *S, pusilla* and *S. europaea* egg. and apparently two distinct forms of *Suaeda maritima*. The interfluves (away from the creeks) have generally small, reddish-coloured individuals, whereas the major creek banks have large, green, vigorous individuals. Some lower-lying areas are populated by *Pucinellia maritima*, and there are pure stands of *Elytrigia atherica* on the uppermost parts,(Jefferies 1977, Davy and Smith 1985, Noble et al. 1992) and (Boorman and Ashton 1997).

In addition to the main sites at Stiffkey, plants were also collected on one occasion from another salt marsh and managed realignment site in Essex for comparison. The Tollesbury salt marsh (TL958118) of 1103 ha of salt marsh fringing the Blackwater Estuary, Essex and is in close proximity to the Abbots Hall managed realignment (Boorman and Ashton 1997). The creeks in this location are up to 1.5 m deep and often drain to larger creeks close to sea walls (Paramor and Hughes 2004). Abbotts Hall managed realignment salt marsh is located on the Salcott Channel, a side channel of the Blackwater Estuary, Essex (TL962133). The 80 ha site was historically salt marsh prior to reclamation for agriculture (Hughes et al. 2009). A scheme to restore this marsh was initiated in 2002 in order to mitigate flood risk; sea defences were breached and tidal inundation was restored to the site allowing salt marsh plants to colonise (Defra 2003).

2.2 Material and methods

The environmental conditions, including elevation in relation to the tidal frame, sediment redox potential, salinity, and water and organic contents, of the four Stiffkey sites were characterized in April - May 2012. Sediment conditions were characterized following a neap and a spring tide.

2.2.1 Elevation

In each of the four sites, elevation relative to the UK reference mean sea level (Ordance Datum Newlyn, ODN), was measured at 25 random points, using a differential GPS (Topcon, Newbury, UK), with an accuracy of <2 cm and precision of <1.5 cm.

The annual number of tidal inundations was calculated from elevation and predicted tidal heights that were calibrated by local measurements of tidal height, based on measurements obtained from depth-sensing fish tag technique; see (Mossman et al. 2012b) for a description of methods.

2.2.2 Sediment water and organic content, and salinity

Three soil samples (10 cm ±2 cm deep, 5 cm diameter) were taken at each site following a spring tide and three samples following a neap tide. Soil samples were placed in sealed polythene bags and stored at 4 °C within 48 h. Subsamples were analysed in the laboratory for gravimetric soil water content and organic matter (percentage loss on ignition after 16 h at 390 °C) in accordance with standard methods (Matter 1982). Two replicates of gravimetric water content and loss on ignition were made for each sample and the mean used.Water extractions were prepared (1:5 sediment:water; two replicate extractions for each sample; Matter, 1982) for electrical conductivity measurement by a conductivity electrode and meter (Jenway 470, Barloworld Scientific Ltd. Essex, UK).

2.2.4 Redox potential

In order to obtain reliable characterization of the redox conditions, specially constructed field electrodes were used for semi-permanent installation which allowed for sufficient time for equilibration before recordings were made and thereby offered a significant advantage over spot redox measurements.

Platinum tipped electrodes were constructed based on established designs (Mann and Stolzy 1972, Mueller et al. 1985, Faulkner et al. 1989) using 24 gauge (diameter 0.5 mm) platinum wire soldered to one end of a 300 mm (ø 3.2 mm) brass alloy rod. A short length of insulated Ag–coated Cu wire was soldered on the other end to serve as a connection terminal to a portable pH/mV meter (Hanna Instruments HI 9025). To prevent water ingress junctions were sealed with two layers of marine-grade epoxy resin and the entire electrode was encapsulated with adhesive heat-shrink tubing leaving exposed approximately 3mm of platinum wire at one end and 10mm of the terminal connection at the opposite end. The Pt tips were polished with a fibreglass abrasion pen to remove any oxidised layer and tested for correct functioning both before and after installation in the field using Zobell’s calibrating solution. Acceptable electrodes were all within 231±30 mV with a Ag/AgCl reference electrode (Sentek, UK). A double-junction Ag/AgCl reference electrode was used to reduce the possibility of interferences by sulphides that may be present in the sediment (Kolling 2000).

Twenty electrodes were installed at each of the four sites (upper marsh, creek bank, upper low marsh and low marsh) at locations close to where *Suaeda maritima* was sampled and at two depths (5 and 10 cm) spanning the bulk of the rooting zone. The electrodes were allowed approximately two weeks to stabilise in the sediment before the first redox recordings were taken. Redox potential measurements were taken once within 2 hours of high tide during one neap and once during two spring tide phases. Standardisation of measurements was obtained by applying the appropriate +200mV correction factor for the Ag/AgCl reference electrode. Using this technique the majority of the redox measurements were found to be immediately stable on recording or displayed short-term drift which stabilised within 30 minutes. Less than 10% of the measurements continued to show drift after 30 minutes at which point a reading was taken and assumed to represent one of four broad redox categories: aerobic at values ≥ +350 mV, hypoxic and moderately reducing between < +350 and 0 mV and anoxic and highly reducing at <0 mV.

2.3 Results

2.3.1 Elevation and inundation frequency

As expected the high marsh had the highest elevation followed by the top of low marsh, the creek bank and the low marsh (Fig. 2.2). Although the creek banks are potentially the highest parts of the marsh, the creek bank site was on the slumped inner bank of the levee and therefore at lower elevation than the high marsh interfluve. The four sites were significantly different from each other in terms of elevation.

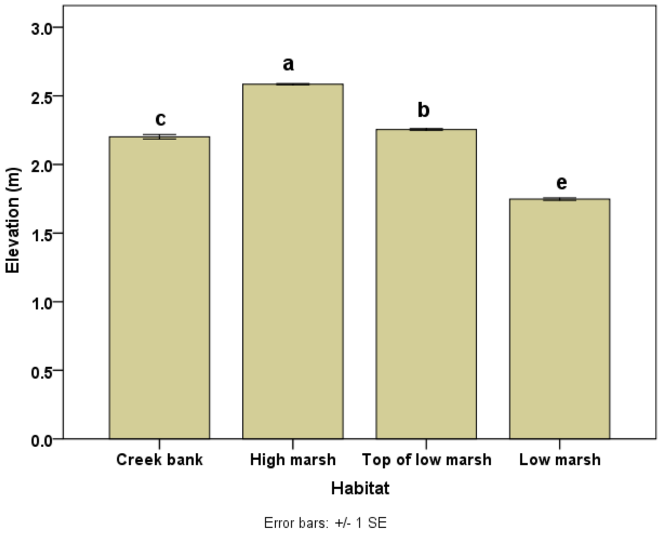


Figure 2.2 Comparisons (means ±S.E) of elevation above ODN (m) among four sites on Stiffkey salt marsh; Creek bank (*n* = 30), High marsh (*n* = 30), Top of low marsh (*n* = 20) and Low marsh (*n* = 30). ($F_{3, 101}$ = 1037.9, $p < 0.001$). Bars with shared letters are not significantly different, as determined by two-way ANOVA (Tukey tests, $p < 0.05$).

The annual number of tidal inundations at the four sites, derived from elevation and recorded tidal heights inevitably closely reflected the differences in elevation (Fig. 2.3). The greatest number of annual inundations was found to occur on the low marsh and the fewest on the high marsh, with creek bank and top of low marsh intermediate. All these differences were found to be significantly different.

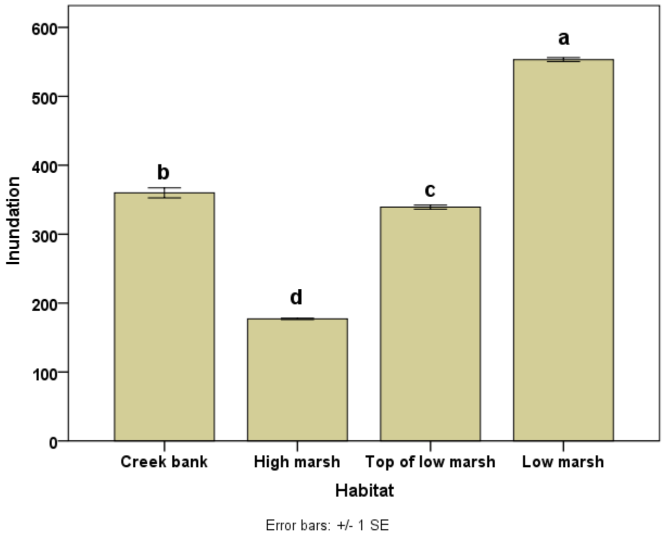


Figure 2.3 Comparisons (means ±S.E) of annual number of inundations **per year** among four sites on Stiffkey salt marsh; Creek bank (*n* = 30), High marsh (*n* = 25), Top of low marsh (*n* = 20) and Low marsh (*n* = 30). ($F_{3, 101}$ = 1167.9, $p < 0.001$). Bars with shared letters are not significantly different, as determined by Two-way ANOVA (Tukey tests, $p < 0.05$).

2.3.2 Sediment water and organic contents

Water content was higher in the sediments at the high marsh sites than in those of all the other sites; water content was significantly lower at the top of the low marsh and significantly lowest on the low marsh and creek bank (Fig. 2.4).

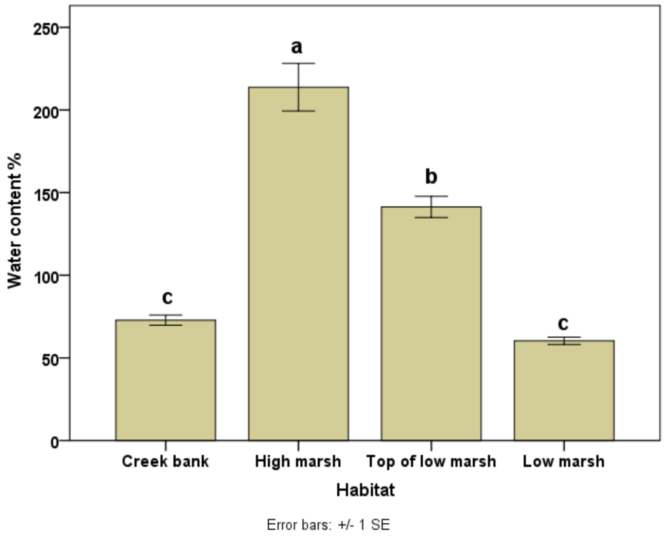


Figure 2.4 Comparisons (means ±S.E of spring and neap tide samples) of soil water content (%) among four sites on Stiffkey salt marsh; Creek bank (*n* = 6), High marsh (*n* = 6), Top of low marsh (*n* = 6) and Low marsh (*n* = 6). ($F_{3, 20}$ = 76.19, $p < 0.001$). Bars with shared letters are not significantly different, as determined by Two-way ANOVA (Tukey tests, $p < 0.05$).

Sediment organic matter content ranged from 9.7 to 12.4% of dry mass and was not significantly different between the four sites (Fig. 2.5).

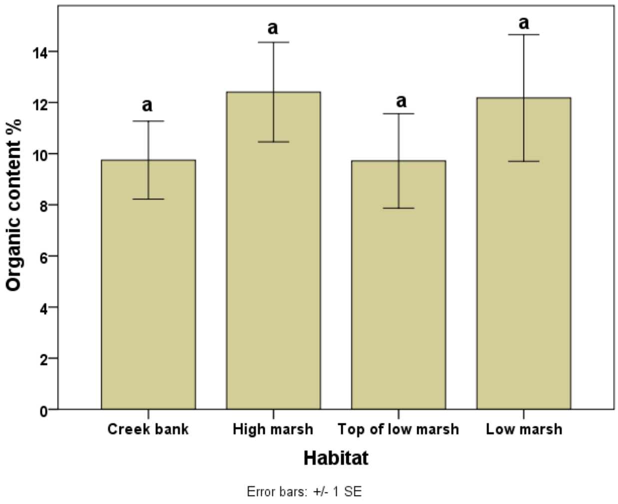


Figure 2.5 Comparisons (means ±S.E of spring and neap tide samples) of sediment organic content (%) among four sites on Stiffkey salt marsh; Creek bank (*n* = 6), High marsh (*n* = 6), Top of low marsh (*n* = 6) and Low marsh (*n* = 6). ($F_{3,\,20} = 0.56$, $p < 0.647$). Bars with shared letters are not significantly different, as determined by Two-way ANOVA (Tukey tests, $p < 0.05$).

2.3.3 Sediment salinity

Sediment salinity was found to be highest on the high marsh and top of the low marsh, which were not significantly different (Fig. 2.6). Similarly, the rather lower salinities on the creek bank and low marsh were not significantly different.

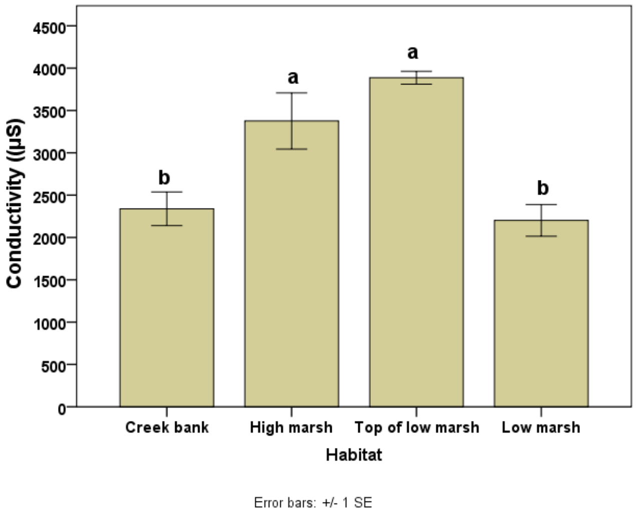


Figure 2.6 Comparisons (means ±S.E of spring and neap tide samples) of soil conductivity (µS) among four locations on Stiffkey salt marsh; Creek bank (*n* = 6), High marsh (*n* = 6), Top of low marsh (*n* = 6) and Low marsh (*n* = 6). ($F_{3,\,20} = 13.98$, $p < 0.001$). Bars with shared letters are not significantly different, as determined by Two-way ANOVA (Tukey tests, $p < 0.05$).

2.3.4 Sediment redox potential

Sediment redox potential (Fig. 2.7) varied considerably between the four sites, over the spring/neap tidal cycle and even between consecutive spring tidal periods. There were only small differences in readings between the two depths of measurement. The effects of site (habitat) and date of measurement were highly significant (Table 2.1). Overall, the low marsh and the creek bank sediments were the most oxidising root environments and the top of low marsh was generally the most reducing. Redox potentials were generally lower with more extreme spring tides.

Table 2.1 Three-way ANOVA for sediment redox potential at the four sites (Creek bank, High marsh, Top of low marsh and Low marsh) at Stiffkey salt marsh

Source of variation	**Type lll SS**	**df**	**F**	**P**
Date	1706965.9	2	13.27	0.001
Habitat	3337911.6	3	17.29	0.001
Depth	65884.4	1	1.02	0.313
Date*Habitat	266443.9	6	0.69	0.658
Date*Depth	19928.3	2	0.16	0.857
Habitat*Depth	577721.3	3	2.99	0.032
Date*Habitat*Depth	63016.9	6	0.16	0.986
Error	1.4	211	-	-

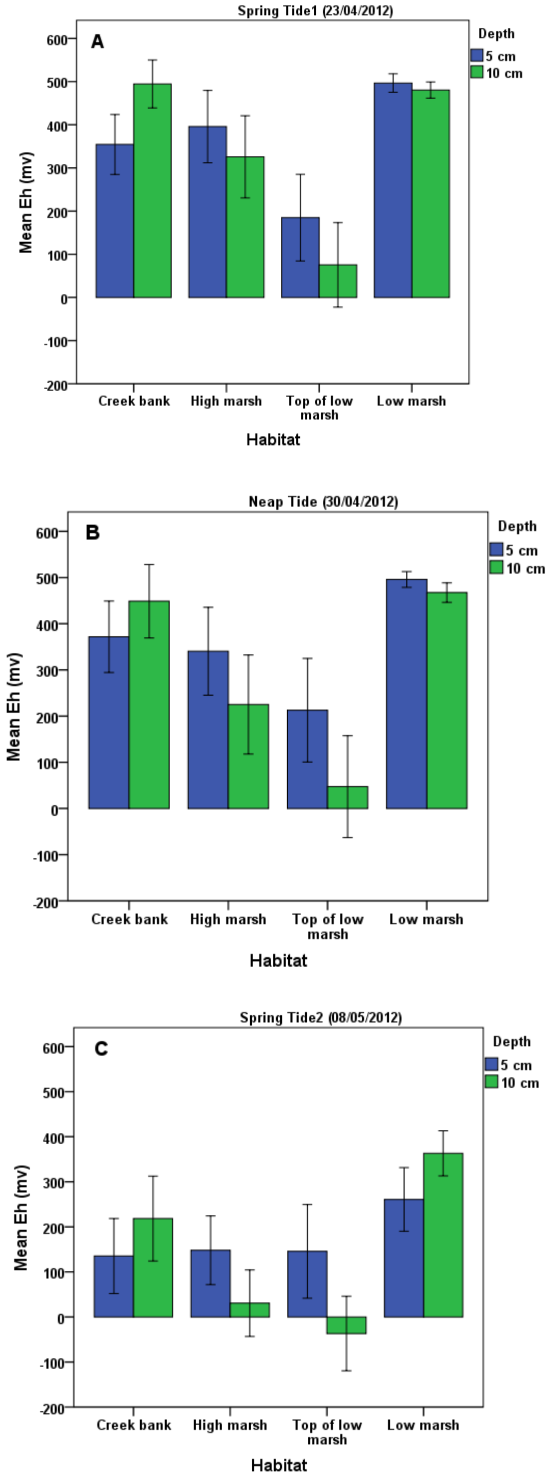


Figure 2.7 Comparisons (means ±S.E) of sediment redox potential among four locations (Creek bank, High marsh, Top of low marsh and Low marsh) on Stiffkey salt marsh taken over three consecutive spring/neap tidal regimes; (a) spring tide 1, (b) neap tide and (c) spring tide 2. Readings are means of sample taken at two depths (5 and 10 cm).

2.4 Discussion

The relative elevations of the high marsh interfluve, top of low marsh and low marsh sites were in line with previous estimations (Jefferies, Davy & Rudmik 1979; Davy & Smith 1985, 1988) and recent measurements (Mossman et al. 2012b) of the structure of Stiffkey marsh. Despite the presence of raised creek levees, the creek bank site was rather lower than the high marsh interfluve area because the characteristic population of *Suaeda maritima* was mainly on the slumped sides of the creek. Altogether there was a range of 83 cm in the tidal frame between low and high marsh sites. These elevations translated into large, corresponding differences in the number of tidal inundations per year, ranging from 177 yr^{-1} on the high marsh to 553 yr^{-1} on the low marsh. This emphasizes the large differences in local environmental conditions experienced by *S. maritima* on the marsh.

The water content of the sediments did not entirely follow elevation. The high marsh site had much the highest water content, reflecting relatively poor drainage that is largely the result of raised levees separating this site from the creek system. The creek bank and low marsh sites had lower water contents as a result of their good drainage.

There were no significant differences between sites in sediment organic matter content and so this could not have been contributing to the differences in water retention.

The low marsh and creek bank sites would be expected to maintain sediment salinities close to those of sea water, given their close proximity to tidal exchanges. The high marsh and top of low marsh sites, however, had higher salinity than the other two sites, even in spring when evapotranspiration would be unlikely to be greatly exceeding precipitation to produce hypersalinity. Clearly there is the potential for significant hypersalinity here later in the growing season, particularly in sunny, dry summers, as recorded previously by Jefferies, Davy & Rudmik (1979).

Sediment redox potential is increasingly recognized as a possible determinant of plant distribution on salt marshes (Armstrong et al. 1985; Davy et al. 2011). It has been found to vary in time and space in a complex manner. Similar variation in redox potential was also found to occur at Stiffkey during the sample period April and May of 2012. It showed considerable variation between neap and spring tidal phases and even between successive spring tides, as would be expected from the influences of tidal inundation flooding the sediment. Redox potential tended to be lowest during the spring tides, when the more extreme high tides would have delayed tidal water draining off the marsh. There was little variation between measurements at two depths in the rooting zone though. Overall, and perhaps counter-intuitively, the most reducing conditions were found to occur on the high marsh interfluve area at this time of year but this is in agreement with the much higher sediment water content at that site; such a high degree of saturation would limit diffusion of oxygen from the atmosphere. However, near the summer solstice, when spring tides do not cover these higher elevations and evapotranspiration is high, conditions are likely to be very different and highly oxidising. Since soil redox potentials are microbially driven and hence likely to be temperature dependent and salinity variation is linked to evapotranspiration, these two factors are bound to vary seasonally and the high marsh could be expected to experience greater seasonal extremes than at the other three sites.

A complete characterization of the rooting environments at the field sites at Stiffkey would require detailed monitoring over the spring/neap tidal cycles of several growing seasons. However, the measurements of elevation and consequent frequency of inundation, along with representative measurements of water content, salinity and redox potential serve to demonstrate that *Suaeda maritima* populations at these sites do experience divergences between their environments that could both affect the phenotypic responses of developing plants and constitute selection pressures for the evolution of genetically differentiated populations.

Chapter 3

Field characterization of local populations of *Suaeda maritima*

Abstract

- ***Background and Aims:*** *Suaeda maritima* is an annual species of halophyte inhabiting coastal and inland salt marshes. It is known to exhibit clear variability in morphology, manifested in the development of markedly distinctive forms of growth in distinct parts of the marsh. The main aim of this study is to characterize the phenotypic variations within and between *Suaeda maritima* populations at Stiffkey salt marsh on the north Norfolk coast, and two other, different creek bank populations (from the Tollesbury and Abbot's Hall salt marshes), UK.
- ***Methods:*** Six morphological traits were characterised (plant height, shoot width, number of primary branches, number of secondary branches, main root length and root system width) within and between four local populations of *S. maritima* (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey during three consecutive summers (2008-10). Also, the same traits were investigated in 2008 in creek bank populations of *S. maritima* from two salt marshes in Essex: Tollesbury and Abbot's Hall.
- ***Key Results:*** All Stiffkey populations are morphologically distinctive in accordance with their distribution, which is strongly correlated with marsh topography. The creek bank population exhibits the most vigorous growth, followed by the low marsh, the top of the low marsh and the high marsh, respectively. Both the Stiffkey and Tollesbury creek bank populations showed more vigorous growth than the Abbot's Hall one.
- ***Conclusions:*** The current study suggests that there are considerable variations between the Stiffkey populations and between the creek bank populations from Tollesbury and Abott's Hall; these variations are assumed to be controlled by the biotic and abiotic factors of the local sites of each population. Consistent variations between the Stiffkey populations have been observed in all years, raising the question as to whether it might be explained as genetically driven.

3.1 Introduction

The various patterns of halophyte distribution and apparent zonation in salt marsh ecosystems have been the subject of considerable research over recent decades (Engels et al. 2011). The low species diversity and distinct patterns of zonation that characterise salt marshes make them an ideal subject for investigations that aim to explain how plant community patterns may be related to environmental gradients and competitive interactions with other plant species on salt marshes. The relative simplicity of these ecosystems readily permits opportunities for experimental manipulations in order to investigate how the interplay between these abiotic and biotic factors may explain the development and persistence of these distributional patterns (Vince and Allison 1984, Bertness 1991, Pennings et al. 2003, Silvestri and Marani 2004, St. Omer 2004).

The abiotic component is strongly related to the topographic elevation of the salt marsh since this governs the frequency and duration of tidal inundations which, in turn, determines the extent and degree of soil anoxia and salinity in the sediment porewaters (Davy et al. 2011, He et al. 2011). Soil anoxia not only manifests as an oxygen deficit in the rhizosphere but also as the low redox potentials that develop under anaerobic conditions which can result in the accumulation of potential phytotoxins in the form of reduced chemical species such as Mn(II), Fe(II) and sulphides (Rozema et al. 1985, Pearson and Havill 1988). These may either be directly toxic to plants if sufficient concentrations accumulate or may inhibit nutrient uptake and plant growth at lower concentrations. In addition to the sea-landward elevational gradient, the network of drainage creeks and smaller-scale topographic features on the marsh surface reflect the considerable spatial heterogeneity in terms of soil drainage conditions and hence anoxia that are independent of elevation (Crooks et al. 2002).

Although the abiotic factors imposed by environmental gradients play a key role in zonation, there have been a number of studies based on experimental manipulations of salt marsh plants which have demonstrated that the interplay of biotic factors may be as important. Central to this biotic consideration are the plant species' differential abilities to tolerate salt stress, waterlogging and the effects of anaerobiosis on its competitive ability (Pennings and Callaway 1992, Crain et al. 2004).

Halophytic species growing in the heterogeneous salt marsh environment respond to abiotic and biotic stress factors through various mechanisms. There is strong evidence among salt marsh plants of the existence of phenotypic plasticity, which reflects the ability of an organism to produce different physiological and morphological phenotypes in response to changing environmental conditions (Wu et al. 2004, Valladares et al. 2007). Most plants are highly plastic within certain parameters, and this plasticity is manifested in varying growth rates, sizes at maturity, reproductive characteristics and rates, and tissue chemistry. Furthermore, phenotypic plasticity, once established in a genotype, may be temporary, being exploited in a fixed manner on a seasonal scale or in a life cycle stage, or it may be dynamic, reflecting, for example, the changing levels of photosynthetic activity in response to daylight (Callaway et al. 2003).

Early work on phenotypic variation among *Suaeda maritima* populations in British salt marshes by (Chapman 1947) and (Clapham et al. 1962) resulted in the identification of three different varieties: var. *flexilis* Focke, var. *macrocarpa* Moq. and var. *vulgaris* Moq. However, both authors found it difficult to be certain about the taxonomic status of those varieties and they are not recognized in modern treatments (Stace 2010). (Boucaud 1962), (Boucaud and Ungar 1973) and (Boucaud and Ungar 1976) studied the phenotypic variation of local varieties of *Suaeda maritima* in France, and concluded that they can be distinguished morphologically and according to their seed germination behaviour. (Metzing 1996) conducted a study on the seed heteromorphism of two *Suaeda maritima* populations in Germany (one growing on the upper parts of the low marsh and the other on the lower parts), and concluded that there was considerable variation between the seed sizes of the two populations.

Suaeda maritima is considered to have a wide tolerance to edaphic conditions (Chapman 1947) and can be found over a wide amplitude in the salt marsh and frequently abundant in areas that are particularly challenging to perennials such as the edges of hollows and along tidal creeks, as well as pioneer low, middle and high marsh zones (Tessier et al. 2000b). Therefore, I hypothesized that a halophyte species that exhibits such a wide ranging ability to colonize across the salt marsh elevational gradient, and within different positions at the same elevation, would express greater variability in terms of its phenotype. Accordingly, this chapter will satisfy the following objectives:

1. To quantify the phenotypic variations (in terms of growth traits including; plant height, shoot width, number of primary branches, number of secondary branches, main

root length and root system width) within and between *Suaeda maritima* populations at Stiffkey, Tollesbury and Abbots' Hall salt marshes.

2. To study the relationships between the different plant morphological traits.

This will serve to deepen our understanding of how the observed phenotypic variations apparent in *Suaeda maritima* may act as an adaptive response to environmental heterogeneity and will provide a basis for further field and laboratory experiments that should assess whether these variations are a plastic response or there might be an element of population differentiation.

3.2 Materials and Methods

3.2.1 Sampling

Mature *Suaeda maritima* individuals were sampled from four locations within Stiffkey salt marsh (see Chapter 2 for details) with the aim to characterize important morphological traits and the relative abundance of seed morphs at each location. Initial field visit to the Stiffkey salt marsh were conducted in order to choose the possible study zones which look ecologically distinctive in light of their description in the relevant literature, and the four zones chosen were: low marsh, top of low marsh, high marsh and creek bank. Samples were collected randomly from the most abundant and homogenous *Suaeda maritima* individuals at each location (Fig. 3.1) during the fruiting periods of 2008, 2009 and 2010. The dates of sampling and the number of individual plants sampled varied between years as described below:

In 2008, sampling took place between the middle of October and the middle of November. On a weekly basis thirty plants in total were collected in this period from each of the creek bank, the high marsh, and the top of the low marsh habitats. Sampling began too late in the season to collect low marsh *Suaeda maritima* plants, which had senesced by the end of October. In 2009, sampling therefore began earlier, starting at the end of August until the middle of November. Sampling was conducted four times on fortnightly basis during this period and on each occasion twenty plants from each location were collected.

Insufficient brown seeds were collected in 2008 and 2009 to conduct the intended experimental work. Sampling intensity was therefore increased in 2010, with sampling undertaken weekly at the four locations between 22 of August and 21 of November. Twenty plants were collected from each location each week.

Suaeda maritima samples from creek bank habitats in Essex (Tollesbury and Abbotts Hall) were collected in November 2008 by Joanna Chitty. Ten *Suaeda maritima* plants from each location collected.

All collected plant material was placed in polythene bags and stored at 5° C immediately on return to the laboratory. Stored samples were measured and processed within few weeks of collection.

3.2.2 Phenotypic Trait Characterization

Each plant was characterized for a range of phenotypic traits. These were: plant height (the above-ground growth height), measured from the soil surface to the terminal bud; shoot width (the above-ground shoot width), measured as the maximum horizontal width of the branches; number of primary branches (defined as the largest stems growing near the base); number of secondary branches (defined as stems grew from nodes of primary branches); number of black seeds; number of brown seeds; total number of seeds; and number of immature seeds. A number of root trait measurements were also taken; these were main root length and root system width (defined as the maximum horizontal spread of lateral roots).

3.2.3 Statistical Analysis

Statistical analysis was carried out using PASW version 18, Chicago, USA for Windows. The main comparisons of the plant height, shoot width, number of primary branches, number of secondary branches within and between populations and years were expressed as mean ± standard error. General linear model (GLM) analysis of co-variance (ANCOVA; $P < 0.05$) was used to compare the treatment effects; collection date was added as a covariate. One-way ANOVA was applied to assess the differences among populations and years.

Linear regression was used to assess relationships between the plant morphological traits within and between populations, and years.

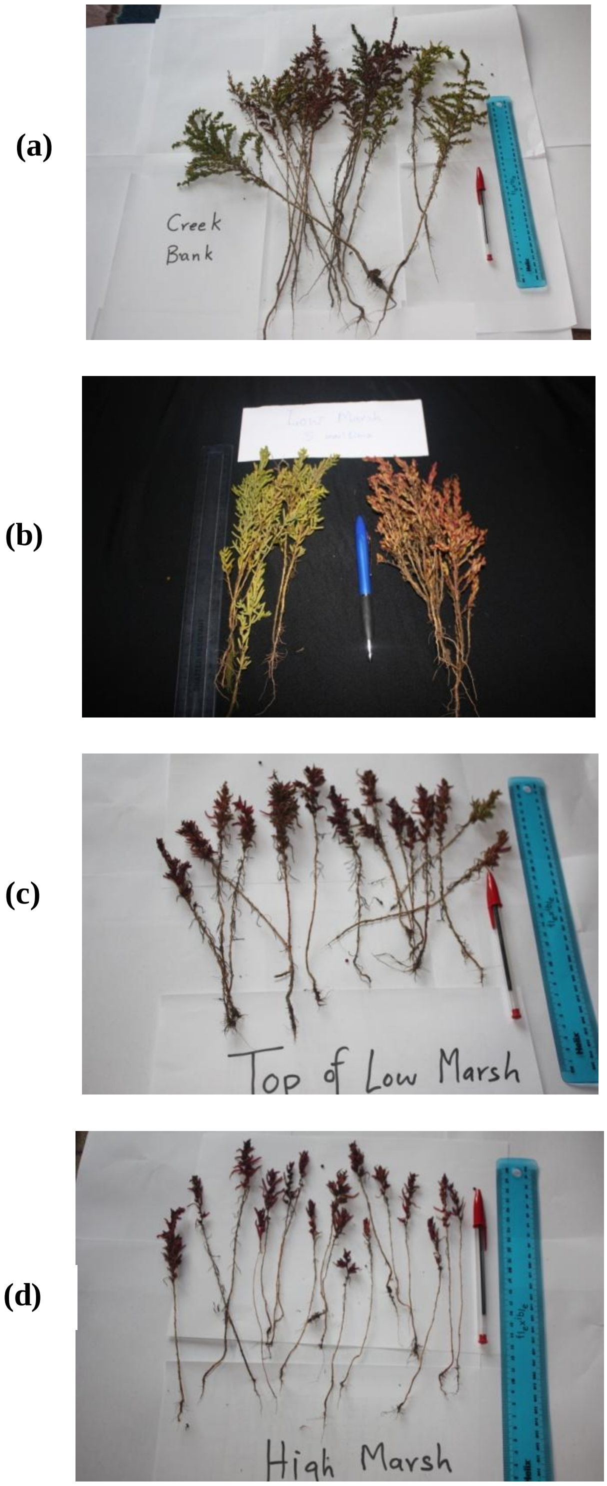


Figure 3.1: Samples of *Suaeda maritima* populations from Stiffkey salt marsh; (a**)** Creek bank, (b**)** Low marsh, (c**)** Top of low marsh, (d**)** High marsh.

3.3 Results

3.3.1 Plant height

In 2008 *Suaeda maritima* plants of the Stiffkey creek bank population were significantly the tallest, followed by both plants from Abbots Hall and Tollesbury populations (Figure 3.2). Also, with regards to the Stiffkey salt marsh populations, the creek bank plants were significantly taller than the top of low marsh and the high marsh plants (Figure 3.3).

In 2009, among the Stiffkey salt marsh populations, the significantly tallest plants were found in the creek bank, followed by plants from the top of low marsh, the low marsh, and finally from the high marsh populations. In 2010 the tallest the plants were again found to be from the creek bank population, followed by plants from the low marsh, the top of low marsh and finally of the high marsh (Fig. 3.3).

Finally, table 3.1 shows the result of the two-way ANCOVA model revealing significant variations at the levels: population, year and the interaction between population and year. Collection date was considered here as a covariate in order to allow examining the effect of the main variables (population and year) and their interaction.

Table: 3.1: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the plant height trait per plant.

Source of Variation	**Plant Height (cm)**			
	Type lll SS	**df**	**F**	**P**
Population	28904.88	3	577.60	<0.001
Year	478.84	2	14.35	<0.001
Population*Year	3634.26	5	43.57	<0.001
Error	17981.89	1078	-	-

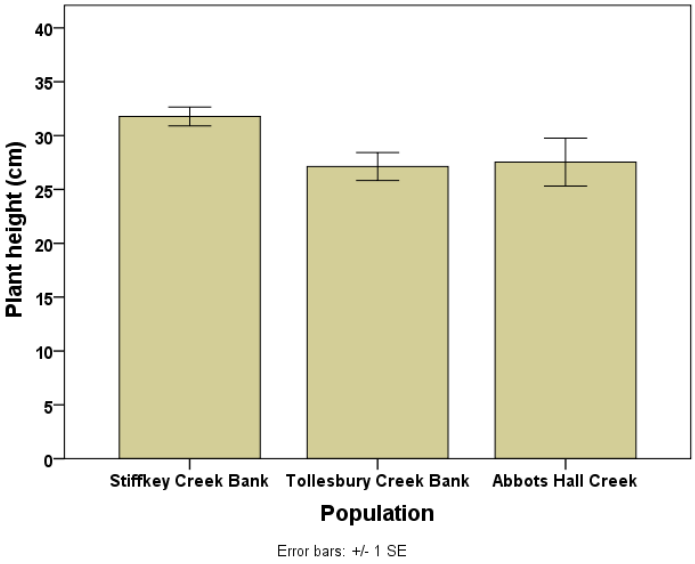


Figure 3.2: Comparisons (mean±s.e.) of *Suaeda maritima* plant height trait per plant of three different creek bank populations in 2008.

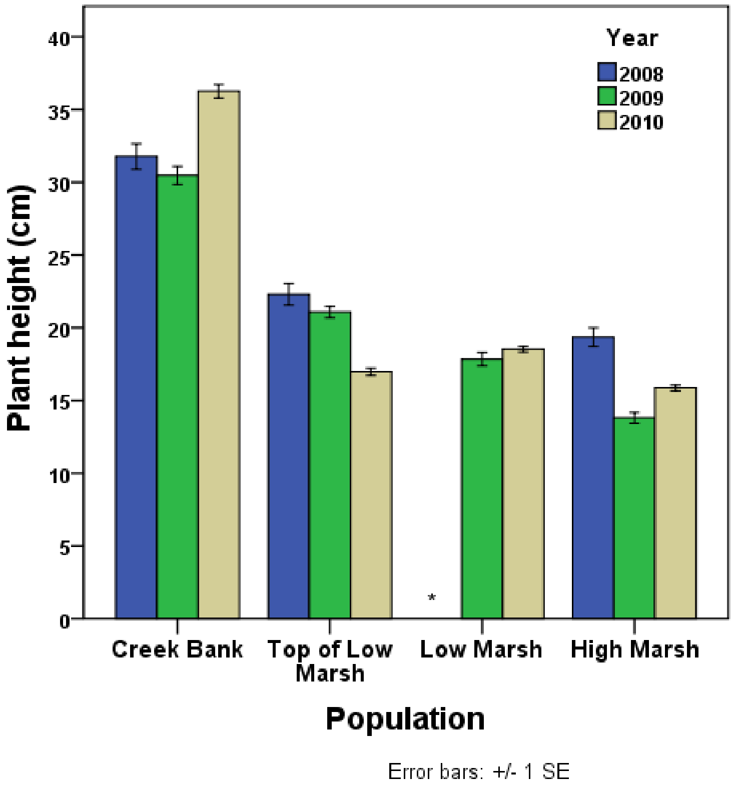


Figure 3.3: Comparisons (mean±s.e.) of *Suaeda maritima* plant height trait per plant for the Stiffkey salt marsh populations in 2008, 2009 and 2010. (* Insufficient data).

3.3.2 Shoot width

In 2008, *Suaeda maritima* plants of the Stiffkey, Tollesbury and Abbot's Hall creek banks were found to have insignificant variation between them in terms of the shoot width (Fig. 3.4). However, with regards to the Stiffkey salt marsh populations, the creek bank plants had the significantly largest shoot width, followed by top of low marsh and finally high marsh plants (Fig. 3.5).

In 2009 and 2010: between the Stiffkey salt marsh populations, significantly the largest shoot widths were achieved by plants from the low marsh, followed by plants from the creek bank, the top of low marsh, and finally from the high marsh.

The two-way ANCOVA model revealed significant variations at all levels; population, year and the interaction between population and year (Table 3.2). Collection date was considered here as a covariate in order to allow examining the effect of the main variables (population and year) and their interaction.

Linear regression was used to examine the relationship between the shoot width and the plant height traits (Fig. 3.6). There was a significant positive relationship within creek bank populations from Abbots Hall and Stiffkey in 2008. Also, the Stiffkey creek bank, the top of low marsh and the low marsh populations had significant positive relationships in all years, while within the high marsh this association was found to be significant only in 2010. Finally, for all seasons, positive associations were found at an inter-population level.

Table: 3.2: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the shoot width trait per plant.

Source of Variation	**Shoot Width (cm)**			
	Type lll SS	**df**	**F**	**P**
Population	16145.52	3	164.49	<0.001
Year	1122.12	2	17.14	<0.001
Population*Year	1171.39	5	7.16	<0.001
Error	35269.54	1078	-	-

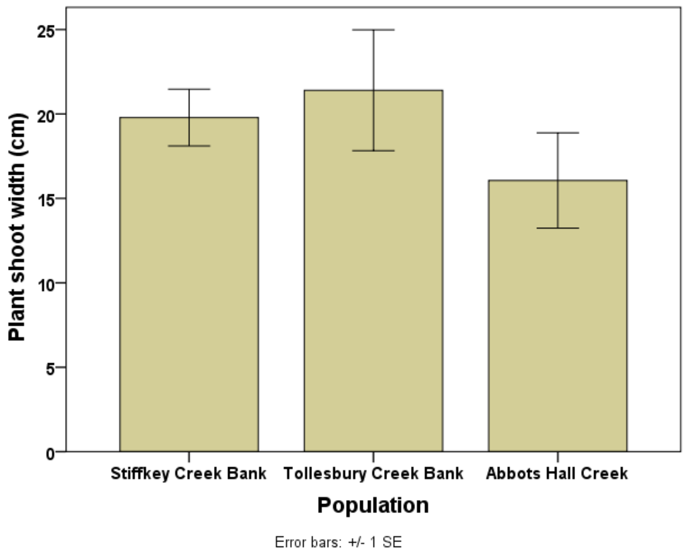


Figure 3.3: Comparisons (mean±s.e.) of *Suaeda maritima* plant width trait per plant of three different creek bank populations in 2008.

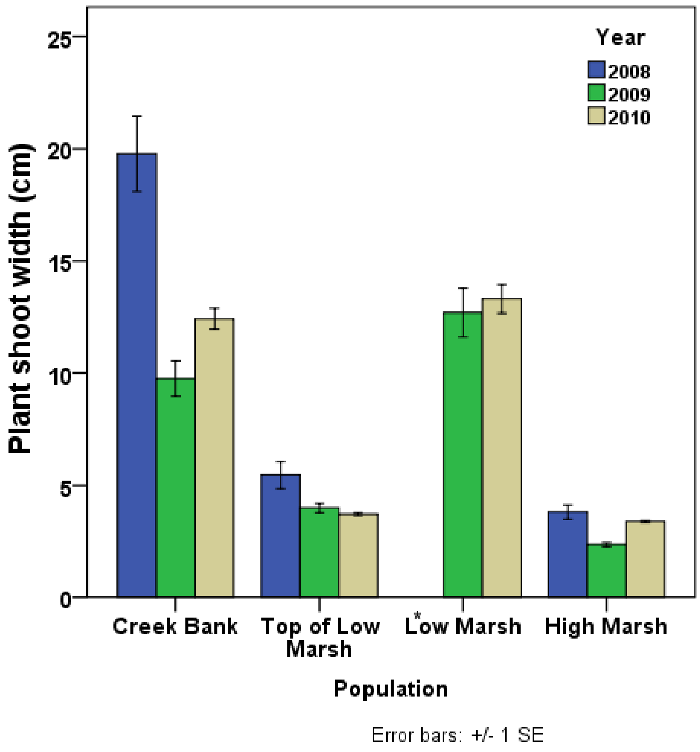
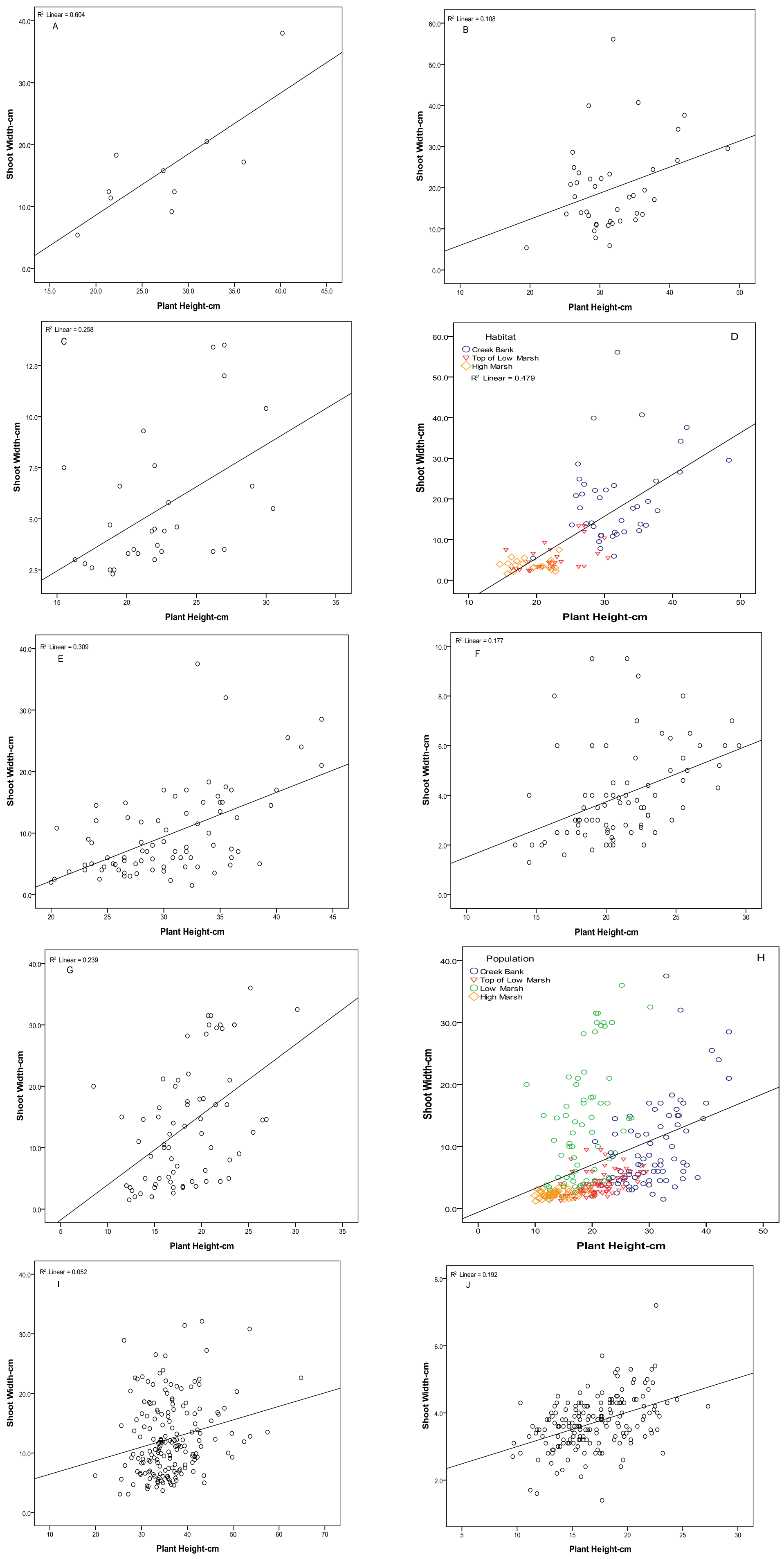


Figure 3.5: Comparisons (mean±s.e.) of *Suaeda maritima* plant width traits per plant for the Stiffkey salt marsh populations in 2008, 2009 and 2010.



A
R² Linear = 0.604
Shoot Width-cm
Plant Height-cm
B
R² Linear = 0.108
Shoot Width-cm
Plant Height-cm
C
R² Linear = 0.258
Shoot Width-cm
Plant Height-cm
D
Habitat
Creek Bank
Top of Low Marsh
High Marsh
R² Linear = 0.479
Shoot Width-cm
Plant Height-cm
E
R² Linear = 0.309
Shoot Width-cm
Plant Height-cm
F
R² Linear = 0.177
Shoot Width-cm
Plant Height-cm
G
R² Linear = 0.239
Shoot Width-cm
Plant Height-cm
H
Population
Creek Bank
Top of Low Marsh
Low Marsh
High Marsh
Shoot Width-cm
Plant Height-cm
I
R² Linear = 0.052
Shoot Width-cm
Plant Height-cm
J
R² Linear = 0.192
Shoot Width-cm
Plant Height-cm

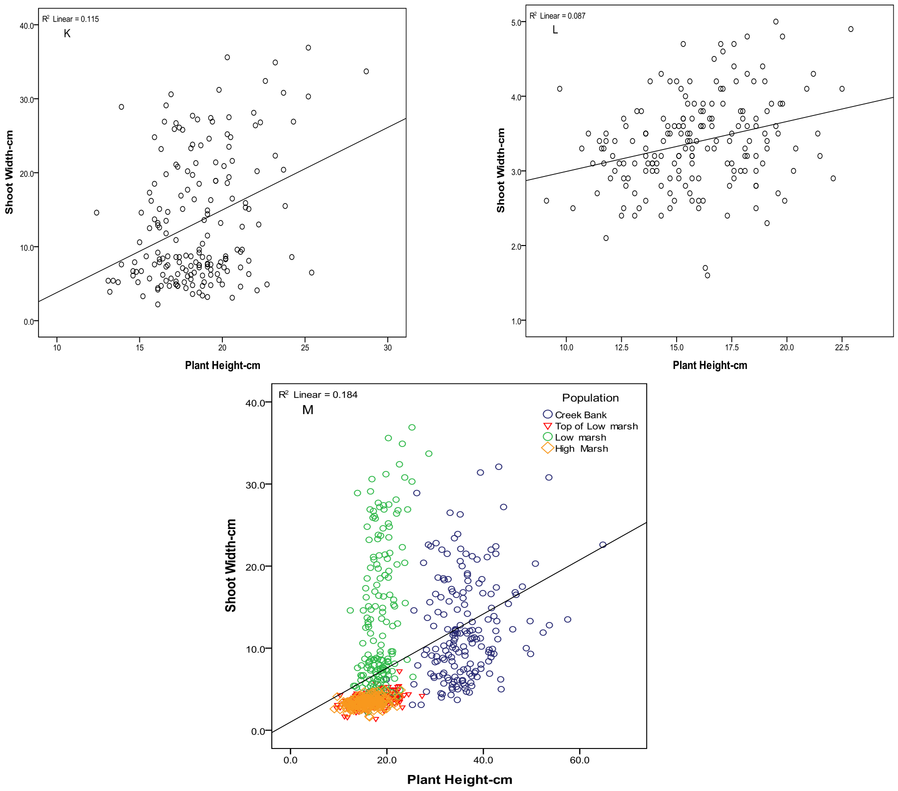


Figure 3.6: Relationship between plant height and shoot width (cm) per plant. **(a)** Within Abbots Hall Creek bank 2008 (r^2=0.604, *P*<0.008). **(b)** Within Stiffkey Creek bank 2008 (r^2=0.108, *P*<0.038). **(c)** Within Stiffkey Top of low marsh 2008 (r^2=0.258, *P*<0.004). **(d)** Between all Stiffkey populations 2008 (r^2=0.479, *P*<0.001). **(e)** Within Creek bank 2009 (r^2=0.309, *P*<0.001). **(f)** Within Top of low marsh 2009 (r^2=0.177, *P*<0.001). **(g)** Within Low marsh 2009 (r^2=0.239, *P*<0.001). **(h)** Between all populations 2009 (r^2=0.136, *P*<0.001). **(i)** Within Creek Bank 2010 (r^2=0.052, *P*<0.002). **(j)** Within Top of low marsh 2010 (r^2=0.192, *P*<0.001). **(k)** Within Low marsh 2010 (r^2=0.115, *P*<0.001). **(l)** Within High marsh 2010 (r^2=0.087, *P*<0.001). **(m)** Between all populations 2010 (r^2=0.184, *P*<0.001).

3.3.3 Number of primary branches

In 2008, significantly more primary branches were produced by both the Stiffkey and the Tollesbury creek bank populations than the Abbots Hall one (Fig. 3.7). Also, with regards to the Stiffkey salt marsh populations, the creek bank plants produced significantly the most primary branches, followed by top of low marsh and finally by the high marsh plants (Fig. 3.8).

In 2009, among the Stiffkey salt marsh populations, the plants producing significantly the highest number of primary branches were found in the creek bank followed by plants from the low marsh, the top low marsh, and finally from the high marsh. In 2010 plants from low marsh produced significantly the most primary branches, followed by the creek bank population, the top of low marsh, and finally of the high marsh (Fig. 3.8).

The two-way ANCOVA revealed significant variations at all levels: population, year and the interaction between population and year (Table 3.3). Collection date was considered here as a covariate in order to allow examining the effect of the main variables (population and year) and their interaction.

The relationship between the plant height and the number of primary branches traits per plant (Fig. 3.9) was significantly positive within the Stiffkey creek bank and top of low marsh populations in year 2008, but not significant within the high marsh. Again, in 2009 and within all populations from the Stiffkey salt marsh showed a significant positive association, with the exception of the high marsh. In 2010 significant positive associations were found between traits within all populations, except for the top of low marsh this time. Positive associations were also found in all years when all populations were taken together.

Finally, very similar trends (to those found between the shoot width and the plant height traits) were found in the associations between the plant shoot width and the number of primary branches, within and between populations (Fig. 3.10).

Table 3.3: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the number of primary branches trait per plant.

Source of Variation	**Number of Primary branches**			
	Type lll SS	**df**	**F**	**P**
Population	13698.04	3	218.31	<0.001
Year	5721.84	2	136.78	<0.001
Population*Year	2334.66	5	22.32	<0.001
Error	22546.2	1078	-	-

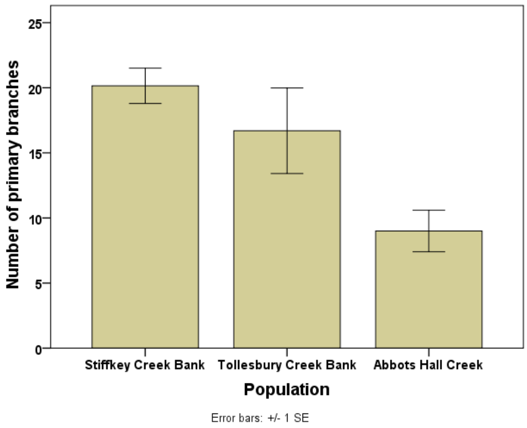


Figure 3.7: Comparisons (mean±s.e.) of *Suaeda maritima* number of primary branches trait per plant of three different creek bank populations in 2008.

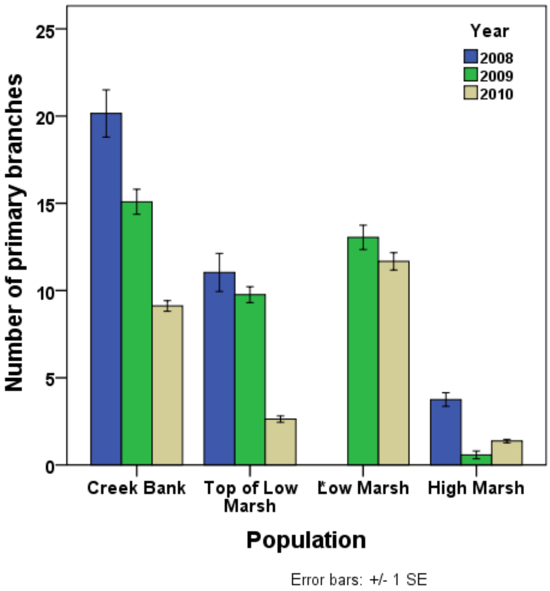


Figure 3.8: Comparisons (mean±s.e.) of *Suaeda maritima* number of primary branches traits per plant for the Stiffkey salt marsh populations in 2008, 2009 and 2010.

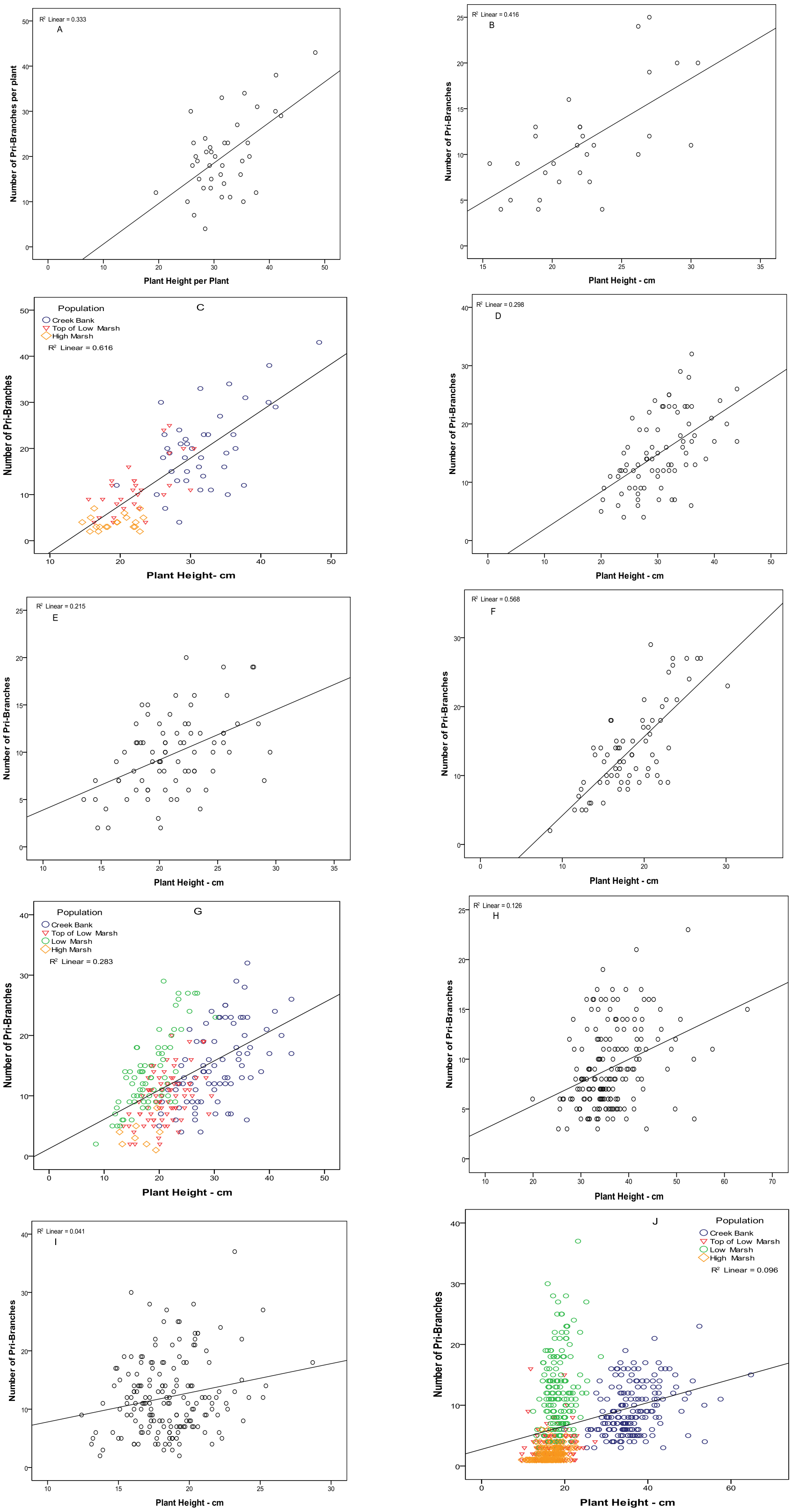


Figure 3.9: Relationship between number of primary branches and plant height per plant. **(a)** Within Stiffkey Creek bank 2008 (r^2=0.333, *P*<0.001). **(b)** Within Stiffkey Top of low marsh 2008 (r^2=0.416, *P*<0.001). **(c)** Between all Stiffkey populations 2008 (R^2=0.616, *P*<0.001). **(d)** Within Creek bank 2009 (r^2=0.298, *P*<0.001). **(e)** Within Top of low marsh 2009 (r^2=0.215, *P*<0.001). **(f)** Within Low marsh 2009 (r^2=0.568, *P*<0.001). **(g)** Between all populations 2009 (r^2=0.283, *P*<0.001). **(h)** Within Low marsh 2010 (r^2=0.041, *P*<0.007). **(i)** Within High marsh 2010 (r^2=0.064, *P*<0.005). **(j)** Between all populations 2010 (r^2=0.096, *P*<0.001).

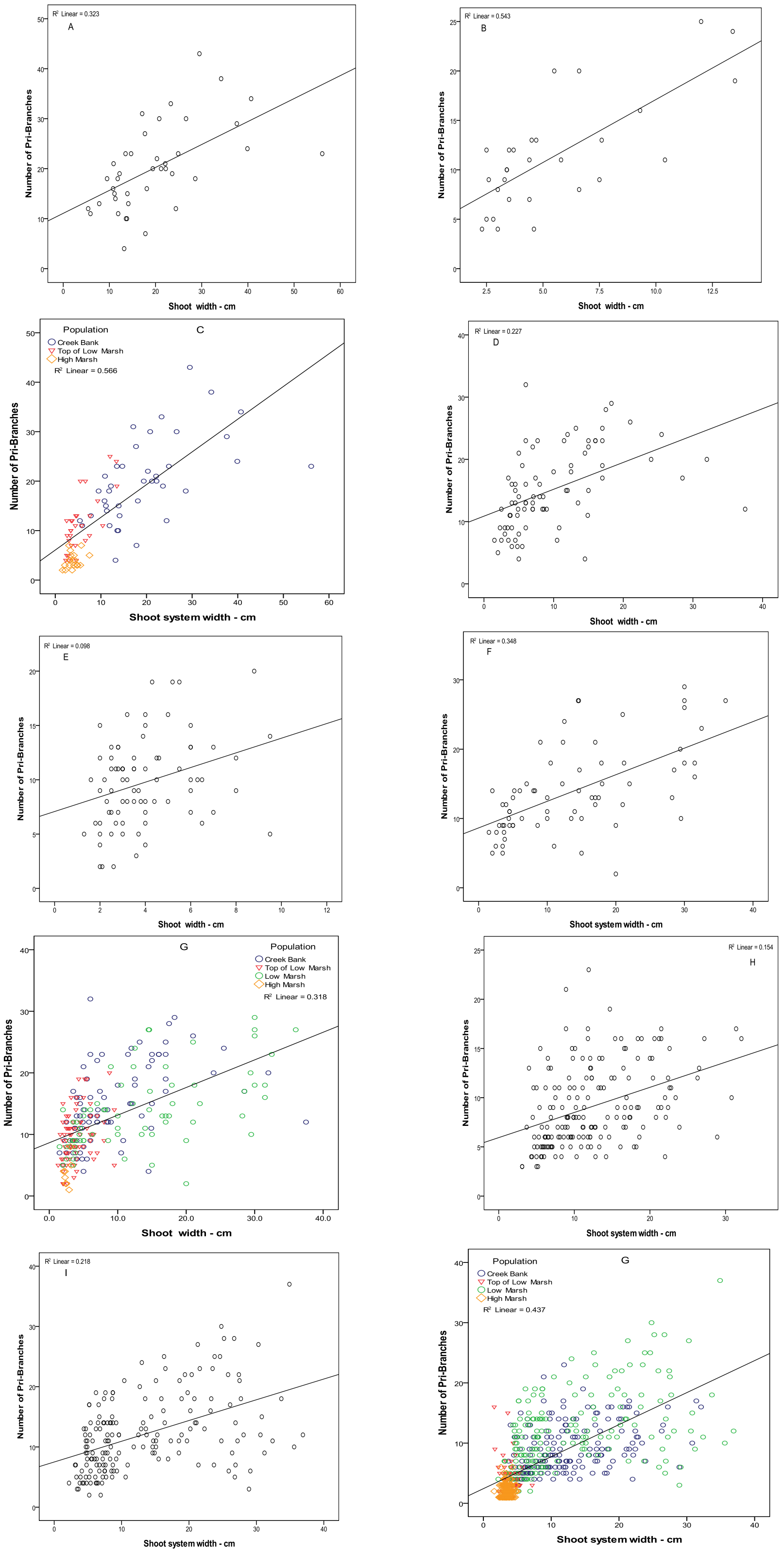


Figure 3.10: Relationship between plant shoot width and number of primary branches per plant. **(a)** Within Stiffkey Creek bank 2008 (r^2=0.323, *P*<0.001). **(b)** Within Stiffkey Top of low marsh 2008 (r^2=0.543, *P*<0.001). **(c)** Between all Stiffkey populations 2008 (r^2=0.566, *P*<0.001). **(d)** Within Creek bank 2009 (r^2=0.227, *P*<0.001). **(e)** Within Top of low marsh 2009 (r^2=0.098, *P*<0.005). **(f)** Within Low marsh 2009 (r^2=0.348, *P*<0.001). **(g)** Between all populations 2009 (r^2=0.318, *P*<0.001). **(h)** Within Creek bank 2010 (r^2=0.154, *P*<0.001). **(i)** Within Low Marsh 2010 (r^2=0.218, *P*<0.001). **(j)** Between all populations 2010 (r^2=0.437, *P*<0.001).

3.3.4 Number of secondary branches

In 2008, no significant differences were found between the Stiffkey, Tollesbury and Abbot’s Hall populations (Fig. 3.11). Among the Stiffkey salt marsh populations, only the creek bank plants produced secondary branches in that season (Fig. 3.12).

In 2009, there were no significant differences between the Stiffkey low marsh and the creek bank populations, while in the top low marsh and the high marsh secondary branches were not produced during this year. In 2010 the pattern of variation among populations was similar to that in 2009 (Fig. 3.12).

The two-way ANCOVA model revealed significant variations at all levels; population, year and the interaction between population and year (Table 3.4). Collection date was considered here as a covariate in order to allow examining the effect of the main variables (population and year) and their interaction.

An investigation of the relationship between the plant height and the number of secondary branches revealed only one positive association, within creek bank population in 2010 (Fig. 3.13). Also, the relationship between the shoot width and the number of secondary branches traits was found to be significantly positive in a few cases, such within the Stiffkey creek bank population in 2008, and within the low marsh in 2010. Between populations, there was an association only in 2010 and it was positive (Fig. 3.14). Furthermore, the relationship between the number of primary branches and the number of secondary branches was found to be significantly positive only in year 2010 within the creek bank, the top of low marsh and the low marsh populations (Fig. 3.15).

Table: 3.4: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the number of primary branches trait per plant.

Source of Variation	**Number of Secondary branches**			
	Type lll SS	**df**	**F**	**P**
Population	1239.39	3	32.19	<0.001
Year	248.82	2	9.69	<0.001
Population*Year	723.15	5	11.27	<0.001
Error	13833.56	1078	-	-

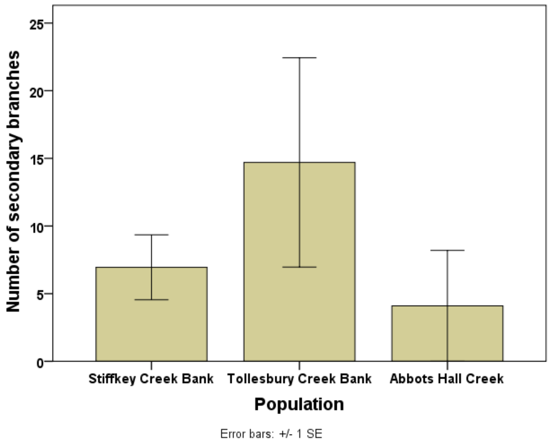


Figure 3.11: Comparisons (mean±s.e.) of *Suaeda maritima* number of secondary branches trait of three different creek bank populations in 2008.

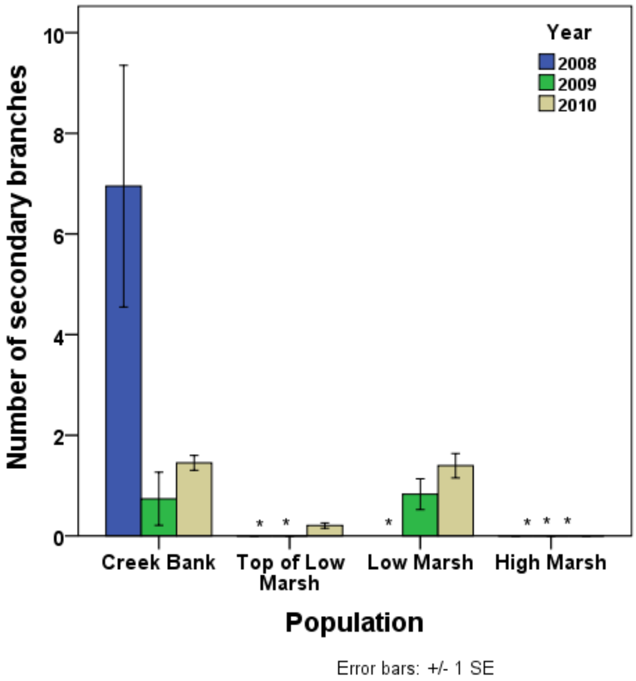


Figure 3.12: Comparisons (mean±s.e.) of *Suaeda maritima* number of secondary branches traits for the Stiffkey salt marsh populations in 2008, 2009 and 2010.

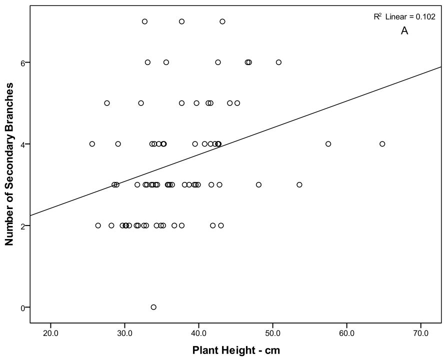


Figure 3.13: Relationship between the plant height and number of secondary branches per plant within Creek bank population 2010 (r^2=0.102, *P*<0.006).

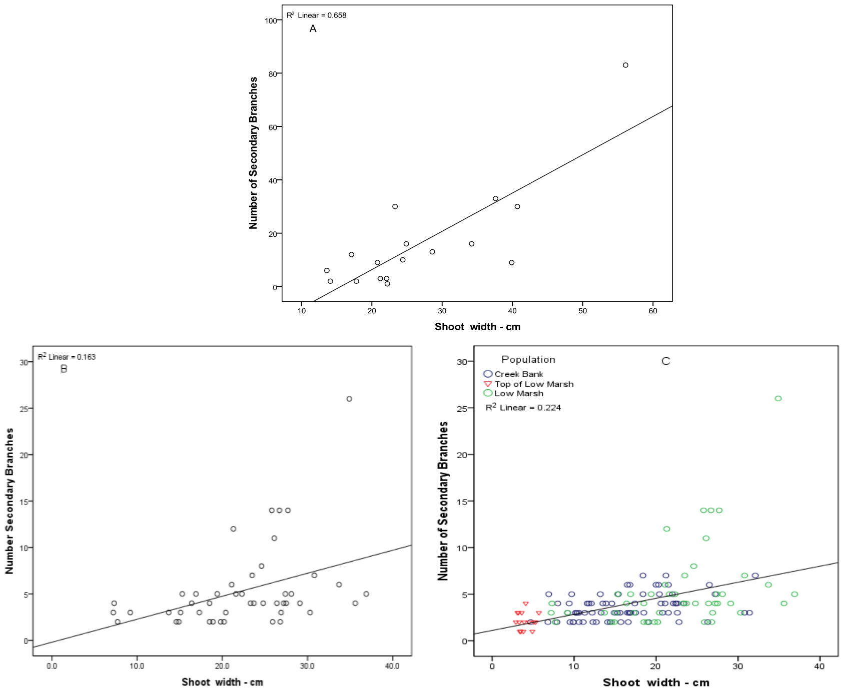


Figure 3.13: Relationship between shoot width and number of secondary branches per plant. **(a)** Within Stiffkey Creek bank 2008 (r^2=0.658, *P*<0.001). **(b)** Within Low marsh 2010 (r^2=0.163, *P*<0.005). **(c)** Between all populations 2010 (r^2=0.224, *P*<0.001).

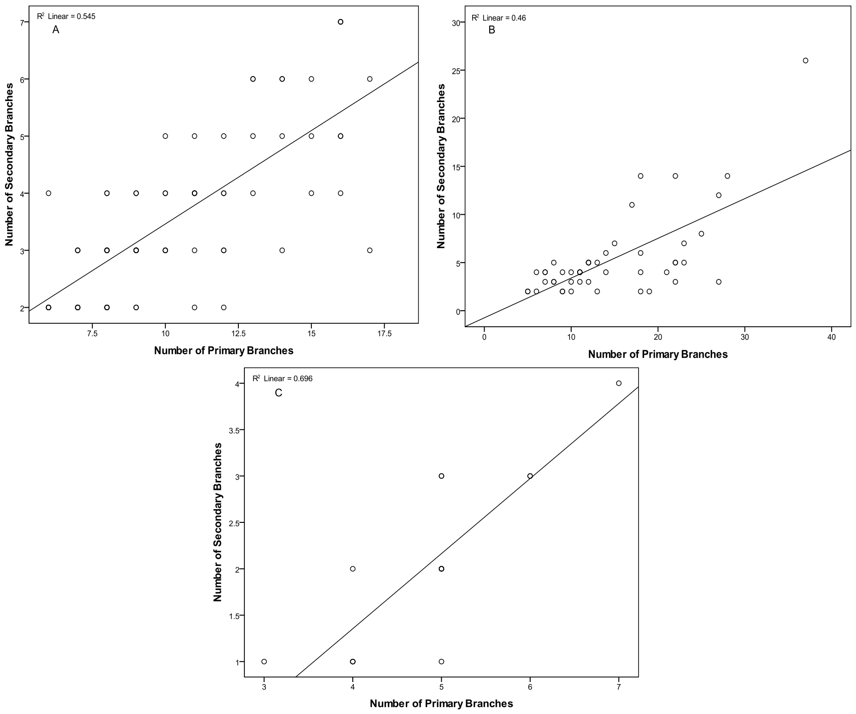


Figure 3.15: Relationship between the number of primary branches and the number of secondary branches per plant. **(a)** Within Creek bank 2010 (r^2=0.545, *P*<0.001). **(b)** Within Low marsh 2010 (r^2=0.460, *P*<0.001). **(c)** Within Top of low marsh 2010 (r^2=0. 696, *P*<0.001).

3.3.5 Root length

In 2008, no significant differences were found between the Stiffkey, Tollesbury and Abbot's Hall populations in the main root length trait (Fig. 3.16). At Stiffkey, the creek bank plants had significantly the longest main root, followed by high marsh and finally top of low marsh plants (Fig. 3.17).

In 2009, the creek bank plants at Stiffkey significantly again had the longest main root, followed by plants from the low marsh, followed by the top of low marsh, which did not differ significantly from the high marsh one. In 2010 plants from the creek bank and the low marsh populations had significantly longer main roots than plants from the high marsh and the top of low marsh (Fig. 3.17).

The two-way ANCOVA model revealed significant variations at three levels; population, year and the interaction between population and year (Table 3.5). Collection date was considered here as a covariate in order to allow examining the effect of the main variables (population and year) and their interaction.

Significant positive associations between plant height and root length traits were found in many cases; within Tollesbury creek bank in 2008, Stiffkey creek bank in both years 2009 and in 2010, and high marsh in 2010 populations. One negative association found within the top of low marsh population in 2009. Finally, taking all seasons together, positive associations were found at an inter-population level (Fig. 3.18).

The relationship between shoot width and root length traits was significant and positive in many cases; within the Abbots Hall population in 2008, within the creek bank population in 2010 and within the low marsh population in 2010. Finally, across all seasons, positive associations were found at inter-population level (Fig. 3.19).

Table: 3.5: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the main root length trait.

Source of Variation	**Root Length (cm)**			
	Type lll SS	**df**	**F**	**P**
Population	1337.01	3	102.15	<0.001
Year	283.08	2	32.44	<0.001
Population*Year	415.02	5	19.02	<0.001
Error	4703.20	1078	-	-

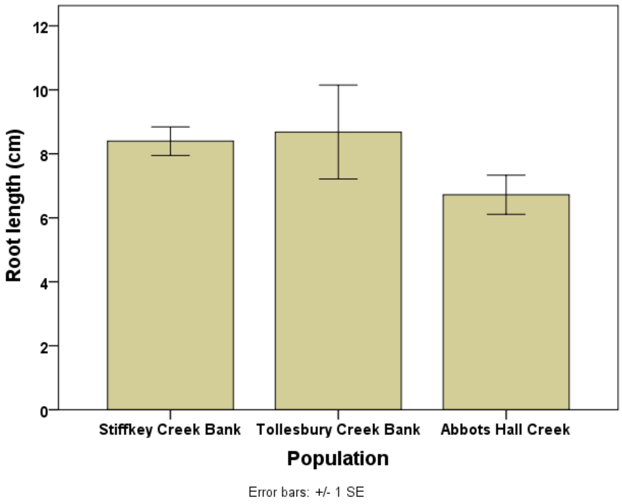


Figure 3.16: Comparisons (mean±s.e.) of *Suaeda maritima* main root length trait per plant of three different creek bank populations in 2008.

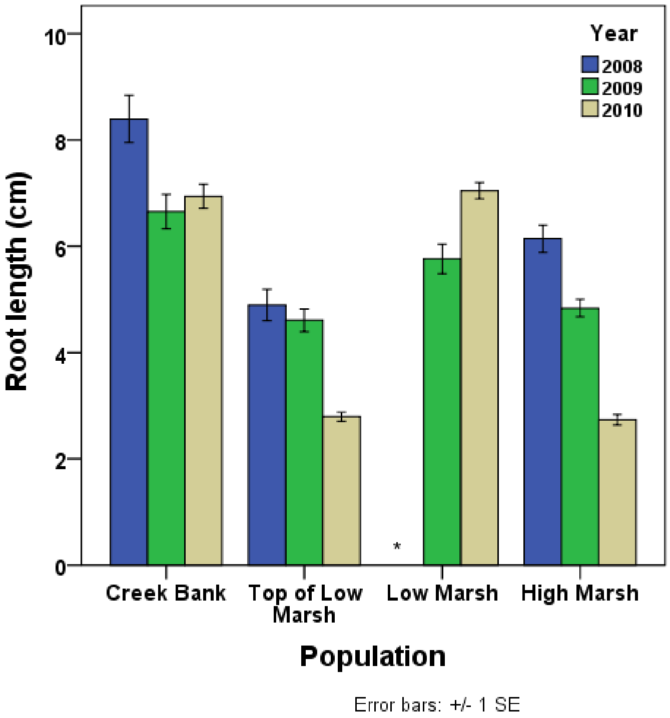


Figure 3.17: Comparisons (mean±s.e.) of *Suaeda maritima* main root length traits per plant for the Stiffkey salt marsh populations in 2008, 2009 and 2010.

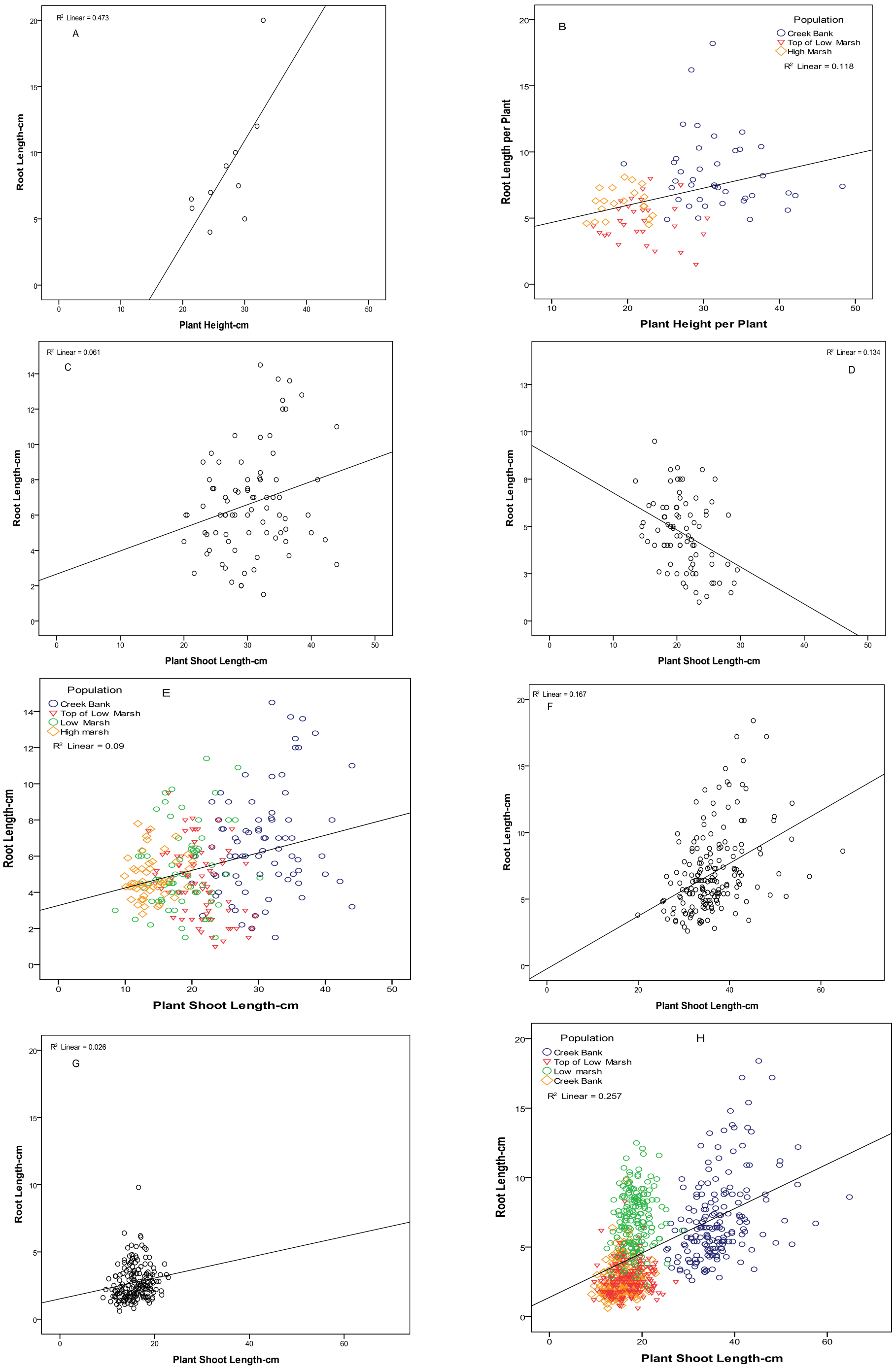


Figure 3.18: Relationship between plant height and root length (cm) per plant. **(a)** Within Tollesbury Creek bank 2008 (r^2=0. 473, *P*<0.028). **(b)** Between all Stiffkey populations 2008 (r^2=0. 118, *P*<0.001). **(c)** Within Creek bank 2009 (r^2=0. 061, *P*<0.027). **(d)** Within Top of low marsh 2009 (r^2=0. 134, *P*<0.001). **(e)** Between all populations 2009 (r^2=0. 090, *P*<0.001). **(f)** Within Creek bank 2010 (r^2=0. 167, *P*<0.001). **(g)** Within High marsh 2010 (r^2=0. 026, *P*<0.032). **h)** Between all populations 2010 (r^2=0. 257, *P*<0.001).

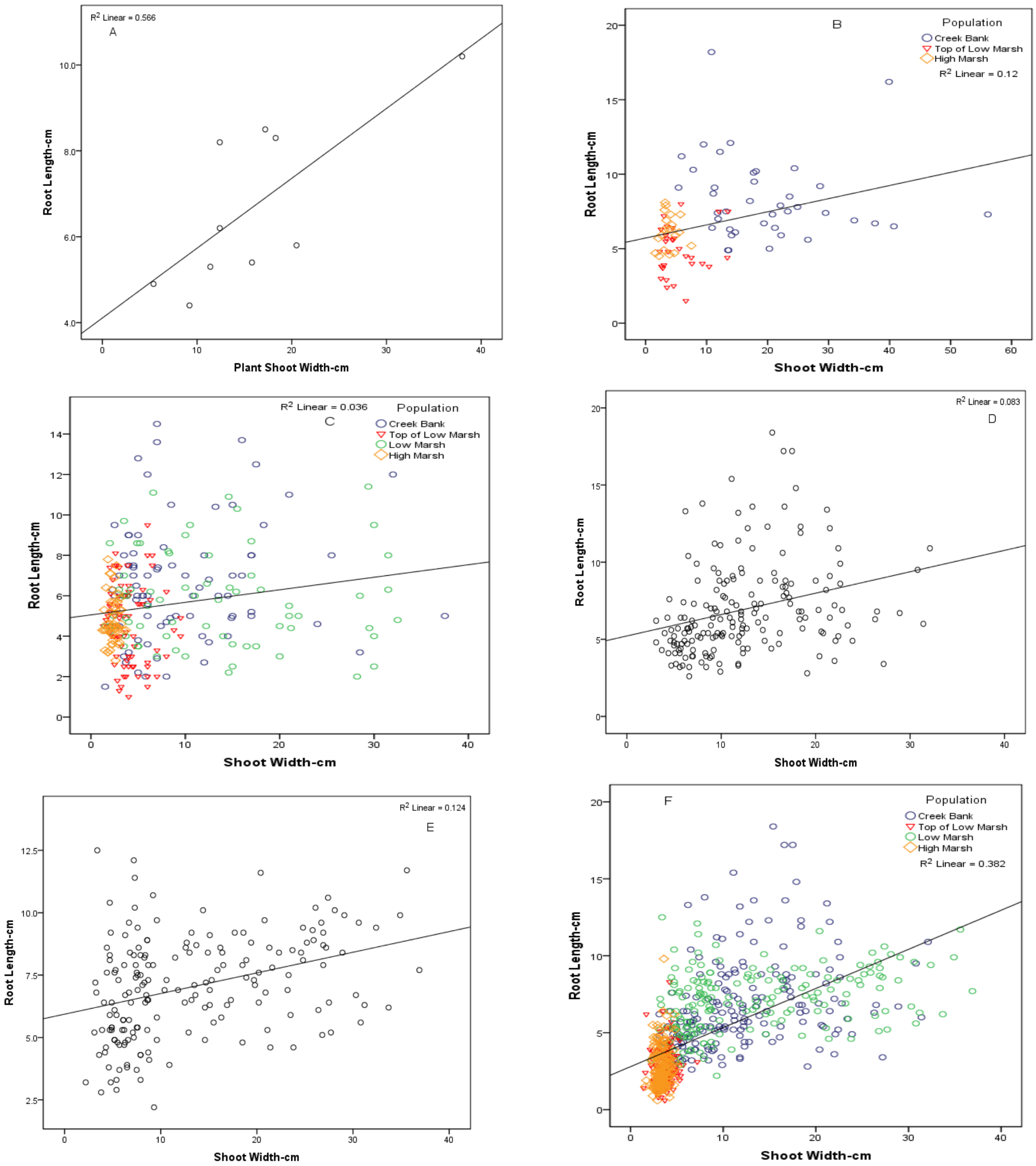


Figure 3.19: Relationship between plant shoot width and root length (cm) per plant. (a) Within Abbots Hall Creek bank 2008 (r^2=0. 566, *P*<0.012). (b) Between all Stiffkey populations 2008 (r^2=0. 120, *P*<0.001). (c) Between all Stiffkey populations 2009 (r^2=0. 032, *P*<0.002). (d) Within Creek bank 2010 (r^2=0. 083, *P*<0.001). (e) Within Low marsh 2010 (r^2=0. 124, *P*<0.001). (f) Between all Stiffkey populations 2010 (r^2=0. 382, *P*<0.001).

3.3.6 Root system width

In 2008, no significant differences were found between the Stiffkey, Tollesbury and Abbot’s Hall populations (Fig. 3.20). Among the Stiffkey populations, the creek bank and the top of low marsh plants had significantly larger root system width than the high marsh plants (Fig. 3.21).

In 2009, at Stiffkey salt marsh, plants from the top of low marsh and the low marsh had significantly larger root system width than those of the creek bank population, or the high marsh. In 2010 significantly the largest root system was found in the low marsh population, followed by plants from the creek bank, the top of low marsh and finally by the high marsh (Fig. 3.21).

The two-way ANCOVA model revealed significant variations at three levels; population, year and the interaction between population and year (Table 3.6). Collection date was considered here as a covariate in order to allow examining the effect of the main variables (population and year) and their interaction.

Significant and positive relationships between the plant height and the root system width were found in many cases; within the top of low marsh population in all years, the low marsh population in 2009 and 2010 and the high marsh only in 2010. Also, for all seasons, positive associations were found at the inter-population level (Fig. 3.22).

Significant and positive associations between shoot width and the root system width traits were found in many cases; within the Tollesbury creek bank in 2008, the Abbot’s Hall creek bank in 2008, the Stiffkey top of low marsh in 2008 and 2009, the creek bank in 2009 and 2010 and finally the low marsh in 2009 and 2010. For all seasons, positive associations were found at the inter-population level (Fig. 3.23).

Table: 3.6: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the main root length trait per plant.

Source of Variation	**Shoot Width (cm)**			
	Type lll SS	**df**	**F**	**P**
Population	16145.52	3	164.49	<0.001
Year	1122.12	2	17.14	<0.001
Population*Year	1171.39	5	7.16	<0.001
Error	35269.54	1078	-	-

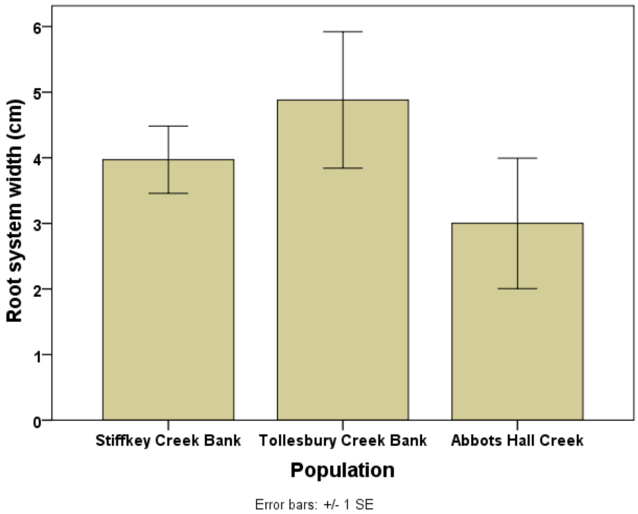


Figure 3.20: Comparisons (mean±s.e.) of *Suaeda maritima* root system width trait per plant of three different creek bank populations in 2008.

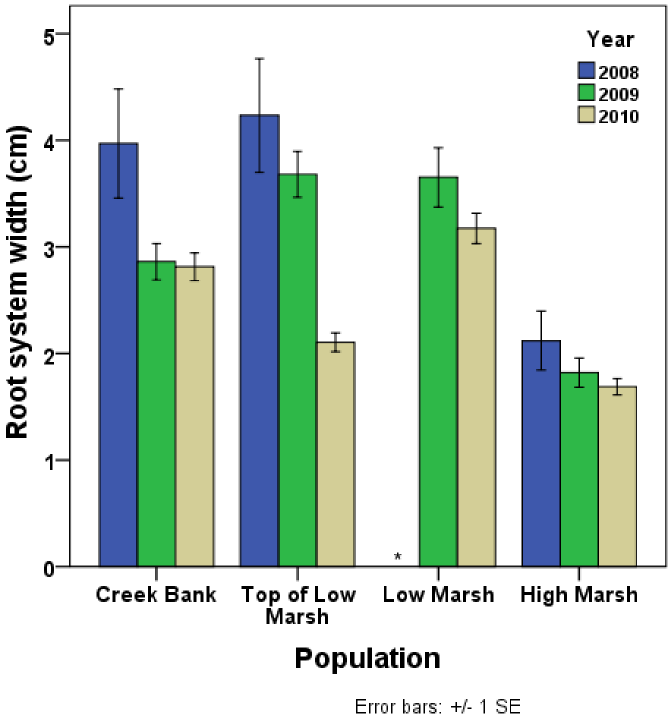
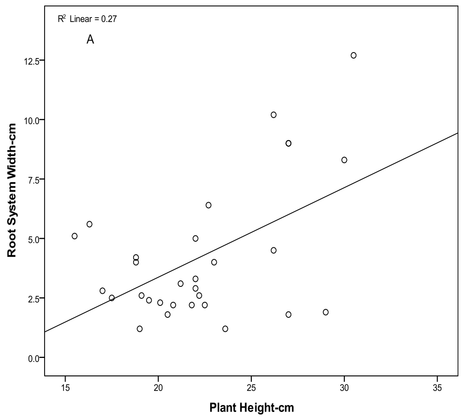
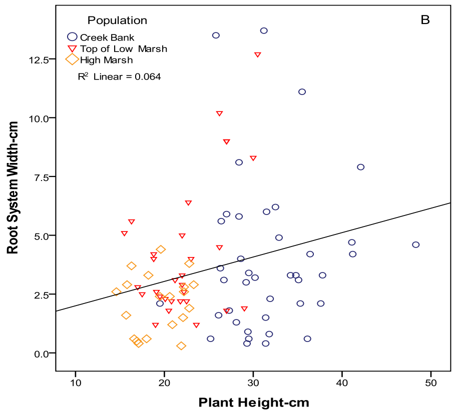
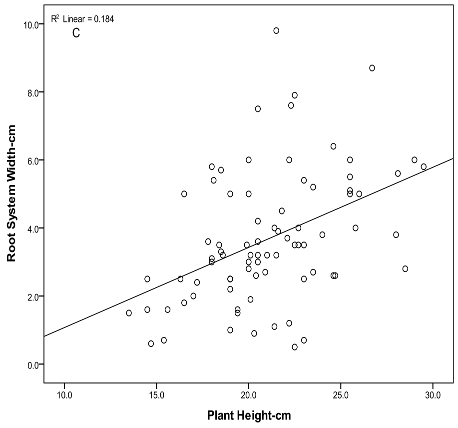
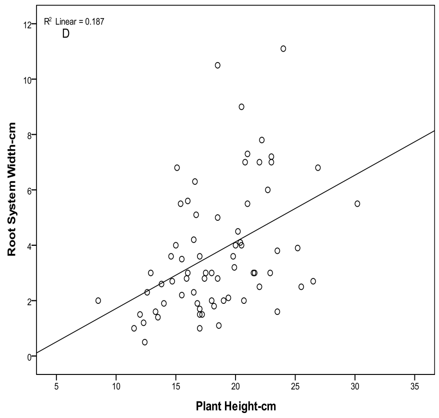


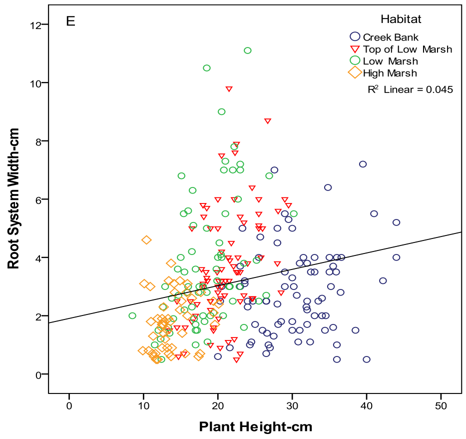
Figure 3.21: Comparisons (mean±s.e.) of *Suaeda maritima* root system width traits per plant for the Stiffkey salt marsh populations in 2008, 2009 and 2010.

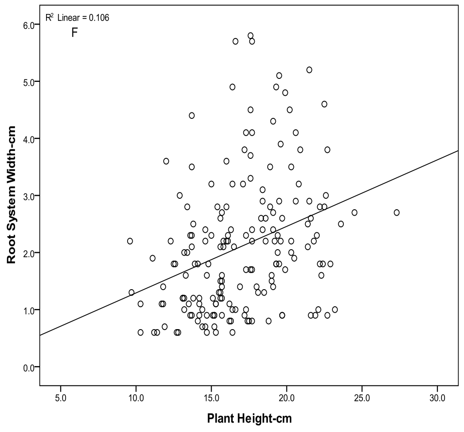

R² Linear = 0.27
A
Root System Width-cm
0.0
2.5
5.0
7.5
10.0
12.5
15
20
25
30
35
Plant Height-cm

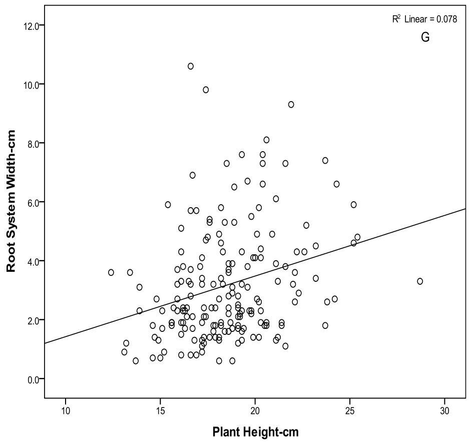

Population
B
Creek Bank
Top of Low Marsh
High Marsh
R² Linear = 0.064
Root System Width-cm
0.0
2.5
5.0
7.5
10.0
12.5
10
20
30
40
50
Plant Height-cm

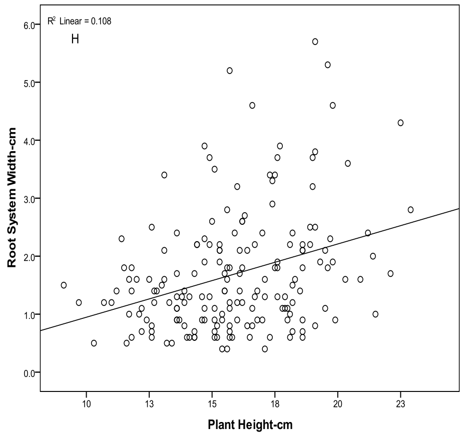

R² Linear = 0.184
C
Root System Width-cm
0.0
2.0
4.0
6.0
8.0
10.0
10.0
15.0
20.0
25.0
30.0
Plant Height-cm


R² Linear = 0.187
D
Root System Width-cm
0
2
4
6
8
10
12
5
10
15
20
25
30
35
Plant Height-cm


E
Habitat
Creek Bank
Top of Low Marsh
Low Marsh
High Marsh
R² Linear = 0.045
Root System Width-cm
0
2
4
6
8
10
12
0
10
20
30
40
50
Plant Height-cm


R² Linear = 0.106
F
Root System Width-cm
0.0
1.0
2.0
3.0
4.0
5.0
6.0
5.0
10.0
15.0
20.0
25.0
30.0
Plant Height-cm


R² Linear = 0.078
G
Root System Width-cm
0.0
2.0
4.0
6.0
8.0
10.0
12.0
10
15
20
25
30
Plant Height-cm


R² Linear = 0.108
H
Root System Width-cm
0.0
1.0
2.0
3.0
4.0
5.0
6.0
10
13
15
18
20
23
Plant Height-cm

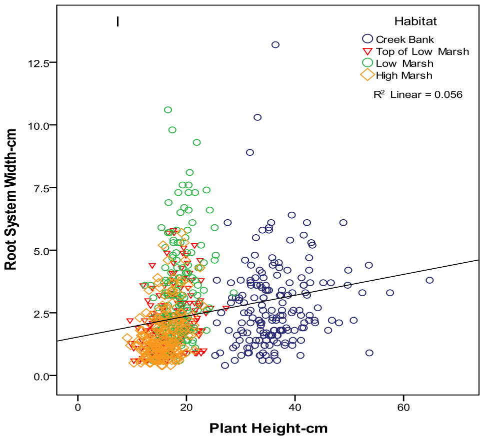
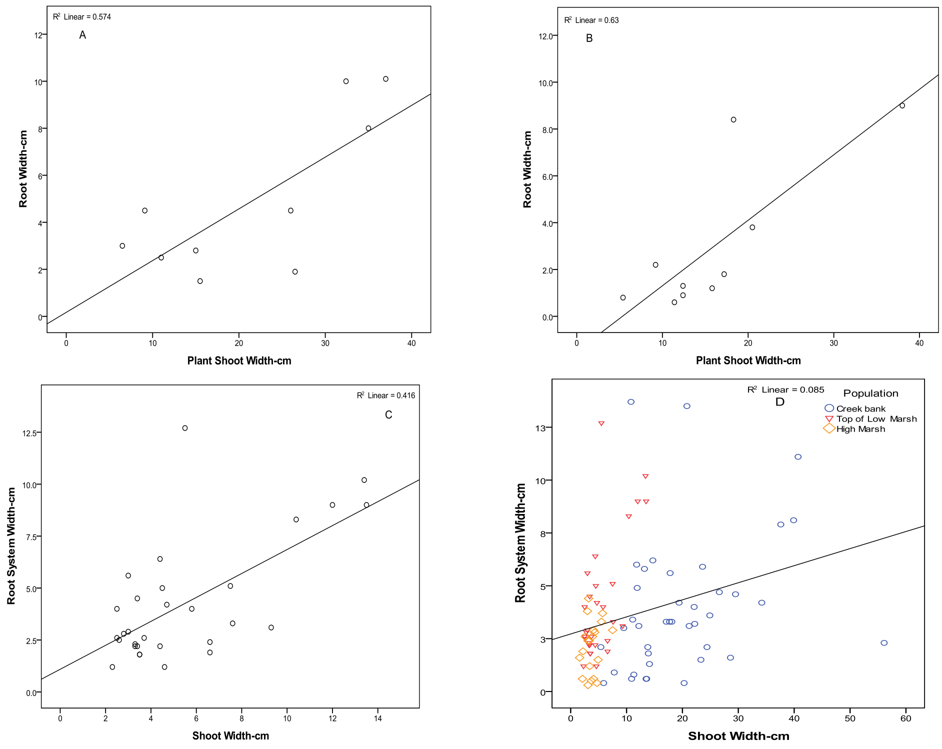
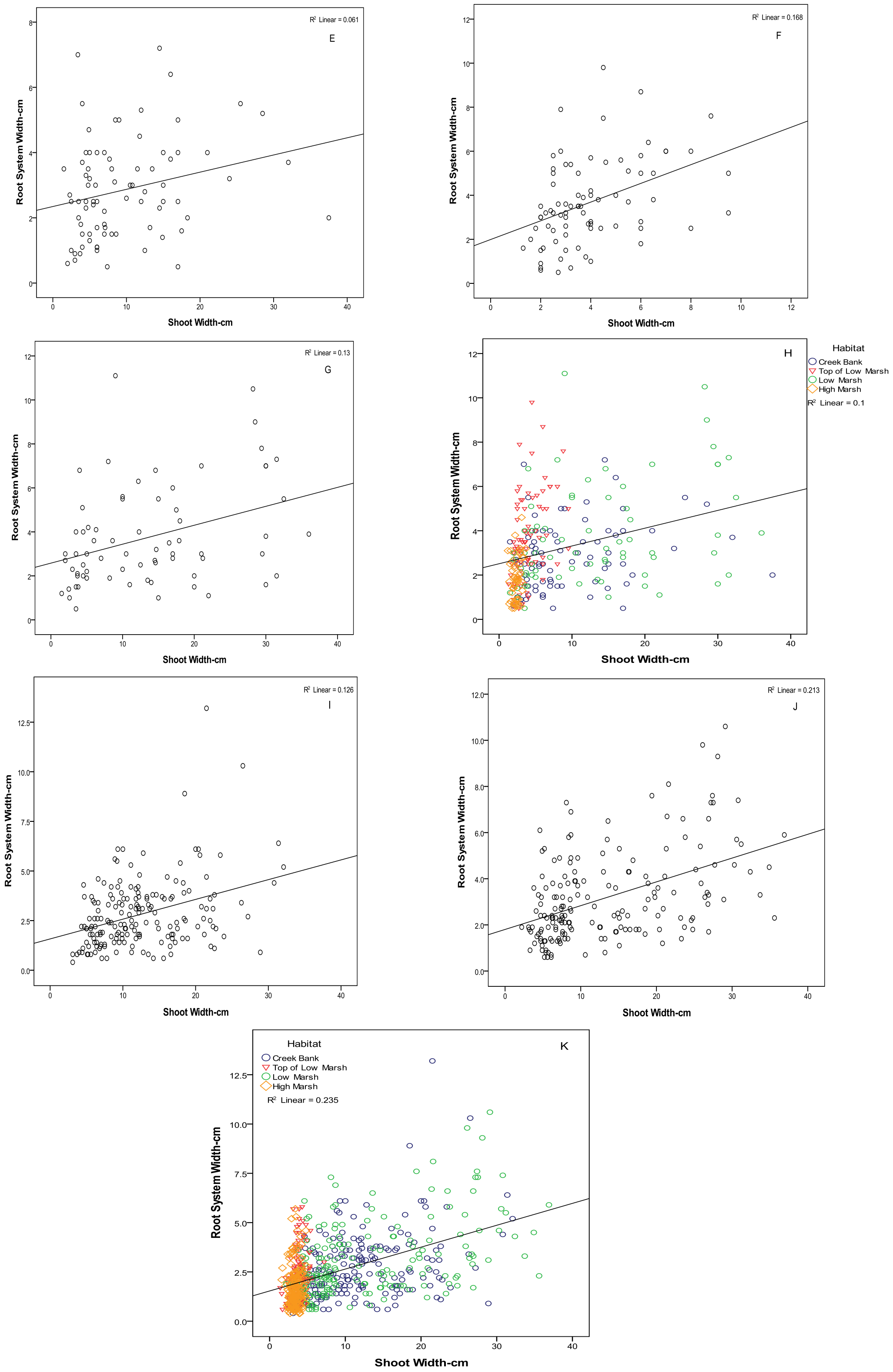


Figure 3.22: Relationship between plant height and root system width. **(a)** Within Stiffkey Top of low Marsh 2008 (r^2=0. 270, *P*<0.003). **(b)** Between all Stiffkey populations 2008 (r^2=0. 064, *P*<0.016). **(c)** Within Top of low marsh 2009 (r^2=0. 184, *P*<0.001). **(d)** Within Low marsh 2009 (r^2=0. 187, *P*<0.001). **(e)** Between all populations 2009 (r^2=0. 045, *P*<0.001). **(f)** Within Top of low marsh 2010 (r^2=0. 106, *P*<0.001). **(g)** Within Low marsh 2010 (r^2=0. 078, *P*<0.001). **(h)** Within High marsh 2010 (r^2=0. 108, *P*<0.001). **(i)** Between all populations 2010 (r^2=0. 560, *P*<0.001).





Figure(3.23: Relationship between plant shoot width and root system width (cm). **(a)** Within Tollesbury Creek bank 2008 (r^2=0. 574, *P*<0.011). **(b)** Within Abbots Hall Creek bank 2008 (r^2=0. 63, *P*<0.006). **(c)** Within Stiffkey Top of low marsh 2008 (r^2=0. 416, *P*<0.001). **(d)** Between all Stiffkey populations 2008 (r^2=0. 085, *P*<0.005). **(e)** Within Creek bank 2009 (r^2=0. 061, *P*<0.028). **(f)** Within Top of low marsh 2009 (r^2=0. 168, *P*<0.001). **(g)** Within Low marsh 2009 (r^2=0. 130, *P*<0.002). **(h)** Between all populations 2009 (r^2=0. 1, *P*<0.001). **(i)** Within Creek bank 2010 (r^2=0. 126, *P*<0.001). **(j)** Within Low marsh 2010 (r^2=0. 213, *P*<0.001). **(k)** Between all populations 2010 (r^2=0. 235, *P*<0.001).

3.4 Discussion

This investigation of the in situ local populations of *Suaeda maritima* at Stiffkey salt marsh has revealed that all of them are morphologically distinctive in accordance with their distribution, which is strongly correlated with marsh topography.

As *S. maritima* is an annual, the size attained during the growing season to a larger extent reflects the vigour of its growth. However, there were also certain differences in the length of growing season between populations. The creek bank population has been identified as the most vigorous population in terms of growth traits, and this is the more so because it was the population with earliest maturity date. Among populations, the creek bank was the tallest in all years; it had the largest shoot width in 2008, it produced the highest numbers of primary branches in 2008 and 2009, it produced the highest numbers of secondary branches in 2008 and 2009, it had the longest main root in all years, and finally it had the largest shoot system width in 2008.

The low marsh population was the second most vigorous in terms of growth traits. It was the second tallest population in 2009 and 2010, it had the largest shoot width in 2009 and 2010, it was the second biggest producer of primary branches in 2009 and the first in 2010, it had the second longest main root in 2009 and the first in 2010, and finally it had the largest root system width in both years (in 2009 and 2010).

The top of the low marsh population exhibited (significantly) the third most vigorous growth traits in most cases. In 2008 and 2009, the top of the low marsh plants were found to be the second tallest population but in 2010 it was the third, it achieved the second largest shoot width in 2008 but the third in 2009 and 2010, it was the second largest producer of primary branches in 2008 but the third at 2009 and 2010, it had the third longest main root in 2008 but it had similar main root length to the high marsh population in both years (2009 and 2010), and it had the biggest root system width in 2008 and 2009 but the third in 2010.

Finally, the high marsh population was found to be in the last position in terms of plant growth traits. It was the shortest in all years, it had the smallest shoot width in all years, it produced the smallest number of primary branches, the secondary branches were absent in all years in this population, it had the second longest main root in 2008 but the shortest in 2009 and 2010, and finally it had smallest root system width in all years.

This clear phenotypic variation between populations in all years is strong evidence for the influence of a group of abiotic and biotic factors, which cause this pattern of definitive distribution for each population. Numerous studies have emphasized the role of abiotic and biotic factors in salt marsh plant distribution, in particular, nutrient availability (Jefferies and Perkins 1977, Álvarez Rogel et al. 2001), inter- and intra-specific competition (Bertness 1989, 1991, Pennings and Callaway 1992, Pennings et al. 2003), salinity (Khan et al. 2002, Song et al. 2006, Li et al. 2010) and soil anoxia (Crooks et al. 2002, Davy et al. 2011, He et al. 2011).

The particular vigour of the *Suaeda maritima* population may be due to the suitability of the local conditions in the creek bank zone of the marsh. Creek banks are by definition associated with good drainage and often have raised levees which further enhance drainage. Due to this unique topography, the creek bank habitat is the most aerated zone of the marsh (Silvestri and Marani 2004). Therefore, environmental factors such as hypersalinity, waterlogging, low redox potential (with associated iron and sulphide toxins), which inhibit the growth of salt marsh plants, may be alleviated along the edges of the creeks (Morzaria-Luna et al. 2004). Work on a newly developing salt marsh at Brancaster in Norfolk, where there were variations in both elevation and drainage (but little natural creek development) showed that *S. maritima* did not colonize the most reducing sediments, even though it could colonize low-lying, well-drained areas (Davy et al. 2011). However, no distinct microhabitats or plant phenotypes were evident at this new site.

Differences in elevation across the salt marsh has been proved to be a determinant of species and population distribution (Pennings and Bertness 2001, He et al. 2011), and the higher the elevation, the lower the amount of available water, total nitrogen, phosphate and organic matter can be (Ihm et al. 2004, Howard 2010). Therefore, the growth of high marsh plants would be expected to be affected, resulting in more or less stunted plants (Pennings and Bertness 2001). The distribution of *Suaeda maritima* populations across the Stiffkey salt marsh may have been affected by these factors because stunted growth was exhibited by the high marsh and the top of the low marsh populations, where such factors were probably stressful; however, in less stressful areas such as the creek bank and the low marsh, larger plants were evident. Similar findings about some other halophytic populations on the Stiffkey salt marsh have been proved previously by (Jefferies and Perkins 1977, Jefferies et al. 1981) and they conluded that the slower growth response to nitrogen of plants from the high marsh away from creek banks is the result of selection for plants with relatively low growth rates, which are able to survive the period of stress during the summer when the soil is hypersaline. Also, (Jefferies et al. 1981) found similar supportive findings when they found that stunt growth rates of *Salicornia europaea* plants on the high marsh are maintained when nitrate or ammonium salts, or sea water, are added to permanent field plots, therefore, this type of growth of individuals is attributed to a genetic response to the adverse effects of hypersalinity in this part of the marsh.

Comparing the creek bank populations that were collected from the three different salt marshes (Stiffkey, Tollesbury and Abbot's Hall) in 2008 showed no significant differences in most plant growth traits, except in plant height; the Stiffkey population was the tallest, and the number of primary branches was greater in the Stiffkey and Tollesbury populations than at Abbot's Hall. Short stature and fewer primary branches on the part of the Abbot's Hall population could be attributed to the inferior quality of its physical conditions, as it is a recently restored marsh. These recently developed restoration marshes provide abiotic soil conditions that differ markedly from natural marshes, and have been shown to differ in terms of patterns of colonisation and development (Mossman et al. 2012a). It would therefore be difficult to determine if the reduced growth can be attributed to the stresses imposed from increased competition for limited nutrients (Emery 2001) or to the effects of a harsher abiotic environment resulting from the prolonged anaerobic conditions and the accompanying low redox potentials that are frequently encountered in the relatively waterlogged soils of restoration marshes (Mossman *et al*., 2012).

Investigating the relationships between different growth traits in the current study revealed many significant and mostly positive associations. To a considerable extent these would reflect allometric relationships between the sizes of plant organs (Weiner et al. 2009, Price and Weitz 2012), but variations in branching pattern and development should explain the ability of the *Suaeda maritima* populations to respond temporally and spatially with the unpredictable environmental conditions; this inherent characteristic is common in halophytes (Noe and Zedler 2001).

The current study suggests that there are considerable variations between the four populations studied; these variations are assumed to be controlled by the biotic and abiotic factors of the local sites of each population. Consistent variations between populations have been observed in all years, raising the question as to whether it might be explained as genetically driven. The variations observed within populations on many occasions could equally well be plastic responses (Wetson et al. 2012) to the local variations in one or more of the local environmental conditions. Finally, ecological field experiments are usually conducted at single sites for one or two years, which creates a predicament for ecologists who are interested in generalizing their findings to larger spatial and temporal scales (Pennings et al. 2003). Therefore, a major strength of the current study comes from the considerable number of observations of those four different populations, which were collected on different occasions within and between three consecutive years. Also, the outcomes of the current study suggest that the small-scale and large-scale differences in both biotic and abiotic factors present in halophyte communities should be considered in ecological studies

Chapter 4

Seed production and heteromorphism in *Suaeda maritima* populations

Abstract

- ***Background and Aims:*** *Suaeda maritima* is an annual species of halophyte inhabiting coastal and inland salt marshes. It is known to exhibit seed heteromorphism, where the seed morphs produced by a single individual differ in terms of mass and colour; it is expected that these morphs differ in dispersal and germination characteristics. The main aim of this study is to quantify this seed heteromorphism mainly within and between local populations of *S. maritima* on Stiffkey salt marsh on the north Norfolk coast, and two other, different creek bank populations (from the Tollesbury and Abbot's Hall salt marshes), UK.
- ***Methods:*** Two seed heteromorphism traits (mass and colour) were recorded within and between four local populations of *S. maritima* (creek bank, high marsh, top of low marsh and low marsh) at Stiffkey during three consecutive summers (2008-10). Also, the same traits were investigated in 2008 in creek bank populations of *S. maritima* from two saltmarshes in Essex: Tollesbury and the Abbot's Hall.
- ***Key Results:*** For *S. maritima* on Stiffkey salt marsh, with respect to seed mass, the creek bank population consistently produced the heaviest seeds, while the low marsh consistently produced the smallest seeds. The high marsh and the top of the low marsh populations tended to produce the second heaviest seeds. With respect of seed colour, the low marsh population was found to produce the highest numbers of black seeds over the three years studied and was the highest producer of brown seeds in the final season (2010). The creek bank population was the second highest producer of black seeds over the three years and the highest producer of brown seeds in 2009. The high marsh and the top of the low marsh populations were found to produce similar numbers of black and brown seeds on many occasions, and their pattern of production would put them in third place with respect to the seed colour trait. Further variations between populations were observed, such as the timing of seed production, the variability of seed heteromorphism over the years, the variability of seed heteromorphism between natural and man-made restoration salt marshes, and the capacity of

populations to make trade-offs between seed mass, seed colour, and other growth trait parameters.

- ***Conclusions:*** At least three separate populations of *Suaeda maritima* at Stiffkey salt marsh were identified to exhibit distinctive seed heteromorphism, which was manifested in variation in seed mass and colour characteristics. These represent important observations as theoretical models describing and predicting how seeds adapt and evolve in terms of dispersal and germination can be tested through knowledge of species that demonstrate seed heteromorphism such as *S. maritima*. An improved understanding of the prevalence of seed heteromorphism in *S. maritima* should provide a foundation for the development of future studies in terms of variability of seed tolerance with respect to the abiotic environment and can therefore act as a useful aid to monitor and predict how species that occupy vulnerable coastal habitats might respond to particular climate change scenarios.

4.1 Introduction

Plant environments are not stable; they vary spatially as well as temporally, and this places a pressure on plants to adjust accordingly. All plants that thrive in particular habitats do so because they are evolutionarily adapted to the local conditions; however, species must have some element of morphological and physiological plasticity, allowing them to cope with the stresses associated with germinating and then surviving in unpredictable heterogeneous environments (Valladares et al. 2007).

Such adaptive plasticity is evident not only in vegetative morphology and physiology but also in seed characteristics (Baskin and Baskin 1988). Generally speaking, each plant species produces seed that is consistent in terms of morphology and physiology. However, some intraspecific seed variability has been observed and this phenomenon is known as seed heteromorphism; it is particularly evident in annuals. Assessing seed heteromorphism is a matter of quantifying seed size and structure, and this has been addressed at length in the literature, although seed dispersal, dormancy and germination strategies are also factors that reflect phenotypic plasticity (Imbert 2002, Wang et al. 2012).

Seed heteromorphism is particularly widespread in halophytes, reflecting the considerable spatial and temporal variability that is a typical feature of their saline environments (Khan et al. 2001). Over 200 halophytic species have been observed to exploit seed heteromorphism, but it is particularly evident in the families Asteraceae and Chenopodiaceae (mostly in annual species) (Table 4.1 and 4.2), and in particular halophytic species including *Arthrocnemum*, *Atriplex*, *Chenopodium*, *Cakile*, *Salicornia*, *Salsola*, *Spergularia*, *Suaeda* and *Trianthema* (Li et al. 2005). Halophytes exhibited seed heteromorphism as some seeds weighed more than others and some had brown testa while others had black ones, and differing germination characteristics reflecting this heteromorphism have been reported (Baskin JM 1976, Khan et al. 2001, Redondo-GÃ³mez et al. 2008, Redondo-Gómez et al. 2008, Wang et al. 2012).

Table 4.1: Species and genera with heteromorphic seeds within halophytic families, based mainly on (Imbert 2002) with more recent additions indicated by an asterisk

Family	Seed heteromorphic species		Total diversity	
	No. species	No. genera	No. species	No. genera
Apiaceae	3	3	3540	446
Asteraceae	138	52	22750	1528
Brassicaceae	12	8	2350	365
Caryophyllaceae	11	2	2300	87
Chenopodiaceae	18+8*	10	1300	103
Cistaceae	4	1	175	8
Commelinaceae	1	1	640	39
Euphorbiaceae	1	1	8100	313
Fabaceae	5	5	18000	642
Fumariaceae	1	1	530	17
Nyctaginaceae	9	1	390	30
Papaveraceae	2	2	230	23
Plantaginaceae	1	1	275	3
Poaceae	7	7	9500	668
Polygonaceae	1	1	1100	46
Rubiaceae	1	1	10220	630
Thymelaceae	1	1	750	53
Valerianaceae	2	1	300	10

Table 4.2: List of seed heteromorphic species within the family Chenopodiaceae based mainly on (Imbert 2002) with more recent additions indicated by an asterisk

Species	References
Aellenia autrani (Post) Zoh.	Negbi & Tamari (1963), Werker & Many (1974)
Arthrocnenum macrostachyum (Moric.) Moris	Khan et al. (1998), Khan & Gul (1998) (*A. indicum* Willd.)
Atriplex dimorphostegia Kar & Kir	Koller (1957)
Atriplex hortensis L.	Becker (1913), Frankton & Basset (1968)
Atriplex micrantha Ledeb.	Frankton & Basset (1968) (*A. heterosperma* Bunge)
Atriplex oblongifolia Waldst. & Kit	Frankton & Basset (1968)
Atriplex patula L.	Ungar (1971)
Atriplex prostrata (Boucher) ex. DC	Wertis & Ungar (1986) (*A. triangularis* Willd.), Ellison (1987) (*A. triangularis*)
Atriplex sagittata Borkh.	Becker (1913) (*A. nitens* Schkuhr), Mandák & Pysˇek (1999a, b, 2001a, b)
Atriplex semibaccata R.Br.	Beadle (1952)
Axyris amaranthoides L.	Becker (1913)
Blackiella inflata (F. Mueller) Aaelen	Beadle (1952) (*Atriplex inflata*)
Chenopodium album L.	Williams & Harper (1965)
Halogeton glomeratus (Bieb.) C.A. Mey.	Williams (1960)
Salicornia europaea L.	Ungar (1979), Philipupillai & Ungar (1984), Austenfeld (1988)
Salsola komarovii Iljin	Takeno & Yamaguchi (1991)
Salsola volkensii Asch. & Schw.	Negbi & Tamari (1963)
Sennellia spongiosa (F. Mueller) Aellen	Beadle (1952) (Atriplex spongiosa)
Suaeda acuminata *	(Wang et al. 2012)
Suaeda aralocaspica *	Wang, Huang et al. (2008)
Suaeda depressa *	Williams and Ungar (1972)
Suaeda fruticosa *	Khan M Ajmal (1998)
*Suaeda maritima**	(Boucaud and Ungar 1973, Ungar and Boucaud 1974, Boucaud and Ungar 1976, Metzing 1996)
Suaeda moquinii *	Khan, Gul et al. (2001)
*Suaeda salsa**	Li et al (2005)
Suaeda splendens *	Redondo-GÃ³mez, Mateos-Naranjo et al. (2008)

Early work on phenotypic variation among *Suaeda maritima* populations in British salt marshes (Chapman 1947) (Clapham et al. 1962) resulted in the identification of three different putative varieties with characteristic seed dimensions (although these varieties are no longer recognized in current British Floras e.g. (Stace 2010): var. *flexilis* Focke (seed diameter 1.1-1.4 mm), var. *macrocarpa* Moq (seed diameter c. 2 mm) and var. *vulgaris* Moq (seed diameter 1.5 mm). Other early work on polymorphic *Suaeda maritima* was conducted by (Boucaud 1962) who assessed the seed size of three proposed varieties of *Suaeda maritima* in France and found differences in the average seed diameter; mean diameter of *S. maritima* var. *flexilis* was 1.5 mm; *S. maritima* var. *macrocarpa* was 2.2 mm, and *S. maritima* var. *vulgaris* was 1.7 mm. Variation in requirements for germination has also been reported for these varieties; var. *macrocarpa* has been found to have no dormancy period, but var. *flexilis* and var. *vulgaris* depended on scarification or stratification (Ungar and Boucaud 1974). (Metzing 1996), also, found that seed size was not a stable feature of morphology. Seeds harvested from laboratory-grown plants were smaller than those found in the field and it was suggested this was a reflection of the nutrient-enriched laboratory environment. Indeed, regardless of seed size, all cultivated plants produced seeds that were smaller than their wild parent plants, thus, although the genetic base is an important factor, environmental conditions can determine seed size (Metzing 1996).

Seed mass, as a function of seed size, is crucial, as it is key in the provisioning for the early establishment stages of seedlings and, as such, it is closely associated with viability; plant fitness, i.e. the rate of successful year-on-year germination, has been shown to be a function of seed size (Braza et al. 2010). Mean seed size was being considered the least plastic variable of a species' seed characteristics, with alteration of the numbers of seeds produced rather than their size, with changing environmental conditions. However, more recent evidence is that the ability to adjust seed size and shape is a key function of the ecology and evolution of vascular plants (Silvertown 1989, Westoby et al. 1992, Telenius 1999). Environmental pressures are a key driver of seed morphology; the effects may be immediate and local, reflecting fluctuations in soil fertility, or they may be based on pressures that have been sustained over generations (Greipsson and Davy 1995). Both of these are a measure of the plant's plasticity, which in turn, is a function of the genetic base.

The central aspect of research in this chapter is to quantify the seed heteromorphism in *Suaeda maritima* populations and to do so in the context of the polymorphism evident in the parent plants in various locations on Stiffkey salt marsh, i.e. to establish whether there is any correlation between seed heteromorphism and plant characteristics.

I hypothesize that variation in the relative proportions of the different seed morphs (in terms of mass and colour) in heteromorphic annual species, such as *Suaeda maritima*, may be related to temporally unpredictable or spatially heterogeneous environments.

In order to address these issues, I seek to satisfy the following three objectives:

1. To quantify the variation in seed mass within and between populations of *Suaeda maritima* in the field at Stiffkey, Tollesbury and Abbots' Hall salt marshes.
2. To quantify the variations in seed number and colour within and between populations of *Suaeda maritima* in the field at Stiffkey, Tollesbury and Abbots' Hall salt marshes.
3. To study the relationships between seed mass, seed number and colour variations, and other plant morphological traits.

4.2 Materials and methods

4.2.1 Plant sampling and sites

Seeds were extracted from the plants collected for the morphometric work described in chapter 3. The sites are described in more detail in chapter 2. In summary, mature *Suaeda maritima* individuals were sampled from four locations with Stiffkey salt marsh with the aim to characterize the abundance and size of seed morphs at each location. Locations chosen were: low marsh, top of low marsh, high marsh and creek bank. Samples were collected randomly from the most abundant and homogeneous *Suaeda maritima* stands at each location during the fruiting periods of 2008, 2009 and 2010.

4.2.2 Seed sampling

In 2008, 30 plants were collected weekly between the middle of October and the middle of November from the creek bank, the high marsh, and the top of the low marsh habitats. Sampling began too late in the season to collect low marsh *Suaeda maritima* plants, which had senesced by the end of October. In 2009, sampling therefore began earlier, at the end of August, collecting 20 plants from each location on a fortnightly basis. Insufficient brown seeds were obtained in 2008 and 2009 to conduct the intended experimental work and so sampling intensity was increased in 2010 to 20 plants weekly from the four locations between 22 August and the 21 November. In addition, ten plants were also collected from creek bank habitats at Tollesbury and Abbotts Hall, Essex, in November 2008 by Joanna Chitty.

All collected plant material was placed in polythene bags and stored at 5 °C immediately on return to the laboratory. Stored samples were measured and processed within a few weeks of collection.

4.2.3 Characterisation of seed heteromorphism

Seeds were dissected out of all of the *Suaeda maritima* plants, classified on the basis of colour as black or brown and counted. Black seed have a thick black, resistant, shiny outer testa, which is missing in the brown ones. Ten randomly selected seeds of each morph (where available) from each plant were weighed using a micro-balance (Metler Toeldo UMX2). All seeds were then placed in sealed plastic vials and then kept for further experiments (Chapter 7). Seeds from 2008 fruiting season were kept in the cold room (5 °C) whereas seeds from the 2009 and 2010 seasons were stored in a dry dark room.

4.2.4 Statistical analysis

Statistical analysis was carried out using PASW Statistics version 18, Chicago, USA for Windows. The main comparisons of the numbers of both seed morphs, as well as seed mass, between populations and years were expressed as mean ± standard error. The general linear model (GLM) analysis of co-variance (ANCOVA; $P < 0.05$) was used to compare the treatment effects; collection date was added as a co-variable. The one-way ANOVA was applied to assess the differences among populations and years. Black seed mass trait between and within populations was investigated in two different approaches; black seed mass per plant (seed masses were averaged by weighting each plant in the population equally (i.e. per each individual plant, then all averages were averaged again) (i.e. the replicate is the plant), and individual black seed mass (seed masses were averaged by weighting all seeds in the population equally (i.e. per seed per population) (i.e. the replicate is the seed). Individual seed mass was investigated in three consecutive seasons; 2008, 2009 and 2010. While, seed mass per plant was investigated in two seasons 2008 and 2010 only, since sufficient data of seed weight (mass) was achieved in both years in relation to the actual collections dates.

Linear regression was used to assess relationships between black seed mass and other morphological traits, including plant height, shoot width, number of black seeds and number of brown seeds. Simple and multiple linear regression were also used to assess relationships between the numbers of each seed morph with other morphological traits.

4.4 Results

4.3.1 Seed mass

In 2008, the creek bank population from Tollesbury salt marsh produced significantly the biggest seeds followed by the Stiffkey and finally the Abbots Hall creek bank populations (Fig. 4.1). In the same season and among Stiffkey populations the creek bank produced significantly the largest black seeds followed by the top of low marsh and the high marsh, respectively (Fig. 4.2).

In the year 2009, the creek bank produced significantly the largest black seeds followed by the high marsh, the top of low marsh and the low marsh, respectively (Fig. 4.2).
In 2010, the creek bank population produced the largest black seeds followed by the top of low marsh, the high marsh, and finally by the low marsh population. Also, the same test revealed that the greatest black seed mass achieved was in 2009 followed by 2008 and finally 2010 (Fig. 4.2).

Taking in consideration the collection date as a covariate, the two-way ANCOVA model revealed that there was significant variability of both individual black seed mass and black seed mass per plant traits at all levels; population, year and also in the interaction between population and year (Table 4.3).

Furthermore, seasonal variability of the black seed mass in 2010 showed that seed mass in all populations generally increased gradually during the season until it reached approximately twice the initial mass. Only the top of low marsh population showed a slight decrease in the black seed mass in the last three collections (Figure 4.3).

The linear regression was used to assess the relationship between black seed mass and plant height and no significant associations were found between traits in 2008 within and between populations. However, in 2010 and within the creek bank population there was one significant positive association in 4th collection (Fig. 4.4a) and one significant negative association as well in 7th collection (Fig. 4.4b). Within the top of the low marsh two significant positive associations (Fig. 4.4c and 4.4d) and one significant negative association were identified (Fig. 4.4e). Within the low marsh there were no significant associations between traits, while there was only one significant positive association within the high marsh. Finally there was a significant positive relationship at the inter-population level only in year 2010 (Fig. 4.4f).

Table: 4.3 Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations black seed mass trait. **a)** Individual black seed mass (mg). **b)** Black seed mass (mg) per plant.

Source of Variation		**Population**	**Year**	**Population*Year**	**Error**
a Individual black seed mass (mg)	**Type lll SS**	81.7	38.1	8.14	454.5
	df	3	2	5	6528
	F	391.0	273.7	23.4	-
	P	<0.001	<0.001	<0.001	-
b Black seed mass (mg) per plant	**Type lll SS**	2.33	0.80	0.79	22.5
	df	3	2	4	612
	F	21.19	10.9	5.43	-
	P	<0.001	<0.001	<0.001	-

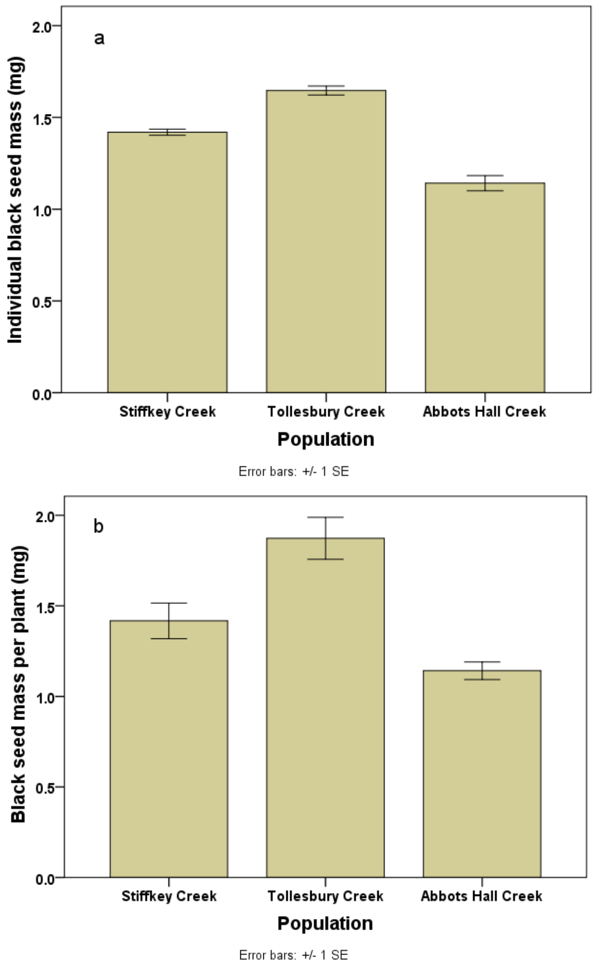


Figure 4.1: Comparisons (mean ±s.e.) of black seed mass of three different creek-bank *Suaeda maritima* populations in 2008. (**a)** Individual black seed mass. (**b)** Black seed mass per plant.

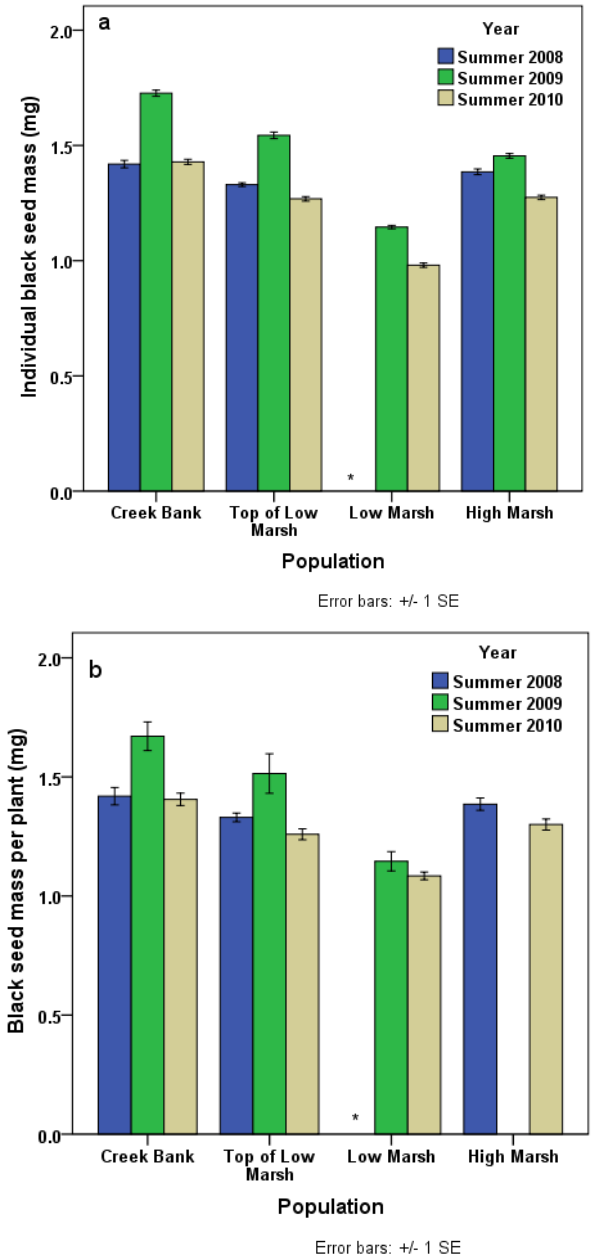
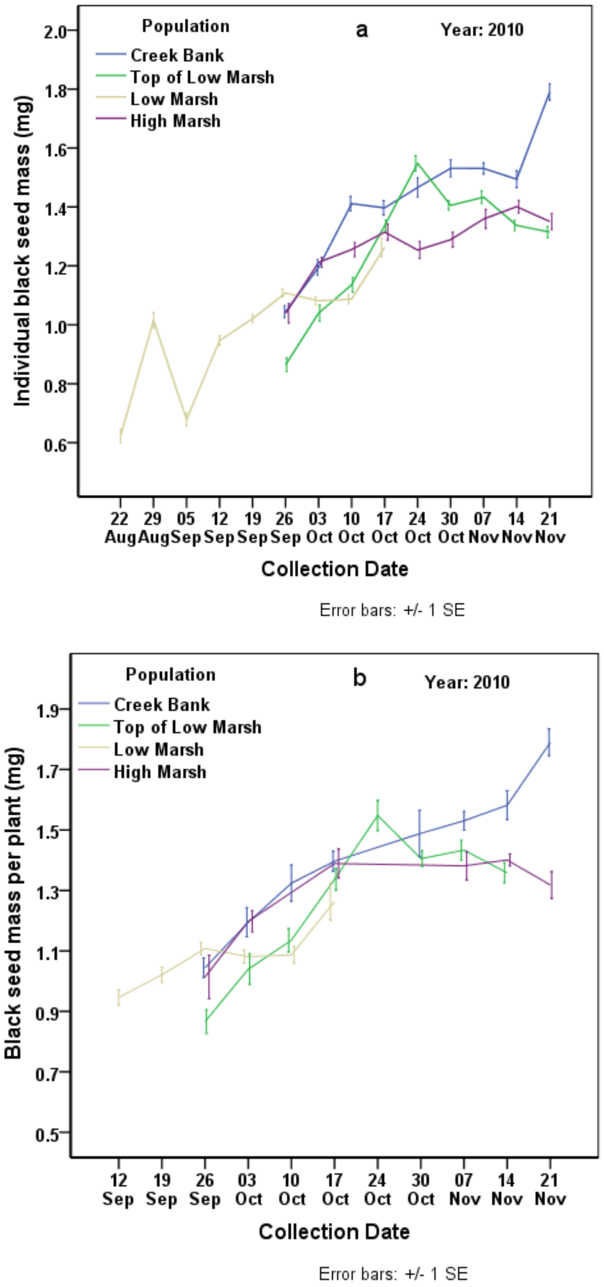


Figure 4.2 Comparisons (means±s.e.) of black seed mass trait of Stiffkey *Suaeda maritima* populations. (**a)** Individual black seed mass**. (b)** Black seed mass per plant. (* Insufficient data).



Error bars: +/- 1 SE

Error bars: +/- 1 SE

Figure 4.3: Temporal changes (mean ±s.e.) of the Stiffkey *Suaeda maritima* populations' black seed mass trait. (**a)** Individual Black seed mass. (**b)** Black seed mass per plant.

No significant relationship between black seed mass and the number of primary branches was found within and between populations in 2008. However, in 2010 two significant associations were identified; one positive within the creek bank 9th collection (Fig. 4.5a) and one significant negative relationship at the inter- population level (Fig. 4.5b).

No significant association was found between black seed mass and the number of black seeds in 2008. However, in 2010 two negative associations were found; one within the 1st collection of the creek bank population (Fig. 4.6a) and the second one at the inter-population level (Fig. 4.6b).

No significant association was found between black seed mass and plant shoot width traits in 2008. However, in 2010 two significant associations were found; one positive within the 9th collection of the creek bank population (Fig. 4.7a) and the second one is a negative relationship at the inter- population level (Fig. 4.7b).

Investigating the relationship between the above traits using the multiple regression approach has revealed that plant height and the number of black seeds influenced the black seed mass positively, but the number of primary branches affected it negatively. However, the plant shoot width variable was negligible and the variance explained by this model was 18.9% ($P < 0.001$).

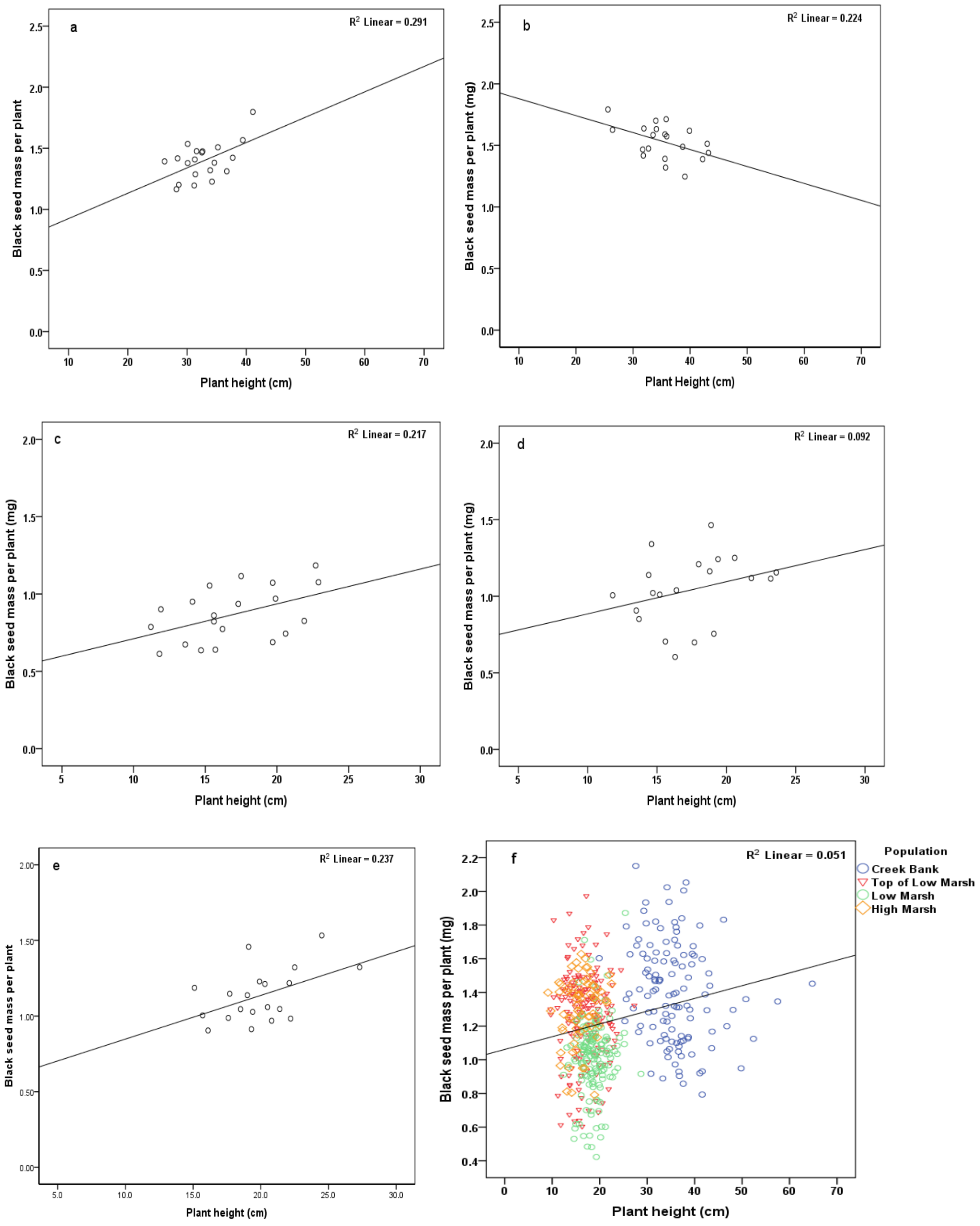


Figure 4.4: Relationship between black seed mass and plant height (per plant). (**a)** Within 7th collection Creek Bank 2010 ($R^2=0.291$, $P<0.014$). (**b)** Within 7th collection Creek bank 2010 ($r^2=0.224$, $P<0.035$). (**c)** Within 1st collection Top of low marsh 2010 ($r^2=0.217$, $P<0.038$). (**d)** Within 2nd collection Top of low marsh 2010 ($r^2=0.252$, $P<0.024$). (**e)** Within 3rd collection Top of low marsh 2010 ($r^2=0.237$, $P<0.029$). **f)** Between populations 2010 ($r^2=0.051$, $P<0.001$).

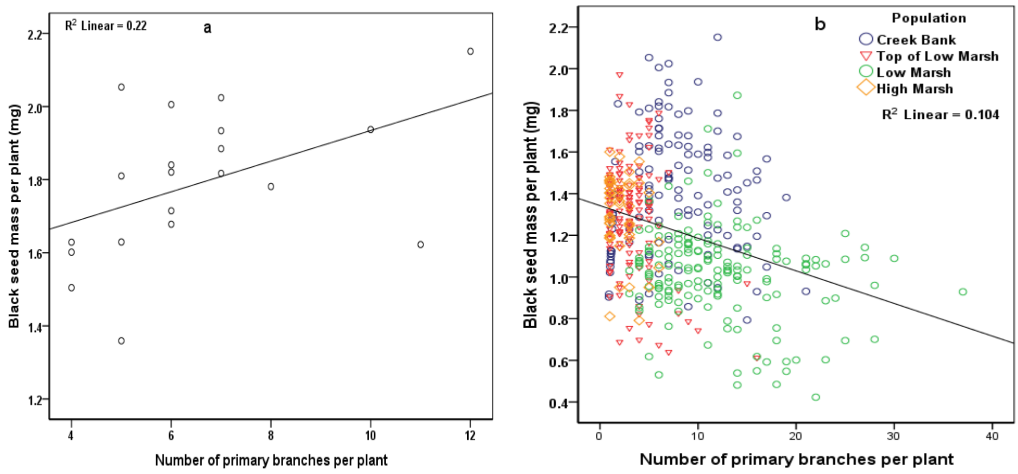


Figure 4.5: Relationship between black seed mass and number of primary branches (per plant) (**a**) Within 9th collection Creek Bank 2010 (r^2=0.220, *P*<0.037). (**b**) Between populations 2010 (r^2=0.104, *P*<0.001).

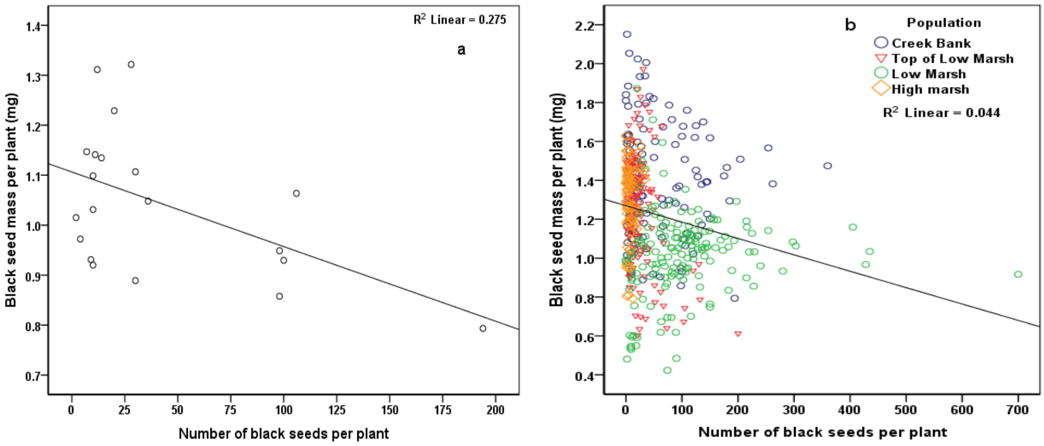


Figure 4.6: Relationship between black seed mass and the number black seeds (per plant) (**a**) Within 1st collection Creek Bank 2010 (r^2=0.275, *P*<0.018). (**b**) Between populations 2010 (r^2=0.044, *P*<0.001).

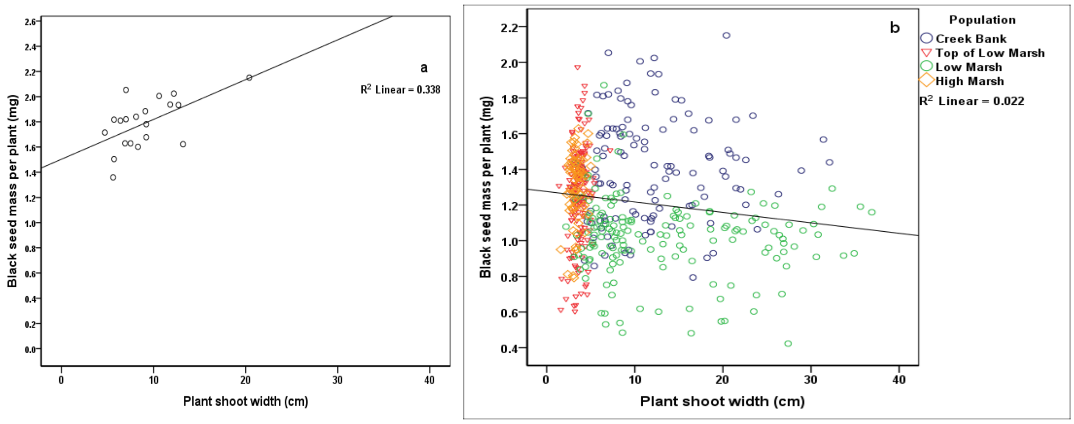


Figure 4.7: Relationship between black seed mass and shoot width (per plant) (**a**) Within 9th collection Creek bank 2010 (r^2=0.338, *P*<0.007) (**b**) Between populations 2010 (r^2=0.022, *P*<0.001).

Brown seed mass was assessed in the Stiffkey populations in two consecutive seasons (2009 and 2010). In 2009 creek bank was found to be significantly producing the largest brown seeds followed by the high marsh and finally by the low marsh. In 2010 the creek bank produced significantly the largest brown seeds followed by the top of low marsh, the high marsh and the low marsh respectively (Fig. 4.8). The two-way ANCOVA (Table 4.4) revealed that variation of brown seed mass was significant at all levels; population, year and between years and in the interaction between population and year. Also, the test revealed the brown seed mass to be significantly heavier in 2009 than in 2010.

Table: 4.4 Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the individual brown seed mass (mg).

Source of Variation		**Population**	**Year**	**Population*Year**	**Error**
Individual brown seed mass (mg)	**Type lll SS**	183.2	11.3	21.36	96.7
	df	3	1	2	1740
	F	1094.8	202.6	191.5	-
	P	<0.001	<0.001	<0.001	-

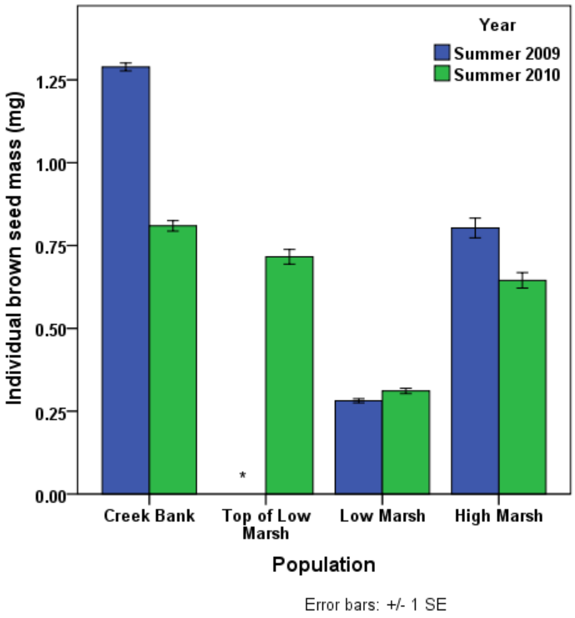


Figure 4.8 Comparisons (means ±s.e.) of individual brown seed mass trait of Stiffkey *Suaeda maritima* populations at 2009 and 2010. (* Insufficient data).

The three-way ANCOVA model (Table 4.5) revealed that the black and the brown seed masses in 2009 and 2010 differ significantly at all levels; population, year, seed colour, interaction between population and year, interaction between population and seed colour and finally interaction between population, year and seed colour, but the interaction between year and seed colour was insignificant.

Table: (4.5) Results of the three-way ANCOVA of Stiffkey *Suaeda maritima* populations for the individual black brown seed mass trait (mg).

Source of Variation		**Population**	**Year**	**Seed colour**	**Error**
Individual black brown seed mass (mg)	**Type lll SS**	315.9	47.3	328.2	598.9
	df	3	2	1	8262
	F	1452.9	326.1	4528.2	-
	P	<0.001	<0.001	<0.001	-

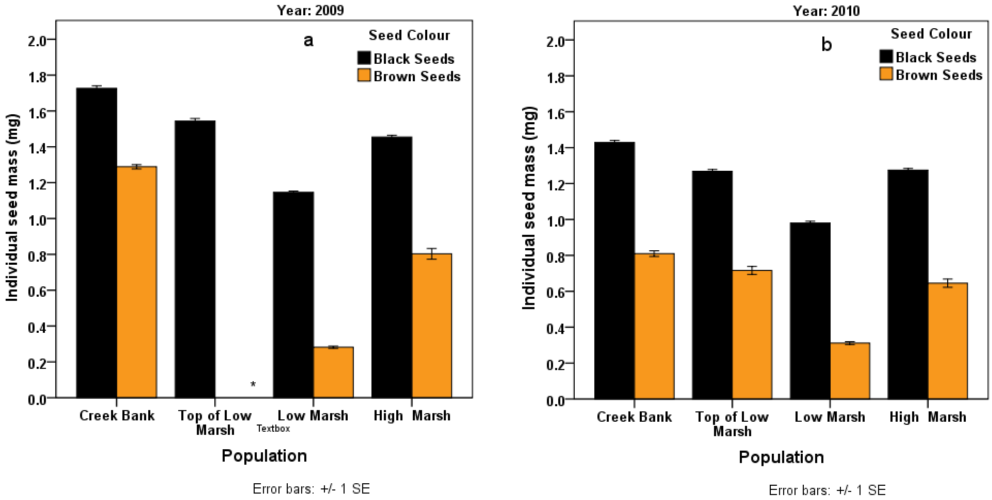


Figure 4.9: Comparisons (means ±s.e.) of black and brown seed masses of the Stiffkey *Suaeda maritima* populations. (**a**) Individual black brown seed mass 2009. (**b**) Individual black brown seed mass 2010. (* Insufficient data).

4.3.1 Fecundity:

4.3.2.1 Total number of seeds

This trait was investigated specifically because the total number of seeds produced by each population included black seeds, brown seeds and immature seeds especially in the early season when it was difficult to distinguish black from brown.

In the year 2008 (Fig. 4.10), *Suaeda maritima* plants of the Stiffkey creek bank population were significantly the biggest producer of seeds, followed by plants from Tollesbury creek and finally by the Abbots Hall creek plants. Among Stiffkey salt marsh populations in the same season (Fig. 4.11), creek bank population produced significantly the largest number of seeds followed by the top of low marsh and the high marsh populations.

In the year 2009 Stiffkey creek bank population was significantly the largest seed producer followed by the low marsh, the top of low marsh and the high marsh populations respectively. However, in 2010 the low marsh population produced significantly the largest amount of seeds followed by the creek bank, the top of low marsh and the high marsh populations respectively (Fig. 4.11).

The two-way ANCOVA revealed the variation of the total number of seeds to be significant at all levels; population, years and even in the interaction between years and populations (Table 4.6).

The investigation of seasonal variability within the season 2010 (Fig. 4.12) has revealed that low marsh population started producing seeds on 22 July (five weeks earlier than other populations) with an average of (mean±s.e,140.40±12.75) seeds per plant. This number fluctuated through the season forming two peaks (at 2nd and 7th collections) and two troughs (at 4th and 9th). The variability of total seeds production throughout the season was also apparent in the creek bank and the top of low marsh populations, but in smaller numbers, and finally significantly less but more stable total seed production was observed by the high marsh population.

Table: 4.6: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the total number of seeds per plant trait (mg).

Source of Variation		**Population**	**Year**	**Population*Year**	**Error**
Total number of seeds	**Type lll SS**	1257368.6	223014.4	418810.9	4267259.3
	df	3	2	5	1078
	F	105.9	28.2	21.2	-
	P	<0.001	<0.001	<0.001	-

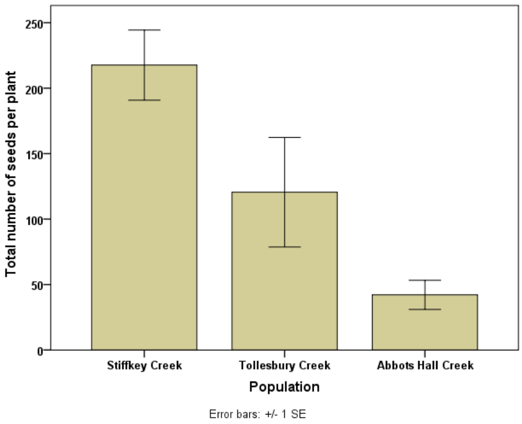


Figure 4.10: Comparisons (mean±s.e.) of the total number of seeds produced per plant by *Suaeda maritima* from different creek bank populations in 2008 (Stiffkey, Tollesbury and Abbot's Hall).

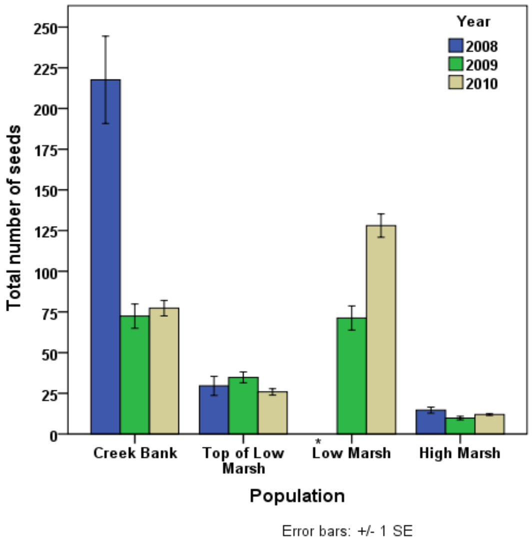


Figure 4.11: Comparisons (mean ±s.e.) of the total number of seeds per plant produced by the Stiffkey *Suaeda maritima* populations in 2008, 2009 and 2010.

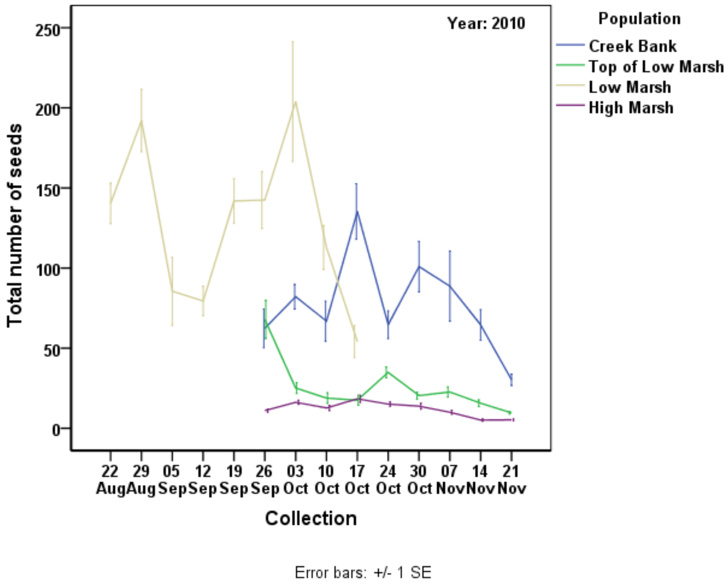


Figure 4.12: Temporal changes (mean ±s.e.) of the total number of seeds per plant of the Stiffkey *Suaeda maritima* populations at 2010.

4.3.2.2 Number of black seeds

In 2008 (Fig. 4.13), the *Suaeda maritima* creek bank populations at Tollesbury and Stiffkey saltmarshes significantly produced higher numbers of black seeds than the creek bank population at Abbots Hall. At Stiffkey (Fig. 4.14), the highest significant number of black seeds was produced by the creek bank population, followed respectively by the top of low marsh and the high marsh populations; no low marsh samples were collected from Stiffkey in 2008.

In 2009 and 2010, the Stiffkey *Suaeda maritima* populations from the low marsh produced significantly the highest number of black seeds followed by plants from the creek bank, the top of the low marsh and, the high marsh population, respectively (Fig. 4.14).

Table 4.7 shows the result of the two-way ANCOVA model which was used to assess the variation of black seed production among populations and years. It revealed that this variation was significant at two levels; population and interaction between population and year.

The variation in the production of black seeds throughout the season was explored in the Stiffkey populations in 2010 (Fig. 4.15). The production of black seeds was observed in the low marsh population five weeks earlier than in the other populations. Also this population stopped producing black seeds and senesced five weeks earlier than the other three populations. There were significant fluctuations of black seed production by the creek bank and the top of low marsh populations. This production reached the peak at the middle of the season before it declined to the lowest level by the end of each population life cycle. However this pattern was more stable with the high marsh population over the season.

Table: 4.7: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the number of black seeds per plant trait (mg).

Source of Variation		**Population**	**Year**	**Population*Year**	**Error**
Number of black seeds	**Type lll SS**	396876.8	16241.5	65576.7	3188510.3
	df	3	2	5	1078
	F	44.7	2.74	4.43	-
	P	<0.001	0.065	<0.001	-

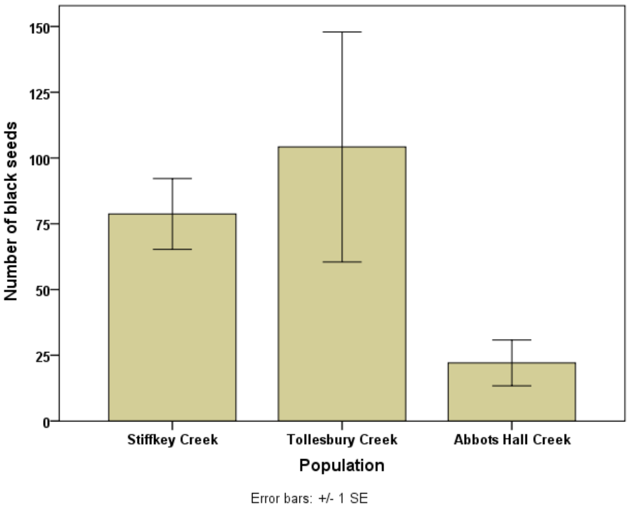


Figure 4.13: Comparisons (mean ±s.e.) of the number of black seeds per plant produced by the *Suaeda maritima* from different creek bank populations in 2008 (Stiffkey, Tollesbury and Abbot’s Hall).

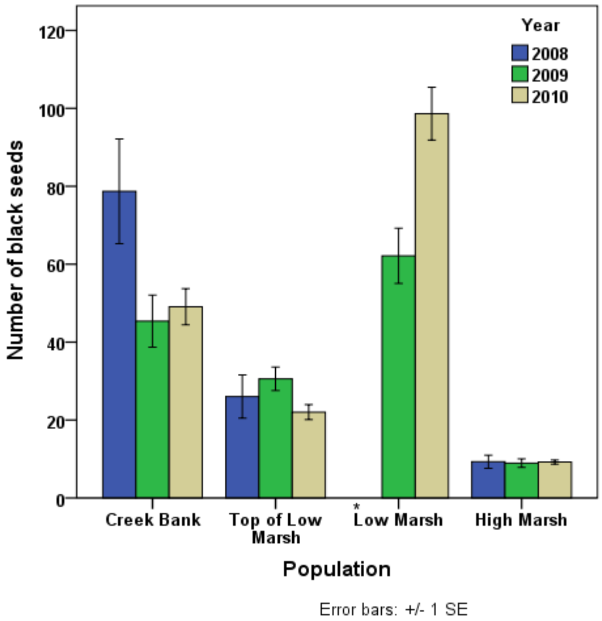


Figure 4.14: Comparisons (mean ±s.e.) of the number of black seeds per plant produced by the Stiffkey *Suaeda maritima* populations in 2008, 2009 and 2010.

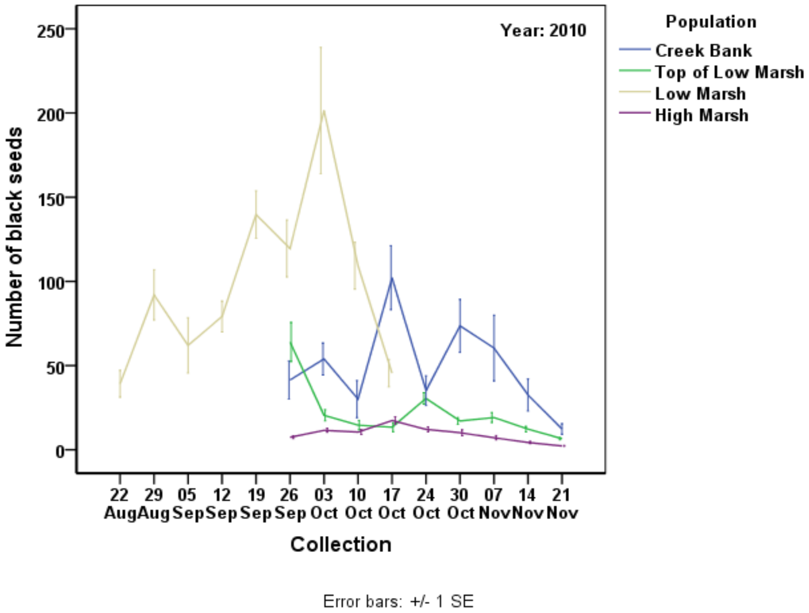


Figure 4.15: Temporal changes (mean ±s.e.) of the number of black seeds per plant of the Stiffkey *Suaeda maritima* populations at 2010.

There was no significant relationship between the number of black seeds and plant height in the creek bank population in all seasons. However, in the low marsh population this association was significant and positive in both seasons 2009 (Fig. 4.16a) and in many collections in 2010 (Fig. 4.16b-d). Also in the top of low marsh there was a significant positive relationship between traits in both seasons (Fig. 4.16e-h). In the high marsh there was no significant relationship in 2009, but in 2010 some significant weak positive associations were evident. Furthermore, between populations there is evidence of the existence of significant positive relationships between the number of black seeds and plant height in all years (Fig. 4.16i-k).

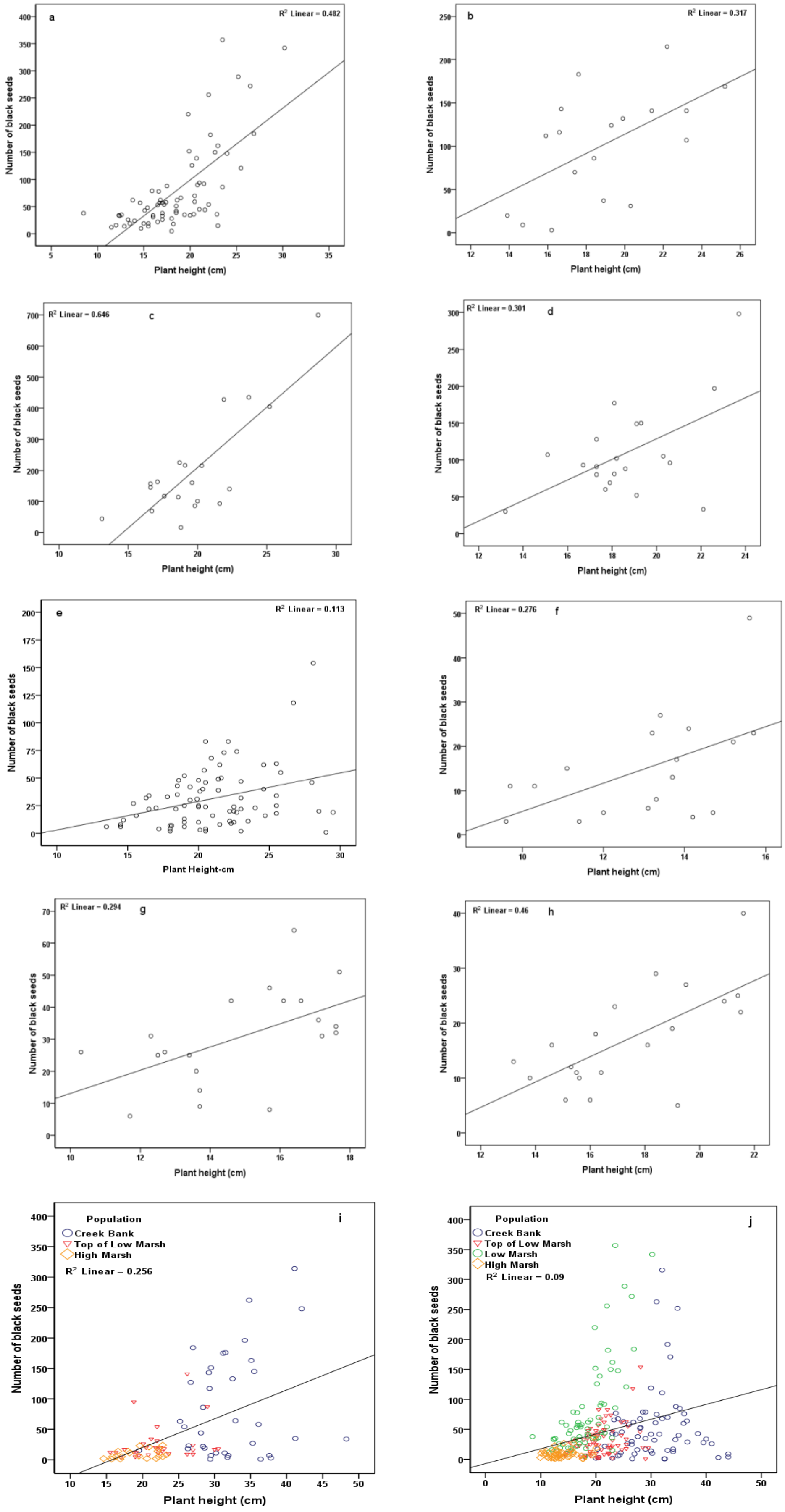
No significant relationship was found between the number of black seeds and the shoot width within the creek bank population in years 2008 and 2009, however, one significant relationship was found in 2010 (Fig. 4.17d). Within the low marsh population this association was found to be significantly positive in both seasons 2009 (Fig. 4.17c) and 2010 (Fig. 4.17g-i). A positive relationship was found in the top of low marsh between traits in 2008 (Fig. 4.17b) and a few significant and positive relationships in the 2010 collections (Fig. 4.17e-f). In the high marsh no significant relationship was found for all years. Finally, for all seasons, significant and positive associations were found at the inter- population level (Fig. 4.17i-k).

In general there were significant relationships between black seed number and the number of primary branches within and between populations in all years (Fig. 4.18). This relationship was positively evident within Tollesbury creek bank 2008 (Fig. 4.18a), within top of low marsh 2008 (Fig. 4.18b), within creek bank 2009 (Fig. 4.18c), within top of low marsh 2009 (Fig. 4.18d), within low marsh 2009 (Fig. 4.18e), within high marsh 2009 (Fig. 4.18f), within 4th collection creek bank 2010 (Fig. 4.18g), within 1st collection top of low marsh 2010 (Fig. 4.18h) and within 8th collection low marsh 2010 (Fig. 4.18i). Finally, for all seasons, significant and positive associations were found at the inter- population level (Fig. 4.18 j-l).

No significant relationship was found between the number of black seeds and the number of secondary branches traits within and between populations in years 2008 and 2009. However, in year 2010, significant positive relationships were found in only three instances; within low marsh 7th collection (Fig. 4.19b), within creek bank population 5th collection (Fig. 4.19a), and finally between populations 2010 (Fig. 4.19c).

There is evidence for a significant association between the number of black seeds and the number of brown seeds traits within and between populations (Fig. 4.20). In 2009 the low marsh and the high marsh populations showed no significant association between traits, while the other two populations showed significant positive associations (Fig. 4.20a-b). In 2010 significant positive associations occurred within the 5th collection of the creek bank population (Fig. 4.20c), within the 9th collection of the low marsh population (Fig. 4.20d), finally no significant association was found within the high marsh population. Finally for both seasons, significant and positive associations were found as well between populations (Fig. 4.20e-f).

Multiple linear regression was used to assess the relationship between the number of black seeds and other related traits, including number of brown seeds, plant height, plant shoot width, number of primary branches and number of secondary branches collectively. The outcome model explained 9.5% of the variation ($P < 0.009$) and revealed that, when interactions between different traits occurred, the only trait that significantly influenced the production of black seeds was plant shoot width.


a
R² Linear = 0.482
b
R² Linear = 0.317
c
R² Linear = 0.646
d
R² Linear = 0.301
e
R² Linear = 0.113
f
R² Linear = 0.276
g
R² Linear = 0.294
h
R² Linear = 0.46
i
Population
Creek Bank
Top of Low Marsh
High Marsh
R² Linear = 0.256
j
Population
Creek Bank
Top of Low Marsh
Low Marsh
High Marsh
R² Linear = 0.09
Number of black seeds
Plant height (cm)
Plant Height-cm

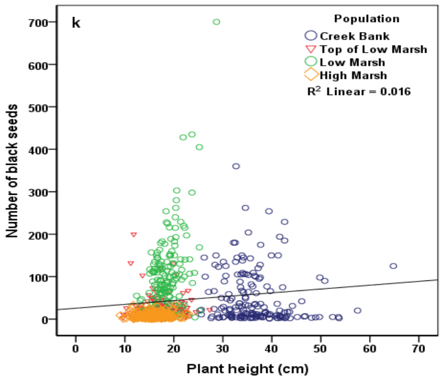
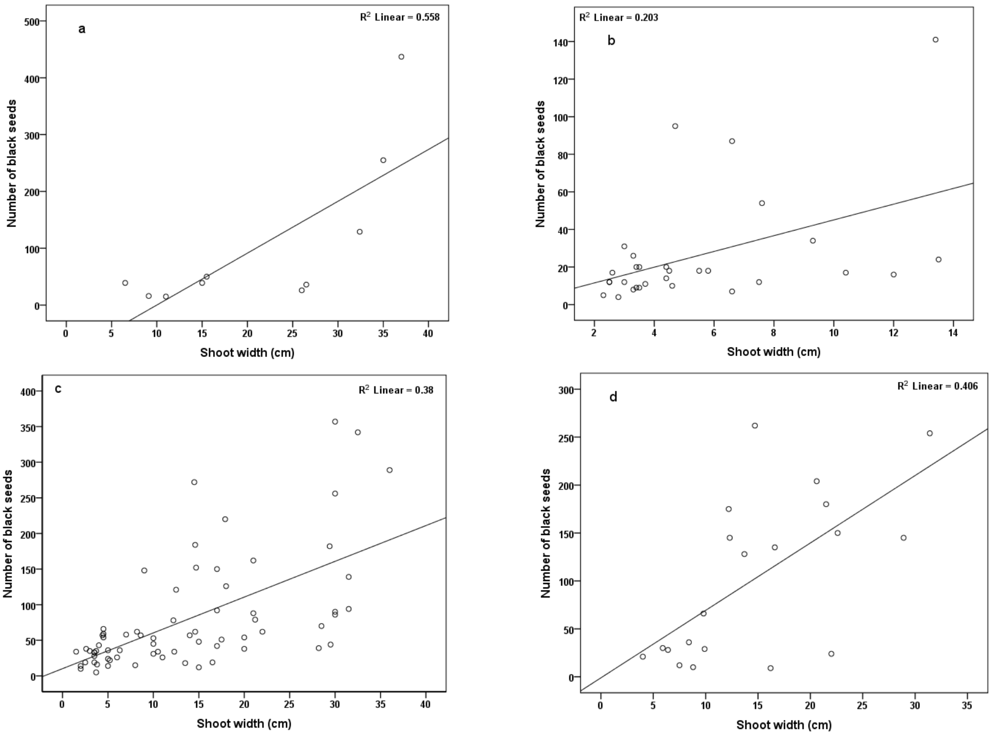


Figure 4.16: Relationship between the number of black seeds and plant height (per plant). (**a**) Within Low marsh 2009 (r^2=0.482, P<0.001). (**b**) Within 2nd collection Low marsh 2010 (r^2=0.317, P<0.015). (**c**) Within 7th collection Low marsh 2010 (r^2=0.646, P<0.001). (**d**) Within 8th collection Low marsh 2010 (r^2=0.301, P<0.012). (**e**) Within Top of low marsh 2009 (r^2=0.113, P<0.003). (**f**) Within 4th collection Top of low marsh 2010 (r^2=0.276, P<0.025). (**g**) Within 5th collection Top of low marsh 2010 (r^2=0.254, P<0.014). (**h**) Within 6th collection Top of low marsh 2010 (r^2=0.460, P<0.001). (**i**) Between populations 2008 (r^2=0.256, P<0.001). (**j**) Between populations 2009 (r^2=0.090, P<0.001). (**k**) Between populations 2010 (r^2=0.016, P<0.001).



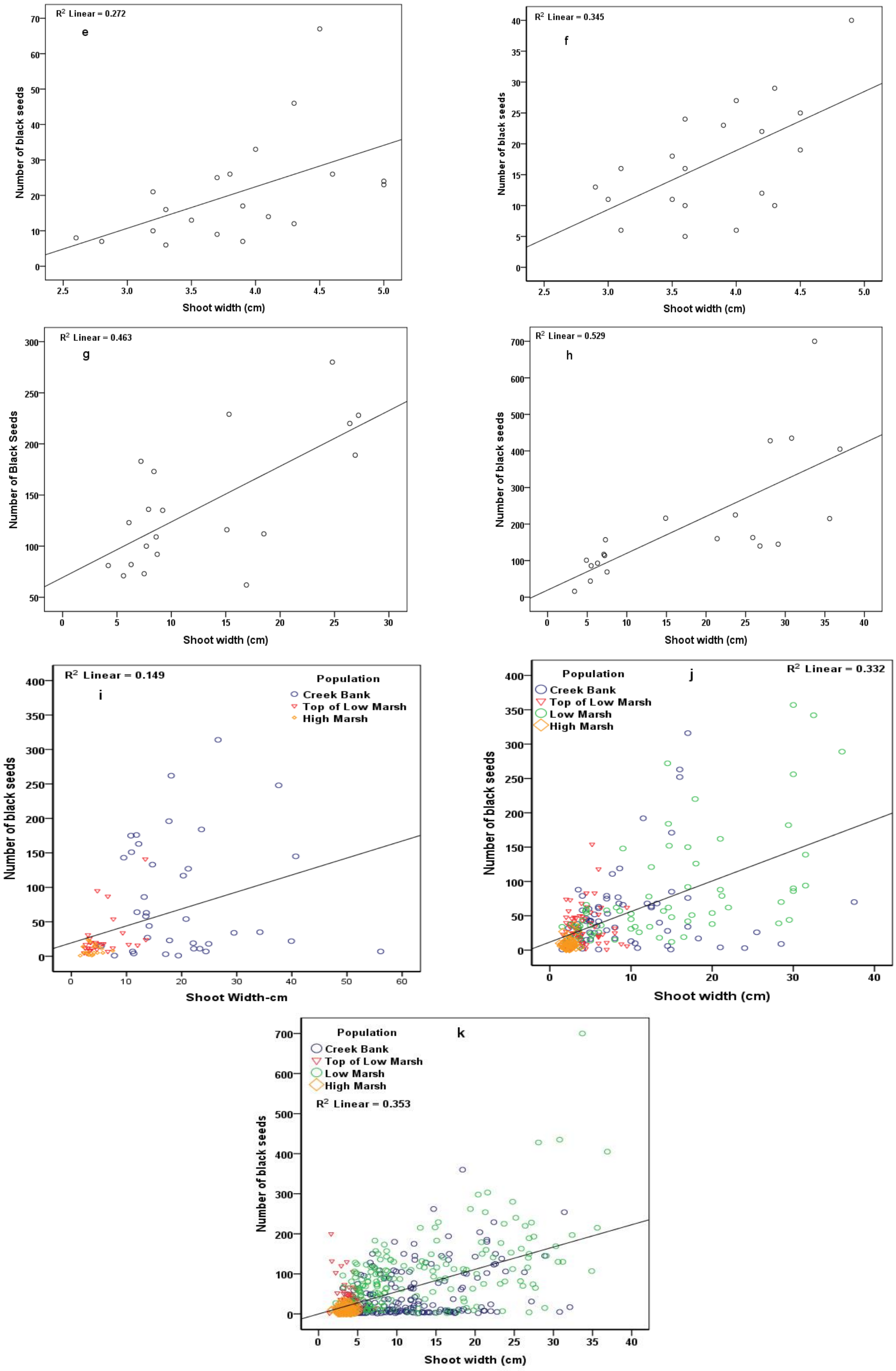
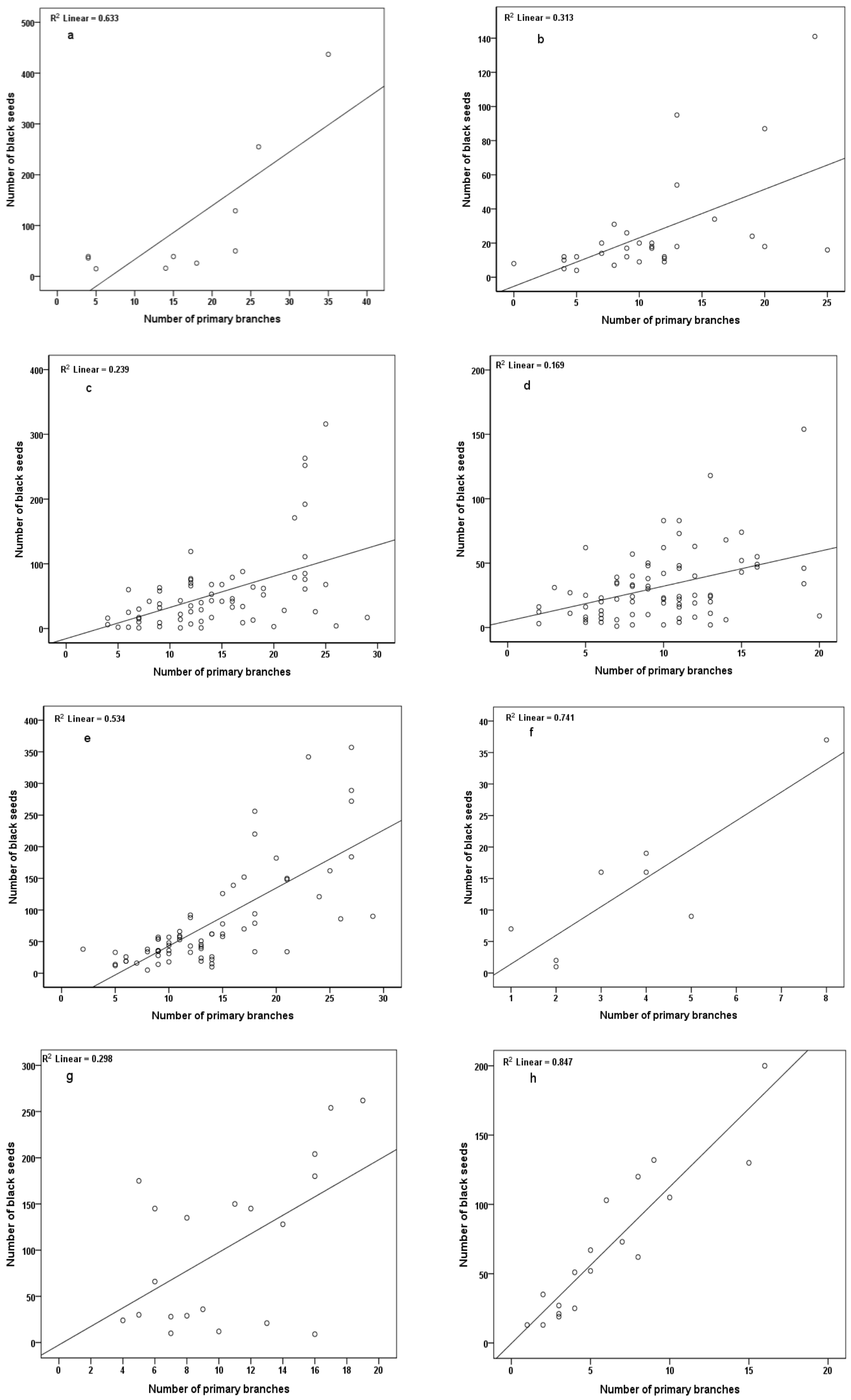


Figure 4.17: Relationship between the number of black seeds and shoot width (per plant). (**a**) Within Tollesbury Creek bank 2008 (r^2=0.558, *P*<0.013). (**b**) Within Stiffkey Top of low marsh 2008 (r^2=0.203, *P*<0.012). (**c**) Within Low marsh 2009 (r^2=0.380, *P*<0.001). (**d**) Within 4th collection Creek bank 2010 (r^2=0.406, *P*<0.003). (**e**) Within 2nd collection Top of low marsh 2010 (r^2=0.272, *P*<0.018). (**f**) Within 6th collection Top of low marsh 2010 (r^2=0.345, *P*<0.006). (**g**) Within 5th collection Low marsh 2010 (r^2=0.463, *P*<0.001). (**h**) Within 7th collection Low marsh 2010 (r^2=0.529, *P*<0.001). (**i**)

Between populations 2008 (r^2=0.149, P<0.001). (**j**) Between populations 2009 (r^2=0.332, P<0.001). **(k)** Between populations 2010 (r^2=0.335, P<0.001).



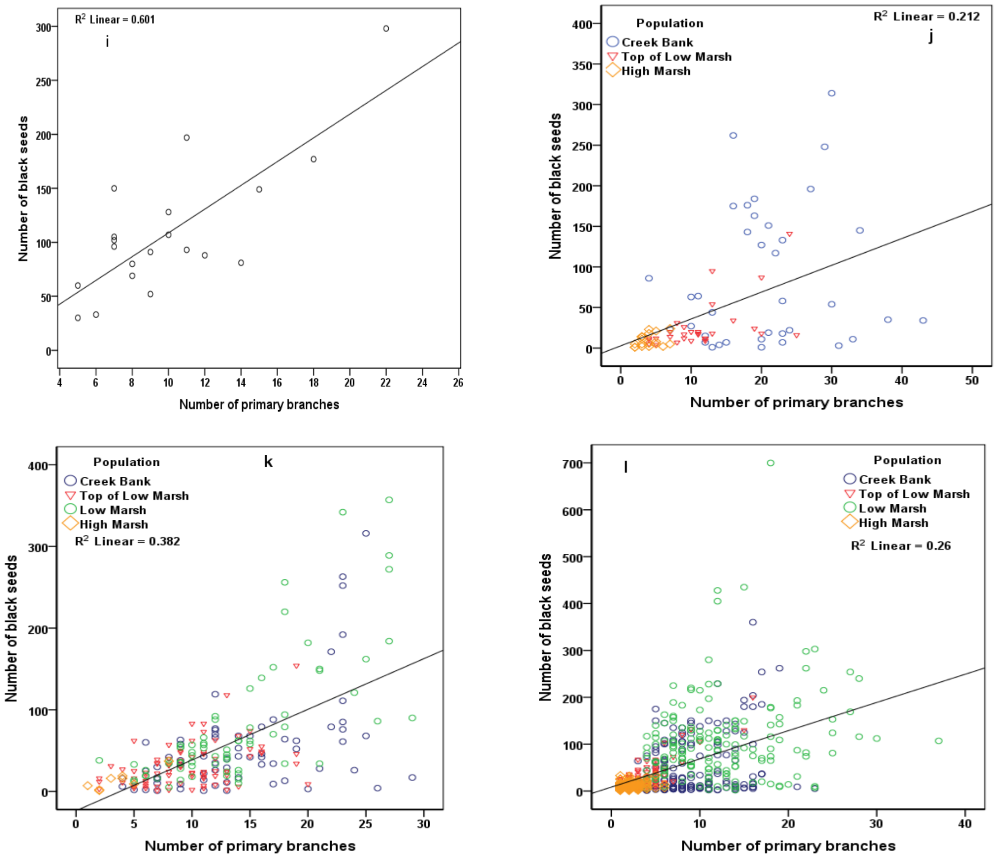


Figure 4.18: Relationship between number of black seeds and number of primary branches (per plant). (**a**) Within Tollesbury Creek bank 2008 (r^2=0.633, *P*<0.006). (**b**) Within Stiffkey Top of low marsh 2008 (r^2=0.313, *P*<0.001). (**c**) Within Creek bank 2009 (r^2=0.239, *P*<0.001). (**d**) Within Top of low marsh 2009 (r^2=0.169, *P*<0.001). (**e**) Within Low marsh 2009 (r^2=0.534, *P*<0.001). (**f**) Within High marsh 2009 (r^2=0.741, *P*<0.001). (**g**) Within 4th collection Creek bank 2010 (r^2=0.298, *P*<0.013). (**h**) Within 1st collection Top of low marsh 2010 (r^2=0.847, *P*<0.001). (**i**) Within 8th collection Low marsh 2010 (r^2=0.601, *P*<0.001). (**j**) Between Stiffkey populations 2008 (r^2=0.212, *P*<0.001). (**k**) Between populations 2009 (r^2=0.382, *P*<0.001). (**l**) Between populations 2010 (R^2=0.260, *P*<0.001).

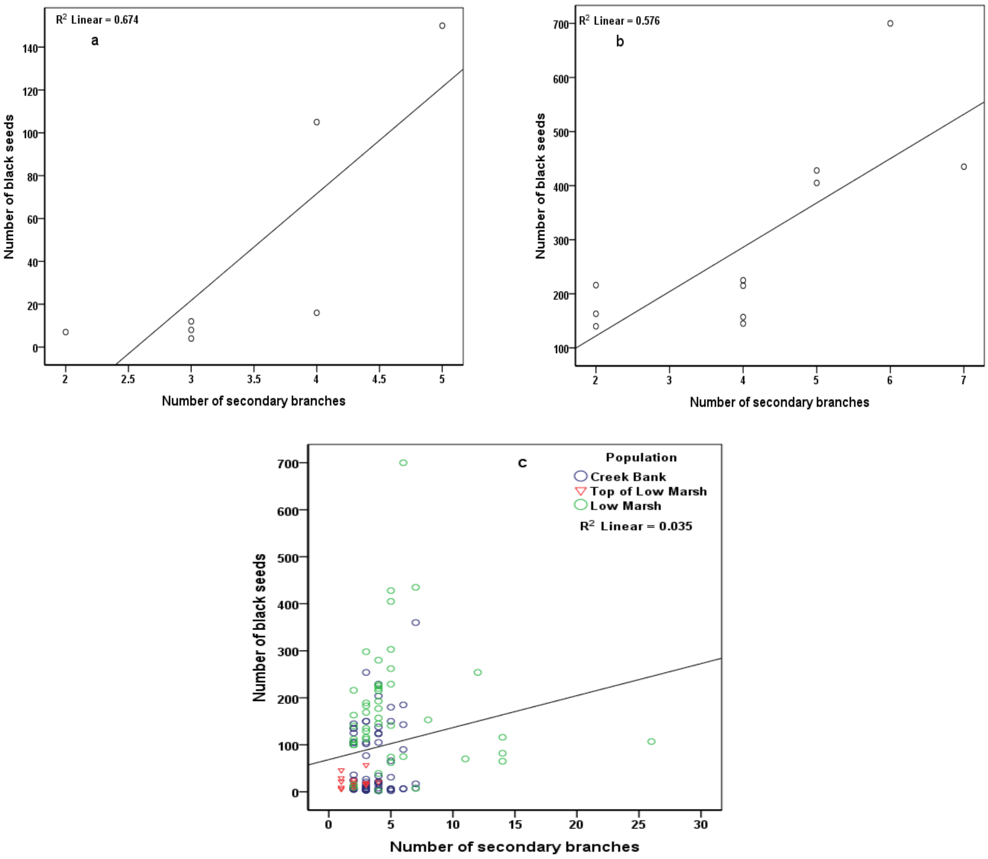


Figure 4.19: Relationship between number of black seeds and number of secondary branches (per plant). (**a**) Within 5th collection Creek bank 2010 (r^2=0.674, P<0.024). (**b**) Within 7th Low marsh 2010 (r^2=0.576, P<0.007). (**c**) Between populations 2010 (r^2=0.035, P<0.032).

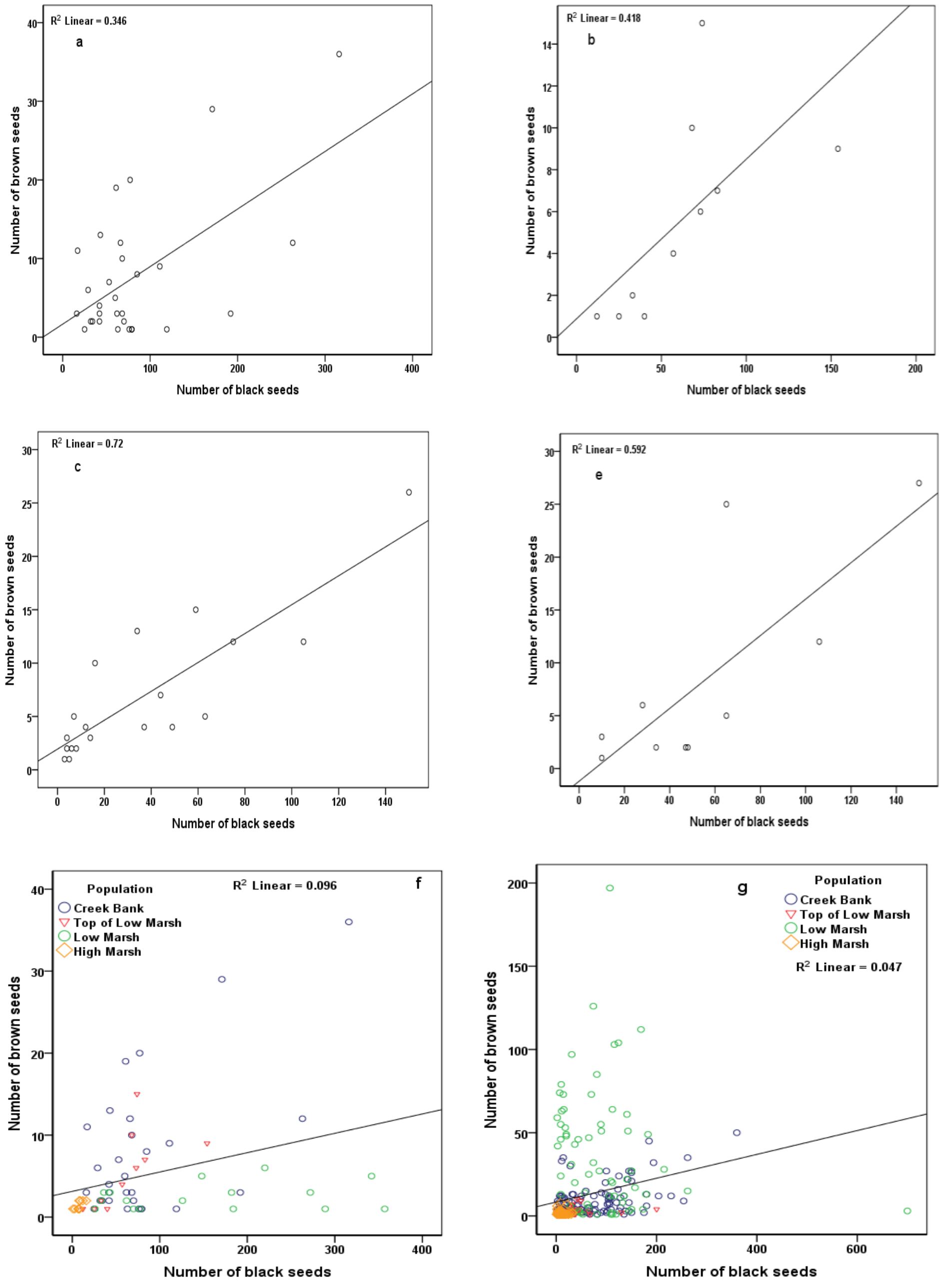


Figure 4.20: Relationship between number of black seeds and number of brown seeds (per plant). (**a**) Within Creek bank 2009 (r^2=0.346, *P*<0.001). (**b**) Within Top of low marsh 2009 (r^2=0.418, *P*<0.043). (**c**) Within 5th collection Creek bank 2010 (r^2=0.720, *P*<0.001). (**d**) Within 9th collection Low marsh 2010 (r^2=0.592, *P*<0.009). (**e**) Between Stiffkey populations 2009 (r^2=0.0.096, *P*<0.011). (**f**) Between populations 2010 (r^2=0.047, *P*<0.001).

4.3.2.3 Number of Brown Seeds:

In 2009 (Fig. 4.21) the creek bank population produced significantly the highest number of brown seeds followed by the low marsh, top of low marsh and high marsh respectively. However, the low marsh population was significantly the largest producer of brown seeds in 2010 followed by the creek bank, top of low marsh and high marsh respectively. Also, the number of brown seeds produced was significantly larger in 2010 than in 2009.

Considering the collection date as a covariate, the two-way ANCOVA (Table 4.8) revealed that the number of brown seeds varied significantly at three levels; population and the interaction between population and year, but at the year level this variation was insignificant.

In 2010 (Fig. 4.22) the low marsh population started brown seed production on 22 July (five weeks earlier than the other populations) with an average of (mean±s.e, 58.18 ±6.26) seeds per plant. This number decreased dramatically until it reached (9.20 ±2.71) seeds at the third collection (5 September) before it declined again to the lowest level and remained stable until the end of the plants’ life cycle. The seasonal fluctuation of brown seed production was significantly lower and more stable in the creek bank, the top of low marsh and the high marsh populations.

Generally a significant relationship was found between the number of brown seeds and total number of seeds traits within and between populations (Fig. 4.23). The more significant and positive associations were found to be within the creek bank population in both seasons followed by the top of the low marsh population. Low marsh and high marsh populations showed no significant associations between traits in 2009, but each exhibited one significant positive association in 2010. For both seasons, significant and positive associations were found at the inter- population level.

The relationship between number of brown seeds and plant height traits appeared to be significant only in very few cases within and between populations in both years 2009 and 2010 (Fig. 4.24). Only within the top of low marsh did significant positive association occur in 2009. In 2010 only two positive associations were found within the low marsh population; within 1st and within 2nd collections, but no significant association was identified for the other three populations. Finally, a significant positive relationship between populations was found only in the season 2009.

Significant associations between the number of brown seeds and plant shoot width were identified only in 2010 (Fig. 4.25). A significant and positive association was identified within the creek bank, one within the top of low marsh and three within the high marsh, but within the high marsh no significant relationship was found. Finally, a significant and positive association between traits at the inter-population level was found only in year 2010.

The relationship between the number of brown seeds and number of primary branches traits was found to be significant in many cases within and between populations in both seasons 2009 and 2010 (Fig. 4.26). In 2009, significant and positive associations were evident within the creek bank and within the top of low marsh, but no significant associations were identified within the low and the high marsh populations. In 2010, significant positive associations were identified within populations as follows; two within the creek bank, one within the top of low marsh and four within the low marsh, but no significant association within the high marsh. For both seasons, significant and positive association was found at the inter- population level.

Investigation of the relationship between the number of brown seeds and number of secondary branches was found difficult to assess via simple linear regression because of the smaller number of samples after neglecting all plants without secondary branches and without brown seeds. As a result in 2009 it was difficult to assess this relationship within and between populations. However, in 2010 (Fig. 4.27) two significant positive associations were identified; one within the creek bank population 6th collection 2010 (Fig. 4.27a) and one positive association at the inter- population level 2010 (Fig. 4.27b).

Assessing the relationship between the production of brown seeds and all the other traits mentioned above, using multiple regression has revealed that the number of primary branches and number of secondary branches influenced the production of brown seeds positively, but the plant height trait affected it negatively. The plant shoot width trait was found to have an insignificant effect, and this is opposite to the result of the multiple regression for number of black seeds. The multiple regression model explained 78% of the variance ($P< 0.001$).

Table: 4.8: Results of the two-way ANCOVA of Stiffkey *Suaeda maritima* populations for the number of brown seeds per plant.

Source of Variation		**Population**	**Year**	**Population*Year**	**Error**
Number of Brown Seeds	**Type lll SS**	1769.03	211.81	2785.11	156550.51
	df	3	1	3	991
	F	3.73	1.34	5.87	-
	P	0.011	0.247	<0.001	-

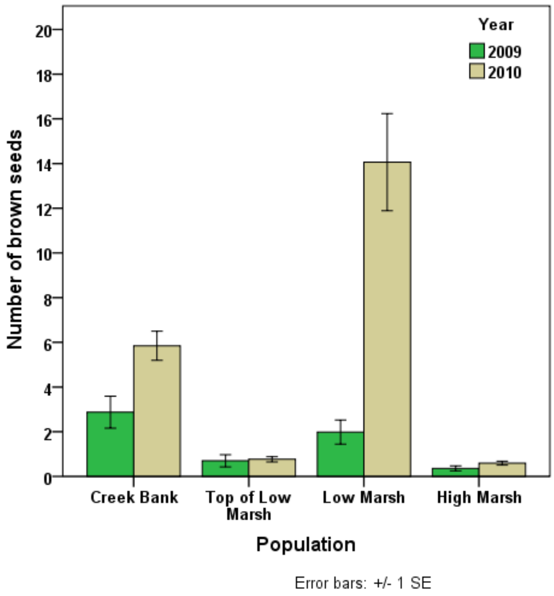
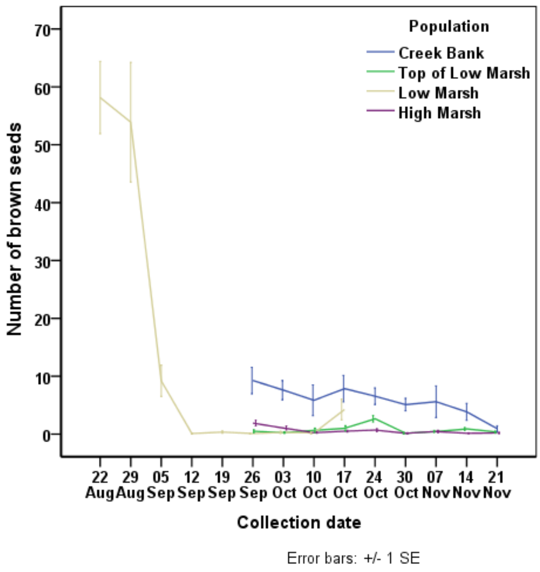
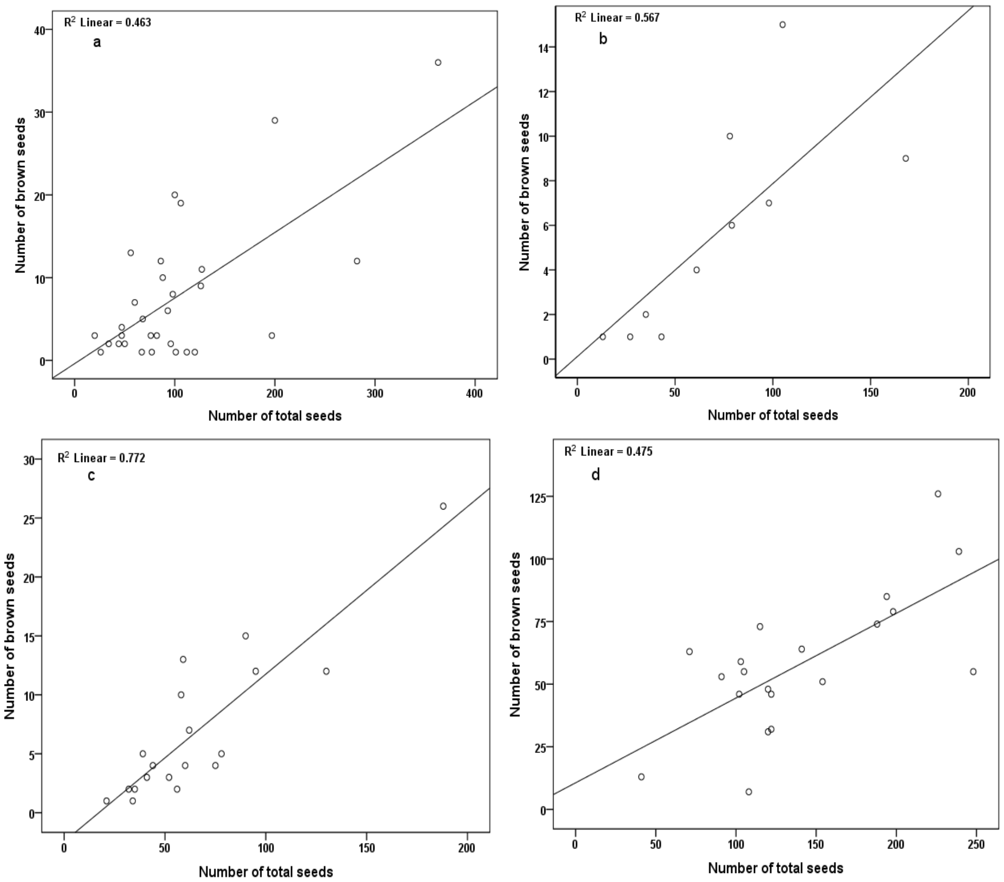


Figure 4.21: Comparisons (mean ±s.e.) of the number of brown seeds per plant produced by the Stiffkey *Suaeda maritima* populations in 2008, 2009 and 2010.



Error bars: +/- 1 SE

Figure 4.22: Temporal changes (mean ±s.e.) of the number of brown seeds per plant of the Stiffkey *Suaeda maritima* populations at 2010.



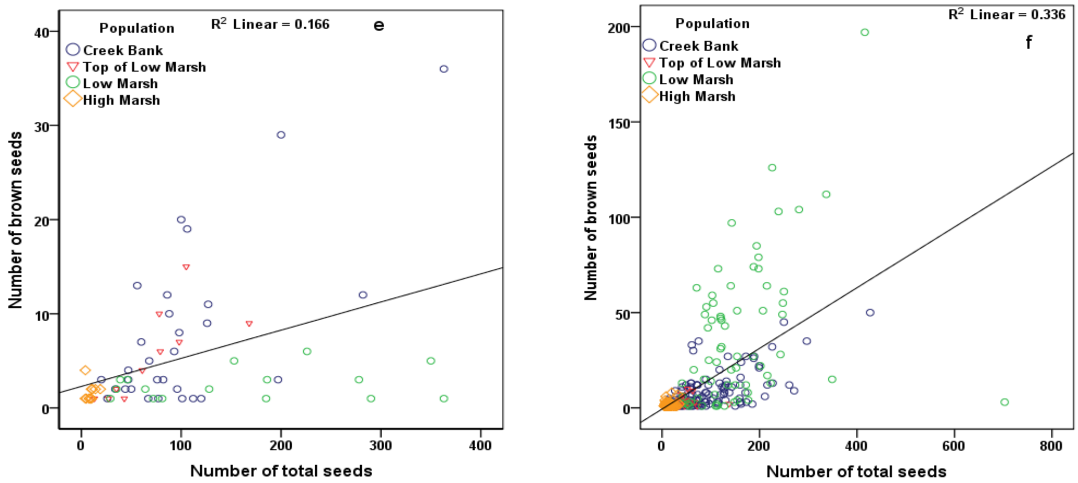


Figure 4.23: Relationship between the number of brown seeds and total number of seeds per plant. **(a)** Within Creek bank 2009 (r^2=0.463, P<0.001). **(b)** Within Top of low marsh 2009 (r^2=0.567, P<0.012). **(c)** Within 5th collection Creek bank 2010 (r^2=0.772, P<0.001). **(d)** Within 1st collection Low marsh 2010 (r^2=0.475, P<0.001). **(e)** Between populations 2009 (r^2=0.166, P<0.001). **(f)** Between populations 2010 (r^2=0.336, P<0.001).

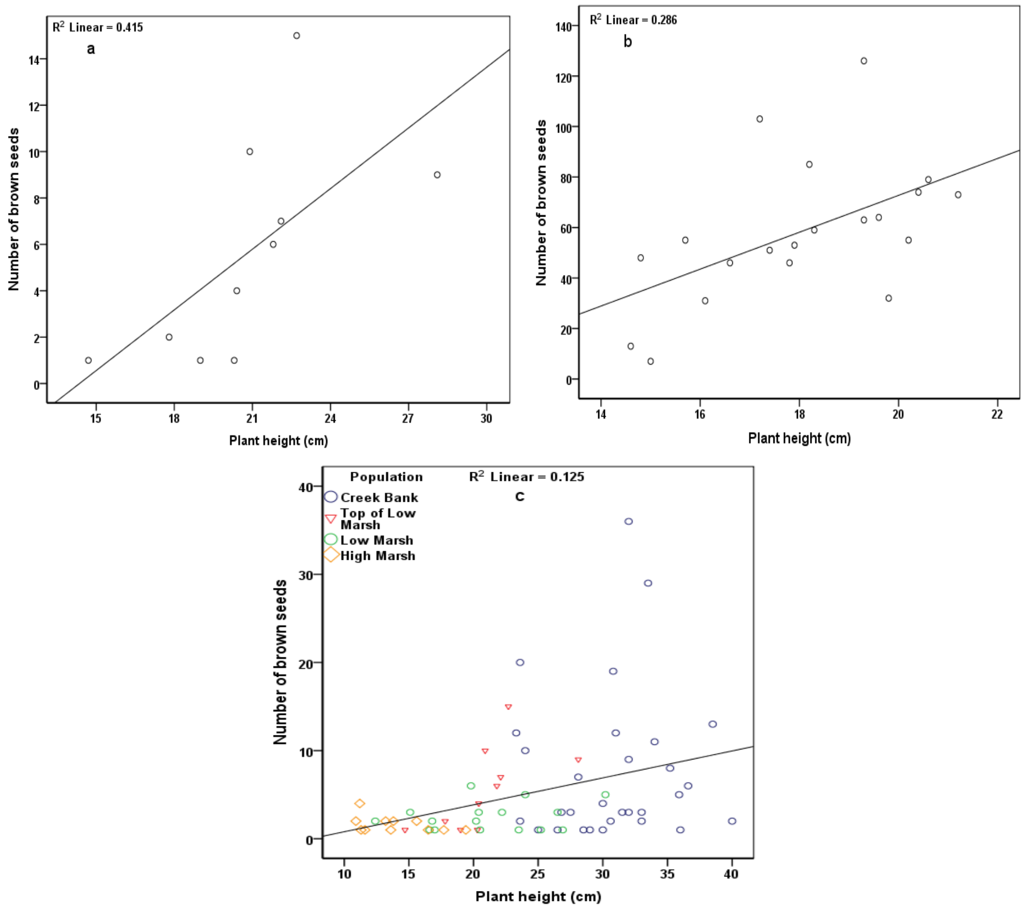


Figure 4.24: Relationship between the number of brown seeds and plant height per plant. **(a)** Within Top of low marsh 2009 (r^2=0.415, P<0.044). **(b)** Within 1st collection Low marsh 2010 (r^2=0.286, P<0.015). **(c)** Between populations 2009 (r^2=0.125, P<0.003).

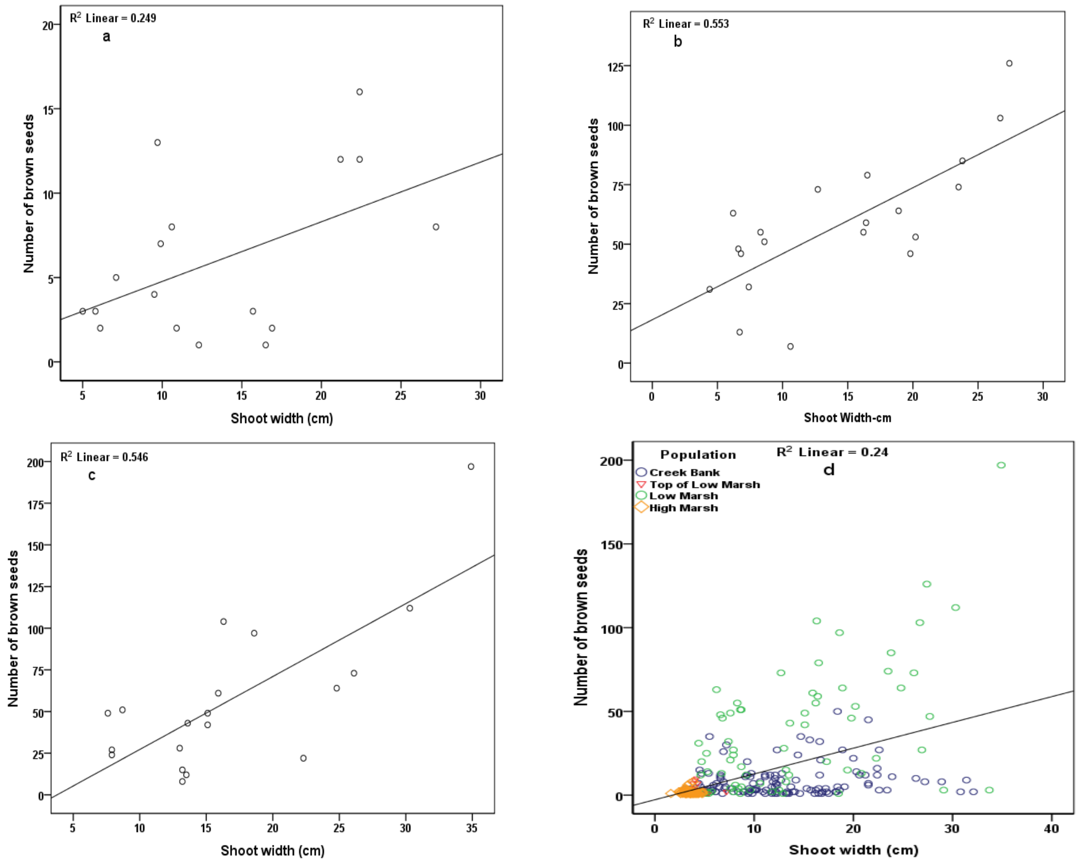
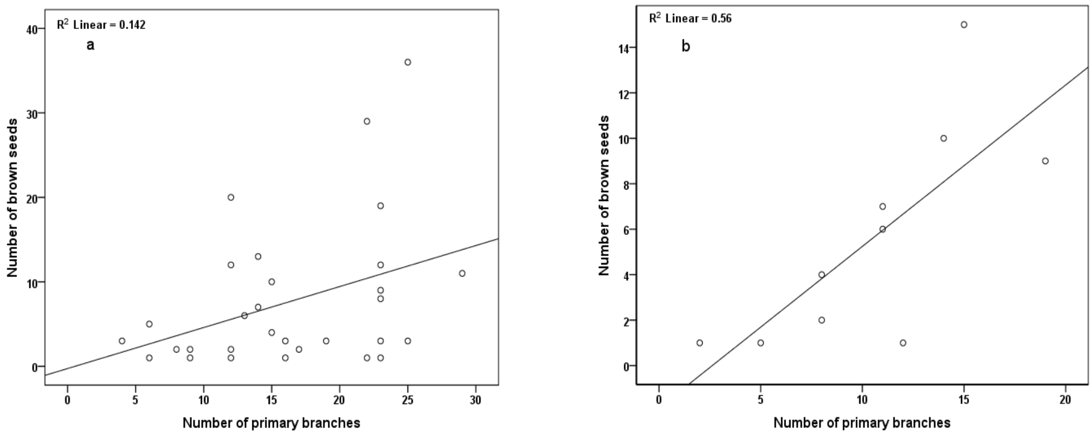


Figure 4.25: Relationship between the number of brown seeds and shoot width per plant. (**a**) Within 6th collection Creek bank 2010 (r^2=0.249, *P*<0.041). (**b**) Within 1st collection Low marsh 2010 (r^2=0.553, *P*<0.001). (**c**) Within 2nd collection Low marsh 2010 (r^2=0.546, *P*<0.001). (**d**) Between populations 2010 (r^2=0.240, *P*<0.001).



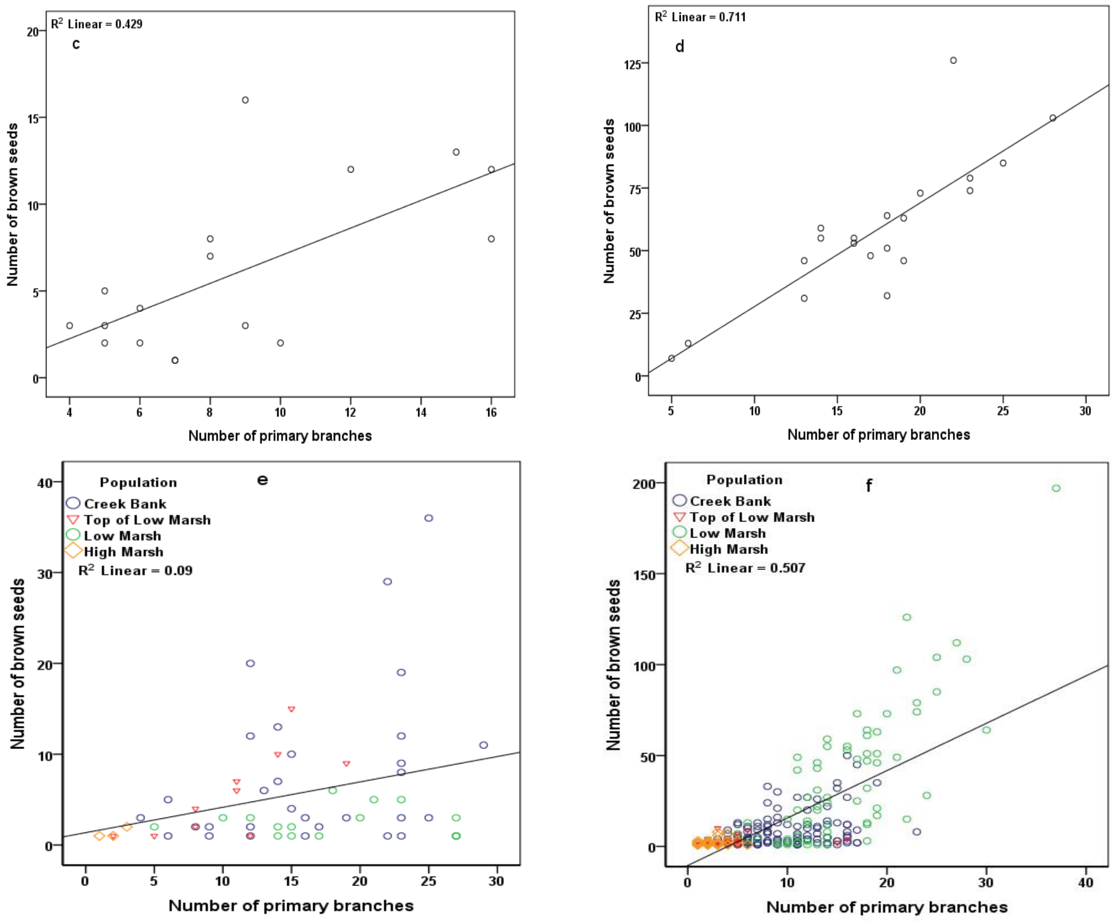


Figure 4.26: Relationship between the number of brown seeds and number of primary branches per plant. (**a**) Within Creek bank 2009 (r^2=0.142, *P*<0.040). (**b**) Within Top of low marsh 2009 (r^2=0.560, *P*<0.013). (**c**) Within 6th collection Creek bank 2010 (r^2=0.429, *P*<0.004). (**d**) Within 1st collection Low marsh 2010 (r^2=0.711, *P*<0.001). (**e**) Between populations 2009 (r^2=0.090, *P*<0.021). (**f**) Between populations 2010 (r^2=0.507, *P*<0.001).

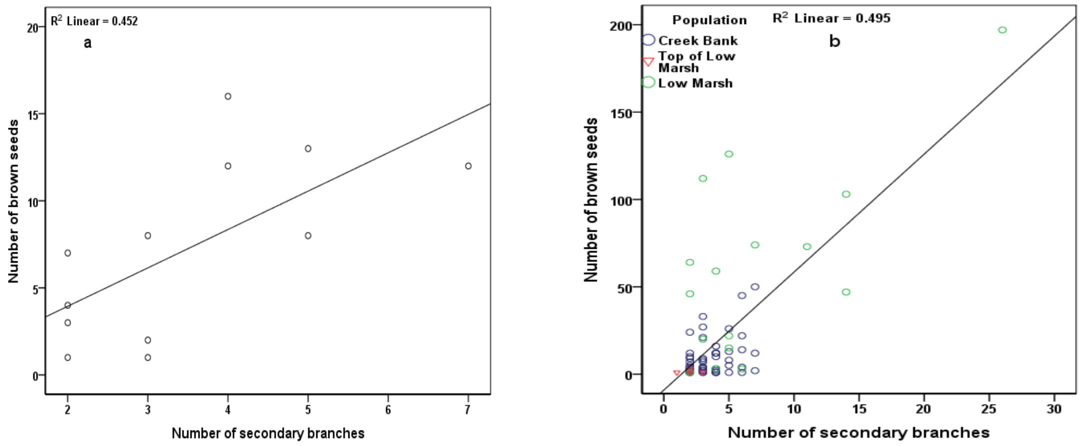


Figure 4.27: Relationship between number of brown seeds and number of secondary branches per plant. (**a**) Within 6th collection Creek bank 2010 (r^2=0.452, *P*<0.017). (**b**) Between populations 2010 (r^2=0.495, *P*<0.001).

4.4 Discussion

Plant species such as *Suaeda maritima* have evolved various mechanisms in order to maximise their chances of reproduction, colonization and survival in unpredictable and stressful environments. One such mechanism is the development of seed heteromorphism, which is the ability of a single individual plant to produce two or more morphologically and ecologically distinct types of seed (Venable 1985, Imbert 2002). The family Chenopodiacae in general and the genus *Suaeda* in particular are well known to exhibit this phenomenon (Li et al. 2005). This study based on *S. maritima* on Stiffkey salt marsh has further developed our understanding of this phenomenon by showing that this heteromorphism is found to be present in seeds sampled from distinct local populations differentiated by marsh topography. Two characteristics of seed heteromorphism have been quantified in this study both within and between these discrete *Suaeda maritima* populations: seed mass and seed morphology with respect to seed colour.

Seed mass from both black and brown traits were analysed at the intra- and inter-population levels, but the work was concentrated on the black seed types as they were found to comprise the majority of local seed production. Mean seed mass has been traditionally regarded as the least plastic variable of a species' seed characteristics and these earlier studies (Harper 1977) tended to concentrate on the capacity of plants to vary the number of seeds produced rather than mass, in response to changing environmental conditions. However, more recent work has emphasized the ability of a plant species to adjust seed mass and shape as key factors in the ecological and evolutionary strategies of vascular plants (Silvertown 1989, Westoby et al. 1992, Telenius 1999). The results from this study of the seed characteristics of *Suaeda maritima* add support to the latter hypothesis.

Among the four populations that were studied, the creek bank population was consistently found to produce significantly the largest black seeds in all three seasons investigated (2008-10). Both the high marsh and the top of the low marsh populations were found to produce the second largest black seeds while the smallest seeds were consistently produced by the low marsh population. These populations were also found to exhibit the same pattern in relation to the brown seed mass trait, which was investigated for 2009 and 2010. Again, it was found that the creek bank plants produced the largest sized brown seeds and the low marsh plants produced the smallest;

while seeds sampled from the high marsh and the top of the low marsh exhibited an intermediate range of sizes.

Variation in seed mass within and between individuals, populations and species has been well documented (Braza et al. 2010). The causes for this variation are evidently complex but they could be attributed to environmental variation (e.g. soil nutrient status) or to induced maternal effects resulting from competition for limited resources (Greipsson and Davy 1995) or a combination of factors. (Metzing 1996) in a study on the German *Suaeda maritima* concluded that seed size is not a stable character and soil nutrition is a significant factor in seed size. Seeds taken from the field and grown in the laboratory usually produce seeds that are smaller than those typically found in the field and thus, there are environmental influences as well as genetic determinants governing seed size. Consequently, parental selection pressures for either larger or smaller seeds can occur at both species and population levels (Ungar 1987a); smaller seeds can be produced in greater numbers than larger ones, and larger seeds may have a better chance of giving rise to well-established seedlings (Westoby et al. 1992). In addition, soil salinity stress could be another selective pressure, where large seeds have been well documented to be less sensitive to this kind of stress than smaller seeds, for example in: *Atriplex rosea* (Khan 2004), *Atriplex centralasiaatica* (Liu et al. 2008), *Suaeda aralocaspica* (Wang et al. 2008) and *Chenopodium album* (Yao et al. 2010).

The seed mass of *Suaeda maritima* harvested from different creek bank populations in 2008 showed that the Tollesbury population produced the largest black seeds, followed by Stiffkey and finally by the Abbot's Hall population. The variation between the Tollesbury and Stiffkey seeds appeared slight compared to the more evident variation exhibited by the Abbot's Hall seeds. The Tollesbury and Stiffkey sites are both natural and mature salt marshes, while Abbot's Hall is a young restoration marsh developed from managed realignment in 2002. These recently developed restoration marshes provide abiotic soil conditions that differ markedly from natural marshes, which have been shown to inhibit patterns of colonization and development (Mossman et al. 2012a). It would therefore be difficult to determine if the reduced seed mass for plants harvested from the Abbot's Hall creek bank could, for example, be attributed to the stresses imposed from increased competition for limited nutrients (Emery 2001) or to the effects of a harsher abiotic environment resulting from the prolonged anaerobic conditions and the accompanying low redox potentials that are frequently encountered in the relatively waterlogged soils of restoration marshes (Mossman et al 2012). Also, it is not clear whether these are directly plastic responses or whether there has been a degree of genetic differentiation.

The evidence from this study suggests the existence of temporal, year-to-year, seed mass variation. This was reflected in the finding that *Suaeda maritima* populations produced larger black seeds in 2009, followed by 2008 and finally in 2010. Also, larger brown seeds were produced in 2009 than in 2010. Furthermore, the seasonal variability of the black seed mass in 2010 shows that seed mass in all populations generally increased gradually during the season until it reached a peak of approximately twice the initial mass. This temporal variation both within season and inter-annually is poorly documented in the literature, and the few studies that have been undertaken are related to the dune-building grass *Leymus arenarius* (Greipsson and Davy 1995) and to the perennial *Lupinus polyphyllus* (Aniszewski et al. 2001). One explanation for this scarcity is presumably because most investigators have underestimated the potential of temporal variation in environmental conditions to exert such a large influence on seed mass. However, a few workers such as (Busso and Perryman 2005) have argued that environmental factors, particularly monthly temperature and precipitation, can be significant determinants of mean seed size.

The relationship between seed mass and other plant traits is an issue of evolutionary interest (Westoby et al. 1992). In this study, seed mass was correlated both in 2008 and 2010 to a number of phenotype traits, in particular: plant height, shoot width, number of primary branches and number of black seeds; a few weak relationships were identified only in 2010.

The relationship between black seed mass and plant height tended to vary within populations; this relationship varied in many cases from significantly positive to significantly negative. Moreover, the relationship was also found to be significantly positive at the inter-population level. In addition to seed mass and plant height, a similar pattern of associations was found between seed mass and the number of primary branches and seed mass and shoot width traits. Taken together, plant height, number of primary branches and shoot width represents the overall form of plant growth. A possible hypothesis for the identified relationships is that having larger seed mass (associated with larger plants) could support their achievable dispersal distance by gravity (Leishman et al. 2000), in other words, in terms of maximising dispersal distances, it seems it is better that species increase their height rather than decrease their seed size to gain an advantage over other species (Thomson et al. 2011).

The relationship between black seed mass and number of black seeds was negative in two cases: within the creek bank population and at the inter-population level. This relationship apparently supports the trade-off hypothesis between these two traits, which is considered to be a hedge-betting strategy of halophytes occurring in temporally and spatially unpredictable habitats (Westoby et al. 1992).

Two distinctive seed colours were observed: black and brown; black seeds have a thick black, resistant, shiny outer testa, which is missing in the brown ones. This also explains why brown seeds do not survive long and must germinate soon after dispersal (as shown in Chapter 7). Both black and brown seed numbers were analysed at the intra- and inter-population levels. The majority of work was concentrated on the black seed type as they were clearly found to comprise the majority of seed production. The *Suaeda maritima* populations in this study were found to exhibit a highly variable annual pattern of seed production in terms of number for both black and brown types. The low marsh population was the most prolific producer of black seeds in 2009 and 2010, followed by the creek bank, the top of the low marsh and the high marsh, respectively. In 2008, when the low marsh plants were absent, the plants from creek bank populations produced the highest number of black seeds, followed by the top of the low marsh and then by the high marsh.

For brown seed production in 2009, the highest production was found in the creek bank population, followed by the low marsh, the top of the low marsh and the high marsh, respectively. However, this pattern differed in 2010: the low marsh produced the highest number of brown seeds, followed by the creek bank, the top of the low marsh, and finally by the high marsh. The trait for total seed production showed a very similar pattern to the trait for black seed colour but this is not an unexpected result considering the fact that the majority of seed production constituted the black variety. The same pattern of variation between numbers of black and brown seeds was maintained in both years (2009 and 2010).

Some studies have linked such variation between populations (in terms of the relative proportions of the different seed morphs in heteromorphic species) to environmental heterogeneity in the field (Venable 1985). (Telenius 1999), for example, suggested annual mean precipitation as a significant environmental determinant for such variation. For the creek bank populations in 2008, the plants from Tollesbury and Stiffkey produced higher numbers of black seeds than those from the Abbot's Hall population. Such variation between those populations agrees with their variation in terms of growth traits which has been discussed in Chapter 3.

Of the four distinct populations studied, the low marsh population exhibited the shortest lifecycle, representing a faster growth rate. The seed production of the low marsh population started five weeks earlier and ended five weeks earlier than the three other populations. The faster growth rate of the low marsh populations (significantly more so than that of the upper marsh ones) could be attributed to the more favourable conditions that are likely to exist there - of low salinity and the availability of sufficient nitrogen (Davy et al. 2001). Interestingly, the two chenopods, *Salicornia europaea* and *Salicornia dolichostachya*, which are closely related to *Suaeda maritima*, were found in another study to exhibit similar rapid growth rates on the same low marsh at Stiffkey (Jefferies 1977, Jefferies et al. 1981).

Variation in reproductive components has been related to different life history strategies (Braza et al. 2010). This study has demonstrated how this relationship is manifest in *Suaeda maritima*. Populations of the low marsh were found to have a shorter lifespan than the other the populations investigated and to be consistently the highest producer of small sized seed morphs. Also, as the low marsh population occupies the zone under the greatest influence of the sea the characteristics of plants from these populations are likely to reflect their ability as an early colonizer. These findings strongly support the hypothesis that early successional plants should be short-lived and have small, readily dispersed seeds (Greipsson and Davy 1995). Such a bet-hedging strategy on the part of the low marsh population is more evident within annual than perennial halophytes and can be interpreted as an adaptation to cope with the variability and unpredictability of conditions in the field (Imbert 2002).

The low marsh population in particular produced relatively high numbers of brown seeds in the first two weeks of the seed production period, and then a very dramatic decrease occurred in the following week until it reached its lowest production rate in the subsequent week; it then remained stable until the end of the lifecycle. Conversely, there was a dramatic increase in the number of black seeds from the start of sampling, reaching a peak in the middle of the seed production period, before falling sharply in the subsequent two or three weeks to its lowest production rate at the end of the lifecycle. Seed dimorphism in *Suaeda corniculata* subsp. have been reported where brown seeds form a transient soil seed bank and black seeds form a persistent seed bank (Cao et al. 2012). Therefore, such seed heteromorphism exhibited by *Suaeda maritima* populations in the low marsh plants could be interpreted as an investment strategy to maximise successful germination in an unpredictable environment as brown seeds tend to germinate much earlier when unfavourable conditions persist and black seeds tend to germinate later on when conditions are more favourable.

The relationship between the number of black seeds and the number of brown seeds produced within and between populations was found to be significantly positive in many cases. This association was found to be strongest within the low marsh and the creek bank populations. As these populations tend to produce more seeds in total than the others, and as both of them live in more unstable and unpredictable environmental conditions resulting from the physical, erosive effects imposed by regular tidal inundation, the association mentioned above should increase the seed germination opportunities for these two populations in particular.

Other plant traits, including plant height, shoot width, number of primary branches and number of secondary branches, are all considered to represent the plant growth form. The nature of the relationship between each of these traits and either number of black seeds or number of brown seeds has been shown to be significant in many cases both within and between populations. Such relationships can be explained by a previous similar argument to that of the relationship between seed mass and growth traits.

In conclusion, this study was conducted to compare the seed heteromorphism phenomenon in four local populations of the halophyte *Suaeda maritima* found on Stiffkey salt marsh. The first main finding was that variation in seed heteromorphism within and between populations was manifested in seed mass. The creek bank population consistently produced the heaviest seeds, while the low marsh consistently produced the smallest seeds. The high marsh and the top of the low marsh populations tended to produce the second heaviest seeds, and variation between both populations was usually found to be insignificant. Thus, in terms of the seed mass trait, the current study has defined at least three distinctive populations of *Suaeda maritima* at Stiffkey salt marsh.

The other manifestation of seed heteromorphism observed within and between populations over two consecutive summers was in seed morphology with respect to the characteristic of seed colour. Two distinctive seed colours were observed: black and brown; black seeds have a thick black, resistant, shiny outer testa, which is missing in the brown ones. The low marsh population was found to be the highest producer of black seeds over the three year period investigated and the highest producer of brown seeds in the final investigated season (2010). The creek bank population was the second highest producer of black seeds over the three year period and the highest producer of brown seeds in 2009. The high marsh and the top of the low marsh populations were found to produce similar numbers of black and brown seeds on many occasions, and their pattern of production would put them in third place with respect to the seed colour trait.

Further variations between the populations were observed, such as the timing of seed production, the variability of seed heteromorphism over the years, the variability of seed heteromorphism between natural and man-made restoration salt marshes, and the capability of populations to make trade-offs between seed mass, seed morphology and other growth traits.

The major strength of this study is the considerable number of scales at which observations of seed heteromorphism were collected, on different occasions during three consecutive summer seasons. Only over such an extended period of study (as opposed to observations over a single season) would it be possible to detect the considerable variation in both seed mass and seed morphology that was observed.

Finally, the attribution of these different variations to phenotypic plasticity or to genetic differentiation is still an open question at this point.

Chapter 5

Field reciprocal transplantation of local populations of *Suaeda maritima*

Abstract

- ***Background and Aims:*** *Suaeda maritima* is an annual halophyte species that inhabits coastal and inland salt marshes. It is known to exhibit clear phenotypic variation in terms of growth and fecundity. The main aim of this study is to examine the extent to which *Suaeda maritima* populations maintain morphological and life-history traits when transplanted in the field, and the extent to which these traits are plastic adaptations to local microenvironments.
- ***Methods:*** Field reciprocal transplantation of seedlings was carried out between three parts of the Stiffkey salt marsh: creek bank, high and low. Life-history traits (manifested in terms of survival, growth and fecundity) were assessed within and between the three populations.
- ***Key Results:*** Interspecific phenotypic variation of the three populations was maintained when they were transplanted within home and into alien sites. Also, intraspecific population phenotypic variation was observed, particularly when populations were grown in alien habitats.
- ***Conclusions:*** The creek bank, the high and the low marsh populations demonstrated substantial genetic differentiation in their survival, growth and fecundity. The intraspecific population variation demonstrated different degrees of phenotypic plasticity in response to local conditions in each part of the marsh. Therefore, the inter- and intraspecific population variation of the *Suaeda maritima* species in the current study could be considered as a mixed strategy of phenotypic plasticity and genetic differentiation in order to tolerate local environmental heterogeneity in the Stiffkey salt marsh.

5.1 Introduction

Phenotypic variation among individuals within a species is a commonly observed phenomenon, which ecologists argue is an adaptation measure, allowing individuals to thrive in slightly varying conditions or regimes (Imbert 2002). Although this observed variability may appear random, phenotypic matching may be an evolutionary survival mechanism that is driven either through natural selection, producing genetically-differentiated ecotypes or through phenotypic plasticity, producing different morphologies from the same genotype in different environments (Richards et al. 2005).

Early work on phenotypic variation among *Suaeda maritima* populations in British salt marshes by (Chapman 1947) and (Clapham et al. 1962) resulted in the identification of three different varieties: var. *flexilis* Focke, var. *macrocarpa* Moq. and var. *vulgaris* Moq. However, both authors found it difficult to be certain about the taxonomic status of those varieties and they are not recognized in modern treatments (Stace 2010). (Boucaud 1962), (Boucaud and Ungar 1973) and (Boucaud and Ungar 1976) studied the phenotypic variation of local varieties of *Suaeda maritima* in France, and concluded that they can be distinguished morphologically and according to their seed germination behaviour.

Metzing 1996 conducted a study on the seed heteromorphism of two populations of *Suaeda maritima* in Germany (one growing on the upper parts of the low marsh and the other on the lower parts), and concluded that there was considerable variation between the seed sizes of the two populations. (Ihm et al. 2004) investigated phenotypic variation between three *Suaeda maritima* populations inhabiting the Muangun sand dunes in South Korea (on low, middle and high parts) and found them to be morphologically distinctive. (Polić et al. 2009) conducted a morpho-anatomical differentiation study on the Mediterranean *Suaeda maritima* subsp. *prostrata* and the Serbian inland *Suaeda maritima* subsp. *maritima*, and they found that those two subspecies can be separated based on their quantitative anatomical characteristics. A similar study to that one was conducted on coastal and inland *Suaeda maritima* populations in Central Europe using amplified fragment length polymorphism (AFLP), and it found that the genetic variation values were not significantly different in populations from natural and anthropogenic inland salt sites, when compared with coastal habitats (Prinz et al. 2009). In a recent study by (Wetson et al. 2012), a reciprocal transplantation experiment of *Suaeda maritima* plants was conducted between the low and high parts of the salt marsh of the River Adur estuary (Essex, UK), and found that the transplanted plants, either in home or alien positions, developed similar morphological characteristics, indicating phenotypic plasticity in terms of plant height and mass.

The reciprocal transplantation technique is considered to be a powerful method for assessing the variation in life history traits, the fitness and the significance or otherwise of genetic differentiation of plant species. The technique involves the introduction of alien forms of a native species into a habitat under investigation, and observing whether those native forms outperform the aliens in terms of survival and fecundity. Should the resident forms do so, it may be concluded that this particular form has adapted to that locality through evolutionary processes, i.e. there is evidence of natural selection in favour of the current resident phenotype (Anna 2000).

Reciprocal field transplants have the advantage over laboratory experiments in that the subjects are exposed to the full range of biotic factors that may affect the development and adaptation of a species to local conditions; this affords more realistic analyses. However, reciprocal transplantation techniques, in the context of salt marshes, suffer from certain drawbacks; freshly transplanted specimens may simply be washed away before they have had sufficient time to become established, and it may also become difficult to differentiate between the transplanted specimens and other similar specimens that have been introduced by transport from the normal action of the tides and waves. In order to address these difficulties, certain specific techniques have evolved, each of which has its own peculiar advantages and disadvantages, but the most appropriate in the context of this research is seedling transplantation (Davy and Smith 1985).

Seedling transplantation involves extracting seedlings in cores before they have become fully established and transferring them to another location in the field. However, it should be noted that this technique necessitates the removal and transference of the sediment in which the seedling has germinated, and this sediment will continue to support the seedling in its early growth stages. On the other hand, this effect may be quickly countered by the continual effects of the local conditions (chiefly driven by the inundation regime) (Davy and Smith 1985). In order to further support the reliability of the experiment, and in order to address this problem of the core soil affecting the growth of the seedlings, adequately replicated transplants can be applied (Davy 1988).

In Chapters 3 and 4, field surveys of *Suaeda maritima* at Stiffkey revealed that all populations exhibited considerable phenotypic variations in three consecutive years in terms of growth and fecundity. This pattern of consistent phenotypic variation between populations might be explained as plasticity or local genetic differentiation. In addition, phenotypic variation within each of the *Suaeda maritima* populations was observed as a plastic response to the unpredictable conditions of these habitats. This work in this chapter is aimed at investigating the phenotypic variation of the annual halophyte *Suaeda maritima* with respect to manifestly differing habitats found within the Stiffkey salt marsh, and the extent to which it represents phenotypic plasticity or genetic differentiation of local populations.

Accordingly, the current study has been designed to examine the variations in survival, morphology and fecundity of three *Suaeda maritima* closely-related populations in a reciprocal transplant experiment at Stiffkey salt marsh. It is anticipated that this approach will provide a powerful tool for identifying the relative importance of genetic variation and phenotypic plasticity in terms of the variations observed in these natural populations, enabling us to address the following questions:

1) To what extent do populations of *Suaeda maritima* maintain morphological and life-history traits when transplanted in the field, and to what extent are these traits plastic adaptations to local micro-environments?

2) To what extent are variations in these traits modified by variations in plant density in these environments?

5.2 Materials and methods

The field sites and their environmental characteristics have been described in Chapter 2.

5.2.1 The field reciprocal transplantation experiment

This experiment was designed to transplant seedlings of *Suaeda maritima* populations reciprocally between the creek bank, high marsh and low marsh habitats at Stiffkey salt marsh. This experiment was conducted in three zones on the marsh; two of these zones were on the high marsh (one within the high marsh itself and the other on the creek bank), and the third was conducted on the low marsh site (Fig. 5.1). The zones were chosen subjectively as typical of the microhabitat in which each local population of *Suaeda maritima* had been growing.

For each population, a total of 60 random soil cores (6 cm diameter × 10 cm deep) were extracted using a bulb planter and then transplanted to randomly located holes of the same size in April 2009. Twenty of these cores were transplanted within their original habitat, and the other 40 cores were transplanted into the other two habitats. The cores of each zone were identified using canes. Very young seedlings at the post-cotyledon stage were targeted (1-1.5 cm in height). The initial number of seedlings in each core was recorded, and then their mortality was recorded during weekly visits until the end of the experiment (Fig. 5.2).

During the fieldwork in the previous chapter, the low marsh population was observed to have a shorter lifespan than the creek bank and the high marsh ones. Therefore, in the current study, the plants from the low marsh population were regularly checked for maturity and when they were found to have produced a sufficient number of seeds, they were collected for the characterisation work before they senesced. In addition, before the end of the experiment (at week twenty), the high marsh population transplants in the low marsh zone were subject to significant interference from some unknown agent. The plants were mechanically removed, together with the marker canes. As few cores survived at this site, they were excluded from the phenotypic characterization results. Moreover, at week twenty the creek bank plants were collected for the phenotypic characterization before they could be subjected to any further damage in this part of the marsh. Therefore, by week twenty, the experiment was over at the low marsh site.

5.2.2 Phenotypic trait characterization

At the end of the reciprocal experiment, the surviving plants from within each core were collected in polyethylene bags and brought back for laboratory investigation. The characterization work began immediately in the lab; plants awaiting characterization were kept in the cold storage room (at 5 ºC). Each plant was measured for the following parameters: shoot traits, including plant height (the above-ground growth height), shoot width (the above-ground shoot width), number of primary branches, number of black seeds, number of brown seeds, number of immature seeds, total number of seeds and finally main root length. Furthermore, measurements of individual seed mass were taken individually using a micro-balance (Metler Toeldo UMX2).

5.2.3 Statistical analysis

The statistical analysis was conducted using PASW Statistics version 18, Chicago, USA for Windows. Survival curves of the three populations were plotted as percentages and one-way ANCOVA was employed to assess the significance of the survival, taking into consideration the collection date as a co-variable. Relative survival was assessed in terms of percentage mean survival for twenty cores per population. Comparisons of morphological traits (including black seed weight, plant height, shoot width, main root length, root system width and number of primary branches) were expressed in graphs, and one-way ANOVA was conducted to assess the variations between populations in each habitat. Two assessment methods were applied to gain a wider understanding about the seed mass trait within and between populations: assessing seed mass per seed (seed masses were averaged by weighting all seeds in the population equally (i.e. per seed per population) (i.e. the replicate is the seed), and assessing seed mass per core (seed masses were averaged by weighting seeds of each plant on the core equally and each core in the set of replicate cores equally (i.e. per each individual plant, then averages of all plants per core were averaged, and finally core averages were averaged) (i.e. the experimental replicate is the soil core).. The latter approach is the main one that is used to assess all the work in this chapter, as soil core is considered to be the basic replication unit (the cores are independent of each other).

The correlation between plant density and the other morphological traits (including plant height, shoot width, main root length, number of black seeds and black seed mass) was analysed using simple linear regression. Also, the same approach was used to identify whether there were any correlations between plant density and final survival percentages. General and specific examples of significant correlations were recorded.

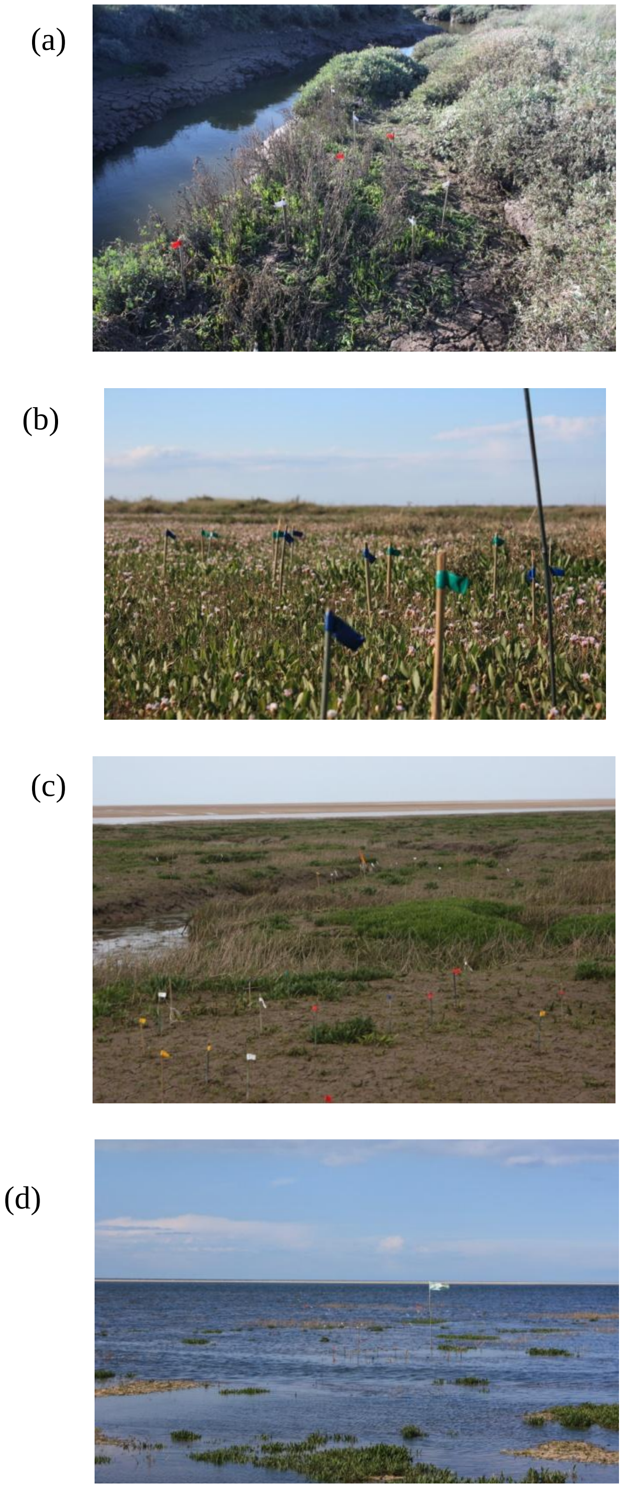


Figure 5.1: (a) Creek bank transplants. (**b)** High marsh transplants. (**c)** Low marsh transplants during low tide. **(d)** Low marsh transplants during high tide.

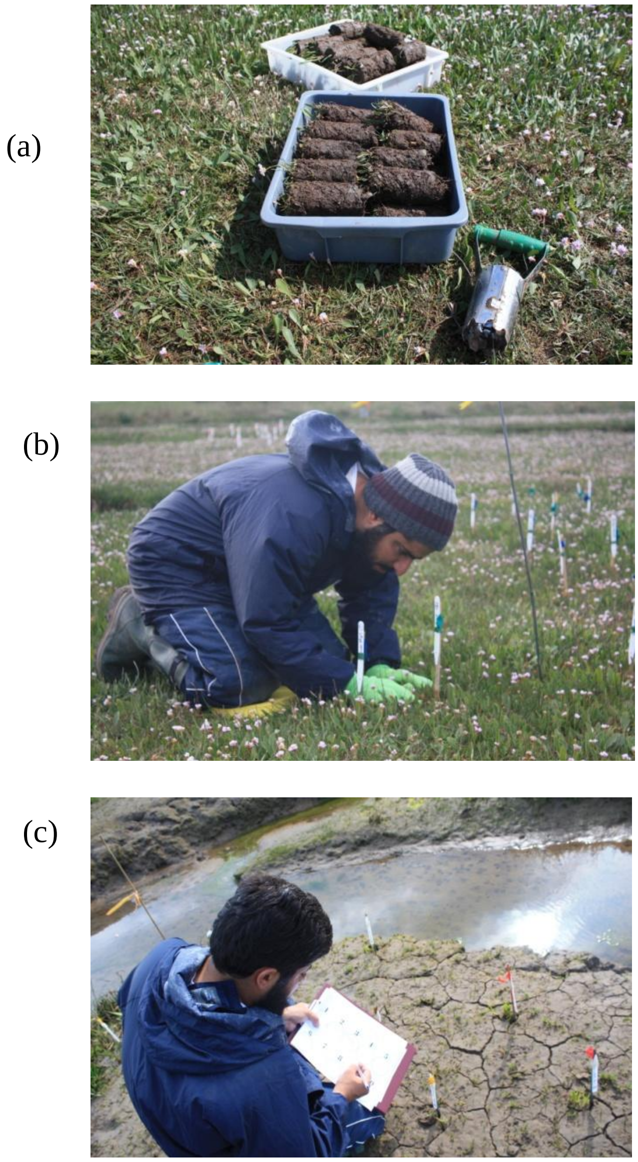


Figure 5.2: (a) soil cores were extracted using a bulb planter. **(b)** Inserting cores into their new host holes. **(c)** Drawing a map and recording positions and numbers of seedlings.

5.4Results

5.3.1 Survival

The overall survival of the creek bank population at the end of the growing seasosn was high (80-90%) when transplanted to all three habitats (Fig. 5.3a) However; it survived least well when transplanted to the low marsh and, marginally, best on the high marsh.
The high marsh population exhibited overall the lowest survival rates when grown in all habitats, compared with the other two populations (Fig. 5.3b). It survived by far the best in its own habitat (c. 60% at the end of the growing season) but rather poorly after transplantation to the creek bank habitat (c. 20%) and very poorly in the low marsh habitat (c. 10%).

The low marsh population (Fig. 5.3c) showed very high survival by the end of the growing season after it was transpanted to its own environment (98%) and survival was similarly high on the high marsh (95%). However final survival was substantially lower when transplanted to the creek bank (68%). In addition, the lifespan of the low marsh population was evidently shorter, when grown in either home or alien habitats, than those of the other two populations.

Table 5.1 shows the one-way ANCOVA output which revealed significant differences for the survival of the creek bank population grown in home and alien habitats. When considering the weekly observations as a covariable in this model, it has been found that the survival rate of the creek bank population was decreased significantly differently among the habitats (i.e. in every habitat this population lost some individuals as a result of the new environmental conditions, but the loss varied among habitats as described above). Finally, the same pattern of outcomes is evident in the high marsh and the low marsh transplants, either in their own or alien habitats.

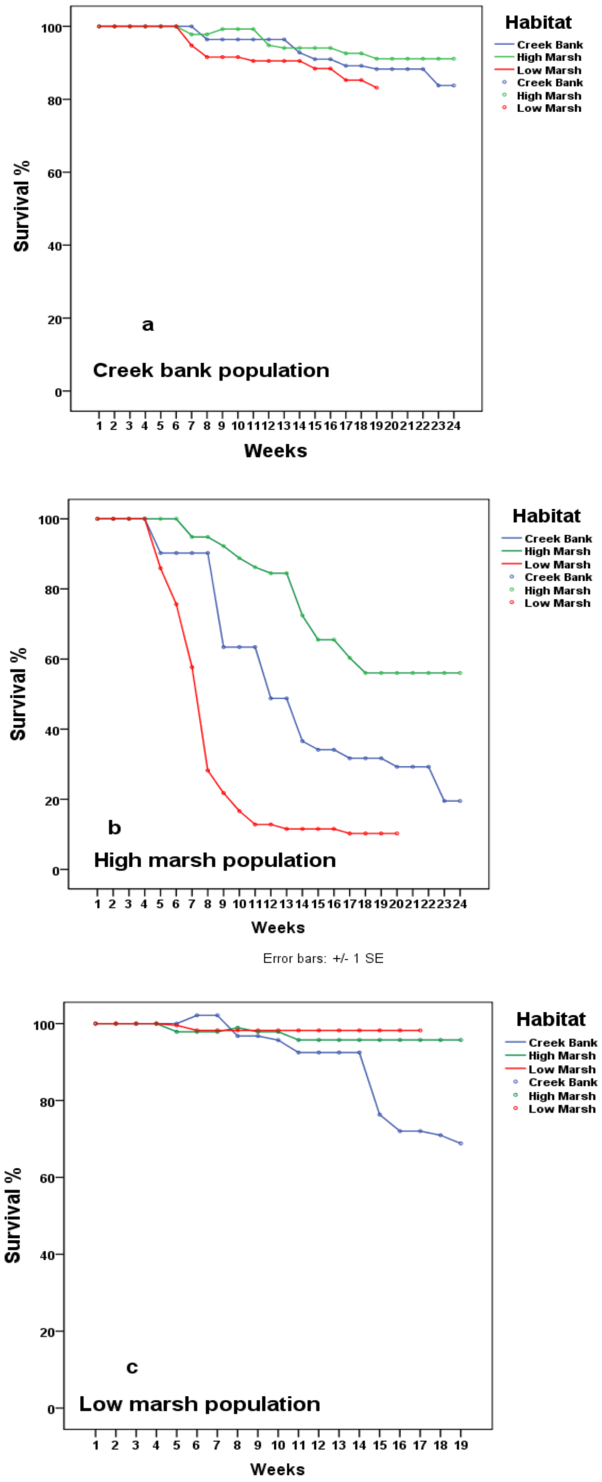


Figure 5.3: Survival percentages of three populations of *Suaeda maritima* reciprocally transplanted between the creek bank, the high and the low marsh positions at Stiffkey salt marsh. **(a)** Creek bank population, **(b)** High marsh population and **(c)** Low marsh population. (Week number refers to field weekly data collection starting from 1st week of April 2009).

Table 5.1: One-way ANCOVA for survival of three *Suaeda maritima* populations transplanted reciprocally between three habitats at Stiffkey.

Source of Variation		Habitat	Error
Creek Bank Population	**Type lll SS**	1018.61	22580.5
	df	2	1356
	F	30.59	-
	P	< 0.001	-
High Marsh Population	**Type lll SS**	331.65	1867.8
	df	2	1356
	F	119.8	-
	P	< 0.001	-
Low Marsh Population	**Type lll SS**	326.8	11204.7
	df	2	1156
	F	16.9	-
	P	< 0.001	-

5.3.2 Fecundity

Fecundity is expressed in as the total number of seeds produced by individuals of *Suaeda maritima* surviving to maturity. Both the high marsh and the low marsh populations showed insignificant differences for the total production of seeds, either when grown in home or alien habitats (Fig. 5.4b and 5.4c). However, the creek bank population produced fewer seeds when grown in its own and on the low marsh habitat, but more seeds when grown on the high marsh habitat (Fig. 5.4a). Generally, the total number of seeds produced was significantly the highest for the low marsh population, followed by the creek bank population, and finally by the high marsh population (Fig. 5.4).

As the majority of the seed production by the *Suaeda maritima* plants was observed to be black seeds, the findings for the number of black seeds were similar to those of the total number of seeds (Fig. 5.5). Figure (5.5a) shows the creek bank population having insignificant differences in terms of the production of black seeds, either when grown in its home or alien habitats. Similar outcomes were achieved by the high marsh and the low marsh populations when they were grown in their home and alien habitats (Fig. 5.5b and 5.5c). In general, significantly more black seeds were produced by the creek bank and the low marsh populations than by the high marsh population.

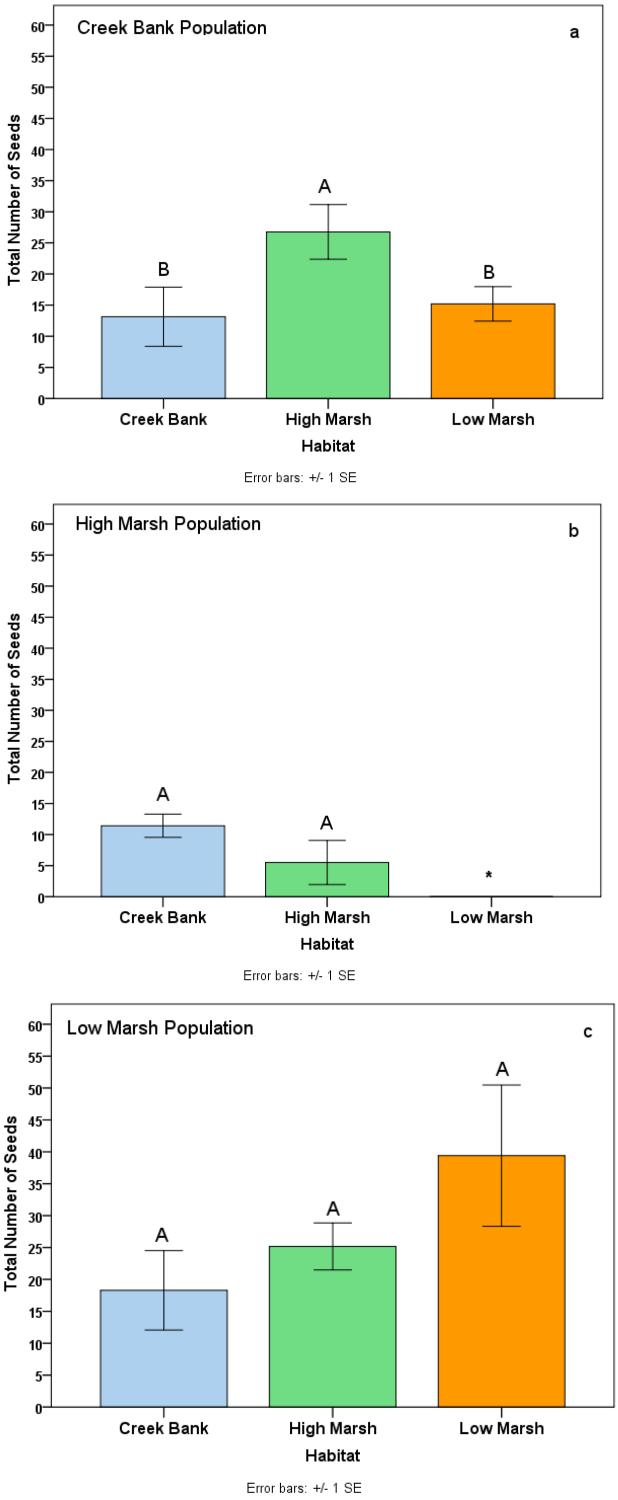


Figure 5.4: Fecundity (total number of seeds per plant) per core. **(a)** Creek bank population ($F_{2,\ 49}$ = 3.61, $p < 0.035$). **(b)** High marsh population ($F_{1,\ 25}$ = 1.16, $p < 0.292$). **(c)** Low marsh population ($F_{2,\ 40}$ = 2.3, $p < 0.116$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data).

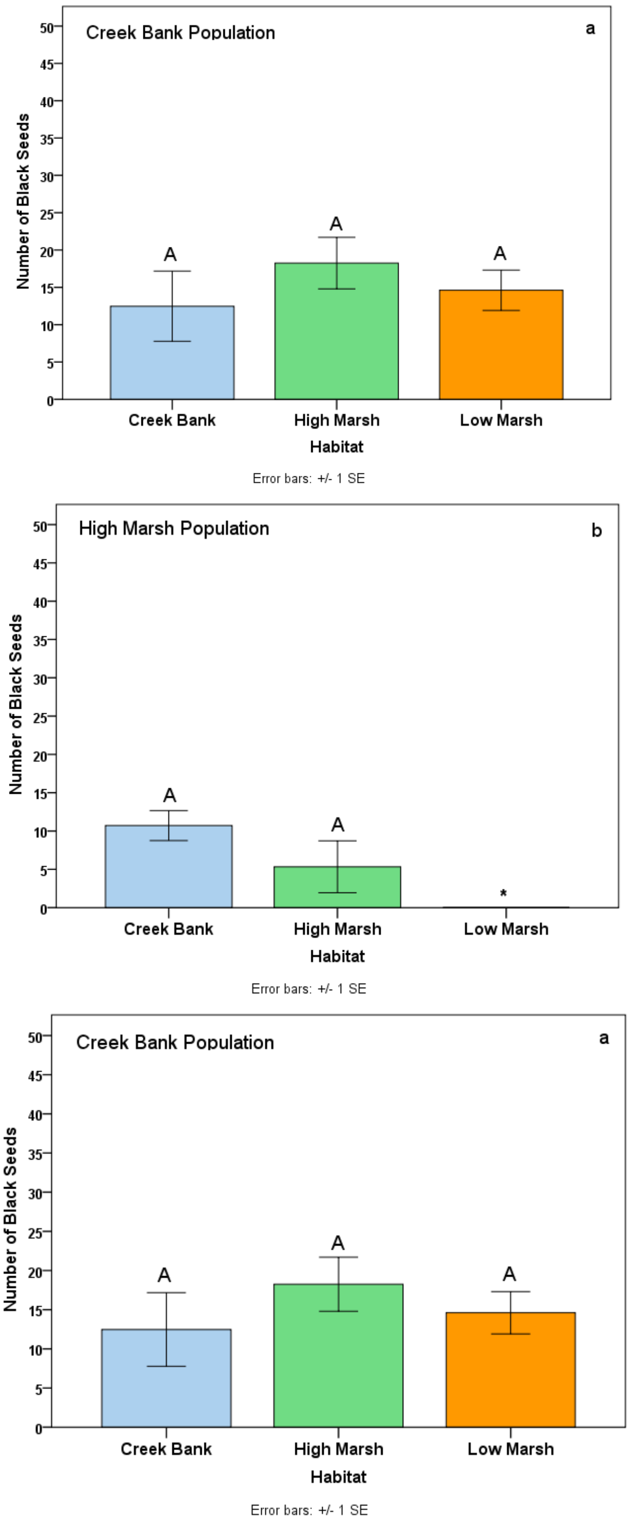


Figure 5.5: Number of black seeds per plant per core. **(a)** Creek bank population ($F_{2,48} = 0.64$, $p < 0.531$). **(b)** High marsh population ($F_{1,25} = 0.88$, $p < 0.356$). **(c)** Low marsh population ($F_{2,\,40} = 2.17$, $p < 0.127$). Within each population, bars with shared letters are not significantly different as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data)

There was very little evidence of brown seed production (Fig. 5.6), although small numbers were produced by the creek bank population, both in its own habitat and in the high marsh habitat; no significant difference was found between them.

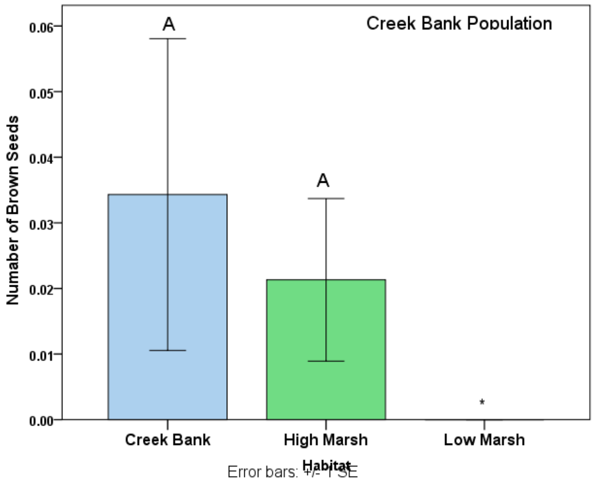


Figure 5.6: Number of brown seeds per plant per core. Creek bank population ($F_{2, 48} = 1.03$, $p < 0.364$). Bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data).

5.3.2 Seed mass

Figure 5.7a shows the creek bank population having significantly higher individual black seed mass (the mean of all seeds produced) in its own habitat and in the high marsh habitat, but smaller seeds in the low marsh habitat. No significant differences between the seed masses of the high marsh population were found when grown in their own habitat and in the creek bank habitat (Fig. 5.7b). Figure 5.7c shows significant differences between the seed masses of the low marsh population in all habitats; the biggest seeds were found in the home habitat and then in the low marsh and the creek bank, respectively.

Assessing mean individual seed mass per core (Fig. 5.8) shows slightly different results. No significant differences between the seed masses of the creek bank population were found when they were grown in home or alien habitats (Fig. 5.8a). The high marsh population exhibited significantly slightly higher seed mass when grown in its own habitat than in the creek bank one (Fig. 5.8b). The low marsh population seed mass was significantly higher when this population was grown in home and creek bank habitats, but they were slightly smaller when grown in the high marsh one (Fig. 5.7c).

Both Figure 5.7 and Figure 5.8 show a common finding; the creek bank and the high marsh populations produced significantly and consistently bigger seeds than the low marsh plants.

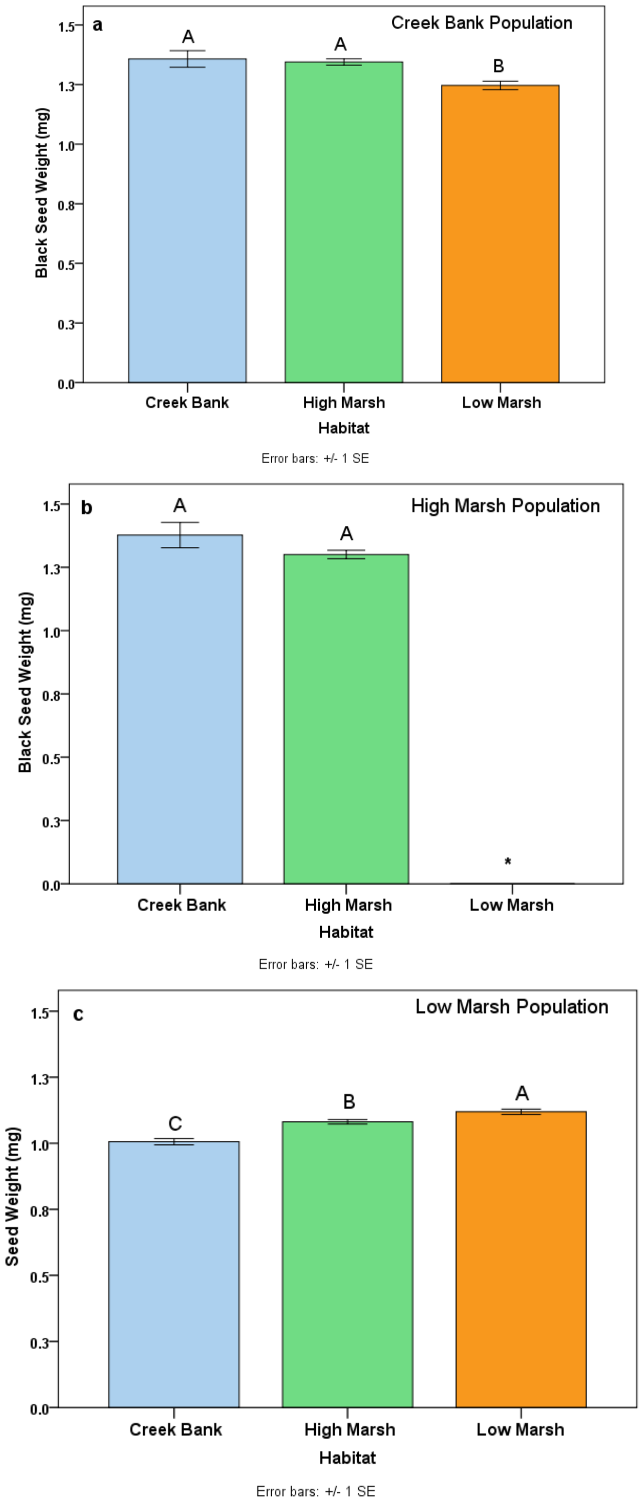


Figure 5.7: Individual black seed mass per seed (mg). **(a)** Creek bank population ($F_{2, 679} = 8.96$, $p < 0.001$). **(b)** High marsh population ($F_{1, 267} = 1.88$, $p < 0.172$). **(c)** Low marsh population ($F_{2, 1046} = 29.03$, $p < 0.001$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data)

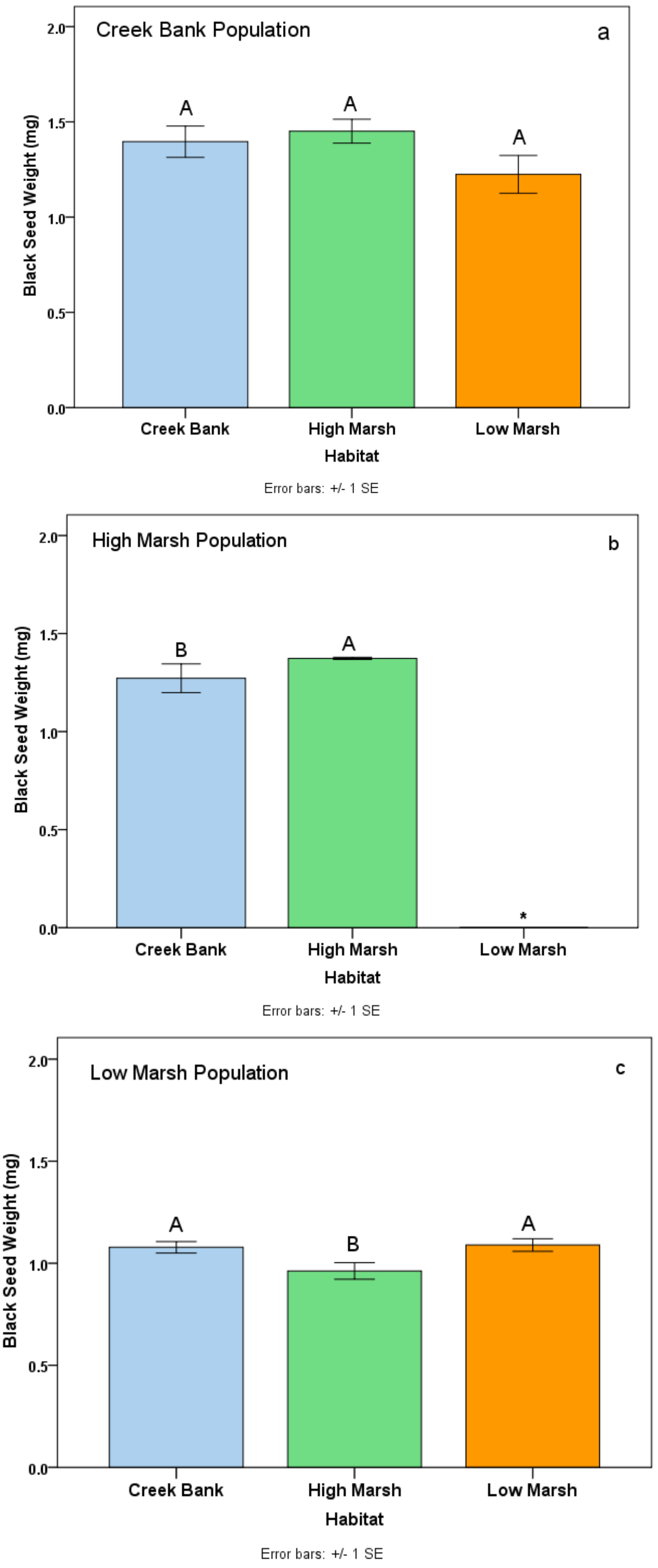


Figure 5.8: Black seed mass (mg) per seed per plant per core. **(a)** Creek bank population ($F_{2,\ 26} = 1.88$, $p < 0.173$). **(b)** High marsh population ($F_{1,\ 16} = 9.77$, $p < 0.002$). **(c)** Low marsh population ($F_{2,\ 37} = 4.3$, $p < 0.021$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data)

5.3.4 Plant height

Figure 5.9a shows that the creek bank population was signficantly taller when grown in the home and the high marsh habitats, but shorter when grown in the low marsh habitat. No significant differences were found regarding the height of the high marsh population, either when grown in its own or in the creek bank habitats (Fig. 5.9b). On the other hand, the low marsh population plants were similar in height when grown in their own habitat and in the creek bank habitat, but significantly shorter when grown in the high marsh zone (Fig. 5.9c).

In general, the creek bank population was the tallest, followed by the low marsh and the high marsh populations, respectively (Fig. 5.9).

5.3.5 Shoot width

The plants of the creek bank population exhibited a similar range of shoot widths when grown in their own and in the alien habitats (Fig. 5.10a), and a similar trend was apparent in the high marsh plants (Fig. 5.10b). The shoot width for the low marsh population was markedly greater when it was grown in its own habitat than when grown in the alien habitats (Fig. 5.10c).

In general, the low marsh population exhibited the greatest shoot width, followed by the creek bank and the high marsh populations, respectively (Fig. 5-10).

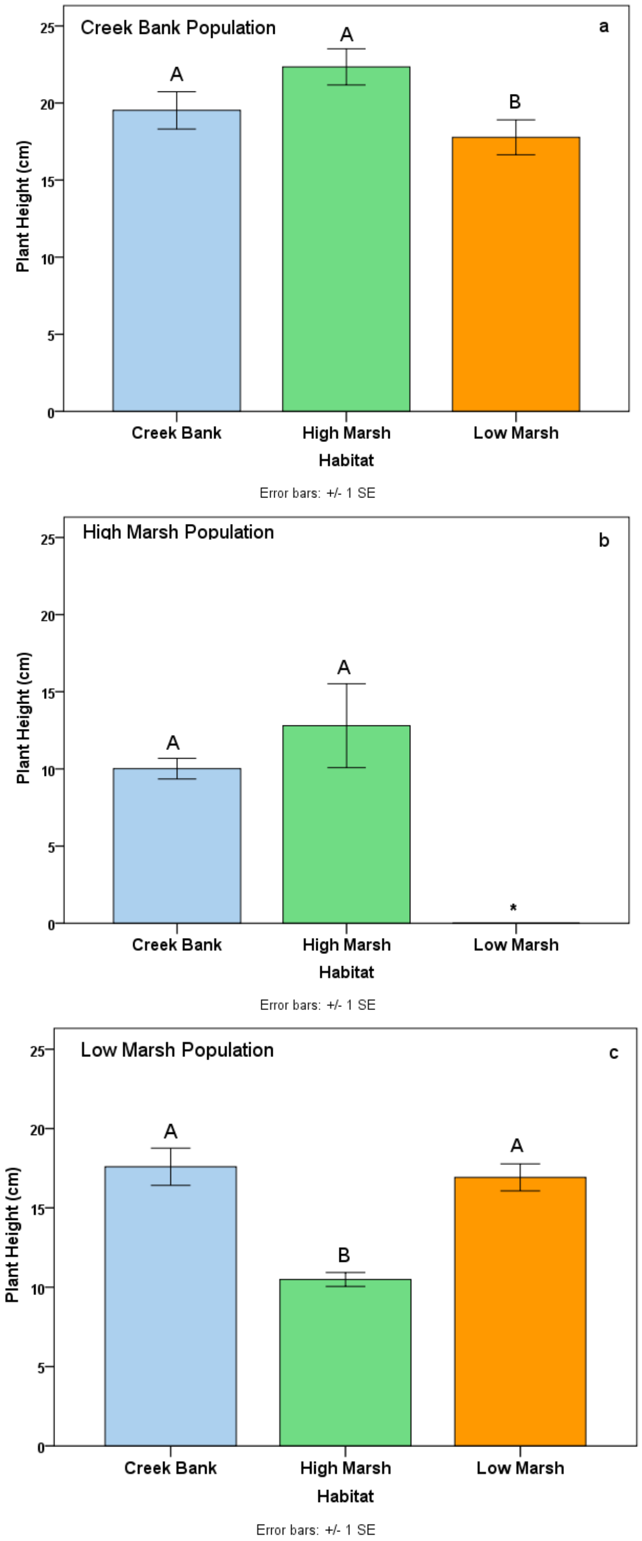


Figure 5.9: Plant Height (cm) per plant per core. **(a)** Creek bank population ($F_{2, 51} = 3.7$, $p < 0.032$). (**b)** High marsh population ($F_{1, 27} = 1.67$, $p < 0.207$). (**c)** Low marsh population ($F_{2, 41} = 23.8$, $p < 0.001$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data)

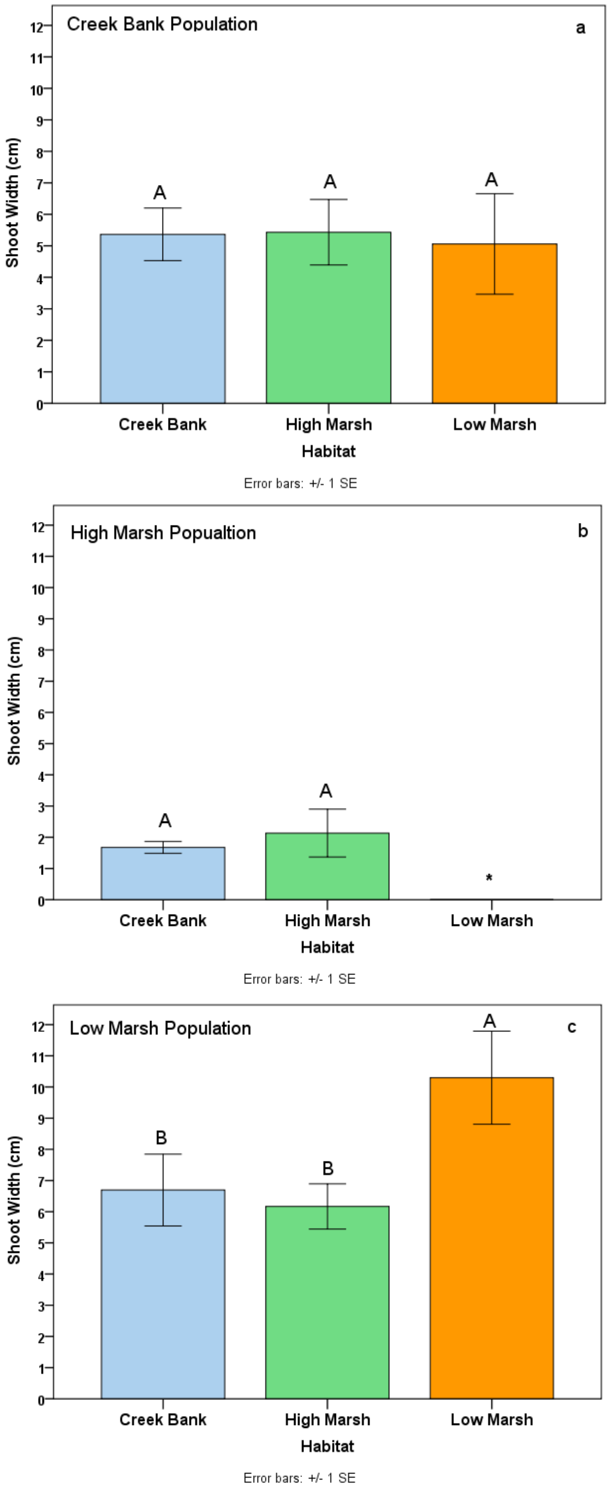


Figure 5.10: Shoot Width (cm) per plant per core. **(a)** Creek bank population ($F_{2,\ 51}$ = 0.28, $p < 0.973$).(**b)** High marsh population ($F_{1,\ 27}$ = 0.56, $p < 0.462$). (**c)** Low marsh population ($F_{2,\ 41}$ = 3.8, $p < 0.031$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data)

5.3.6 Number of primary branches

The creek bank population produced significantly fewer branches when grown in its own and in the low marsh habitats, and the more branches found when grown in the high marsh habitat (Fig. 5.11a). The high marsh population produced a similar number of branches either when grown in its own or alien habitats (Fig. 5.11b). Finally, the low marsh population produced more branches when grown in its home habitat compared with the alien ones (Fig. 5.11c).

In general, the low marsh population produced the largest number of primary branches, followed by the creek bank and the high marsh populations, respectively (Fig. 5.11).

5.3.7 Main root length

No significant differences in the main root length were observed for the creek bank and the low marsh populations, whether when grown in their home or alien habitats (Fig. 5.12a and 5.12c). However, the high marsh population had significantly slightly shorter root when they were grown in their home than in the creek bank habitat (Fig. 5.12b).
In general, the creek bank and the low marsh populations tended to exhibit a longer main root than the high marsh population (Fig. 5.12).

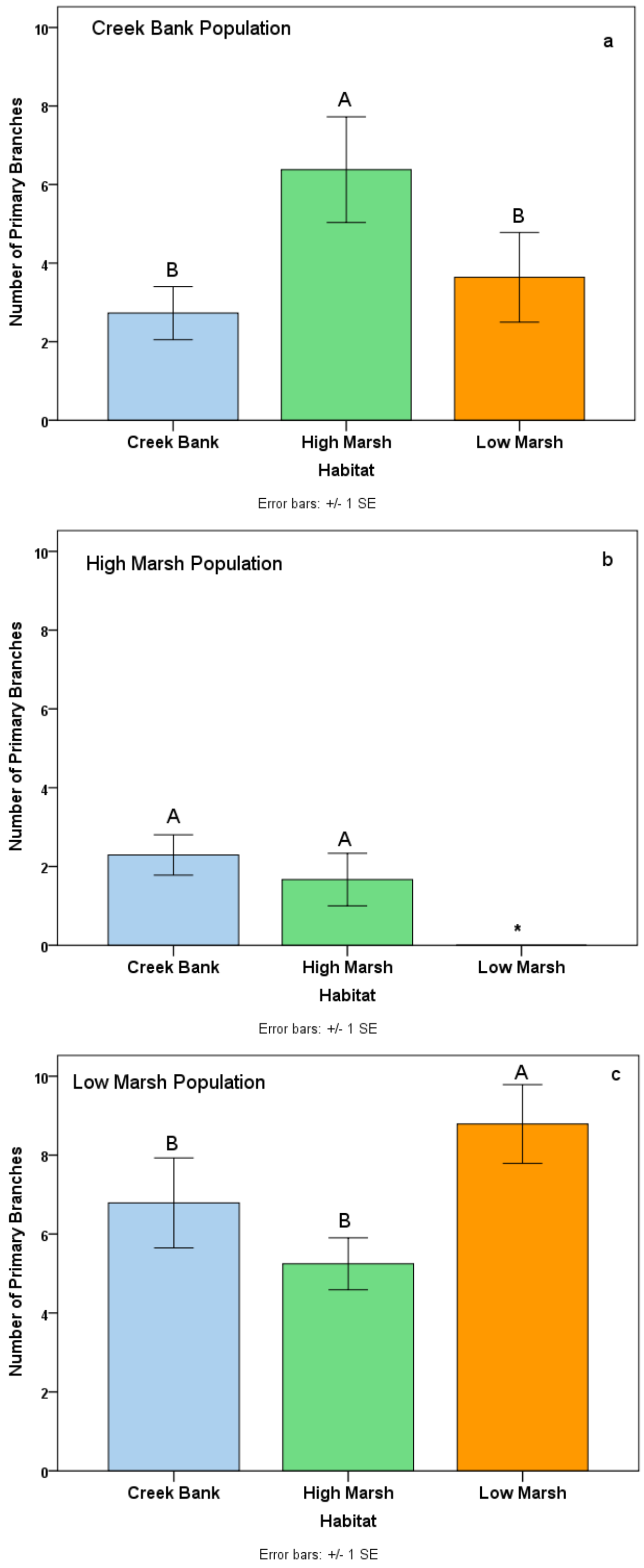


Figure 5.11: Number of primary branches per plant per core**. (a)** Creek bank population ($F_{2, 51} = 3.19$, $p < 0.049$). **(b)** High marsh population ($F_{1, 25} = 0.49$, $p < 0.619$). **(c)** Low marsh population ($F_{2, 39} = 3.77$, $p < 0.032$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* insufficient data)

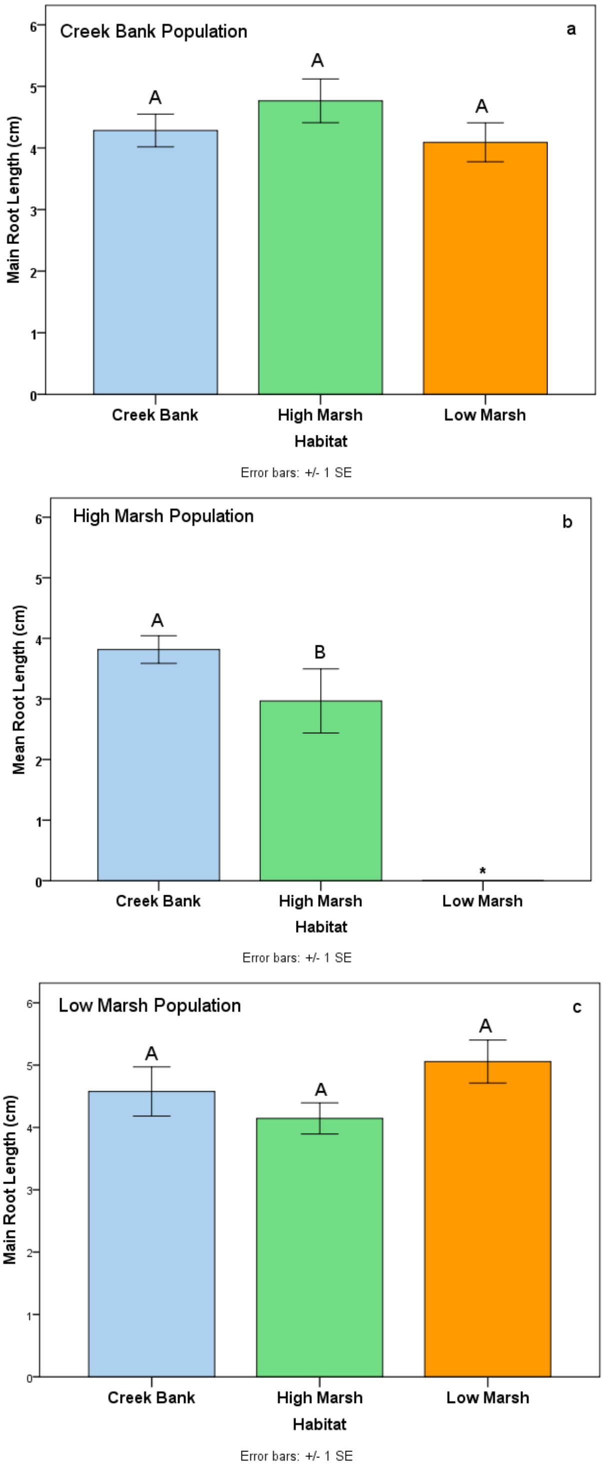


Figure 5.12: Main root length (cm) per plant per core. **(a)** Creek bank population ($F_{2, 51} = 1.19$, $p < 0.312$). **(b)** High marsh population ($F_{1, 27} = 1.47$ $p < 0.235$). **(c)** Low marsh population ($F_{2, 41} = 1.82$, $p < 0.175$). Within each population, bars with shared letters are not significantly different, as determined by one-way ANOVA (Tukey tests, $p < 0.05$). (* Insufficient data)

5.3.8 Density dependence

All of the plant traits presented previously might potentially be subject to negative density-dependent effects, because of local competition for resources. As there was some variation in the initial density of plants on the cores, such effects where detected, are presented here. Figure 5.13 shows that plant density negatively affected the final survival rate in just one case; within the high marsh population, when it was grown in its home habitat, but for the other populations and habitats, survival appeared to be density-independent.

Plant density affected the black seed mass per seed negatively, but very weakly in many situations, including the creek bank population when grown in home and high marsh habitats, the high marsh population when grown in its home habitat, and finally the low marsh population when grown in the high marsh habitat. In addition, only one weak positive relationship was found: within the low marsh population when grown in its home habitat (Fig. 5.14). However, assessing the plant-density effect on black seed mass per core revealed only one weak positive association: for the low marsh population when grown in the creek bank habitat (Fig. 5.15).

No significant associations were observed when investigating the relationship between density and fecundity (total number of seeds). However, when investigating the relationship between density and the number of black seeds, one negative association was observed: within the low marsh population when grown in the high marsh habitat (Fig. 5.16).

Quite strong negative relationships between plant density and plant height occurred on three occasions: within the creek bank population when grown in the high marsh habitat, and within the low marsh population when grown in the creek bank and in the high marsh habitats (Fig. 5.17).

Several negative relationships have been identified between plant density and plant shoot width, including the creek bank population when grown in the high marsh, and the low marsh population when grown in alien habitats (Fig. 5.18).

Only one negative relationship occurred between plant density and the number of branches: for the creek bank population when grown in the high marsh habitat (Fig. 5.19).

Finally, investigating the relationship between plant density and main root length revealed two negative associations: within the low marsh population when grown in home and in creek bank habitats (Fig. 5.20).

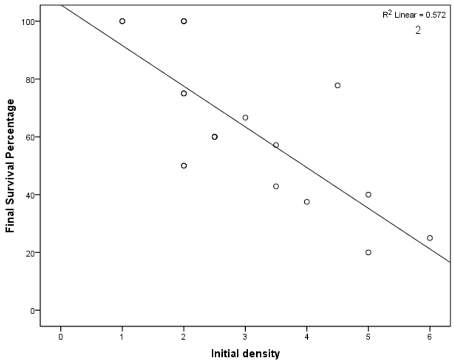


Figure 5.13: Relationship between final survival percentage and initial density shown by the high marsh population in high marsh habitat ($r^2 = 0.572$, $p < 0.001$).

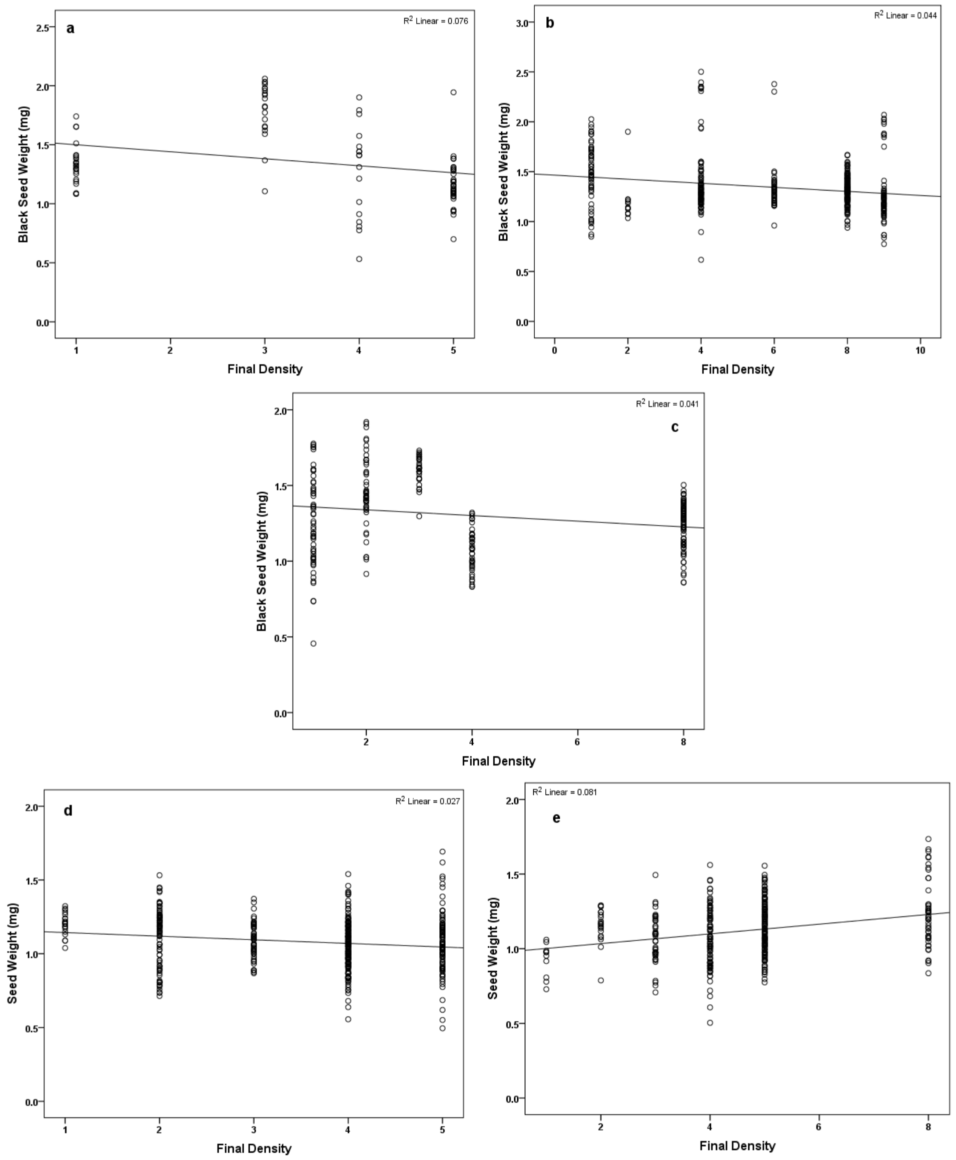


Figure 5.14: Relationship between final density and black seed mass (mg) per seed. (**a)** Creek bank population in creek bank habitat ($r^2 = 0.076$, $p < 0.007$), (**b)** Creek bank population in high marsh habitat ($r^2 = 0.044$, $p < 0.001$), (**c**) High marsh population in high marsh habitat ($r^2 = 0.041$, $p < 0.001$),(**d)** Low marsh population in high marsh habitat ($r^2 = 0.027$, $p < 0.001$) and **e)** Low marsh population in low marsh habitat ($r^2 = 0.081$, $p < 0.001$).

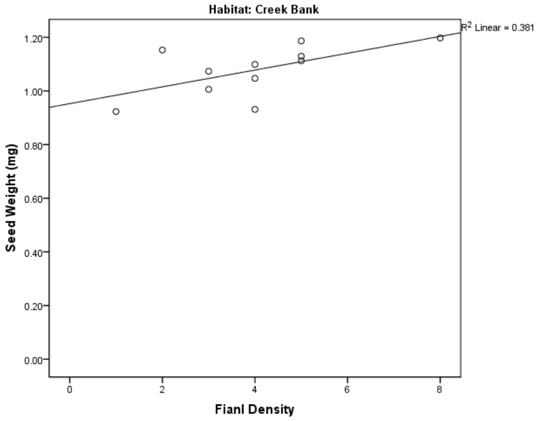


Figure 5.15: Relationship between final density and black seed mass (mg) per seed per plant per core shown by the low marsh population in creek bank habitat ($r^2 = 0.381$, $p < 0.043$).

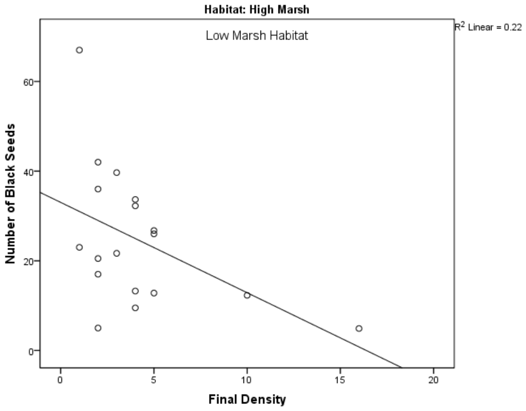


Figure 5.16: Relationship between final density and number of black seeds per plant per core shown by the low marsh population in high marsh habitat ($r^2 = 0.220$, $p < 0.050$).

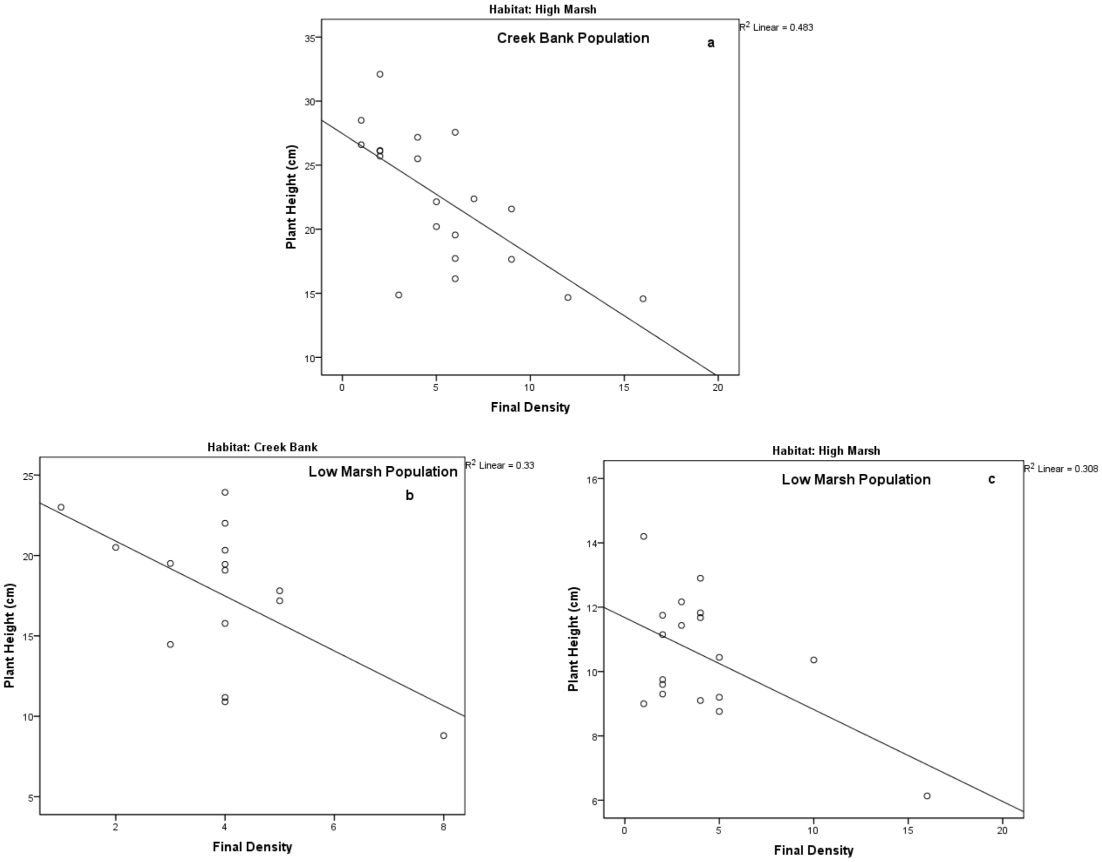


Figure 5.17: Relationship between final density and plant height (cm) per plant per core. (**a)** Creek bank population in high marsh habitat ($r^2 = 0.483$, $p < 0.001$), (**b)** Low marsh population in creek bank habitat ($r^2 = 0.330$, $p < 0.025$) and (**c)** Low marsh population in high marsh habitat ($r^2 = 0.308$, $p < 0.017$).

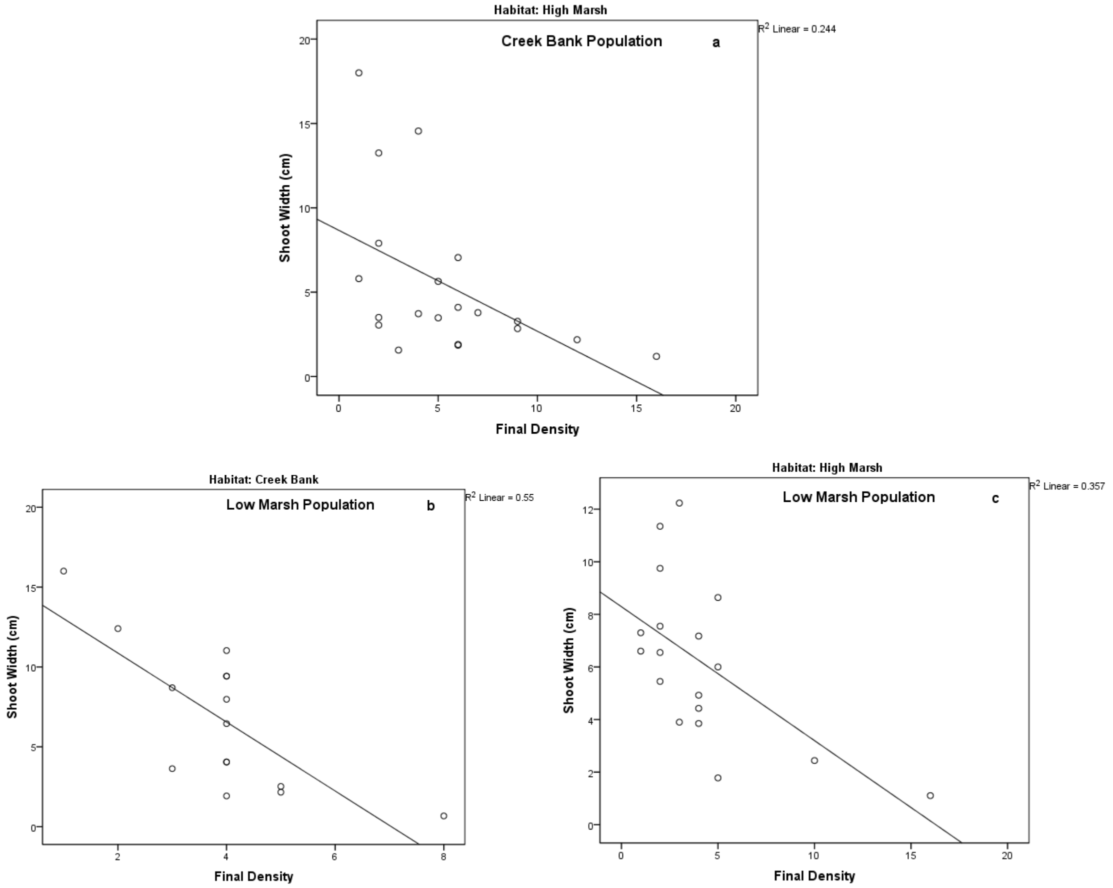


Figure 5.18: Relationship between final density and plant shoot width (cm) per plant per core. (**a)** Creek bank population in high marsh habitat ($r^2 = 0.244$, $p < 0.027$), (**b)** Low marsh population in creek bank habitat ($r^2 = 0.550$, $p < 0.002$) and (**c)** Low marsh population in high marsh habitat ($r^2 = 0.357$, $p < 0.009$).

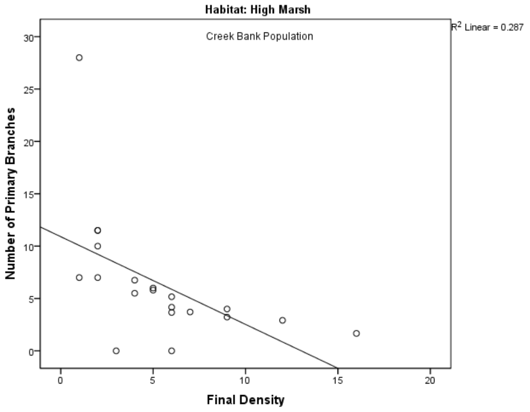


Figure 5.19: Relationship between final density and number of primary branches per plant per core shown by the creek bank population in high marsh habitat ($r^2 = 0.287$, $p < 0.015$).

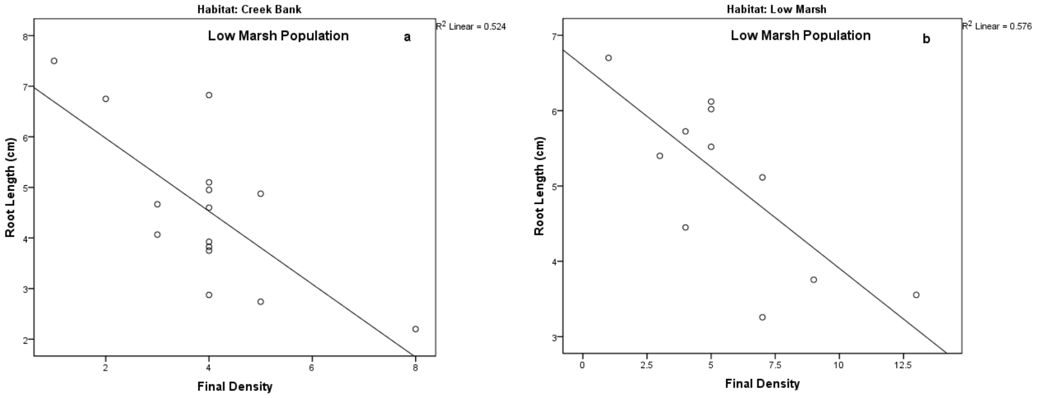


Figure 5.20: Relationship between final density and main root length per plant per core. (**a)** Low marsh population in creek bank habitat (r^2 = 0.524, $p < 0.002$) and (**b)** Low marsh population in low marsh habitat (r^2 = 0.576, $p < 0.007$).

5.4 Discussion

Although several closely-related forms have been recognized within the *Suaeda maritima* species in British salt marshes (Chapman 1947, Clapham et al. 1962), little work has been conducted to explain whether or not their phenotypic variation is simply phenotypic plasticity or whether there is an element of genetic differentiation to local microhabitat conditions,. The current field reciprocal approach revealed considerable population differentiation in some of their life-history traits, in particular in terms of survival, growth and fecundity.

The most pronounced finding on the survival is that both the creek bank and the low marsh populations had high overall survival rates compared with the high marsh population. Also, the high marsh plants were severely affected when they were transplanted to the creek bank site and even more severely affected when transplanted to the low marsh site. It is well known that populations on the higher parts of a salt marsh may be better adapted to hypersaline conditions (especially in summer) and that populations on the lower parts of a marsh may be better adapted to long periods of submergence, waterlogging and tidal action (Jefferies 1977, Davy et al. 2001). Therefore, the high marsh population in the current study is believed to be less well adapted to the local abiotic conditions of the alien habitats, i.e. in the creek bank and in the low marsh. In addition, the low marsh population suffered from some unfavourable conditions in the creek bank site, which increased its mortality. The creek bank habitat should be suited to the low marsh population as it is a more favourable environment in terms of certain abiotic factors, including soil aeration and nutrient availability, and therefore, such factors might not be the cause for the lower survival rate of the low mash transplants in that habitat, but biotic factor such as competition for resources between plants could be the main stressor in this site (compared with the low marsh habitat). However, it is not entirely clear that the explanation above is sound because according to some researchers survival was found to be to a great extent a density-independent trait (Jefferies and Perkins 1977). The survival density-independent hypothesis in this case is supported to a large extent by the investigation carried out in the current study into the relationship between survival and density per core; only one significant association was identified among all the populations and habitats. Finally, the high and the low marsh populations exhibited higher survival rates when they were grown in their home habitats than in the alien ones. This could be the result of a home site advantage, which serves as a natural selection force against alien populations (Wang 1996).

The current survival findings made it difficult to predict which part of the marsh is the most stressful to plant life, and similar doubts have been mentioned previously (Pennings and Callaway 1992). In addition, there is the risk to generalising about the survival trends of a particular species, as survival rates can vary for instance between years (Watkinson and Davy 1985). Anyway, survival is only one measure of the local adaptation of a species and its populations, and the fecundity and growth findings in this study may further and more reliably explain the variation drivers among the *Suaeda maritima* populations.

The low marsh population tended to have a shorter lifespan in home and alien habitats than the other two populations. This phenomenon was observed during the field surveys in the previous chapters, and this population in particular could be considered as an opportunist, exploiting the temporarily favourable conditions of low salinity and high nitrogen availability that are likely to exist on the low marsh (Davy et al. 2001). In addition, the shorter lifespan of the low marsh plants in home and alien habitats may support the argument that there is a genetic component inherited by this population, because it behaves as an early short-lived successor population that has small, readily dispersed seeds, which are suited to unstable primary colonization sites (Greipsson and Davy 1995).

The current study showed two prominent patterns regarding the growth (plant height, shoot width, number of primary branches and main root length) and the fecundity (total number of seeds, number of black seeds and black seed mass) traits of the three populations. The first one is that the general patterns of variation regarding the growth and fecundity characteristics of the populations were retained, even when they were grown in alien sites. For example, the creek bank and high marsh populations produced consistently bigger seeds than the low marsh plants. In addition, the total number of seeds produced was significantly higher in the low marsh population, followed by the creek bank, and finally by the high marsh population. Furthermore, the creek bank population was the tallest, followed by low marsh and high marsh populations, respectively, and the low marsh population exhibited the greatest shoot width, followed by the creek bank and the high marsh populations, respectively. Finally, this general pattern of phenotypic variation between those transplanted populations strongly coincides with the pattern of phenotypic variations between the same populations that were growing naturally and were surveyed in the previous chapters.

The second prominent pattern is that much of the phenotypic variations observed in the current study regarding the growth and the fecundity traits were within each population, especially when grown in alien habitats. For instance, the high marsh population exhibited slightly bigger seed mass when grown in its own habitat than in the creek bank one, and the low marsh population produced bigger seeds when grown in home and creek bank habitats, but slightly smaller ones when grown on the high marsh. In addition, the creek bank population produced fewer seeds when grown in its own and on the low marsh, but more seeds when grown in the high marsh habitat. Furthermore, the creek bank population was taller when grown in the home and the high marsh habitats, but slightly shorter when grown in the low marsh habitat; also, the low marsh population plants had a similar height when grown in their own habitat and in the creek bank habitat, but they were shorter when grown on the high marsh. Finally, the shoot width for the low marsh population was markedly greater when it was grown in its own habitat than when grown in the alien habitats.

The low marsh population exhibited much variation when grown in the high marsh habitat; it exhibited smaller seed mass, shorter height, smaller shoot width and fewer primary branches when grown in the high marsh than in its own habitat. These changes represents a plastic response to the adverse abiotic factors prevalent in the high marsh site, particularly the summer hypersaline conditions (Jefferies et al. 1979, Partridge and Wilson 1988). On the contrary, the creek bank population produced more seeds and primary branches when grown in the high marsh than in its own habitat. Such enforced phenotypic plastic changes therefore represent either losses for the low marsh or gains for the creek bank populations in terms of fitness within the high marsh site. This is an indication that differences in salt tolerance are the key driver behind population responses in this particular part of the marsh.

Investigating the relationship between plant density per core and growth as well as fecundity traits revealed negative associations in many cases. The negative density-dependent effect on the growth and fecundity traits was expected, as those traits are known to be highly plastic in responding to adverse conditions in micro-environments

(Watkinson and Davy 1985). Such findings have been confirmed by (Tessier et al. 2000a); they examined the effect of density on reproduction for a population of *Suaeda maritima* in the Mont Saint-Michel Bay salt marsh, and they concluded that when density increases, biomass and seed production decreases, and individuals tend to be less-branched. Researchers consider such density relationships as trade-offs in the halophytes' competitive ability and stress tolerance along stress gradients in salt marshes (Vince and Allison 1984, Crain et al. 2004).

The phenotypic variations of the growth and fecundity characteristics among the *Suaeda maritima* populations, which were maintained even when grown in alien sites, could be explained as a carry-over effect from maternal resources to descendents, which is evidence of a substantial genetically determined component in those populations (Davy and Smith 1985). However, the phenotypic variations occurring within populations demonstrated differing degrees of phenotypic plasticity in response to local conditions in each part of the marsh. Therefore, the inter- and intraspecific variations of the *Suaeda maritima* populations in the current study could be considered as a mixed strategy (of phenotypic plasticity and genetic differentiation) in order to tolerate the local environmental heterogeneity of the Stiffkey salt marsh (Jefferies and Rudmik 1991).

The findings of the current study in agreement with the findings of (Jefferies et al. 1981); in their reciprocal transplantation experiments, the *Salicornia europea* populations of the Stiffkey high and low marsh sites showed that distinctive phenotypic variations were maintained, indicating that genetic differentiation among populations was evident, particularly with regards to growth. In addition, similar supportive findings are reported in a study conducted on the Edge and Tall populations of *Salicornia europea* from the Ohio Salt Pan; when they were reciprocally transplanted, they resembled plants from their source more closely than those in the new site (Ungar 1987b). However, in a recent reciprocal transplantation experiment of *Suaeda maritima* plants, conducted between the low and high parts of the salt marsh on the River Adur estuary (Essex, UK), it was found that the transplanted plants, either in home or alien positions, developed similar morphological characteristics, indicating phenotypic plasticity in terms of plant height and mass (Wetson et al. 2012). The opposing findings in the latter study could be due to site-specific topographic variation, as the Stiffkey salt marsh is naturally more complex (within it, there is an ancient shingle ridge that physically separates the marsh into upper and lower parts, with a unique drainage system that greatly inhibits gene flow, i.e. seeds floating between marsh parts) (Davy 1988). Therefore, the topographic complexity of Stiffkey may act as a determinant on the life-history characteristics of these *Suaeda maritima* populations, more so than in the River Adur estuary.

A study conducted by (Richards et al. 2005) confirmed the complexity of plant responses to local environmental gradients. It found that intraspecific phenotypic variation in relation to environmental variables was a common trend, demonstrated in twelve species of south-eastern USA coastal salt marshes, with negative associations between plant traits such as height, leaf size and number, and the environmental variables of salinity and waterlogging.

In conclusion, phenotypic variation between *Suaeda maritima* populations in terms of survival, growth and fecundity is evident and most likely because of the selection of genotypes that best fit the micro-environmental conditions in their local habitats. The field reciprocal transplants of the current study resembled the populations from which they had been taken more than those in their new habitats. Therefore, the reciprocal transplantation technique has proved to be a powerful approach in revealing the selection pressures that possibly maintain the genetic differentiation of life-history traits (Davy 1988). However, as this technique measured their response to all environmental variables acting together, a further laboratory study is needed for these three populations, to be conducted under similar conditions in growth chambers; this could assist in more fully comprehending the genetic component of variability through isolating the variables affecting plant phenotypic variation in the field, such as salinity and waterlogging (Partridge and Wilson 1988).

Chapter 6

Life-history variation of local populations of *Suaeda maritima* under controlled growth conditions

Abstract

- ***Background and Aims:*** *Suaeda maritima* is an annual halophyte species that inhabits coastal and inland salt marshes. It is known to exhibit clear phenotypic variation in terms of growth and fecundity. The main aim of this study is to examine the extent to which *Suaeda maritima* populations maintain morphological and life-history traits when transplanted under controlled conditions, and the extent to which these traits are plastic adaptations or genetic differentiation.
- ***Methods:*** Young seedlings of *Suaeda maritima* populations (the creek bank, the high marsh, the top of low marsh and the low marsh) were transplanted in two growth chambers under two temperature regimes: 20-15 ºC and 25-20 ºC. Life-history traits (manifested in terms of survival, growth and fecundity) were assessed within and between those populations.
- ***Key Results:*** Under the 20-15 ºC regime, the four populations demonstrated substantial differentiation in their survival, growth and fecundity, resembling their maternal populations in the field. However, under the 25-20 ºC regime, insignificant differences among the populations were observed.
- ***Conclusions:*** Under the low temperature (and therefore low salinity) of the first regime (20-15 ºC), the *Suaeda maritima* populations were able to maintain the inherited significant differences between them. However, under the much more severe conditions of temperature (and therefore salinity) of the second regime (25-20 ºC), those populations showed insignificant plastic response differences among them, indicating a striking ability to survive and complete the lifecycle by producing seeds for future stability purposes.

6.1 Introduction

The field surveys (Chapter 3) and the field transplantation experiment (Chapter 5) strongly suggested the presence of at least three differentiated populations of *Suaeda maritima*, located in discrete populations in specific areas of the marsh. These populations exhibit specific morphological traits that were found to be related to differences in the elevations and salinities that characterise the three different microtopographic locations investigated: the lower marsh, the upper marsh and on the creek bank. Each particular phenotype is dominant within its locality and thus can be assumed to have developed distinctive evolutionary strategies for coping with the range of biotic and abiotic variations that characterise these specific sites on the marsh (Wu et al. 2004, Valladares et al. 2007).

Both biotic and abiotic conditions, within which each population thrives, are key determinants of the fecundity and morphology of individual plants, which may become manifest in the form of either phenotypic plasticity (Callaway et al. 2003) or genetic differentiation (Jefferies 1977, Davy and Smith 1985, Davy et al. 1990, Howard and Rafferty 2006, Baythavong and Stanton 2010a), or through a combination of both of them (Jefferies and Rudmik 1991, Richards et al. 2005).

In order to distinguish between variation in fecundity and morphology resulting from phenotypic plasticity or genetic heterogeneity, two principal approaches can be employed: field reciprocal transplantation of the populations (described in Chapter 5) and transplanting the populations to controlled conditions in growth chambers (Kawecki and Ebert 2004). The former is a valuable technique because it allows experimental investigations under conditions that include the full range of naturally occurring abiotic and biotic factors, which are likely to be the key influences of fecundity and morphology, given the distinct phenotypic or genetic characteristics (Davy and Smith 1985). However, the latter technique can serve as an important complementary approach to field-based experimentation, as growth chambers can assist in more fully comprehending the genetic component of variability through isolation of the factors affecting variability in the field (i.e. abiotically driven factors in the form of marsh elevation gradients that influence inundation frequencies/durations, soil drainage properties and salinities, and biotically driven factors in the form of plant competition (Jefferies et al. 1981).

In Chapter 5, the reciprocal transplantation experiment was conducted to examine the variations in survival, morphology and fecundity of the three main *Suaeda maritima* populations at Stiffkey salt marsh on the creek bank zone, on the upper marsh, and on the lower parts of the marsh. This experiment revealed the extent to which these populations responded to environmental variability, which was found to be a mixed strategy of genetically driven responses between the three populations and plastic responses within each population.

In order to establish if the variation in performances found in the field experiments in *Suaeda maritima* populations are plastic and/or genetic responses, it became necessary to conduct similar investigations under controlled growth chamber conditions. These conditions are intended to eliminate any abiotic factors that may have affected the findings of the previous chapter, and to provide a stable control in which the impact of the key biotic parameters on the transplanted specimens can be assessed in terms of fecundity and morphology.

Accordingly, this chapter seeks to address the following two questions:

1. To what extent do the *Suaeda maritima* populations maintain their variations, in particular, with respect to morphology and fecundity?
2. To what extent does plant density affect certain life-history components (survival, morphology and fecundity), and therefore, can this be used to predict changes in the genotype level?

6.2 Materials and Methods

In order to replicate the field transplant experiments under controlled conditions, two growth cabinets (Fitotron SGC097, Weiss Gallenkamp, UK) at UEA were used from April to October 2009 (Fig. 6.1).

6.2.1 Sampling and experiment design

Suaeda maritima seedlings from the creek bank, the high marsh, the top of low marsh and the low marsh populations from Stiffkey were chosen as typically representative of the microhabitat in which each local population had been growing. For each population, a total of 20 random soil cores (6 cm diameter × 10 cm deep) were extracted using a bulb planter and then transferred to the laboratory. Areas with young seedlings at the post-cotyledon stage were targeted (1-1.5 cm in height) (Fig. 6.2). Ten soil cores of each population were placed in a tray containing moderately saline water (200 mM NaCl) and therefore, a total of four trays were placed in each growth cabinet (i.e. 10 cores per population per cabinet).

Two growth cabinets were used to provide two different temperature regimes; the first was 20-15 ºC (day/night) and the second was 25-20 Cº. The photoperiods in both chambers were: 16 hours of daylight and 8 hours of darkness. The first temperature regime was chosen to resemble the average natural conditions of the field in spring and summer. The second regime was warmer in order to assess the growth behaviour of the different populations under a more stressful environment.

The medium was changed every two weeks and the level of water was maintained at 5 cm in all trays. After two months, commercial fertilizer (liquid vegetable fertilizer product of B&Q UK) was added once fortnightly to the medium (15 ml fertilizer to 7 l of the medium) in order to support the nutritional needs of the plants in case the original nutrient sources in the soil cores had started to deplete.

The growth chambers were monitored and the number of individuals in each core was recorded on a weekly basis throughout the course of the experiment. In addition, the plants were harvested when they produced mature seeds before senescence, when it was still possible to carry out phenotypic characterisation.

Figure 6.1: Two growth chambers at UEA were used in the current study.

Figure 6.2: Young seedlings targeted for the purpose of the current study.

6.2.2 Phenotypic trait characterization

At the end of the experiment, the surviving plants from within each core were collected in polyethylene bags and brought back for laboratory investigation. The characterization work began immediately in the lab; plants awaiting characterization were kept in the cold storage room (at 5 ºC). Each plant was measured for the following parameters: shoot traits, including plant height (the above-ground growth height), shoot width (the above-ground shoot width), number of primary branches, number of black seeds, number of brown seeds, number of germinated seeds, and total number of seeds.

Also, main root length trait measurements were taken,. Furthermore, measurements of seed mass were taken individually using a micro-balance (Metler Toeldo UMX2).

6.2.3 Statistical analysis

The statistical analysis was conducted using PASW Statistics version 18, Chicago, USA for Windows. Survival curves of the three populations were plotted as percentages, and ANCOVA was employed to assess the significance of the survival curves, with collection date as a covariate. Comparisons of morphological traits (including plant height, shoot width, main root length and number of primary branches) and fecundity (total number of seeds, number of black seeds, number of brown seeds and black seed mass) were expressed in figures and Two-way ANOVA was conducted to assess the variations between populations. Two assessment methods were applied to gain a wider understanding about the seed mass trait within and between populations: assessing seed mass per seed and assessing seed mass per core. The latter approach is the main one used to assess all the work in this chapter because the soil core is considered to be the basic replication unit, as the cores are independent of each other.

The correlation between plant density and the morphological traits (including plant height, shoot width, main root length, number of black seeds and black seed mass) was analysed using linear regression. Also, the same approach was used to identify whether there were any correlations between plant density and final survival percentages.

6.3 Results

6.3.1 Survival

Survival curves based on the numbers of seedlings per core are shown in Figure 6.3. The relative survival (percentage mean survival for ten cores per treatment per population) of the creek bank population (Fig. 6.3a) exhibited a slight decrease in survival percentage under the 20-15 ºC regime. This decrease started gradually from the second week until the seventh week, when it reached 90%; then it stabilised until the end of the experiment at week twenty-six. Under the 25-20 ºC regime (Fig. 6.3b), a similar pattern of gradual decrease is evident in the creek bank survival percentage, but it was more severe, falling to 65% in the twelfth week; then it stabilised until the end of the experiment at week twenty.

The high marsh population (Fig. 6.3a) exhibited a gradual decrease regarding survival percentage under the 20-15 ºC regime, from the beginning until the fourteenth week, when this percentage reached 80%; it then remained stable until the end of the experiment at week twenty-six. However, a more severe decrease occurred vis-à-vis the survival percentage of this population under the 25-20 ºC regime (Fig. 6.3b), falling as low as 50% in the fourteenth week; then it remained steady until the end of the experiment at week twenty-three.

In the top of the low marsh population under the 20-15 ºC regime (Fig. 6.3a), there was a slight decrease in numbers during the first four weeks until it reached 90%, and then it remained steady until the end of the experiment at week twenty-six. However, under the 25-20ºC regime, this population exhibited a more severe decrease in survival percentage (Fig. 6.3b), falling to 70% by the tenth week; then it stabilised until the end of the experiment at week twenty-three.

Generally, under the 25-20ºC temperature regime, the decline in the survival percentages of the three populations immediately above was much in evidence during the first five weeks of the experiment.

However, the equivalent-to-survival pattern of the low marsh population (Fig. 6.3a) was in stark contrast to the other three populations under both temperature regimes. Under the 20-15 ºC regime, this pattern started with a slight decrease, staying as high as 95% in the first three weeks and then there was a gradual increase in numbers in the following four weeks until it was equivalent to 110%; it then remained steady until the end of the experiment at week twenty-two. Under the 25-20 ºC temperature regime, this population (Fig. 6.3b) exhibited a gradual increase in the numbers as well for the first nine weeks, until it reached the equivalent of 115% equivalent-to-survival; then it stabilized until the end of the experiment at week nineteen.

Table 6.1 shows the results of the two-way ANCOVA model, which was used to assess whether or not differences are maintained between populations through their whole lifecycle under both regimes. Considering week as a covariate, this model revealed that the number of *Suaeda maritima* individuals differ significantly among populations. The temperature regime did not affect this number significantly; whereas, the interaction between the population and the temperature regime has affected this number of individuals.

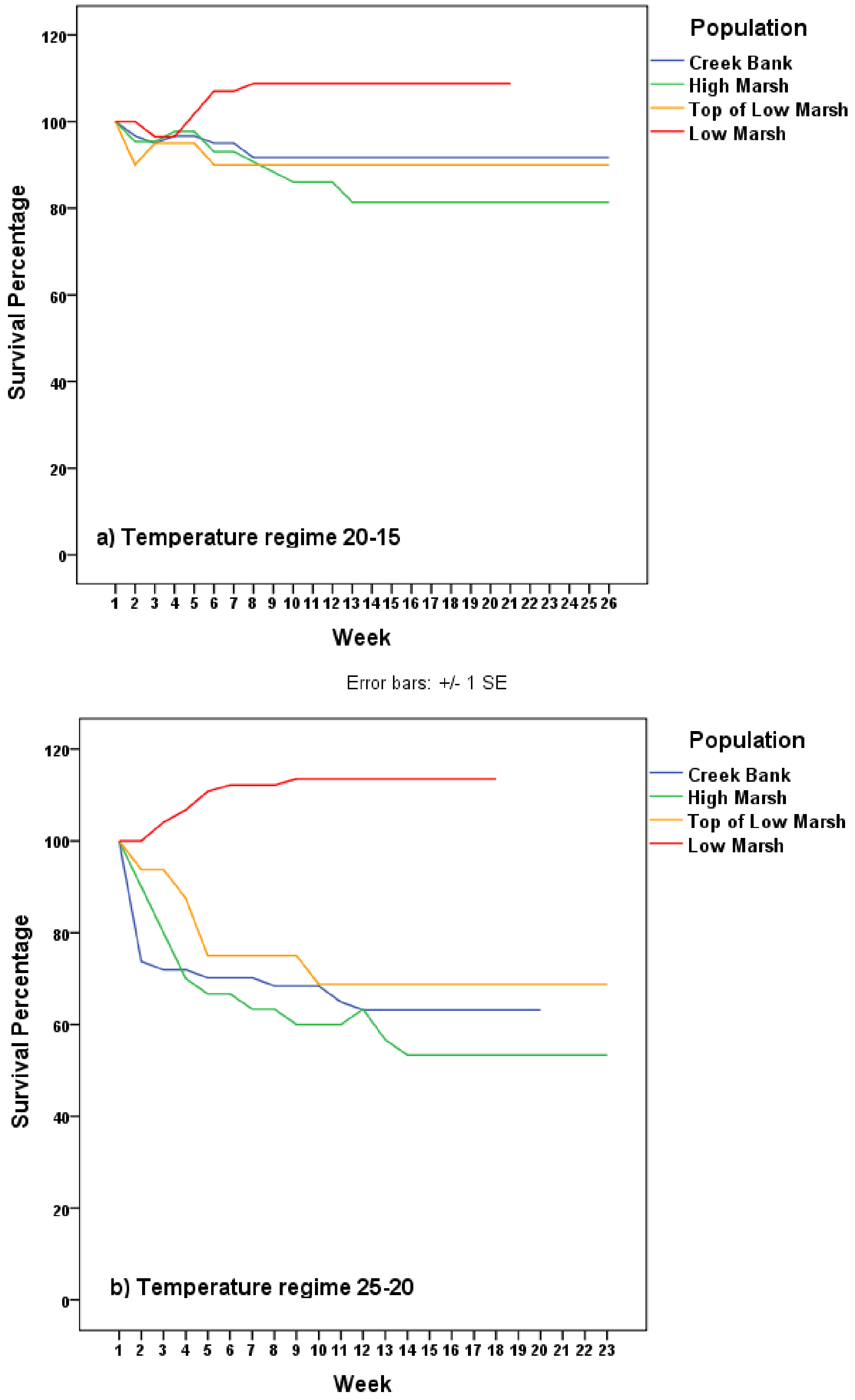


Figure 6.3: Survival of four populations of *Sueada maritima* (creek bank, high marsh, top of low marsh and low marsh) transplanted under controlled conditions in growth cabinets at UEA. Week number refers to field weekly data collection starting from the 3rd week of April 2009). **(a)** Temperature regime 20-15 ºC; **(b)** Temperature regime 25-20 ºC.

Table 6.1: Two-way ANCOVA for the survival of the *Suaeda maritima* populations (creek bank, high marsh, top of low marsh and low marsh) transplanted to two temperature regimes (20-15 ºC and 25-20 ºC) in growth cabinets at UEA.

Source of Variation	**Population**	**Temperature regime**	**Population*Temperature regime**	**Error**
Type lll SS	334500.83	1.87	267.86	724.79
Df	3	1	3	174
F	26767.98	0.45	21.44	-
P	<0.001	0.504	<0.001	-

6.3.2 Fecundity

6.3.2.1 Total number of seeds

Figure 6.4 shows the low marsh population having a significantly higher total number of seeds per plant under the 20-15 ºC temperature regime, followed by the creek bank population and finally by both the high and the top of the low marsh populations. However, under the 25-20ºC regime, all the populations have similar total numbers of seeds. In addition, the Two-way ANOVA (Table 6.2) reveals that the total number of seeds varied significantly at the population level but it varied insignificantly at the temperature regime level and at the interaction between population and temperature regime level.

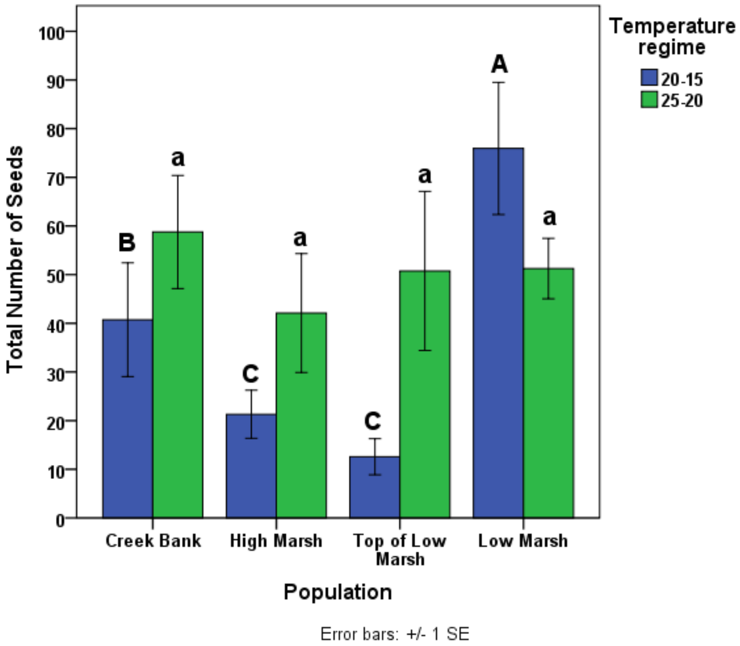


Figure 6.4: Comparisons for the total number of seeds of the *Suaeda maritima* populations per core (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 ºC; **(b)** Temperature regime 25-20 ºC.

Table 6.2: Two-Way ANOVA results for the total number of seeds of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 ºC and 25-20 ºC.

Source of Variation	**Type lll SS**	**df**	**F**	**P**
Population	10975.26	3	3.77	0.016
Temperature regime	2156.77	1	2.22	0.142
Population* Temperature	7962.87	3	2.74	0.053
Error	50458.1	52		

6.3.2.2 Number of black seeds

As the majority of the seed production by the *Suaeda maritima* plants are now known to be black seeds, the figures for the number of black seeds per plant are quite similar to those of the total number of seeds per plant. Figure 6.5 shows the low marsh population having a significantly higher number of black seeds under the 20-15 °C temperature regime than the other three populations, which have no significant differences between their numbers of black seeds. However, under the 25-20 ºC regime, all populations have similar numbers of black seeds. In addition, the two-way ANOVA (Table 6.3) reveals that the number of black seeds varied significantly at the population level and at the interaction between population and temperature regime level, but it varied insignificantly at the temperature regime level.

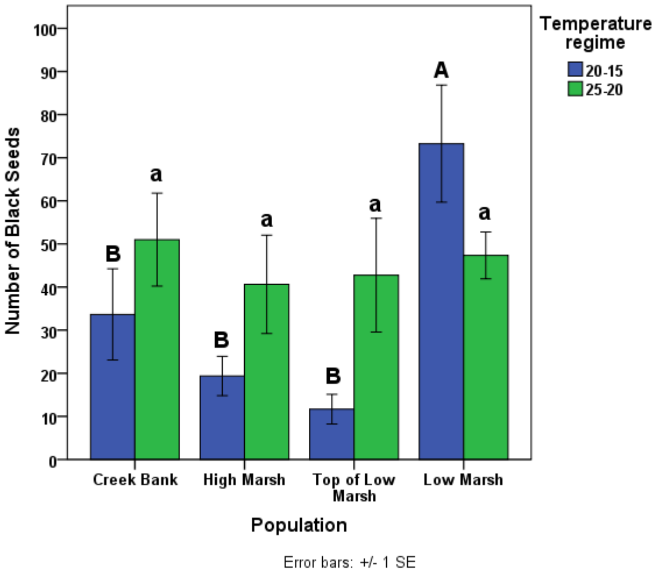


Figure 6.5: Comparisons for the number of black seeds per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 ºC; **(b)** Temperature regime 25-20 ºC.

Table 6.3: Two-way ANOVA results for the number of black seeds of the *Suaeda maritima* populations (Creek bank, High marsh, Top of the low marsh and Low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 ºC and 25-20 ºC.

Source of Variation	Type lll SS	df	F	P
Population	10403.01	3	4.06	0.011
Temperature regime	1506.82	1	1.77	0.190
Population* Temperature	7646.13	3	2.99	0.039
Error	44402.88	52		

6.3.2.3 Number of brown seeds

There were no significant differences between the populations under both regimes regarding the production of brown seeds (Fig. 6.6). In addition, the two-way ANOVA (Table 6.4) reveals that the number of brown seeds varied insignificantly at all levels (the population level, the temperature regime level, and the interaction between population and temperature regime level).

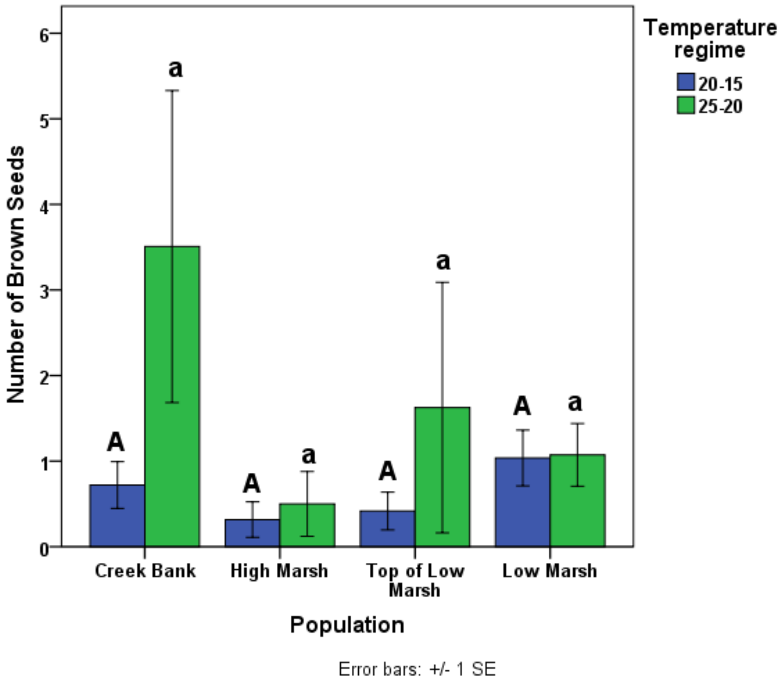


Figure 6.6: Comparisons for the number of brown seeds of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 15-20 ºC; **(b)** Temperature regime 20-25 ºC.

Table 6.4: Two-way ANOVA results for the number of black seeds of the *Suaeda maritima* populations (Creek bank, High marsh, Top of the low marsh and Low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 ºC and 25-20 ºC.

Source of Variation	Type lll SS	df	F	P
Population	24.8	3	1.72	0.174
Temperature regime	13.99	1	2.92	0.094
Population* Temperature	20.9	3	1.45	0.239
Error	249.55	52		

6.3.2.4 Black seed mass

Figure 6.7 shows the creek bank population having the biggest seeds under the 20-15 ºC temperature regime, followed by the top of the low marsh, the high marsh and the low marsh populations, respectively. Under the 25-20 ºC regime, there were no significant differences between the seed masses per seed of the creek bank, the high marsh and the top of the low marsh populations, but the low marsh seeds were significantly smaller than the other ones. In addition, the two-way ANOVA (Table 6.5) reveals that the black seed mass per seed varied significantly at all levels (the population level, the temperature regime level, and the interaction between population and temperature regime level).

Assessing seed mass per core in Figure 6.8 shows slightly different results. Under the 20-15 ºC temperature regime, the biggest seeds were found in the creek bank population, followed by the top of the low marsh population, and finally by both the high marsh and the low marsh populations. Under the 25-20 ºC regime, the seed masses of the creek bank, the high marsh and the top of the low marsh populations were insignificantly different, but the seed mass of the low marsh population was significantly smaller than the other ones. In addition, the two-way ANOVA (Table 6.6) reveals that the black seed mass per core varied significantly at two levels: the population level and the interaction between population and temperature regime level (i.e. insignificantly at the temperature regime level).

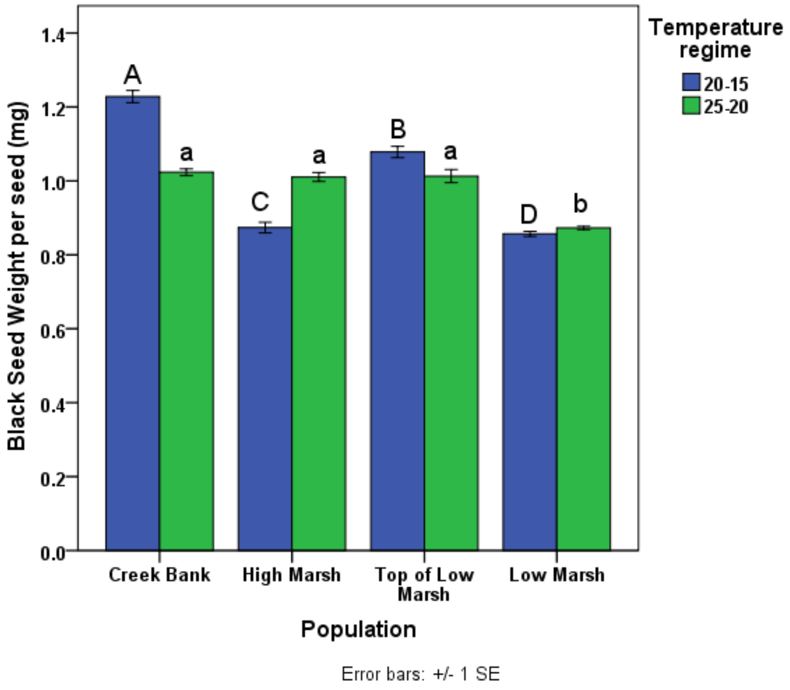


Figure 6.7: Comparisons for the black seed mass per seed of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 ºC; **(b)** Temperature regime 25-20 ºC.

Table 6.5: Two-Way ANOVA results for the black seed mass per seed of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA Temperature regimes: 20-15 ºC and 25-20 ºC.

Source of Variation	**Type lll SS**	**df**	**F**	**P**
Population	30.68	3	322.29	<0.001
Temperature regime	0.35	1	11.11	<0.001
Population* Temperature	7.68	3	80.71	<0.001
Error	79.22	2497		

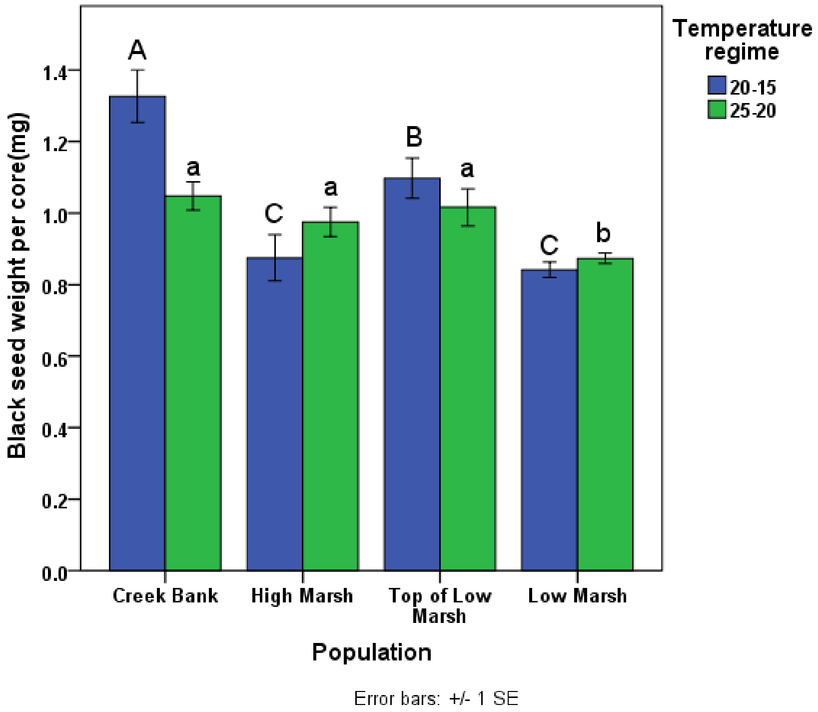


Figure 6.8: Comparisons for the black seed mass per seed per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 °C and (**b)** Temperature regime 25-20 °C.

Table 6.6: Two-way ANOVA results for the black seed mass per seed per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 °C and 25-20 °C.

Source of Variation	**Type lll SS**	**df**	**F**	**P**
Population	1.09	3	21.81	<0.001
Temperature regime	0.05	1	3.17	0.080
Population* Temperature	0.33	3	6.67	<0.001
Error	0.97	58		

6.3.3 Plant height

Figure 6.9 shows that the creek bank is significantly the tallest population under the 20-15 ºC temperature regime, followed by the top of the low marsh, and finally by the high marsh and low marsh populations. However, under the 25-20 ºC regime, all the populations have similar heights. In addition, the two-way ANOVA (Table 6.7) reveals that the plant height varied significantly at two levels: the population level and the interaction between population and temperature regime level (i.e. insignificantly at the temperature regime level).

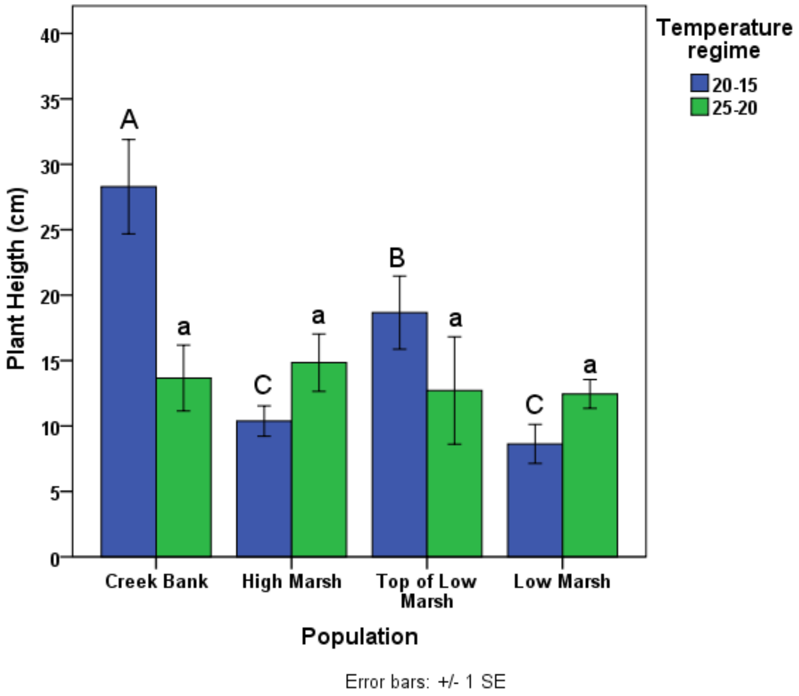


Figure 6.9: Comparisons for the plant height per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 ºC and (**b)** Temperature regime 25-20 ºC.

Table 6.7: Two-way ANOVA results for the plant height per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 ºC and 25-20 ºC.

Source of Variation	**Type lll SS**	**df**	**F**	**P**
Population	869.63	3	7.03	<0.001
Temperature regime	117.15	1	2.84	0.098
Population* Temperature	859.1	3	6.94	<0.001
Error	1979.41	48		

6.3.4 Shoot width

Figure 6.10 shows the creek bank population having a significantly greater shoot width under the 20-15 °C regime, i.e. greater than the other three populations, which exhibited similar shoot widths. However, under the 25-20ºC regime, all the populations have a similar range of shoot widths. In addition, the two-way ANOVA (Table 6.8) reveals that the shoot width varied significantly at the population level but insignificantly at both the temperature regime level and the interaction between population and temperature regime level.

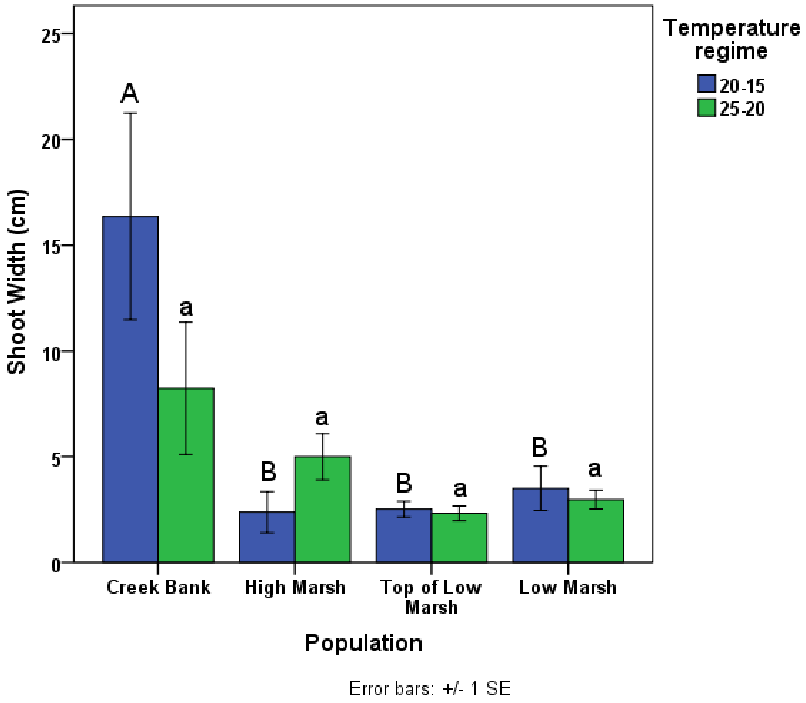


Figure 6.10: Comparisons for the shoot width per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 °C; **(b)** Temperature regime 25-20 °C.

Table 6.8: Two-way ANOVA results for the shoot width per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 °C and 25-20 °C.

Source of Variation	Type lll SS	df	F	P
Population	808.44	3	6.11	<0.001
Temperature regime	30.16	1	0.68	0.412
Population* Temperature	219.15	3	1.66	0.189
Error	2117.93	48		

6.3.5 Number of primary branches

Figure 6.11 shows that under the 20-15 ºC regime, the number of primary branches per core was significantly greater in the low marsh population, followed by the creek bank population, and finally by both the high and the top of the low marsh populations. However, under the 25-20 ºC regime, all the populations have similar numbers of primary branches. In addition, the two-way ANOVA (Table 6.9) reveals that the shoot width varied significantly at the population level but insignificantly at both the temperature regime level and the interaction between population and temperature regime level.

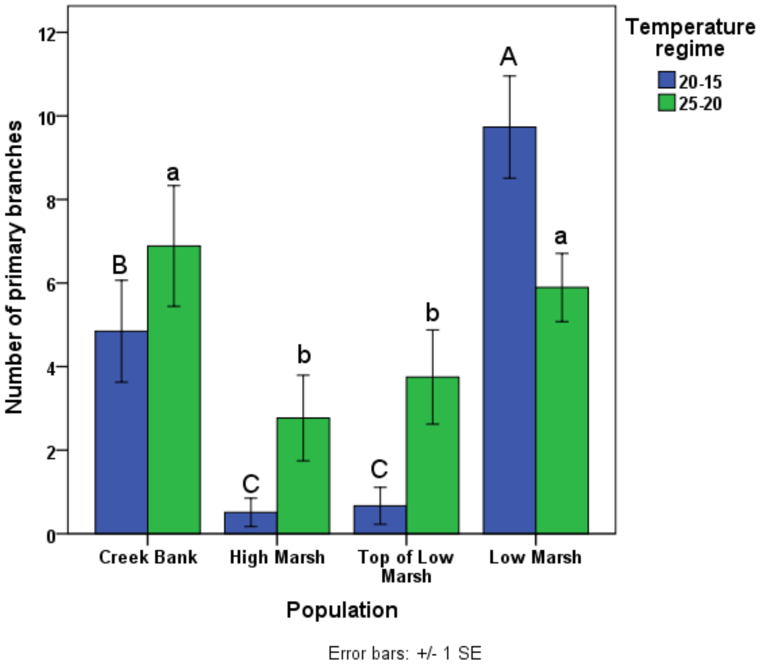


Figure 6.11: Comparisons for the number of primary branches per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 ºC; (**b)** Temperature regime 25-20 ºC.

Table 6.9: Two-Way ANOVA results for the number of primary branches per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 ºC and 25-20 ºC.

Source of Variation	**Type lll SS**	**df**	**F**	**P**
Population	415.49	3	14.2	<0.001
Temperature regime	10	1	1.03	0.316
Population* Temperature	128.07	3	4.38	0.008
Error	2531	61		

6.3.6 Main root length

Figure 6.12 shows that under the 20-15 °C regime, the main root length per plant per core was significantly longer in the creek bank population, followed by the other three populations. However, under the 25-20 °C regime, the top of the low marsh population had longer main roots than the other three populations. In addition, the two-way ANOVA (Table 6.9) reveals that the main root length varied significantly at the population level and at the interaction between population and temperature regime level, but insignificantly at the temperature regime level.

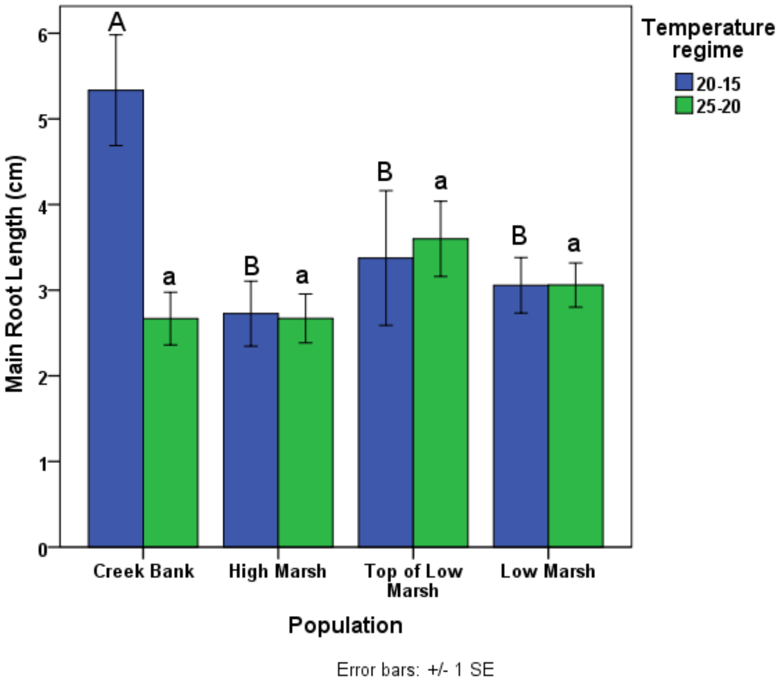


Figure 6.12: Comparisons for the main root length per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. **(a)** Temperature regime 20-15 °C; **(b)** Temperature regime 25-20 °C.

Table 6.10: Two-Way ANOVA results for the main root length per plant per core of the *Suaeda maritima* populations (creek bank, high marsh, top of the low marsh and low marsh) transplanted to controlled conditions in growth cabinets at UEA. Temperature regimes: 20-15 °C and 25-20 °C.

Source of Variation	Type lll SS	df	F	P
Population	13.46	3	3.26	0.029
Temperature regime	4.82	1	3.5	0.067
Population* Temperature	17.84	3	4.32	0.009
Error	66.08	48		

6.3.7 Density-dependence

Investigating the relationship between the final survival percentage and the initial density revealed no association between them.

Figure 6.13 shows that the final plant density affected the black seed weight per seed negatively, but very weakly in many situations, including the creek bank population when grown under both temperature regimes (Figs. 6.13a and 6.13b), the top of the low marsh population under the 20-15 ºC regime (Fig. 6.13d), and finally the low marsh population under the 25-20 ºC regime (Fig. 6.13f). Also, there were two cases of weak positive association: in the high marsh population under the 25-20 ºC regime (Fig. 6.13c) and the low marsh population under the 20-15 ºC regime (Fig. 6.13e). However, there were no associations between the final density and the black seed weight per core in all populations and temperature regimes.

Figure 6.14 shows the relationship between the plant final density and the total number of seeds was found to be strongly negative in two cases: within the low marsh populations when grown under both regimes.

Figure 6.15 shows the relationship between the final density and the black seed number, and two quite strong associations were found: within the low marsh population under both temperature regimes. However, there was no association found between the plant density and the number of brown seeds produced by all populations under both temperature regimes.

Figure 6.16 shows the relationship between the final density and the plant height trait, and two quite strong negative associations were noticed: within the creek bank population under the 20-15 ºC regime and within the high marsh population under the same temperature regime. However, there was no association found between the plant density and the shoot width trait in all populations under both temperature regimes. In addition, there was no association found between the plant density and the number of primary branches produced by all populations under both temperature regimes. Finally, there was no association found between the plant density and the main root length in all populations under both temperature regimes.

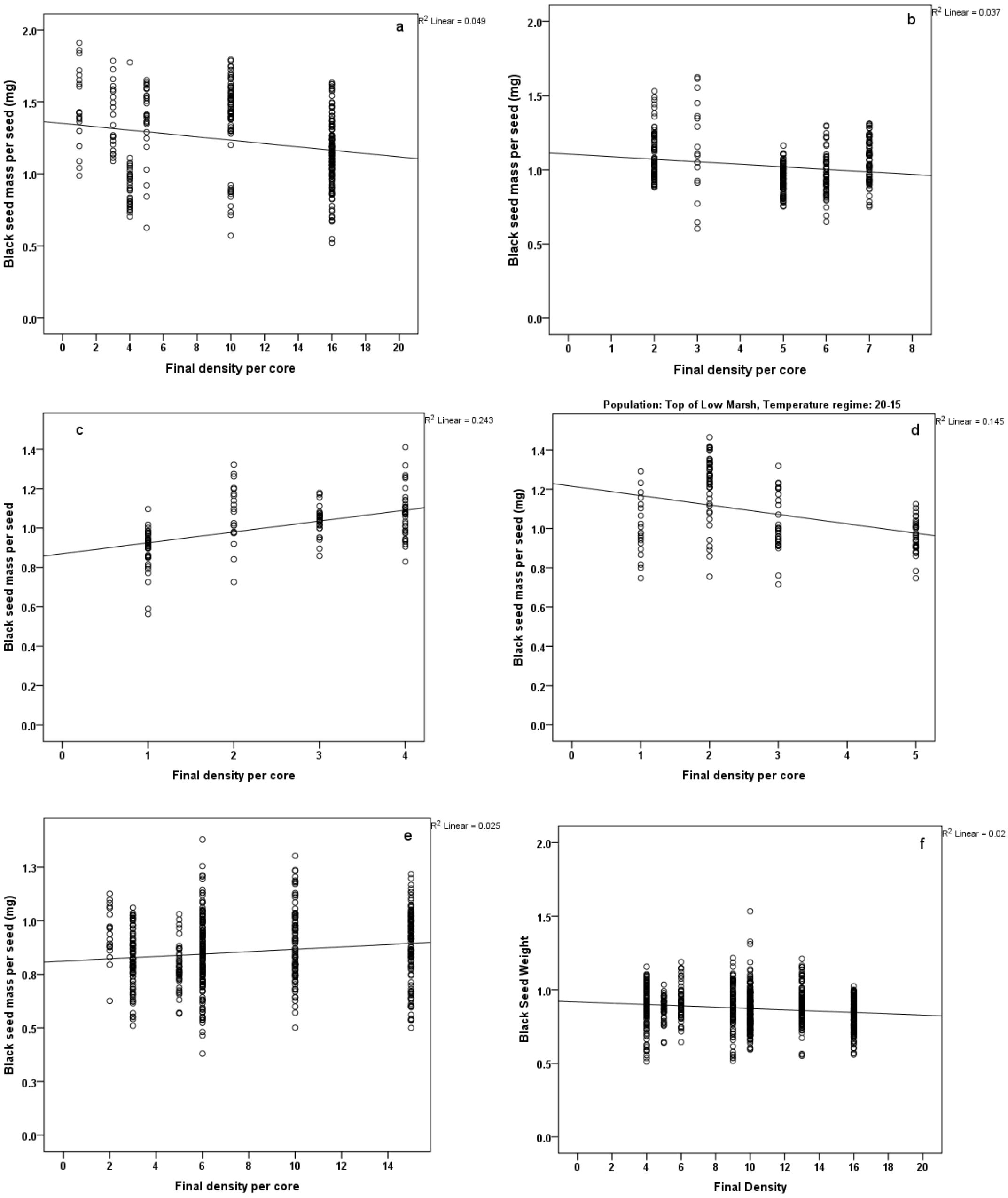


Figure 6.13: Relationship between final density and black seed mass (mg) per seed. (**a)** Creek bank population under the 20-15 ºC regime (r^2 = 0.045, p < 0.001), (**b)** Creek bank population under the 25-20 ºC regime (r^2 = 0.037, p < 0.001), (**c**) High marsh population under the 25-20 ºC regime (r^2 = 0.024, p < 0.001), (**d)** Top of the low marsh population under the 20-15 ºC regime (r^2 = 0.145, p < 0.001), (**e)** Low marsh population under the 20-15 ºC regime (r^2 = 0.025, p < 0.001), and (**f)** Low marsh population under the 25-20 ºC regime (r^2 = 0.020, p < 0.001).

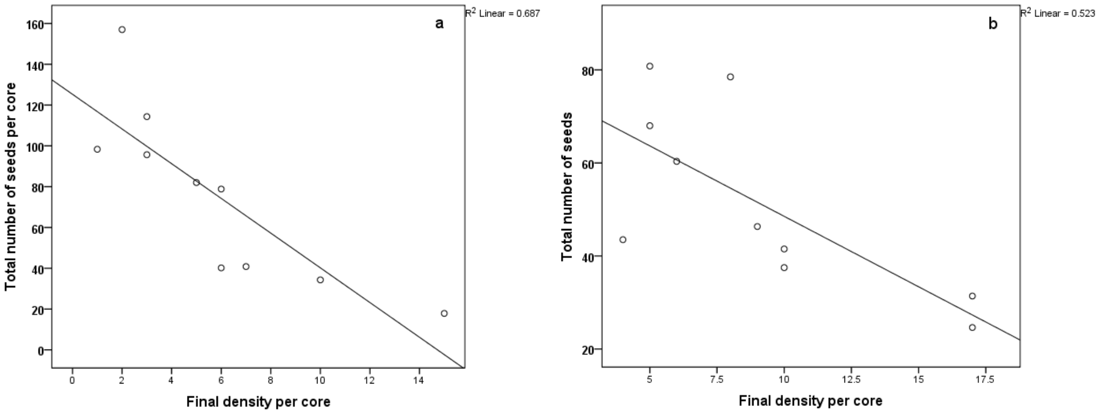


Figure 6.14: Relationship between final density and fecundity per plant per core (total number of seeds). (**a)** Low marsh population under the 20-15 ºC regime (r^2 = 0.687, $p <$ 0.003), and(**b)** Low marsh population under the 25-20 ºC regime (r^2 = 0.523, $p <$ 0.018).

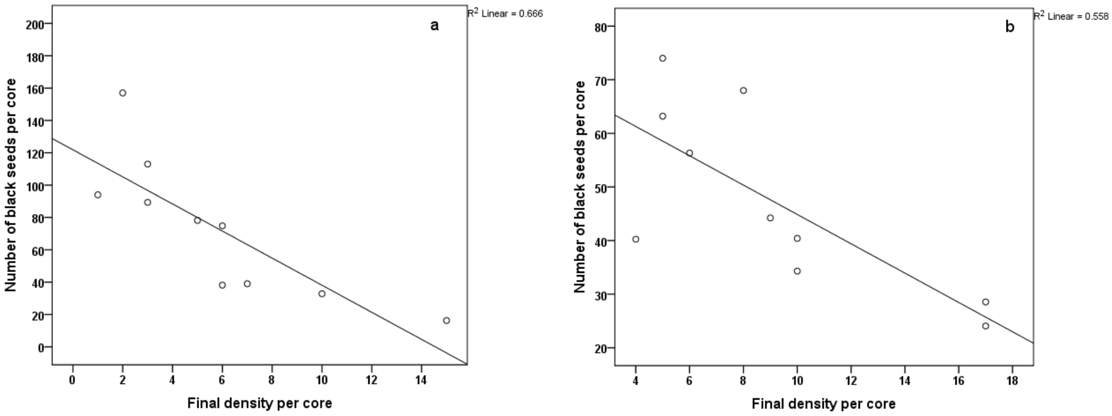


Figure 6.15: Relationship between final density and number of black seeds per plant per core. (**a)** Low marsh population under the 20-15 ºC regime (r^2 = 0.666, $p <$ 0.004), and (**b)** Low marsh population under the 25-20 ºC regime (r^2 = 0.558, $p <$ 0.013).

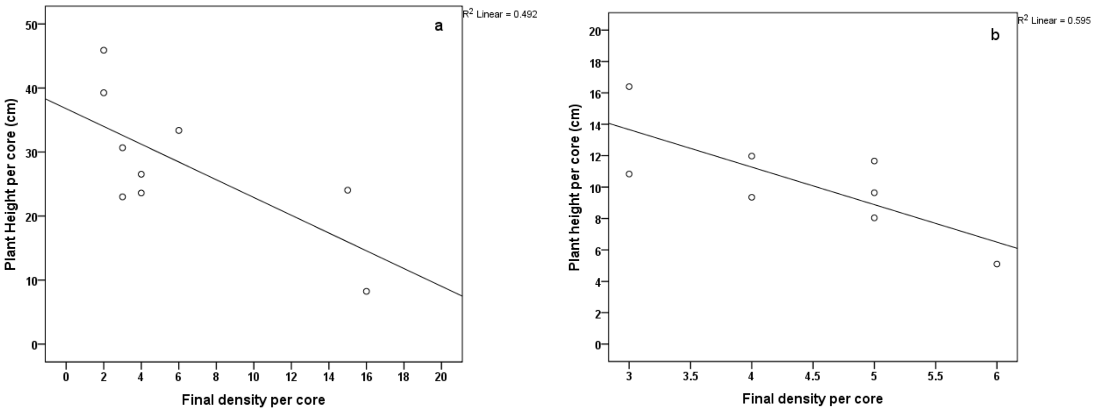


Figure 6.16: Relationship between final density and plant height per plant per core. (**a)** Creek bank population under the 20-15 ºC regime (r^2 = 0.492, $p <$ 0.035), and (**b)** High marsh population under the 20-15 ºC regime (r^2 = 0.595, $p <$ 0.025).

6.5 Discussion

The field surveys in Chapters 3 and 4, and the field transplantation findings in Chapter 5 have suggested that the four *Suaeda maritima* populations identified at Stiffkey exhibit significant phenotypic variations among them in terms of growth and fecundity. In order to further confirm these findings, those populations were grown under controlled conditions in growth chambers under two different regimes of temperature; this has been the subject of investigation in the current chapter. Under the first temperature regime (20-15 ºC), the populations clearly maintained their distinctive phenotypic variations in terms of growth and fecundity, which confirms the presence of genetic differentiation among populations. However, under the second regime (25-20 ºC), the plants were subjected to highly stressful conditions in terms of temperature and salinity, which apparently led to deleterious plastic effects in terms of growth and fecundity to the extent that phenotypic variations were difficult to identify.

The survival findings were significant between populations under both temperature regimes. In first place came the low marsh population, which exhibited unique equivalent-to-survival behaviour under both regimes, as it achieved higher numbers of individuals at the end of the experiment than at the beginning. In second place came the creek bank and the top of the low marsh populations, which lost some individuals but achieved higher and similar survival percentages to the high marsh population (under both regimes). The survival percentages for the creek bank, the top of the low marsh and the low marsh populations declined more severely under the second regime (25-20 ºC), particularly in the high marsh population when it lost half of its individuals by the end of the experiment. Those three populations in general lost more individuals during the seedling stage, particularly under the 25-20 ºC regime, than the population.

For the low marsh population to have higher final percentages than the initial ones is explained by the new unrecorded seedlings that emerged after the experiment had been started. Unfortunately, the current work does not distinguish between original and new seedlings, as it was an unexpected event, and therefore, it was difficult to assess the survival of the initial number of seedlings alone. However, such a pattern exhibited by the low marsh should reflect its ability to maintain a larger persistent seed bank than the other populations, and this phenomenon was observed by (Gul 1998), who concluded that the size of seed bank increased from the upper to the lower parts of a salt marsh. In addition, under the second regime, the low marsh population exhibited a higher equivalent-to-survival percentage than in the first one. This behaviour could be due to the seed size of this population. The low marsh population proved, in Chapters 4 and 5, to produce persistently the smallest sized seeds among the populations, and therefore those seeds are expected to be more dormant and persistent, i.e. as a seed bank (Wang et al. 2008). Therefore, under the second regime (25-20 ºC), the remaining dormant seeds are subjected to higher degrees of temperature, which caused them to initiate germination.

The unique pattern of equivalent-to-survival of the low marsh population in particular under both regimes could be attributed to the inherited ability of this population to tolerate the prevalent waterlogged conditions in the medium during the current experiment. In addition, sensitivity to such waterlogged conditions could be the determining factor for the other three populations in achieving lower survival rates, as it is well observed that the frequency of tidal inundation and therefore waterlogging is greatest in the low marsh zone (Mendelssohn et al. 1981, Pennings and Bertness 2001). In addition, in agreement with the findings of Chapters 4 and 5, the low marsh population in the current study tended to have a shorter lifespan than the other populations. This phenomenon was attributed in those chapters to the fact that there is a genetic component inherited by this population, because it behaves as an early short-lived successor population that has small, readily dispersed seeds, which are suited to unstable primary colonization sites (Greipsson and Davy 1995).

The current study showed two distinctive patterns under both temperature regimes regarding the growth traits (plant height, shoot width, number of primary branches and main root length) and the fecundity (total number of seeds, number of black seeds and black seed mass) traits of the four populations. The first pattern is the phenotypic variation regarding the growth and fecundity characteristics of the populations, which were observed in the field surveys in Chapters 3 and 4; these were retained to a great extent under the 20-15 ºC regime, revealing an element of genetic differentiation at the inter-population level. For instance, the creek bank proved to be the tallest, its main root was the longest, and its black seeds were the biggest in terms of seed mass. Also, the low marsh proved to be the biggest producer of seeds and primary branches. Furthermore, both the top of the low marsh and the high marsh populations proved to produce fewer seeds and primary branches, but were in the middle between the creek bank and the low marsh populations in terms of black seed mass and main root length.

The second striking pattern was observed under the 25-20 ºC regime, when all four populations showed insignificant differences regarding their growth and fecundity traits. The higher temperatures in this regime would have caused higher rates of evapotranspiration, causing salinity to increase, and therefore these populations were expected to be unable to adjust their osmotic potentiality and ion toxicity as well and maintain favourable water realtions in such a harsh unpredictable environment (Khan et al. 2000b). In addition, such harsh conditions could lead to nutrient deficiency, which in turn would be expected to cause deleterious effects on the growth and fecundity parameters (Troyo-Diéguez et al. 1994). A study conducted by (Khan et al. 2000a) under controlled conditions on the halophyte *Atriplex grifithii*, in order to assess its responses to salinity gradients, showed that low levels of salinity do not cause substantial inhibition of growth, as observed here in the first regime; however, low water potentials and low osmotic potentials were correlated to higher salinity concentrations, which led to a substantial reduction in the growth of *Atriplex grifithii*, and this is expected to be the case for the *Suaeda maritima* populations under the second temperature regime (25-20 ºC).

Findings reported in a study conducted on the Edge and Tall populations of *Salicornia europea* from the Ohio Salt Pan, when they were transplanted under common garden conditions, showed that those populations demonstrated some degree of plasticity in their growth characteristics, including height and dry mass (Ungar 1987b). In addition, a recent transplantation experiment in simulated tidal-flow glasshouse tanks was conducted on *Suaeda maritima* populations collected from the low and high parts of the salt marsh on the River Adur estuary (Sussex, UK). It was found that flooding reduced plant height, root length, shoot fresh weight, shoot dry weight and root dry weight; however, those morphological differences appeared to be insignificant between populations, indicating phenotypic plasticity in terms of plant height and mass (Wetson et al. 2012). Therefore, such scenarios of flooding, waterlogging and hyper-saline conditions, as existed in the 25-20 ºC regime, arguably caused the *Suaeda maritima* populations in the current study to exhibit immediate plastic responses in terms of growth and fecundity.

Phenotypic response to controlled environments has been investigated and compared in many studies, and some of these have revealed genetic differentiation among natural populations for a group of adaptive traits, such as salt tolerance, nutrient availability, water availability and light availability (Baythavong and Stanton 2010b). Therefore, the current findings in the 20-15 ºC regime, which confirm the inherited genetic element among *Suaeda maritima* populations, should broaden our understanding of another adaptive trait, which is temperature.

Investigating the relationship between plant density and growth as well as fecundity traits revealed negative associations in many cases, which is in agreement with the findings of Chapter 5. The negative density-dependent effect on the growth and fecundity traits was expected, as those traits are known to be highly plastic in responding to adverse conditions in micro-environments (Watkinson and Davy 1985). Similar supportive studies on density were addressed in the previous chapter.

In conclusion, the current study has found that under the low temperatures (and therefore low salinity) of the first regime (20-15 ºC), the *Suaeda maritima* populations were able to maintain significant differences between them, resembling their maternal resources in nature. However, under the much more severe conditions of temperature (and therefore salinity) of the second regime (25-20 ºC), those populations showed insignificant plastic responses among them, indicating a striking ability to survive and complete their lifecycle by producing seeds for future stability purposes. Therefore, despite the evidence for genetic variation in this species under the first temperature regime, the outcomes under the second regime at least support that this species is well adapted to high temperatures.

Chapter 7

Seed germination characteristics in the annual halophyte *Suaeda maritima*

Abstract

- ***Background and Aims:*** *Suaeda maritima* is an annual species of halophyte inhabiting salt marshes and its populations are known to exhibit distinctive phenotypic variations, including the production of heteromorphic seeds that differ in size and colour. This study investigates the strategies exploited by those seeds during the dormancy and the germination stages that allow them to successfully become established in their local heterogeneous microhabitats.
- ***Methods:*** Different germination experiments are conducted in the laboratory, including salinity effects on fresh black and brown seeds, salinity and thermoperiod (15-5, 15-10 and 10-5 °C) effects on black seeds, salinity and photoperiod (light/dark and constant darkness) effects on black seeds, and salinity and storage conditions (long cold storage 2008, long dry room storage 2009 and short dry room storage 2010) effects on black seeds. Cold storage 2008 included black seeds from the Stiffkey populations and two other, different creek bank populations (from the Tollesbury and Abbot's Hall salt marshes). Five germination traits are assessed: final germination percentage, mean time-to-germination, final recovery from salinity percentage, final dormant seed percentage and final non-viable seed percentage.
- ***Key Results:*** All populations germinated best under non-saline treatment and their germination decreased with the increase in salinity gradients. Ungerminated seeds germinated well when transferred to non-saline solutions and their percentage increased with the increase in the original salinity gradients. The secondary dormancy and the non-viable seed percentages progressively increased with the increase in the original salinity gradients. The different populations exhibited distinctive germination behaviour in all experiments. The creek bank population exhibited the highest germination and the lowest recovery, dormancy and non-viabilty rates, followed by both the high marsh and the top of the low marsh, and finally by the low marsh population. Seed heteromorphism, temperature, light and the different storage conditions were shown to affect the germination behaviour of those seeds.

- ***Conclusions:*** The ability of seeds to germinate well in non-saline solutions, decreasing with increasing salinity and recovering when transferred from the original saline to non-saline solutions indicates that their germination is inhibited by osmotic stress rather than ion toxicity. The distinctive germination behaviours among the populations suggest that they possess varying degrees of salt tolerance. The influence of the abiotic and biotic factors tested indicates that the germination and early establishment of those populations are affected by a combination of factors and that salinity is not the only suppressor. However, such complex germination behaviour in the lab should reflect the adaptive ability of those populations in the natural field.

7.1 Introduction

The germination of halophytic seeds has been widely studied but there is still much to learn (Khan 1999); of the 2,400 or so species that have been investigated (Lieth *et al.* 2008), the germination characteristics have been studied in depth for only a few hundred species (Baskin and Baskin 1988, Wetson et al. 2008).

The development and germination of seeds is a central aspect in the reproductive cycle of all higher plants. In the case of halophytes, a reduced level of salinity is desirable for germination to be initiated, and in most temperate saline environments, seed germination usually occurs in spring, when the levels of precipitation are still sufficiently high to dilute soil salinity (in other climates this may refer to the rainy season) (Pujol *et al.* 2000). In salt marsh habitats, the levels of salinity vary greatly, in accordance with the inundation regime, but in the upper reaches of a marsh (where there is exposure to evaporation as well as to dilution by rainfall), this variability can be exacerbated (Wetson *et al.* 2008). Thus, successful establishment for populations of halophytes in such unpredictable environments depends hugely on the ability of their seeds to tolerate salinity (Woodell 1985). Generally, it is well documented that *Suaeda maritima* is an extreme halophyte that thrives in 100-350 mM (Flowers *et al.* 1977) (Yeo and Flowers 1980).

Notwithstanding the fact that a key factor in halophytic seed germination is salinity, temperature, light, plant form, habitat, etc. are also important drivers (Khan and Ungar 1997). Ecologists and geneticists would be greatly interested to know whether there are any patterns or dependencies among these factors, and so this study endeavours to identify any such germination characteristics. In the natural environment, germination is controlled in particular by light (Qu *et al.* 2008) and temperature (Baskin JM 1976) as well as by salinity (Khan *et al.* 2002). The interaction between temperature and salinity and its effect on halophytic seed germination has been investigated (Baskin and Baskin 1988, Gulzar et al. 2001); in some species, higher temperatures promote germination but the reverse has been found in others. It is thus evidently the case that temperature affects the salinity tolerance of seeds or possibly their dormancy processes. It has also been found that seeds germinate best under conditions of alternating temperatures (Al-Hawija *et al.* 2012). The other key factor in halophytic seed germination is light; germination is suppressed by darkness or the deep shade of dense vegetative cover. However, the relative effects of light in combination with salinity vary among species and different responses have been reported but it has generally been reported that the combined effect of darkness and high salinity levels is to greatly reduce the germination of halophytic seeds (Saira Saeed 2011).

In addition to these abiotic considerations, one particular biotic factor could be a key determinant of germination behaviour, that of seed heteromorphism. As found in Chapter 4, *Suaeda maritima* exhibits a considerable degree of seed heteromorphism; the plants in this study showed variations in terms of size and colour, i.e. some weighed more than others and some had brown testa while others had black ones. Differing germination characteristics reflecting this heteromorphism have been reported (Baskin JM 1976, Khan et al. 2001, Redondo-GÃ³mez et al. 2008, Redondo-Gómez et al. 2008, Wang et al. 2012). Such heteromorphism is an adaptation strategy designed to exploit different windows of opportunity as they present themselves in the highly varying and heterogeneous salt marsh environment, and thence to thrive in this harsh and unpredictable environment (Redondo-Gómez et al. 2008). Thus the different morphs respond differently to the prevailing conditions, and it has now been established that large or brown seeds have a higher degree of salt tolerance than small or black ones (Wang *et al.* 2008). The ability of a halophytic species to produce more than one seed morph could be an adaptation strategy by either establishing a dormancy phase in order to extend the period of germination or varying the dispersal distance of seeds from mother plants (Khan *et al.* 2001).

The investigations detailed in Chapter 4 have characterized the seed production of the local populations of the annual salt-tolerant *Suaeda maritima* on Stiffkey salt marsh, in terms of number, size and colour, with at least three distinctive *Suaeda maritima* populations being identified, which could relate to specific local environmental conditions. Accordingly, in the current chapter, we hypothesize that the ability of *Suaeda maritima* populations to colonize and thrive in all the different levels of the salt marsh is to an extent due to their seed heteromorphism and to their varying germination strategies for coping with the changing conditions of this stressful environment. Thus, the objectives of the work described in this chapter are:

1. To characterize the variations in seed germination behaviour of the local populations of *Suaeda maritima* at Stiffkey salt marsh, and two further creek bank populations from Tollesbury and Abbot’s Hall salt marshes.

2. To evaluate the interacting effects of salinity and other abiotic factors, including temperature, light and different storage conditions, on *Suaeda maritima* seed germination.

7.2 Materials and Methods

7.2.1 Seed Collection

The germination of seeds collected from populations at various sites at Stiffkey (and the other marshes), as described in Chapter 4, is investigated in this chapter.

7.2.2 Experiment designs

7.2.2.1 Experiment 1: Salinity effects

For this purpose, seeds from the latest harvest (summer 2010) were used. Eight replicates of 25 black seeds and four replicates of 25 brown seeds for each treatment of each population were subjected to the germination experiment.

The thermoperiod regime used was: 16 hours of light at 15 °C and 8 hours of darkness at 5 °C, which were chosen specifically to mimic the spring mean temperatures on the Stiffkey salt marsh, when those populations start to germinate.

7.2.2.2 Experiment 2: Salinity and temperature effects

To determine the interaction of salinity and temperature on germination, two further thermoperiod regimes were used; the first was 16 hours of light at 10 °C and 8 hours of darkness at 5 °C. The second regime was 16 hours of light at 15 °C and 8 hours of darkness at 10 °C. For each regime, four replicates of 25 black seeds for each salinity treatment of each population were used. The thermoperiod regimes used here (10-5 °C and 15-10 °C) were chosen to mimic the beginning and the end means-of-temperatures in spring on the Stiffkey salt marsh, when those populations naturally germinate.

7.2.2.3 Experiment 3: Salinity and light effects

To determine the interaction of salinity and light on germination, four replicates of 25 black seeds for each treatment of each population were used for this experiment. The temperature regime was 15 °C for 16 h and 5 °C for 8 h. Those seeds were placed in an incubator where light was excluded during the whole period of this experiment. Transferring the seeds to the incubator and daily seed counting were performed under green incandescent light bulb (25 W). Beside this photoperiod regime, the data for the black seeds from the thermoperiod regime in Experiment 1 were used to achieve a wider comparison.

7.2.2.4 Experiment 4: Salinity and storage effects

To determine the interaction of salinity and storage on germination, two different storage conditions were tested: cold storage for two years (black seeds from summer 2008) and dark dry storage at normal lab temperature (black seeds from summer 2009). For each experiment, four replicates of 25 black seeds for each treatment of each population were used. The thermoperiod used for this experiment was: 16 h of light at 15 °C and 8 h of darkness at 5 °C. Beside those regimes, the data for the black seeds from the thermoperiod regime in Experiment 1 were used to achieve a wider comparison.

7.2.3 Experiment methods

7.2.3.1 The germination experiments

All these experiments were initiated in February 2011. The seeds were exposed to solutions containing 0 (distilled water), 100, 200, 300, 400, 500 and 600 (mM) of NaCl. The seeds were placed on two layers of Whatman No. 1 filter paper in a 5 cm Petri dish, to which was added 5 mL of the appropriate solution. The dishes of each experiment were placed in a programmed incubator and subjected to specific alternating diurnal regimes, as described above.

The whole period planned for the germination experiments was 30 days. The dishes were inspected daily and germinated seeds were recorded and removed. The water level was adjusted every few days with appropriate solutions to compensate for evaporation. Seeds were considered to have germinated when the emerging radical was at least 2 mm.

7.2.3.2 The recovery–from-salinity experiment

This experiment was carried out to determine whether high salinities inhibit or damage the seeds. Ungerminated seeds were rinsed with distilled water and transferred to new Petri dishes containing 5 mL of distilled water, and then returned to the incubator under the common diurnally alternating light conditions (16 h of light at 15 °C and 8 h of darkness at 5 °C) for a further 30 days. At two-day intervals, germinated seeds were recorded and removed.

7.2.3.3 The viability experiment

Seeds that had not germinated in those experiments were subjected to a tetrazolium test in order to assess the viability of their embryos. All seeds for tetrazolium testing were soaked in distilled water at 25 °C for 16 hours. Then, they were incubated in a 1% aqueous solution of 2, 3, 5-triphenyl-tetrazolium chloride (TTC) (Redondo-Gómez et al. 2008), at pH 7 in the dark, and subsequently the seeds were dissected, and the number of embryos that either were stained pink or not in the TTC was recorded.

7.2.3.4 Statistical analysis

Five germination characteristics were determined: final germination percentage, mean time-to-germination (MTG), percentage of final recovery from salinity, percentage of dormant seeds and percentage of non-viable seeds.

MTG was calculated using the equation: $\mathrm{MTG} = \sum_i(n_i \ \mathrm{x}\ d^i) / N$

where n is the number of seeds germinated on day$_i$, d is the incubation period in days, and N is the total number of seeds germinated in the treatment (Redondo-Gómez et al. 2007b).

All data are expressed as mean ±se and the data were analysed using PASW Statistics version 18, Chicago, USA for Windows. Percentages were arcsin transformed before statistical analysis to ensure homogeneity of variance. General linear model (GLM) analysis of co-variance (ANCOVA; $P < 0.05$) was used to compare the treatment effects. One-way or two-way ANCOVA were used when appropriate (using salinity gradients as a covariate) to test the significance of the main effects and their interaction on the germination, recovery, dormancy and non-viability of the seeds. The Bonferroni adjusted Post-hoc test was applied to assess the differences among treatments.

7.3 Results

7.3.1 Experiment 1

7.3.1.1 Effects of salinity on germination

Table 7.1 shows that all populations germinated best under non-saline treatment, and that the final germination percentage of black seeds generally declined in all populations with increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the black seeds' final germination percentage for all populations (Table 7.2), and the Post-hoc test shows the creek bank black seeds having significantly the highest germination percentage, followed by the high marsh and the top of the low marsh seeds, and finally by the low marsh seeds (Table 7.1).

Rate of germination, expressed here as mean time-to-germination (MTG), increased with salinity in all populations (Table 7.3). Also, significant differences occurred between populations in terms of MTG and the Post-hoc test shows the mean MTG to be significantly shorter in both the creek bank and the high marsh black seeds, than in both the top of the low marsh and the low marsh ones (Table 7.3).

Table 7.1 Final germination percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ± SE (n = 8). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	92±0.7	82.5±1.38	84.5±0.48	63±1.08	53.5±2.14	46.5±1.29	45.5±1.85
High marsh ^{b}	56.6±0.9	50±0.38	32±1.18	25±0.79	23.5±0.99	15±0.96	9.5±0.59
Top low marsh ^{b}	75.4±1.62	53.5±1.02	44±1.35	35.5±1.23	26.5±1.15	9±0.56	10±0.65
Low marsh ^{c}	50.9±1.32	39.5±0.81	28±1.61	18.5±1.49	8.5±0.64	1±0.164	15.5±0.95

Table 7.2 Results of the one-way ANCOVA for the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	1.01	3	53.81	<0.001
Error	0.14	23	-	-

Table 7.3 Mean time-to-germination (MTG) (d) of the *Suaeda maritima* populations under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	8.2±0.5	8.5±0.2	9.8±0.5	11.3±0.8	11.4±0.6	11.7±0.5	11.9±0.7
High marsh ^{b}	13.5±0.6	12.6±0.5	11.3±0.8	12.9±0.5	13.5±0.5	11.5±1.5	12±2.2
Top of low marsh ^{a}	14.3±0.7	15.1±0.8	13.2±0.6	14.9±0.7	17±1.3	17.4±2.7	14.4±1.02
Low marsh ^{a}	14.9±0.9	14±0.4	15.2±2.4	12.9±0.8	14.6±2	22±7	17.2±1.7

Table 7.4 shows that the final germination percentage of the brown seeds fluctuated with the increases in salinity in all populations.

Significant differences occurred between populations in terms of the brown seeds' final germination percentage (Table 7.5), and the Post-hoc test reveals that the low marsh brown seeds achieved a lower final germination percentage than the other three populations (Table 7.4).

Table 7.4 Final germination percentage of brown seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size, and values are means ± SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, *p* < 0.05).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	18±6	26±2	36±16	38±2	14±2	16±0	2±2
High marsh ^{a}	36±16	28±12	26±14	16±0	4±4	0	0
Top of low marsh ^{a}	36±16	28±12	26±14	16±0	4±4	0	0
Low marsh ^{b}	1±1	4±2.8	0	1±1	0	0	0

Table 7.5 Results of the one-way ANCOVA of the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	1.11	3	19.7	<0.001
Error	0.43	23	-	-

Finally, two-way ANCOVA was used here to compare the final germination percentages of both morphs among the different populations. It reveals that the final germination percentage differed significantly between both seeds morphs at all levels; salinity, seed colour, population and the interaction between seed colour and population (Table 7.6). The Post-hoc test reveals that the black seeds achieved higher significant means of final germination percentage in all salinity treatments than the brown ones.

Table 7.6 Results of the two-way ANCOVA of the germination percentage of black-brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Seed colour	1.22	1	90.88	<0.001
Population	1.64	3	40.82	<0.001
Seed colour*population	0.48	3	11.86	<0.001
Error	0.63	47	-	-

7.3.1.2 Recovery from salinity

The transfer of ungerminated black seeds from the salinity treatments to distilled water resulted in considerable further germination, and it increased significantly by the late original salinity treatments (Table 7.7). Also, the one-way ANCOVA reveals significant differences between populations in terms of the ability of their black seeds to recover from the salinity treatments (Table 7.8). The Post-hoc test reveals the black seeds of the low marsh population achieving significantly the highest final percentage of recovered seeds from salinity, followed by both the high marsh and the top of the low marsh, and finally by the creek bank ones (Table 7.7).

Table 7.7 Recovery percentages from salinity of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{c}	0	2.5±0.26	4±0.5	12±0.8	27±1.33	33±1.37	36.5±1.71
High marsh ^{b}	11.5±0.72	15±0.53	22.5±0.94	25±0.59	29±0.82	34±1.66	41.5±1.29
Top low marsh ^{b}	9.5±0.65	16.5±0.44	24±0.33	34.5±1.35	40±0.91	50±0.98	56.5±1.04
Low marsh ^{a}	16±0.82	23.5±1.22	39±1.01	46.5±1.28	36±1.89	47±1.64	37.5±1.53

Table 7.8 Results of the one-way ANCOVA of the recovery percentage from salinity of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of Variation	**Type lll SS**	**df**	**F**	**P**
Population	0.31	3	16.2	<0.001
Error	0.15	23	-	-

Table 7.9 shows that the final percentages of recovery from salinity of the brown seeds generally increased with the level of the original increases in the saline treatments. The one-way ANCOVA reveals significant differences between populations in terms of the ability of their brown seeds to recover from the salinity treatments (Table 7.10). The Post-hoc test reveals that the low marsh brown seeds achieved significantly a lower percentage of recovery from salinity than the other three populations (Table 7.9).

Table 7.9 Recovery percentages from salinity of brown seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	0	2±2	2±2	10±2	16±4	22±6	42±14
High marsh ^{a}	2±2	6±2	4±4	10±6	8±0	24±8	10±2
Top of low marsh ^{a}	2±2	6±2	4±4	10±6	8±0	24±8	10±2
Low marsh ^{b}	0	0	0	0	0	0	0

Table 7.10 Results of the one-way ANCOVA of the recovery percentage from salinity of brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (Creek bank, High marsh, Top of the low marsh and Low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.31	3	16.2	<0.001
Error	0.15	23	-	-

Finally, the two-way ANCOVA was used here to compare the recovery from salinity percentage of both seed morphs among the populations. It reveals significant differences between the two seed morphs in terms of final percentage of recovery from salinity at the levels of salinity, seed colour and the interaction between seed colour and population; however, this percentage was insignificant at the population level (Table 7.11). The Post-hoc test shows the black seeds having significantly higher recovery percentages from salinity than the brown ones.

Table 7.11 Results of the two-way ANCOVA of the recovery percentage from salinity of black-brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Seed Colour	1.52	1	158.31	<0.001
Population	0.08	3	2.7	0.055
Seed colour*population	0.66	3	22.94	<0.001
Error	0.45	47	-	-

7.3.1.3 Dormancy

The percentage of black dormant seeds increased with the increases in saline treatments in all populations (7.12). The variation of the percentage of black dormant seeds between populations was significant (7.13), and the low marsh population showed a significantly higher percentage of black dormant seeds than the other three populations (7.12).

Table 7.12 Dormancy percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	5±0.41	11.5±1.26	9±0.53	18.5±0.86	16±1.38	18.5±1.49	15.5±0.95
High marsh ^{b}	16.5±1.2	20±0.78	33±1.19	44±0.98	36±1.08	43.5±1.33	43.5±1.19
Top low marsh ^{b}	14.5±1.32	27.5±1.01	29.5±1.15	29±088	26±0.57	38.5±1.19	29±1.15
Low marsh ^{a}	30.5±1.32	34±1.74	27.5±1.71	29±1.8	53±2.09	45±1.63	40±1.13

Table 7.13 Results of the one-way ANCOVA of the dormancy percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.33	3	24.2	<0.001
Error	0.11	23	-	-

The percentage of brown dormant seeds fluctuated with the increases in the salinity treatments (Table 7.14). Also, significant variation occurred between populations in terms of the percentage of brown dormant seeds (Table 7.15); the creek bank population showed the highest percentage of brown dormant seeds, followed by both the high marsh and the top of the low marsh, and finally by the low marsh population (Table 7.14).

Table 7.14 Dormancy percentages of brown seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	62±14	48±4	34±10	26±2	28±0	48±0	34±6
High marsh ^{b}	22±2	22±10	34±6	22±6	20±4	12±0	16±4
Top of low marsh ^{b}	22±2	22±10	34±6	22±6	20±4	12±0	16±4
Low marsh ^{c}	5±1	2±1.1	7±4.1	4±1.6	4±1.6	5±1.9	3±3

Table 7.15 Results of the one-way ANCOVA of the dormancy percentage of brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
NaCl Treatment	0.05	1	6.98	0.015
Population	0.80	3	39.97	<0.001
Error	0.15	23	-	-

The two-way ANCOVA was used here to compare the dormancy percentage of both seed morphs among populations. It reveals significant differences between the two seed morphs in terms of the percentage of dormant seeds at the levels of seed colour, population and the interaction between the seed colour and the population (Table 7.16), while the population level was insignificant. The Post-hoc test shows the black seeds having a significantly higher percentage of dormant seeds than the brown ones.

Table 7.16 Results of the two-way ANCOVA of the dormancy percentage of black-brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Seed morph	1.52	1	158.31	<0.001
Population	0.08	3	2.7	0.055
Seed morph*Population	0.66	3	22.94	<0.001
Error	0.45	47	-	-

7.3.1.4 Viability

The percentage of non-viable black seeds fluctuated with the increases in salinity (Table 7.17). This percentage differed significantly between populations (7.18); the low marsh population achieved a higher percentage than the other three populations, which do not differ significantly from each other (7.17).

Table 7.17 Non-viable seed percentage of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	4±0.5	3.5±0.39	2.5±0.26	6.5±0.46	3.5±0.23	2±0.27	2.5±0.26
High marsh ^{b}	14.5±0.9	15±0.79	12.5±1.01	6±0.5	11.5±0.72	5±0.59	5.5±0.26
Top low marsh ^{b}	4.5±0.39	2.5±0.32	2.5±0.32	1±0.16	7.5±0.52	3.5±0.44	4.5±0.35
Low marsh ^{a}	4±0.27	3±0.31	5.5±0.42	6±0.63	2.5±0.49	7.5±0.52	7.5±0.85

Table 7.18 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.08	3	8.15	<0.001
Error	0.07	23	-	-

Table 7.19 shows that the percentage of non-viable brown seeds generally increased with the increases in salinity. The variation between populations in this percentage was found to be significant (Table 7.20). The Post-hoc test shows that the highest significant percentage of non-viable brown seeds was achieved by the low marsh population, followed by both the high marsh and the top of the low marsh populations, and finally by the creek bank population (Table 7.19).

Table 7.19 Non-viable seed percentage of brown seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{c}	20±8	24±4	28±4	26±6	42±6	14±6	22±10
High marsh ^{b}	40±16	44±4	36±4	52±0	68±8	64±8	74±6
Top of low marsh ^{b}	40±16	44±4	36±4	52±0	68±8	64±8	74±6
Low marsh ^{a}	94±1.2	94±2	93±4.1	95±1.9	96±1.6	95±1.9	97±3

Table 7.20 Results of the one-way ANCOVA for the non-viable seed percentage of brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
NaCl Treatment	0.13	1	14.65	<0.001
Population	2.45	3	89.81	<0.001
Error	0.21	23	-	-

The percentage of non-viable seeds of both morphs differed significantly at all levels: salinity, seed colour, population and the interaction between seed colour and population (Table 7.21). The Post-hoc test reveals that the brown seeds achieved a significantly higher percentage of non-viable seeds than the black ones.

Table 7.21 Results of the two-way ANCOVA of the non-viable seed percentage of black-brown seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Seed colour	5.95	1	763.27	<0.001
Population	1.34	3	57.46	<0.001
Seed colour*population	1.18	3	50.57	<0.001
Error	0.37	47	-	-

7.3.2 Experiment 2

7.3.2.1 Effects of salinity on germination

Table 7.22 shows that all populations germinated best under the non-saline treatment, and that the final germination percentage of the thermoperiod 10-5 °C generally declined in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages among all populations under the thermoperiod 10-5 °C (Table 7.23). The Post-hoc test shows the creek bank seeds having significantly the highest germination percentage, followed by both the high marsh and the top of the low marsh seeds, and finally by the low marsh seeds (Table 7.22).

Rate of germination, expressed here as mean time-to-germination (MTG), increased with salinity in all populations (Table 7.24). Also, significant differences occurred between populations in terms of MTG for the thermoperiod 10-5 °C, and the Post-hoc test shows the mean MTG to be significantly shorter in the creek bank, than in both the high and the top of the low marsh, and finally in the low marsh (Table 7.24).

Table 7.22 Final germination percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n*= 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	88±1.6	84±1.6	85±1.9	69±5.3	64±2.8	57±7.5	52±2.8
High marsh ^{b}	71±5.3	70±7.6	55±3	51±3.4	49±4.4	45±1	17±5.3
Top of low marsh ^{b}	78±3.8	68±2.3	58±3.4	60±3.7	47±5	29±6.8	19±3.8
Low marsh ^{c}	45±4.7	41±5.6	24±2.4	21±2.5	6±1.2	22±6	13±5.3

Table 7.23 Results of the one-way ANCOVA for the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.93	3	50.28	<0.001
Error	0.14	23	-	-

Table 7.24 Mean time-to-germination (MTG) (d) of the *Suaeda maritima* populations under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank^{c}	7.5±0.5	8.6±0.3	8.9±0.7	10.9±0.5	11.9±0.4	13.3±1	14.1±0.4
High marsh^{b}	9.8±0.8	11±0.9	11.8±0.4	11.6±0.3	14.6±1	15.4±0.8	13.2±1.2
Top of low marsh^{b}	8.7±0.1	10.3±0.7	12.1±0.5	12.6±0.4	13.9±0.7	15.5±1.4	15.1±2.6
Low marsh^{a}	12.8±1.1	15.7±0.7	16.2±0.8	17.7±2.1	18.4±2.5	16.7±1.7	17.4±1.9

Table 7.25 shows that all populations germinated best under the non-saline treatment, and that the final germination percentage under the thermoperiod 15-10 °C generally declined in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages among all populations under the thermoperiod 15-10 °C (Table 7.26). The Post-hoc test shows the creek bank seeds having significantly the highest germination percentage, followed by both the high marsh and the top of the low marsh seeds, and finally by the low marsh seeds (Table 7.25).

Rate of germination, expressed here as mean time-to-germination (MTG), increased with salinity in all populations under the thermoperiod 15-10 °C (Table 7.27). Also, significant differences occurred between populations in terms of MTG; the Post-hoc test shows the mean MTG to be significantly shorter in the creek bank, in the high and the in the top of the low marshes, than in the low marsh (Table 7.27).

Table 7.25 Final germination percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank^{a}	84±2.8	80±4.3	67±4.1	67±5.5	72±3.6	64±9.7	60±7.5
High marsh^{b}	82±7.4	82±2	60±4.3	63±3.4	46±4.8	39±4.1	20±6.9
Top of low marsh^{b}	69±6.6	69±4.1	59±4.4	56±5.9	53±3	34±3.8	17±3.8
Low marsh^{c}	42±4.2	48±1.6	36±5.4	29±7.7	10±3.8	2±1.2	2±1.2

Table 7.26 Results of the one-way ANCOVA for the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	1.05	3	39.09	<0.001
Error	0.21	23	-	-

Table 7.27 Mean time-to-germination (MTG) (d) of the *Suaeda maritima* populations under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank^{b}	4.9±0.3	5.3±0.3	6.3±0.4	5.2±0.2	6.3±0.2	7.3±0.4	7.1±0.1
High marsh^{b}	5.5±0.3	6.3±0.3	6.1±0.5	6.4±0.4	7±0.6	9.4±0.9	8.1±2.9
Top of low marsh^{b}	5.1±0.5	5.4±0.3	7.4±0.6	6.5±0.3	7.5±0.7	7.9±0.2	8.2±1.8
Low marsh^{a}	8.2±0.5	11.3±1.5	9.7±1	10.6±2.2	12.4±1.1	5	9.8±6.8

The two-way ANCOVA model reveals the effect of salinity and thermoperiod (15-5 °C, 10-5 °C and 15-10 °C) to be significant on the black seed final germination percentage at all levels: salinity, thermoperiod, population, and the interaction between thermoperiod and population (Table 7.28). The Post-hoc test shows the black seed final germination percentage was significantly higher in both 10-5 °C and 15-10 °C than in 15-5 °C.

Table 7.28 Results of the two-way ANCOVA for the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under three thermoperiods (15-5 °C, 10-5 °C and 15-10 °C).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Thermoperiod	0.27	2	18.93	<0.001
Population	2.81	3	129.89	<0.001
Thermoperiod* population	0.19	6	4.38	<0.001
Error	0.51	71	-	-

7.3.2.2 Recovery from salinity

Table 7.29 shows that in all populations the recovery percentage from salinity under the thermoperiod 10-5°C increased with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the recovery percentages from salinity among the populations under the thermoperiod 10-5 °C (Table 7.30). The Post-hoc test shows the low marsh seeds having a significantly higher recovery percentage from salinity than the other three populations (Table 7.29).

Table 7.29 Recovery percentages from salinity of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	2±1.2	5±1	5±1.9	14±4.8	23±3	33±5.3	31±3
High marsh ^{b}	3±1	8±2.8	12±2.8	19±4.1	23±4.1	31±4.1	54±1.2
Top of low marsh ^{b}	8±1.6	11±1	15±1.9	12±3.7	19±4.7	33±5.7	28±3.7
Low marsh ^{a}	13±1.9	14±2.6	30±8.4	24±2.8	38±2.6	29±4.4	33±4.1

Table 7.30 Results of the one-way ANCOVA for the recovery percentage from salinity of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.08	3	4.7	0.011
Error	0.13	23	-	-

Table 7.31 shows that in all populations the recovery percentage from salinity under the thermoperiod 15-10°C increased with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the recovery percentages from salinity among the populations under the thermoperiod 15-10 °C (Table 7.32). The Post-hoc test shows the low marsh seeds having a significantly higher recovery percentage from salinity than the other three populations (Table 7.31).

Table 7.31 Recovery percentages from salinity of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	1±1	0	4±0	5±1.9	7±1	10±2.6	18±4.2
High marsh ^{b}	0	2±2	4±2.8	6±2.6	17±5	21±3	32±2.8
Top of low marsh ^{b}	2±1.2	1±1	7±3.4	14±4.8	7±3.4	24±6.3	30±5.3
Low marsh ^{a}	4±2.3	5±1.9	10±3.8	12±2.8	24±5.1	32±5.7	25±3

Table 7.32 Results of the one-way ANCOVA for the recovery percentage from salinity of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.10	3	8.88	<0.001
Error	0.09	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effect of both salinity and thermoperiod (15-5 °C, 15-10 °C and 10-5 °C) on the black seed recovery percentage from salinity. It reveals that the effect of both salinity and thermoperiod was significant on the black seed recovery percentage from salinity at all levels: salinity, thermoperiod, population, and the interaction between thermoperiod and population (Table 7.33). The Post-hoc test shows the black seed recovery percentage from salinity to be significantly the highest in the thermoperiod 15-5 °C, followed by the thermoperiods 10-5 °C and 15-10 °C, respectively.

Table 7.33 Results of the two-way ANCOVA for the recovery percentage from salinity of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under three thermoperiods (15-5 °C, 10-5 °C and 15-10 °C).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Thermoperiod	0.68	2	67.19	<0.001
Population	0.41	3	26.86	<0.001
Thermoperiod* Population	0.07	6	2.43	0.034
Error	0.36	71	-	-

7.3.2.3 Dormancy

Table 7.34 shows that in all populations the dormancy percentage under the thermoperiod 10-5 °C generally increased with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the dormancy percentages among the populations under the thermoperiod 10-5 °C (Table 7.35). The Post-hoc test shows the low marsh seeds having a significantly higher dormancy percentage than the other three populations (Table 7.34).

Table 7.34 Dormancy percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	3±1.9	4±2.3	3±1.9	4±1.6	4±1.6	6±3.5	6±1.2
High marsh ^{b}	5±1	10±2.6	10±1.2	11±3	13±3.4	8±2.8	16±2.3
Top of low marsh ^{b}	2±1.2	4±0	10±3.5	9±4.1	16±1.6	15±2.5	18±2
Low marsh ^{a}	14±2.6	15±2.5	15±4.7	18±4.8	18±2.6	15±1.9	15±1

Table 7.35 Results of the one-way ANCOVA for the dormancy percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.14	3	19.75	<0.001
Error	0.06	23	-	-

Table 7.36 shows that in all populations the dormancy percentage under the thermoperiod 15-10°C generally increased with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the dormancy percentages among the populations under the thermoperiod 15-10 °C (Table 7.37). The Post-hoc test shows the low marsh seeds having a significantly higher dormancy percentage than the other three populations (Table 7.36).

Table 7.36 Dormancy percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	6±1.2	6±2.9	14±1.1	15±5.5	11±3.8	20±5.4	17±6.8
High marsh ^{b}	8±3.7	5±1.9	18±3.4	14±3.5	19±1.9	17±4.4	21±3
Top of low marsh ^{b}	13±3.8	5±1	7±1.9	12±1.6	15±4.4	14±5.8	20±3.7
Low marsh ^{a}	26±3.5	26±4.8	26±5	36±1.6	38±2	42±4.8	42±3.5

Table 7.37 Results of the one-way ANCOVA for the dormancy percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.34	3	46.6	<0.001
Error	0.07	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effect of both salinity and thermoperiod (15-5 °C, 15-10 °C and 10-5 °C) on the black seed dormancy percentage. It reveals the effect of both salinity and thermoperiod to be significant on the black seed dormancy percentage at all levels: salinity, thermoperiod, population, and the interaction between thermoperiod and population (Table 7.38). The Post-hoc test shows the black seed dormancy percentage to be significantly the highest in the thermoperiod 15-10 °C, followed by the thermoperiods 10-5 °C and 15-5 °C, respectively.

Table 7.38 Results of the two-way ANCOVA for the dormancy percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under three thermoperiods (15-5 °C, 10-5 °C and 15-10 °C).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Thermoperiod	0.43	2	59.34	<0.001
Population	0.55	3	51.25	<0.001
Thermoperiod* Population	0.15	6	6.84	<0.001
Error	0.26	71	-	-

7.3.2.4 Viability

Table 7.39 shows that in all populations the non-viable seed percentage under the thermoperiod 10-5°C fluctuated with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the non-viable seed percentages among the populations under the thermoperiod 10-5 °C (Table 7.40). The Post-hoc test shows the low marsh seeds having significantly the highest non-viable seeds percentage, followed by both the high marsh and the top of the low marsh, and finally by the creek bank population (Table 7.39).

Table 7.39 Non-viable seed percentage of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, p < 0.05).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{c}	7±1.9	7±1.9	7±1.9	13±2.5	9±2.5	4±1.6	11±4.1
High marsh ^{b}	21±4.4	12±5.2	23±4.7	20±4.9	15±5	17±1.9	13±3.4
Top of low marsh ^{b}	12±3.3	17±1.9	17±5.3	19±5.7	18±1.2	23±1.9	35±3
Low marsh ^{a}	28±2.8	30±5.8	31±7.7	37±6.6	38±2	34±3.8	39±4.4

Table 7.40 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.39	3	38.03	<0.001
Error	0.08	23	-	-

Table 7.41 shows that the non-viable seed percentage under the thermoperiod 15-10°C fluctuated with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between non-viable seed percentages among the populations under the thermoperiod 15-10 °C (Table 7.42). The Post-hoc test shows the low marsh seeds having significantly the highest non-viable seed percentage, followed by both the high marsh and the top of the low marsh, and finally by the creek bank population (Table 7.41).

Table 7.41 Non-viable seed percentage of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{c}	9±2.5	14±3.5	15±3.4	13±1.9	10±1.2	6±3.8	5±3.8
High marsh ^{b}	10±3.8	11±3.4	18±3.5	17±4.7	18±6.2	23±3.8	27±3.8
Top of low marsh ^{b}	16±7.1	25±5.3	27±5.7	18±2.6	25±4.7	14±5.8	33±5
Low marsh ^{a}	28±4	21±1.9	28±7.5	23±5	28±3.3	24±2.3	31±3.4

Table 7.42 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 10 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.18	3	12.76	<0.001
Error	0.11	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effect of both salinity and thermoperiod (15-5 °C, 15-10 °C and 10-5 °C) on the non-viable black seed percentage. It reveals the effect of both salinity and thermoperiod to be significant on the non-viable black seed percentage at all levels: salinity, thermoperiod, population, and the interaction between thermoperiod and population (Table 7.43). The Post-hoc test shows that there were insignificant differences among the different thermoperiods (15-5 °C, 10-5 °C and 15-10 °C) in terms of the non-viable black seed percentage.

Table 7.43 Results of the two-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under three thermoperiods (15-5 °C, 10-5 °C and 15-10 °C).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Thermoperiod	0.01	2	1.004	0.371
Population	0.97	3	82.89	<0.001
Thermoperiod* Population	0.05	6	2.04	<0.001
Error	0.28	71	-	-

7.3.3 Experiment 3

7.3.3.1 Effects of salinity on germination

Table 7.44 shows that all populations germinated best under the non-saline treatment, and that the final germination percentages under the constant darkness regime generally declined in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages of all populations under the constant darkness regime (Table 7.45). The Post-hoc test shows the creek bank seeds having significantly the highest germination percentage, followed by both the high marsh and the top of the low marsh seeds, and finally by the low marsh seeds (Table 7.44).

Rate of germination, expressed here as mean time-to-germination (MTG), increased with salinity in all populations under the constant darkness regime (Table 7.46). Also, significant differences occurred between the populations in terms of MTG; the Post-hoc test shows the mean MTG to be significantly the longer in the low marsh than in the other three populations (Table 7.46).

Table 7.44 Final germination percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	92±2.8	94±2.6	93±3	92±3.7	80±5.4	73±6.6	64±4.3
High marsh ^{b}	79±2.5	74±3.5	79±4.1	74±3.5	67±4.4	46±7.7	35±2.5
Top of low marsh ^{b}	83±3	84±2.8	69±5.7	61±6.6	62±4.2	45±4.1	38±1.2
Low marsh ^{c}	57±2.5	49±4.4	54±1.2	39±3	32±4.3	33±3	13±5.7

Table 7.45 Results of the one-way ANCOVA for the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.89	3	63.61	<0.001
Error	0.11	23	-	-

Table 7.46 Mean time-to-germination (MTG) (d) of the *Suaeda maritima* populations under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank^{b}	6.2±0.6	6±0.2	6.4±0.4	8.2±0.5	9.8±0.5	9.3±0.1	10.1±0.6
High marsh^{b}	6.4±0.2	6.6±0.2	7.2±0.4	7.5±0.4	8.3±0.9	9.7±1.1	11.1±0.7
Top of low marsh^{b}	6.1±0.3	7.6±0.3	8.4±0.5	7.8±0.4	8.4±0.6	10±0.7	11.8±0.9
Low marsh^{a}	10.9±0.3	14.1±0.7	14.4±0.7	13.4±0.9	14.2±0.4	15.9±1.1	17±1

Finally, the two-way ANCOVA model was used here to compare the effects of both photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness) on the final black seed germination percentage. It reveals that the final seed germination percentage was affected significantly by both salinity and photoperiod at all levels: salinity, photoperiod, population, and the interaction between photoperiod and population (Table 7.47). The Post-hoc test shows the constant darkness regime achieving a significantly higher final germination percentage than the light/dark regime.

Table 7.47 Results of the two-way ANCOVA for the final germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under two photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Photoperiod	1.05	1	172.97	<0.001
Population	1.84	3	101.39	<0.001
Photoperiod* Population	0.07	3	3.8	0.016
Error	0.28	47	-	-

7.3.3.2 Recovery from salinity

Table 7.48 shows that the final recovery from salinity percentage under the constant darkness regime increased in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages of all populations under the constant darkness regime (Table 7.49). The Post-hoc test shows the creek bank seeds having a significantly lower recovery from salinity percentage than the other three populations (Table 7.48).

Table 7.48 Recovery from salinity percentage of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	0	0	1±1	3±1.9	7±2.5	14±3.7	27±4.4
High marsh ^{a}	1±1	3±1.9	2±2	7±1	12±2.3	28±4.3	41±2.6
Top of low marsh ^{a}	1±1	1±1	6±3.5	6±2	9±1.9	13±3	15±3
Low marsh ^{a}	1±1	9±4.1	7±3	12±4.3	21±1	25±5.3	42±9.3

Table 7.49 Results of the one-way ANCOVA for the recovery from germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C.

Source of variation	**Type III SS**	**df**	**F**	**P**
Population	0.13	3	10.65	<0.001
Error	0.09	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effects of both photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness) on the final recovery from salinity percentage. It reveals that the final recovery from salinity percentage was affected significantly by both salinity and photoperiod at all levels: salinity, photoperiod, population, and the interaction between photoperiod and population (Table 7.50). The Post-hoc test shows the light/dark regime achieving a significantly higher final recovery from salinity percentage than the constant darkness regime.

Table 7.50 Results of the two-way ANCOVA for the final recovery from salinity percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under two photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Photoperiod	0.75	1	145.17	<0.001
Population	0.36	3	23.4	<0.001
Photoperiod* Population	0.08	3	4.86	0.005
Error	0.24	47	-	-

7.3.3.3 Dormancy

Table 7.51 shows that the dormancy percentage under the constant darkness regime generally increased in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the dormancy percentages among all populations under the constant darkness regime (Table 7.52). The Post-hoc test shows the creek bank seeds having a significantly lower dormancy percentage than the other three populations (Table 7.51).

Table 7.51 Dormancy percentages of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	0	3±1	3±1	2±1.2	2±2	3±1.9	0
High marsh ^{a}	4±1.6	3±1	6±2.6	4±2.3	10±4.8	12±3.7	6±2.6
Top of low marsh ^{a}	4±2.3	5±3.8	11±4.1	13±1	9±1.9	17±3	14±3.8
Low marsh ^{a}	12±2.8	15±1	8±1.6	18±3.4	7±2.5	9±1	10±2.6

Table 7.52 Results of the one-way ANCOVA for the dormancy percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.22	3	14.22	<0.001
Error	0.12	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effects of both photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness) on the dormancy percentage. It reveals that the dormancy percentage was affected significantly by both salinity and photoperiod at all levels: salinity, photoperiod, population, and the interaction between photoperiod and population (Table 7.53). The Post-hoc test shows the light/dark regime achieving a significantly higher dormancy percentage than the constant darkness regime.

Table 7.53 Results of the two-way ANCOVA for the final dormancy percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under two photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Photoperiod	1.18	1	219.3	<0.001
Population	0.51	3	31.5	<0.001
Photoperiod* population	0.04	3	2.39	0.080
Error	0.25	47	-	-

7.3.3.4 Viability

Table 7.54 shows that the non-viable seed percentage under the constant darkness regime generally increased in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the non-viable seed percentage among all populations under the constant darkness regime (Table 7.55). The Post-hoc test shows the creek bank seeds having significantly the lowest non-viable seed percentage, followed by both the high marsh and the top of the low marsh, and finally by the low marsh populations (Table 7.54).

Table 7.54 Non-viable seed percentage of black seeds harvested in summer 2010 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{c}	8±2.8	3±1.9	3±1.9	3±1	11±3.4	10±3.8	9±1.9
High marsh ^{b}	16±0	20±4.9	13±2.5	15±3.4	11±3	14±3.7	19±3.4
Top of low marsh ^{b}	12±4.3	10±1.2	14±2	20±7.1	20±5.9	25±3.8	33±3
Low marsh ^{a}	30±3.8	27±1.9	31±4.4	31±6	40±4.3	33±3.4	35±1.9

Table 7.55 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under constant darkness and the thermoperiod of: 16 h at 15 °C and 8 h at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.44	3	41.56	<0.001
Error	0.08	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effects of both photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness) on the non-viable seed percentage. It reveals that the non-viable seed percentage was affected significantly by both salinity and photoperiod at three levels: photoperiod, population, and the interaction between photoperiod and population (Table 7.56). The Post-hoc test shows the constant darkness regime achieving a significantly higher non-viable seeds percentage than the light/dark regime.

Table 7.56 Results of the two-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under two photoperiods (15 °C light and 5 °C dark, and 15-5 °C constant darkness).

Source of variation	**Type lll SS**	**df**	**F**	**P**
Photoperiod	0.55	1	141.37	<0.001
Population	0.29	3	24.44	<0.001
Photoperiod* population	0.23	3	19.74	<0.001
Error	0.18	47	-	-

7.3.4 Experiment 4

7.3.4.1 Effects of salinity on germination

Table 7.57 shows that all populations from the 2008 cold storage germinated best under the non-saline treatment, and that the final germination percentage generally declined in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages among all populations from the 2008 cold storage (Table 7.58). The Post-hoc test shows both the creek bank and the top of the low marsh seeds having significantly higher germination percentages than the high marsh ones (Table 7.57).

Rate of germination, expressed here as mean time-to-germination (MTG), increased with salinity in all populations from the 2008 cold storage, and insignificant differences occurred between populations in terms of MTG (Table 7.59).

Table 7.57 Final germination percentage of black seeds harvested in summer 2008 from the *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	100	97±3	97±1	93±1.9	93±1	58±20.5	72±6.9
High marsh ^{b}	96±1.6	88±9.3	78±4.7	61±3.4	56±4	20±8.8	11±6.2
Top of low marsh ^{a}	100	100	96±1.6	100	95±1	95±3.8	64±16.9

Table 7.58 Results of the one-way ANCOVA for the germination percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.94	2	24.71	<0.001
Error	0.33	17	-	-

Table 7.59 Mean time-to-germination (MTG) (d) of the *Suaeda maritima* populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl Treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank^{b}	6.3±0.1	7.1±0.5	7.6±0.2	8.4±0.5	9.7±0.2	11.3±0.7	14.6±0.1
High marsh^{b}	5.8±0.3	6.7±1.9	8.3±0.9	12±0.5	13.3±1.1	14±1.1	18.2±0.9
Top of low marsh^{b}	5.9±0.3	6.1±0.2	7.7±0.8	7.3±0.2	8.4±0.4	8.3±0.1	13.8±1.9

Table 7.60 shows that all 2008 creek bank populations germinated best under the non-saline treatment, and that the final germination percentage generally declined in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages among all 2008 creek bank populations. The Post-hoc test shows both the Stiffkey and the Tollesbury seeds having significantly higher germination percentages than the Abbot’s Hall ones (Table 7.60).

Rate of germination, expressed here as mean time-to-germination (MTG), generally increased with salinity in all 2008 creek bank populations. Also, the Post-hoc test shows both the Stiffkey and the Tollesbury seeds having significantly shorter MTG values than the Abbot’s Hall ones (Table 7.62).

Table 7.60 Final germination percentage of black seeds harvested in summer 2008 from three different creek bank populations (Stiffkey, Tollesbury and Abbot’s Hall) of *Suaeda maritima* under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Stiffkey^{a}	100	97±3	97±1	93±1.9	93±1	58±20.5	72±6.9
Tollesbury^{a}	98±2	95±3	99±1	96±2.8	100	99±1	91±3.8
Abbot’s Hall^{b}	94±4	78±3.7	62±11.6	98±2	66±9.3	94±4	60±7.7

Table 7.61 Results of the one-way ANCOVA for the germination percentage of black seeds harvested in summer 2008 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.29	2	4.49	0.027
Error	0.56	17	-	-

Table 7.62 Mean time-to-germination (MTG) (d) of black seeds harvested in summer 2008 from three different creek bank populations (Stiffkey, Tollesbury and Abbot's Hall) of *Suaeda maritima* under the thermoperiod of: 16 h of light at 10 °C and 8h of darkness at 5 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank^b	6.3±0.1	7.1±0.5	7.6±0.2	8.4±0.5	9.7±0.2	11.3±0.7	14.6±0.1
High marsh^b	7.2±0.4	6.5±0.4	5.8±0.2	6.6±0.04	5.9±0.1	7.7±1	7.2±0.1
Top of low marsh^a	13.5±1.7	12.4±1.3	14.1±0.9	7.3±0.2	9.3±1.3	8.2±0.6	15±2.2

Table 7.63 shows that all populations collected in 2009 germinated best under the non-saline treatment, and that the final germination percentage generally declined in all populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the final germination percentages among all populations collected in 2009 (Table 7.64). The Post-hoc test shows the low marsh seeds having a significantly lower germination percentage than the other three populations (Table 7.63).

Rate of germination, expressed here as mean time-to-germination (MTG), generally increased with salinity in all populations collected in 2009. Also, insignificant differences occurred between populations in terms of MTG (Table 7.65).

Table 7.63 Final germination percentages of black seeds harvested in summer 2009 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl Treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	95±1.9	40±4.3	38±6.8	58±22	30±7.7	3±3	3±1.9
High marsh ^{a}	79±3.8	74±3.8	86±4.1	87±4.1	36±7.1	17±3	7±2.5
Top of low marsh ^{a}	94±2.6	89±3.4	85±3.8	5±3	53±1.9	41±4.4	27±5.9
Low marsh ^{b}	21±12.3	36±7.8	17±10.6	13±6.8	1±1	0	0

Table 7.64 Results of the one-way ANCOVA for the final germination percentage of black seeds harvested in summer 2009 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	1.47	3	10.62	<0.001
Error	1.06	23	-	-

Table 7.65 Mean time-to-germination (MTG) of black seeds harvested in summer 2009 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C in six salinity treatments for 30 days. The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	10.6±0.2	18.8±2.1	21.8±0.7	18.2±1.8	14.6±1.6	27	27±1
High marsh ^{a}	16.2±0.9	16.8±0.2	11.6±0.8	10.9±0.2	15.2±1.5	15.8±0.5	19±3
Top of low marsh ^{a}	10.4±0.3	10.3±0.5	10.1±0.4	23.5±1.5	14.7±0.9	17.3±1	17±2
Low marsh ^{a}	15.2±1.1	18±2.1	18.5±0.5	25.3±1.1	29	0	0

Finally, the two-way ANCOVA model was used here to compare the effects of both salinity and the different storage conditions (2008 cold storage, 2009 dark room storage and 2010 fresh seeds) on the final germination percentage of the three populations (creek bank, high marsh and top of the low marsh at the Stiffkey salt marsh) (Table 7.65). It reveals that the final germination percentage was affected significantly by both salinity and storage condition at all levels: salinity, storage, population, and the interaction between storage and population. The Post-hoc test shows the 2008 cold storage achieving a significantly higher final germination percentage than the either 2009 or 2010.

Table 7.65 Results of the two-way ANCOVA for the final germination percentage of black seeds harvested in the summers of 2008, 2009 and 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Storage	2.61	2	47.84	<0.001
Population	0.52	2	9.54	<0.001
Storage*population	1.29	4	11.82	<0.001
Error	1.45	53	-	-

7.3.4.2 Recovery from salinity

Table 7.66 shows that the recovery from salinity percentage generally increased in all 2008 Stiffkey populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the recovery from salinity percentages among all 2008 Stiffkey populations (Table 7.67). The Post-hoc test shows both the creek bank and the high marsh seeds having significantly higher recovery from salinity percentages than the top of the low marsh ones (Table 7.66).

Table 7.66 Recovery from salinity percentage of black seeds harvested in summer 2008 from the *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl Treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	0	1±1	0	1±1	2±1.2	32±17	21±7.5
High marsh ^{a}	0	1±1	2±1.2	5±3	17±3.8	18±6.6	28±6.3
Top of low marsh ^{b}	0	0	1±1	0	0	3± 3	20±7.3

Table 7.67 Results of the one-way ANCOVA for the recovery from salinity percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.09	2	3.77	0.044
Error	0.22	17	-	-

Table 7.68 shows that the recovery from salinity percentage generally increased in all 2008 creek bank populations with the increasing salinity concentrations.

The one-way ANCOVA model reveals insignificant differences between the recovery from salinity percentages among all the 2008 creek bank populations (Table 7.69).

Table 7.68 Recovery from salinity percentage of black seeds harvested in summer 2008 from three different creek bank populations (Stiffkey, Tollesbury and Abbot’s Hall) of *Suaeda maritima* under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Stiffkey ^{a}	0	1±1	0	1±1	2±1.2	32±17	21±7.5
Tollesbury ^{a}	0	1±1	0	0	0	1±1	1±1
Abbot’s Hall ^{a}	0	6±2.4	2±2	0	30±8.4	0	14±8.7

Table 7.69 Results of the one-way ANCOVA for the recovery percentage of black seeds harvested in summer 2008 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.11	2	2.02	0.163
Error	0.47	17	-	-

Table 7.70 shows that recovery from salinity percentage generally increased in all populations harvested in 2009 with the increasing salinity concentrations.

The one-way ANCOVA model reveals insignificant differences between the recovery from salinity percentages among all populations harvested in 2009 (Table 7.71).

Table 7.70 Recovery from salinity percentage of black seeds harvested in summer 2009 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	1±1	8±1.6	26±7.4	5±1.9	15±7.5	58±10.6	63±8.4
High marsh ^{a}	1±1	2±2	2±1.2	2±1.2	34±8.2	31±3.4	41±5
Top of low marsh ^{a}	1±1	2±2	2±1.2	20±2.8	15±4.1	30±4.8	31±7.9
Low marsh ^{b}	4±4	16±4.9	14±5.3	21±9.9	36±6.5	29±14.7	37±3.8

Table 7.71 Results of the one-way ANCOVA for the recovery percentage of black seeds harvested in summer 2009 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.12	3	2.54	0.082
Error	0.36	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effects of both salinity and the different storage conditions (2008 cold storage, 2009 dark room storage and 2010 fresh seeds) on the recovery from salinity percentages of the three populations (creek bank, high marsh and top of the low marsh at the Stiffkey salt marsh) (Table 7.72). It reveals that the recovery from salinity percentage was affected significantly by both salinity and storage conditions at the levels of salinity, storage, and the interaction between storage and population. The Post-hoc test shows the fresh seeds of 2010 achieving significantly the highest recovery from salinity percentage, followed by the 2009 dark room storage, and finally by the 2008 cold storage.

Table 7.72 Results of the two-way ANCOVA for the final germination percentage of black seeds harvested in the summers of 2008, 2009 and 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Storage	0.98	2	42.19	<0.001
Population	0.014	2	0.60	0.552
Storage*population	0.37	4	7.95	<0.001
Error	0.61	53	-	-

7.3.4.3 Dormancy

Table 7.73 shows that the dormancy percentage generally increased in all Stiffkey populations harvested in 2008 with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the dormant seed percentages among all Stiffkey populations harvested in 2008 (Table 7.74), and the Post-hoc test shows both the creek bank and the top of low marsh seeds having significantly lower dormant seed percentages than the high marsh ones (Table 7.73).

Table 7.73 Dormancy percentages of black seeds harvested in summer 2008 from the *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	0	0	1±1	0	2±1.2	4±2.3	5±1.9
High marsh ^{a}	0	3±3	5±1.9	14±7.7	15±2.5	17±3.4	27±11.4
Top of low marsh ^{b}	0	0	1±1	0	1±1	1±1	11±8.5

Table 7.74 Results of the one-way ANCOVA for the dormant seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.21	2	20.12	<0.001
Error	0.09	17	-	-

Table 7.75 shows that the dormancy percentage generally increased in all creek bank populations harvested in 2008 with the increasing salinity concentrations.

The one-way ANCOVA model reveals insignificant differences between the dormancy percentages among all creek bank populations harvested in 2008 (Table 7.76).

Table 7.75 Dormancy percentages of black seeds harvested in summer 2008 from three different creek bank populations (Stiffkey, Tollesbury and Abbot's Hall) of *Suaeda maritima* under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Stiffkey [a]	0	0	1±1	0	2±1.2	4±2.3	5±1.9
Tollesbury [a]	2±2	3±1.9	0	1±1	0	0	1±1
Abbot's Hall [a]	2±2	6±4	16±5.1	2±2	2±2	0	10±3.2

Table 7.76 Results of the one-way ANCOVA for the dormancy percentage of black seeds harvested in summer 2008 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.07	2	2.75	0.092
Error	0.20	17	-	-

Table 7.77 shows that dormancy percentage generally increased in all populations harvested in 2009 with the increasing salinity concentrations.

The one-way ANCOVA model revealed significant differences between the dormancy percentages among all populations harvested in 2009 (Table 7.78), and the Post-hoc test reveals that the low marsh seeds achieved a higher dormancy percentage than the other three populations (Table 7.77).

Table 7.77 Dormancy percentages of black seeds harvested in summer 2009 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (*n* = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank [b]	3±1.9	40±5.4	29±10.1	32±21.2	42±7.4	36±12.8	32±10.5
High marsh [b]	11±1	23±5.3	9±3	9±3.8	19±1	42±4.2	27±4.4
Top of low marsh [b]	1±1	1±1	8±2.8	56±6.7	11±1.9	8±0	14±4.2
Low marsh [a]	46±10.9	34±10.5	42±8.9	46±14	57±10.2	69±13.9	61±4.7

Table 7.78 Results of the one-way ANCOVA for the dormancy percentage of black seeds harvested in summer 2009 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.81	3	10.97	<0.001
Error	0.56	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effects of both salinity and the different storage conditions (2008 cold storage, 2009 dark room storage and 2010 fresh seeds) on the dormancy percentages of the three populations (creek bank, high marsh and top of the low marsh at the Stiffkey salt marsh) (Table 7.79). It reveals that the dormancy percentage was affected significantly by both salinity and storage condition at all levels: salinity, storage, population and the interaction between storage and population. The Post-hoc test shows both the 2009 dark room storage and the 2010 fresh seeds achieving significantly higher dormancy percentages than the 2008 cold storage.

Table 7.79 Results of the two-way ANCOVA for the dormancy percentage of black seeds harvested in the summers of 2008, 2009 and 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Storage	1.44	2	53.86	<0.001
Population	0.21	2	7.98	<0.001
Storage*Population	0.42	4	7.85	<0.001
Error	0.71	53	-	-

7.3.4.4 Viability

Table 7.80 shows that the non-viable seed percentage generally increased in all Stiffkey populations harvested in 2008 with the increasing salinity concentrations.

The one-way ANCOVA model reveals significant differences between the non-viable seed percentages among all Stiffkey populations harvested in 2008 (Table 7.81), and the Post-hoc test shows both the creek bank and the top of low marsh seeds having significantly lower non-viable seed percentages than the high marsh ones (Table 7.80).

Table 7.80 Non-viable seed percentage of black seeds harvested in summer 2008 from the *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE (n = 4). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{b}	0	2±2	2±1.2	6±1.2	3±1	6±3.8	2±2
High marsh ^{a}	4±1.6	8±5.4	15±4.4	20±7.1	12±3.3	45±16.2	34±7.4
Top of low marsh ^{b}	0	0	2±1.2	0	4±1.6	1±1	5±5

Table 7.81 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2010 from *Suaeda maritima* local populations (Creek bank, High marsh and Top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.47	2	27.2	<0.001
Error	0.15	17	-	-

Table 7.82 shows that the non-viable seed percentage generally increased in all creek bank populations harvested in 2008 with the increasing salinity concentrations.

The one-way ANCOVA model reveals insignificant differences between the non-viable seed percentages among all creek bank populations harvested in 2008 (Table 7.83).

Table 7.82 Non-viable seed percentage of black seeds harvested in summer 2008 from three different creek bank populations (Stiffkey, Tollesbury and Abbot's Hall) of *Suaeda maritima* under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Stiffkey ^{a}	0	2±2	2±1.2	6±1.2	3±1	6±3.8	2±2
Tollesbury ^{a}	0	1±1	1±1	3±1.9	0	0	7±3.4
Abbot's Hall ^{a}	4±2.5	10±3.2	20±7.7	0	2±2	6±4	16±6.9

Table 7.83 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2008 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 10 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.1	2	3.41	0.057
Error	0.24	17	-	-

Table 7.84 shows that dormancy percentage fluctuated in all populations harvested in 2009 with the increasing salinity concentrations.

The one-way ANCOVA model reveals insignificant differences between the dormancy percentages among all populations harvested in 2009 (Table 7.85).

Table 7.84 Non-viable seed percentage of black seeds harvested in summer 2009 from the *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C. All percentages relate to the original sample size and values are means ±SE ($n = 4$). The different superscript letters indicate characteristic means for populations that are significantly different (Adj. Bonferroni test, $p < 0.05$).

Population / NaCl treatment	**0 mM**	**100 mM**	**200 mM**	**300 mM**	**400 mM**	**500 mM**	**600 mM**
Creek bank ^{a}	1±1	12±6.3	7±4.7	5±3	13±7.7	3±1.9	2±2
High marsh ^{a}	9±5.3	1±1	3±1.9	2±2	11±5.3	10±3.8	25±1
Top of low marsh ^{a}	4±1.6	8±4	5±3	19±9	21±4.4	21±2.5	28±4.3
Low marsh ^{a}	29±11.2	14±9	27±12.9	21±18.4	6±3.5	2±1.2	2±1.2

Table 7.85 Results of the one-way ANCOVA for the non-viable seed percentage of black seeds harvested in summer 2009 from *Suaeda maritima* local populations (creek bank, high marsh, top of the low marsh and low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Population	0.104	3	1.58	0.222
Error	0.51	23	-	-

Finally, the two-way ANCOVA model was used here to compare the effects of both salinity and the different storage conditions (2008 cold storage, 2009 dark room storage and 2010 fresh seeds) on the dormancy percentages of the three populations (creek bank, high marsh and top of the low marsh at Stiffkey) (Table 7.86). It reveals that the dormancy percentage was affected significantly by both salinity and storage condition at the levels of salinity, population and the interaction between storage and population. The Post-hoc test shows insignificant differences between the three different storage conditions in terms of non-viable seed percentage.

Table 7.86 Results of the two-way ANCOVA for the non-viable seed percentage of black seeds harvested in the summers of 2008, 2009 and 2010 from *Suaeda maritima* local populations (creek bank, high marsh and top of the low marsh) at Stiffkey under the thermoperiod of: 16 h of light at 15 °C and 8 h of darkness at 5 °C.

Source of variation	**Type lll SS**	**df**	**F**	**P**
Storage	0.06	2	2.96	0.061
Population	0.26	2	12.67	<0.001
Storage*Population	0.36	4	8.68	<0.001
Error	0.55	53	-	-

7.4 Discussion

The present study shows how closely related but discrete *Suaeda maritima* populations (colonising contrasting habitats) exhibit differences in germination patterns in response to salinity, temperature, light and storage condition. In addition, some common characteristics were recorded in all populations, which have been previously for other halophytes: reduction of final germination percentage, enforcement of seed dormancy and reduction of seed viability all caused by increasing salinity.

7.4.1 Final germination percentage

In all experiments and among all populations in the current study with no exception, the seeds germinated best under the non-saline treatment (distilled water). In addition, the final germination percentage progressively decreased with the increasing salinity levels in all cases. These findings are not unexpected, as it is well documented that increasing salinity inhibits the seed germination of halophytes (Ungar 1978, Woodell 1985, Baskin and Baskin 1988). This suggests that *Suaeda maritima* does not necessarily have a physiological requirement for salt in order to initiate germination. Generally, the inhibitory effect of salinity on seed germination is mainly due to an osmotic effect and/or specific ion toxicity, depending on the plant species (Katembe *et al.* 1998). This reduction in germination can be attributed as an osmotic effect only for halophytes (Song *et al.* 2005), but non-halophytes are more likely to suffer from ion toxicity (Bajji *et al.* 2002).

Considerable differences between populations were observed in the current study in terms of final germination percentage. In most cases, the black seeds of the creek bank population exhibited a higher germination percentage, followed by both the high marsh and the top of the low marsh populations, and finally by the low marsh population. A very early study by (Boucaud 1962) addressed the behaviour of black seeds germination in certain populations of *Suaeda maritima* in France, concluding that the *S. maritima* var. *macrocarpa* seeds are non-dormant, while var. *vulgaris* and var. *flexilis* are dormant and require scarification or stratification to induce germination. Similar findings have been obtained among populations of many halophytic species (Mauchamp and Mésleard 2001, Khan and Gulzar 2003b, Rubio-Casal et al. 2003, Redondo et al. 2004).

The intensity of the salinity effects on seed germination for the different populations could be attributed to differences in their level of salt tolerance to varying concentrations of salinity in their natural microhabitats. This hypothesis is supported by the findings of the current research; genetic differentiation between populations was observed in the field and in the laboratory. Therefore, the ability of these populations to show different degrees of salt tolerance should be considered as an adaptation strategy for surviving in heterogeneous environments, particularly for coping with the varying levels of salinity in salt marshes. The low marsh population achieving lower germination percentages, particularly so at higher salinities, may be a reflection of the adverse and complex conditions of anoxia and salinity in the field, which inhibit germination to a certain extent (Wijte 1996).

The black seeds of the Stiffkey and Tollesbury populations achieved higher germination percentages than the Abbot's Hall ones. In Chapters 3 and 4, the phenotypic variations between those populations were addressed, and the same patterns of differentiation were observed and explained by the topographic variations between ancient natural and newly developed man-made salt marshes (Mossman et al. 2012a). Therefore, the current finding vis-à-vis final germination percentage would probably support the phenotypic variation capacity among those populations.

The brown seeds achieved a lower final germination percentage than the black seeds. This finding was not expected, as brown seeds normally germinate more rapidly than black seeds; they were observed previously to be non-dormant and more salt-tolerant in halophytic species (Metzing 1996, Khan et al. 2004, Wang et al. 2008). The brown seeds are well known for having a soft seed coat, which eases water uptake, gas diffusion and radical emergence, and which therefore facilitates rapid germination (Mohamed-Yasseen *et al.* 1994). In fact, a previous comparison between both *Suaeda maritima* seed morphs was conducted by (Metzing 1996), who concluded that large brown seeds achieved more rapid and higher germination rates than small black ones. Furthermore, the brown seeds of some other *Suaeda* species, such as *Suaeda aralocaspica* (Wang *et al.* 2008), *Suaeda splendens* (Redondo-Gómez et al. 2008) and *Suaeda acuminata* (Wang *et al.* 2012), have also been found to achieve higher germination percentages than their black counterparts.

The opposing results achieved by the brown seeds in the current study could be attributed to the length of storage period, which might have caused many of them to lose their viability. In order to collect a sufficient number of brown seeds to conduct reliable germination experiments, more mother plants and more time would have been necessary than was required for the black seeds. It was noted in Chapter 4 that all populations produced many fewer brown seeds than black ones, especially in the high marsh and the top of the low marsh populations. Therefore, the storing of those brown seeds for a few weeks before starting the experiment might be at the root of the problem; such a problem was mentioned in a similar case by (Redondo-Gómez et al. 2008). However, the limited findings available here vis-à-vis the brown seeds' final germination percentage revealed significant variation among populations. The brown seeds of the creek bank, the high marsh and the top of the low marsh achieved higher final germination percentages than the low marsh brown seeds. This finding would undoubtedly support the population differentiation that has been confirmed throughout the current research.

Seed mass is another seed heteromorphism trait, which was examined thoroughly in Chapter 4 as well as in the current chapter. In Chapter 4, the creek bank population produced the largest black seeds, followed by both the high marsh and the top of the low marsh, and finally by the low marsh population. It is well documented now that different seed masses often exhibit different germination behaviour, and that large seeds are expected to be less dormant than small ones (Baskin and Baskin 1988), and therefore germinate more rapidly and achieve higher germination percentages (Carter and Ungar 2003). This behaviour could be due to the characteristics of the seed coat (Mohamed-Yasseen *et al.* 1994), which could play a major role in facilitating rapid germination on the part of the large seeds and delayed germination on the part of the small ones; besides this is the fact that large seeds have a high reserve mass (Mandák and Pysek 2005), which should provide enough energy for the radicle to break the seed coat. The current findings in this study agree with those that have been documented about the germination behaviour of both seed morphs. The largest black seeds of the creek bank population consistently achieved the highest final germination percentage, followed by both the high marsh and the top of the low marsh populations, and finally by the low marsh population, which produced the smallest seeds among the populations. Very similar findings were reported in a recent study conducted on *Suaeda maritima*, which confirmed that the germination of large seeds was 100-150% better than that of small seeds (Wetson *et al.* 2008).

The ability of *Suaeda maritima* populations to produce different seed morphs could be a strategy for extending the germination period, and probably to provide a seed bank for the long-term recruitment of seedlings (Yao et al. 2010); these could facilitate responding differently to different environmental stresses in the field at the time of germination (Khan *et al.* 2004).

The final germination percentage varied according to the temperature regimes employed in the current study. Higher percentages were achieved by both the 10-5 °C and the 15-10 °C thermoperiods than by the 15-5 °C one. In fact, the actual differences between these three regimes are slight, as they were designed in the first place to reflect the range of temperatures in the field at the germination time of those seeds. However, finding differences among these regimes in terms of final germination percentage should give an indication of how temperature is an important regulating factor in germination and how the *Suaeda maritima* seeds are able to respond to different environmental conditions. (Khan 2008) provided a list of temperature requirements for halophytic species during the seed germination stage, and concluded that the 15-5°C temperature regime is much better than higher regimes for the germination of halophytic seeds from moist temperate regions. However, the seeds of some halophytes, such as *Salicornia europeaea*, are documented to tolerate temperatures fluctuating between 15/5 and 20/30 °C, although germination is faster at higher temperatures (Davy *et al.* 2001).

The assessment of the differences between the light/dark and constant darkness treatments in terms of the final germination percentage revealed that this percentage was higher in the dark than in the light/dark regime, and this result was consistent among all populations. This is a striking finding because light is considered to be a key regulatory environmental signal for the seed germination of halophytes (Khan and Ungar 1997). (Baskin and Baskin 1988) reported the light/dark requirements for germination for twenty-three halophytic species. The seeds of four species require light, four species germinate to higher percentages in light than in dark, thirteen germinate equally in both light and dark, and two species germinate more highly in dark than in light. The light/dark requirements for the germination of the *Suaeda maritima* species in particular and the *Suaeda* genus in general are not recorded within the above list, which probably means that there is no strong evidence yet pertaining to the light requirements for the germination of its seeds. However, such findings could be explained as a photo-inhibition mechanism, which is known to be exhibited by many Mediterranean halophytes, such as *Allium staticiforme*, *Brassica tournefortii*, *Cakile maritima* and *Otanthus maritimus* (Thanos et al. 1991). This mechanism is explained as a halophytic seed germination adaptation strategy against surface seedling establishment at unfavourable times in harsh open-sea coastal habitats. Seeds of salt marsh annuals are known to remain near the soil surface, meaning that the light requirement could be risky; it could initiate germination and produce seedlings that could then be damaged by any one of the stress factors prevalent in the environment such as cold and wave action. Therefore, the current findings would support the hypothesis that the photo-inhibition strategy is probably designed to provide seeds with caution against the light factor. In addition, it has been found that low winter temperatures (cold stratification) removed the light requirement for the germination of *Spegularia media* (Ungar and Binet 1975), and that seeds kept for several months in dry storage at room temperature may lose this ability as well, as noticed in *Ocimum americanum* (Baskin and Baskin 1988). Thus, the seeds used in this study might be subjected to similar effects, as they normally experience low winter temperatures and were kept dry stored for a few months in dark room temperatures before the start of the germination experiment. Moreover, some halophytic seeds are well adapted to burial in the superficial surface of the soil to the extent that they would achieve better germination in total darkness than in light/dark, as observed in *Cyperus capitatus* (Redondo-Gómez *et al.* 2011); this could be one of the scenarios experienced by the *Suaeda maritima* seeds in the field, which caused them to show such findings in the lab in the current study. (Redondo *et al.* 2004) explained why the germination of some halophytic seeds are affected adversely by darkness deep in the substrate, as "species that live in highly specific habitats, such as salt marshes, often produce seeds with highly specialized adaptations". Therefore, in light of the current study findings, further investigations are needed to determine whether or not *Suaeda maritima* seeds require light in order to trigger their germination, thereby providing a better understanding of the ecology of species thriving in coastal habitats.

Investigating the effects of the different storage conditions on seed germination revealed that the seeds collected in summer 2008 and kept in cold storage at 5 °C for about two years achieved a higher final germination percentage than either the seeds harvested in 2009 and kept in dark room storage for about one year or those newly collected seeds that were kept in dry storage for a few months. Similar findings were observed recently by (Wetson *et al.* 2008) when they conducted various germination experiments on *Suaeda maritima* seeds; they concluded that seeds that had been stored in cold wet conditions germinated better than those stored dry. The current cold storage findings in terms of final germination percentage could be due to the fact that cold storage (stratification) resembles the winter dormancy period that is experienced in the field by the *Suaeda maritima* seeds, which is in fact a prerequisite condition for halophytic seeds in order to initiate germination in the spring (Baskin and Baskin 1988). For example, (Davy *et al.* 2001) addressed the importance of cold stratification to relieve dormancy in the *Salicornia* species, which is closely related to *Suaeda maritima*. According to (Baskin and Baskin 1988), low temperatures or high temperatures will break the dormancy depending on the species; it seems therefore that low temperatures regulate the dormancy-breaking process at least according to the current findings, as those seeds stored at 5 °C germinated much better than those stored at room temperature (ranging from 20 to 25 °C). This result in fact is an indication of the ecological limits for those seeds to be germinated and therefore for the new progeny to be established in the field.

The rate of germination trait, expressed in the current study as mean time-to-germination (MTG), is another window for exploring how seeds respond to external environmental factors during the time of germination, and it is considered as a strategy for improving long-term reproductive success by spreading risk over time (Venable 2007). In the current study, the mean time-to-germination trait increased significantly with the increases in salinity. This is an expected behaviour, as it has already been established that higher salinities inhibit the speed of germination (Woodell 1985). In addition, significant differences occurred between populations in terms of MTG, and the low marsh exhibited the highest mean time-to-germination values, followed by both the high marsh and the top of the low marsh populations, and finally by the creek bank population. These differences support the previous findings achieved in Chapters 3 and 4 about the phenotypic variation between populations in terms of growth and fecundity. Also, such variation between populations in terms of MTG is the reverse of the final germination percentage findings; together, they should reflect the relationship between germination dynamics and the natural distribution of those populations (Woodell 1985).

The creek bank seeds achieved a higher final germination percentage and a shorter MTG, which means that this population might be better adapted to high levels of soil salinity. These two traits together could facilitate the plants in becoming quickly established in the salt marsh; their seeds are deposited on what can generally be described as a safe site, whereas the low marsh seeds may have to take a risk-avoiding strategy, i.e. delaying germination until lower salinities prevail in their microhabitat. Similar supportive findings were reported by (Zhang *et al.* 2011); for restoration purposes, for example, they concluded that selecting species with a high final germination percentage and a short mean time-to-germination would be highly advantageous.

7.4.2 Recovery from salinity percentage trait

All ungerminated samples in all experiments exhibited considerable germination when transferred to distilled water, and the recovery from salinity trait increased progressively with the increasing levels in the original salinity treatments. This was not an unexpected trend, as most halophytes show considerable recovery from salinity when stress conditions are alleviated (Ungar and Binet 1975, Ungar 1978, Woodell 1985). This trend in recovery indicates that those seeds were in a state of dormancy (induced by salinity), and the main factor behind this is apparently related to osmotic potential. For those seeds to possess the ability to remain dormant under higher low water potentials and to recover successfully after being transferred to fresh water is an indication that those populations are more salt tolerant than actively growing plants (Pujol *et al.* 2000). In addition, the ability of those seeds to accelerate germination after being removed from saline solutions is ecologically advantageous (Redondo *et al.* 2004), as it should reflect a quick establishment ability when rainfall decreases the salt levels on the marsh surface during spring. This ability to recover from salinity suggests that seed survival rather than germinability (Huang *et al.* 2003) is the key to success for halophytic seeds under saline conditions, i.e. those seeds possess another important trait, which is the ability to prioritize strategies.

The recovery from salinity trait differed significantly between populations and treatments. The low marsh population exhibited the highest percentage, followed by both the high marsh and the top of the low marsh, and finally by the creek bank. As for whether any particular salinity level is a key primer of germination, there is significant variation among species, with some stimulated into germination and others failing to germinate altogether by exposure to high salinity levels; thus, recovery is also highly species-specific (Saira Saeed 2011), and the current findings support the latter hypothesis among the *Suaeda maritima* populations.

The thermoperiod 15-5 °C achieved the highest percentage of recovery from salinity, followed by the 10-5 °C and finally by the 15-10 °C thermoperiods. Also, the light/dark treatment achieved a higher recovery percentage than the constant darkness one. In addition, the black seed morph achieved a higher recovery percentage than the brown seed morph. Moreover, the short dark room storage of 2010 achieved the highest recovery percentage, followed by the long dark room storage of 2009 and finally by the cold storage of 2008. This indicates that the precise requirements for inducing germination in seeds that have been subjected to various environmental conditions in general and salinity in particular are highly complex (Pujol *et al.* 2000). However, such complicated variations and interactions in the recovery from salinity trait should at least reflect significant ecological conditions in terms of when seeds germinate under field conditions (Ungar 2001).

7.4.3 Dormancy percentage and viability percentage traits

The viability of the remaining seeds that did not germinate was assessed using the TTC test. Both the dormant seeds and the non-viable seed percentages increased significantly with the increasing levels in their original salinity treatments. This is an expected finding, as salinity is reported to enforce seeds to enter secondary dormancy (Pujol *et al.* 2000) and to reduce seed viability (Redondo *et al.* 2004). In the field, if the salinity stress level did not fall in the germination season, dormant seeds would be forced to either enter a secondary dormancy phase or to enter the seed bank to be cold stratified by the following spring (when they might be released from dormancy and initiate germination) (Wang *et al.* 2008). Therefore, the ability of seeds of the *Suaeda maritima* populations to maintain viability even under high salinities may reflect an adaptive strategy to salt stress conditions.

Significant differences between populations in terms of both dormant and non-viable seed percentages were observed. The low marsh population achieved higher percentages of dormant and non-viable seeds than the creek bank, the high marsh and the top of the low marsh. One possible explanation for the low marsh population having the highest percentage of non-viable seeds is related to the particularly harsh conditions of the lower parts of the salt marsh, where waterlogging and therefore soil hypoxia might lead to higher levels of mortality (Ungar 1978).

In addition, the non-viable brown seed percentage was observed to be higher than the non-viable black seed percentage. In the current study, this is clearly due to the issue of age and the loss of viability at the start of the experiment. A similar difficulty was reported by (Redondo-Gómez et al. 2008) when they dealt with the brown seeds of *Suaeda splendens*.

In conclusion, this study was conducted to examine the germination characteristics of four populations of the salt-tolerant *Suaeda maritima* in order to assess the factors that affect their establishment in the field. In general, all populations germinated best under non-saline treatment and their germination levels decreased with the increasing salinity gradients. Ungerminated seeds generally germinated well when transferred to non-saline solutions, indicating that their germination had been inhibited osmotically by salinity. Salinity increased the secondary dormancy and non-viable seed percentages progressively with the increasing salinity gradients.

The populations exhibited distinctive behaviours in all the experiments, which reflect the fact that they have differing degrees of salt tolerance adaptation. Seed heteromorphism, temperature and light as well as the different storage conditions proved to affect the germination behaviour of those seeds, reflecting the fact that salinity is not the only factor affecting seed germination in the field. Finally, the current laboratory germination responses have proved to be a useful confirmation tool in terms of the noticeable variations between populations (reflecting the complex heterogeneity in their natural microhabitats), and therefore, it can be suggested that this differentiation reflects adaptive ability on the part of the *Suaeda maritima*.

Chapter 8

General discussion

The main aim of this dissertation has been to examine the phenotypic variations within and between populations of the halophyte *Suaeda maritima*. It is anticipated that the current research will add to our understanding of the strategies they utilise in order to cope with the steep environmental gradients and heterogeneity, and to determine whether any phenotypic variations are the result of plasticity or genetic differentiation. Also, it is anticipated that this is the first comprehensive study assessing the phenotypic variation of *Suaeda maritima* at the inter- and intra-population level in the natural field and under uniform laboratory conditions.

Some specific characteristics of *Suaeda maritima* were exploited to conduct the current research. It is a fast-growing plant with annual life history that produces seeds in abundance; this facilitated its study under laboratory conditions and in the field. There is also an appropriate body of knowledge available, affording the opportunity to compare and complement this research with other work. In addition, the particular characteristics of coastal salt marshes facilitated the current research, as the selection pressures within such marshes can act as a natural field laboratory (Davy and Smith 1988) for investigating the relative roles that plasticity and genetic differentiation play in determining phenotypic variation; the plants live across severe gradients of environmental stress and display clear differences in phenotype that can be correlated to these conditions (Richards et al. 2005).

The work in this thesis focused firstly on investigating the main environmental characteristics of four contrasting field study sites at Stiffkey salt marsh. The spatial heterogeneity of the study sites was evident; altogether there was a range of 83cm in the tidal frame between the low and high marsh sites, with the number of tidal inundations per year ranging from 177yr^{-1} on the high marsh to 553yr^{-1} on the low marsh. The high marsh site had much higher water content than the creek bank and the low marsh sites. The high marsh and the top of the low marsh sites had higher salinity levels than the other two sites. Sediment redox potential varied considerably between the four sites, over the spring/neap tidal cycle and even between consecutive spring tidal periods. Therefore, these measurements should serve to demonstrate that the *Suaeda maritima* populations at these sites do indeed experience divergences between their environments that could both affect the phenotypic responses of developing plants and constitute selection pressures for the evolution of genetically differentiated populations.

Notwithstanding the extensive research in salt marshes that has examined how these environmental gradients affect phenotypic variation at the community level (Pennings and Bertness 2001), little research has addressed how these gradients have shaped the genetic make-up of salt marsh plant species and populations (Richards et al. 2010). Therefore, it is hoped that the findings of the main chapters in the current study will provide valuable evidence for explaining how *Suaeda maritima* exhibits such a wide ranging ability to colonize across the salt marsh elevational gradient, and even within different positions at the same elevation.

Besides providing an overview of the local environmental gradient characteristics at Stiffkey salt marsh, it was vital to provide a detailed assessment of the phenotypic variation and seed production of the local populations of *Suaeda maritima*. This has been achieved (in Chapters 3 and 4). At Stiffkey salt marsh and over three consecutive seasons (2008-2010), considerable variations between the four populations studied were observed. The creek bank population has been identified as the most vigorous population in terms of growth traits (plant height, shoot width, number of branches, main root length), followed by the low marsh population, and finally by both the top of the low marsh and the high marsh populations. Also, comparing the creek bank populations that were collected from three different salt marshes (Stiffkey, Tollesbury and Abbot’s Hall) in 2008 showed no significant differences in most plant growth traits, except in plant height; the Stiffkey population was the tallest, and the number of primary branches was greater in the Stiffkey and Tollesbury populations than at Abbot’s Hall.

Therefore, such consistent variations between populations (observed in all years) raised the question as to whether it might be explained as genetically driven. However, in a recent study, it has been found that the variations observed between populations of *Suaeda maritima* in the River Adur estuary (Essex, UK) could be plastic responses to the local variations in one or more of the local environmental conditions (Wetson et al. 2012).

Chapter 4 provided a comprehensive evaluation of the seed production and heteromorphism between and within the *Suaeda maritima* populations. On the Stiffkey salt marsh and over three consecutive seed production seasons, the creek bank population consistently produced the heaviest seeds, followed by the high marsh and the top of the low marsh populations, and finally by the low marsh population. The low marsh population was found to produce the highest numbers of black seeds over the three years studied, and was the highest producer of brown seeds in the final season (2010). The creek bank population was the second highest producer of black seeds over the three years and was the highest producer of brown seeds in 2009. The high marsh and the top of the low marsh populations were found to produce similar numbers of black and brown seeds on many occasions, and their pattern of production would put them in third place with respect to the seed colour trait.

Black seed mass variation among *Suaeda maritima* populations has been observed previously by many researchers, including (Chapman 1947, Boucaud 1962, Clapham et al. 1962, Metzing 1996) and more recently by (Wetson et al. 2012). However, although seed heteromorphism in terms of producing different seeds with different colours is particularly evident in annual species of the families Asteraceae and Chenopodiaceae (Imbert 2002, Li et al. 2005), little work has been done on *Suaeda maritima* brown seeds and (Metzing 1996) is one of the few researchers who conducted germination experiments on the brown seeds of two populations from the lower and upper parts of a salt marsh in Germany. Most of the extensive works conducted on *Suaeda maritima* seeds have been mainly on the black seeds, and this could be due to the fact that they comprise the majority of the seed production by the populations under investigation. Therefore, it is believed that the current study has provided the first comprehensive evidence pertaining to brown seed production by all *Suaeda maritima* populations in terms of seed number and mass.

In order to distinguish between variations in morphology (Chapter 4) and fecundity (Chapter 4) resulting from phenotypic plasticity or genetic heterogeneity, two principal approaches were employed: field reciprocal transplantation of the populations (Chapter 5) and transplanting the populations to uniform conditions in growth chambers (Chapter 6) (Kawecki and Ebert 2004).

Field reciprocal transplantation of seedlings was carried out between three parts of the Stiffkey salt marsh: creek bank, high and low. All populations demonstrated substantial genetic differentiation in their survival, growth and fecundity. The most pronounced finding in terms of survival is that both the creek bank and the low marsh populations had high overall survival rates, compared with the high marsh population. Also, the high marsh plants were severely affected when they were transplanted to the creek bank site and even more severely affected when transplanted to the low marsh site. It is well known that populations on the higher parts of a salt marsh may be better adapted to hypersaline conditions (especially in summer), and that populations on the lower parts of a marsh may be better adapted to long periods of submergence, waterlogging and tidal action (Jefferies 1977, Davy et al. 2001). Therefore, the high marsh population in the current study is believed to be less well adapted to the local abiotic conditions of the alien habitats, i.e. in the creek bank and in the low marsh. The growth and fecundity characteristics of the populations were retained, even when individuals were grown in alien sites. For example, the creek bank and high marsh populations produced consistently bigger seeds than the low marsh plants. In addition, the total number of seeds produced was significantly higher in the low marsh population, followed by the creek bank, and finally by the high marsh population. Furthermore, the creek bank population was the tallest, followed by the low marsh and the high marsh populations, respectively, and the low marsh population exhibited the greatest shoot width, followed by the creek bank and the high marsh populations, respectively.

The findings of the current seedling transplantation experiment are in agreement with the findings of (Davy and Smith 1985, Davy and Smith 1988) on *Salicornia europea* populations at the same salt marsh on the north Norfolk coast, and with the findings of (Ungar 1987b) on *Salicornia europea* populations from the Ohio Salt Pan. However, they do not support the very recent findings of (Wetson et al. 2012), who conducted research on two populations of *Suaeda maritima* on a salt marsh in the River Adur estuary (Essex, UK). Such opposing findings in the latter study could be due to site-specific topographic variation, as the Stiffkey salt marsh is naturally more complex (within it, there is an ancient shingle ridge that physically separates the marsh into upper and lower parts, with a unique drainage system that greatly inhibits gene flow, i.e. seeds floating between marsh parts) (Davy and Smith 1988). Therefore, the topographic complexity of Stiffkey may act as a determinant on the life-history characteristics of these *Suaeda maritima* populations, more so than in the River Adur estuary. There are other precedents for genecological variation in salt-marsh plants that may be adaptive, particularly in *Aster tripolium* and *Puccinellia maritima* (Gray 1974; Gray, Parsell & Scott 1979; Gray & Scott 1980).

Besides the benefits of the reciprocal transplantation experiment for confirming the genetic element of the phenotypic variation among populations, it also suggest that the mechanisms that control *Suaeda maritima* distribution in a single site at the salt marsh (for example physiological tolerance) are not entirely appropriate for describing its distribution along all the salt marsh gradients.

To further confirm whether or not phenotypic variations among populations are genetically driven, the growth chambers experiment was conducted using two different temperature regimes; the first resembled the average range of temperature in the field during the growing season, and the other was much higher in terms of temperature. Under the low temperature (and therefore low salinity) of the first regime (20-15ºC), the *Suaeda maritima* populations were able to maintain the inherited significant differences between them. However, under the much more severe conditions of temperature (and therefore higher salinity) of the second regime (25-20 ºC), those populations showed insignificant plastic response differences among them, probably indicating a striking ability to survive and complete the lifecycle by producing seeds for future stability purposes. Therefore, despite the evidence for genetic variation in this species under the first temperature regime, the outcomes under the second regime do not support the hypothesis that this species is well adapted to high temperatures. However, the plastic responses in the latter regime should be appreciated as a buffer against extinction (Valladares et al. 2007), which is a possible scenario if the current and future rates of global warming continue or are exacerbated.

Both the field transplantation and the growth chamber experiments proved to be informative approaches to assessing the variations in the life-histories of the *Suaeda maritima* populations. Those populations were found to differ significantly in many life-history characteristics, particularly survival, growth and fecundity, as revealed by those experiments. Life-history characteristics have frequently been used as measures of performance, as they would be expected to affect fitness directly (i.e. the relative capacity to leave offspring) (Davy and Smith 1988).

In both the field transplantation and the growth chamber experiments, the relationship between plant density per core and growth as well as fecundity traits was investigated, revealing negative associations in most cases. The negative density-dependent effect on the growth and fecundity traits was expected, as those traits are known to be highly plastic in responding to adverse conditions in micro-environments (Watkinson and Davy 1985). Such findings confirm those by (Tessier et al. 2000a); they examined the effect of density on the reproduction of a population of *Suaeda maritima* in the Mont Saint-Michel Bay salt marsh in France, and they concluded that when density increases, biomass and seed production decreases, and that individuals tend to be less-branched. Researchers consider such density relationships as trade-offs in the halophytes' competitive ability and stress tolerance along stress gradients in salt marshes (Vince and Allison 1984, Crain et al. 2004).

As a complementary but important step, the final approach of the current study was to investigate the strategies exploited by the seeds of the different *Suaeda maritima* populations during the dormancy and germination stages that allow them to successfully become established in their local heterogeneous microhabitats. In general, all populations germinated best under the non-saline treatment, and their germination levels decreased with the increasing salinity gradients. Ungerminated seeds generally germinated well when transferred to non-saline solutions, indicating that their germination had been inhibited osmotically by salinity. Salinity increased the secondary dormancy and non-viable seed percentages progressively with the increasing salinity gradients. The populations exhibited distinctive behaviours in all the experiments, which reflect the fact that they have differing degrees of salt tolerance adaptation. Seed heteromorphism, temperature and light as well as the different storage conditions proved to affect the germination behaviour of those seeds, reflecting the fact that salinity is not the only factor affecting seed germination in the field. Finally, the current laboratory germination responses have proved to be a useful confirmation tool in terms of the noticeable variations between populations (reflecting the complex heterogeneity in their natural microhabitats), and therefore, it can be suggested that this differentiation reflects adaptive ability on the part of *Suaeda maritima*.

The brown seeds achieved a lower final germination percentage than the black seeds at all saline treatments. This finding was not expected, as brown seeds normally germinate more rapidly than black seeds; they have been observed previously to be non-dormant and more salt-tolerant in halophytic species (Metzing 1996, Khan et al. 2004, Wang et al. 2008). The brown seeds are well known for having a soft seed coat, which eases water uptake, gas diffusion and radicle emergence, and which therefore facilitates rapid germination (Mohamed-Yasseen et al. 1994, Wang et al. 2008). The different results achieved by the brown seeds in the current study could be attributed to the length of storage period, which might have caused many of them to lose their viability. In order to collect a sufficient number of brown seeds to conduct reliable germination experiments, more mother plants and more time would have been necessary than was required for the black seeds. It was noted in Chapter 4 that all populations produced many fewer brown seeds than black ones, especially in the high marsh and the top of the low marsh populations. Therefore, the storing of those brown seeds for weeks before starting the experiment might be at the root of the problem; such a problem was mentioned in a similar case by (Redondo-Gómez et al. 2008). However, the limited findings available here vis-à-vis the brown seeds' final germination percentage revealed significant variation among populations. The brown seeds of the creek bank, the high marsh and the top of the low marsh achieved higher final germination percentages than the low marsh brown seeds. This finding would undoubtedly support the population differentiation that has been confirmed throughout the current research.

Although the *Suaeda maritima* populations of the current study may correspond approximately with varieties recognized in older British floras, such as described in (Chapman 1947, Clapham et al. 1962), they are now no longer recognized as taxonomic entities within modern treatments (Tutin et al. 1993, Stace 2010). The loss of confidence in the existence of such diverse populations of the *Suaeda maritima* species may be due to the very limited research conducted on them. The current research did not aim to tackle the taxonomy of the *Suaeda maritima* populations, but it revealed an element of genetic differentiation that should encourage experts in the field of taxonomy to reconsider the taxonomic status of those populations.

As the current findings have demonstrated the ability of the *Suaeda maritima* species to adapt well to diverse environmental conditions, it is therefore a promising annual salt-tolerant species that can be exploited with potential for the purposes of the desalination and restoration of saline soils, as they can withstand high soil salinity and saline water irrigation, phytoremediation and wetland restoration.

The current study demonstrated that *Suaeda maritima* populations exhibit clear phenotypic variation in terms of their seed dormancy, germination, and seedling survival in the field reciprocal transplantation and under uniform laboratory conditions, as well as in terms of morphology, seed production and heteromorphism. All the different approaches in the current study support that there is an element of genetic differentiation between populations and phenotypic plasticity within populations. Both strategies appear to be exploited by the *Suaeda maritima* species in order to cope with the steep environmental gradients in their natural habitats.

Finally, there are a number of research recommendations that could be followed to assist similar work in the future. Many of these involve modifying the sampling techniques utilized to assess environmental factors over longer periods that cover all the life-history stages of *Suaeda maritima* populations, including seed dispersal, dormancy, survival, growth and fecundity. In addition, the current study should encourage molecular ecologists to conduct further molecular assessment approaches using sophisticated techniques in order to reveal the genetic factors underlying the variations among those populations.

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