The redox potential characteristics of saltmarsh sediments and their influence on the restoration of perennial halophytes in coastal realignment

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Abstract

Sediment anoxia and associated low redox potential (Eh) are important influences on the distribution of saltmarsh plants. Nevertheless, the detailed variation of Eh in space and time is poorly understood, especially on managed realignment (MR) saltmarshes where anoxia may be a significant constraint on the establishment of characteristic perennial halophytes. This thesis examines the distribution of Eh in natural and MR saltmarshes of North Norfolk and Lincolnshire, UK and its implications for *Armeria maritima*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima*, species important in general saltmarsh (GSM) communities.

Detailed measurements of Eh were made using arrays of fixed electrodes and a field datalogger, at different sites/seasons/sediment depths, over tidal cycles, and in relation to microtopography (including on an existing experiment with manipulated microtopography). Laboratory apparatus was devised to examine responses of halophytes to controlled Eh in water culture.

Relationships between vegetation and Eh proved complex. Prolonged low-Eh episodes occurred both at MR and natural GSM sites, particularly in summer. *Atriplex portulacoides* and *Puccinellia maritima* tended to dominate where low-Eh episodes were more transient, generally near drainage channels or at higher elevations. *Atriplex*, *Armeria* and *Plantago* previously planted in experimental plots survived poorly at low Eh, while *Limonium* and *Triglochin* showed higher survival. In cultivation, *Atriplex* tolerated anoxia for only two weeks, and low Eh did not impose any additional stress. In contrast, *Triglochin* survived the ten-week duration of the experiment even at low Eh.

Thus laboratory results suggested mechanistic bases to explain those in the field. Although MR and GSM sediments can exhibit similar Eh measurements, they may differ with respect to redox capacity; MR sediments sites with high reduction capacities may exert additional stresses that could limit colonisation. Conversely, consistently high Eh could also limit colonisation on MR sites by promoting vigorous competitive growth of *Atriplex* and *Puccinellia*.

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CHAPTER 1: Introduction

1.1 Saltmarshes – nature and importance

Coastal saltmarshes are vegetated areas distinguished from terrestrial ecosystems by the defining feature of periodic exposure to tidal flooding (Adam 1990). These environments occur high in the intertidal zone, roughly between mean high water neap tides and mean high water spring tides. They can develop sediments with variable silt, clay and sand composition supporting a varied range of specially adapted halophytic species. Saltmarshes are widely distributed ecosystems, predominantly occurring at high latitudes, but also forming locally extensive communities at sub-tropical and tropical latitudes.

Characteristically, saltmarsh formation follows the establishment of a mudflat where a source of suspended sediment and a low energy environment provide favourable conditions for the net accumulation of sediment (Allen & Pye 2002). Once this reaches the level of mean high water neap tides (MHWN) initial colonisation by pioneer plants occurs. Suitable conditions for saltmarsh formation are typically found on low-energy coasts such as sheltered embayments, estuaries and fringing open coasts where sufficient protection from wave energy occurs from the presence of extensive shallow sand banks, or in the lee of offshore barrier islands (Allen & Pye 2002). Pioneer vegetation comprises a small number of species notable for their particular tolerances to stresses associated with regular tidal inundation, which include algae and vascular halophytes, such as the common saltmarsh grass *Puccinellia maritima* and certain species *Salicornia* spp. and *Spartina* spp. (Davy 2000) (Note: Flora nomenclature hereafter based on Stace (2010)). As these species become established the process of sediment accretion is enhanced and the height of the marsh is raised by the capacity of these plants to exert a frictional drag and reduce the speed of tidal flow, which limits sediment resuspension, while the roots act to stabilise the sediment (Langlois *et al*. 2003). The process facilitates the creation of suitable conditions for the subsequent colonisation of species that are less tolerant of inundation stresses but are able to competitively displace the early colonists and alter the composition of the saltmarsh community during a process of succession. The number of plant species that will eventually colonise a saltmarsh will depend on its location and a range of biotic and abiotic factors. Out of a potential pool of more than 60 species of higher plant that can potentially occur below the level of high water spring (HWS) on British saltmarshes (Adam 2015, pers. comm.), those occurring in the south-eastern part of the country typically have between 10 and 20 species present below the mean high water spring (MHWS) tide level (Burd 1989).

Although evidence from MR sites shows that saltmarshes can form rapidly where suitable conditions prevail (Brown 2008; Mossman *et al*. 2012a,b), their persistence is largely determined by the continuation of an effective degree of protection from erosive wave energy and the availability of a supply of suspended sediment. Rather than a slow evolution toward geologic maturity, the attainment of marsh maturation is estimated to occur over a relatively rapid timescale of centuries (Steers 1977; Allen 2000). At this stage, the morphological maturity of minerogenic saltmarshes is generally represented when a near to dynamic equilibrium is reached with sediment supply, vegetative growth and relative sea level (Friedrichs & Perry 2001). A number of studies that have quantified sedimentation rates with local sea level rise have demonstrated the importance of biological and physical feedbacks between salt marsh plant growth and geomorphology in maintaining marsh vertical accretion in pace with high rates of sea level rise (Kirwan & Megonigal 2013; Fagherazzi *et al*. 2012; Morris *et al*. 2002). This is further supported by historical investigations of depositional stratigraphy indicating the persistence of saltmarshes throughout Holocene transgressions suggesting the tight coupling of these systems to longer-term fluctuations in sea level (Allen 2000). While these biophysical processes may promote relative stability in vertical salt marsh development, processes responsible for lateral marsh erosion and expansion are more likely to be far from equilibrium. Accordingly, Fagharazzi *et al*. (2013) proposed that saltmarshes naturally tend to be ephemeral landforms on a geological timescale with average lifecycles typically of the order of a few thousands of years since they are constantly reworked and rejuvenated by the very dynamic coastal environment that operates at the marsh boundary.

There are some 45000 ha of saltmarsh in Britain (Burd 1989) representing a major area of essentially natural habitat of significant wildlife conservation value. They are home to a unique fauna and flora that are adapted to survive the particular conditions that result from interactions between the marine and terrestrial environments (Boorman 2003). These habitats are a vital refuge for a range of birds and wintering wildfowl including many species of conservation concern (Atkinson *et al*. 2001; Greenberg *et al.* 2014; Norris 2000), and act as an important feeding and nursery grounds for a range of marine invertebrate and fish species (Green *et al.* 2009). In some places, tidal marshes have been identified as highly productive ecosystems, providing a valuable source of organic matter and nutrients to the adjacent marine environment (Valiela & Teal 1979). The value of these habitats for biodiversity has been officially recognised in the UK being of ‘principal importance’ under UK legislation as a Priority Habitat and a focus for conservation action under the Natural Environment and Communities Act 2006. All European saltmarshes are also afforded a high level of protection under the EU Habitats Directive (92/43/EEC). Furthermore, saltmarshes generate some of the most valuable ecosystem services among all natural ecosystems (Costanza *et al.* 2014). Saltmarshes can serve as nitrogen sinks that filter runoff water and reduce nitrogen loading to estuaries that could otherwise contribute to eutrophication (Valiela & Teal 1979). The value of these systems as refuges for juvenile fish and crustaceans that support commercial fisheries has been globally recognised (UNEP 2006). Furthermore, the ability of saltmarsh vegetation to stabilise sediments and attenuate wave energy has become increasingly appreciated for its utility as natural sea barriers that limit shoreline erosion and help mitigate flooding of built up areas (King & Lester 1995; Möller *et al.* 2014).

1.2 Saltmarsh loss and restoration

Global losses of coastal wetlands have been extensive with an estimated disappearance of 67% of these habitats during human history, based on a study of 12 of the world’s largest estuaries (Lotze *et al.* 2006). Anthropogenic impacts on saltmarshes continue to be multiple with the main direct threats originating from land conversion to agriculture, resource extraction and exploitation (particularly from livestock grazing), species introductions (notably the invasive cordgrass hybrid *Spartina anglica*), alterations to hydrology and sedimentology, pollution and climate change (Bromberg-Gedan *et al.* 2009; Silliman *et al.* 2009). Saltmarsh loss in Britain has a long history with a reported area of some 56000 ha claimed for agricultural conversion over the past millennium (Doody 2002, 2008). While conversion to agriculture ceased from around 1980, relatively small areas continue to be lost for port and other coastal developments. The main contemporary threat has arisen from recent increases in the rates of coastal erosion (van der Waal & Pye 2004; Wolters *et al.* 2005). Although decreases in saltmarsh extent have not been shown at all regional scales, surveys have estimated a net rate of loss since the 1960s of around 100 ha yr^{-1} mainly concentrated in south-east England (Cooper *et al.* 2001, UK Environment Agency 2011).

The most often cited threat for potential loss of intertidal habitats in the future is from the process of 'coastal squeeze' along coastlines fixed by hard engineered structures that prevent the landward migration of saltmarshes as a dynamic natural response to the increase in relative sea levels (Doody 2013). A rise in sea levels will gradually increase the frequency and duration of tidal inundation, and ultimately result in a narrowing of the width of the saltmarsh area as the lower areas effectively become drowned. It would therefore be expected that increased exposure would lead to a shift in the types of marsh communities. However the loss of plant communities at the seaward edge has not generally been accompanied by the loss of upper marsh species predicted by the coastal squeeze hypothesis (Hughes & Paramour 2004). Moreover, the mean relative sea level remains modest a 1.22-2.14 mm yr^{-1} in the region and some sites have experienced an increase in marsh elevation relative to the mean sea level increase (Cahoon *et al.* 2000; van der Wal & Pye 2004). The causes of erosion may therefore be more complex and a number of factors, both anthropogenic and natural, need to be considered. In many areas marshes have retreated laterally and other possible causes have been put forward, including sediment destabilisation by invertebrate bioturbation and herbivory (Hughes & Paramor 2004) and a documented increase erosive wave energy as a result of changing climatological conditions (van der Wal & Pye 2004).

The protection given to saltmarsh ecosystems under the EU Habitats Directive requires maintenance of key habitats in a 'favourable status' with a policy of *no net loss* placing governments under a legal obligation to implement measures for compensatory habitat restoration for areas lost to development. In addition, increasing recognition of threats posed by sea level rise and extreme weather events to the economic viability of maintaining hard engineered coastal defences, the UK has placed increasing emphasis on the concept of sustainable flood and erosion risk management in the form of managed realignment (hereafter, MR) (Doody 2013; Esteves 2014; French 2006). In the UK to date, over 50 MR schemes have been implemented through removal, breaching or realignment of hard defences to reintroduce tidal inundation usually onto formerly reclaimed saltmarsh (Esteves 2014). MR sites tend to be low in the tidal frame (French 2006), allowing them to become a sink for sediments, initiating the development of saltmarsh and thereby providing the dual function of cost-effective natural coastal protection through dissipation of wave energy and reinstatement of potentially valuable intertidal habitat.

In essence MR attempts to replicate artificially the process of landward migration of saltmarsh that would naturally develop in response to rising sea levels. However, the development of natural saltmarsh and that on managed realignment have very different starting points, with landward movement occurring in one large ‘jump’ over a large area of land at lower elevation, compared with the gradual natural adjustment along a natural profile (French 2006). The unnatural starting point may therefore require sufficient sediment accretion and morphological adjustment before saltmarsh and ecological functioning can be restored (French 2006). Consequently considerable uncertainty remains concerning the extent it is ever possible fully to restore functional equivalency with naturally developed saltmarshes. Surveys of older sites that have undergone an analogous process of saltmarsh reactivation from historical storm breaches of sea defences indicate plant communities on these sites often remain significantly different compared to their natural counterparts, even after a century of development (Garbutt & Wolters 2008; Mossman *et al*. 2012a). Such differences in vegetation development have important implications for achieving wider ecological functioning of these systems and bring into question the capacity of MR projects to satisfy the requirements the EU Habitats Directive (Mossman *et al.* 2012a). The prospects for improving adherence to the Directive in the future therefore depends on a fuller understanding of how to address the limitations to restoring functional equivalency. To date, the evidence indicates that the altered edaphic environment on restoration sites creates conditions of impeded drainage and excessive waterlogging and this factor is likely to be a key abiotic influence that explains the divergence of vegetation development on restored and natural marshes (Crooks *et al.* 2002; Davy *et al.* 2011).

1.3 Abiotic influences on vegetation zonation in saltmarshes

The specialised halophytes that occupy saltmarshes often exhibit discrete patterns of spatial zonation in species composition, productivity and distribution that generally follows a topographic elevational gradient extending from land to sea. The extent that this zonation represents the legacy of past succession is often unclear (Davy 2000). More relevant may be the influence of contemporary abiotic and biological factors in determining these patterns; this has been the subject of debate over a number of years and currently remains unresolved (Mahall & Park 1976; Pennings & Callaway 1992; Pennings *et al.* 2005; Snow & Vince 1984; Vince & Snow 1984). Since elevation in the tidal range determines the frequency and duration of tidal inundation and therefore is a primary determinant of edaphic gradients it is, however, widely accepted that salinity and waterlogging exert a major influence on the patterns of saltmarsh plant distribution (Adam 1990).

In general, plant communities in saltmarshes exhibit a gradient of species diversity from low to high elevation (Adam 1990), largely reflecting interspecific differences in physiological tolerances that determine the absolute limits of their distribution. However, from casual observation it is evident that many species have a broad distribution along an elevational gradient indicating a degree of tolerance to a range of abiotic conditions. This appears to be a consequence of the considerable variation in ecotypes exhibited by many species that have a wide distribution on saltmarshes (Jefferies 1977). While in general terms it is often possible to delineate species distributions in British saltmarshes based on the broad elevational categories of lower, mid and upper elevational zones, considerable heterogeneity in species abundances can often be found within these zones.

In many situations the interaction of tidal flooding with variations in sedimentology, microtopography and the complex network of drainage channels that are frequently an important feature of marsh geomorphology, creates large but localised variations in sediment drainage and waterlogging characteristics. Such effects may be superimposed on the wider influence of spatial and temporal variations in salinity to produce a complex mosaic of distributional patterns of vegetation that confound any straightforward interpretation in terms of elevational gradients (Cooper 1982; Silvestri *et al.* 2005). Patterns of zonal composition may also reflect the outcome of complex competitive interactions that are strongly modulated by the responses of species to the prevailing abiotic conditions (Adam 1990). The extent to which species distributions are determined directly by absolute limits to physiological tolerances or are modified by positive or negative inter-specific interactions remains poorly understood.

The exact physiological mechanisms that determine species distributions have not been determined largely because of the difficulty of disentangling environmental factors that are often inter-correlated. Although only a small number of studies have investigated the combined effects of salinity and waterlogging on a range of saltmarsh species, it widely assumed that while waterlogging plays an important role, variation in species tolerances to salinity is the main driver of saltmarsh zonation (Cooper 1982; Rozema *et al*. 1988). Nevertheless, for saltmarsh plants the two factors are inseparable since the ability of saltmarsh plants to tolerate waterlogging has important implications for root functioning particularly in relation to the capacity of plants to meet the high metabolic requirements associated with maintaining root osmotic potential in response to variations in sediment salinity (Colmer & Flowers 2008).

1.4 Waterlogging and low redox potentials

The development and persistence of sediment waterlogging can vary considerably both within and between saltmarshes as a consequence of the effects of frequency and duration of tidal inundations, and restricted drainage, collectively influenced by elevation, marsh topography and the physical characteristics of the sediment. The displacement of air contained in interstitial spaces and its replacement with water leads to severe inhibition of respiratory gas fluxes between the sediment-atmosphere interface as a consequence of the inherently low solubility of oxygen and carbon dioxide, and the major reduction in their aqueous diffusion coefficients $< 10^4$ relative to the gaseous phase (Armstrong 1975).

The rate of oxygen diffusion is influenced by temperature and sediment type since the oxygen status of the soil is largely governed by the proportion of soil pores filled with water relative to air filled porosity. Fine textured saltmarsh sediments with a high silt and clay content can support a relatively high volume of water and remain saturated for longer because of the intrinsically high water-holding capacity compared to coarse textured sediments with a high sand content (Bradley & Morris 1990; Rabenhorst 2001). However, the degree and extent of anaerobic soil volume is contingent on a balance between the rate of oxygen diffusion into the sediment; radial oxygen loss (ROL) and its consumption by both plant roots and microbial activity. Microbial respiration in sediments is influenced by the availability of labile organic matter and temperature, and where rates are sufficiently high, depletion of oxygen can occur in waterlogged sediments over a period of hours (Catallo 1999). This leads to the accumulation of carbon dioxide, organic acids and profound redox transformations of sediment biogeochemistry (Reddy & DeLaune 2008).

The severity of the anaerobic condition, as measured by the sediment redox potential, is a measure of electron activity from compounds in the sediment undergoing electron transfer from anaerobic microbial respiration. In the absence of oxygen, facultative and obligate anaerobic microorganisms use a variety of alternative terminal electron acceptors during electron transfer in respiration. This results in a change in the valence state to an extent that causes a transformation of the overall redox status of the sediment from an oxidised to a chemically reduced state (Bartlett 1981; 1998; Ponnamperuma 1972, 1984; Gambrell *et al.* 1991).

Reduction of saturated soils is a sequential process governed by laws of thermodynamics and mainly controlled by microbial respiration, which uses labile organic matter as an electron source in respiratory electron transport chains to extract energy via redox reactions (Ponnamperuma 1972, 1984; Gambrell *et al.* 1991). The electron affinity of an accepting oxidant is measured by its energy released when it accepts an electron and changes valency to a reduced state. Molecular oxygen generates the greatest free energy change when it accepts an electron and is reduced to water. Therefore, when oxygen is present in sediments, it will always be used as the preferred electron acceptor since its high reduction potential makes it a strong oxidising agent. In the absence of oxygen alternative less oxidising electron acceptors with smaller reduction potentials that progressively release less energy for each oxidised molecule of organic matter are sequentially used in the less efficient anaerobic respiration in the order:

$$NO_3^- > MnO_2 > Fe(OH)_3 > SO_4^{2-}$$

While the sediment chemistry of well drained and aerobic soils is dominated by oxidised compounds, waterlogged and anaerobic sediments are distinguished by a chemistry variously dominated by the accumulation of reduced ions: NH_4^+, Mn^{2+}, Fe^{2+}, S^{2-}/HS^-.

The relative amounts of reduction and oxidation are associated with changes to the sediment environment which has important implications for plant functioning and survival in terms oxygen stress, nutrient availability and phytotoxicity (Pezeshki 2001; Pezeshki & DeLaune 2012). Clearly, plants that are exposed to waterlogged sediments therefore not only require adaptations to survive oxygen deprivation, but also require mechanisms and strategies to deal with the additional stresses imposed by chemically reduced sediments.

The exchange of electrons between oxidised and reduced species creates a measurable electrical potential in the sediment – the redox potential. This parameter not only gives an indication of the aeration status of the sediment but also provides an indication of the intensity of the anaerobic condition that is determined by the degree of microbially mediated biogeochemical transformations that occur in the absence of oxygen (Fiedler *et al*. 2007; Reddy & DeLaune 2008). Although the oxygen status of sediments can be measured directly using amperometric or oxygen optode sensors (e.g. Colmer *et al.* 2013), measurement of redox potentials provides much more information about the anaerobic condition concerning factors likely to affect plant growth and survival in waterlogged soils than simply the concentration of oxygen alone. The importance of redox potential as a master variable analogous to pH has long been recognised in wetland ecology (Gillespie 1920; Pearsall 1938; Pearsall & Mortimer 1939). However, relatively few studies have attempted to elucidate its influence on the distribution of saltmarsh plant species (Armstrong *et al*. 1985; Castellanos *et al.* 1994; Castillo *et al*. 2000; Sanchez *et al.* 1998) and their physiological responses (Delaune & Pezeshki 1991; DeLaune *et al.* 1983; Howes *et al.* 1981; Mendelssohn & Morris 2000), consequently knowledge in this area remains limited.

A greater prevalence of low redox potentials in sediments of MR sites has been correlated with the differences in vegetation development relative to areas of natural marshes at equivalent elevations (Mossman *et al.* 2012b). MR sites may experience reduced drainage efficiency as a consequence of a lower density of drainage channels compared to natural saltmarshes (Nottage & Robertson 2005). In addition, newly accreting marine sediments on MR marshes occur on soils that were formerly saltmarsh sediments but may have a significantly restricted capacity for drainage as a consequence of the physicochemical transformations during their period under land claim (Crooks & Pye 2000; Hazelden & Boorman 2001; Tempest *et al.* 2015). The absence of an effective drainage system and altered soil structure can result in a decoupling of the relationship between elevation and waterlogging on MR sites (Davy *et al.* 2011). The imperfect correlation between sediment oxygenation and elevation, particularly at intermediate levels, allowed the quantification of their independent effects on species distributions. This demonstrated that, while some species are influenced by both elevation and redox potentials (e.g. *Elytrigia atherica*), other species are more influenced by redox potentials than elevation *per se* (e.g. *Atriplex portulacoides*, *Puccinellia maritima*, *Suaeda maritima*) (Davy *et al.* 2011). This suggests that the low elevations relative to the tidal frame that characterise many MR sites may not necessarily be the primary constraint for colonisation by a number of species rather than the prevailing redox status of the sediments. Species that are frequently abundant components on natural saltmarshes, such *as Armeria maritima*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima*, are often much less common on both recent MR sites and accidentally restored saltmarshes even after more than a century of development (Garbutt & Wolters 2008; Mossman *et al.* 2012a). On the other hand, the shrub *Atriplex portulacoides* was reported to be more abundant on these older sites and may inhibit colonisation by other species (Mossman *et al.* 2012a).

1.5 Thesis outline

Redox potentials in saltmarsh sediments have been shown to exhibit considerable variation both spatially and temporally in response to periodic tidal flooding and drainage (Armstrong *et al*. 1985; Catallo 1999). Measurements of redox potentials that are limited to intervals between data collection of days or weeks may fail to capture the ecologically relevant information in spatial and temporal variations of true redox conditions. A fuller understanding of the influence of redox potentials on the colonisation of restoration marshes requires measurements that can adequately capture the dynamic nature of this variable. To date, the most detailed understanding of saltmarsh redox dynamics is based on 32 sampling events over one season on a saltmarsh in the Humber estuary (Armstrong *et al.* 1985). This study demonstrated the large fluctuations in redox potentials can occur in well drained sediments reflecting the disappearance of oxygen and its subsequent replacement in response to cycles of tidal flooding and drainage.

No comparable measurements have been undertaken for sediments of restoration saltmarshes so there is little understanding of how redox behaviour and hence drainage characteristics differ from natural marshes. Furthermore, there is little available information on seasonal changes in redox potentials in saltmarsh sediments. Measurements are usually taken during the growing season but it is thought that the most adverse and persistent anoxic conditions can occur in winter when soils are most at their most saturated state (Crawford 2003). The limitations imposed by low redox potentials on colonising plants may be more significant at an earlier stage in the year than later in the growing season when plants may be physiologically better developed with the necessary mechanisms to escape such stresses (Crawford 2003).

Elucidation of the factors that influence the colonisation of intertidal sediments by halophyte species requires both measurements of long-term redox dynamics in sediments in response to cyclical patterns of flooding and drainage and also an experimental approach to gain a better understanding of response of individual species to the stresses imposed by low redox potentials. Plant survival under such conditions over prolonged periods usually necessitates specific adaptations that enable rhizosphere oxidation in order to acquire nutrients and avoid phytotoxicity from reduced substances (Armstrong *et al.* 1994; Vartapetian & Jackson 1997). In addition to oxygen deprivation, plants exposed to low redox potentials also experience a high demand for the oxygen of the rhizosphere created by highly reduced sediments (Pezeshki & DeLaune 2000). Such conditions necessitate adaptations that enables plants to maintain a balance between efficient internal transport of oxygen to the rhizosphere while limiting its diffusive loss from the rest of the root system (Armstrong *et al.* 1994; Pezeshki & DeLaune 2000). Saltmarsh species are likely to show considerable variation in their ability to oxygenate their rhizosphere and this could be an important determinant of their ability colonise a site. However, the response of the majority of species to prolonged anoxia and low redox potentials remains unknown.

This thesis was therefore undertaken to further our understanding of the limitations of halophyte colonisation on restoration saltmarshes. In particular, to investigate the influence of redox potential on a number of plant species that tend to be common components of natural saltmarsh communities but are frequently poor colonists on MR sites in the same geographical region. Specific objectives were:

1) To characterise in detail redox potential conditions in response to tidal flooding on a natural saltmarsh with relatively diverse plant communities;
2) To characterise redox potential conditions in response to tidal flooding on two contrasting MR sites;
3) To investigate the effect of artificially altering sediment drainage on redox potential and plant survival by experimental manipulations of microtopography;
4) To undertake laboratory based experimental investigations on the effect of redox potentials on the growth and physiological responses on saltmarsh plants.

The following provides an outline of the remaining chapters:

Chapter Two identifies and describes the locations where fieldwork was undertaken over the period 2011-2014. Natural and MR marshes at two locations on the North Norfolk coast, Stiffkey and Brancaster, and natural and MR marshes at Freiston Shore on the south Lincolnshire coast were the focus of this study. The sites on the North Norfolk coast are representative of the open coast and back barrier saltmarshes of this region. They were selected since they support a high abundance of halophyte species in communities at upper elevations that are scarce on MR sites. The MR sites are of similar age with tidal flows restored in 2002 but have different reclamation histories: Freiston Shore MR from 1982, formerly arable agriculture; Brancaster MR from the 1800s, formerly livestock grazing. Descriptions are also given of the target species that were the focus of subsequent chapters in this study: *Armeria maritima*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima*.

Chapter Three investigates redox potentials in natural saltmarshes of the North Norfolk coast. The main aim was record short-term and long-term redox conditions in sediments where the target species are abundant. Simultaneous measurements were made mainly in sediments of the general saltmarsh community and an adjacent creek bank community of the upper marsh. A smaller number of redox potential measurements was also taken from a low marsh community for comparison. Measurements were made using a total of 48 electrodes attached to a datalogger for continuous hourly sampling. The aim was gain insights into the drainage characteristics of sediments from changes in redox potentials over one or more spring-neap tidal cycles for periods ranging from about two weeks to four months. Measurements were undertaken on separate occasions from late summer to mid winter to investigate seasonal changes in redox potentials. The effect of precipitation on redox potentials is also examined from measurements taken during the unusually wet summer of 2012 and the unusually dry summer of 2013.

Chapter Four describes redox potentials on MR marshes at Brancaster and Freiston Shore. The aim was to characterise the redox conditions of sediments where the target species show a limited ability to colonise. Continuous hourly measurements were made with the datalogger over one or more spring-neap tidal cycles both early in the year and later in the growing season to investigate seasonal variations. Sampling at Brancaster MR was taken from a *Spartina anglica – Aster tripolium* (mainly var. *discoideus*) dominated low marsh site and a *Puccinellia maritima* dominated mid elevation site in close proximity to a drainage channel. Sampling at Freiston Shore MR was taken from a similar *Spartina anglica – Aster tripolium* (mainly var. *discoideus*) site, and *Atriplex portulacoides – Puccinellia maritima* dominated communities at a mid elevation site and a low elevation site close to a drainage channel. Measurements were also taken from an *Atriplex portulacoides* dominated natural marsh at Freiston for comparison.

Chapter 5 describes a field experiment to measure redox potentials in a series of 1 m^2 plots constructed on the Brancaster and Freiston Shore MR sites in which individuals of the 4 target species and *Atriplex portulacoides* were experimentally planted. An attempt was made to artificially alter sediment drainage conditions and hence the redox potentials by manipulating the microtopography (raising and lowering the surface elevation) of the plots. Twice monthly measurements of redox potentials during spring tide and neap tide phases were made from March 2012 to October 2013. Sampling was undertaken manually from 200 permanently installed electrodes. The aim of the experiment was to address the hypothesis that survival of the target species could be related to the average redox conditions that prevail on MR sites over the long-term rather short-term variations or exposure to low redox *per se*.

Chapter 6 describes the design of a laboratory experiment based on a hydroponic method for the long-term cultivation of plants under controlled low redox potentials using titanium(III) citrate as a non toxic redox buffer. The aim was to test the target species to the stresses imposed by anoxia and high oxygen demands at low redox potentials. Due to time constraints, experimentation was limited to two species. *Atriplex portulacoides* and *Triglochin maritima* were selected since they were expected to demonstrate sharply contrasting responses. An evaluation was made of the effects of differing redox potentials under controlled conditions on growth, photosynthetic functioning based on chlorophyll fluorescence parameters, root porosity and ADH activity. It was anticipated that insights gained from the abilities of plants to survive exposure to low redox potentials and the mechanisms they employ to tolerate or avoid this condition would contribute to understanding their limitations to establishment on restoration marshes.

Chapter 7 brings together the findings of the other chapters to discuss the implications of redox potentials for the establishment of halophytes on restoration saltmarshes. In addition, a number of recommendations are made for further research in this area.

CHAPTER 2: Sites and species studied

Continuous measurements of redox potential on natural marshes were taken simultaneously in sediments occupied by the two distinct types of vegetation communities that predominate the upper marshes of the North Norfolk coast. Initial measurements were taken from a site at Brancaster in 2012 (British Ordnance Survey National Grid Reference TF7773 4493) but, due to equipment disturbance, the majority of measurements (from 2013) were subsequently undertaken at a less accessible upper marsh site of similar vegetation composition at Stiffkey (TF9519 4408). A single set of measurements was also undertaken at an undrained low marsh site at Stiffkey (TF9619 4496). Measurements were taken from two managed realignment sites over the period 2011 – 2014. These sites were breached in 2002 but have very different reclamation histories. The first site is located at Brancaster (TF7679 4492) and the second at Freiston Shore, Lincolnshire (TF4068 4293).

2.1 Natural saltmarsh sites – Stiffkey and Brancaster

Figure 2.1. Stiffkey saltmarsh site showing distinct mosaic of general saltmarsh (GSM) communities (dark) and creek bank vegetation (light) in the upper marsh. Arrows indicate locations where continuous redox measurements were made in the upper and lower marshes (Image: Google Earth).

Both the Brancaster and Stiffkey sites are typical of the upper saltmarshes of the North Norfolk barrier coast. These comprise a 3000 ha belt up to 2 km wide extending some 40 km along the North Norfolk coast from Hunstanton to Weybourne. Saltmarshes of this coast appear to be of ancient origin as their inception has been found to coincide with periods of marine incursion in response to eustatic and isostatic fluctuations in sea levels that occurred during the Holocene transgression (Pethick 1980). Carbon dating of upper saltmarsh stratigraphy extending to depths of almost -7.0 ODN (Ordnance Datum Newlyn) at Stiffkey for example shows marine incursion occurred here some 4600 years ago and despite experiencing periods of regression much of the present day arrangement of major features of the marsh topography appears to have been established for over 4000 years (Funnell & Pearson 1984, 1989). The relative rate of sea level rise over the last 2800 years appears to have remained stable at 1.5 mm year^{-1} approximating the rate of crustal subsidence at 1-2 mm year^{-1} (Funnell & Pearson 1989). Historical evidence indicates that some of the current upper saltmarshes on this coast may have continuity dating back to over 2000 years (Pethick 1980) suggesting present patterns of zonation on these ancient saltmarshes may have remained more or less unchanged for a considerable period.

The saltmarsh systems of the North Norfolk coast in conjunction with extensive areas adjacent intertidal mudflats and sandflats together comprise a coastal ecosystem of nationally and internationally recognised conservation value. Its importance is reflected in the list of designations that include: Site of Special Scientific Interest (SSSI); Norfolk Coast Area of Outstanding Natural Beauty (AONB); Special Area of Conservation (SAC); and Special Protection Area (SPA) under the EU Habitats Directive and EU Birds Directive thus providing an important contribution to the Natura 2000 network of the EU's most important nature protected areas. It is also designated as a wetland of international importance on the Ramsar List and much of the area is designated as a UNESCO Biosphere Reserve.

The general topography of Stiffkey saltmarsh comprises two distinct areas separated by a shingle ridge that runs parallel to the coast. On the seaward side lies a submergence or low marsh (the undrained site) estimated to be less than a few hundred years old (Steers 1960) that has formed on a relatively open stretch of coast protected by a wide zone of intertidal sandflats and sand bars. The lower marsh is physiographically simple comprising of an area of well drained silt-clay consolidated accretions overlying sandy sediments interspersed with a high density of shallow drainage channels and pans containing unconsolidated sediments that frequently remain undrained. The lower limit of the vegetation on the low marsh extends between +2.50 to + 1.65 ODN (Smith 1985) and is flooded by most semi-diurnal tides each year. The better drained raised areas are variously colonised by *Aster tripolium*, *Atriplex portulacoides*, *Limonium vulgare*, *Puccinellia maritima*, *Salicornia europaea* agg., and *Suaeda maritima* while the undrained pools are predominantly colonised by *Spartina anglica*.

In contrast, the upper or emergence saltmarsh (the main measuring site) that lies on the landward side of the shingle ridge is a relatively ancient area of mature marsh with a complex physiography. Both this area and the Brancaster upper marsh are physically representative of the upper saltmarshes of the North Norfolk coast comprising a sinuous network of dendritic drainage creeks with slightly raised banks separating topographically flat interfluve areas interrupted to varying degrees by generally undrained salt pans and shallow depressions. Most of the high marsh is at an elevation of 2.5-3.0 m ODN (Smith 1985) and is flooded by higher spring tides.

The vegetation and physical form of the upper marshes on the North Norfolk coast have been described in detail elsewhere (Chapman 1938, 1939, 1960). One community type borders the edge of drainage creeks and variously ranges from less than one metre to several metres in width forming an abrupt transition to the adjacent very different type referred to as the general saltmarsh community (hereafter GSM) that forms topographically flat expanses of the inner marsh interspersed with numerous often undrained salt pans (Figure 2.2). The former is variously dominated by the perennial species *Atriplex portulacoides*, dense and vigorous stands of *Puccinellia maritima* and *Elytrigia artherica* interspersed with frequent *Artemisia maritima*, large growth forms of *Plantago maritima* and occasional individual plants of *Suaeda vera*. The GSM site at both the Stiffkey and Brancaster sites comprises a similar assemblage forming a relatively diverse community composed of plants with stunted morphologies of the perennials *Armeria maritima*, *Aster tripolium*, *Atriplex portulacoides*, *Limonium vulgare*, *Plantago maritima*, *Puccinellia maritima* and

Triglochin maritima and the annuals *Salicornia europaea* agg. and *Suaeda maritima* of similarly restricted growth form.

Figure 2.2. Stiffkey upper marsh GSM site with creek bank vegetation in background.

2.2 Managed realignment sites

2.2.1 Brancaster West Marsh

Figure 2.3. Brancaster Managed realignment site with locations where continuous redox measurements were taken in the lower marsh and the creek site (Image: Google Earth).

The Brancaster west marsh MR site (Figure 2.3) is located on the North Norfolk coast 20km west from Stiffkey Marsh. In contrast to the Freiston Shore which was claimed for agriculture for a relatively short period from 1983, this history of land-claim of this site was considerably longer dating from the 18th century when it was converted to freshwater grazing marsh (Jude *et al.* 2006). A decision was made by the U.K. Environment Agency to reintroduce tidal flooding in August 2002 to a 7.5 ha site of the marsh with the aim to protect the remaining area that was at increasing risk of being lost from ongoing erosion of sand dunes and the general poor condition of other flood defences along this part of the coast. Tidal flooding was enabled by the construction of a culvert within a lowered section of the sea wall that connects to a large drainage creek of the adjacent tidal marsh. In order to facilitate drainage of the MR site a series of branching linear creeks was excavated prior to flooding. The elevation of the site extended over a greater range that the Freiston Shore MR from 2.16-3.36 m ODN with the majority exceeding 2.4 m ODN (Mossman *et al*. 2012b).

The colonisation and development of vegetation communities in this site for the initial 5 year following the restoration of tidal flooding are described in detail by Mossman *et al.* (2012b). Similar to the Freiston Shore, site colonisation in the first year at this site was dominated by the annuals *Salicornia europaea* agg. and *Suaeda maritima* with a much lower abundance of eight perennials, including *Puccinellia maritima*, *Atriplex portulacoides* and *Triglochin maritima*. In the following year *Limonium vulgare* and *Spartina anglica* were recorded as present while *Plantago maritima* and *Armeria maritima* were recorded in the fourth year. After 5 years of colonisation 26 species were found to be present representing 76% of the local species pool and a higher number than found in mature reference saltmarshes. The higher number of species compared to Freiston Shore likely reflects differences in the availability of propagules, the larger elevational range and the differing substrates where aeolian derived sand from nearby dunes has an influence on a significant part of this site. However, like Freiston Shore the frequency and abundances remained substantially different from the natural reference marshes with large areas remaining unvegetated. Remarkably while *Puccinellia maritima* and *Atriplex portulacoides* were the two most abundant colonists at mid-high elevations and *Aster tripolium* and *Spartina anglica* at lower elevations at Freiston Shore by this stage, the Brancaster MR was still dominated by the annuals *Salicornia europaea* and *Suaeda maritima*.

More recently, much of site has been overtaken by a dense meadow of *Spartina anglica* and *Aster tripolium* at the lower elevations (Figure 2.5) while various combinations of *Atriplex portulacoides, Puccinellia maritima*, *Aster tripolium* and *Spartina anglica* to a lesser degree dominate much of the mid-upper elevations. *Limonium vulgare* and *Triglochin maritima* have also increased in parts of the low elevation site and are fairly abundant at higher elevations. Similarly to the plants occurring at Freiston Shore, the growth forms of *Limonium vulgare* and *Triglochin maritima* at Brancaster MR are also often found to be much larger and more robust than those in the GSM communities of the natural saltmarshes. The other notable similarity with Freiston Shore is the development of vegetation communities with a noticeable absence of *Plantago maritima* and *Armeria maritima*, both of which remain extremely scarce over almost the entire site.

2.2.2 Freiston Shore

Figure 2.4. Freiston Shore managed realignment showing locations where continuous redox measurements were taken in the upper marsh, lower marsh, low marsh creek site and the natural marsh (Image: Google Earth).

Freiston Shore is situated on the west margin of The Wash embayment (Figure 2.4). This embayment is a relatively low-energy coastal feature where fluvial deposition is minimal and instead acts as a sink for marine sedimentation which has accumulated in line with sea-level rise over the last 6500 years (Ke *et al.* 1996). Although there has been a long-term trend of general seaward advance around The Wash, saltmarsh near Freiston has shown considerable historical variation with retreat of 165 m to the north and seaward advance of 60 m to the south between 1971 and 1985 (Pye 1995) An extensive shallow intertidal zone fronts the MR forming a gradient ranging from a mudflat at 1.5 m ODN, an algal dominated upper mudflat up to 2.4 m ODN and saltmarsh to 3.3 m ODN (Freiss *et al.* 2012). The saltmarsh community distribution in the upper intertidal zone comprises *Salicornia europaea* agg. at the lowest extent transitioning to localised areas of *Spartina anglica-Aster tripolium* and communities dominated at the most landward extent by dense *Atriplex portulacoides*-*Aster tripolium* to the south of the site and *Puccinellia maritima* to the south.

The Freiston Shore managed realignment was one of the largest saltmarsh restoration sites to be created in the UK at the time of its inception in 2002. Prior to its realignment it was one of the last areas around the Wash to be claimed for arable cultivation in 1983 extending 300 m seaward beyond the former shoreline. Its extreme seaward position made the seawall vulnerable to erosion (Doody 2012) since the narrow strip of saltmarsh fronting the embankment, composed of less well developed vegetation, primarily of low marsh and pioneer zone species, is not sufficient to effectively attenuate incoming wave energy, compared to the more mature and extensive marshes to the north and south. Coastal defences were thus realigned back to an earlier position and tidal flooding was reintroduced to 66 ha of formerly arable land in August 2002 (Nottage & Robertson 2005). In addition to creating a sustainable flood defence, the aim of the project was also to restore a saltmarsh community of botanical value and provide a suitable habitat for invertebrates and birds. Prior to inundation an attempt was made to recreate the primary drainage channels that were infilled during land claim with the expectation that a network of secondary and tertiary channels would develop naturally over time. However, to date these have generally failed to materialise and the MR site currently has a much lower creek density than the relatively dense dendritic network that characterises the adjacent natural marsh. The elevation of the site extends over a landward-seaward gradient of 3.3 m – 2.7 m ODN so the entire site lies at elevations suitable for vegetation colonisation (Brown 2008). Numerical modelling indicated the site would fully inundate 150 times a year, with 50% of the site inundating 467 times a year, allowing the development of mid to upper marsh communities (just above MHW) (Nottage & Robertson 2005).

Detailed annual surveys for the initial 5 years of vegetation colonisation were undertaken by Brown (2008). Following initial colonisation by the pioneering annuals *Salicornia europaea* agg. and *Suaeda maritima*, different patterns of colonisation by perennial vegetation generally followed three elevation ranges. At the upper elevation category (3.16 – 3.3 m ODN) *Puccinellia maritima* rapidly replaced the annuals after 2003 followed by *Atriplex portulacoides*. By 2007 *Puccinellia maritima* was dominant and *Atriplex portulacoides* was the second most abundant but much lower level than in the nearby natural marsh, where it predominant at equivalent elevations. *Puccinellia maritima* again dominated at intermediate elevations (3.0 – 3.15 m ODN) by 2007 with *Atriplex portulacoides* occurring at lower abundances than the upper elevation areas. Lower elevations (2.7 – 2.99 m ODN) on both the natural marsh and the MR are colonised mainly by *Aster tripolium* and *Spartina anglica* but with lower abundances of the latter after 5 years of colonisation. During this short time, 16 out of the 17 species found on the outside of the realignment were found inside the MR and all common and abundant species on the natural marsh were also common and abundant in the MR (Brown 2008).

Observations over the following years in the current study, indicate *Atriplex portulacoides* cover has increased considerably in parts of the upper marsh to similar levels as those in the natural marsh although *Puccinellia maritima* continues to persist over large areas in a vigorous and highly stoloniferous form. At lower elevations *Spartina anglica* has continued to spread where it occurs as dense monoculture in large areas while in other places it is co-dominant with *Aster tripolium* resembling the vegetation occurring at lower elevations on the natural marsh. *Puccinellia maritima* still dominates much of the intermediate elevations and there are signs that *Atriplex portulacoides* continues spread here, albeit at a slower rate, while in other areas *Spartina anglica* and *Aster tripolium* have become more abundant at this elevation.

Some species that are abundant on the upper marshes on the North Norfolk coast are generally uncommon or absent on the natural marsh at Freiston shore. Interestingly, there is evidence of an increase in diversity at the mid to upper elevations, where large and vigorous individual examples of *Limonium vulgare* and *Triglochin maritima* are increasingly encountered. A number of large *Plantago maritima* plants has also established near the base of the new embankment but has not been encountered further inside the MR. There is no evidence of *Armeria maritima* colonisation on any part of the MR.

Figure 2.5. Low marsh on Brancaster (top) and Freiston Shore (bottom) managed realignment sites dominated by similar *Spartina anglica -Aster tripolium* communities which occupy substantial areas of these restoration marshes.

2.3 The target plant species

The species focused on in this study, *Armeria maritima*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima* comprise major components of the GSM communities (British Plant Community SM13c sub-community; Rodwell 2000) typical of the upper saltmarshes of the North Norfolk coast. In this area the term GSM refers to a distinctive herb dominated community where neither grasses nor any other species occur as a clear dominant (Chapman 1960).

2.3.1 *Triglochin maritima* L. (Juncaginaceae)

A detailed description of *Triglochin* is provided by Bishop & Davy (1991) which is summarised here. The distribution of *Triglochin* is more or less continuous on saltmarsh around the coast of Britain. There is some evidence indicating ecotypic variation in populations sampled as seed from different parts of Stiffkey saltmarsh which showed significant differences in growth under uniform glasshouse conditions (Jefferies 1977). Although the centre of its distribution is on the upper elevation GSM communities on north Norfolk with less than 360 submergences its distribution has a wide elevational range estimated at 3.1-2.4 m ODN on marshes of Scolt Head Island (Chapman 1960). It is most abundant on wetter areas of the marsh on generally saturated silt-clay sediments and appears to tolerate a range of substrates. *Triglochin* has been recorded as present with a high frequency in nine types of British Plant Community (Rodwell 2000) generally representative of upper marsh elevations including the general saltmarsh SM13c sub-community in where it prominent amongst the more vigorous vegetation fringing salt pans. However, it is commonly found in communities at lower elevations where it is present at reduced abundances in a wide range of other associations including well drained *Atriplex portulacoides* (SM14a) communities, the relatively poorly drained transitional low marsh communities (SM10) and in communities generally associated with more anoxia tolerant species such as those dominated by *Aster tripolium* (SM12) stands and the *Puccinellia maritima – Spartina anglica* (SM13f) sub-community (Davy & Bishop 1991).

Information about interactions with other species under conditions of flooding is scant. However, Groenendijk *et al.* (1987) reported niche differentiation and one-sided interference competition between *Triglochin* and *Plantago maritima* enabled coexistence at varying equilibrium frequencies that developed under different flooding regimes. *Triglochin* increased cover at the expense of *Plantago maritima* in experimental transplants of mixed stands sited at lower elevations while the reverse was true at higher elevations. A degree of tolerance to lower marsh conditions was also demonstrated by the increases in abundance of *Triglochin* that resulted from an artificial increase in tidal range from coastal barrage construction (Beeftink 1979).

Plants on the lower marsh often have a taller and more robust morphology than plants suggesting the low growth rates in *Triglochin* plants in the GSM is likely to be an adaptation to high salinity (Jefferies 1977). Investigations into the combined effects of experimental waterlogging and salinity found that productivity (dry mass yield) was greatly reduced in saline compared to non-saline waterlogging (Cooper 1982; Rozema *et al.* 1985b) but neither reported a lethal effect from prolonged waterlogging for the 8-10 week duration of these experiments. Evidence for tolerance to waterlogging is further supported by the presence of aerenchyma in the rhizomes and roots of *Triglochin* that was first described in detail by Hill (1900) and the volume of fractional porosity was later shown to be extensive (Justin and Armstrong 1987; Rozema *et al.* 1985b). A degree of sensitivity to low redox potentials was suggested by a significant reduction in shoot and root dry weight yield in response to elevated concentrations of Mn^{2+} (10 mM l^{-1}) in saline water culture (Cooper 1984).

Given the ability of *Triglochin* to tolerate prolonged periods of waterlogging, it is unclear why it is an infrequent coloniser of many restoration saltmarshes. This is an important consideration since a noteworthy feature of *Triglochin* is the possibly significant ecological role it plays in community development by facilitating colonisation of other species. Heslop-Harrison & Heslop-Harrison (1958) and Fogel *et al.* (2004) reported from observations and experimental work that *Triglochin* was able to modify its local physical environment by creating elevated rings that effectively ameliorate waterlogging stress and support, through a process of succession, an increase in species diversity compared to nearly bare adjacent substrate. This suggests the possibility that *Triglochin* may share a similar functional role with

Puccinellia maritima (Langlois *et al* 2003) as a pioneer coloniser in some circumstances.

2.3.2 *Limonium vulgare* Miller (Plumbaginaceae)

Limonium vulgare is the commonest of three *Limonium* species found in saltmarshes around the coasts of Britain and Ireland northwards to southern Scotland although it does not grow on coasts of Ireland where it is replaced by *Limonium humile*. It occurs on some marshes mixed with the closely related and morphologically similar *L. humile* where hybridisation may be extensive in some locations (Dawson & Ingrouille 1995). Populations on the East Anglian coast appear to be mainly pure *L. vulgare* and are morphologically distinct from *L. humile*, indicating little evidence of widespread hybridisation and introgression between these species on the saltmarshes of the North Norfolk coast (Dawson & Ingrouille 1995).

Detailed descriptions of the morphology are given by Boorman (1966, 1967) and Dawson & Ingrouile (1995). The rosette leaves are attached to a stout branched woody stock with a deep taproot and short laterals; horizontal rhizomes up to 5 cm length run a few centimetres below the surface that can develop new rosettes and taproots that can grow independently of the parent plant. Flowers are insect pollinated and flowering extends from late June to the beginning of October reaching a peak in the second half of July.

L. vulgare is highly variable, although Dawson & Ingrouille (1995) suggest this variability is unlikely to be environmentally determined. Boorman (1966) used comparative cultivation and transplant experiments to demonstrate that some of the variation is genotypic origin and this variation tends to be emphasised in marshes because of the extent of clonal reproduction and rhizomatous spread. Thus large homogeneous patches with distinct morphologies are found growing next to each other.

The most important factor determining its distribution on saltmarshes of the North Norfolk coast is a tidal range of approximately 1 m, as determined in a number of different sites (Boorman 1967). Chapman (1938) for example, gives the absolute range of *L. vulgare* at Scolt Head Island at 2.40 – 3.11 m ODN. In eastern England *L. humile* is a much a rarer species restricted to a narrow zone in the middle range of the *L. vulgare* (Boorman 1966, 1967). Although *L. humile* was found to be more frequent on the lower marsh at Scolt Head Island than *L. vulgare* there is no evidence that *L. humile* is more tolerant of submersion and waterlogging. Both species are plants of the mid to upper marsh although *L. vulgare* has a wider amplitude and occurs at higher and lower elevations than *L. humile* and the lower limit of *L. humile* is well above *L. vulgare* (Boorman 1966, 1967).

Seed production in an area dominated by *L. vulgare* was estimated to be 700 m^{-2} (Boorman 1967). Seeds are dispersed by a persistent calyx and can be transported by sea currents, although laboratory studies have shown that they are unable to float for more than a few days this would still be sufficient for them to be transported considerable distances (Boorman 1967). Germination and survival of seedlings was found to be highest and occurred much more rapidly in freshwater after seeds were pre-treated with saline water suggesting establishment by seed depends on the occurrence of a sufficient period of non-flooding low neap tides coinciding with high rainfall. It was suggested that this factor could be an important determinant of the vertical range of *L. vulgare* colonisation. Germination was found to be possible under hypoxic (2.5% O_2) conditions after 45 days in saltwater but at much reduced rates; germination was achieved in anoxic conditions (0% O_2) in freshwater after 25 days (Boorman 1968, 1971).

L. vulgare seedlings have been shown to sustain growth for at least one month in glasshouse conditions of experimental waterlogging. and for at least 3 months in one-year-old plants, while higher growth rates were achieved under partial waterlogging to a depth of 5cm (Boorman 1971). Growth was least in waterlogging under full strength seawater compared to 50% seawater. Interestingly Boorman (1971) noted that, although both high water table and salinity seriously decrease seedling growth, water table was found to be more critical than salinity in restricting growth. However, this experiment was conducted under conditions of a static water table and the effect of fluctuating conditions is difficult to predict from such an experiment. Enhanced root porosity development under conditions of waterlogging was reported Justin &

Armstrong (1987), indicating that *L. vulgare* possesses the anatomical capacity for avoidance of a degree of anoxia under waterlogged conditions.

2.3.3 *Plantago maritima* (Plantaginaceae)

Plantago maritima is a herbaceous plant with dense rosette of stemless leaves of 2-22cm long and an inflorescence of small green-brown wind-pollinated flowers produced on a dense spike 0.5-10 cm long on top of a stem 3-20 cm tall. It is a widely distributed species of the mid to upper marsh found more or less continuously around the coast of Britain occurring on mid to upper saltmarshes and also sea cliffs (Gregor 1938, 1939).

Like other plants that make up the GSM, *Plantago*demonstrates a high degree of variation in morphological characteristics in terms of plant size and growth form (erect / prostrate) and reproductive characteristics (generative / vegetative). Intraspecific variation in *Plantago* has been studied extensively (Gregor 1939; Gregor & Lang 1950). Variation based on edaphic ecotypes was demonstrated to be present in coastal populations of *Plantago* (Gregor 1939). These were distinguished at the extreme ends of an ecocline of intraspecific variation in characters such as density of spike, habit of growth, length of scape which varied genetically within a coastal region differentiated by plants growing under poor drainage conditions of a saltmarsh or waterlogged mud and those growing under better drained coastal sites. Evidence for ecotypic differentiation was demonstrated between the dwarf and prostrate morphologies of populations of *Plantago* and other species in the GSM and populations from different parts of the low marsh site on the Stiffkey saltmarsh (Jefferies 1977). Plants from GSM and the lower marsh populations grown under uniform glasshouse conditions in response to nitrogen additions showed differences in growth indicating a significant degree of genetic differentiation between the two populations. This ecotypic differentiation was interpreted as an adaptive response to adverse edaphic conditions of drought and hypersalinity rather than waterlogging. It was found that the progeny of *Plantago* from these two populations contained both prostrate and upright forms suggesting that selection operates at the seedling stage so that one form predominates at each site. It was thought that high rates of clonal vegetative spread of *Plantago* in the GSM site and differences in flowering phenology between plants in the upper and lower marsh could limit gene flow between the two populations sufficient to maintain separate ecotypes. Jerling (1985) also reported genetic differentiation in fitness related characters in *Plantago* along gradients of both grazing intensity and flooding frequency.

Although there is evidence of genetic differentiation in some physiological processes and the existence of ecotypes in separate areas, later work by Gregor (1956) indicated that differences in morphological characters between *Plantago* plants in close proximity at different microtographical settings in the upper marsh were due to phenotypic plasticity. Morphological and reproductive differences in *Plantago* were also attributed to phenotypic plasticity in more widely separated populations of *Plantago* on a saltmarsh in the Netherlands (Blom 1983). This study described a prostrate form with higher rates of clonal reproduction on a site that received more frequent tidal flooding compared to an erect form with higher rates of generative reproduction that flooded only on the highest spring tides. Differences were attributed to seedling emergence which was found to be inhibited by higher salinities in more frequently flooded site and vegetative reproduction was found increase under saline conditions.

Experimental treatments of increased salinity and waterlogging severely limited growth in *Plantago* from upper marsh populations while no indication was given for lethality indicating a degree of tolerance to waterlogging for at least the 2 month duration of the experiment (Cooper 1982). Tolerance to oxygen stress is also inferred from the capacity of *Plantago* to produce a similar volume of aerenchyma to *Triglochin maritima* (Justin & Armstrong 1987) while Rozema *et al.* (1985b) reported both an increase in root porosity under waterlogged conditions and a degree of tolerance to Mn^{2+} and Fe^{2+} treatments. Anoxia inhibition of electrogenic pumps in root cells may adversely affect cell membrane potential in *Plantago* suggesting oxygen stress may limit its capacity to tolerate salinity (De Boer *et al.* 1983).

2.3.4 *Armeria maritima* (Plumbaginaceae)

A detailed description of *Ameria maritima* is provided by Woodell & Dale (1993). Highly variable morphology, growth rates and flowering both within and between populations exhibiting both genetic differentiation with several ecotypes described and phenotypic plasticity (Coulaud *et al*. 1999; Philipp 1974; Philipp *et al*. 1992). Four races of *Armeria* sub-species have been described in Britain of which two occur on saltmarshes (Baker 1953). One is restricted to the saltmarshes of the Shetland Islands while the other has a widespread distribution on western European saltmarshes. The form of *Armeria* was found to vary considerably between habitats in a detailed study of populations of *Armeria* on Scolt Head Island (Preston 1981). In saltmarsh populations leaf production was considerable reduced and the biomass only a quarter of that compared to dune populations. Some of these differences in morphology were retained when grown under glasshouse conditions indicating a degree of ecotypic differentiation.

Armeria is able to tolerate extreme exposure to severe winds, extreme drought, high salinities and high concentrations of calcium, magnesium and heavy metals (Woodell & Dale 1993). The occurrence on saltmarshes was found to be limited to the narrowest elevational range of all species of around 10 cm in a total range of 2 m in Scolt Head Island (Chapman 1960) and this is reflected in its generally restricted distribution to the GSM communities on the upper saltmarshes of the North Norfolk coast. The low growth habit and slow rates of growth limits its tolerance to shade which probably explains its general exclusion from the areas tall growing vegetation that dominates the banks of drainage creeks in the upper marsh. In general it appears at greater abundances on sites where taller growing vegetation is suppressed by edaphic conditions on saltmarshes likely related to higher levels salinity during the growing season (Jefferies 1977). Competition studies between *Armeria* and a common species of saltmarsh grass, *Festuca rubra*, showed the former had a competitive advantage in treatments with salt water (Goldsmith 1978). In other locations it occurs in varying abundances in communities ranging from transitional low-marsh vegetation (SM10) to high elevation *Inula crithmoides* marsh (SM26) (Woodell & Dale 1993). However, it is particularly abundant on *Puccinellia maritima* dominated (SM13) and all SM13 sub-communities which represent the most widespread and extensive perennial community of British saltmarshes. Reproduction is normally by seed and vegetative propagation is uncommon although it may occur on saltmarshes when ramets form adventitious roots (Woodell & Dale 1993). Colonies of *Armeria* can produce high numbers of viable seeds (> 10 000 m^{-2}) and it was reported to be a rapid coloniser of suitable bare areas in an experiment on saltmarshes of Scolt Head Island (Woodell & Dale 1993).

Growth of *Armeria* in experimental conditions of waterlogging and salinity treatments was found to be considerably restricted (Cooper 1982; Rozema *et al*. 1985a) although there no indication given that conditions of experimental waterlogging of up to 2 months was lethal. There is little information on the presence of root aerenchyma in *Armeria* although Rozema *et al*. (1985a) measured low porosity for the smaller adventitious roots in response to waterlogging while porosity of the tap root was not reported.

CHAPTER 3: Redox characteristics of natural saltmarshes

3.1 Aims

The redox characteristics of saltmarsh sediments may be influenced by a number of drivers operating at different timescales. These can occur in the form of short-term changes in response to photosynthetic activity, periodic hydrological fluctuations and seasonal temperature variations, all of which have yet to be fully described for saltmarshes of the North Norfolk coast. The aim of the current study was to improve the current understanding of the dynamic nature of redox potentials by using a datalogger to continuously measure hourly changes in redox potentials in sediments different from vegetation communities on natural saltmarshes. Rather than providing a detailed analysis of the various possible influences on redox dynamics in saltmarsh sediments, which is beyond the scope of the current study, the aim was to provide a descriptive assessment of spatial and temporal patterns of Eh in response to cycles of tidal flooding and drainage in greater detail than has previously been attempted.

Detailed measurements were therefore undertaken in order to attempt to describe the dynamic nature of Eh and by inference the variable oxygen status prevalent in natural saltmarsh sediments that support a range of perennial halophyte species a number of which tend to be uncommon or absent on many restoration saltmarshes (Garbutt & Wolters 2008; Mossman *et al*. 2012). It was hoped that the information acquired would assist in elucidating possible explanations for the patterns of vegetation establishment on restoration saltmarshes which frequently differ from nearby natural saltmarshes. In particular, to provide a baseline description of redox conditions on natural saltmarshes for use in further analyses to investigate if limited colonisation could be attributed to differences in the sediment redox conditions.

3.2 Introduction

The depletion of oxygen that is often a consequence of waterlogging can initiate a sequence of profound changes to the oxidation-reduction (redox) status of the sediment by increasing the speciation and solubility of various elements thus promoting a transformation of the sediment from an aerobic and a chemically oxidised state to an anaerobic and chemically reduced state (Gambrell *et al.* 1991; Ponnamperuma 1972, 1984) The severity of the anaerobic condition is commonly obtained by measurements of redox potential (Eh) as an indication of the degree of electron activity from compounds in the sediment undergoing electron transfer from anaerobic microbial respiration. The relative amount of reduction and oxidation is associated with important changes to the sediment environment in ways that have been shown experimentally to have variable affects on wetland plant functioning and survival in terms of its influence on oxygen stress, nutrient availability and phytotoxicity (Pezeshki 2001; Pezeshki & DeLaune 2012).

A number of previous studies have attributed intraspecific and interspecific variation in responses to low redox potentials as explanations for the occurrence of zonal patterns in plant productivity and species distribution respectively. The existence of two distinct growth forms (tall/short) of *Spartina alterniflora* dominating the Atlantic coastal marshes of North America, for example, has received particular attention and has been linked both in the field and experimentally to site specific differences in prevailing redox conditions (DeLaune *et al*. 1983; Howes *et al*. 1981; Linthurst & Seneca 1981; Mendelssohn & Morris 2000; Mendelssohn & Seneca 1980; Wiegert *et al*. 1983). More generally, a number of studies have reported a correlation between the intensity of the anaerobic condition from measurements of redox potential in the field and the distribution of plant communities in both natural and restoration saltmarshes (Armstrong *et al*. 1985; Davy *et al*. 2011; de la Cruz *et al*. 1989; Sánchez *et al*. 1998).

Measurements of Eh in the field are commonly based on single time interval readings of the voltage difference between a platinum-tipped measuring electrode and a reference electrode using a handheld voltmeter. However, this manual method suffers from a number of limitations. Newly installed electrodes often show variable drift in mV values before more or less stable readings are taken which limits the time available for sampling opportunities. Moreover, there is no standard protocol for sampling and a wide range of times required for electrode "equilibration" has been reported varying from 5-10 minutes (Ponnamperuma 1972), overnight (Patrick *et al.* 1996), 24-48 hours (Hunting & van der Geest 2011) and 90-200 hours (Eshel & Benin 2002). Probably the main constraint in using manual measurements is the limited ability of this method to adequately characterise the dynamic nature of redox potentials in natural systems that can show large variations both spatially and temporally over hourly to seasonal time scales in response to changing hydrological regimes and temperatures (Fiedler *at al.* 2007; Rabenhorst *et al.* 2009; Shoemaker *et al.* 2012; Unger *et al.* 2008). Although the installation of permanent electrodes is more likely to provide a greater degree of accuracy, manual methods will always be impractical in remote locations and at inaccessible times when a site experiences periodic flooding. Consequently sampling using this approach is usually limited to intervals between data collection of days or weeks that may fail to capture the ecologically relevant information in spatial and temporal variations of true redox conditions.

This is a particularly relevant consideration in systems that experience fluctuating hydrological conditions that often demonstrate rapid changes in Eh largely driven by dynamic microbial metabolic activities. Changes of 50 mV for example can correspond to important biogeochemical changes involving the processing of organic matter, nutrient availability and sulphate reduction (Reddy & DeLaune 2008). The development of anoxic conditions on flooding and certain biogeochemical processes can occur rapidly, such as the onset of denitrification that was recorded to occur in the order of 15-50 h in sediments (Reddy *et al.* 1976). Furthermore, rates of nitrogen loss from denitritication can be signficantly enhanced in soils undergoing frequent oscillations between aerobic and anaerobic conditions (Reddy & Patrick 1975).

Currently, the most detailed understanding of redox dynamics on saltmarshes is based on monitoring undertaken by Armstrong *et al.* (1985) in the Humber estuary which was mainly acquired by manual measurements (32 sampling occasions over 4 months) during the summer of 1979. This was supplemented by a six week period of daily Eh measurements using an early version of a redox datalogger (Armstrong & Wright

1976) which was able to demonstrate that sediments can experience rapid changes in aeration status in response to variations in tidal hydrology. More recent use of a redox datalogger for continuous hourly recording over 12 days showed rapid and significant changes sufficient to alter biogeochemical processes were possible on hourly and diel scales (e.g. 40-300 mV/12 h) in response to cycles of flooding and drainage on saltmarsh sediments experiencing diurnal tides (Catallo 1999). Large changes measured using similar methodology (600 mV/36 h) were also reported in response to tidal flooding over a 2 month summer period in coastal marshes by LaRiviere *et al.* (2004).

Although the number of studies that have used continuous measurements to characterise spatial and temporal redox dynamics in saltmarsh sediments is small, results from studies undertaken in other systems using this method to record Eh at high sampling frequencies have reported a wide range of fluctuations that are not always clearly associated with hydrological conditions. Large fluctuations documented in experimental mesocosms, for example, were attributed more to soil temperature changes than to changing hydrologic regimes (Vorenhout *et al.* 2004) while Shoemaker *et al.* (2012) reported strong diel fluxes of 100mV from field sampling in aquatic systems and mesocosms that appeared to be correlated with both temperature and photosynthetic activity. The influence of the latter was reported in other studies using redox datalogging that recorded similarly large diel Eh fluctuations which were also associated with diurnal patterns of photosysnthesis (Flessa 1994; Nikolausz *et al.* 2008).

3.3 Theoretical considerations

Detailed descriptions of theoretical aspects of redox potentials relevant to saltmarsh sediments are provided elsewhere and are summarised here (Bohn *et al.* 2001; Fiedler *et al.* 2007; DeLaune & Reddy 2005; McBride 1994; Rabenhorst 2009; Reddy & DeLaune 2008; Vepraskas & Faulkner 2001).

Measurements of redox potentials provide an indication of the intensity of reduction between redox couples which is equivalent to the electron activity based on the relative concentrations/activities of oxidants (oxygen, nitrate, nitrite, manganese and iron oxides and sulphate) and reductants (various organic substrates and reduced compounds). This electron activity is a consequence of microbial oxidation of organic matter in the absence of oxygen that results in the release of electrons which creates an electron pressure or intensity that increases as alternative electron acceptors are sequentially reduced. Thus highly reduced sediments that occur under sulphate reduction have a high electron pressure or high reduction intensity relative to that created by nitrate reduction, for example. Measurement of the redox potential of the sediment gives an indication availability of free electrons and therefore the relative oxidising or reducing tendency of the system.

Measurements of redox potential are made by quantifying gradients of electron pressure in units of electromotive force (emf) or millivolts (mV) in order to provide an indication of the reduction (or oxidation) intensity of the sediment. The measured electrode potential can be related to the redox potential value calculated from the Nernst equation that is intrinsic to the reduction reaction of particular redox couple based on the free energy and electron activity at equilibrium. This relationship demonstrates how redox potential values increase with increasing activity of the oxidised component and decrease with increasing activity of the reducing component. In this sense, Eh provides a measure of the availability of electrons in a solution in a way that is analogous to pH, which is a measure of proton availability in a solution. Accordingly, oxidised sediments are depleted of electrons while reduced sediments are rich in electrons since, in the latter, the proportion of each redox couple present in its reduced state will be greater.

The standard redox potential (Eh) of a sediment is determined by measuring its electron affinity using an inert platinum electrode as an electrical potential difference relative to a reference electrode of a known potential. Microbial oxidation of organic matter results in transfer of electrons to a suitable oxidant resulting in the solution having a definite electron pressure that reflects the intensity with which these electrons are transferred to the acceptor. The inert electrode provides a surface onto which electrons flow at the same electron pressure in the soil solution which can be measured by determining the voltage required to neutralise the charge on the platinum electrode. Waterlogged sediments that are depleted of oxygen generally have low redox potentials.

However, since Eh is also governed by the concentrations of reductants, this means that final value relates to the dominant redox couple that is present in excess concentration relative to other redox couples. Minerogenic saltmarsh sediments tend to be highly poised (i.e. buffered) by high concentrations of manganese, iron and sulphate (Reddy & DeLaune 2008). Since the reduction of these oxidants is assumed to attain a steady state that approximates Nernst equilibria in highly reduced sediments, measured Eh values are considered to provide a reasonable qualitative indication of the presence of the dominant reduced forms of reversible redox couples under such conditions (Bohn *et al*. 2001; Reddy & DeLaune 2008).

The utility of using platinum electrodes as a relatively simple and straightforward method for acquiring field Eh measurements that can be used as an operational parameter to assess the intensity of the anaerobic condition and characterise the oxidation-reduction status of wetland soils has long been recognized (Gillespie 1920; Pearsall 1938; Pearsall & Mortimer 1939) and remains widely used as a valid parameter (Fiedler *et al*. 2007; Rabenhorst *et al*. 2009; Reddy & DeLaune 2008). At a minimum Eh provides a quantitative measure of the range of Eh values indicating the presence of processes affecting the fate of nutrients and potentially phytotoxic inorganic and organic compounds.

The redox values in wetland soils that experience periodic waterlogging and drainage range between Eh +700 to -300 mV, with progressively negative values indicating more intense anaerobic conditions with increasingly higher electron activity (i.e. electron pressure). Measurements of Eh values obtained in the field can be compared with the critical redox potential values derived from the Nernst equilibrium potential for individual chemical species in its oxidised state to become unstable for a qualitative indication of the redox status of sediments. Thus the critical value at which oxygen becomes thermodynamically unstable is +300 mV (Ponnamperuma 1972, 1984) and soils are generally considered aerobic at values between +400 to +700 mV (Patrick *et al*. 1996; Reddy & DeLaune 2008). The critical redox potentials for the most important redox couples in wetland soils at roughly neutral pH are shown in Figure 3.1.

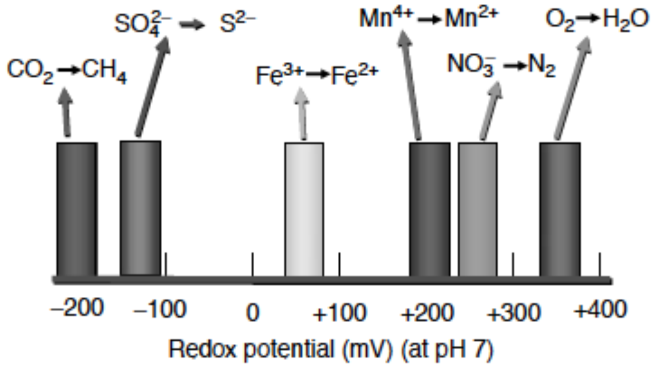


Figure 3.1. Critical redox potential at which oxidised inorganic redox systems begin to undergo reduction in wetland soils (Reddy & Delaune 2008)

3.4 Methods

3.4.1 Equipment specification

Measurements of redox potential were made in the field using a multichannel redox datalogger (HYPNOS III, MVH Consult, Utrecht, The Netherlands) automated for continuous *in situ* monitoring. The instrument incorporates a control unit, a multiplexer, storage memory, Eh, reference and temperature probes into a single unit. (Figure 3.2). (For further technical details see Vorenhout *et al*. 2004, 2011) The datalogger has a high input impedance > 2 T Ohms (Ω) and measuring range ±1000 mV and is simultaneously able to measure 48 redox potentials at pre-set intervals of 1-60 minutes, with data stored on 2 GB SD card. A 9 volt battery pack allowed the datalogger to be deployed in the field for up to 6 months of continuous measurements. In order to make the datalogger able withstand tidal submersion, the unit was modified by the addition of a second IP68-rated polycarbonate enclosure (Model: H10086H-6P, Bernstein Ltd, Aldridge UK). As a precaution to prevent any possibility of moisture contamination, the inner enclosure was encased in a re-enterable potting gel (MagicGel, RayTech SRL, Milan, Italy) which provides > IP68 protection against moisture ingress. A portion of the gel can be removed to access the datalogger after recording and resealed with the gel again.

Platinum tipped electrodes were constructed based on established designs (Mueller *et al*. 1985; Faulkner *et al*. 1989), using 10 mm length of 24 gauge (0.5 mm diameter) platinum wire (99.95% purity /hard tempered, Advent metals, UK) soldered to one end of a 300 mm (3.2 mm diameter) brass alloy rod. A 400 mm length of 3mm diameter insulated copper cable was soldered to the other end of the rod for connection to the longer 4-core signal cables attached to the datalogger. To prevent moisture contamination the soldered junctions were sealed with three layers of marine grade epoxy-ceramic barrier resin (Reactive Resins, Bodmin, UK) and the entire electrode body was encapsulated with adhesive heat shrink tubing leaving exposed approximately a 3 mm section of platinum tip. The platinum tips were polished with an abrasive fiberglass pen to remove any oxidised layer and tested for correct functioning both before and after installation in the field using quinhydrone redox buffers at pH 4 and pH 7 (HM Digital, Culver City, USA) and also plain tap water. These were deemed to be acceptable if they were within the range of ±10 mV.

Electrodes were soldered in groups of four to a 10 m length of 4-core screened signal cable (Unistrand, UK). Exposed joins between electrode cable and signal cable were sealed with marine grade epoxy resin and contained in a 100mm section of 15mm diameter PVC tubing into which additional resin was poured to prevent moisture ingress and was further weatherproofed by sealing the entire tube enclosed junction with adhesive heat shrink tubing. The 12 signal cables were permanently connected to the logger through holes drilled in the outer enclosure and fitted with IP68 cable glands while the reference electrode and a temperature thermocouple were connected to the logger using similar methods with all joins sealed with epoxy resin and adhesive heat shrink tubing.

The reference electrode used was a Ag/AgCl type (Model: InLab ARGENTHAL, Mettler Toledo, Switzerland), with built-in silver ion trap to prevent electrode junction contamination in sulphide-bearing sediments, since sulphide can precipitate the Ag ions released through porous diaphragm of a standard reference electrode causing electrolyte blockage and faulty readings (Kölling 2000). The reference electrode filling solution was renewed before each deployment of the datalogger.

3.4.2 Field measurements

Redox measurements were taken from three distinct types of vegetation communities in the upper saltmarshes of the north Norfolk coast at Brancaster and Stiffkey. Initial measurements were at Brancaster (TF7773 4493) in 2012 but due to disturbance of the equipment, subsequent measurements were taken in 2013 and 2014 at a less accessible site at Stiffkey (TF9519 4408).

The vegetation communities were: 1) creek bank - community bordering the edge of drainage creeks, with the width ranging from less than one metre to several metres, and forming an abrupt transition to adjacent communities; 2) general saltmarsh community (GSM) that occurs on flat expanses of the inner marsh, interspersed with numerous often undrained salt pans; and 3) undrained low marsh dominated by *Spartina anglica* (single set of measurements taken from Stiffkey (TF9619 4496). The vegetation and physical form of these upper marshes have been described in Chapman (1960). All creek bank measurements were taken from sediments underlying mixed stands of dense and vigorous *Puccinellia maritima* and *Atriplex portulacoides*.

Hourly redox measurements in the upper marsh sediments (creek bank and GSM communities) were undertaken simultaneously, with 24 electrodes installed in each site within a 5m distance of the datalogger arranged in six groups of four in each site (i.e. six replicates at four depths = 48 electrodes total). One electrode from each group of four was inserted into the sediment at each of four depths, 2cm, 5cm 10cm and 20cm, with care taken to avoid transporting organic matter down with the electrode during insertion. In hard, resistant sediments, a rod of slightly smaller diameter than the electrode was used to make a pilot hole in order to avoid physical damage to the platinum tip. The logger enclosure was anchored to the sediment surface using camouflage netting and tent pegs.

Recordings were made over one or more spring-neap tidal cycles of approximately fortnightly duration from early summer to winter to investigate redox behaviour following tidal flooding, to estimate the propensity of a site to develop anoxia, and its capacity recover to an oxic condition during the subsequent post-inundation drainage period.

A tide logger was devised to record time and duration of tidal inundation. This was constructed using a small event logger (EL-USB-5, Lascar Electronics Ltd., Salisbury, UK) connected to a float switch via a small cable (Figure 3). In order to withstand repeated submersion, the tide logger was housed in a waterproof enclosure and encased with IP68-rated re-enterable gel.

All redox potential readings were corrected against a standard hydrogen electrode reference electrode (SHE) by adding a 210 mV correction factor to give standardised redox potential (Eh) values. No adjustment to the Eh value was made for pH which was measured to consistently range pH 6.9-7.6 at 5 cm depth in the sediment at the start and end each recording period using a glass pH electrode calibrated with buffers adjusted to seawater salinities.

3.4.3 Data Analysis

The majority of electrodes worked with high degree of reliability, but occasional readings were lost as a result of electrode failure and these were omitted from the analysis.

Following installation, there was a long period (24-48 hours) of stabilisation, with individual electrodes showing a wide degree of variation (Figure 3.4). There was an appreciable delay in achieving stable readings. This was also commonly experienced on other occasions using the datalogger. All subsequent analysis of redox therefore omitted values obtained during the initial 50 hours from the commencement of recording.

For each vegetation community type a time series of average redox values for all electrodes with standard errors was plotted for each depth to summarise the measurements from individual electrodes made over the course the sampling period using R software (R Core Team (2013). An examination of differences in Eh values at depth was made using a one-way ANOVA test and the Games Howell or Tukey HSD post hoc tests at the $p < .05$ level (SPSS, IBM, New York).

(a)

(b)

Figure 3.2. (a) The HYPNOS III redox datalogger and secondary waterproof enclosure before encapsulation with a re-enterable potting gel for added protection against moisture ingress. (b) Datalogger as set up on the undrained low marsh site at Stiffkey with electrode and support

arrangements in foreground. 48 electrodes were connected to the datalogger and installed in groups of four set at different depths in the sediment (2, 5, 10, 20 cm).

Figure 3.3. The tide gauge installed on the upper marsh at Stiffkey comprising of an event datalogger attached to a float switch which measures the time and duration of tidal flooding. The netting on the float switch case is to prevent fouling by *Hydrobia* molluscs.

3.5 Results

3.5.1 Low marsh site

Redox measurements from the *Spartina* dominated low marsh site, taken from 14 – 27 March 2014, were found to be severely reducing over entire duration of the sampling period with the mean values consistently around -200 mV at all measured depths (Figure 3.4). During this sampling period, the site experienced semidiurnal tidal flooding over 8 days of the spring tide phase, resulting in 16 inundations, with durations of up to about 3 hours. The sediment temperature recorded at hourly intervals at 5cm depth average 8°C (min 5.4C, max 9.8C). With the exception of one electrode at the 2cm depth, no electrodes positioned close to *Spartina* plants exhibited noticeably higher values than electrodes placed in unvegetated areas of the site (Appendix Figure 1), indicating that rhizophere oxygenation by *Spartina* roots is insufficient to increase the redox potential of the bulk sediment.

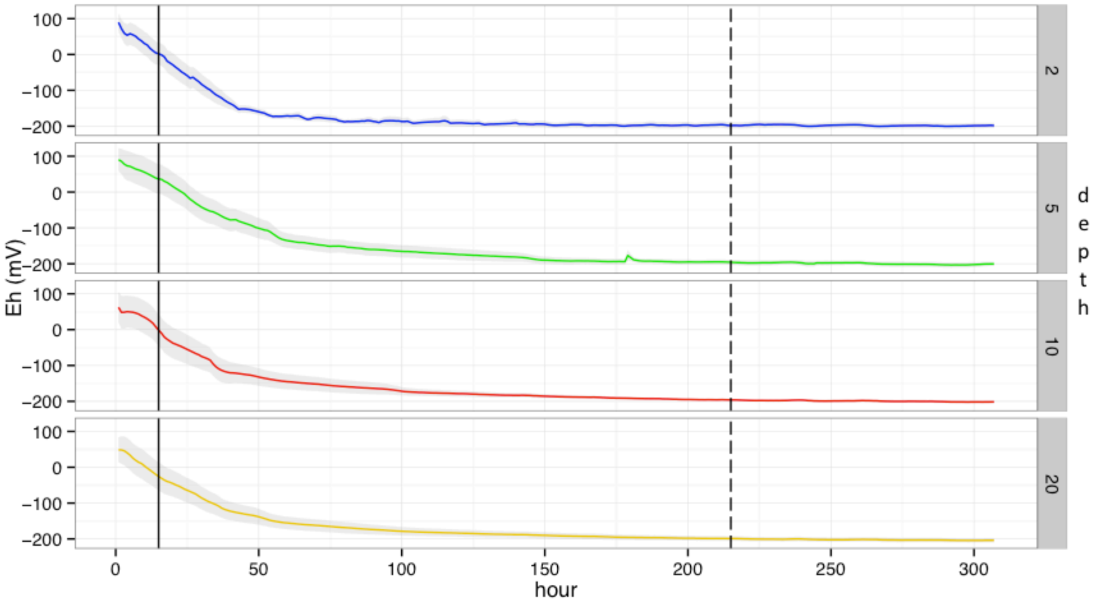


Figure 3.4. Time series of mean ±S.E. (shaded area) hourly redox potential measurements from a minimum of 10 electrodes at four depths for the period 14 – 27 March 2014. The solid and dashed vertical lines delineate the start and end points of spring tidal flooding phase respectively although the site comprises of a hollow (pan) colonised by *Spartina anglica* from the periphery that remains largely undrained and ponded throughout the relatively short unflooded neap tide phase. Sediment depth (cm) is shown on right hand side of each panel.

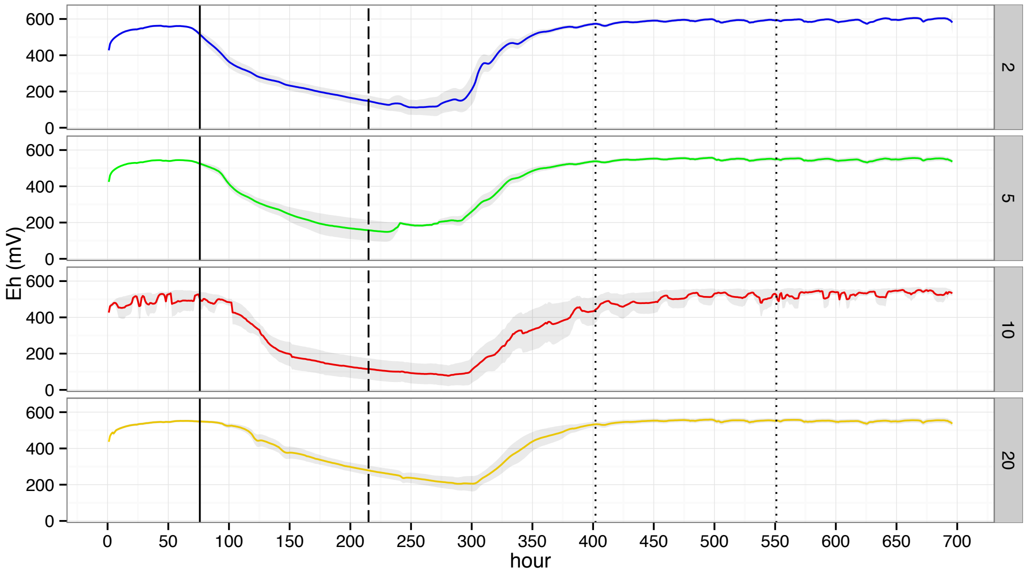
3.5.2 Development of seasonal anoxia in creek site sediments

In contrast to the low redox potentials observed in the *Spartina* dominated low marsh, mean redox potentials in the creek bank sites were much higher (Figures 3.5 – 3.7). However, creek bank sites regularly experienced conditions of temporary anoxia (< +350 mV) lasting several days shortly after the upper marsh is flooded by spring tides. This period of anoxia was found to be present at all depths, even the shallowest (Figure 3.5).

Late spring and early summer

Patterns of redox potential over the late spring (25 May – 19 June 2013) and early summer (22 June – 16 July) sampling periods were similar. The presence of a consistent anoxic phase (mean over all probes < +350 mV) was well established (Figure 3.5). In late spring, the period of anoxia ranged from 7 days at 2 cm depth and 8 days at all other measured depths (Figure 3.5). These anoxic conditions extended well into the neap tide phase with anoxic conditions persisting 4-5 days after the final tidal inundation (Figure 3.5a). During the next sampling period (22 June – 16 July), the length of the anoxic phase increased markedly up to 12 days at all measured depths (Figure 5).

The transition from oxic to anoxic conditions following flooding occurred within less than 24 hours (Figure 3.6). However, there was considerable variation between electrodes, with some taking longer to register anoxic Eh values (Appendix Figure 2). The onset of anoxia tended to take longer at greater depths (Figure 3.6) based on average Eh values. For the period 21 May – 19 June for example, average times taken were: 29 h at 2 cm; 37 h at 5cm; 52 h at 10 cm and 91 h at 20 cm.



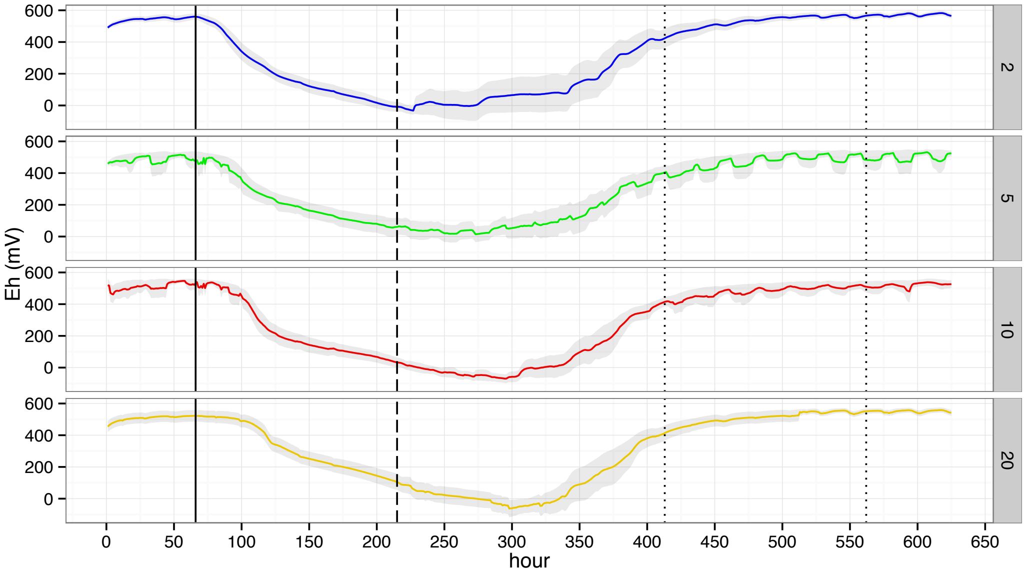


Figure 3.5. Mean ± S.E. redox potential at a creek bank site during the sampling period 21 May – 19 June 2013 (top), and 22 June – 16 July (bottom). This period covers the last spring tide inundation delineated by the solid and dashed vertical lines before the apogee tidal phase prior to the summer solstice when the upper marsh remain in an unflooded state for an extended period. This phase is delineated by dotted vertical lines spanning three days either side of the mid point of the apogee tide to give an indication of the approximate period of flooding under circumstances of a normal spring tide. Sediment depth (cm) is shown on right hand side of each panel.

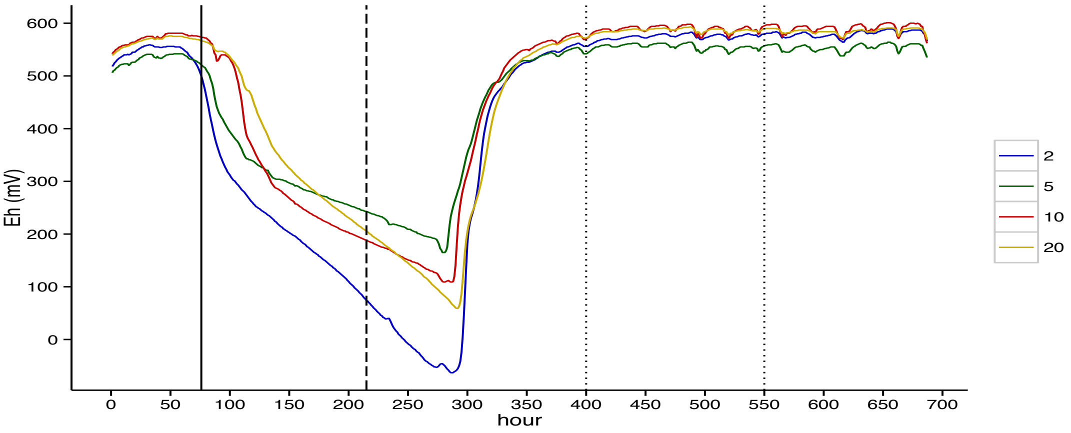


Figure 3.6. A typical time-series of actual hourly Eh recordings from the period 21 May – 19 June from one group of electrodes at four depths showing the high degree of consistency at each depth. Sediment depth (cm) is shown on right hand side key.

Late summer

The trajectory of increasing prevalence of anoxia continued into late summer (22 July – 14 August) at Stiffkey, with the period of anoxia continuing for 11-12 days at depths up to 5 cm. However, unlike in earlier sampling periods when the period of anoxia was similar at all depths, the duration of anoxia was longer at greater depths, extending to 17 days at 10 cm and no recovery in the mean redox conditions at the 20 cm depth before the start of the next phase of tidal flooding (Figure 3.7).

The anoxia phase was considerably longer in duration than the intervening period of recovery to aerobic conditions (as little as 3-4 days). This pattern occurred at the creek bank site at Brancaster during the recording period 17 – 26 August 2012 (Figure 3.7).

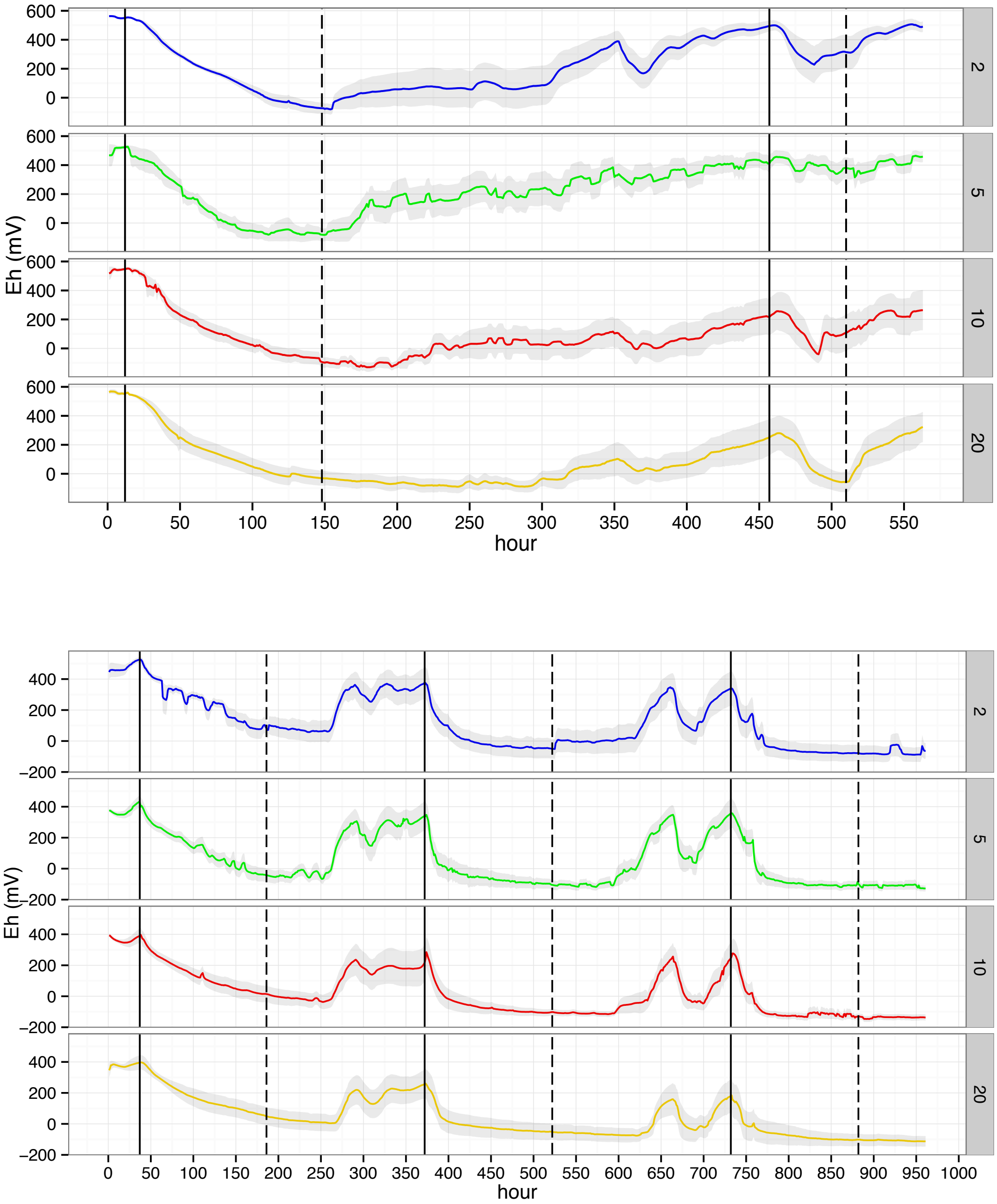


Figure 3.7 Stiffkey creek site mean ± S.E. hourly recordings for 22 July – 14 August 2013 (top) and Creek site (Brancaster) for 17 July – 26 August 2012 (bottom). Sediment depth (cm) is shown on right hand side of each panel.

3.5.3 Development of seasonal anoxia in general saltmarsh sediments

Simultaneous measurements at the same periods for the creek site were made for the GSM site and are described below.

Late spring / early summer

The period of anoxia based on average Eh values was much longer than those for the creek reflecting the poorly drained nature of the general saltmarsh sediments, despite the extended period (25 days) resulting from the non-flooding apogee spring tide that occurs before the summer solstice. The average period of anoxia following tidal flooding at 2 cm was close to 8 days and at the intermediate depths, 5 cm and 10 cm, extended to 16-17 days while only one electrode at the 20 cm depth indicated a return to oxic conditions suggesting very limited oxygen availability at this depth.

During the post flooding phase, individual electrodes exhibited a higher degree of variation at the same depth compared to the creek site indicating a more heterogeneous redox environment at this time, with areas of oxic and anoxic sediments co-occurring at the same time although the proportion of anoxic readings increased with depth. While the 2 cm depth was largely oxidising during this phase the 20 cm depth appears to be predominantly anoxic with mild-moderately reducing characterising the intermediate depths is less certain. Averaging Eh values at these depths somewhat exaggerates the extent and degree of anoxia during the post-flooding phase since it is clear from at least half of the electrodes at the 5 cm and 10 cm depths indicate that a degree of oxic conditions returned to the sediment profile.

Measurements during this second extended period without flooding from the apogee spring tide that occurs after midsummer (22 June – 16 July) are shown in Figure 3.8. Although temperatures recorded at the nearest Met Office synoptic observation station (Weybourne 15 km distance from Stiffkey) during this period were either close to or above average and a near absence of precipitation (Appendix Figure 11) would suggest high rates of evapotranspiration, the measured sediments of the GSM community nevertheless showed very limited capacity for recovery to oxic conditions at all measured depths despite the extended mid-summer period free from flooding.

The variation in readings between electrodes was far less than recorded for the previous period with only 3 registering oxic Eh values and only at 2 cm and 5 cm depths in the final few days of the recording period suggesting that anoxia was well established at all measured depths by this stage. However, a single anomalous electrode at the 5 cm depth recorded continuously oxic conditions throughout the recording period suggesting that the presence of oxic microsites at the upppermost depths cannot be discounted. Furthermore there was a clear increase in the number of electrodes recording severely reducing Eh values at all depths. In contrast to the previous recording period where severely reducing Eh values were transient and restricted to the 20 cm depth they were much more prevalent during this recording period. While these low Eh values tended to be transient to varying extents at the 2 cm and 5 cm depths, they were found to continuous in the the majority of electrodes at greater depths.

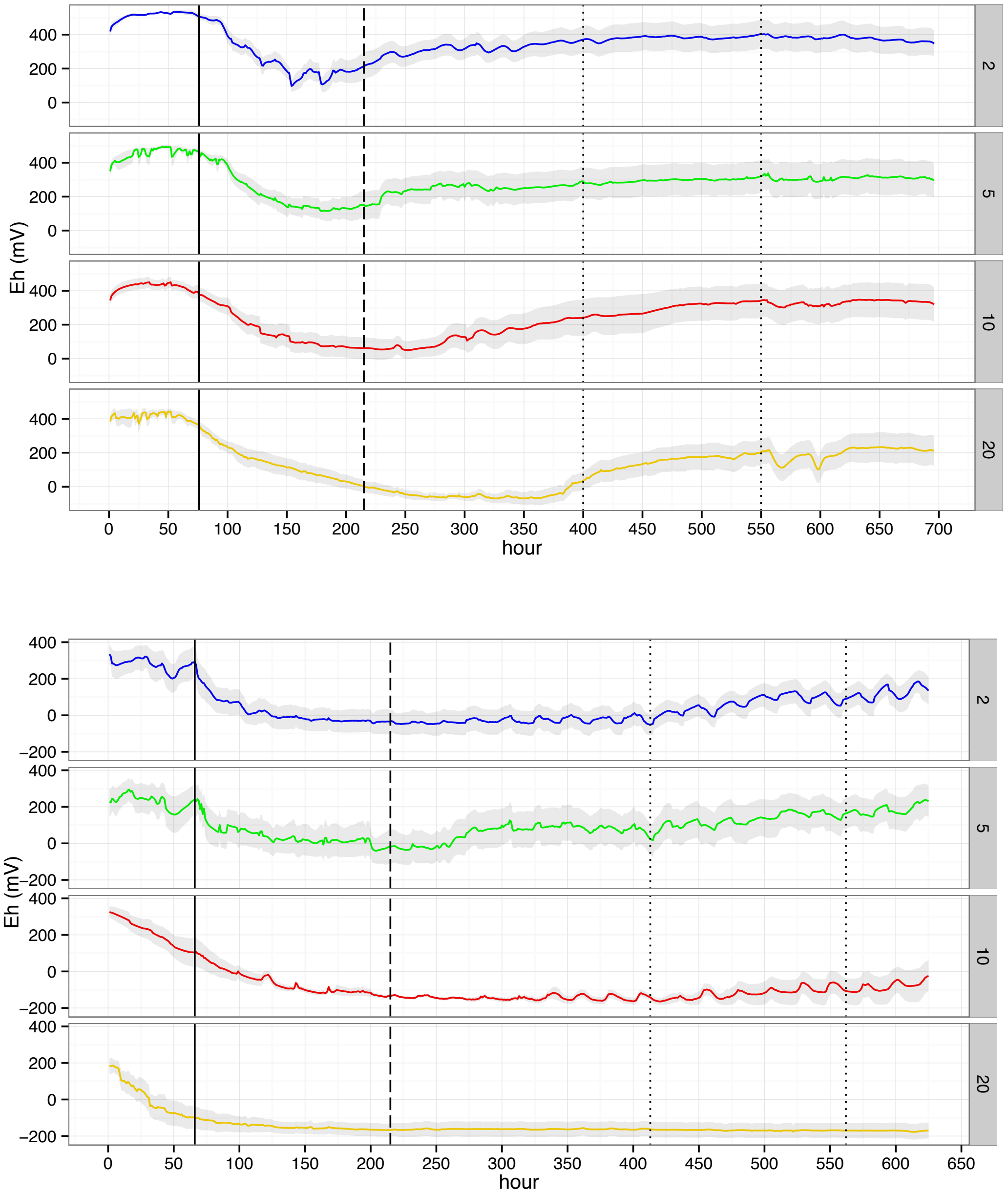


Figure 3.8. Time series of mean ± S.E. redox potential recordings for the general saltmarsh community at Stiffkey for the period 21 May – 19 June 2013 (top) and 22 June – 16 July (bottom). Sediment depth (cm) is shown on right hand side of each panel.

A notable feature revealed by higher resolution measurements of Eh by continuous hourly recording was the existence of persistent diel Eh fluctuations. These were observed in electrodes from both the creek site and the GSM site but tended to be more pronounced in the latter. These oscillating Eh patterns exhibit large variations in peak-peak amplitude ranging from tens of mV to over 400 mV generally occurring over a 24 hour cycle. These fluctuations occurred at all measured depths but found to be more prevalent to 10 cm. It is presumed that these fluctuations result from the close proximity of the Pt tip of the electrode to an oxygenating rhizosphere although the interpretation is complicated by an absence of a clear photosynthetic pattern to the peaks. For example, the peaks after the flooding phase occurred close to sunrise in a 5 cm plot and around midday in the 10 cm plot from 22 June – 16 July recording (Figure 3.9).

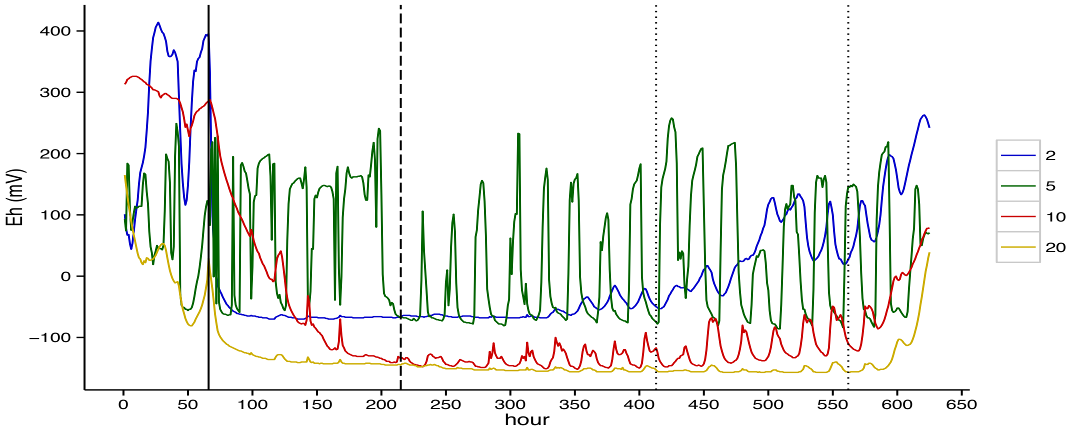


Figure 3.9. Time series of actual Eh recordings for a group of electrodes at 4 depths from the GSM site (22 June – 16 July) showing varying degrees of diel fluctuations presumably as a consequence of rhizosphere oxidation effects. Sediment depth (cm) is shown on right hand side key.

Late summer

Measurements for this period (20 July – 14 August) at Stifkey showed a continuation of predominanly anoxic Eh values (Figure 3.10). Only three electrodes registered a return to oxic Eh levels in the uppermost depths for any reasonal length of time (Appendix Figure 6). Continuously severe reducing Eh values were recorded by 2 electrodes at 2 cm and 5 cm while these values were observed in all electrodes at the 10 cm and 20 cm depths.

In contrast to the drier summer of 2013, where the absence of significant precipitation enabled a degree of recovery in the uppermost parts of the sediment of the GSM from severe-moderately reducing to mildly conditions, the high levels of precipitation that occurred during the summer of 2012 at the Brancaster site appeared to prevent any significant increase in Eh values between phases of tidal flooding and more or less constant conditions of severely reducing conditions persisted at all depths for the six week duration of the period 17 July – 26 August (Figures 3.11).

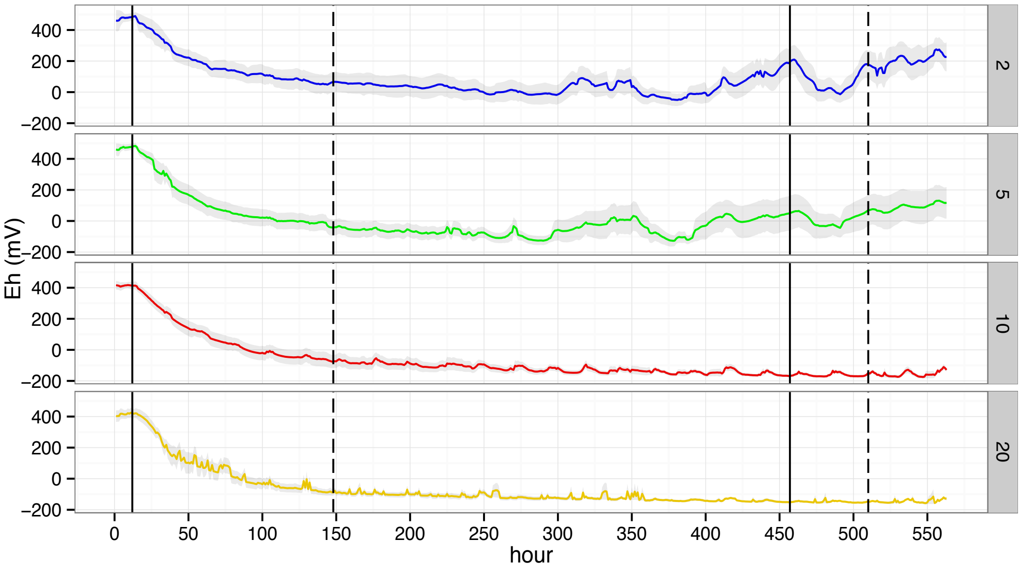


Figure 3.10. Mean ± S.E. Eh recordings for the GSM at Stiffkey for the period 20 July – 14 August 2013 again showing only a limited evidence of a recovery to oxic Eh values by the end of the recording period at the uppermost depths during an exceptionally warm and dry season. Sediment depth (cm) is shown on right hand side of each panel.

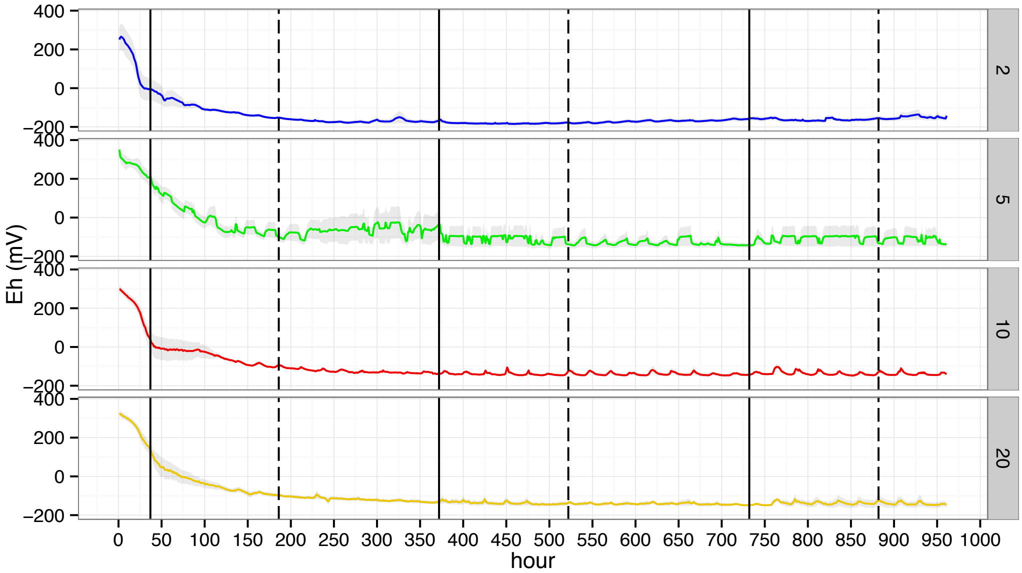


Figure 3.11. Mean ± S.E. Eh recordings for the GSM at Brancaster for the period 17 July – 26 August 2012 showing no evidence of an oxic recovery even at the uppermost depths in an usually wet season. Sediment depth (cm) is shown on right hand side of each panel.

Autumn

Results from a subsequent recording from the Brancaster general saltmarsh site for this period showed that moderate-severe reducing conditions can persist at all depths until well into the autumn months (Figures 3.12). Although there were clear signs of an increase in Eh values by the start of November there was no evidence of a widespread return to oxic conditions at the uppermost depths. Overall the evidence from results of extensive recordings of continuous Eh measurements suggests that sediment at depths that encompass much of the rooting zone of the general saltmarsh community experiences seasonal conditions that are predominantly anoxic and reducing for a period of about 4-5 months from July to November and such conditions may persist for a longer period at the lower measured depths of the sediment profile.

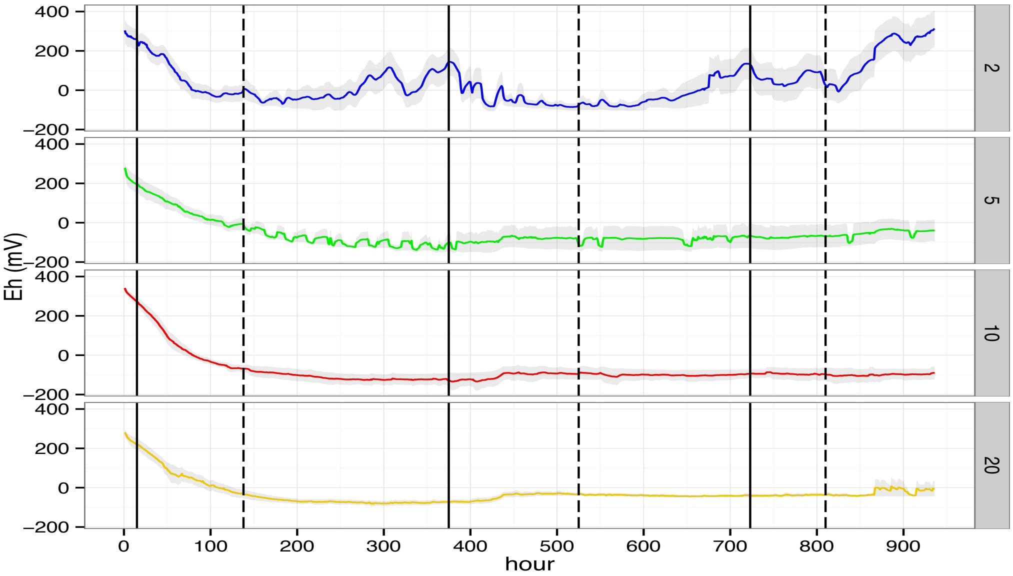


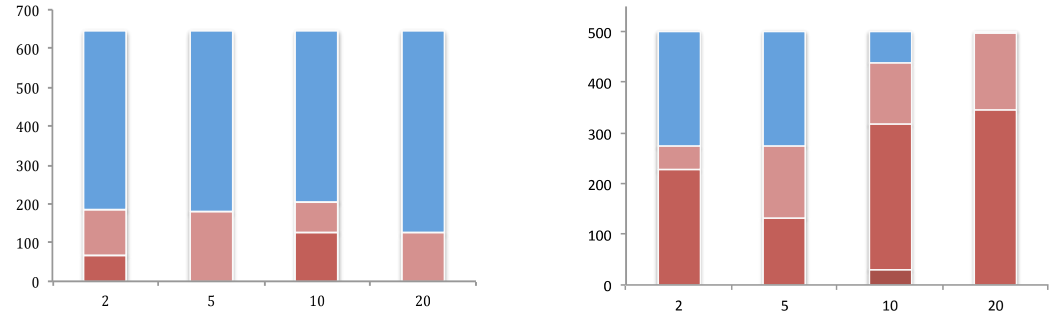
Figure 3.12. Mean ± S.E. Eh recordings for the GSM at Brancaster for the period 28 September – 6 November 2012 showing the persistence of anoxic Eh values into the autumn months with limited evidence of recovery to oxic Eh levels in surficial sediments by the end of this period. Sediment depth (cm) is shown on right hand side of each panel.

3.5.4 Variation in mean redox potential with depth

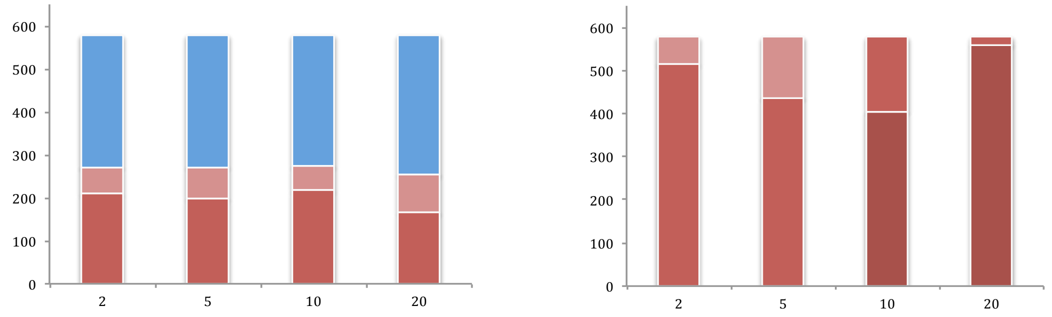
Categorising mean redox potentials by critical ranges of mildly reducing, (+300 mV to +151 mV), moderately reducing (+150 mV to -100 mV), severely reducing (< -100 mV) and oxic conservatively at (> + 300 mV) illustrates the distribution of redox intensities with depth more clearly (Figure 3.13). Redox intensities increased with the progression of summer in both the creek and the GSM sites. Mild to moderate redox intensities were present throughout the summer at the creek site at Stiffkey. The similar time period for Brancaster shows how elevated levels of precipitation may exacerbate the levels of redox intensity even in relatively well drained creek bank communities with mild-moderate Eh conditions predominant at all depths and the appearance of significant periods of severe reducing conditions at depths below 5cm. At such time plants growing in these habitats need to tolerate periods moderate-severely reducing Eh can be more than twice as long as period when oxygen is available in the uppermost depths and continuous periods of moderate-severe Eh conditions at depths at least to 10 cm.

In comparison to the creek site the impression of the GSM site is one of a considerably harsher sediment environment where moderately reducing Eh values are more prevalent at an earlier stage at all depths and dominate depths at least from 10 cm by early summer. The severity of the redox condition rapidly escalates in the following months with the majority of the prolonged anoxic phase at a moderately reducing state to depths of 5 cm and predominantly severely reducing state at depths from 10 cm. Again, high rates of precipitation exacerbates the levels of redox intensity with severely reducing Eh conditions becoming widespread in the upper few cm of the sediment profile. Furthermore, the recordings from Brancaster indicate that moderate-severe Eh values can persist at all depths well into November.

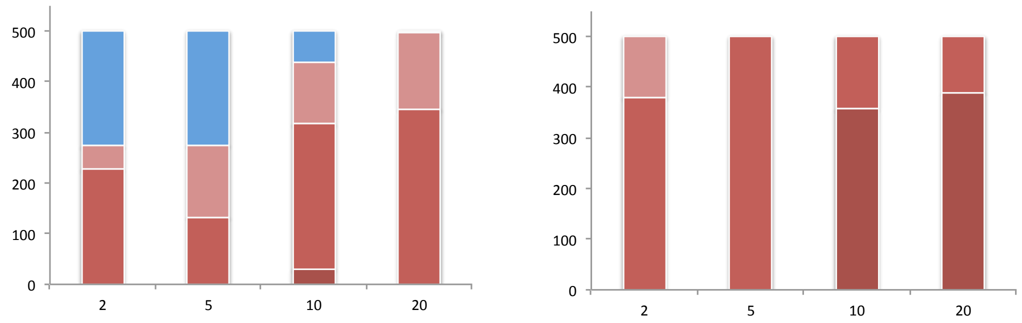
a) 21 May – 19 June



(b) 20 June – 16 July



(c) 22 July – 14 August 2013



(d) 16 July – 27 August 2012 (Brancaster)

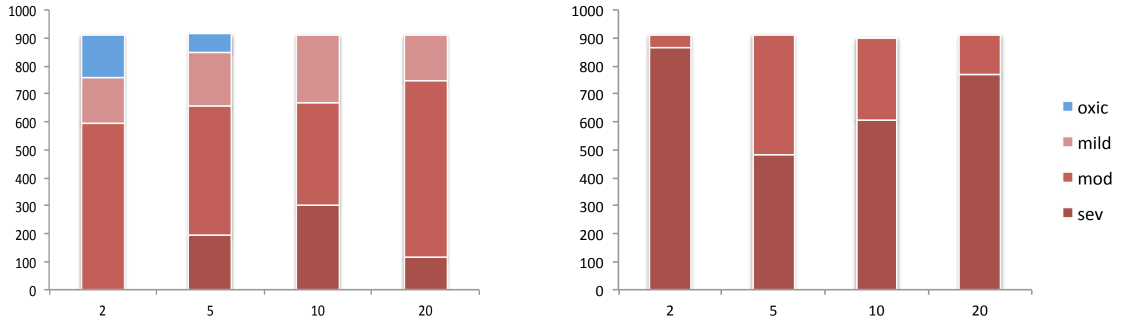


Figure 3.13. Hourly distribution of redox intensity categories based on average Eh values at each depth are shown for the summer recording periods at the creek site (left) and the GSM site (right).

3.5.5 Minimum and maximum redox potentials with depth

In order to investigate differences in redox potential with depth the minimum mean Eh value (i.e. the lowest mean Eh from all electrodes at each depth) from the start of flooding and the maximum mean Eh value (i.e. the highest mean Eh from all electrodes at each sampled depth) 4 days after the end of flooding were subjected to a one way ANOVA test.

Undrained Spartina dominated low marsh site

At the undrained low marsh, there were no significant differences between the four sampled depths in the minimum [($F_{(3,41)} = 0.063$, $p = 0.979$] and maximum [($F_{(3,41)} = 0.062$, $p = 0.980$] redox values (Figure 3.14). Almost all values from individual electrodes at the minimum and maximum points were well within the highly reducing range indicating a high degree of consistency with respect to redox intensity at all depths and all stages of the tidal cycle.

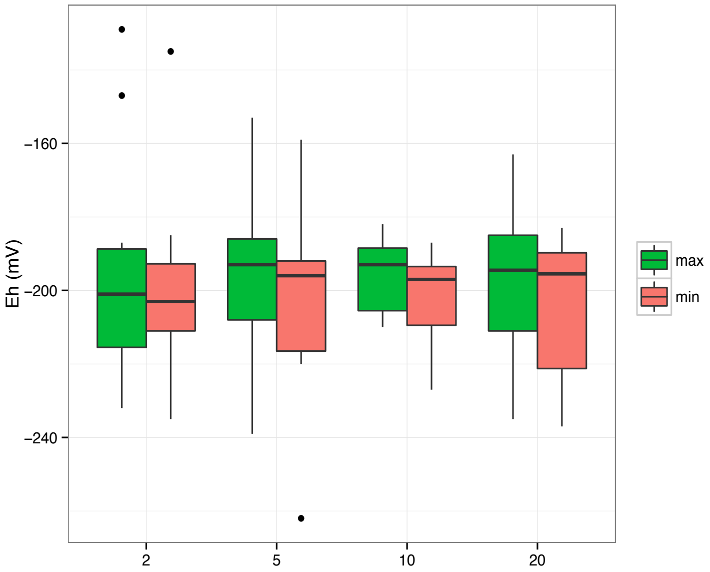


Figure 3.14. The distribution of individual electrode readings for average maximum and minimum Eh values measurements at four depths for the *Spartina* dominated undrained low marsh site.

Creek site and GSM

Late spring: 21 May – 19 June

The distribution of the mean maximum and minimum Eh values recorded from the period for the creek site (Figure 3.15) shows that the electrodes at the minimum Eh point were all within the mild-moderately reducing range (+300 to -100 mV). At the maximum point, Eh values indicated by a number of electrodes at each depth registered oxic Eh values as evidence of the return of aerobic conditions at all sampled depths by the fourth day after the end of the flooding phase. At the creek bank site, there were no significant differences between depths in either the minimum [($F_{(3,19)} = 1.294$, $p = 0.979$] or maximum [($F_{(3,19)} = 1.896$, $p = 0.165$] average Eh values. In contrast, there were signficant differences between the depths for both minimum [($F_{(3,19)} = 3.817$, $p = 0.027$] and maximum [($F_{(3,19)} = 5.251$, $p = 0.008$] values suggesting the presence of a signficant redox gradient with depth at this site.

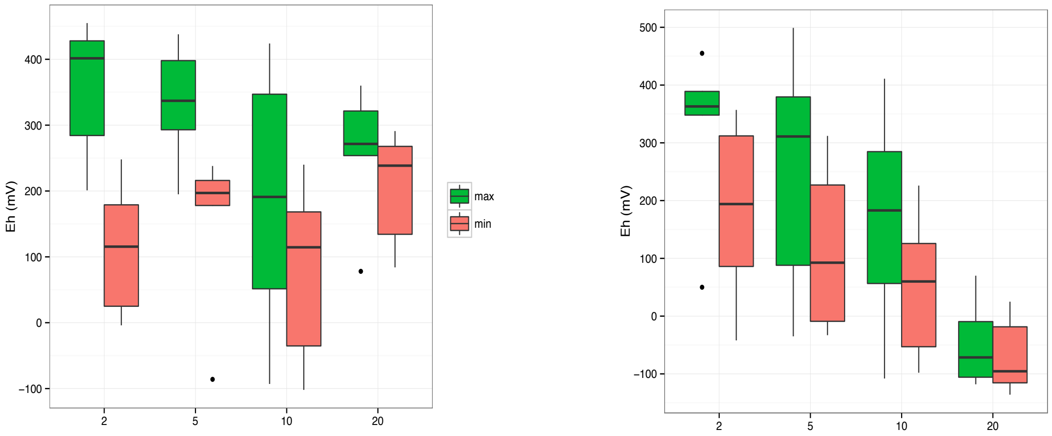
20 Jun – 16 July

The distribution of the average maximum and minimum Eh values recorded from the period 20th Jun – 16th Jul for the creek site (a) showed the distributions of the Eh readings at the minimum point were predominantly in the mild-moderately reducing range although a number of electrodes at each depth registered values within the severe reducing range (< -100 mV) and these low values persisted for at least several days in to the post flooding phase. The slower recovery time to oxic conditions was confirmed by the ANOVA test which found no significant differences for the mean values at both the minimum and maximum points [($F_{(3,20)} = 0.464$, $p = 0.710$] indicating the absence to a significant redox gradient with depth at this time. The distribution for the GSM site reflected a sharp increase in redox intensity compared to the previous period with Eh values predominantly within the moderate-severe reducing range (+150 mV – 100 mV) in the 2 cm and 5 cm depths and largely severe in the lower sampled depths. Only one electrode at the 10 cm depth recorded consistently aerobic Eh values throughout the recording period indicating the possible existence of limited aerated micro-sites within the sediment profile. Although the results indicate sediments from the lower sampled depths were more consistently and highly reducing this was not refelcted in the results of the ANOVA test which failed to detect significantly differences between depths for the minimum [($F_{(3,20)}$ = 1.992, p = 0.148] or maximum [($F_{(3,20)}$ = 0.464, p = 0.094] Eh reflecting the high degree of overlapping variation in Eh values from individual electrodes in a relatively small sample size (N=6) at each depth.

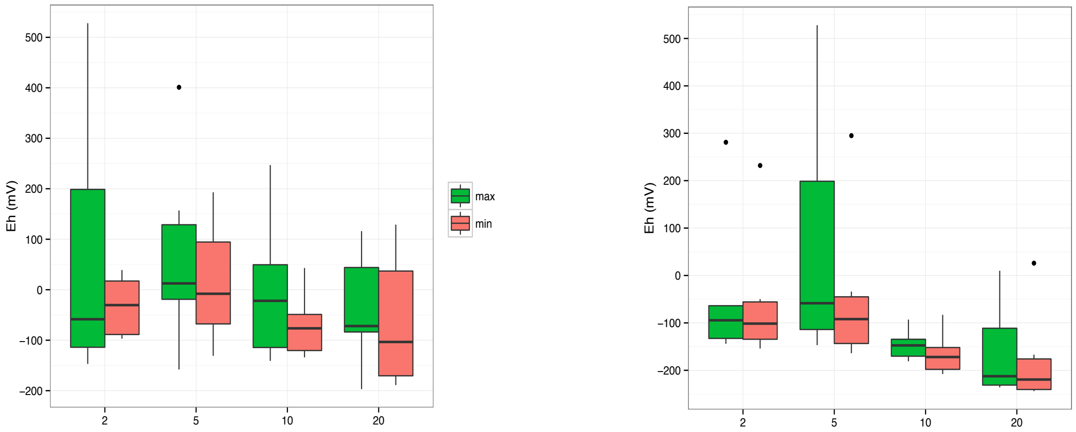
22 Jul – 14 August

The distribution of electrode readings at average maximum and minimum points recorded from this period for the creek site (a) showed that although a proportion of the electrodes recorded a return to aerobic Eh within four days of the end of flooding at the 2 and 5 cm depths overall differences in recovery times between depths were not found to be statistically significant [($F_{(3,20)}$ = 1.244, p = 0.320] again due to the high degree of variation in recovery times between electrodes in the upper depths. No significant differences were found between minimum Eh values at different depths reflecting the presence of transient moderate-severely reducing Eh values at all sampled depth. GSM site (b) showed no significant differences ($p < .05$) at either minimum [($F_{(3,20)}$ = 4.034, p = 0.210] or maximum [($F_{(3,20)}$ = 1.550, p = 0.233] Eh levels between sampled depths reflecting the very limited redox recovery by the end of the 4 day post flooding period in these sediments and the prolonged occurrence of moderate-severely reducing Eh conditions particularly at the lower sampled depths.

(a) 21 May – 19 June



(b) 20 June – 16 July



(c) 22 Jul – 14 August

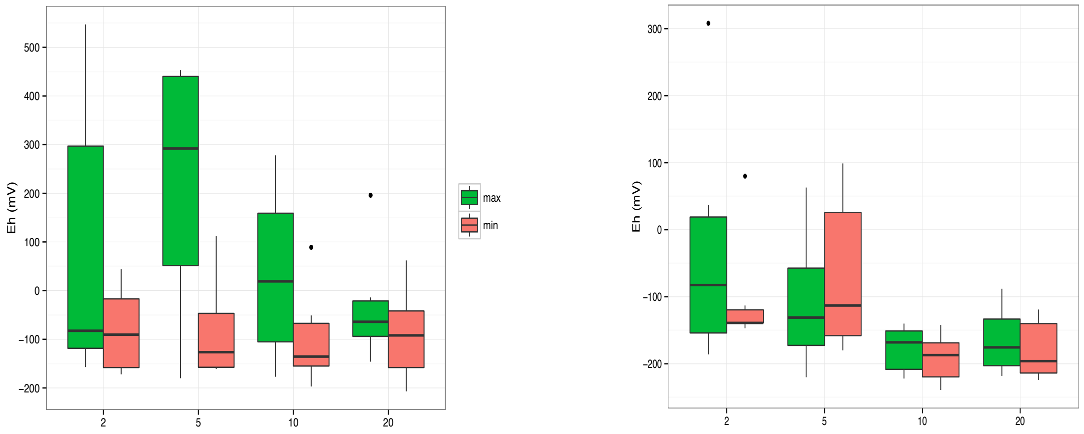


Figure 3.15. The distribution of individual electrode readings for mean maximum and minimum Eh values measurements at 4 depths for creek site (left) and the GSM site (right).

3.5.6 The return to oxic conditions

Measurement of Eh in the autumn periods of three years (2012 - 2014) show that redox conditions in the upper marsh sediments experienced a marked transformation to largely oxic conditions (Figures 3.16 – 3.17) at a time when the sediments are at their most saturated due to more consistent flooding and far lower rates of evapotranspiration.

Measurements of mean Eh ±S.E. for the creek site (2 – 29 November 2013) are shown in Figure 16. There is evidence to suggest that this period represents a transitional phase from the period of lowest redox potentials in the upper marsh sediments that develops in late summer to early autumn. The prevalence of oxic conditions was particularly pronounced in the uppermost sediments with the period of average anoxia following the initial spring tide lasting for around 2.5 days at 2 cm and 4 days at 5 cm. Electrodes during this anoxic phase reached a wide range of Eh minima from mild to severely reducing values (Appendix Figure 10). The second tidal flooding showed less influence with the majority of electrodes registering oxic Eh values throughout the period of flooding. Average anoxic conditions at 10 cm lasted for around 10 days during the first flooding phase and 8 days for the second flooding phase while anoxic conditions at 20 cm was found to be continuous until at least the 25th November.

Measurements of Eh for the general saltmarsh sediments over this period showed stronger evidence of a return to aerobic conditions in the uppermost sediment depths which was concealed in the mean Eh values (Figure 3.16 and Appendix Figure 10) due to severely reducing values recorded by two electrodes which lowered the mean Eh to the mildly reducing category. This broad variation in Eh values suggests the presence of a degree of redox heterogeneity persists in the general saltmarsh sediments at a late stage of the year with highly reducing and aerobic sites co-occurring in the uppermost sediment depths. Without more extensive measurements it is not possible to make a general inference regarding the nature of this heterogeneity. It possible that these severely reducing Eh values are restricted to limited microsites within the bulk sediment or alternatively they are a more general and widespread feature in the uppermost parts of the sediment profile at this time of year. There is little evidence of a return to aerobic conditions at greater depth with only one electrode returning to aerobic Eh values after the end of the second period tidal flooding while continuation of severely reducing conditions was found to persist at the 20 cm depth.

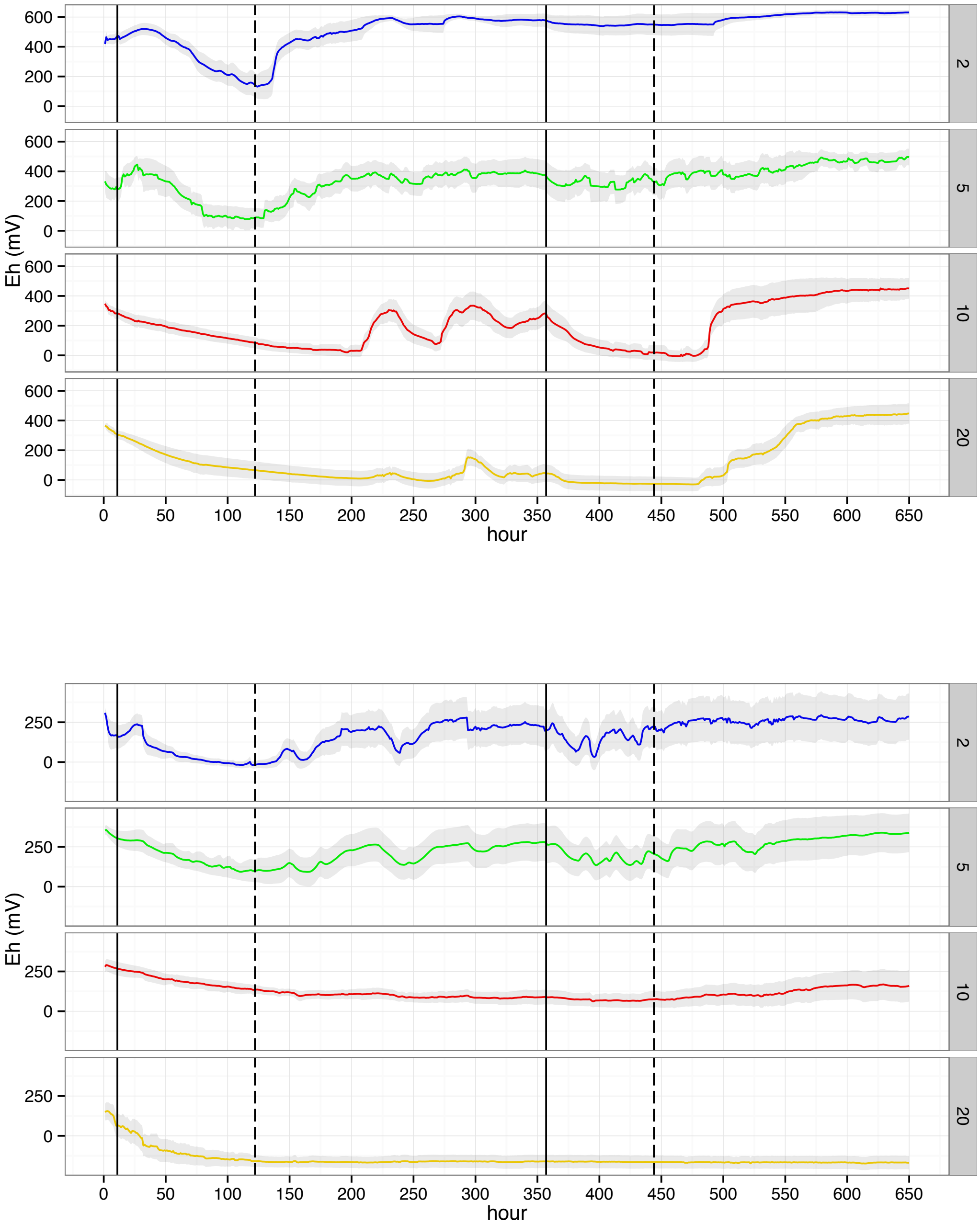


Figure 3.16. Mean ± S.E. Eh recordings for the creek site (top) and GSM site (bottom) at Stiffkey for the period 2 – 29 November 2013.

A longer period of recording over 4 months 6 October 2014 – 4 February 2015 illustrates more clearly the anoxic-oxic transition in the GSM site (Figure 17). This showed again predominantly oxic conditions were attained in the uppermost sediments by the end of November and that the delay in reaching this status at greater depth. Predominantly oxidising conditions were not attained at the 10cm depth until well into January and although there is a clear upward trajectory at the 20cm depth is evident overall sediments at this depth appear to be predominantly anoxic with many electrodes still registering highly reduced conditions. However a few electrodes at this depth did show the return to an oxic state had occurred in parts of the sediment at this depth and it is likely that a generally oxidised state would be reached some weeks later. Actual measurements for the period 20 December 2014 – 29 January are shown in Figure 3.18 to illustrate the development of oxidised sediments which contrasts markedly to the Eh reading obtained from these sediments during the summer-autumn period.

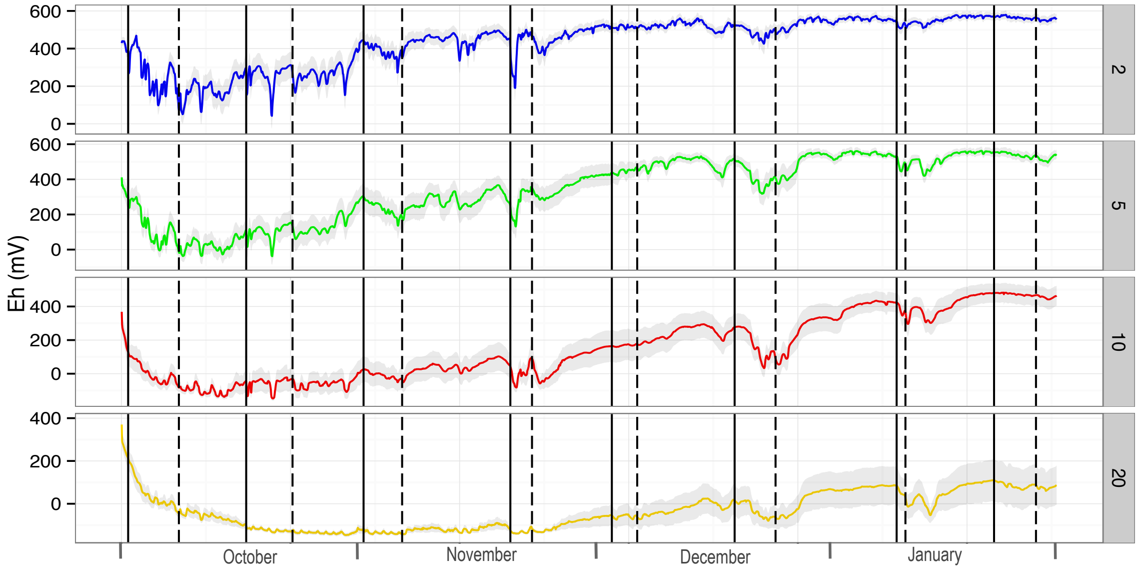
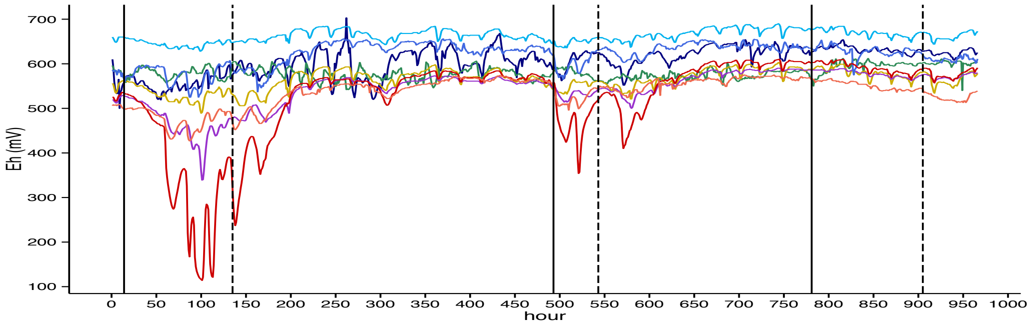
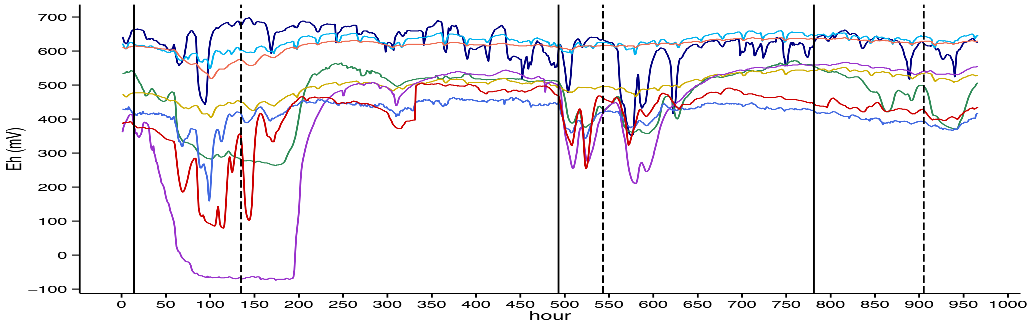


Figure 3.17. Mean ± S.E. Eh recordings for the GSM site at Stiffkey for the period 6 October 2014 – 4 February 2015 showing a similar pattern to measurements at the same time at this site in 2013 (Figure 17) with generally anoxic Eh values at all depths throughout October and a clear transition to progressively oxic values at the uppermost depths from November while generally anoxic conditions persisted at 10 cm at least until the end of December. By the start of January although the majority of readings remained predominantly anoxic and highly reducing at 20 cm a small number of electrodes recorded oxic Eh values indicating the return of oxic microsites at this depth. Sediment depth (cm) is shown on right hand side of each panel.

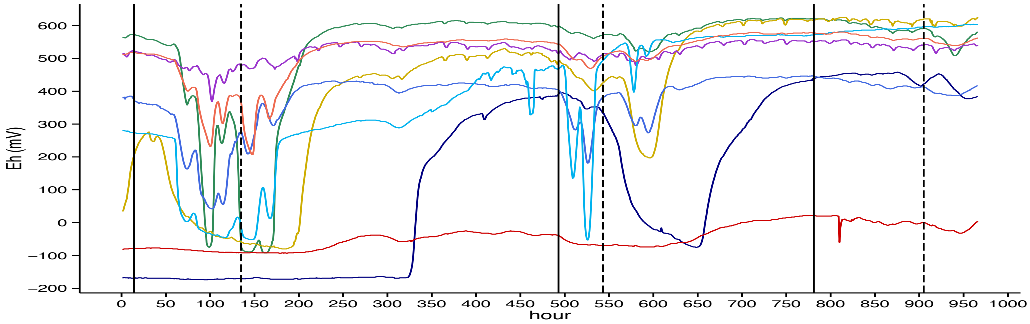
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

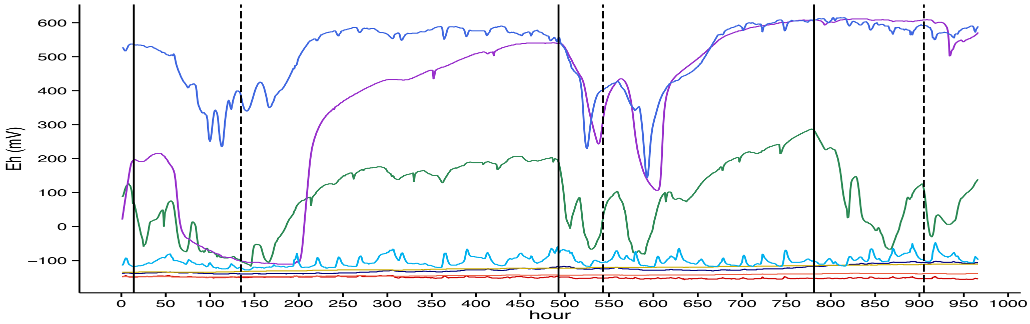


Figure 3.18. Eh measurements from replicate electrodes at four depths for the period 20 December 2014 – 29 January 2015 showing the return to generally oxic conditions in the GSM site at least down to 10cm while more heterogeneous redox conditions continue to persist at greater depths with many electrodes still registering anoxic and highly reducing Eh values.

3.6 Discussion

Using a datalogging methodology that allowed more detailed *in situ* field measurements of redox potential than previously undertaken in saltmarshes, this study revealed distinct soil oxygen conditions in three plant communities. A number of species that are frequent or abundant in two of these communities tend to be uncommon or absent in many restoration saltmarshes. An improved knowledge of the redox characteristics of natural saltmarsh communities gained in the current study may therefore assist in understanding processes that contribute to the success or failure of these species to establish on restoration saltmarshes.

This method allowed collection of long-term continuous hourly field measurements of sediment redox potential at a much greater resolution and duration than has been previously reported for saltmarsh sediments. Prior to this study the most detailed understanding of redox dynamics on saltmarshes was based on monitoring undertaken by Armstrong *et al*. (1985) in the Humber estuary that was mainly acquired by manual measurements.

Measurements of redox potential identified distinct and different patterns of sediment aeration in three vegetation zones, low marsh dominated by *Spartina anglica*, creek bank dominated *by Atriplex portulacoides* and *Puccinellia maritima,* and a general saltmarsh community (mainly comprised of *Armeria maritima*, *Limonium vulgare, Plantago maritima* and *Triglochin maritima*).

3.6.1 Low marsh site

Spartina dominated low marsh sediments did not vary with season. Measurements of redox potential at this site were taken in March and found to be highly reducing at a time when sediments measured elsewhere were generally found to be highly oxidising within much of the depth that encompasses the rooting zone. Comparable redox conditions were reported by Armstrong *et al*. (1985) for a similar *Spartina* dominated low marsh during the summer, suggesting sediments that experience hydroperiods close to a continuous semi-diurnal regime of twice daily tidal inundation often with relatively small number of days free from flooding are likely to be in a more or less contnuous highly reduced state at Eh levels that theoretically should provide conditions conducive for the accumulation of sulphides.

It is not clear why these low marsh sediments respond indifferently to seasonal decreases in temperatures and this requires further investigation. One possibility is that these more frequently flooded and undrained sediments have higher rates of sulphate replenishment allowing dominance by different types of microbial community, such as certain groups of sulphate reducing bacteria (Psychrophilic) that have been found to be adapted to low temperatures (Knoblauch *et al.* 1999; Sawicka *et al*. 2012). However, Nedwell & Abram (1978) reported a seasonal pattern of sulphate reduction rates in creek beds and salt-pans of Essex saltmarshes that increased in May and was followed by a sharp decline in October.

Out of all saltmarsh plants *Spartina* is probably the best adapted to permanent waterlogging and low redox potentials since it is likely to possess specific mechanisms to tolerate sulphidic sediments, although they have yet to be fully elucidated. Possible mechanisms that have been identified include a high capacity for internal oxygen transport to oxidise sulphides in the rhizosphere (Koop-Jacobsen & Wenzhöfer 2015; Maricle & Lee 2002, 2007), internal transport and volatilisation of H_2S to the atmosphere (Lee 2003), and processes of sulphide detoxification by the production of high concentrations of dimethylsulphoniopropionate (DMSP) (Otte *et al*. 2004).

There was no evidence in the current study to suggest that rhizosphere oxidation by *Spartina* is sufficient to increase the redox potential of the bulk sediment and any alteration is likely to be highly localised and limited to the immediate vicinity of the rhizosphere. This is supported by recent high resolution studies that have reported sediment oxygenation by *Spartina* is limited to a narrow zone about 1.5mm wide and restricted to a distance of just 16mm from the root apex (Koop-Jacobsen & Wenzhöfer 2015).

This does not mean that *Spartina* is unable to influence the redox potential of the bulk sediment in general, rather it suggests that such undrained sites can be highly poised (i.e. highly buffered) with respect to redox potential and resistant to small changes in

Eh that may be induced by root radial oxygen loss (ROL) in less well poised sediments (Reddy & DeLaune 2008). The high degree of redox poise is well illustrated in the charts of the actual measurements which clearly shows the development of a high degree of stability with uniform readings following the period of initial physical disturbance. Less well poised sediments such as those encountered in the GSM sediments show varying degrees of fluctuation in Eh readings usually reflecting diel cycles of photosynthetically-driven patterns of sediment oxidation from ROL which is frequently detected with the measuring electrodes in the bulk sediment.

3.6.2 Creek site

While the *Spartina* dominated low marsh represents one end in the spectrum of hydrological gradients colonised by saltmarsh plants, the other end is represented by the well drained sediments of the creek site that are dominated by *Atriplex* and vigorous stands of *Puccinellia*. Sediments underlying these communities are characterised by strongly oscillating redox conditions in response to cyclical patterns of tidal flooding and drainage. No evidence was found of redox fluctuations in response semi-diurnal tidal flooding during the spring tide phase. Rather the measurements were found to be comparable to those reported by Armstrong *et al* (1985) for sites occupied by *Atriplex*, *Puccinellia* and *Elytrigia artherica* that experience transient anoxia that persists continuously throughout the spring-tide phase. Furthermore, where *Puccinellia* is dominant, measurements indicated that this period could persist well into the neap tide phase entailing a considerable lag before a resumption of oxic conditions is experienced.

A notable finding in the current study was evidence of a strong seasonal effect in the creek bank sediments. Creek bank sediments, which are often regarded as relatively well drained, experience periods of transient anoxia even at depths of a few cm, but it was found that the duration of this period of anoxia progressively increases through summer and into autumn. This is accompanied by an increase in redox intensity reflected by the increasing number of electrodes registering severely reducing Eh values. The anoxic condition also becomes increasingly pronounced with depth and by late summer little oxygen is available throughout the neap tide phase at the lowest depths that were measured at this site.

Measurements showed that delay in re-oxidation of sediments close to a major drainage creek in the upper marsh could be considerable and extend well into the neap tide phase. A delayed return to anoxia of several days into the neap tide phase was also reported by Armstrong *et al*. (1985) for *Puccinellia* swards in interfluve sites at intermediate elevations but the current study shows that such delayed re-oxidation occurs even in areas close to major creeks in the upper marsh sites.

Moreover, at a certain stage in late summer the duration of the anoxic phase over a spring-neap tidal cycle exceeded the length of oxic phase and there are indications such a condition can extend for several months into autumn. Plants occupying this sites may therefore have to tolerate oxic conditions that are present in relatively short 'pulses' as little as 4-6 days, depending on the prevailing meteorological conditions, that are present between longer phases of flood-induced anoxia. This may explain the restricted distribution of *Atriplex* in many of these creek site communities where it is not always predominant but is often present with dense stands of *Puccinellia*. Of these species *Puccinellia* is thought to be the best adapted to tolerate longer periods of anoxia due to its capacity for enhanced porosity for internal transport of oxygen to the root tips (Justin & Armstrong 1987). In contrast *Atriplex* appears to lack this physiological adaptation and its general intolerance of waterlogging has previously been reported (Mohamed 1998; Cott *et al*. 2013; Crooks *et al*. 2002). This likely explains its distribution where it is often the exclusive dominant close to the very edge of creek banks and where Armstrong *et al*. (1985) recorded the shortest anoxic fluctuations.

It appears that within these well delineated creek site communities gradients in waterlogging and thus extent of anoxia and low redox potentials are likely to persist. This is reflected in the compositions of the vegetation where certain areas are exclusively occupied by *Elytrigia atherica* and localised distributions of *Artemisia maritima* and *Suaeda vera* are encountered. The degree that these species are able to tolerate anoxia is unclear since it is notable that both *Elytrigia atherica* and *Artemisia maritima* were reported to exhibit enhanced root porosity under experimental flooding Rozema *et al*. 1985b)

3.6.3 General saltmarsh site

Perhaps the most notable difference between the measurements made in the Humber estuary saltmarshes and those made in the current study relate to the redox conditions reported for the GSM communities. In the former study these sediments were described as well aerated for much of the summer with highly oxidised Eh values recorded to depths of 30 cm until August. Periods of anoxia were found to be transient in response to tidal flooding from August returning to a highly oxidised state during the intervening periods over much of the measured sediment profile that resembled the strongly fluctuating redox conditions reported for sediments for the relatively well drained *Puccinellia* and the *Atriplex* runnel site in the same study. Simultaneous measurements of the *Puccinellia – Atriplex* creek site and the GSM site in the current study however showed that the period of anoxia in similar GSM communities can be far more extensive and persistent than previously characterised.

The initial recordings taken from a GSM site at Brancaster in 2012 showed firstly, that conditions of anoxia were prevalent at an earlier time in the growing season at least from July, secondly, rather than present as transient phases of anoxia fluctuating in response to periods of tidal flooding and drainage such conditions were largely continuous at all measured depths from the near surface (~2 cm) sediments to a depth of 20 cm, and thirdly, anoxic conditions appeared to persist over several months well into November. Moreover, the majority of Eh values from individual electrodes were continuously in the severely reducing range (< -100 mV) and the time series of Eh recordings more resembled the undrained Spartina low marsh sediments than the well drained *Puccinellia* sites. This large disparity between results obtained in the current study and those from the GSM site in Armstrong *et al*. (1985) was attributed at that time to the unusually high levels of sediment saturation from exceptionally high rates of precipitation in what emerged to be one of the wettest summers on record.

However, measurements taken from the GSM site at Stiffkey in the following year coincided with an exceptionally warm and dry summer and also showed that anoxic conditions were prevalent even during apogee tidal phases where the upper saltmarshes remain in an unflooded state for an extended period of at least 3 weeks either side of the summer solstice. This suggests that even during relatively dry periods sediments in the GSM still appear maintain sufficient saturation to impede oxygen diffusion to cause a deficit at a time of high oxygen demand. The high water content of these sediments relative to low marsh sediments has previously been reported and was attributed to its high organic matter content (Jefferies 1977).

While there was evidence of an increase in Eh values in some electrodes located in the upper portions of the sediment towards the end of extended neap tide phases the majority of electrodes registered continuously anoxic Eh values while at greater depths the prevalence of continuously low redox potentials in the moderate-severely reducing range (+150 mv to < -100 mV) was more evident. Overall the impression of the GSM sediments based on extensive monitoring of redox potentials was one of little available oxygen in the uppermost sediments even during an exceptionally dry summer and its presence was likely restricted to limited areas of microsites. It would therefore be expected that in a typical summer with average temperatures and average rates of precipitation any recovery towards oxic Eh values and presence of oxic microsites in the uppermost sediments would be less apparent than was evident during the unusually dry summer of 2013.

A particularly striking finding in the current study was evidence of a seasonal relationship with sediment redox potentials in the upper marsh communities. Measurements of Eh from both Brancaster and Stiffkey provided a strong indication that anoxic conditions and low redox potentials can extend well into the autumn months. This means that plants growing in the GSM communities are exposed to predominantly anoxic and reducing sediments for at least 4-5 months of the year from around mid-summer to well into the autumn months. Although Armstrong *et al.* (1985) alluded to the possibility of seasonal effects, their assumption was that anoxia was likely to be maximal in the winter and spring when sediments are most saturated. This is not supported in the current study by measurements from the upper marsh communities at Stiffkey and Brancaster, at least in the portion of the sediment depth that comprises much of the rooting zone. Conversely, it is during this period that rates of oxygen consumption in the sediment are likely be lowest due to the cessation of plant growth, the reduction in microbial activity and the increased diffusion coefficient and solubility of oxygen as a consequence of low sediment temperatures.

This accords with similar patterns found in saltmarshes for seasonal variations in sediment oxygen concentrations that were recorded to be lowest in summer and highest in winter months (Baumann *et al*. 2015) and in several studies that have reported a summer-autumn maxima and winter-spring minima for iron and sulphate reduction (Abdollahi & Nedwell 1979; Feijtel *et al.* 1988; Gribsholt & Kristensen 2003; Howarth & Teal 1979; Howarth *et al*. 1983; Kaplan *et al*. 1977; King 1988; Kirwin *et al*. 2014; Koretsky *et al.* 2003; Kostka & Luther 1995; Nedwell & Abram 1978; Nedwell & Floodgate 1972; Oenema 1990b; Rey *et al*. 1992; Senior *et al*. 1982; Thompson *et al*. 1995).

However, other studies of saltmarsh biogeochemistry have reported that different seasonal patterns can occur where plant-induced sediment oxidation in the upper portions of the sediment profile is a significant factor in the summer (Giblin & Howarth 1984; Luther & Church 1988; Otero & Macias 2002; Sundby *et al.* 2003). It appears that under certain conditions, where rates of root oxygen release are high and/or sediment oxygen consumption rates are low, the uppermost portions of sediments can become oxidised during the summer. The development of low redox potentials is delayed until autumn and winter months when the decomposition of seasonally accumulated dead organic matter becomes anaerobic.

Information about diel changes in redox conditions is saltmarsh sediments is scarce although their existence was previously described for *Spartina alterniflora* saltmarshes (Catallo 1999) and recently confirmed for similar saltmarshes by measurements of daily fluctuations in sediment oxygen concentrations (Baumann *et al*. 2015). While sampling frequency in Armstrong *et al*. (1985) was not sufficient to detect diel redox fluctuations, hourly measurements in the current study revealed abundant evidence of oscillations in Eh values which were particularly apparent in individual electrodes in the GSM site which varied in amplitude from tens of mV to over 400mV over a 24-hour cycle. These rapid fluctuations were most prevalent during the summer and the assumption is that they are generated by photosynthetically driven patterns of sediment oxidation is complicated by the observation that the peaks of these fluctuations usually fail to demonstrate a clear relationship with expected times for peak photosynthetic activity. Rather the appearance is of a high degree of randomness with peak Eh values that can occur at any time over a 24 hour cycle although the possibility cannot be discounted that the variable time lag could be caused by the different response times of individual electrodes. The presence of these dynamic redox patterns may, in part, explain the drift in Eh values and the difficulty often encountered in obtaining stable readings from manual measurements of redox potentials in the field.

Although there is a substantial body of evidence confirming the ability of wetland plants to alter the redox status with varying capacities to oxidise anaerobic soils (e.g. Howes *et al*. 1981), measurements in the current study found no evidence to support large-scale plant induced oxidation of the bulk sediment in the GSM site and short-term increases in Eh are is likely be restricted to a narrow region of the rhizosphere. The evidence based on extensive measurements of redox potentials over a three-year period suggests that the GSM site can be characterised by an intermediate degree of anoxia stress which is primarily influenced by season. Such conditions exist between the two ends along a redox continuum in these saltmarshes where one is characterised by a relatively low level of transient anoxia stress in the well drained creek site primarily influenced by the fluctuating hydrology driven by cycles of tidal flooding and drainage, and the other by a high levels of anoxia stress as a consequence year round conditions of continuous anoxia and low redox potentials primarily influenced by a state of more or less permanent flooding.

A number of the species that comprise the GSM community, including *Plantago maritima*, *Limonium vulgare*, *Puccinellia maritima* and *Triglochin maritima*, have been found to possess the anatomical adaptations typically found in waterlogging tolerant wetland plants in the form of enhanced levels porosity for internal transport of oxygen from leaves to the roots (Justin & Armstrong 1987). However, the capacity of these plants to tolerate conditions of prolonged anoxia and low redox potentials remains poorly understood. Moreover, in addition to tolerating oxygen deprivation and low redox potentials, plants of the GSM also experience stress from periods of hypersalinity where the ionic content of sediments from extracted porewater was measured in the current study to be at least 2x seawater concentration during the apogee tidal phases either side of mid-summer when the sediment saturation is at its lowest. There is evidence to suggest that growth of plants in the upper marsh communities is limited by the combined effects of waterlogging and salinity (Cooper

1982; Rozema *et al*. 1985). Rather than simply salinity acting alone (Jefferies 1977), it may be that the peculiar nature of the combined stresses better explains why no one species dominates the GSM and instead permits the co-occurrence of several species to form a relatively diverse and unique community.

It is interesting to note the analogous processes of waterlogging that have been attributed to maintaining the forb panne communities that occur on saltmarshes on the Atlantic coast of North America (Ewanchuk & Bertness 2004a). Forb pannes are described as physically harsh habitats that similarly experience low redox potentials and hypersalinity and appear to resemble GSM communities with high levels of plant diversity dominated by *Plantago maritima* and *Triglochin maritima*. These communities appear to be maintained largely by a degree of waterlogging that is sufficient to exclude competitively dominant clonal turves of *Spartina patens* (Ewanchuk & Bertness 2004b). A knowledge of the tolerances of GSM species to low redox potentials and oxygen stress that results from waterlogging therefore forms an crucial component in understanding the processes involved in structuring these communities and elucidating possible explanations for their limited colonisation on many restoration saltmarshes.

3.7 Conclusion

This study has successfully demonstrated the feasibility of adapting a portable redox datalogger for continuous in situ measurements of redox potentials on saltmarshes subject to periodic tidal inundation. The results of long-term hourly measurements from a range natural saltmarsh communities has revealed previously undescribed patterns of seasonal anoxia and short term variations in redox potentials in response to cycles of tidal flooding and drainage. Little evidence has emerged in this study supporting reports by some workers of the seasonal occurrence of plant driven sediment oxygenation on a scale sufficient to induce widespread alteration of the bulk sediment to an oxidised status in the summer followed by plant induced sediment anoxia in the winter as a consequence of plant dormancy and the anaerobic decay of accumulated organic matter.

While such seasonal cycles may occur in some types of saltmarsh under certain climatic conditions, a contrary pattern has emerged in the current study that may be a more applicable characterisation of more temperate saltmarshes. Here seasonal patterns are instead characterised by generally prevailing conditions of sediment anoxia in the summer months followed by a return to predominantly oxic conditions in the winter. Rather than redox processes being driven by plant growth and decay these sediments appear to be more strongly influenced by temperature driven changes to activities of microbially mediated redox processes that are highest in the summer months. An exception to this seasonal pattern appears to occur in the undrained *Spartina anglica* low marsh site where different redox processes appear to be operating which likely generates generally continuous anoxia and highly reducing sediment conditions throughout the year.

The apparent presence of summer-autumn anoxia in the general saltmarsh communities has potentially important implications for understanding the differential patterns of vegetation establishment on restoration saltmarshes. The evidence from the current study indicates that a number of species which show a limited capacity for colonisation on restoration sites appear to be tolerant of conditions of prolonged anoxia and high reduction intensities in natural saltmarshes at levels that are associated with iron and sulphate reduction. This suggests other processes may be operating to constrain their establishment instead such as differences in the inherent redox capacity characteristics of sediments between natural and restoration saltmarshes. It is this factor that determines how much of the system is undergoing reduction and hence the likelihood of accumulated inorganic reductants to potentially phytotoxic concentrations. Further work is therefore needed to establish experimentally the tolerances of these species to anoxia, low redox potentials and phytotoxicity since it cannot be discounted that these plants are able to survive generally anoxic environments by scavenging oxygen via shallow rooting or exploiting oxic microsites.

While the results obtained in the current study broadly corroborated some of these earlier findings by Armstrong *et al*. (1985), a number of important differences were found particularly in relation to the GSM site. While the GSM site in the Humber estuary was described as being predominantly oxic, measurements undertaken at the same time of year in the current study showed that sediments under similar upper saltmarsh vegetation communities on the North Norfolk coast are largely anoxic at all but the most surficial depths, even during an exceptionally dry summer and at a time when the influence of tidal flooding was at its lowest. Furthermore a strong seasonal pattern was found where anoxia and the resulting redox intensity appears to increase throughout the progression of summer and these conditions persist well into the autumn before the GSM communities return to predominantly oxic conditions.

CHAPTER 4: The redox characteristics of managed realignment saltmarshes

4.1 Introduction and aims

The Brancaster and Freiston managed realignment sites represent two contrasting examples of saltmarsh restoration on sediments that have experienced different reclamation histories. While the former was under reclamation for arable agriculture for a few decades, the latter was under pastoral agriculture for a period extending from the mid-19th century. During this time the original saltmarsh sediment is likely to have experienced considerable physicochemical alterations or ‘ripening’ from permanent drainage and oxidation of the organic matter that causes irreversible consolidation and compaction (Crooks & Pye 2000; Hazelden & Boorman 2001).

The consequent conditions of waterlogging, anoxia and the associated effects of low redox potentials can limit the establishment of certain species either directly or indirectly by negatively affecting the complex sediment microbiology. For example, there may be a range of poorly understood specific interactions between soil microorganisms and plants species that may difficult to restore under such conditions (Burke *et al*. 2002; Carvalho *et al*. 2001). Considerable time may be needed for the edaphic and the hydrological conditions to return to their natural state which may important implications for the establishment of plant communities and ecosystem functions to a level of equivalence to the original saltmarsh (Craft *et al*. 1999; Onaindia *et al*. 2001). The development of accidentally reactivated saltmarshes on formerly reclaimed land following historical storm breaches of sea defences indicates significant differences in vegetation composition can persist after well over a century of development (Garbutt & Wolters 2008; Mossman *et al*. 2012a). An understanding of the abiotic constraints to vegetation establishment relating to waterlogging and low redox potentials in recent MR sites may therefore provide important insights regarding the limitations and prospects for the successful reinstatement of saltmarsh ecosystems.

In order to compare how redox characteristics in sediments of relatively diverse natural saltmarshes of the North Norfolk coast differs from sediments of less diverse restoration saltmarshes continuous measurements of hourly redox potential using the redox datalogger were undertaken to describe the redox dynamics in sediments of MR response to tidal flooding. Measurements were undertaken between 2012-2014 both at an early stage in the year when the average Eh was expected to be highest and at a late stage in the growing season when the average Eh was expected to be lowest for the following sites:

Brancaster MR: (a) *Spartina-Aster* low marsh and (b) *Puccinellia* mid marsh ~3m distance of an artificial drainage channel.

Freiston Shore MR: (a) *Spartina-Aster* low marsh; (b) *Atriplex-Puccinellia* low marsh ~5m distance of an artificial drainage channel; (c) *Atriplex-Puccinellia* upper marsh site without close proximity of a drainage channel and (d) *Atriplex* natural marsh site ~5m distance from a drainage channel.

Measurements were taken from a total of 48 electrodes at four depths: 2 cm, 5 cm, 10 cm and 20 cm and the results are presented as a time series of actual recordings and summary format of mean ±S.E. for electrodes at each depth.

4.2 Results

4.2.1 Brancaster MR - Low Marsh site

Continuous redox potential measurements were taken for three periods, 20th February – 22nd March 2013 over two phases of tidal flooding and two recordings over a single phase of tidal flooding, 1st – 15th October 2013 and 12th – 26th May 2014. Results for first and last recordings are summarised as a time series of mean ±S.E. Eh values in Figure 4.1 and actual measurements are shown in Figures 4.2 & 4.3. The measurements indicate sediments at this site are poorly aerated in all but uppermost parts of the sediment profile. Only at the 2 cm depth were there clear signs of the presence of oxic sediments in four electrodes three of which registered continuously oxic Eh values regardless of the stage of tidal cycle while the fourth electrode showed a slow decrease to mildly reducing values in response to flooding followed by a return to an oxic state during subsequent neap tide phases. The remainder of electrodes registered continuously anoxic conditions throughout the neap tide phase at moderate-severe reducing levels with minimum Eh values of -130 mV. The impression given by the broad range of Eh values at this shallow depth is one of a heterogeneous redox environment with the presence of a mixture of oxic and anoxic areas probably reflecting microtopographical variations in the sediment surface resulting in localised areas of oxic and anoxic conditions. The recordings from 5 cm depth show signs of a return to transient oxic Eh values in the latter half of the neap tide phase by three electrodes showing a degree of redox heterogeneity extending to this depth with the presence of short-lived aerobic conditions of little more than 48 hours. Again, the majority of the electrodes registered continuously reducing conditions at moderate-severe Eh values with similar minimum Eh values as recorded at 2 cm. No oxic Eh values were recorded below 10 cm with all reading with the moderate-severe range minimum values of about -130 mV at 10 cm and -175 mV at 20 cm.

The second recording period showed more consistent conditions with anoxic and moderate-severe reducing conditions in the uppermost sediments. Only one electrode returned to an oxic state some 48 hours after the end of the flooding phase. A greater number of severely reducing Eh values was recorded at each depth with a range of minima of about -150 mV to -185 mV at all depths. The redox profile for this site at this stage appears to resemble that recorded from the Stiffkey *Spartina* dominated low marsh site where almost all readings are highly poised within a limited range of low Eh values. However, average Eh values at all depths at the Stiffkey site are < -200 mV while at this site the lowest average value was -125 mV at 20 cm. It is likely that these values would decrease further into the summer season as the sediment temperature increased. This is supported by subsequent measurements taken in October 2013 showing a continuation of moderate-severe reducing conditions at all measured depths and decrease in average values towards the range -160 mV to -175 mV at the 10 – 20 cm depths. No indication of recovery to oxic conditions during the neap tide phase.

The Stiffkey low marsh site was an unusual case since the sediments were found to be highly reducing at all depths at an early stage in the year. This was attributed to the short periods free from tidal flooding that creates a different redox environment under such conditions. All other measurements taken in the current study from a range of sites at higher elevations show a strong seasonal effect with oxic conditions persisting in the winter-spring period at least in the uppermost sediments. To an extent the redox conditions of the *Spartina-Aster* dominated low marsh site at Brancaster resembles the Stiffkey low marsh site since the poor aeration status of this site early in the year is likely a consequence of the short interval of the non-flooding phase at this low elevation site which can be a little as 4 days before the onset of the next inundation.

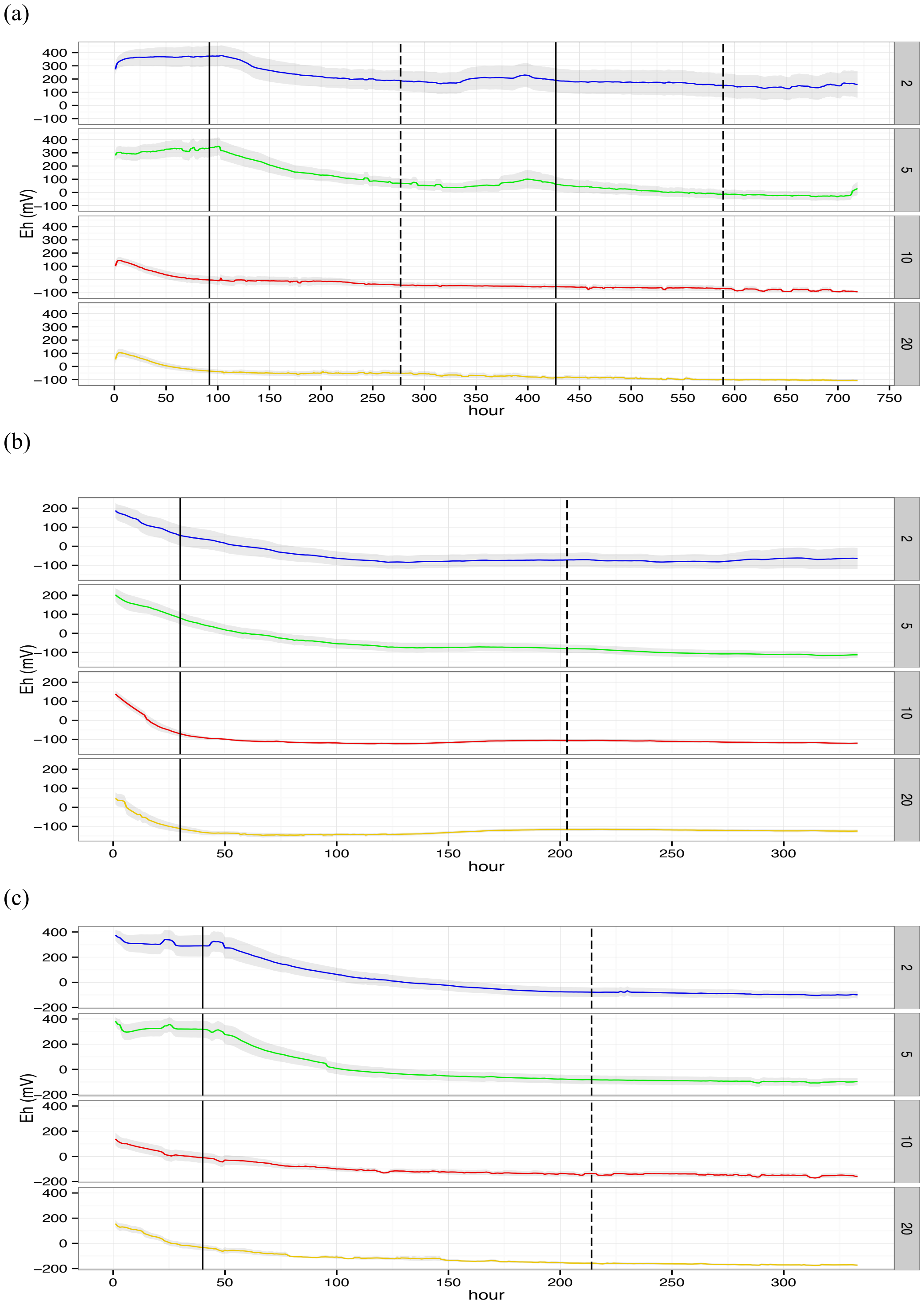
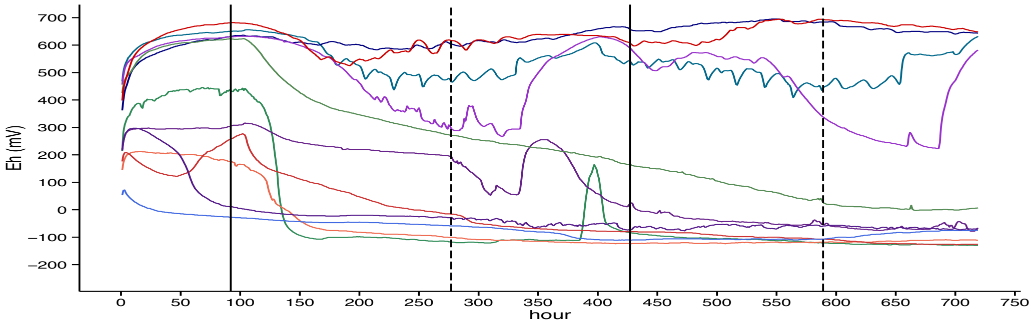
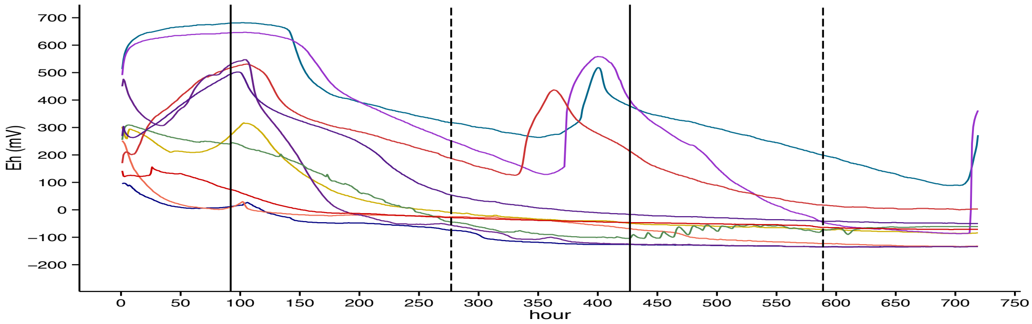


Figure 4.1. Time series of mean ± S.E. for Brancaster MR *Spartina-Aster* dominated low marsh site (a) 20th February – 22nd March 2013, (b) 12h – 26th May 2014 and (c) 1st – 15th October 2013. Average Eh conditions at all depths are predominantly anoxic (< +350 mV) at all depths throughout neap-spring tidal phase with only small seasonal increase in the uppermost sediments in winter. Sediment depth (cm) is shown on right hand side of each panel.

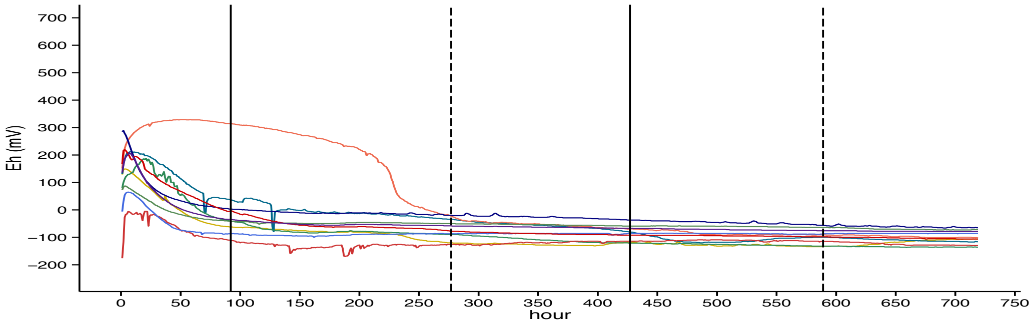
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

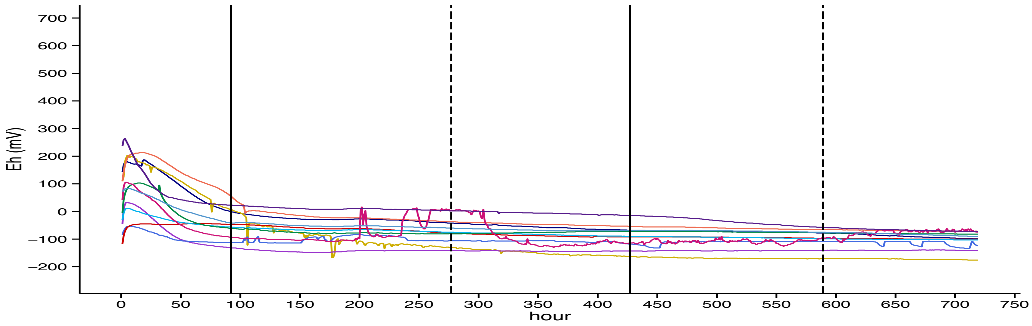
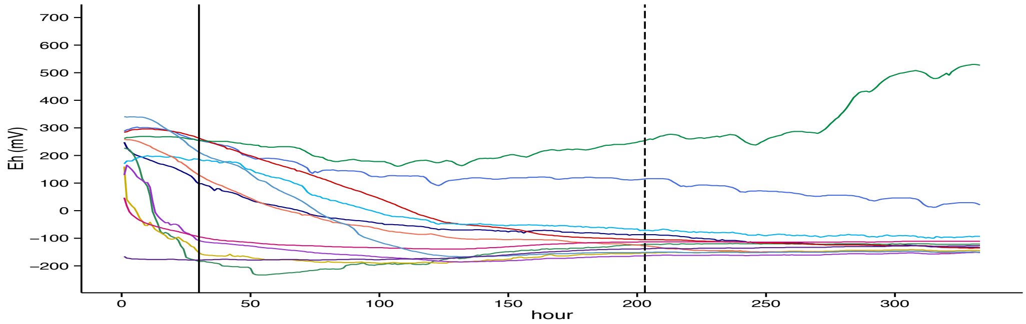
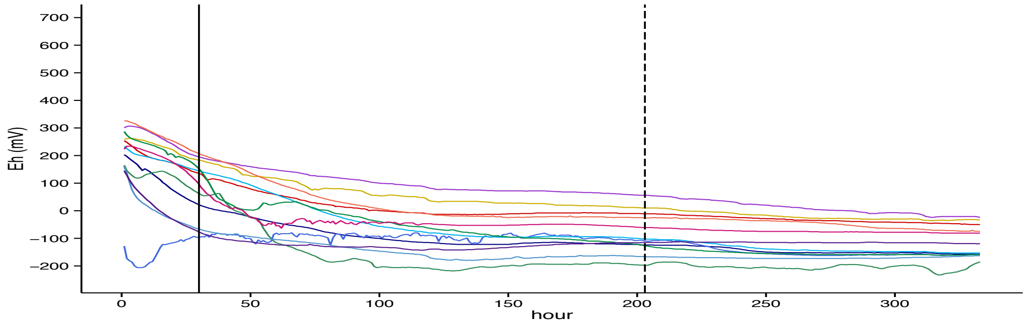


Figure 4.2. Eh measurements from replicate electrodes at four depths for the period 20th February – 22nd March 2013 at the Brancaster MR low marsh site over two flooding phases. The uppermost sediments indicate a degree of heterogeneity with the presence of oxic microsites at Eh values (> +350 mV).

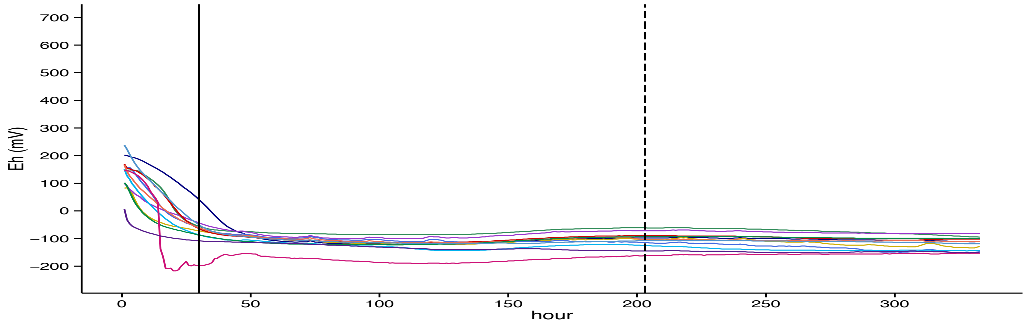
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

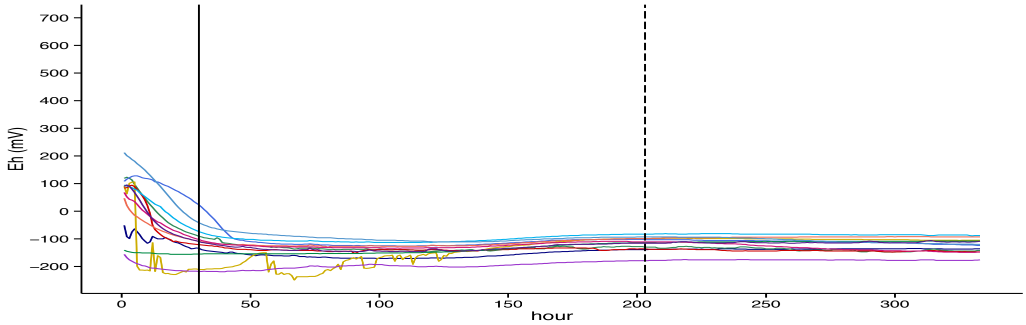


Figure 4.3. Eh measurements from replicate electrodes at four depths for the period 12th – 26th May 2014 at the Brancaster MR low marsh site over a single flooding phase showing prevalence of severe reducing Eh at all depths early in the growing season. Similar results for October suggest highly reducing conditions persist throughout the growing season.

4.2.2 Brancaster MR - Mid Marsh Creek site

Measurements were taken at this site over a single phase of tidal flooding for three periods: 27th March – 6th April 2013, 11th – 25th July 2012 and the 15th – 29th October 2013. Results for these recordings are summarised as a time series of mean ±S.E. Eh values in Figure 4.4 and actual measurements for two periods are shown in Figures 4.5 & 4.6. In contrast to the low marsh site this site showed indications of a seasonal trend of oxic conditions at least in the uppermost sediments extending from the spring to the summer period leading to generally anoxic state at all depths by autumn.

During the spring period the majority of electrodes at the 2cm depth recorded more or less oxic Eh values throughout the flooding phase while a small number showed a gradual decrease to transient mild-moderately reducing conditions followed by a recovery to an oxic state within 48-72 hours after the final inundation. A similar pattern was found to extend to at least the 5cm depth although a higher number of electrodes recorded a decrease to mild-moderately reducing values persisting for 3-4 days into the neap tide phase. Signs of oxic recovery at 10 cm were less evident with only two electrodes showing an increase but still failing to attain oxic status by the end of the recordings session four days post flooding. The majority of electrodes at this depth and all electrodes at 20 cm indicated that the redox environment in this part of the sediment profile resembles that recorded in the lower marsh over the preceding four weeks. Readings taken in July show a more clearly defined development of flood induced transient anoxia at least to the 2 cm depth with the majority of electrodes registering moderate-severe reducing conditions extending well into the neap tide phase before a return to an oxic state after a delay of 4-5 days post flooding. A similar pattern of transient anoxia was less evident at 5 cm with only two electrodes showing oxic recovery with 6 days post flooding while the majority showed the persistence of continuous anoxia at moderate-severe reducing Eh values which was also evident in all the electrodes at 20 cm. A seasonal trajectory of increasing redox intensity at all depths was further indicated in the readings from October which showed a higher prevalence of electrodes recording severely reducing Eh values and only a small increase in post flooding readings at the 2 cm depth was recorded none of which attained oxic recovery before the proceeding flooding tide. At this stage the minimum mean Eh values at all measured depths was in the range -140 to -150 mV which was only slightly higher than the mean values for the low marsh site at this time of year. It is remarkable that despite all measurement sampled within a 3 m proximity from the edge of a large creek that any drainage effect in the Eh values of was only reflected at the uppermost depths of the sediment while at a certain stage in the year the redox activity appears to increase to such an extent that it nullifies any drainage effect even at the uppermost measured depths. Nevertheless, differences between the creek and low marsh sites in redox conditions are apparent in the earlier stages of the year that are evidently sufficient to be attributable to the development of very different vegetation compositions between these two sites.

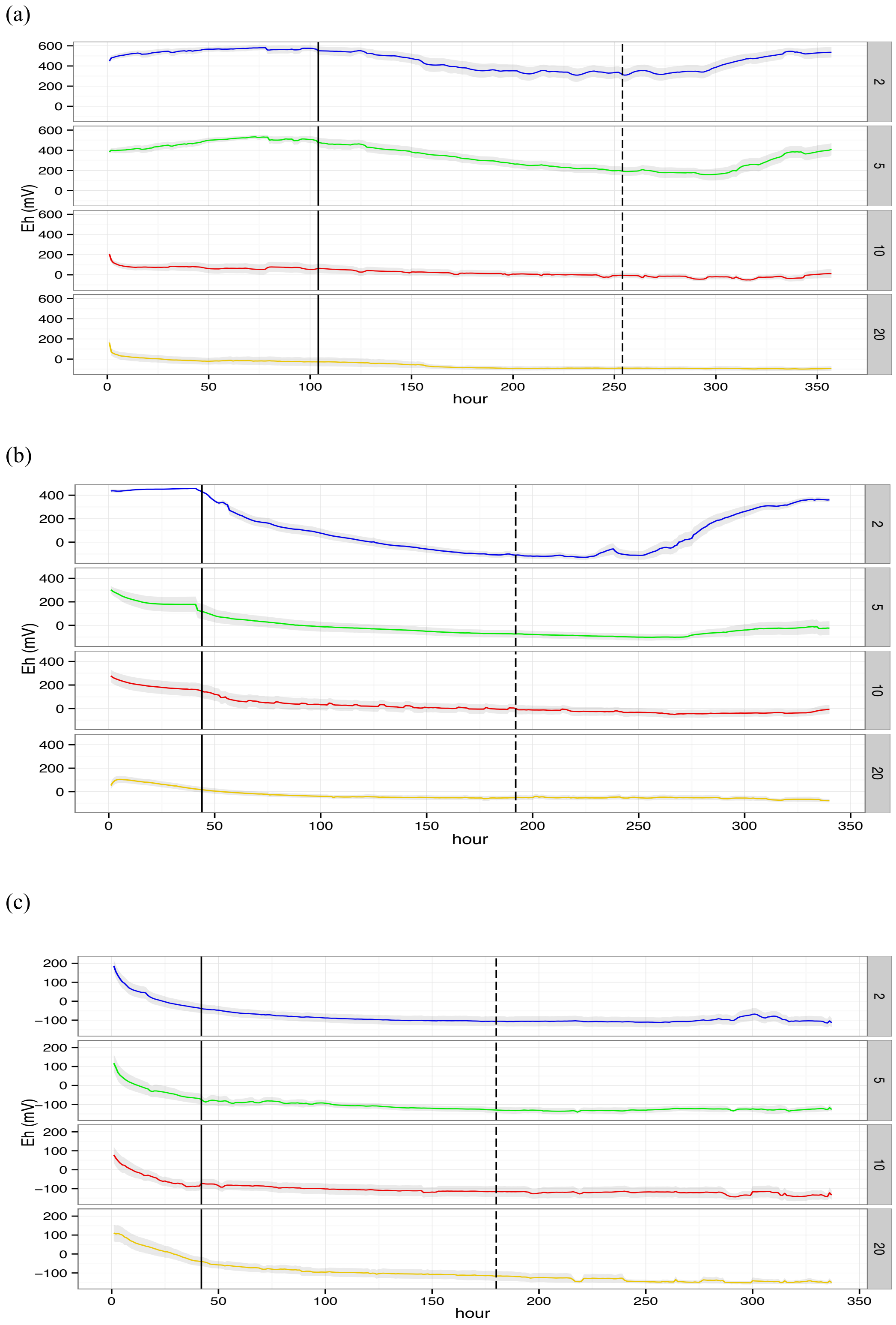
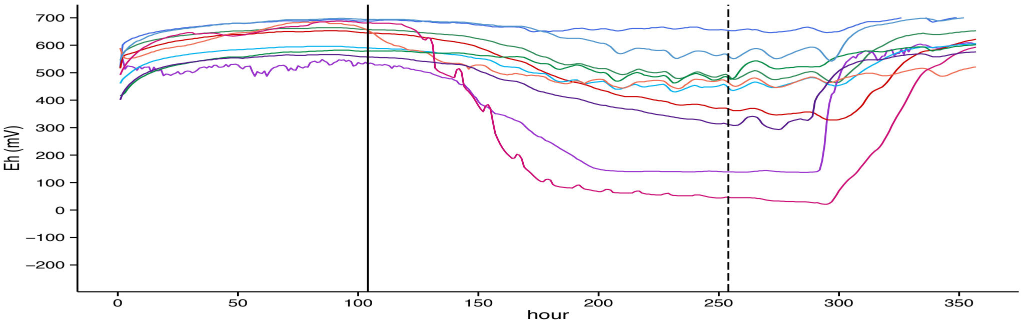
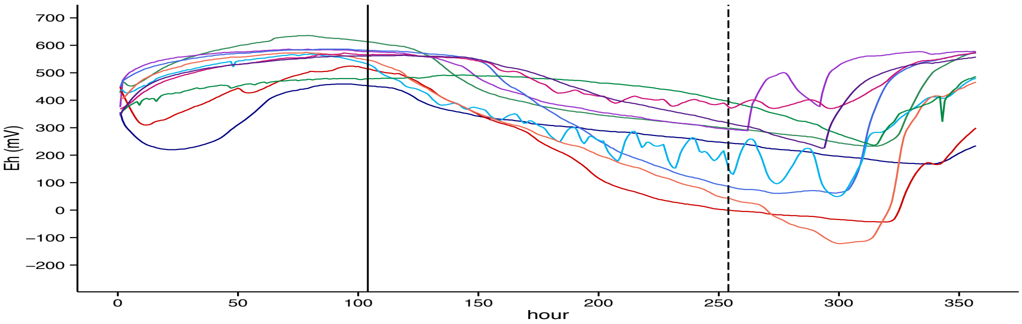


Figure 4.4. Time series of mean ± S.E. for Brancaster MR *Puccinellia* dominated creek site at mid marsh elevation (a) 22th March – 6th April 2013, (b) 11th – 25th July 2012 and (c) 15th – 29th October 2013. Average Eh conditions at all depths are predominantly oxic at uppermost depths early in the year and the presence of post flooding oxic recovery mainly at the 2cm depth in summer. The development progressively reducing conditions extending to the uppermost depths is indicated by the autumn. Sediment depth (cm) is shown on right hand side of each panel.

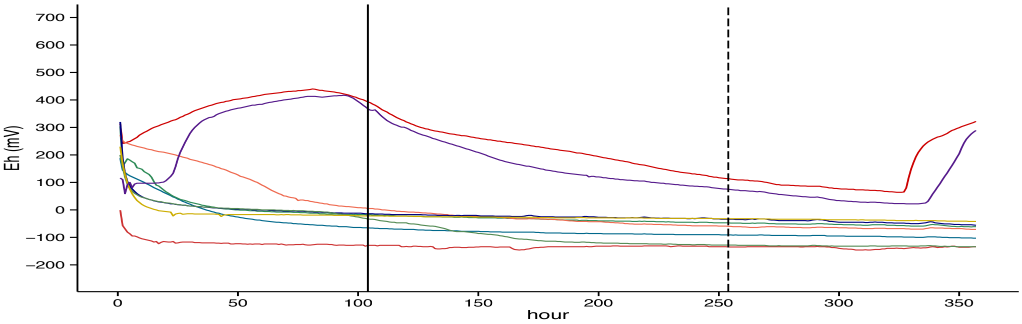
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

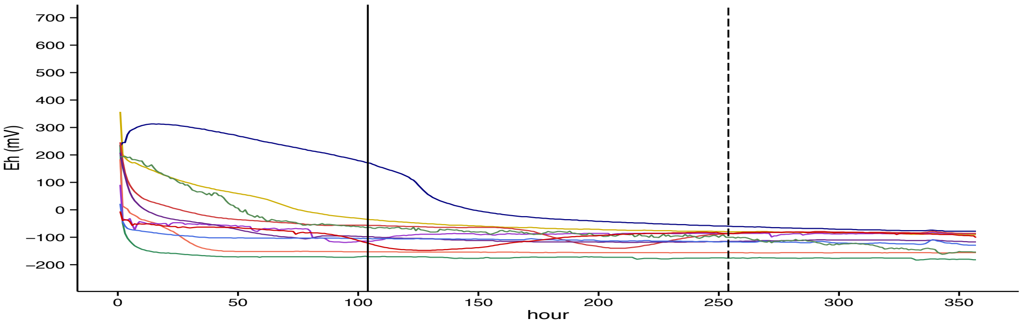
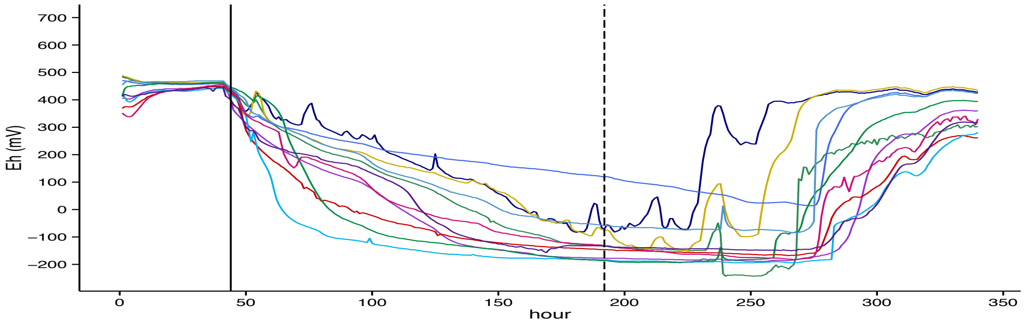
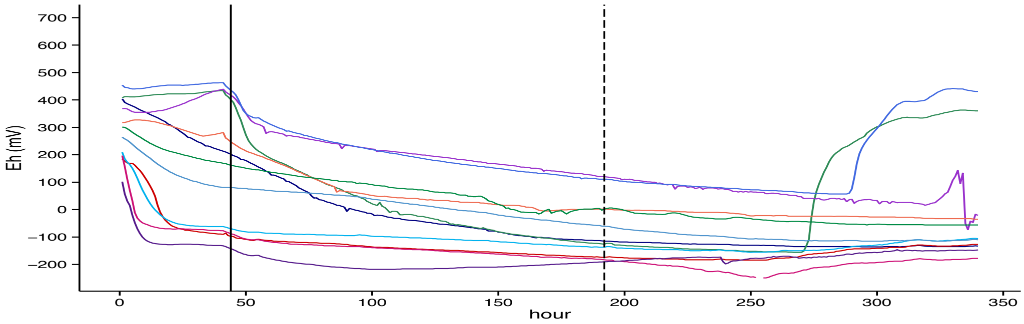


Figure 4.5. Eh measurements from replicate electrodes at four depths for the period 22nd March – 6th April 2013 at the Brancaster MR creek site over a single flooding phase showing prevalence of oxic Eh at uppermost depths early in the year while lower measured depths show predominantly continuous anoxic Eh levels throughout the tidal cycle.

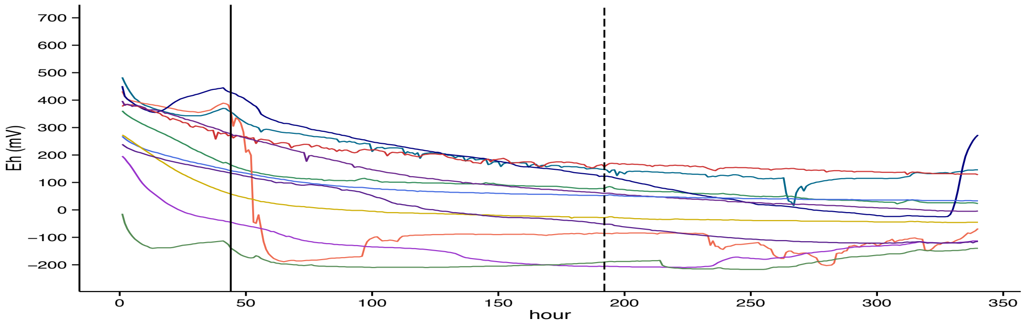
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20cm

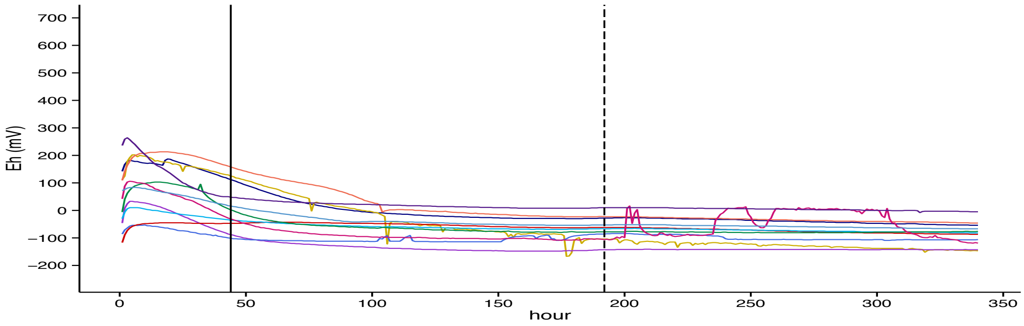
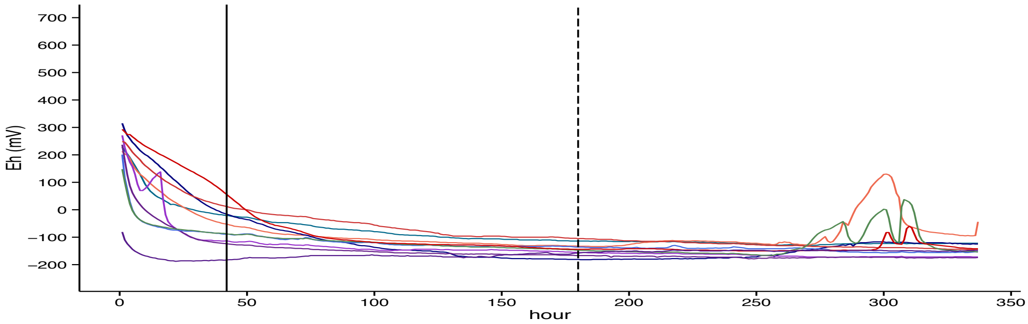
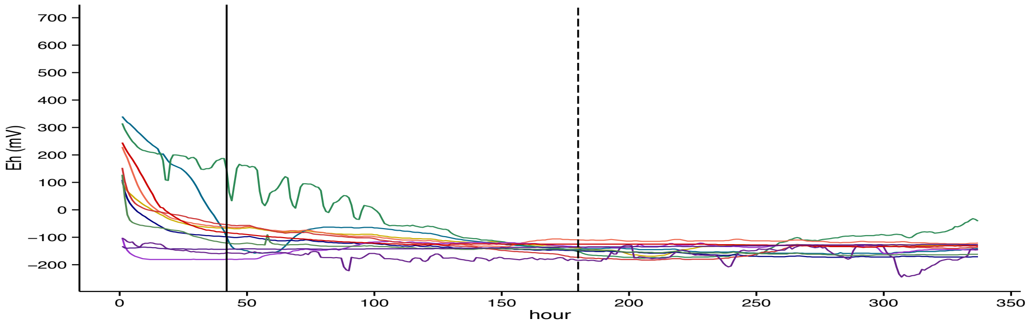


Figure 4.6. Eh measurements from replicate electrodes at four depths for the period 11th – 25th July 2012 at the Brancaster MR creek site over a single flooding phase showing decrease to severely reducing Eh followed by oxic recovery at 2 cm and to a lesser degree at 5 cm. Conditions remain predominantly anoxic at moderate-severe reducing Eh at greater depths.

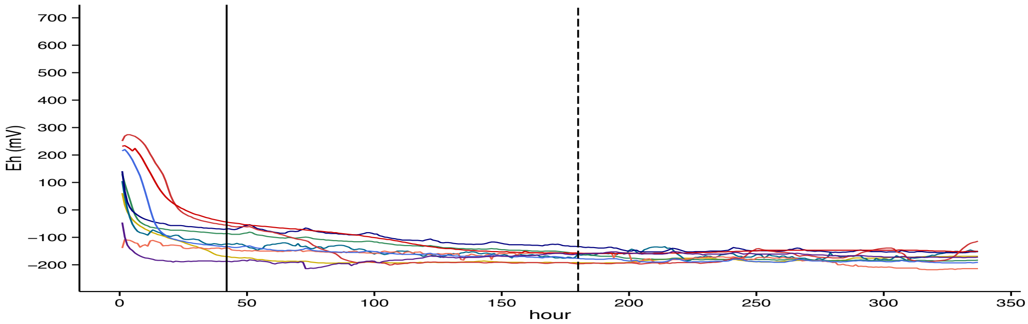
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

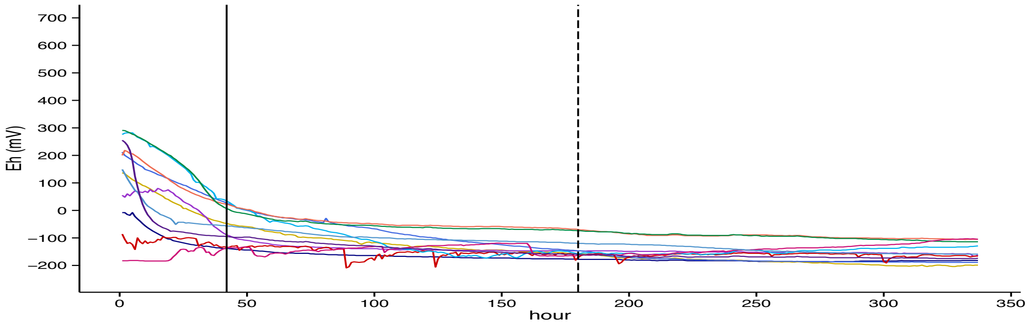


Figure 4.7. Eh measurements from replicate electrodes at four depths for the period 1st – 15th October 2013 at the Brancaster MR creek site over a single flooding phase showing prevalence of moderate-severely reducing Eh at all measured depths by early autumn.

4.2.3 Freiston Shore – Natural Reference Marsh

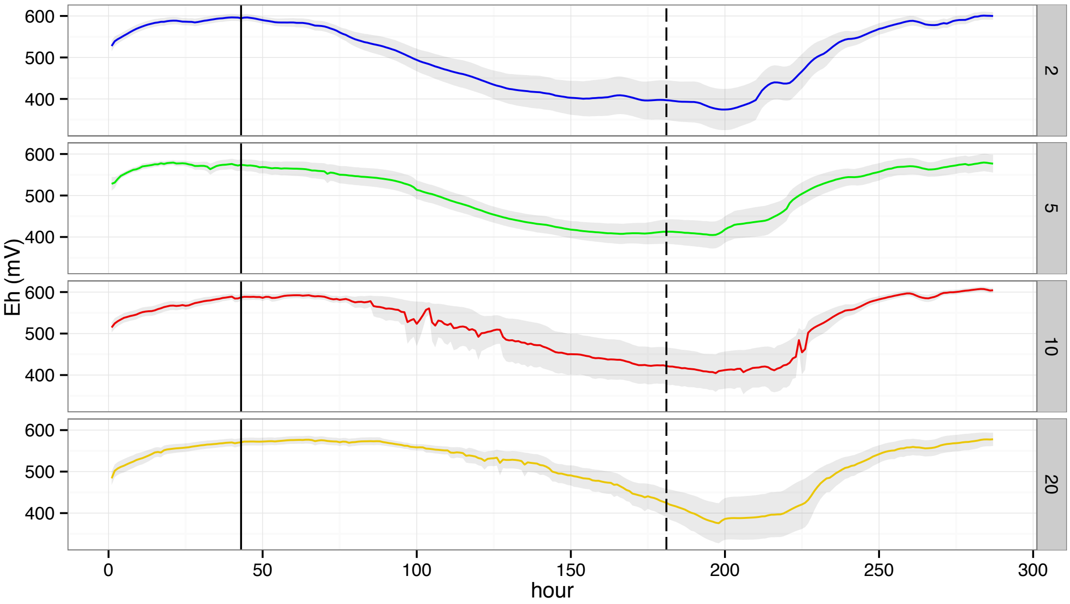
Measurements were taken at this site over a single phase of tidal flooding for the periods: 26th February – 10th March 2014 and two flooding phases for the period 23rd August – 18th September 2014. Results for these recordings are summarised as a time series of mean ±S.E. Eh values in Figure 4.8 and actual measurements for two periods are shown in Figures 4.9 & 4.10. The recordings made early in the year show a remarkably aerobic sediment at this site to measured depths of at least 20 cm. A similar pattern is replicated at all depths with a slow decrease to mildly reducing Eh values following the onset of tidal flooding. The lowest Eh values induced by flooding were recorded at the uppermost depths probably the availability of plant litter which provides the energy source for the limited amount of microbial activity occurring at this time of the year, The charts of actual hourly recordings (Figure 5.9) show how the effect of a low oxygen demand in the sediment at this time of the year can result in a slow decrease in Eh such that minimum values are achieved well after the end of the flooding phase. Almost all electrodes registered a return to highly oxidised Eh values with 2-3 days post flooding regardless of depth.

The subsequent period of measurements taken in late summer revealed striking fluctuations Eh values demonstrated by a number of electrodes with some showing particularly high peak to peak amplitudes of up to 300 mV during the two flooding phases occurring over an approximate 24 hour cycle. The majority of these highly fluctuating values occurred in electrodes at the 2 cm and 5 cm depths only a few exhibiting this behaviour at the deeper depths where the fluctuations tended to be more muted. Similar large fluctuations were encountered in the GSM site where it was noted that the peaks did not always coincide with times of photosynthetic activity and it was suggested that this could be attributable to the variable response times of the electrodes. However, a number of electrodes show a degree of synchrony in the pattern of fluctuation confounding any interpretation with regard to idiosynchratic electrode behaviour. The large fluctuations do not appear to be an artefact from a faulty reference electrode since this was tested for correct functioning after the recordings were taken. Moreover, a similar pattern of fluctuating Eh was observed in subsequent measurements at this site using a new reference electrode (Figure 4.11).

This site is dominated by plants not known for their capacity to oxidise sediments although *Aster tripolium* is abundant and may be responsible if sediments occurring in this part of the marsh are not well poised with respect to redox potential.

Notwithstanding the fluctuating Eh values it is still possible to discern some evidence of a seasonal decrease in Eh values induced by flooding with a number of electrodes at each depth registering moderate-severe reducing conditions with most returning to an oxic state within 48 hours post flooding. Remarkably a number of electrodes at each depth recorded oxic Eh values throughout the flooding phases indicating a relatedly low oxygen demand at all depths. Despite receiving more than twice the frequency on tidal inundations than the upper marsh creek site at Stiffkey, redox measurements in sediments of this marsh indicate a greater prevalence of oxic conditions at all depths at this stage of the year. The particularly oxic state of these sediments is reflected in the composition of the vegetation that is dominated by dense *Atriplex portulacoides* and large, vigorous *Suaeda maritima* – both species are species which lack the anatomical adaptations to thrive in less well oxidised sediments.

(a)



(b)

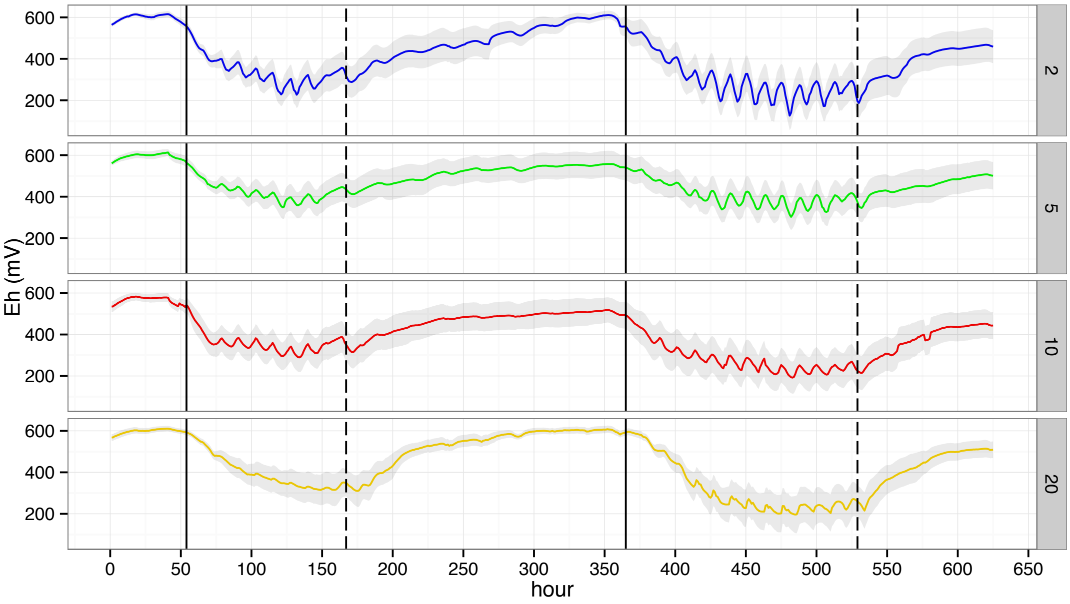
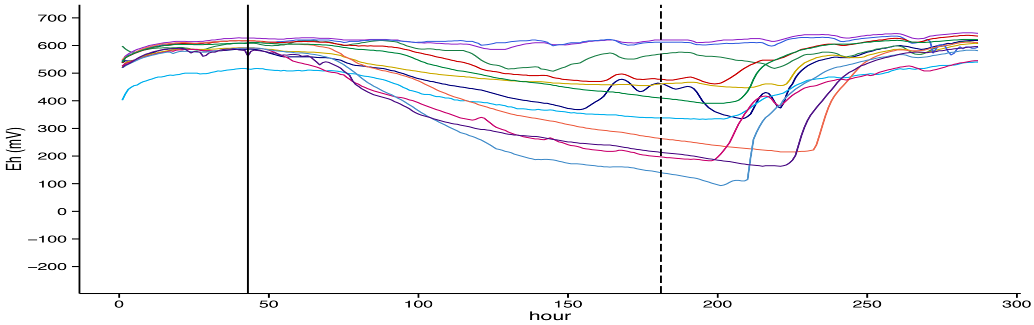
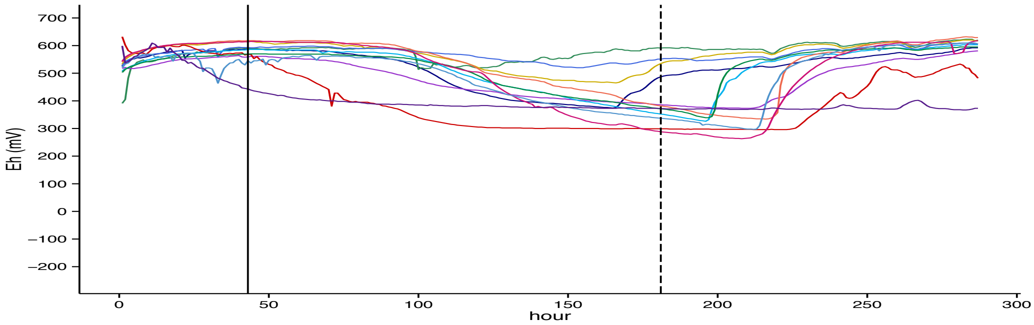


Figure 4.8. Time series of mean ± S.E. for Freiston Shore *Atriplex* dominated natural marsh site at 26th February – 10th March 2014 (a) and 23rd August – 18th September 2014 (b) Average Eh conditions at all depths are predominantly oxic at all depths early in the year even through the flooding phase. Predominantly oxic conditions persist throughout the growing period and well into late summer with only a small post flooding decrease to mildly reducing Eh. Sediment depth (cm) is shown on right hand side of each panel.

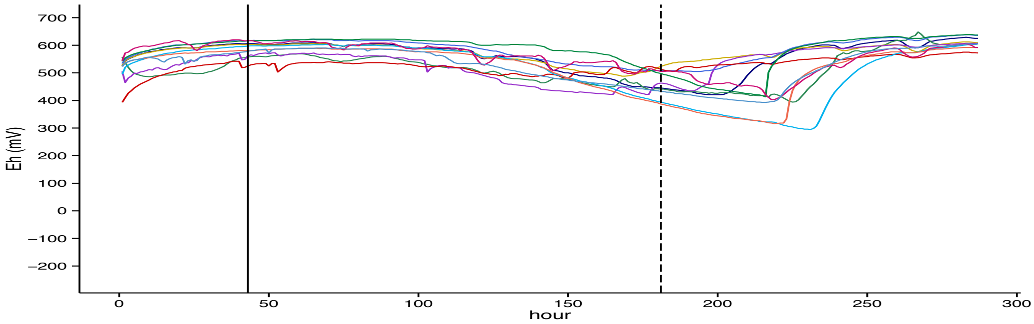
(b) 2 cm



(b) 5 cm



(c) 10 cm



(c) 20 cm

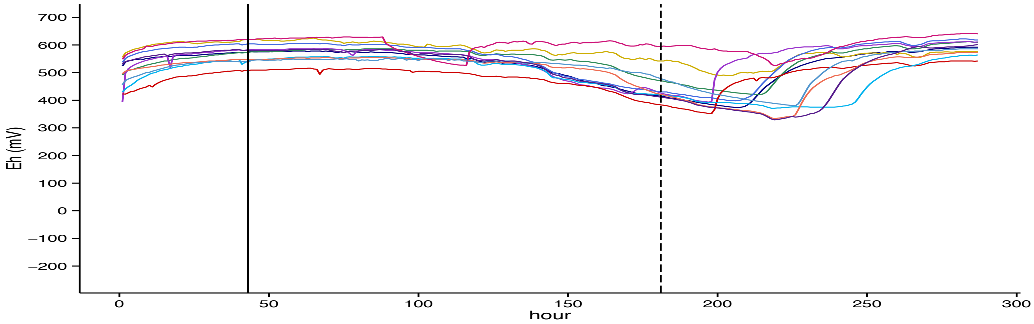
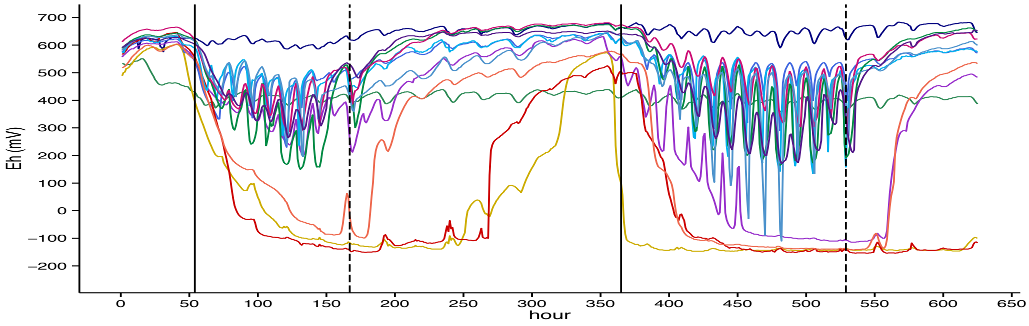
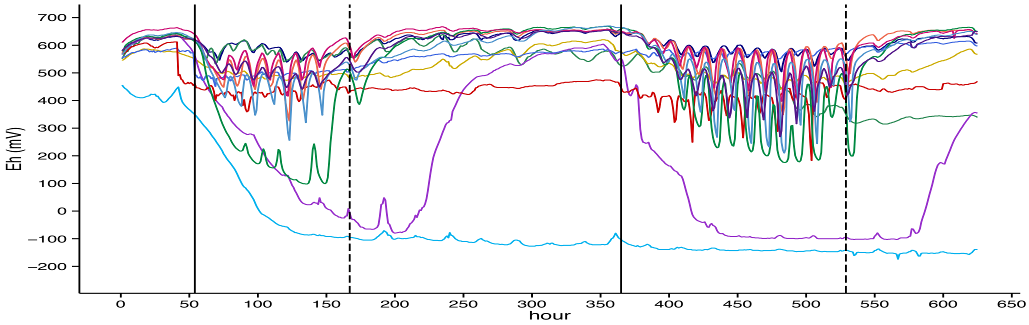


Figure 4.9. Eh measurements from replicate electrodes at four depths for the period 26th February – 10th March 2014 actual Eh at the Freiston Shore natural marsh site over a single flooding phase showing high consistency of oxic Eh with only a small post flooding decrease to mildly reducing Eh at the 2 cm depth.

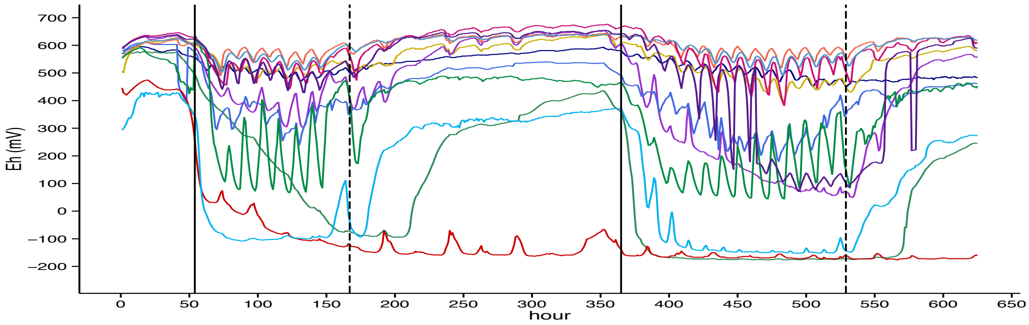
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

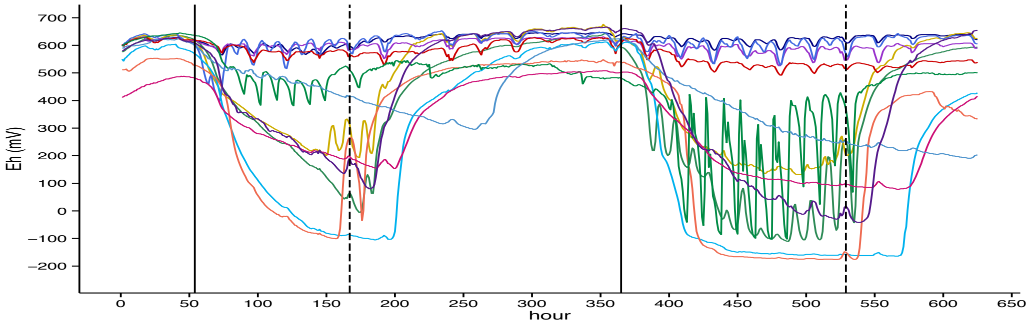
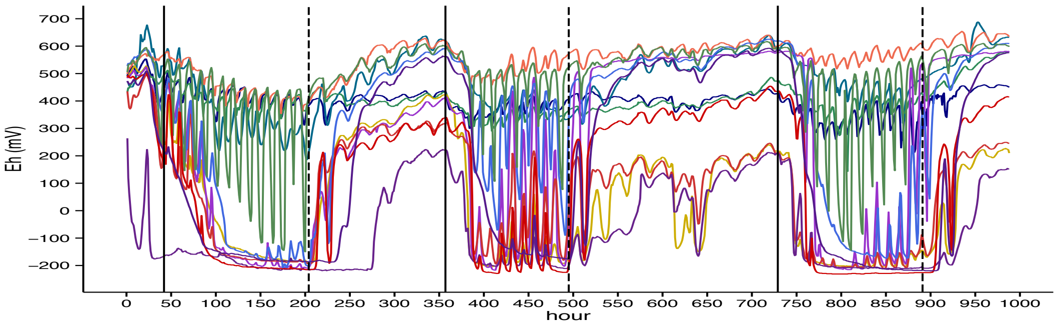
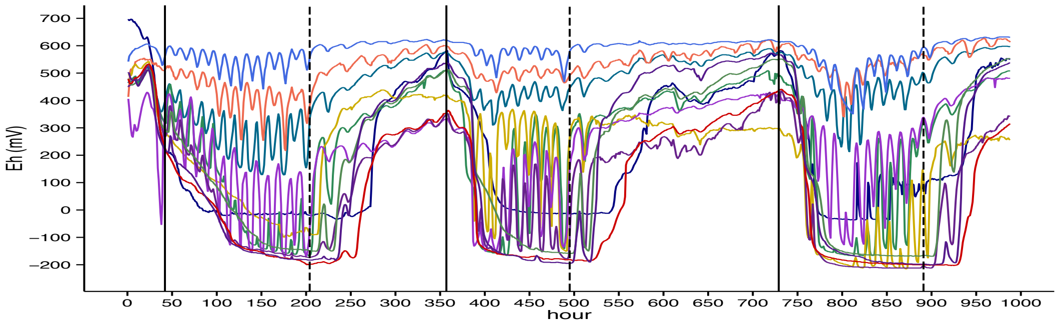


Figure 4.10. Eh measurements from replicate electrodes at four depths for the period 23rd August – 18th September 2014 at the Freiston Shore natural marsh site. This shows the large variation in electrode response to flooding from oxic Eh maintained throughout flooding phase even at 20 cm to decreases to severely reducing Eh followed by relatively rapid post flooding oxic recovery.

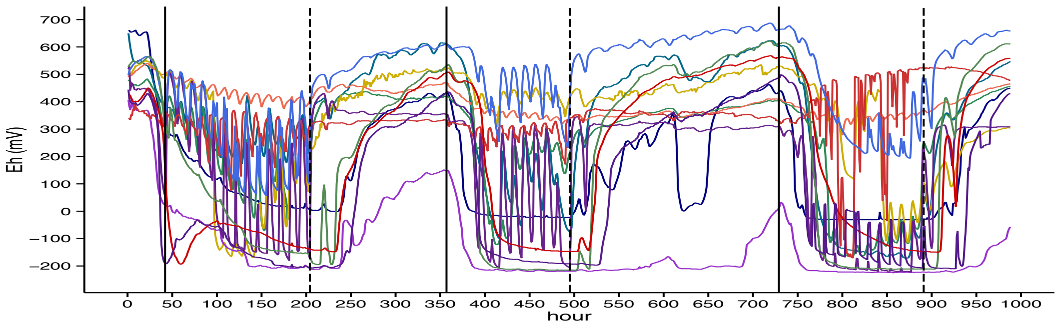
(a) 2 cm



(a) 5 cm



(a) 10 cm



(a) 20 cm

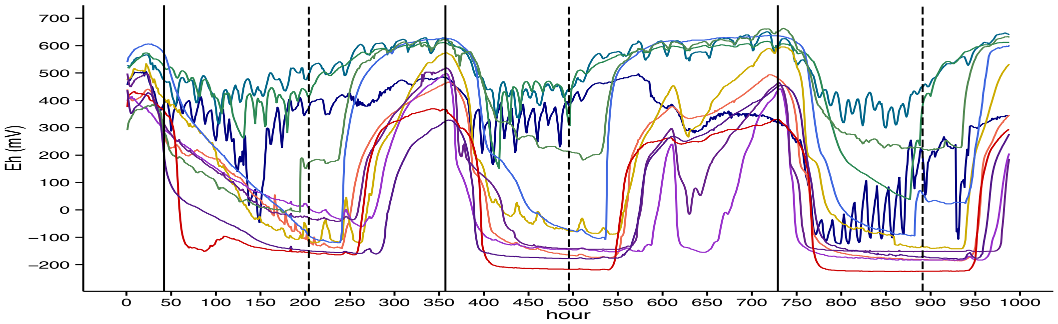


Figure 4.11. Eh measurements from replicate electrodes at four depths for the period 29th July – 7th August 2015 at the Freiston Shore natural marsh site over three flooding phases.

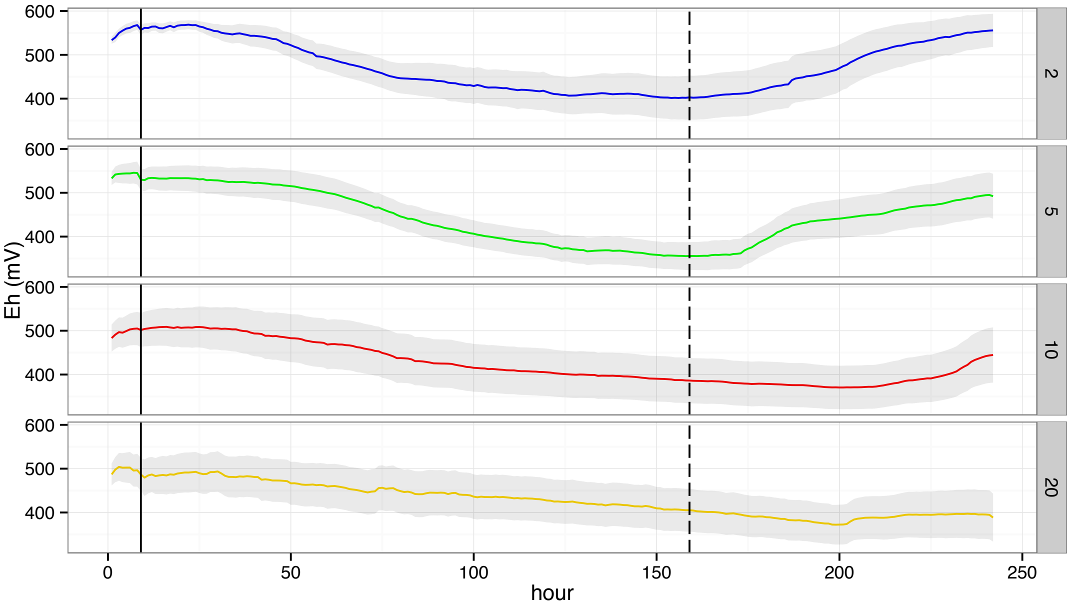
4.2.4 Freiston Shore MR – Upper Marsh site

Measurements were taken at this site over two single phases of tidal flooding for the periods: 9th – 19th January 2012 and 19th August – 3rd September 2013. Results for these recordings are summarised as a time series of mean ±S.E. Eh values in Figure 4.12 and actual measurements for two periods are shown in Figures 4.13 & 4.14. The redox conditions in these sediments below vegetation dominated by a mixture of *Atriplex portulacoides* and vigorous *Puccinellia maritima*, at the beginning of the year much resemble the conditions recorded early in the year for the *Atriplex* dominated natural marsh site with the majority of electrodes recorded more or less continuously oxic conditions throughout the flooding phase. There is however some evidence of a degree of heterogeneity at this site with a small number of electrodes indicating the existence of localised areas of persistent anoxia and mild- moderate reducing conditions at the 5 cm depth and the lower measured depths. Overall the sediments at each depth are on average characterised as oxic with most electrodes showing a similar response to flooding as the natural marsh by exhibiting a slow transient decrease in Eh to mildly reducing conditions before a general return to oxic Eh values within 48 hours post flooding. Again, this effect appears to be somewhat muted at deeper depths where the flood induced decrease in Eh is smaller and the post flood increase is delayed for a longer period.

The recording for the late summer period shows a stronger seasonal effect compared to the equivalent time in the natural marsh site and did not replicate the high levels of fluctuating Eh recorded during the flooded phase in the natural marsh. Additionally, unlike the natural marsh where a number of electrodes remained in an oxic state throughout the flooding phase, all electrodes at this site registered a flood induced decrease to a broad range of values from mild to severely reducing with the average minimum values in the moderately reducing range at all depths. The electrodes showed a high degree of variation at the 2 cm depth in time taken to register a return to an oxic state from under 48 hours at the earliest although the majority attained this status some 4-5 days post flooding. Similar delays of 4-5 days were recorded at the deeper depths however more electrodes failed to reach an oxic state by the end of the recording period some 8 days post flooding. The higher degree of variation in oxic recovery at this site indicates a greater degree of redox heterogeneity in these sediments compared to sediments of the natural marsh. This lack of consistency between electrodes readings is reflected for the average Eh that remain at a sub-oxic values at these depths at the end of the recording period despite the clear presence of a number of electrodes attaining an oxic state by the end of the recording period (Figure 4.12).

This probably explains the patchy distribution of *Atriplex* at this site where it thrives in the more oxic areas and its inability to spread to adjacent areas more prone to anoxia where *Pccinellia* is able to persist. Such conditions may develop from microtopographical differences in the sediment surface where *Atriplex* appears to preferentially colonise areas slightly raised by several cm relative to areas that tend to be occupied by *Puccinellia*. These raised areas appear to be persistent legacy of ridges and furrows from the use of heavy machinery on this site during its time under reclamation and areas of slightly raised mounds inadvertently created by engineering works undertaken in the preparation of this site prior to breaching. The small differences in microtopography are evidently sufficient to create a hydrological gradient between better drained raised areas and depressions underlain with more compacted sediment that likely inhibits drainage and causes a degree of ponding relative to the raised areas. It is, however, notable that apparently well drained and generally oxic sediment conditions recorded by redox measurements from within 5 m distance of a drainage channel can be replicated to a high degree at a location on this restoration marsh isolated by a distance of some 80m from the nearest drainage channel resulting in the development of a comparable cover of *Atriplex* growth in certain areas.

(a)



(b)

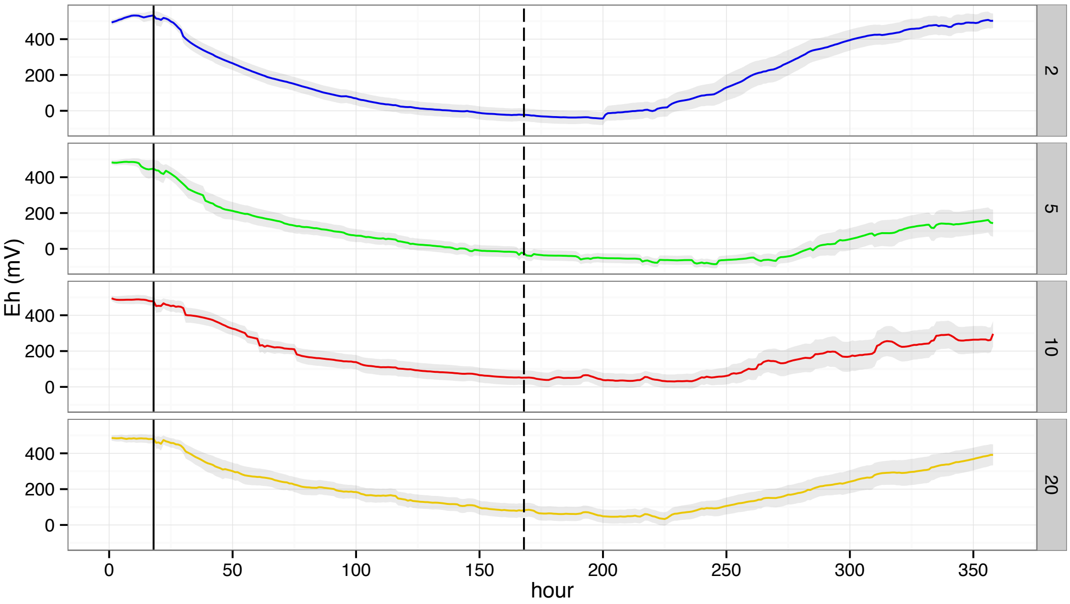
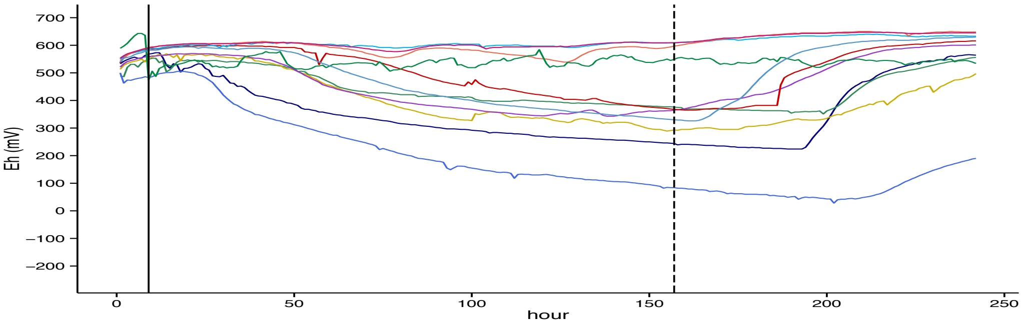
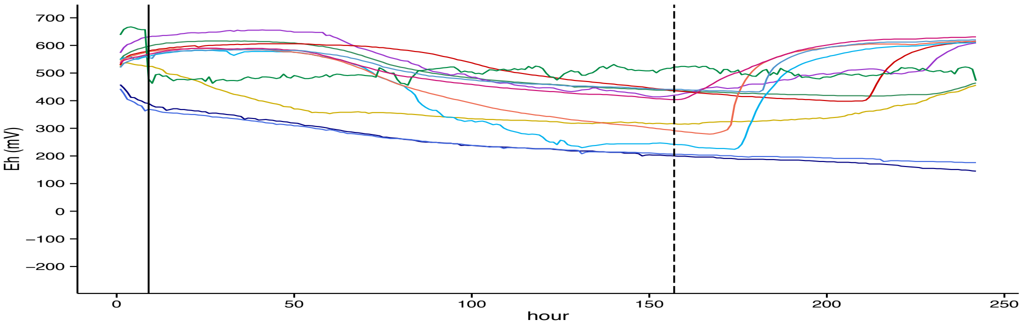


Figure 4.12 Time series of mean ± S.E. for Freiston MR *Atriplex-Puccinellia* upper marsh site for 9th – 19th January 2012 (a) and 19th August – 3rd September 2013 (b). Similar to the natural marsh early in the year oxic Eh prevail throughout the flooding phase compared to late summer when a post flood decrease to moderately reducing Eh followed by oxic recovery to a lesser extent than the natural marsh due to a higher number of electrodes registering continuously reducing Eh indicating a greater heterogeneity in Eh conditions in this area of the MR. Sediment depth (cm) is shown on right hand side of each panel.

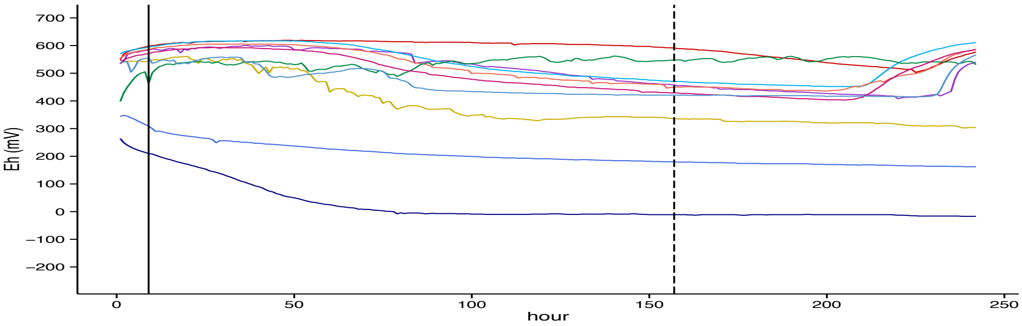
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

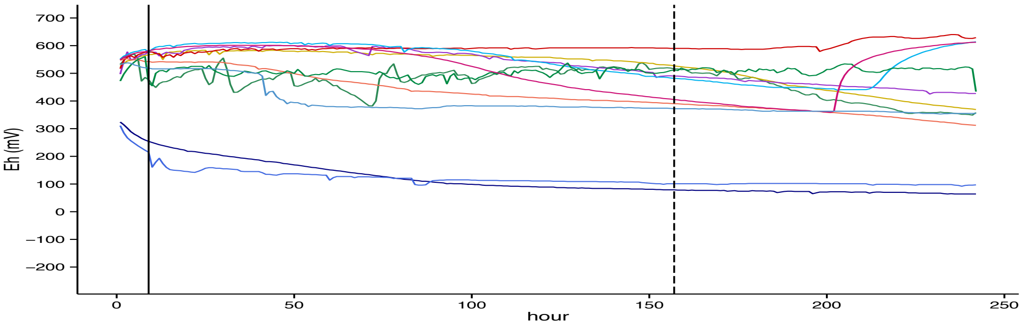
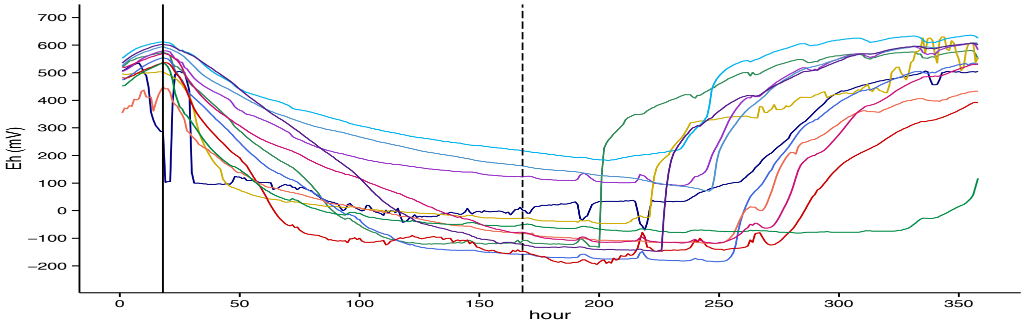
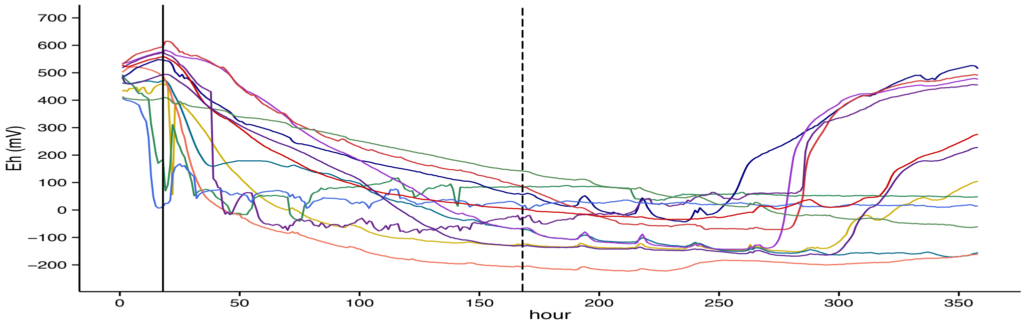


Figure 4.13. Eh measurements from replicate electrodes at four depths for the period 9th – 19th January 2012 actual Eh at the Freiston MR upper marsh site over a single flooding phase showing fairly high consistency of oxic Eh with only a small post flooding decrease to mildly reducing Eh at the uppermost depths. A small proportion of electrodes measured continuously reducing conditions in areas indicating these sediments are not as uniformly oxic as the natural marsh. This could explain the patchy distribution of *Atriplex* here and the much greater prevalence of *Puccinellia* compared to the natural marsh.

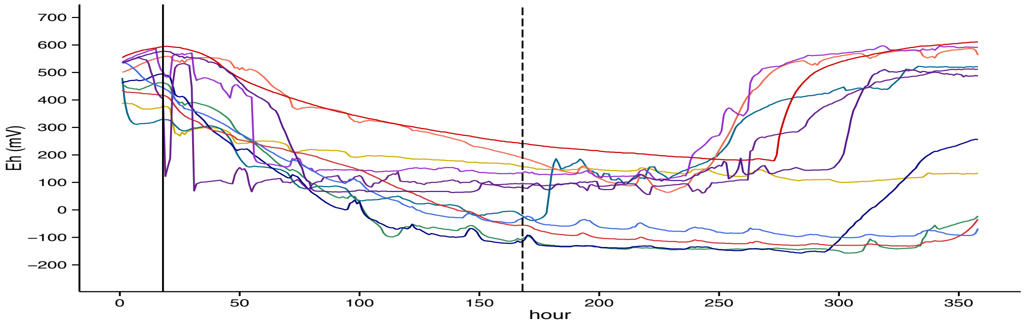
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

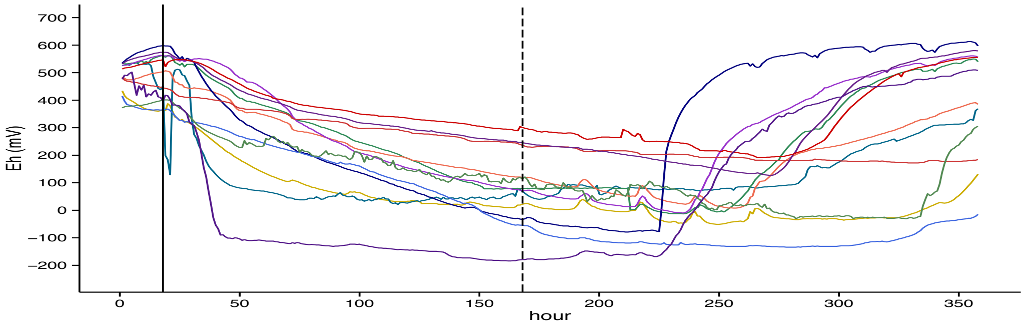


Figure 4.14 Eh measurements from replicate electrodes at four depths for the period 19th August – 3rd September 2013 at the Freiston MR upper marsh site over a single flooding phase with many electrodes demonstrating rapid post flooding oxic recovery at all depths and again indications of the presence of areas that remain continuously reducing at moderate-severe Eh.

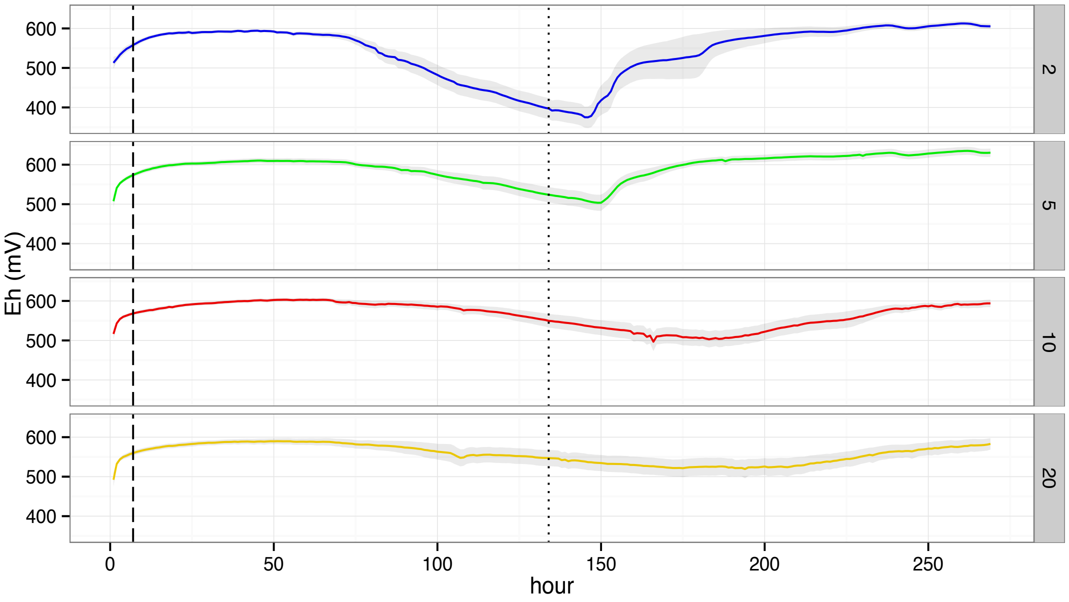
4.2.5 Freiston Shore MR – Low Marsh Creek site

Measurements were taken at this site over two single phases of tidal flooding for the periods: 20th February – 2nd March 2012 and 29th July – 10th August 2012. Results for these recordings are summarised as a time series of mean ±S.E. Eh values in Figure 4.15 and actual measurements for two periods are shown in Figures 4.16 & 4.17. Recordings taken early in the year at this low marsh site that has a similar vegetation composition to the upper marsh site of the MR dominated by a mixture of *Atriplex portulacoides* and vigorous *Puccinellia maritima* demonstrate sediments sampled at a distance between ~5 m from the edge of a major drainage creek are oxic at all measured depth and appear to have a comparable redox status at this time of year to the adjacent natural marsh. A similar response to flooding recorded in the natural marsh was evident at this site with a slow decrease in Eh values in the uppermost sediments to predominantly mildly reducing conditions with minimum values reached some time after the end of the flooding phase. Again, this effect is muted at the deeper measured depths and the majority of electrodes registered oxic Eh values throughout the flooding and post flooding phases.

Subsequent recordings at this site taken during the summer period again showed a strong seasonal effect with clear signs of an increase in redox intensity in response to flooding. This increase in redox activity also clearly indicates these sediments are in general well drained at all depths with flood induced transient anoxia extending for 3 to 3 days into the post flooding phase before rapid return to an oxidised state over a period of 24 hours. Many electrodes at the 2 cm and 5 cm depths showed a rapid return to an oxic state with a measured transition time from a highly reduced to an oxic state of less than 8 hours recorded in some instances. Although the majority of electrodes showed a similar response indicating sediments at this site are largely well drained and readily re-oxidised following flooding an increasing number of electrodes with depth failed to attain oxic recovery by the end of the recording period 5 days post flooding indicating the possible presence of continuous anoxic areas at mild to moderate reducing intensities persisting throughout the neap tide phases at this time of the year. The highest reducing intensities were recorded at shallower depths with less reducing conditions occurring at the deeper depths. The number of electrodes that recorded severely reducing Eh values was small and these were restricted to the uppermost sediments where at least half of the electrodes at the 2 cm depth decreased to highly reducing Eh values for durations up to 48-72 hours. Fewer electrodes registered a highly state at 5cm and all electrodes at the 10 cm and 20 cm depths remained within the mild to moderately reducing category.

The overall impression is that the redox conditions in sediments at this site appear to be less heterogeneous than recorded for the upper marsh site with oxic conditions prevailing to a greater extent. This may be reflected in differences in the vegetation with *Puccinellia* appearing to grow more vigorous at this site generally attaining a greater height and high rates of vegetative spread resulting in a dense mat of stoloniferous runners which may be limiting the coverage of *Atriplex* to a greater degree than spatial differences in redox conditions. However, given enough time the oxic conditions of the sediments in this area of low marsh in close proximity to a major drainage channel may eventually lead to a greater dominance by *Atriplex* at the expense of *Puccinellia*.

(a)



(b)

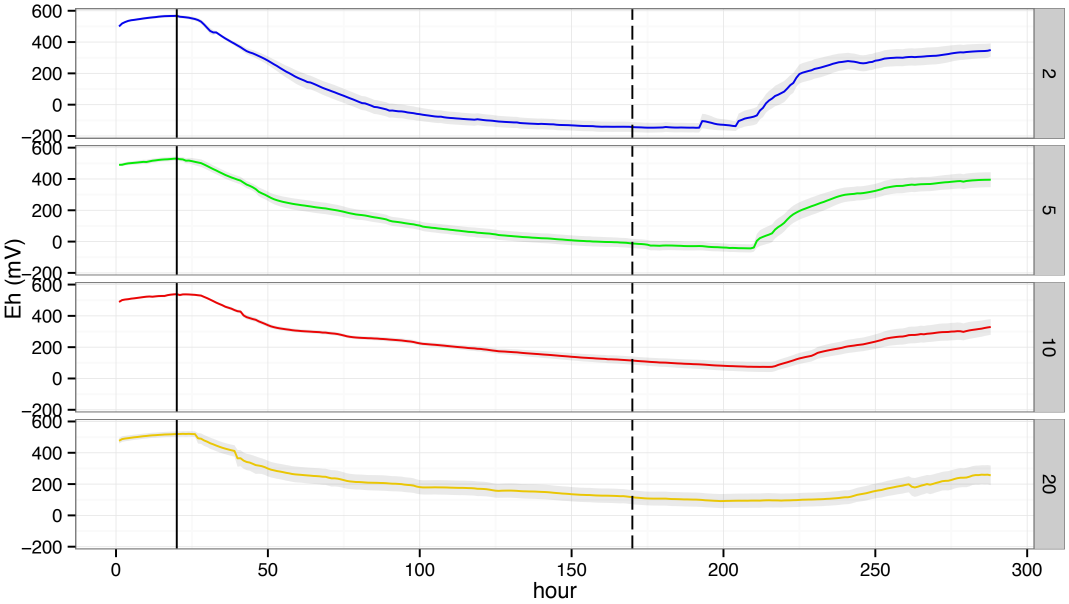
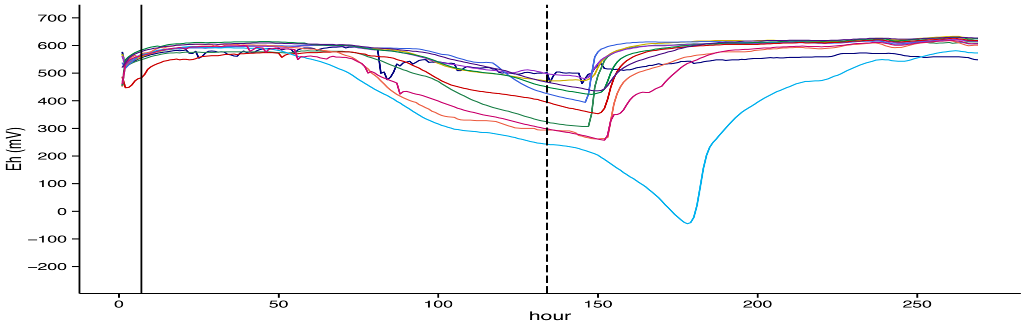
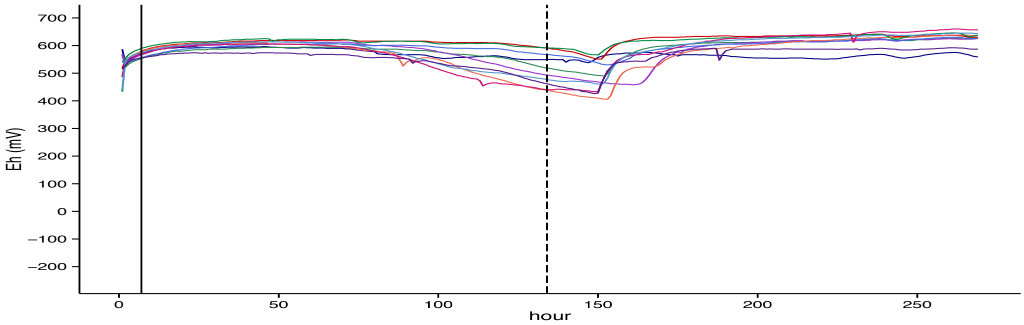


Figure 4.15. Time series of mean ± S.E. for Freiston MR *Atriplex-Puccinellia* low marsh creek site for 20th February – 2nd March 2012 (a) and 29th July – 10th August 2012 (b). Similar to the natural marsh and the upper MR site early in the year oxic Eh prevail throughout the flooding phase and similar conditions to the upper MR in summer when a post flood decrease to generally moderately reducing Eh followed by oxic recovery to a lesser extent at 10 cm and 20 cm depth than the natural marsh due to a higher number of electrodes registering continuously reducing Eh indicating a greater heterogeneity in Eh conditions in this area of the MR. Sediment depth (cm) is shown on right hand side of each panel.

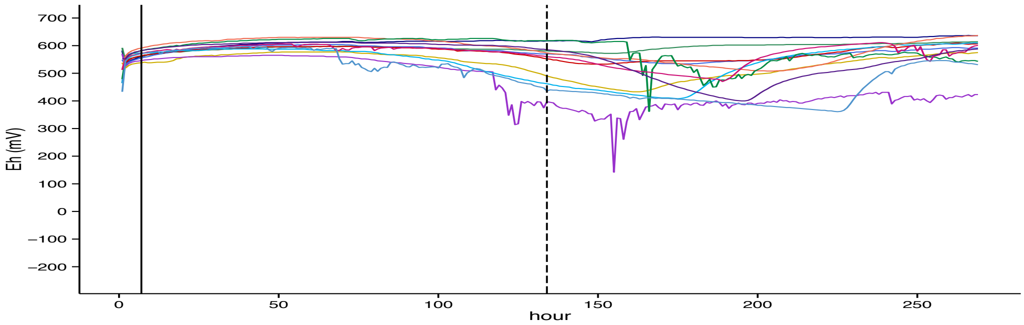
(a) 2cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

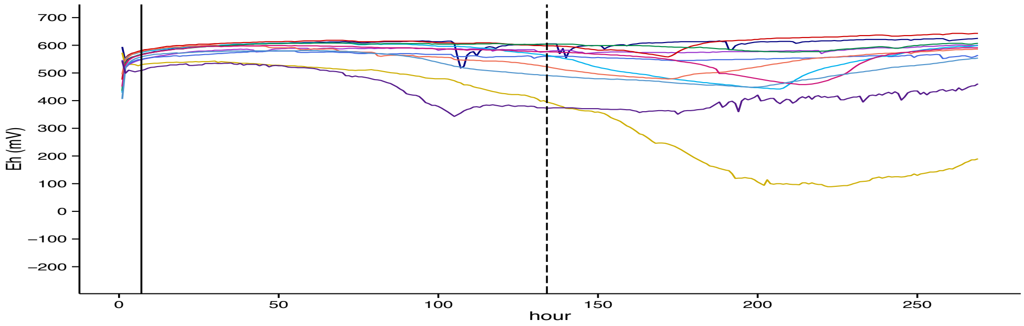
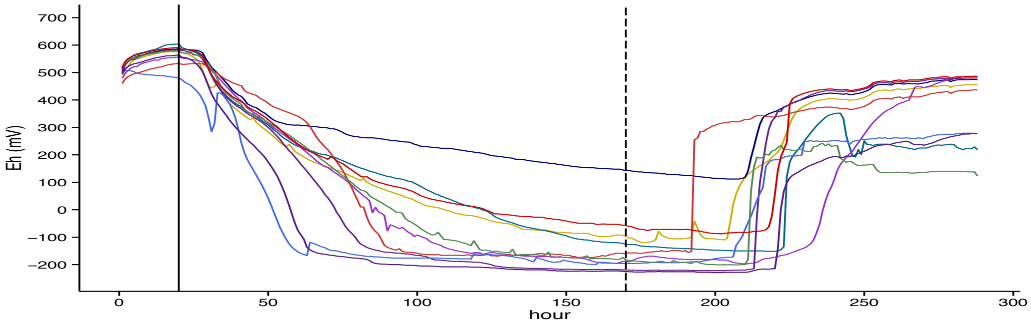
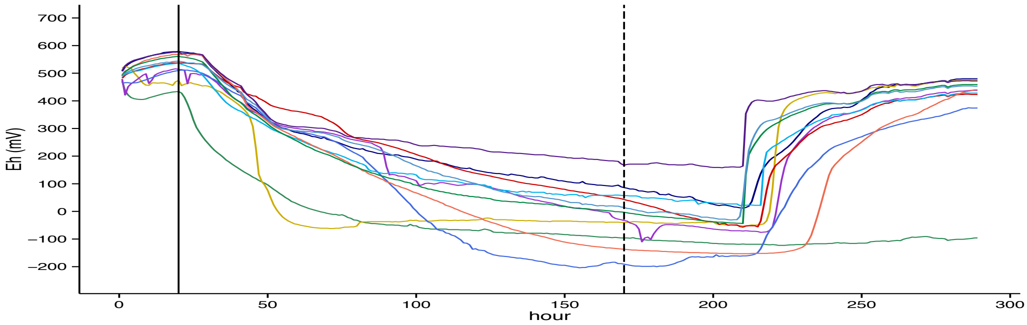


Figure 4.16. Eh measurements from replicate electrodes at four depths for the period 20th February – 2nd March 2012 at the Freiston MR low marsh creek site over a single flooding phase showing high consistency of oxic Eh similar to the natural marsh with only a small decrease to mildly reducing Eh at 2 cm.

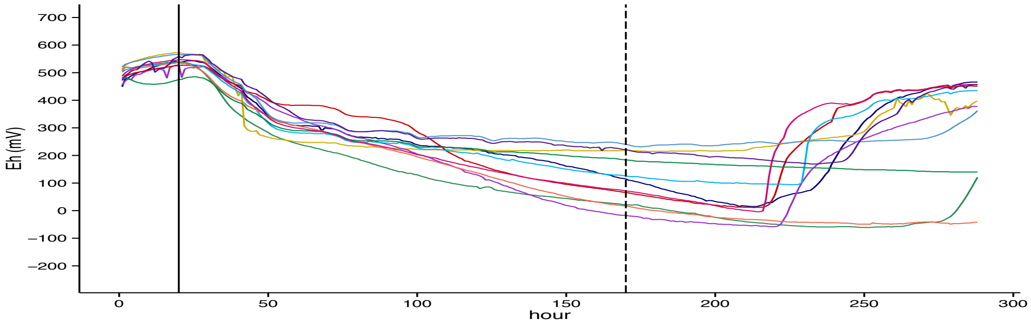
(a) 2cm



(b) 5cm



(c) 10 cm



(d) 20 cm

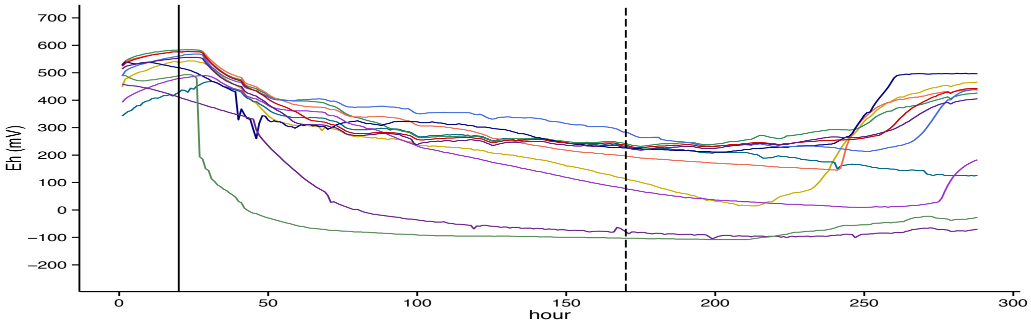


Figure 4.17. Eh measurements from replicate electrodes at four depths for the period 29th July – 10th August 2012 at the Freiston MR low marsh creek site over a single flooding phase showing a decrease to severely reducing Eh followed by a rapid oxic recovery at the upper depths and mild-moderately reducing Eh at lower depths followed by slower oxic recover at lower depth. Again with a number of electrodes recorded the presence of areas with continuously reducing Eh.

4.2.6 Freiston Shore MR – Low Marsh site

Measurements were taken at this site over a single phase of tidal flooding for the periods 13th – 26th February 2014 and two flooding phases for the periods 14th April – 12th May 2014 and 3rd – 27th September 2013. Results for these recordings are summarised as a time series of mean ±S.E. Eh values in Figure 4.18 and actual measurements for two periods are shown in Figures 4.19-4.21. Recordings from early in the year indicate the presence a much higher degree of flood induced redox activity than was evident in sediments in the other sites at Freiston Shore. In contrast to the upper marsh and the creek sites that remained predominantly oxic and indifferent to flooding at a similar stage of the year, the low marsh site showed evidence of a clear decrease in average Eh values to mild-moderate reducing levels at each depth. Since the vegetation composition at this site is similar to the Brancaster MR low marsh site it was expected that the redox conditions would be similar for both sites early in the year. While the Brancaster site showed a general state of continuous anoxia that appeared to be indifferent to, rather than induced by, tidal flooding at all but the uppermost depths with the persistence of continuous moderate-severely reducing Eh values regardless of the state of the tidal cycle, conditions at this low marsh site resembled the better drained sites of Freiston MR at a later stage in the year. Instead, sediments in this low marsh site indicated an appreciable capacity for drainage demonstrated by a rapid return to oxic Eh values with 48 hours post flooding for the majority of electrodes at the 2cm depth. Oxic recovery times at the lower depths were more protracted with electrodes on average taking between 4-5 days. The number of electrodes recording moderate-severely reducing Eh values also increased with depth but only a few failed to show oxic recovery indicating conditions of continuous anoxia are limited even at the deepest measured depths.

Recordings some six weeks later however showed the development of markedly different redox conditions at this site demonstrating a transformation from predominantly oxic to predominantly anoxic and highly reducing state presumably in response to the doubling of the sediment temperature that occurred during this period. Average sediment temperature at 5cm during February was 5.6 C (min. 3.2 / max. 7.4) increased to 11.3 C in April (min. 8.0 / max. 14.0).

The second recording period revealed some aberrant redox behaviour that requires explanation. Firstly, electrodes demonstrated a much higher degree of variation in the time taken to reach stable readings with some electrodes achieving stability, the majority between 10-12 days while a few were still decreasing by the end of the recording period some 4 weeks later. The long equilibration time could be attributed to these sediments being in a state of transition at this time of year from a predominantly oxic to a predominantly reducing state. Secondly, the first neap tide period showed little evidence of oxic recovery with almost all electrodes exhibiting a continuation of reducing Eh throughout this phase while the second neap tide phase showed a clearer degree of oxic recovery by some electrodes at least in the uppermost sediments. A likely explanation for these divergent responses is the differing degree of sediment saturation where conditions during the first neap tide phase were exacerbated by high levels of precipitation occurring at this time. The effect of heavy precipitation depressing Eh values during the neap tide phase was previously encountered and can be seen again in the latter half of the second neap tide phase during this recording period (Figure 4.18b). In contrast, the last recording period in September occurred during drier weather and a less interrupted return to an oxic state is clearly demonstrated by these sediments under certain situations when precipitation is low. Its is notable that a number of electrodes at the uppermost depths can show an extremely rapid return to an oxic state over a period of hours and after a relatively short post flooding delay similar to that observed in electrodes in the natural marsh and the upper marsh of the MR.

While the sediments at this site appear to indicate a higher capacity for drainage compared to those at the Brancaster MR low marsh site the ability of these sediments to return to an oxic state appears tenuous and susceptible to additional hydrological effects that are independent of tidal flooding. Nevertheless the majority of electrodes clearly show that highly reducing Eh values similar to those recorded at the Brancaster MR site can persist to varying extents at all measured depths. The different redox characteristics in this site and the Brancaster MR appear to be sufficient to explain some differences in the vegetation composition. While *Spartina anglica* and *Aster tripolium* are co-dominant at both sites, *Suaeda maritima* is largely absent from the low marsh at the Brancaster MR but is abundant in areas of the low marsh community at Freiston Shore MR where large and vigorous plants develop presumably where they are able to exploit the limited availability of oxygen at shallow rooting depths in these sediments.

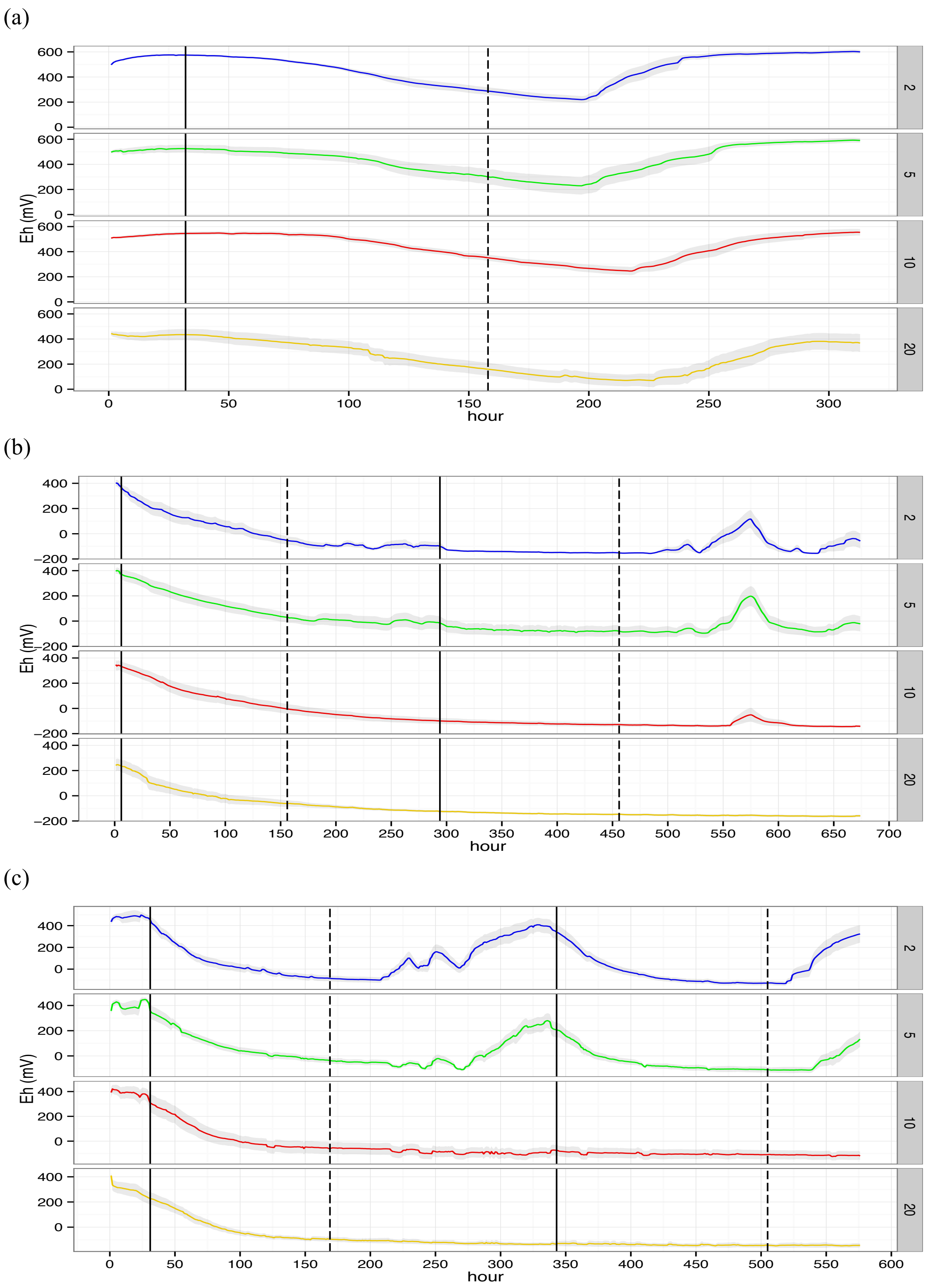
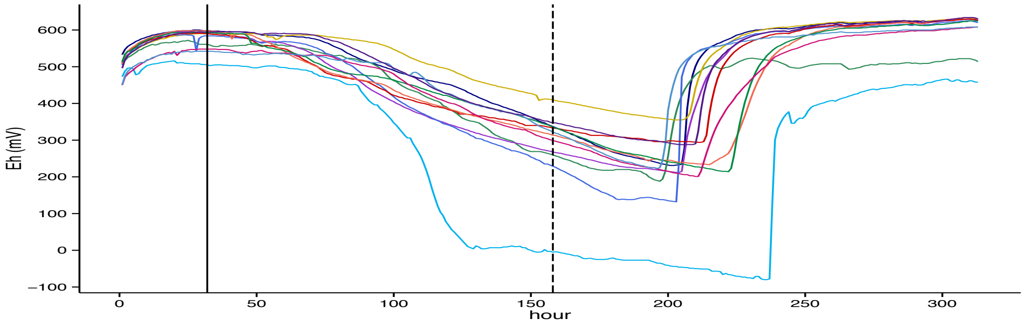
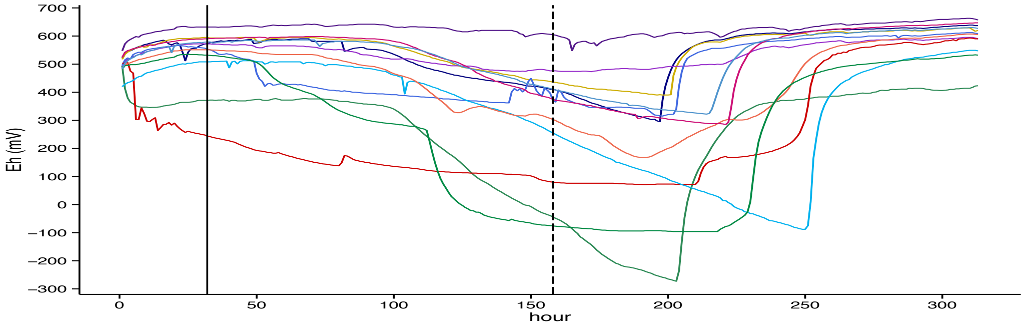


Figure 4.18. Time series of mean ± S.E. for Freiston MR *Spartina-Aster* low marsh site for 13th – 26th February 2014 (a), 14th April – 12th May 2014 (b) and 3rd – 27th September 2013 (c). Despite the similarity in vegetation composition at this site with the Brancaster MR low marsh site oxic Eh early in the year is much more prevalent than the latter site at all depths. Lower Eh conditions appear to be somewhat delayed. Predominantly anoxic Eh returns early in the growing season when sediments become severely reducing during the flooding phase and continuously severely reducing at lower depths.

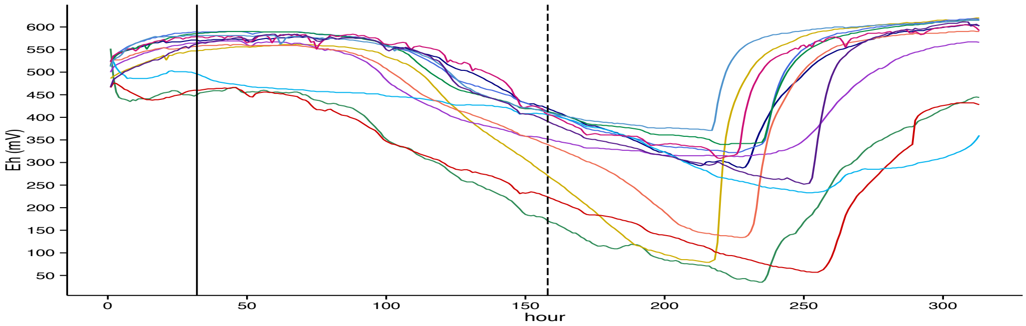
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

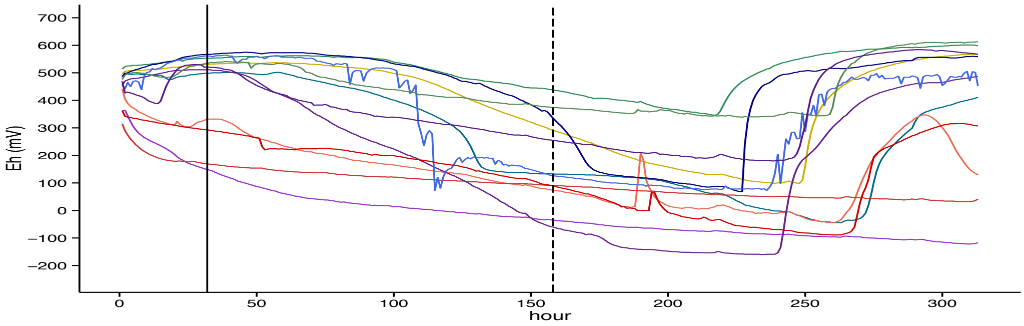
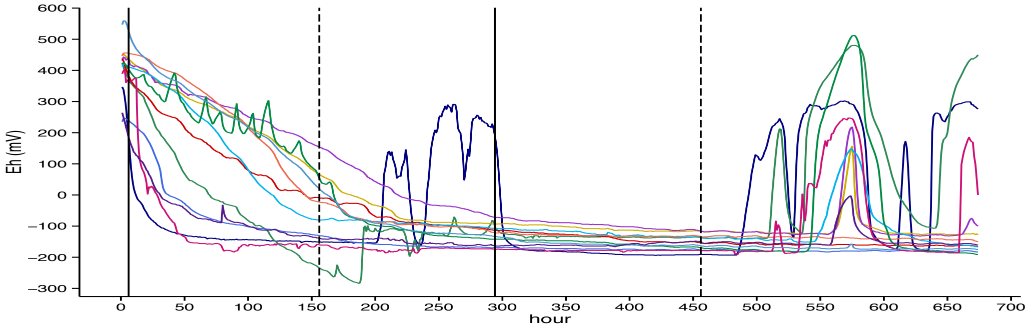
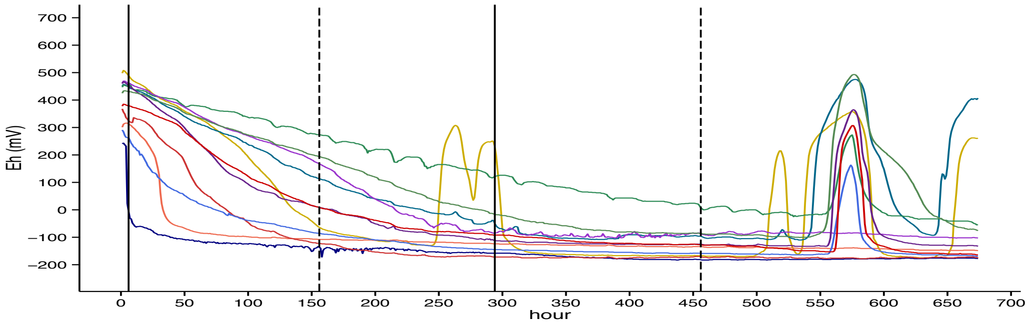


Figure 4.19. Eh measurements from replicate electrodes at four depths for the period 13th – 26th February 2014 at Freiston MR low marsh site showing characteristics of well drained sediments similar to the upper MR marsh with post flood oxic recovery at all depths.

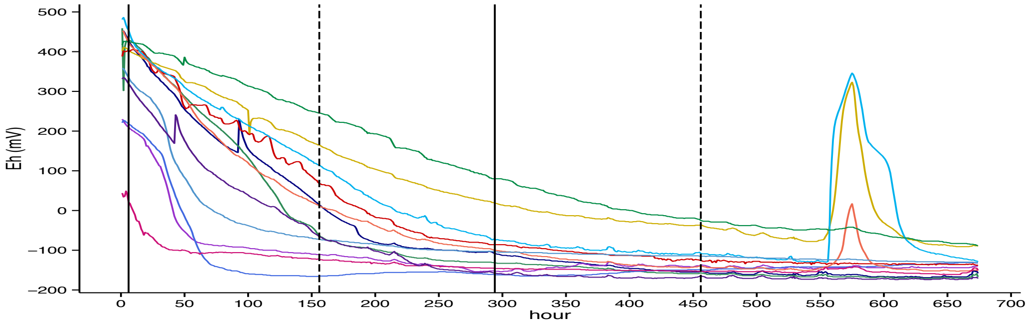
a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

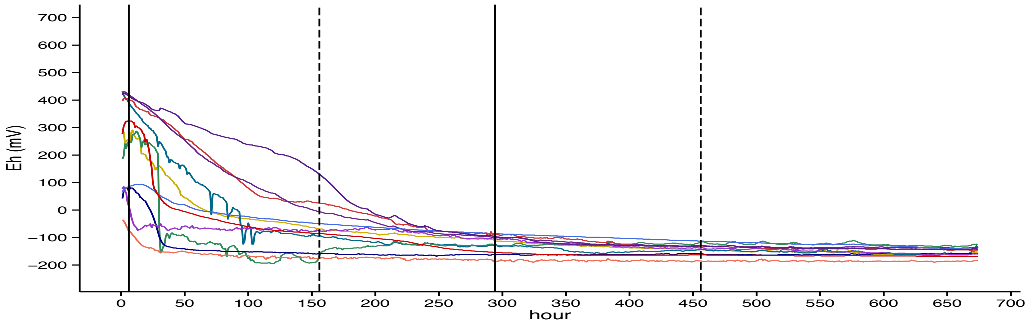
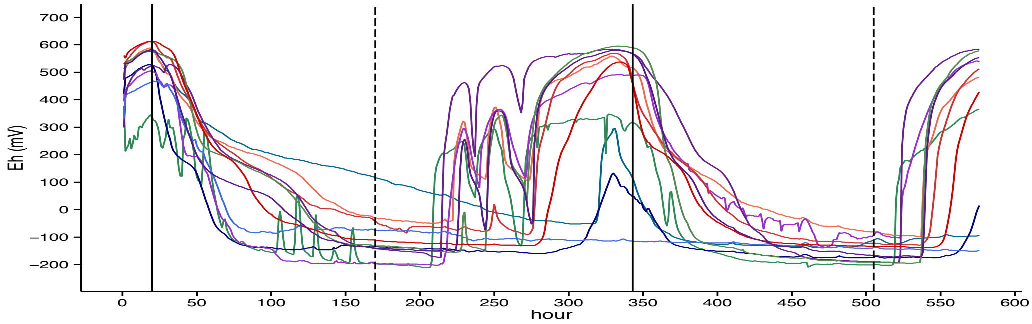
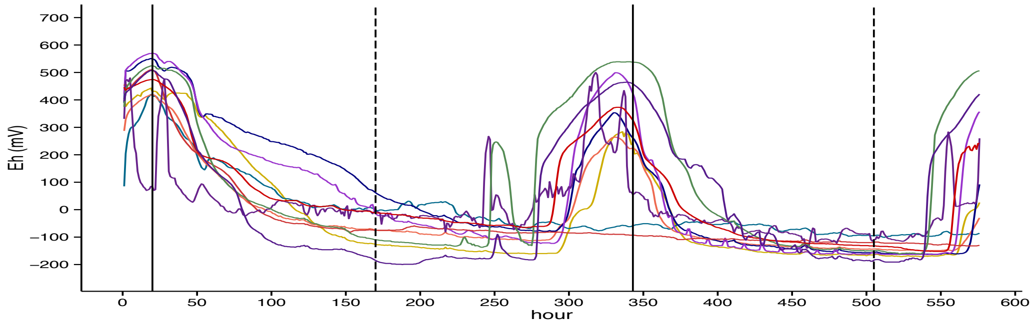


Figure 4.20. Eh measurements from replicate electrodes at four depths for the period 14th April – 12th May 2014 Eh at Freiston MR low marsh site over two flood phases. Limited oxic recovery following the first flood was attributed to high levels of precipitation for much of the post flood phase. The effects of high precipitation of a lesser degree can be seen during the depression of Eh in the second post flood phase. Sediments at all depths readily attain severely reducing Eh conditions similar to the Brancaster MR low marsh site. Long equilibration times indicate areas are under transition from the earlier oxic state.

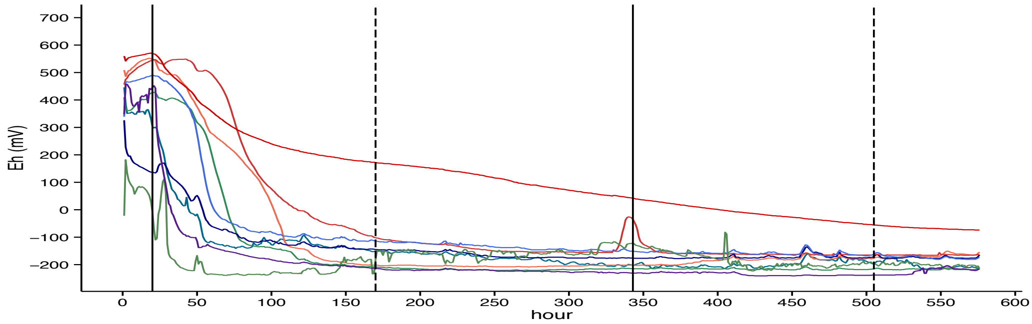
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

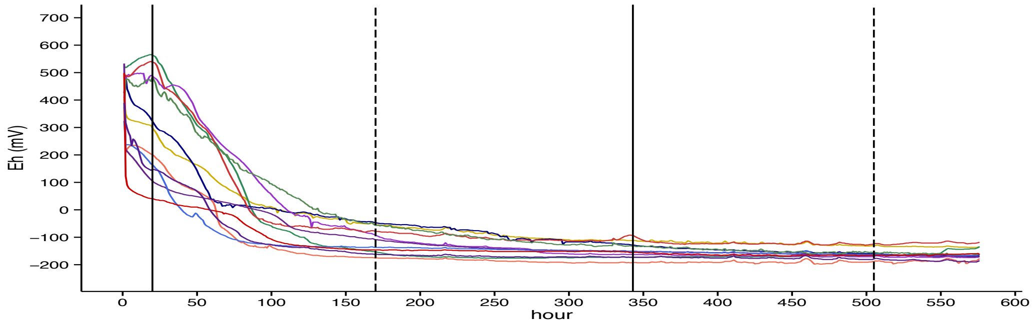


Figure 6.21. Eh measurements from replicate electrodes at four depths for the period 3rd – 27th September 2013 Eh at Freiston MR low marsh site over two flood phases showing a greater capacity for oxic recovery in the upper sediments than the Brancaster MR low marsh site and severely reducing Eh during the flood phase while lower depths show continuously severely reducing Eh similar to the latter site at the same depths.

4.3 Discussion

4.3.1. Predominantly oxic sediments at Freiston Shore natural reference marsh and the MR marsh

A key finding to emerge from the measurements of Eh in sediments of MR marshes is that persistent low redox potentials are not a general characteristic of MR sites with relatively low plant diversity. Where suitable initial conditions are present, MR sites such as Freiston Shore can rapidly (< 10 years) develop a large coverage of communities dominated by a dense cover of vigorous *P. maritima* and *A. portulacoides* that occupy well drained sediments with relatively oxic Eh characteristics. Continuous measurements of Eh in sediments of these communities showed that it was possible to restore Eh conditions similar to those measured on the adjacent *Atriplex*-dominated natural marshes. Much of the *Atriplex-Puccinellia* cover is concentrated at higher elevations areas on Freiston MR. Measurements of Eh for this community type were taken from a location 80m distant from the nearest drainage creek and readily showed typical Eh characteristics of well drained sediments. This demonstrated such conditions could occur under suitable microtopographical/edaphic conditions in interior areas of the marsh in the absence of drainage channels in close proximity.

On the other hand, the functional role of drainage creeks was indicated by the presence of nearby *Atriplex-Puccinellia* communities at lower elevation areas at Freiston MR. Relatively oxic Eh conditions similar to those occurring in the natural reference marsh during the summer were also recorded at this low marsh site at an equivalent distance from a major drainage channel. The apparent restoration of Eh conditions in Freiston MR may be a consequence of the limited physical alteration of the underlying sediment from its relatively short reclamation history. However, surveys of sites restored accidentally by storm breaches of sea walls show that similar *Atriplex-Puccinellia* communities are commonly encountered in older restoration marshes (Burd 1992; Garbutt & Wolters 2008; Mossman *et al.* 2012a). This suggests that suitable elevation or edaphic conditions on a large scale may have been initially present in some accidentally restored marshes enabling the rapid colonisation and dominance of *Atriplex-Puccinellia* communities and these have persisted on timescales, in some instances, extending well over a century. The alternative hypothesis is that such communities gradually evolved on these sites during a process of succession after sufficient time had elapsed for a suitable elevation or edaphic conditions to develop sufficiently oxic sediments (see Langois *et al*. (2003) for possible mechanism).

Similar to the upper marsh sediments at Brancaster and Stiffkey, redox potentials in sediments measured at the Brancaster MR and both the Freiston MR and natural reference marsh sites were frequently found to display a strong seasonal effect. In Chapter 3, it was shown that relatively well drained sediments occurring close to drainage channels on the upper marshes of the north Norfolk coast were characterised by large fluctuations between oxic and anoxic Eh in response to cycles of tidal flooding and drainage. In general, the amplitude of these fluctuations and the duration of the anoxic phase induced by spring tide flooding progressively increased from early summer to late autumn months. It was shown that the phase of anoxia and lowered Eh could extend well into the neap tide phase until this considerable lag was ended by a rapid return to oxic Eh values.

While the presence of oxic sediments in these upper marsh creek sites was expected, continuous measurements of redox potential in the Freiston natural reference marsh revealed comparable redox conditions characterised by large oscillations between oxic and reducing Eh. This showed that such conditions could also occur relatively low in the tidal frame where well drained sediments dominated by *Atriplex* are a common feature. Despite receiving more than twice as many tidal inundations as the upper marsh creek site, the summer anoxic phase in the Freiston natural marsh site, as measured within a 5-10 m distance from a major drainage channel, was found to be appreciably shorter and attained less severely reducing Eh levels. This was demonstrated by measurements taken during the summer when the period of flood induced anoxia generally decreased to mildly reducing mean Eh values at all measured depths for the duration of 5-6 days. In comparison, summer redox conditions measured at equivalent depths at the creek site at Stiffkey attained lower mean Eh values that persisted for over an extended period of 10 days followed by a relatively short duration oxic phase between the subsequent inundation event. The more persistent low Eh at the upper marsh creek bank sediments at Stiffkey is presumed to be a consequence of lateral hydraulic movement of sub-surface porewater draining the adjacent highly saturated and anoxic GSM sediments of the inner marsh areas.

4.3.2 Moderately reducing sediments at Brancaster MR Mid Marsh

Measurements of Eh taken from this site around 3 m from a major artificial drainage creek (cf. 5-10 m Freiston artificial creek site) showed that the oxic phase of the fluctuating Eh was limited to the upper sediments for much of the growing season while continuous moderate-severely reducing Eh prevailed at lower depths spreading to upper depths by the autumn. This suggests that the artificial drainage channel at this site appeared to have a much smaller influence on alleviating waterlogging than the at the Freiston MR site. A reduced drainage efficiency and higher degree of waterlogging would support the possibility that the underlying original sediments at Brancaster MR have experienced a greater degree of physicochemical alteration than in the Freiston MR. Such changes can lead to the irreversible development of a dense and relatively impermeable barrier in the old reclaimed soil underlying newly deposited sediments from the restoration of tidal flooding (Crooks & Pye 2000; Hazelden & Boorman 2001). The resulting over-consolidated horizon effectively forms an aquaclude that acts as a barrier to sub-surface hydraulic conductivity impeding the efficient drainage of overlying newly deposited marine sediments of the developing saltmarsh (Crooks & Pye 2000; Crooks *et al*. 2002). Saltmarshes in south-east Britain commonly have sediments that are deficient in detrital calcium carbonate which are susceptible to clay particle dispersion and deflocculation when exposed to a period of desiccation leading to the breakdown of soil structure and impeded soil drainage (Boorman *et al*. 2002; Crooks & Pye 2000). The presence of such a modified soil horizon at the Brancaster MR could explain the greater persistence of waterlogging and low Eh, even at sites close to drainage creeks in comparison to conditions in the Freiston MR. The development of an aquaclude in the Brancaster MR, while seemingly less evident at Freiston MR, could be attributed to its longer history under reclamation (*ca*. >150 years) compared to the sediments of the Freiston MR (*ca*. 20 years) and/or differences in the agricultural management practices during reclamation: pasture *vs*. arable respectively. The presence of over-consolidated horizon in the form of an aquaclude in MR sites may require further consideration in the design of restoration saltmarshes since it suggests that the construction of artificial drainage creeks may not always be effective in ameliorating conditions of waterlogging and low redox potentials.

The apparent differences in Eh conditions between the *Atriplex-Puccinellia* dominated areas of Freiston MR and the mid-elevation Brancaster MR site which experience a similar tidal regime were sufficient to explain visible differences in the vegetation composition. It was evident that the development of *Atriplex-Puccinellia* communities was less well pronounced at the Brancaster site where the distribution of *Atriplex* was largely limited to the very edge of the drainage creek and, while *Puccinellia* was abundant, its growth form was less vigorous than on Freiston MR allowing a greater abundance of other species, notably *Limonium* and *Triglochin*, to become established in this part of the marsh. The data from extensive Eh measurements at Freiston MR indicates that where sediments tend to be predominantly oxic, such conditions promotes the establishment of vigorous growth of *Atriplex* and *Puccinellia* to the exclusion of other potentially colonising species. This finding is significant since it implies that a certain degree of exposure to low Eh may be beneficial in promoting more diverse vegetation on MR sites.

While this is in agreement with earlier studies indicating *Atriplex* is a strong competitor under favourable conditions (Bockelmann & Neuhaus 1999; Dormann *et al*. 2000), the competitive ability of *Puccinellia* in predominantly oxic sediments is less clear. While *Puccinellia* occurs over a broad tidal amplitude on many saltmarshes, the low marsh has been ascribed as its centre of distribution (Adam 1981). It appears to be anatomically well adapted to survive waterlogging with the formation of extensive aerenchyma enabling the supply of oxygen from shoots to roots exposed to anoxia (Gray & Scott 1977b; Justin & Armstrong 1987). In addition, the capacity of *Puccinellia* to survive waterlogging and low redox potentials is supported by both fieldwork (Davy *et al*. 2011) and glasshouse experiments which showed an increased yield of pot grown plants under waterlogged treatments (Brereton 1971; Cooper 1982; Gray & Scott 1967). Its capacity to tolerate waterlogging and low Eh suggests that *Puccinellia* should be a poorer competitor in better drained sediments as a consequence of the trade-off between the development of adaptations to survive abiotic stress factors and competitive ability under less stressful conditions. However, it was also reported that populations of *Puccinellia* from low and upper marshes showed significant genetic differentiation with selection for higher yielding genotypes and more vigorous morphologies in better drained areas of mature marshes at higher elevations (Gray & Scott 1980; Gray 1985). This is supported by other work showing that the competitive ability of *Puccinellia* is actually enhanced under non-waterlogged conditions (Huckle *et al*. 2000). Rather than the effects of anoxia *per se*, the competitive ability of *Puccinellia* may be more inhibited by its sensitivity to sulphide (Havill *et al*. 1985; Ingold & Havill 1984) and this could be a more important factor limiting its growth in the persistently waterlogged areas low in the tidal frame.

4.3.3. Predominantly reducing sediments at Freiston and Brancaster MR sites

In contrast to the relatively oxic Eh conditions prevalent at the *Atriplex-Puccinellia* dominated areas of Freiston MR, predominantly anoxic and low Eh conditions were found to be associated with *Spartina-Aster* sediments. These communities, also of relatively low floristic diversity, form a major component of the overall vegetation cover at both the Brancaster and Freiston Shore MR where they dominate much of the lower elevation areas. Unlike the *Atriplex-Puccinellia* communities, which were found to be largely oxic over winter months and experienced temporary phases of anoxia of relatively short duration during summer-autumn months in response to tidal flooding, the low marsh sites were typically found to experience anoxia to varying degrees at an early stage in the year (February-March) and extended periods of continuous anoxia at low Eh levels at all measured depths. The presence of anoxia early in the year at these lower elevation MR sites is consistent with the recording of anoxic sediments at a similar stage of the year in the *Spartina* dominated low marsh site at Stiffkey (Chapter 3). However, differences were apparent in the Eh conditions between the three low marsh sites in these same months. Where *Spartina* formed monospecific stands at Stiffkey, sediments were found to be permanently highly reducing at all measured depths and at Eh values commensurate with sulphate reduction. Highly reducing sediments at similarly low Eh levels were frequently encountered in both the Brancaster and Freiston MR low marsh. However, despite their similarity in vegetation composition, continuous measurements of Eh revealed the presence of apparent differences in their redox characteristics. Measurements from Brancaster MR early in the year showed that, while such conditions were uniformly recorded at sediment depths <10 cm, both oxic values and low Eh values were simultaneously recorded at higher depths indicating the presence of a more heterogeneous redox environment in the uppermost portion of the sediment profile. The subsequent occurrence of more uniformly low Eh conditions at all depths resembling those recorded in the Stiffkey low marsh was evident by late spring suggesting a delay in the development of widespread low Eh conditions at this site. A delayed development of low Eh was more evident at the Freiston MR low marsh site where conditions early in the year more resembled the pattern of transient anoxia found in the *Atriplex-Puccinellia* sediments during the summer. Although more widespread low Eh also prevailed at this site by late spring, the uppermost sediments continued to show some capacity to revert to an oxic state during the neap tide phase when rates of precipitation are low. The higher degree of persistence of low Eh in the Brancaster MR site is a likely consequence of its slightly lower position in the tidal frame in combination with the presence of a more developed and effective aquaclude compared to the Freiston MR low marsh site. Nevertheless, the prevalence of low Eh conditions at both MR low marsh sites appears to be sufficiently convergent for the establishment of remarkably similar vegetation communities.

4.3.4. An assessment of the possibility of redox phytotoxicity in MR marshes

Insights into the abiotic factors determining community composition in these MR marshes may be acquired from a consideration of the current understanding of tolerances of the dominant species to both anoxia and the phytotoxicity effects associated with low redox potentials. *Atriplex* is reported to have a limited capacity to develop intercellular air spaces (aerenchyma) in the root cortex as an adaptive mechanism for transporting oxygen to its roots under flooded conditions (Justin & Armstrong 1987; Mohamed 1988). In addition, *Atriplex* was found to be sensitive to iron phytotoxicity at relatively low concentrations (van Diggelen 1991). Both these factors could explain its distribution limited to sites that experience short-term anoxia lasting up to several days. In contrast, *Puccinellia*, *Aster* and *Spartina* appear to be anatomically well adapted to survive long-term exposure to anoxic sediments and the increased root oxygen demands associated with low redox potentials. Each of these species is able to develop extensive aerenchyma under flooded conditions with measured root porosities for *Puccinellia* of 25.5%, *Aster* (25.6%) and *Spartina* (33.3%) (Justin & Armstrong 1987) which should favour survival in more permanently anoxic sediments. However, *Puccinellia* is often sparsely represented or absent from the MR low marsh sites and *Aster* is usually absent in areas prone to ponding from poorly drained microtopographical depressions in the low marsh where *Spartina* often thrives as a monospecific sward.

Previous studies have shown growth in each of these species is unaffected under conditions of experimental waterlogging (Cooper 1982; Lee 2003; Maricle & Lee 2002; Van Diggelen 1991) suggesting that the effects of anoxia *per se* may not be the most important abiotic stress that determines their distribution in MR marshes. However, there is some evidence indicating differential responses to phytotoxicity associated with low redox potentials in the form of bioavailable manganese, iron and sulphides could be a more important influence on growth and survival (Armstrong 1982; Ponnamperuma 1972, 1984). The case for differential Mn^{2+} and Fe^{2+} phytotoxicity is unclear since *Aster*, *Puccinellia* and *Spartina* have all demonstrated a high degree of tolerance to high concentrations of these ions (Cooper 1984; Rozema *et al.* 1985b; Singer & Havill 1985; Van Diggelen 1991). While there is little experimental evidence demonstrating a relationship between differential tolerances to Mn^{2+} or Fe^{2+} in these species and their position in the marsh, there is more convincing evidence to suggest that differential tolerances to sulphide could be an important factor in delineating the dominant communities in these MR marshes. Experimental treatments under hydroculture showed that *Spartina anglica* growth was little inhibited at sulphide concentrations of 500 µM but hampered at higher concentrations of 1mM that can occur locally on lower saltmarsh sites (Van Diggelen *et al.* 1987). Such conditions could explain the absence of *Spartina* in the very lowest parts of Brancaster MR. In comparison, *Aster* was shown to demonstrate a moderate tolerance to sulphide concentrations to 100 µM (Havill *et al.* 1985; Pearson & Havill 1988). Similar concentrations to this were previously reported to be frequently encountered in low marsh sediments occupied by *Salicornia europaea* (Ingold & Havill 1984). In contrast, *Puccinellia* was found to be negatively correlated with sediment sulphide content (Ingold & Havill 1984). The sensitivity of *Puccinellia* to sulphide was corroborated by laboratory studies that demonstrated its tolerance was limited to short-term exposure to sulphide after which it showed significant growth inhibition, chlorosis and impeded root development at concentrations of 100 µM (Ingold & Havill 1984). It therefore follows that the limited degree of tolerance of *Puccinellia* to sulphide offers a plausible explanation for its low abundance in the *Spartina-Aster* communities of the MR marshes.

Due to the slow reaction kinetics of many redox couples, the accumulation of manganese, iron and sulphides may take up to several weeks before potentially phytotoxic concentrations are reached (Ponnamperuma 1972, 1984; Bohn *et al*. 2001). Sufficient time may therefore not be available to allow for this level of accumulation to occur in better drained sediments associated with the *Atriplex-Puccinellia* communities that experience short-term anoxia and low redox potentials over a period of several days. In contrast, phytotoxicity, mainly in the form of sulphide, is more likely to be a significant factor where low redox conditions persist over prolonged periods, such as those found to occur in sediments of the *Spartina-Aster* communities. The importance of this stress factor is therefore likely to be reflected in increasing abundance of *Spartina* to point beyond which exceeds the limits of its tolerance resulting in unvegetated areas of the marsh.

CHAPTER 5: Is the measured mean redox potential more important than redox potential variability?

5.1 Introduction and aims

Saltmarshes restored by managed realignment (MR) tend to be at lower elevations compared to natural marshes (Mossman *et al*. 2012a). These restored marshes often have impeded drainage from physical alterations to the saltmarsh sediment under reclamation (Crooks *et al*. 2002; Hazelden & Boorman 2001; Tempest *et al*. 2015). The combined effect of increased frequency and duration of tidal flooding, due to low elevation, and poor drainage can lead to conditions of increased waterlogging in MR sediments (Crooks *et al*. 2002; Dausse *et al*. 2008; Tempest *et al*. 2015). The resulting anoxia and its associated effects are thought to limit colonisation of restored marshes by a number of species that are common constituents of middle and upper elevations of nearby natural saltmarshes (Davy *et al*. 2011; Mossman *et al*. 2012a).

Extensive measurements of Eh in the current study (Chapter 4) have shown that low redox potentials occur to varying extents within the general rooting zone of sediments on recently restored MR sites. Areas that are low in the tidal frame generally experience prolonged periods of continuously low Eh. However, such conditions can also extend to mid elevation areas, even in close proximity to drainage channels (Chapter 4). On the other hand, measurements at the Freiston Shore MR indicated that it might be possible to restore Eh conditions that resemble those occurring on adjacent well-drained natural marshes in certain situations where the transformation of the physical structure of sediments during reclamation is limited.

However, it was also found that prolonged periods of low Eh also occur in sediments of general saltmarsh communities (GSM), that have greater abundances of plant species than generally found on MR sites (Chapter 3). Not only does this raise interesting questions about the physiological mechanisms that enable these plants to survive such conditions, it also poses an important question concerning the nature of the constraints that inhibit establishment of these plants on MR sites.

Evidently these plants are able to tolerate significant exposure to low redox potentials but the extent of their tolerances is unclear. An experiment was devised to measure the long-term Eh conditions in sediments of a series of plots on the MR sites where individuals of five species that occur at lower abundances on restored saltmarshes were experimentally planted. In addition, an attempt was made to vary the Eh by artificially manipulating the microtopography (raising and lowering the surface elevation) of the plots. The aim of the experiment was therefore to address the hypothesis that survival could be related to the average redox conditions that prevail on MR sites over the long-term rather short-term variations or exposure to low redox *per se*.

5.2 Methods

5.2.1 Microtopographical manipulations

The managed realignment marshes at Brancaster and Freiston used in this study were previously subject to an experiment, to investigate the effect of microtopographical manipulations on plant establishment and growth. The experiment, set up by Dr Hannah Mossman in October/November 2009, involved the construction of ten groups of 1 m^{-2} plots on each of the MR sites. Each group comprised of flat (F) plot at the existing marsh elevation, raised (R) plot to a height of 15 cm from the marsh surface and lowered (L) plot to a depth of 15 cm lower than the marsh surface (Figure 5.1a). In each group one set of three plots at each elevation (i.e. one raised, one flat, one lowered) were planted with plugs of five target species while the other set of 3 plots was left free to colonise naturally. The locations of the plots on the MR sites are shown in Figure 5.2.

Raised plots were established using sediment-retaining wooden frames (1 m x 1 m x 0.15 m) and transferring the top 15 cm of sediment from a lowered plot (also reinforced by sediment retaining wooden frames). Sediment removed to create the lowered plot was cleared of vegetation and transferred to create the raised plots which were allowed to settle naturally. Flat plots were established by removing all surface vegetation where this involved removing turf, sediment from nearby but outside the group of plots (5-10 m) was used to raise the plot level to the same as the marsh surface. Each group of plots had a similar, non-contiguous arrangement (Figure 5.1a). Placement of the groups of plots was stratified across the elevation gradient of each MR site, with plots haphazardly located along the gradient (Figure 5.2). Groups were placed a minimum of 20 m apart.

5.2.2 Planting

Seeds of five target species, *Armeria maritima*, *Atriplex portulacoides*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima* were collected from natural marshes close to the MR sites in late summer and early autumn 2009. Seeds were mixed and so the original of the material is not known. Seeds were given to a commercial nursery for propagation (British Wildflower Nursery, North Burlingham) which germinated and grew the plugs outdoors in peat-free compost. Plugs were watered with tap water until two weeks prior to anticipated field planting when they were watered with 50% strength artificial seawater (Tropic Marin, Aquarientechnik GmbH, Wartenberg, Germany).

Ten plugs of each of five species, *Armeria maritima*, *Atriplex portulacoides*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima*, were planted by Dr Hannah Mossman into planted plots in a regular grid, with individuals randomly assigned to a location. Plugs were watered with locally collected seawater immediately after planting. Planting was undertaken in May 2010. The number of surviving planted individuals was counted in October 2013.

5.2.3 Substrate redox potential and elevation

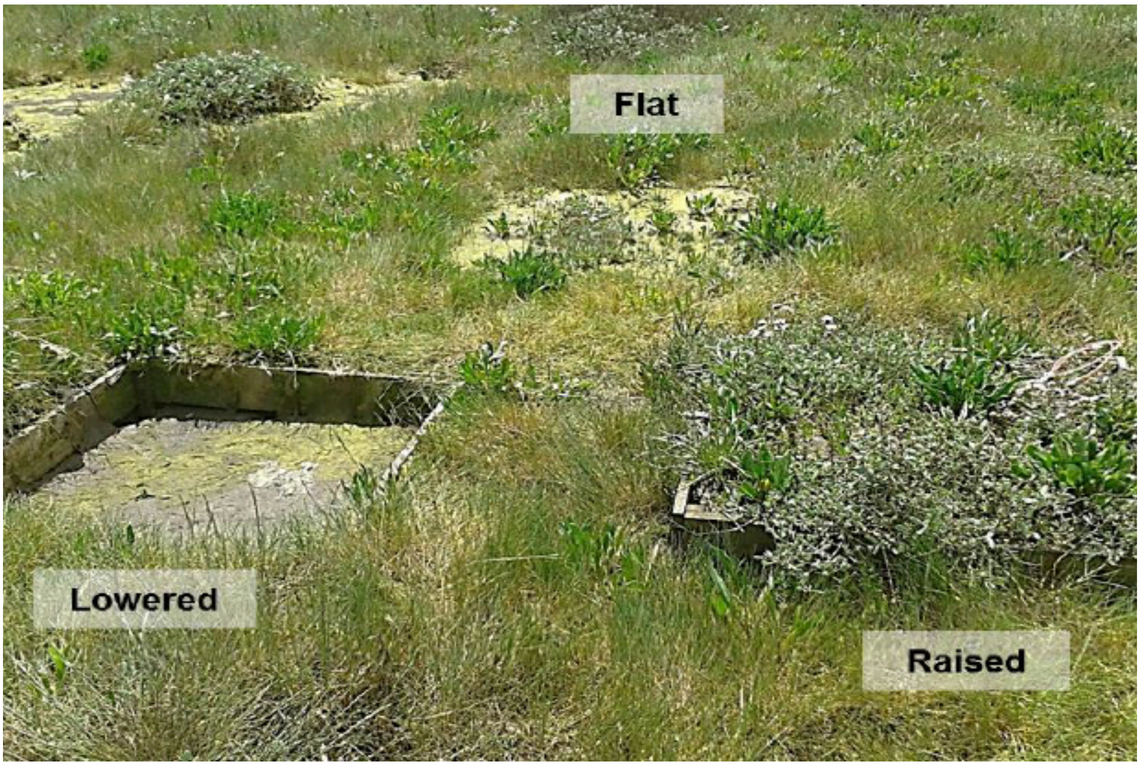
Substrate redox potentials at 2 cm and 10 cm depths were measured at the centre of each plot using two permanently installed electrodes (Figure 5.1b). Platinum tipped electrodes were constructed based on established designs (Mueller *et al.* 1985; Faulkner *et al.* 1989) using 10 mm length of 24 gauge (0.5 mm diameter) platinum wire (99.95% purity /hard tempered, Advent metals, UK) soldered to one end of a 300 mm (3.2 mm diameter) brass alloy rod. A 150 mm length of 3mm diameter insulated copper cable was soldered to the other end of rod. A small section of sliver coated copper wire (20 mm x 1.5 mm diameter) was soldered to the exposed end of the copper cable and to prevent moisture contamination the soldered junctions were sealed with three layers of marine grade epoxy-ceramic barrier resin (Reactive Resins, Bodmin, UK) and the entire electrode body was encapsulated with adhesive heat shrink tubing leaving exposed approximately a 3 mm section of platinum tip. The platinum tips were polished with an abrasive fiberglass pen to remove any oxidised layer and tested for correct functioning both before installation in the field using quinhydrone redox buffers at pH 4 and pH 7 (HM Digital, Culver City, USA) and also plain tap water. These were deemed to be acceptable if they were within the range of ±10 mV.

Redox measurements were made using a handheld millivolt meter (Model HI 9025, Hanna Instruments, USA) relative to a Ag/AgCl type reference electrode (Model: InLab ARGENTHAL, Mettler Toledo, Switzerland). Values were corrected by adding the potential of the reference electrode (210 mV) with respect to a standard hydrogen electrode (Eh). No adjustment to the Eh value was made for pH, which was measured at 5 cm depth in the sediment at the start and end each recording period using a glass pH electrode calibrated with buffers adjusted to seawater salinities, and found to consistently range between pH 6.8-7.4.

Sampling was undertaken twice monthly from March 2012 to October 2013 inclusive, with the exception of January 2013. After the 6 months the electrodes were removed and checked for correct functioning and reinstalled (failure rate was < 5%). Measurements were taken on two occasions each month, but not always in the same tidal cycle: once within the last few days of a spring tide phase (presumed maximum sediment saturation stage) and once within the last few days of the neap tide phase (presumed maximum drainage stage).

In October 2012 and 2013, the surface elevation relative to the UK reference level (Ordnance Datum, Newlyn (ODN)) was measured at the four corners and centre of each plot, using a differential GPS (Topcon, Newbury, UK), with an accuracy of < 2 cm and precision of < 1.5 cm. The mean measurement per plot was used in analyses. In order to compare data between the sites that have differing tidal regimes, the relative tidal height on a scale where 0 = Mean High Water Neap (MHWN) tide level, 1 = Mean High Water Spring (MHWS) tide level was calculated using:
Relative tidal height = Elevation relative to ODN – MHWN/(MHWS-MHWN).
Measurements MHWN and MHWS were obtained from Mossman *et al.* (2012b).

(a)



(b)

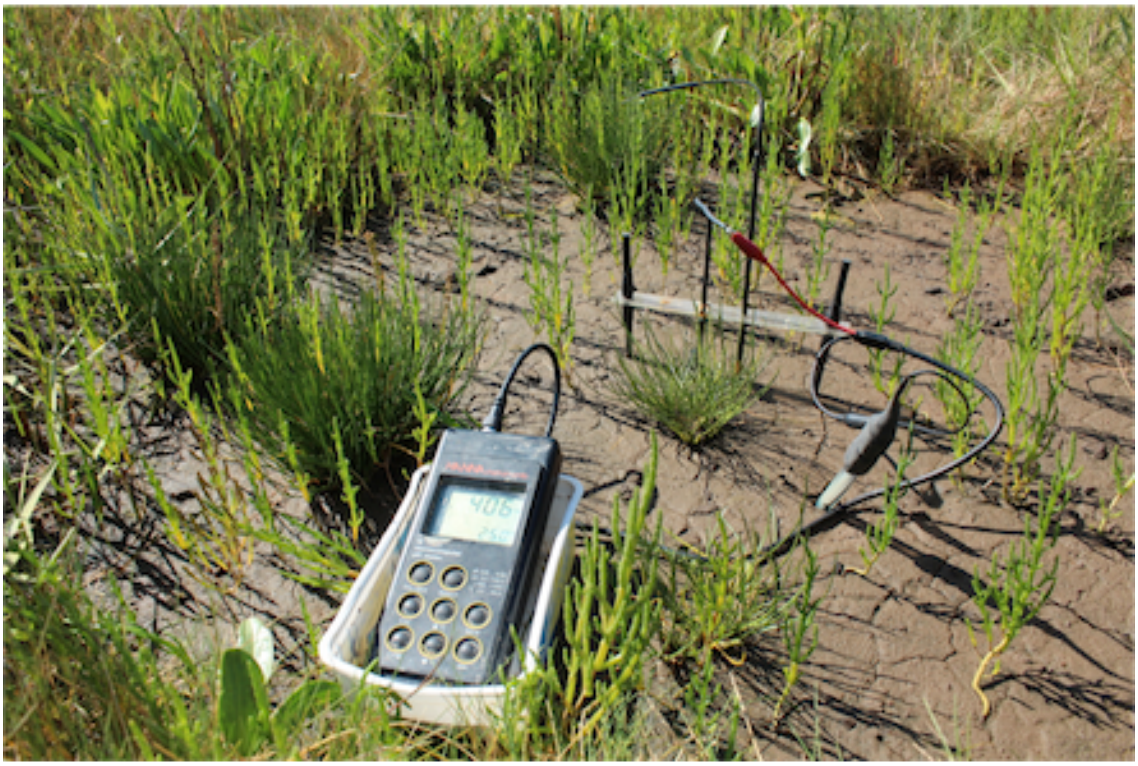
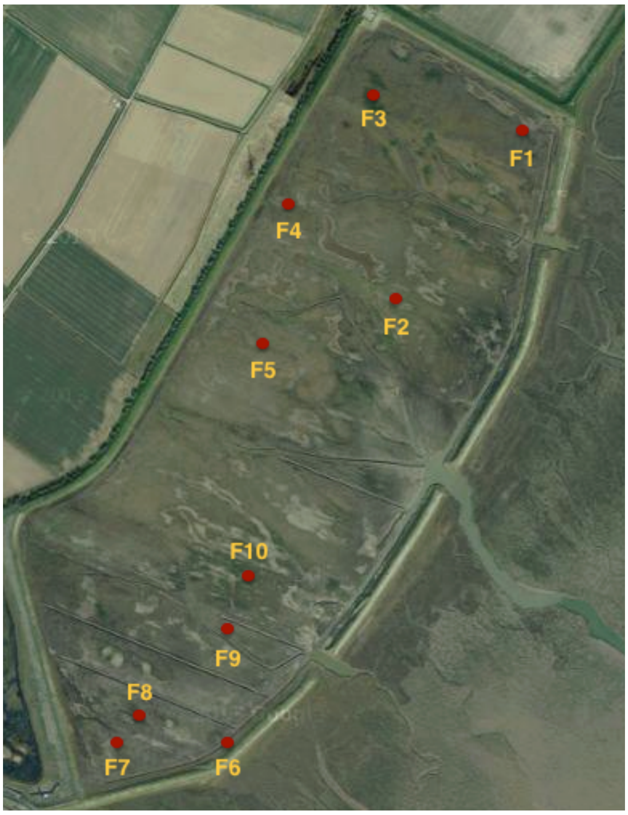


Figure 5.1. The arrangement of the experimental plots (a) and the permanently installed redox electrodes (b).

(a)



(b)

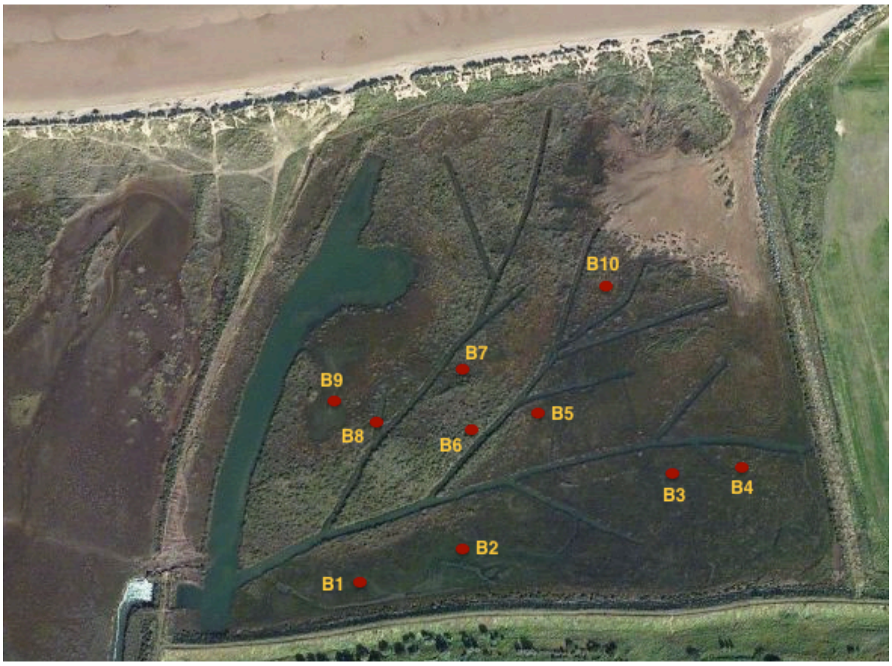


Figure 5.2. Locations of the experimental plots on MR sites at Freiston Shore (a) and Brancaster (b).

5.2.4 Data analysis

Summary statistics were calculated describing the central tendency and variability of redox at each sampling point and at each depth based on an examination of variation in the mean, representing central tendency, standard deviation, skewness and kurtosis in detail. Relationships between mean redox and each measure of variability were examined, with linear models used to relate each measure of variability to the mean, including a quadratic term used to capture non-linear relationships. The effect of relative tidal height and microtopography on each of these variables was examined by constructing linear models with relative tidal height, microtopographic manipulation treatment and their interaction as explanatory variables. A multi-model inference approach was employed using functions in the package MuMin (Barton *et al*. 2014) in order to fit all valid simplifications of this model, calculate AIC_C weights of each variable and perform model averaging. Seasonal variation in redox was examined by calculating the mean and standard error of redox across all samples at a given depth and tide in each month.

The relationship between mean redox and exposure to extreme redox values was examined in more detail. Firstly, an examination was made of the relationship between the mean and minimum redox experienced by a plot, using linear models to model the minimum redox experienced by a plot as a function of mean redox, microtopographic manipulation treatment and their interaction. This model was repeated using relative tidal height in place of mean redox. Secondly, an examination of the relationship between both mean and minimum redox and the number of sampling points where the redox was at or below a given threshold. The thresholds used were -200 mV, 0 mV and 200 mV and visualised relationships using locally weighted polynomial regressions (implemented using the R function loess, with default parameters). Only data from the 2 cm redox depths were used for these analyses.

Plant responses to redox were also investigated, using redox at 2 cm depth as (1) these were correlated with measurements at 10 cm (r = 0.78) and (2) because roots were most exposed to conditions at this depth. The proportion of planted individuals that survived until 2013 in each plot was modelled as a function of mean and minimum redox values, relative tidal height and topographic manipulation treatment using generalised linear models with binomial errors and a logit link function. A separate model was constructed for each species. We used the multi-model inference approach described above to calculate AIC_C weights of each variable and perform model averaging, and also used hierarchical partitioning (using the R package hier.part – Walsh & MacNally 2013) to assess the independent effect of each of these variables. Statistical analysis in R was carried out by Dr Hannah Mossman and Dr Martin Sullivan.

5.3 Results

5.3.1 Relationship between mean redox and measures of redox variability

There was a positive humped relationship between the mean and standard deviation of redox values experienced in plots, indicating that plots with intermediate mean redox values experienced the most variability. This relationship held when only measurements from spring or neap tides were used (Figure 5.3). There was a positive humped relationship between the mean difference between spring and neap redox values, and the standard deviation of these differences. Skewness was negatively related to mean redox values, with plots experiencing low mean redox having positively skewed distributions of redox, and plots with high mean redox values having negatively skewed distributions of redox. Relationships between mean redox and the kurtosis of distributions of redox were weaker than for other measurers of redox variability ($R^2 = 0.05 – 0.3$, compared to $R^2 = 0.2 – 0.74$ for skewness and $R^2 = 0.37 – 0.85$ for standard deviation), and had a negative humped form. Collectively, these results indicate that at low mean redox values (< 0 mV) plots typically experience redox values close to the mean, but with a long tail of values above the mean. At intermediate mean redox values (0 – 300 mV) there is considerable variability in redox values, distributed relatively evenly around the mean. At high mean redox values (> 300 mV) plots typically experience redox values close to the mean, but with a long tail of values below the mean.

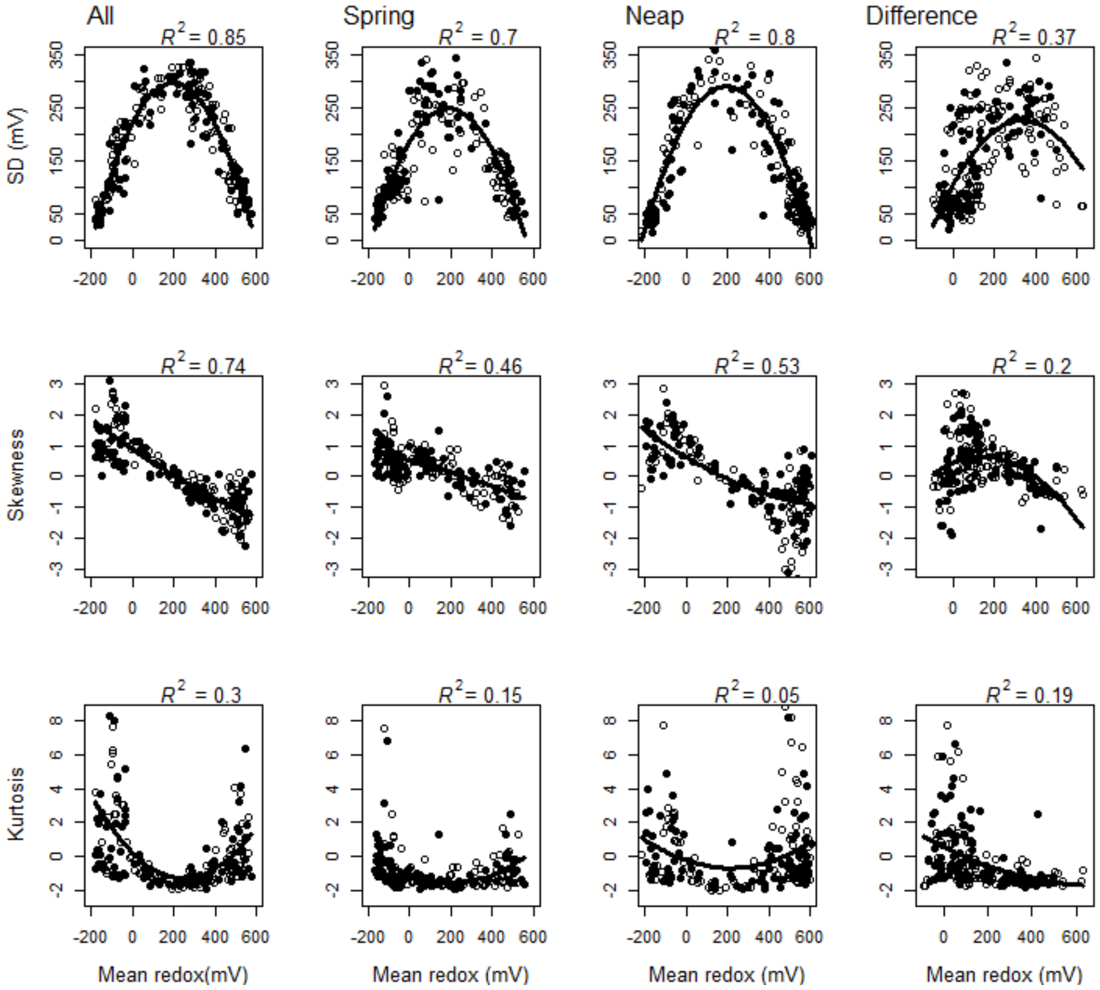


Figure 5.3. Relationship between mean redox and measures of standard deviation (SD), skewness and kurtosis. Measures of redox were calculated pooling samples at spring and neap tides (All), only using samples at spring tides (Spring), only using samples at neap tides (Neap), or taking the difference between each month's spring and neap tides (Difference). Open circles show measurements at 2cm depth, closed circles show measurements at 10cm depth. Solid lines show relationships from regression models modelling each response variable (pooling depths) as a function of mean redox. Models included a quadratic term. The R^2 of each model is show above the relevant plot.

5.3.2 Effect of topography and elevation on redox

Mean redox increased with relative tidal height, while skewness decreased with relative tidal height (Figure 5.4). There was also an additional effect of topographic manipulation treatment on both these variables, with raised plots having higher mean redox potentials and more negatively skewed distributions of redox than plots in different treatments at the same height (Figure 5.4). Interactions between treatment and relative tidal height were not well supported (selection probabilities ≤ 0.15, Table 1). Redox standard deviation differed between treatments, with the lowest values in raised plots (Figure 5.4). There was uncertainty about the importance of relative tidal height and its interaction with treatment (selection probabilities 0.54 – 0.66, Table 5.1), with a slight positive relationship in lowered plots becoming flatter in control plots and negative in raised plots (Figure 5.4). Variation in redox kurtosis was poorly described, with the null model appearing in the 95% confidence set of best supported models. The best supported relationship was with treatment (Table 5.1), with lower kurtosis in raised plots.

Table 5.1. Selection probability of treatment, relative tidal height and their interaction in models explaining variation in redox variables.

Variable	Mean redox	Redox SD	Redox skewness	Redox kurtosis
Treatment	1	1	0.99	0.76
Relative tidal height	0.98	0.66	0.96	0.26
Treatment : Relative tidal height	0.1	0.54	0.15	0.15
R^2	0.516	0.323	0.336	0.099

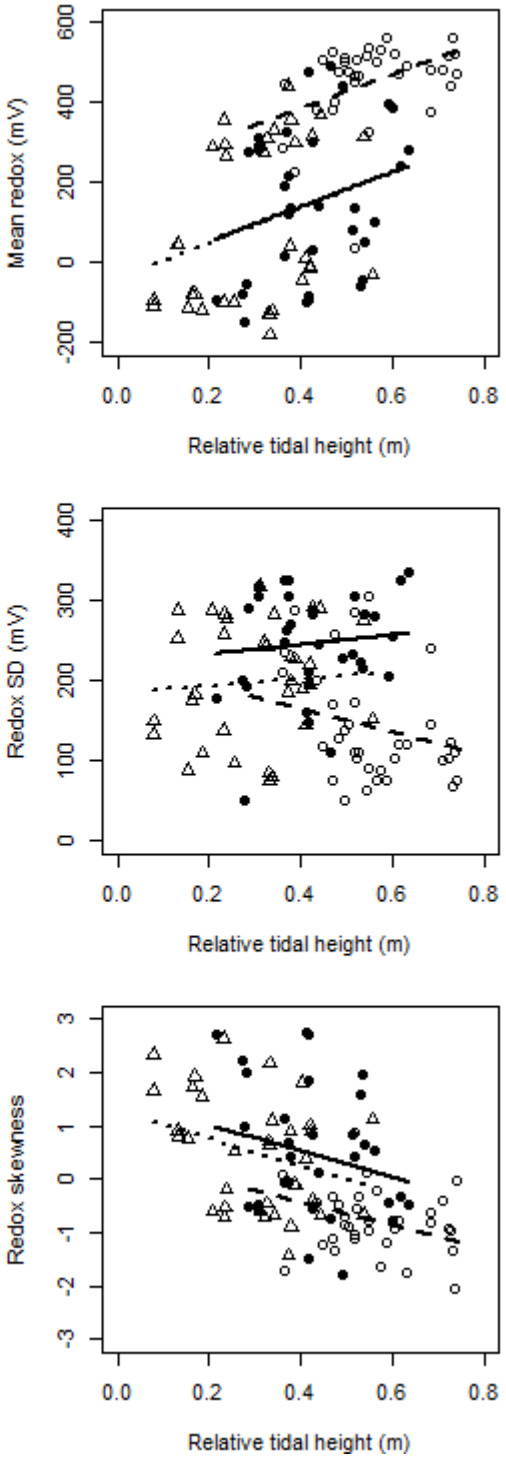


Figure 5.4. Relationship between measures of redox and relative tidal height. Filled circles show measurements from control plots, open circles show measurements from raised plots, and open triangles show relationships from lowered plots. Relationships based on model-averaged coefficients of models presented in Table 5.1 are shown. The solid line shows relationships in control plots, the dashed line shows relationships in raised plots, and the dotted line shows relationships in lowered plots. All measurements were taken at 2 cm.

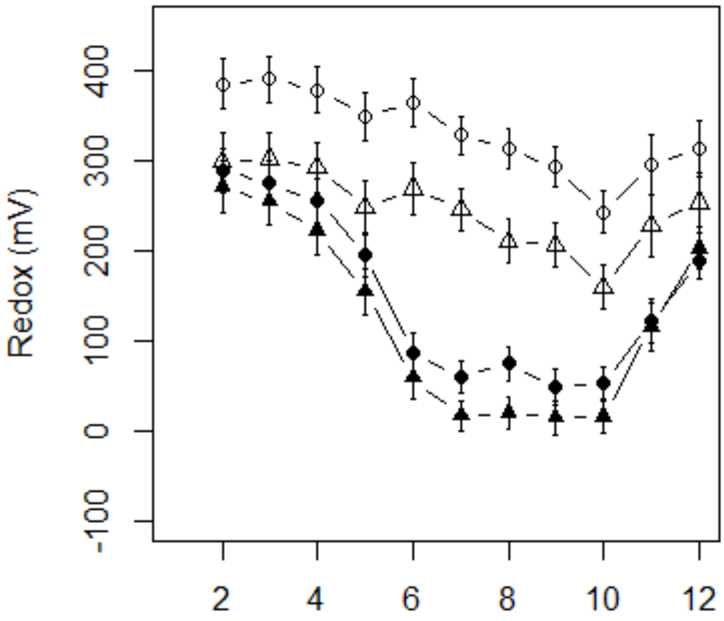
5.3.3 Seasonal variation in redox

Redox values were lower between June and October than between November and May (Figure 5.5). This reduction in redox in summer and early autumn was most pronounced on spring tides. Measurements of redox on neap tides were consistently higher, with redox values slowly declining over the summer to a minimum in October (Figure 5.5). Redox potentials were lower in the 10 cm sample than the 2 cm sample, but patterns of seasonal variation in redox were similar between depths.

5.3.4 Exposure to extreme low redox

The relationship between the minimum redox values experienced by a plot and both relative tidal height and mean redox were positive (selection probability of relative tidal height = 0.98, selection probability of mean redox = 1) but with very shallow gradients (Figure 5.4), indicating that low minimum redox values are experienced by plots high in the tidal frame and with high mean redox values. Despite this, a subset of plots high in the tidal frame experienced higher minimum redox values (Figure 5.6). These were mostly in the raised treatment (selection probability of treatment = 1 for both models). Interactions with treatment were moderately to strongly supported (selection probability for treatment: relative tidal height interaction = 0.66, selection probability for treatment: mean redox interaction = 1), with more positive relationships in raised plots (Figure 5.6).

The length of exposure to low redox was related to both mean and minimum redox. For low thresholds (-200 mV), the relationship with mean redox was represented by a concave curve, with exposure increasing non-linearly with decreasing mean redox. In contrast, for high thresholds (200 mV) the relationship was convex (Figure 5.7). Relationships between exposure and minimum redox showed sharp thresholds, with exposure rapidly increasing as the minimum redox decreased below the threshold (Figure 5.7).



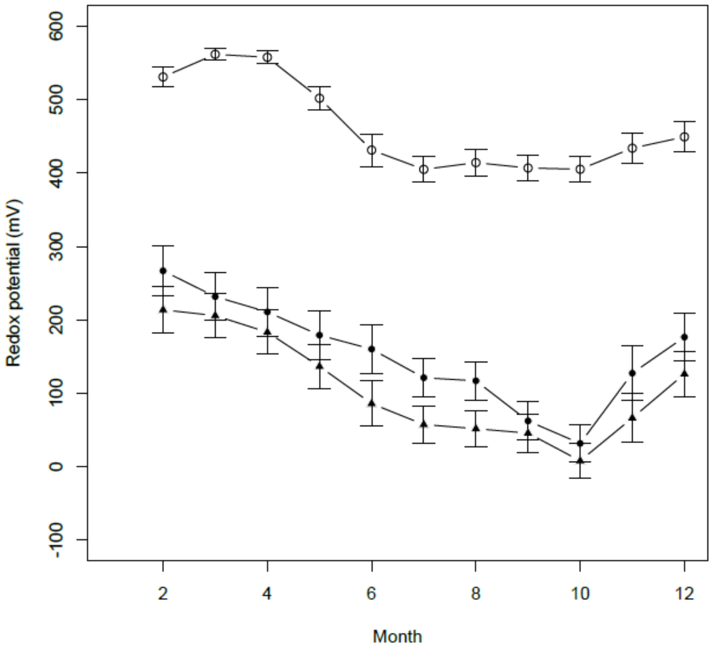


Figure 5.5. Seasonal variation in redox. ***Left:*** Points show mean values across all plots, with error bars showing standard error. Circles denote measurements made at 2 cm, triangles denote measurements made at 10 cm. Open symbols show measurements made on neap tides, filled symbols show measurements made on spring tides. ***Right:*** Points show mean values across all plots, with error bars showing standard error, at 2 cm depth. Open circles denote measurements made on raised plots, triangles denote measurements made in lowered plots, filled circles denote measurements made in flat plots.

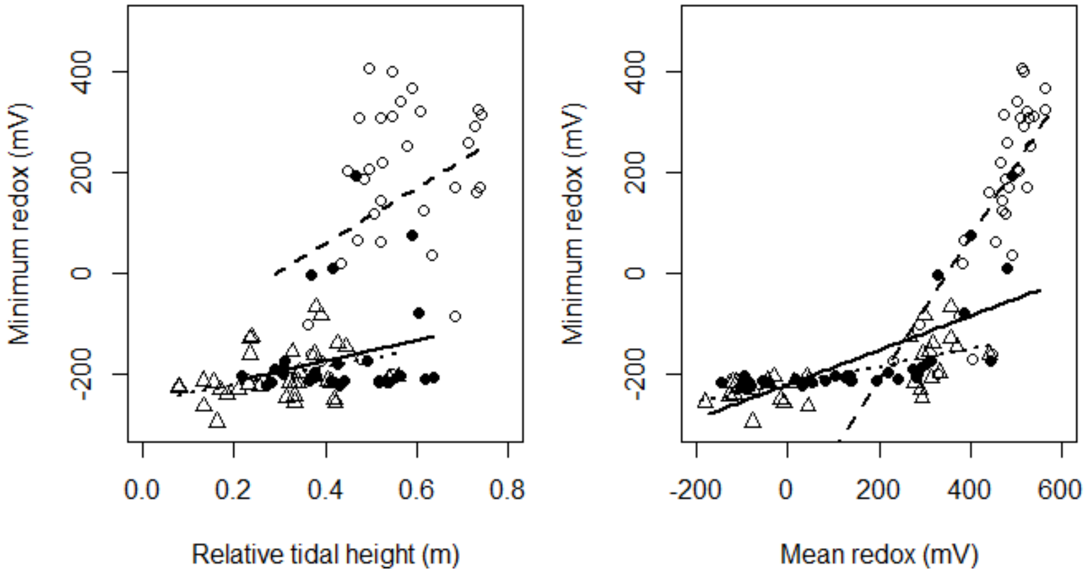


Figure 5.6. Relationship between minimum redox and relative tidal height and mean redox. Filled circles show measurements from control plots, open circles show measurements from raised plots, and open triangles show relationships from lowered plots. The solid line shows relationships in control plots, the dashed line shows relationships in raised plots, and the dotted line shows relationships in lowered plots. All measurements were taken at 2 cm.

5.3.5 Effect of redox mean and redox variability on plant survival

Survival was strongly influenced by the topographic treatment, with survival of all species reduced in the lowered plots and higher in the raised plots (Table 5.2). Survival of all species also increased with increasing mean redox (Figure 5.8), although this was less strong for *Triglochin*, for which survival was > 20% in the lowest mean redox category (Figure 5.8). Survival of *Armeria* and *Atriplex* was very low in the lowest redox category and whilst survival of *Armeria* was generally poor in all conditions, survival of *Atriplex* was almost 100% in the highest redox conditions.

Treatment, and mean redox, explained the most variation in survival of all species except *Armeria*, for which elevation was the strongest influence (Table 5.2). Minimum redox potential explained relatively little of the variation in survival compared to mean redox (Table 5.2).

Table 5.2. Contribution of mean redox, minimum redox, relative tidal height and topographic manipulation treatment to models of plant survival (N = 200 per species / treatment). Model averaged coefficients are shown. Plant survival data courtesy of Dr Hannah Mossman.

Species	Explanatory variable		Estimate	Adjusted SE	Selection probability	Independent effect (%)
Armeria	Intercept		-7.626	1.240		
	Mean redox		0.006	0.002	0.97	20.54
	Minimum redox		-0.004	0.001	0.96	17.61
	Relative tidal height		6.569	1.565	1.00	36.35
	Treatment	Lowered	-0.488	0.651	0.25	25.49
		Raised	0.599	0.590		
	Pseudo R^2					0.348
Atriplex	Intercept		-2.902	0.783		
	Mean redox		0.006	0.001	1.00	27.03
	Minimum redox		0.001	0.002	0.24	19.27
	Relative tidal height		4.176	1.448	0.97	19.35
	Treatment	Lowered	-2.109	0.389	1.00	34.35
		Raised	1.342	0.613		
	Pseudo R^2					0.656
Limonium	Intercept		-1.609	0.513		
	Mean redox		0.005	0.001	1.00	37.5
	Minimum redox		-0.003	0.001	0.97	12.65
	Relative tidal height		1.196	1.020	0.37	14.17
	Treatment	Lowered	-1.522	0.296	1.00	35.69
		Raised	0.146	0.398		
	Pseudo R^2					0.343
Plantago	Intercept		-5.290	0.929		
	Mean redox		0.007	0.002	1.00	30.25
	Minimum redox		-0.005	0.001	1.00	13
	Relative tidal height		3.402	1.237	0.94	20.43
	Treatment	Lowered	-1.939	0.594	1.00	36.32
		Raised	0.619	0.428		
	Pseudo R^2					0.506
Triglochin	Intercept		-0.623	0.461		
	Mean redox		0.002	0.001	0.71	18.7
	Minimum redox		-0.003	0.001	0.94	-2.78
	Relative tidal height		0.863	0.975	0.30	23.03
	Treatment	Lowered	-1.914	0.287	1.00	61.06
		Raised	0.256	0.377		
	Pseudo R^2					0.21

Negative independent effects occur when the joint effect of a variable is greater than their total effect.

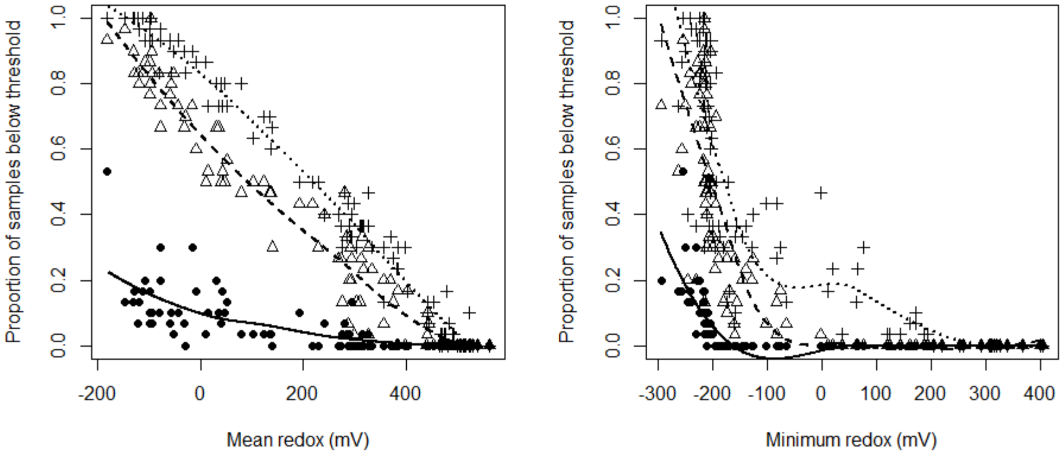


Figure 5.7. Relationship between both mean and minimum redox and proportion of samples with redox at or below a given threshold. Results are shown for thresholds of -200 mV (filled circles, solid line), 0 mV (open triangles, dashed line) and 200 mV (crosses, dotted line). Fitted relationships are from locally weighted polynomial regressions.

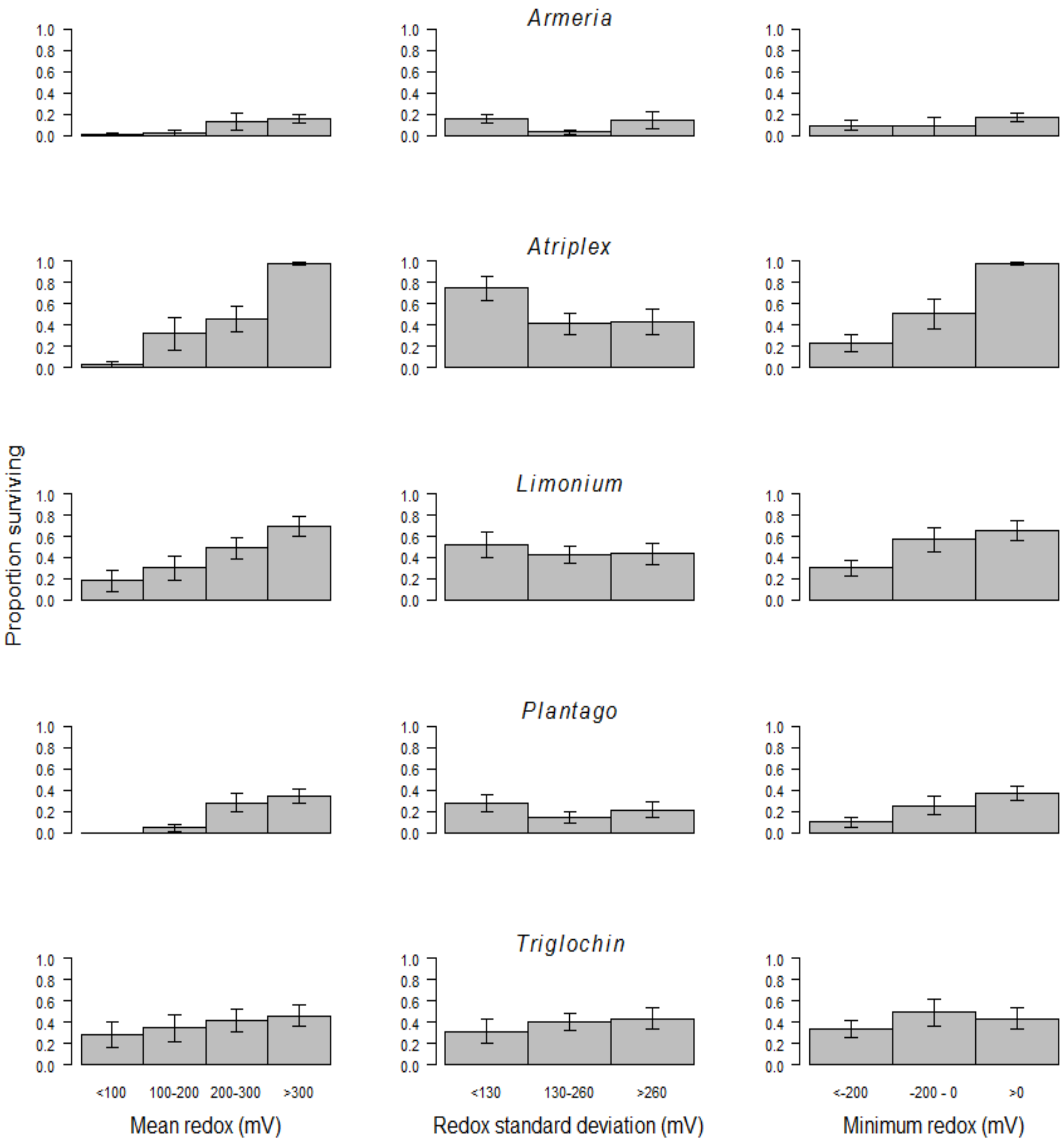


Figure 5.8. Relationship between survival and mean redox, redox standard deviation and minimum redox. Bars show mean survival, with error bars showing standard errors. Plant survival data courtesy of Dr Hannah Mossman.

Figure 5.9. Raised plot at upper elevation site at Freiston MR at year 3 showing vigorous growth of *Atriplex* and potential exclusion of other target species.

Figure 5.10. Raised plot on the low marsh site at Brancaster at year 3 showing restricted growth of *Atriplex* and potentially enabling higher numbers of surviving *Limonium* and *Triglochin* plants as a likely consequence of higher sediment volume experiencing anoxic (< 400mV) and reducing Eh conditions.

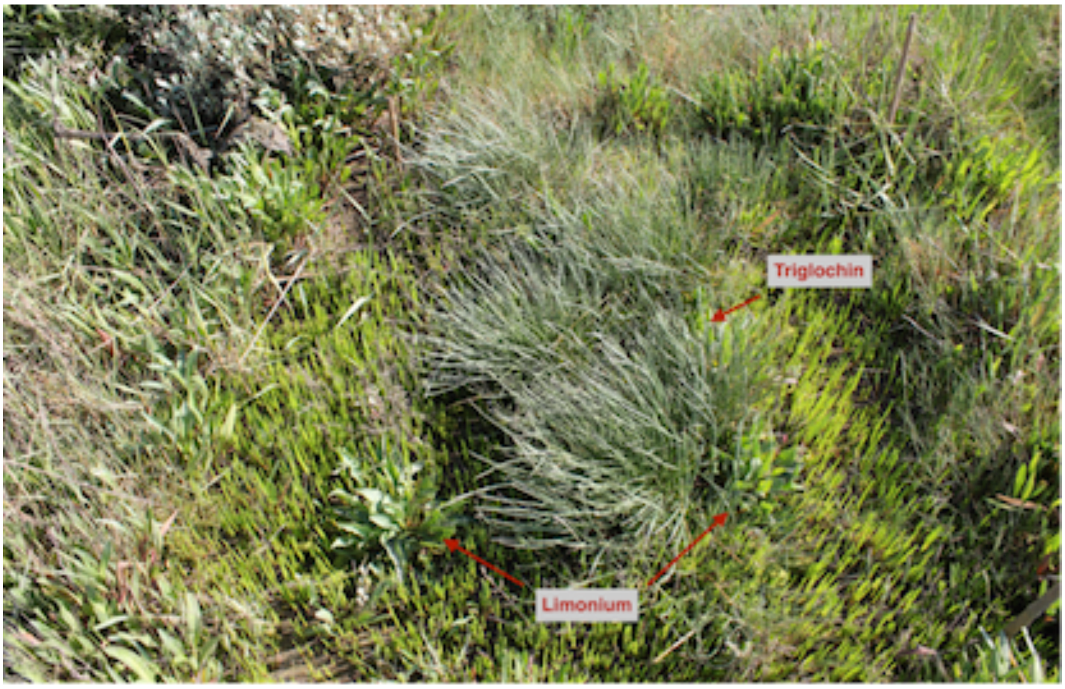


Figure 5.11. Flat plot on the low marsh site at Brancaster at year 3 showing *Limonium* and *Triglochin* as only surviving species from target plants on sediments with very low mean Eh.

Figure 5.12. Flat plot on the upper marsh site at Freiston at year 3 showing restricted growth of *Atriplex* and enabling higher numbers of surviving *Limonium*, *Plantago*, *Triglochin* plants as a likely consequence of higher sediment volume experiencing anoxic (< 400mV) and reducing Eh conditions.

5.4 Discussion

Manual measurements of redox potential were found to be broadly similar to those obtained from the datalogger at the same managed realignment sites (Chapter 4). Mean redox potential varied over the year, with levels lowest between June and October. Seasonal patterns of redox potential were consistent between spring and neap tides, and between depths. Seasonal patterns were also consistent between raised, flat and lowered treatments, with raised plots having higher redox potentials than both flat and lowered plots. The raised plots showed a much higher degree of seasonal consistency in maintaining aerobic conditions, although these plots did experience occasional low redox conditions.

Artificial raising the elevation significantly increased the redox potential of a plot, resulting in predominantly oxic conditions in these localised microsites. Interestingly, the effect was apparent irrespective of elevation. The redox potential was also less variable in raised plots compared to lowered and flat plots, although, redox in raised plots at higher elevations was less variable than plots sited at lower elevations. Although the mean Eh of the L plots was similar to the mean of the F plots most remained in an undrained state for much of the neap tide phase consequently survival for all species was absent in most of these plots due to conditions of prolonged submergence rather than the prevailing Eh *per se*.

Overall, the differences in redox conditions between the raised and flat plots were sufficient to significantly influence the survival of the target species often leading to dramatic differences in the resulting composition and development relative to the target vegetation in the flat plots. This is in agreement with other studies that reported the significance of microtopographical variation in the surface height that can lead to major differences in the rate of drainage of tidal water from the marsh surface which was shown to influence the distributional patterns of different species on saltmarshes (Gray & Scott 1977; Stribling *et al*. 2006, 2007; Varty & Zedler 2008).

5.4.1 *Atriplex*

Of all the target species *Atriplex* proved to be the most efficient at exploiting the most oxic conditions, with almost all planted individuals surviving in plots with the highest mean Eh. This mirrors its natural distribution on saltmarshes where it is rapid coloniser in the more oxidised sediments of MR marshes (Brown 2008). Such conditions tend to occur at relatively well-drained locations, such as the edges of drainage channels and areas of raised microtopography in more central areas of the marsh distant from drainage channel influence (Chapter 4). This distribution is a likely consequence of the absence in *Atriplex* of enhanced root porosity that more waterlogging tolerant wetland plants possess as a structural adaptation to avoid anoxia stress (Justin & Armstrong 1987; Mohamed 1998). Its intolerance of waterlogging and reducing conditions has previously been reported in other work (Crooks *et al.* 2002; Mohamed 1998) so it was not unexpected that manual measurements of Eh from the experimental plots showed that it particularly thrived in the more consistently oxic raised plots where its extensive growth may have competitively excluded other target species (Figure 5.9 compared to Figures 5.10 – 5.12).

This experiment demonstrated *Atriplex* was excluded from plots that potentially experienced long-term anoxia represented by low mean Eh values. However, it also showed that *Atriplex* is tolerant of sediments that might, at best, be only partially oxic, with limited oxygen availability and sediments that fluctuate between oxic and reducing conditions in response to flooding. This is supported by the datalogger measurements of continuous hourly Eh recordings from sites where *Atriplex* is dominant (Chapter 4), which indicated sediments that remained largely oxic during the winter and early spring subsequently experience a wide amplitude of flood-induced Eh fluctuations, with mean reducing conditions in the rooting zone typically extending over a period of 6-8 days well into the neap tide phase. In these sites, the period of anoxia is approximately of equal duration to the period it is at an oxic state (Chapter 4). In sites where *Atriplex* was less dominant, such as the creek site at Stiffkey (Chapter 3) where it where its growth was restricted and co-occurred with *Puccinellia*, the period of mean reducing conditions was found to be considerably longer from late summer when it exceeded the oxic period before the subsequent phase of flooding.

The capacity of *Atriplex* to survive in occasional but relatively prolonged periods of anoxia likely confers it a competitive advantage in colonising well drained sediments that are characterised by strongly fluctuating Eh. Support for this proposition comes from reports of possible niche partitioning with the more upper marsh species *Elytrigia atherica*, which appears to be marginally less tolerant of reducing conditions (Armstrong *et al*. 1985; Davy *et al*. 2011). *Elytrigia atherica* is a better competitor than *Atriplex* in more consistently oxic sediments, but still able to colonise sediments occupied by *Atriplex* after its experimental removal (Bockelmann & Neumann 1999).

5.4.2 *Limonium* and *Triglochin*

Limonium and *Triglochin* demonstrated the highest levels of survival of the target species at the lowest mean Eh. *Triglochin* was found to be the most be weakly related to the independent effect of Eh. Both these species occur over a wide elevational range on natural saltmarshes on the North Norfolk coast estimated at 3.1 – 2.4m ODN, where their centres of distribution are in the upper marsh GSM communities (Chapman 1960). Survival was highest for *Limonium* at higher mean Eh. However, both species were able to survive in F plots even at lowest elevations sited in the *Spartina-Aster* dominated low marsh at Brancaster where mean Eh values were predominantly in the severely reducing range and these conditions remain continuous throughout the growing season. Survival of *Triglochin* was less affected under these conditions and appeared to be better adapted than *Limonium*, which visually appeared to experience a reduction in growth relative to plants on the raised plots (pers. obs. Figures 5.9 – 5.12).

It is unclear why these two species are rare on some areas of MR sites because they appear to be more tolerant than *Atriplex* of anoxic conditions. Furthermore, germination and initial establishment in spring would have occurred at a time when much of the sediment on the MR sites at mid to upper elevations, and particularly at surficial depths, is predominantly oxic. Both species are anatomically adapted to survive conditions of prolonged anoxia, with each species possessing similar volumes of extensive aerenchyma in their root systems (Justin and Armstrong 1987; Rozema *et al*. 1985b). Moreover, experimental investigations of the combined effects of salinity and waterlogging indicated both these could survive these treatments, at reduced growth rates, for 8-10 weeks for *Triglochin* (Cooper 1982; Rozema *et al*. 1985b) and 3 months for *Limonium* (Boorman 1968, 1971). It therefore seems possible that biotic constraints may play a more important role in limiting the establishment of *Limonium* and *Triglochin* rather than abiotic causes at mid to upper elevations on these MR sites.

There is little available information concerning the competitive ability of these species with *Atriplex* and *Puccinellia*, which tend to dominate at mid to upper elevations of the MR sites. A few studies have indicated that the abundance of *Triglochin* could be artificially manipulated by the alteration of the tidal range from coastal barrage construction in the Netherlands. Groenendijk *et al*. (1987) reported an increase in the abundance of *Puccinellia* was achieved at the expense of *Triglochin* by a reduction in tidal amplitude, which supported an earlier study that found an increase in the mean high water level resulted in large increases in the abundance of *Triglochin* within 2 years (Beeftink 1979). Observations of the *Limonium* and *Triglochin* propagated in the laboratory in the current study indicate these species possess slow rates of growth relative to *Atriplex* and *Puccinellia*, supporting the proposition that they may be competitively disadvantaged under favourable (i.e. oxic) conditions. However, recent observations in the field in the current study indicate that the abundance of these two species has increased significantly at the mid elevation areas of the Brancaster MR (pers. obs.), where there is an abundant supply of propagules from adjacent marshes, and at a slower rate at Freiston Shore where neither *Limonium* nor *Triglochin* is abundant on the associated natural marshes at this location. In contrast, measurements of Eh in the current study from low elevation sites at both MR's indicated the presence of low Eh earlier in the growing season supporting previous work suggesting the operation of abiotic constraints may be limiting establishment of these species in large areas of MR sites (Mossman *et al*. 2012b).

5.4.3 *Armeria* and *Plantago*

Overall, *Armeria* and *Plantago* had poorer survival than the other species, with very low levels of survival at the lowest Eh. These species regularly occur at high abundances in the general saltmarsh community. The apparent intolerance of these species of very low mean Eh clearly contradicts the findings from extensive hourly measurements of Eh in the sediments of the GSM, which found this community to experience periods of anoxia (Chapter 3). Eh measurements of the GSM communities indicated that these sites were predominantly anoxic and experienced moderate-severe reducing intensities for a considerable period (4-5 months) of the year, extending from late summer to well into the autumn. The possibility that these species are able to survive prolonged periods of low Eh in the GSM by exploiting relatively oxic microsites cannot be discounted. However, there was little evidence from extensive measurements in the current study to indicate such features are widespread given the high abundances of these species in this community.

There are no studies of which the author is aware that have specifically investigated the tolerance of *Armeria* and *Plantago* to anoxia and low redox potentials. Their tolerance to anoxia can only be inferred from the few studies that investigated the combined effects of salinity and waterlogging under glasshouse conditions, which indicated both *Armeria* and *Plantago* could survive waterlogging for the experimental durations of up to 3 months but at a much reduced rate of growth (Cooper 1982; Rozema *et al*. 1985b). While *Plantago* is able to develop extensive root aerenchyma (Justin & Armstrong 1987; Rozema *et al*. 1985b), the capacity for enhanced porosity in *Armeria* is unclear.

Little information is available on the presence of root aerenchyma in *Armeria* although Rozema *et al* (1985b) measured low porosity for the smaller adventitious roots in response to waterlogging while porosity of the tap root was not reported. Interestingly, the same study reported low porosity in *Puccinellia maritima* while another study reported well developed aerenchyma (Justin & Armstrong 1987).

One explanation for this apparent contradiction in responses might be that while sediments from the GSM and the MR both experience high reducing intensities, these sediments differ with respect to redox capacity. While measurements of Eh give an indication of the intensity of reduction, this metric does not indicate how much reduction is actually occurring, which depends on the concentration of oxidants and the availability of labile organic matter in the sediments (Gambrell *et al*. 1991; Reddy & DeLaune 2008). It may be possible that sediments in MR sites have a higher reduction *capacity* at any given *intensity* of reduction in the root zone that can compete for oxygen transported to the rhizosphere, potentially increasing plant stress above that caused by the redox intensity factor alone (Pezeshki & DeLaune 2012). Sediments with high reduction capacity are also more likely to have high concentrations of reduced compounds with potentially phytotoxic effects (Ponnamperuma 1972; Gambrell *et* a*l*. 1991). A few studies that have investigated the effect of phytotoxicity reported that both *Armeria* and *Plantago* have a degree of tolerance to high concentrations of Mn^{2+} and Fe^{2+} (Rozema *et al*. 1985a; Singer & Havill 1988). Sulphide toxicity also may be a factor in low marsh sediments (Ingold & Havill 1984) and redox measurements at the lower and mid elevations on the MR sites readily attain the critical Eh levels where sulphate reduction occurs.

While it is possible that the lower distributional limits of *Armeria* and *Plantago* may be determined abiotic factors related to redox capacity, these effects may also operate independently of elevation particularly a mid marsh elevations where redox conditions have previously been reported to be highly variable (Davy *et al*. 2011). This may impose additional distributional constraints on these species. Areas of the MR where Eh conditions are more amenable for *Armeria* and *Plantago* survival tend to be occupied by vigorous growth of *Puccinellia* and *Atriplex*, which likely imposes a biotic constraint, further limiting opportunities for their establishment. This may explain why these plants tend to remain particularly scarce, both on recent MR sites and on historically older restored saltmarshes that developed from accidental storm breaches of sea wall defences (Mossman *et al*. 2012a; Wolters *et al*. 2005).

In *natural saltmarshes* of this region, the only community where *Armeria* occurs at high abundances is in the special circumstances of the GSM, where the suppressed growth of other species has been attributed to the effects high levels of sediment salinity (Jefferies 1977). Such conditions may have occurred at the highest elevation plots at Brancaster, which were observed to be very dry, likely due to the high sand content of the substrate at that particular location. The number of surviving *Armeria* was observed to be highest at this site, perhaps because the growth of other target plants was restricted.

Armeria was found to limited to the narrowest elevation of all the saltmarsh species, with a range of approximately 10 cm distribution over a total range of 2 m at Scolt

Head Island on the North Norfolk coast (Chapman 1960). In the experimental plots of the current study, survival of *Armeria* was low even in raised and flat plots at the highest elevations (pers. obs.). The low survival rates appeared to result from the favourable conditions in these plots for promoting vigorous growth of, competitively dominant *Atriplex*, potentially resulting in the exclusion of *Armeria*. *Plantago* survival was similarly also restricted to sites with higher mean Eh although the overall number of surviving plants was higher than *Armeria*. A possible explanation for this could be because *Plantago* appears to have a faster growth rate (pers. obs.) and a larger growth form and therefore was not as readily excluded by *Atriplex*.

5.5 Conclusion

Measurements of redox potential in saltmarshes are frequently undertaken as a key parameter that indicates not only the oxygen status of sediments, but also the potential presence of additional stresses from an increased demand for oxygen in the rhizosphere and phytotoxicity, which is a result of accumulation of reduced compounds in the absence of oxygen. Each of these factors can potentially exert a significant influence on the ability of a plant to survive and therefore provide an important explanatory variable for the patterns of zonation that are characteristic of many saltmarshes. This experiment showed that all the target plants could, to varying degrees, occupy sites that experience low Eh values. Less tolerant species, such as *Armeria*, survived in conditions where low Eh values occur intermittently, while more tolerant species, such as *Triglochin*, are able to survive where low Eh conditions are present with a greater degree of permanence.

Variability in survival was strongly related to mean redox potential, meaning that plots experiencing given redox characteristics had predictable patterns of variability. Plots with intermediate mean redox were the most variable, with variation evenly distribution around this mean. In contrast, plots with high mean redox potential consistently experience high redox potential, with only occasional low values. Thus single, one-off measures of Eh may provide an indication of the rank order redox at locations, particularly at high and low marsh locations. However, single measurements may not be sufficient to discriminate between these temporal patterns, and more ecologically relevant information can be only gained from data relating to average conditions derived from longer term measurements.

This experiment also showed how artificially raising the local sediment surface can dramatically alter the mean redox status of the sediment and significantly increase the survival of species that would otherwise be excluded from sediments where very low mean Eh values are more prevalent. On the other hand, in sediments that experience high mean Eh values, survival of *Atriplex* is high and the vigorous growth of this species may exclude others.

An understanding of the relationship between species and mean Eh clearly has important implications for engineering restoration saltmarshes in order to maximise the diversity of vegetation and increase the conservation value of these habitats. Objectives that aim to maximise drainage by engineering artificial drainage channels to increase mean redox potentials need to be considered with care since these conditions may limit opportunities for colonisation by slower growing species such as *Limonium* and *Triglochin*. More consideration needs to be given to engineering topographic heterogeneity to create a variety of Eh conditions, with the aim of limiting the dominance by a few species that are better able to exploit sediments with a high mean Eh. Other species, particularly *Armeria* and *Plantago* are unlikely to colonise MR sites in high abundance unless the very specific conditions of hypersalinity and Eh that constrains the growth of other species on GSM sites can be artificially replicated.

CHAPTER 6: Laboratory experiments on the effects of redox intensity on *Atriplex portulacoides* and *Triglochin maritima*

6.1 Aims

Plants growing in flooded sediments with low redox potentials experience not only an absence of oxygen but also an environment that imposes an increasing demand for its internal oxygen by promoting oxygen loss from the rhizosphere (Pezeshki & DeLaune 2012). Saltmarsh plants may have a range of metabolic and anatomic adaptations that enable them to tolerate or avoid stresses from anoxia and low redox potentials which is likely to play an important role in distributions. However, the response of most saltmarsh species to anoxia is poorly understood and even less is known about their responses to the effects of low redox potentials. The oxygen demand imposed by low redox potentials is influenced both by the reducing strength, as measured by its Eh value, and also the capacity for reduction which is equivalent to the amount of the system undergoing reduction (Reddy & DeLaune 2008). In order to address the lack of understanding of their response to the increased oxygen demand, a laboratory experiment was devised based on a hydroponic method for long-term cultivation of plants under controlled redox intensities using titanium (III) citrate as a non toxic redox buffer (Lissner *et al.* 2003a). Two species - *Atriplex portulacoides* and *Triglochin maritima* - were selected which were expected to demonstrate sharply contrasting responses. An evaluation was made of the effects of differing redox intensities on growth, photosynthetic functioning, root porosity and ADH activity.

6.2 Introduction

Root oxygen deficiency has long been established as a critical stress factor for plant functioning and survival that has generated a considerable body of research which has attempted to elucidate the complex range of physiological effects and the diversity of plant responses. Much of the current state of knowledge has been gained from detailed investigations on a small number of economically important crop species that, with the exception of *Oryza sativa*, are generally intolerant of oxygen stress. Greater insights have been provided by studies on a number of freshwater wetland species that have illuminated the range of adaptations enabling survival in oxygen deficient conditions (Crawford *et al.* 1994; Hook & Crawford 1978; Jackson *et al.* 1991;

Kozlowski 1984). However, the number of studies that have described such mechanisms in saltmarsh plants is relatively few, despite the fact that these highly specialised species are of considerable ecophysiological interest due to their ability to adapt to or tolerate the concurrently operating stresses from oxygen deficiency, phytotoxicity and high salinity while sometimes supporting high levels of productivity (Colmer & Flowers 2008). Moreover, the response of many saltmarsh species to varying levels of redox potentials has received little attention and remains largely unknown.

A basic understanding of the capacities for oxygen stress tolerance of British saltmarsh species has been inferred from a small number of studies based on the effects of experimental soil waterlogging treatments on growth and survival (e.g. Cott *et al*. 2013; Cooper 1982; Groenendijk *et al*. 1987; Huckle *et al*. 2000; Rozema *et al*. 1985b, 1988; van Diggelen 1991). In addition fewer published studies have investigated specific anatomical and metabolic responses to oxygen deficiency stress in British salt marsh species (Alhdad *et al*. 2013; Justin & Armstrong 1987; Rozema *et al*. 1985b).

A potentially significant limitation of such studies that are based on the method of experimental soil waterlogging is the difficulty entailed in maintaining consistent and therefore replicable soil redox conditions resulting in the possible introduction of artefacts from uncontrolled changes in the soil oxygen demand on plant roots. Few of these investigations appeared to have rigorously monitored the aerobic or redox status of the soil during the course of the experiment. Where this was undertaken it was found that although soil waterlogging can quickly lower the redox potentials from +600 mV to -100 to -300 mV at the start of the experiment, many of the wetland species tested were able to eventually increase redox potentials by as much as 400mV as a result of root oxygen effusion to the rhizosphere. Even at depths of 10cm (the maximum depth recorded) values greater than +300 mV developed for a significant number of species (Justin & Armstrong 1987). Another potential problem arises from the possibility introducing confounding stress effects of phytotoxicity from the accumulation of reduced ions and other anaerobically derived compounds particularly in species that have a low capacity to raise redox potentials in the soil containers (DeLaune *et al*. 1990; Pezeshki & DeLaune 2012). Under these circumstances disentangling and interpreting the plant response to oxygen deficiency becomes highly problematic.

The alternative commonly employed technique to study oxygen stress responses involves pressurised nitrogen purging to deoxygenate the nutrient solution of hydroponically grown plants which has, to date, been tested only on a single British saltmarsh species in a series of detailed investigations of the biochemical, anatomical and morphological responses in *Suaeda maritima* (Wetson & Flowers 2010; Wetson *et al*. 2012; Alhdad *et al*. 2013; Colmer *et al*. 2013). While this method provides improved experimental control and repeatability it suffers from a potentially significant drawback since such a system is able to achieve redox potentials in the culturing medium only slightly below conditions of hypoxia ~Eh +350 mV (DeLaune *et al*. 1990). Clearly, limiting oxygen to hypoxic levels fails to adequately simulate the stresses associated with the true anoxic conditions that are generated at low redox potentials responsible for the high oxygen demand on plant roots commonly encountered in highly reduced saltmarsh sediments. This is an important consideration in plant oxygen stress investigations since responses elicited at the upper range of the anaerobic scale may be significantly different and therefore unrepresentative of responses induced by more reducing environments (DeLaune *et al*. 1990; Brix & Sorrell 1996; Pezeshki 2001).

To address the paucity of information regarding tolerances of British saltmarsh species to the effects of strong oxygen demand created by low redox potentials that readily occurs in saltmarsh sediments an experiment was designed in the current study to investigate the responses of hydroponically grown plants under controlled redox intensities.

6.3 Review of effects of anoxia and low redox potentials on plants

Soil oxygen deficiency, either complete (anoxia) or partial (hypoxia), elicits a detrimental response in most species of higher plants by suppressing, or completely inhibiting, aerobic cellular metabolism in roots. However, plants exhibit a wide range a tolerances with negative effects occurring with a few hours in more sensitive species to several months in better adapted species. Although the decrease in cellular respiration in root tissues and its replacement with the less efficient anaerobic process of energy generation by ethanolic fermentation is the most widely understood response to cellular oxygen deficiency variation in the tolerances of plants is likely to be a reflection of the fact there is no single universal cause of anoxic death for plant tissues. Instead since there is a variety of different ways oxygen deficiency causes injury or mortality there is consequently a commensurate variety of adaptations and strategies employed by plants to counter this stress. The various mechanisms have been described in detail in a number of reviews (Armstrong *et al.* 1994; Blom 1999; Colmer & Voesenek 2009; Pezeshki 2001; Pezeshki & DeLaune 2012; Sairam *et al.* 2008; Vartapetian 2006; Vartapetian & Jackson 1997).

Differential tolerances can be explained, in part, as loss of plant tissue viability when deprived of oxygen which occur in a variety of metabolically induced processes leading to injury during anoxia and also post-anoxia from oxidation of accumulated metabolites. In non-wetland species mortality can take place rapidly. A common early indication of stress in poorly adapted species is wilting within a few hours to a few days of imposing a flooding stress (Jackson & Drew 1984). Wilting results from the loss of ATP synthesis from the inhibition of respiration that blocks the ion transport mechanisms responsible for maintaining an osmotic gradient across the root endodermis. The loss of water and nutrient uptake results in a decline in photosynthetic activity and carbon assimilation, severe chlorosis and early senescence of leaves and injury to vital organs leading to eventual plant mortality. In other species the duration of anoxia will deplete carbohydrate reserves and cause irreversible damage within hours due to metabolic dysfunction causing impairment of membrane operation and of loss of cellular pH homeostasis (Roberts *et al.* 1984). In other cases irreversible damage can occur after a longer period (1-2 weeks) and accelerated on return to post anoxic injury by the inhibition of protein synthesis and cellular damage from the generation of excess free radicals and toxic oxidative products such as acetaldehyde (Crawford 2003).

The following provides an overview of the current state of knowledge regarding stress responses to root oxygen deficiency. While much of this understanding has been gained from a large body of research based on a small number of freshwater wetland and commercially important crop species, with the exception of *Spartina* spp., the responses of many saltmarsh species to oxygen deprivation are largely unknown. Insights gained from the numerous studies on non saltmarsh species may still be of relevance to understanding the different possible mechanisms saltmarsh plants are likely to use to enable survival and growth in conditions of sediment anaerobiosis. However, since the majority of these investigations were based on plants grown using experimental soil waterlogging or nitrogen purging techniques responses at different redox intensities remain unknown for most species.

In general the different adaptive plant strategies are based either on avoidance or tolerance of root oxygen deficiency using a diverse variety of structural and metabolic modifications. Adaptation to short term (hours-days) oxygen deprivation usually relies on the capacity of the plant to supply soluble sugars to fuel anaerobic respiration switch and the ability to provide of antioxidant defences to neutralise oxidative stresses associated with oxygen deficiency. While long term (weeks-months) survival is thought to be possible only with the development of anatomical and morphological features that enable superficial rooting to sequester oxygen confined to the uppermost layers in the soil profile, the development of adventitious roots and increased capacity from transport internal transport of respiratory gases between the shoots and the root tips and the formation barriers to oxygen leakage from the roots to the soil. The diversity of responses in plants to soil oxygen deprivation suggests that depending on the life strategy and habitat many different mechanisms may be involved adaptation to survive periods of inundation and no one mechanism is adequate for ensuring survival (Crawford & Braendle 1996).

6.3.1 The consequences of flood induced anoxia

In aerobic soils plant roots are able to obtain sufficient oxygen for respiration and growth mainly by diffusion through the root epidermis from soil air spaces. The rate of atmospheric oxygen diffusion into saturated soils is not sufficient to replenish the rate of oxygen consumption by the metabolic activities of roots and soil microorganisms. Oxygen deficiency therefore results from the inhibition of aerobic respiration when the plant is unable to supply the roots with sufficient oxygen through an internal transport pathway from the shoots.

Usually roots do not experience a sudden transition from oxic to an anoxic state on flooding, rather it is a more gradual process occurring over a number of hours as the change passes through the intermediate stage of hypoxia that provides an opportunity for acclimation before conditions become injurious (Drew 1997). The capacity of the plant to respond to conditions of oxygen deficiency is likely to depend on its ability for early sensing of the change in soil oxygen availability in order to be able respond accordingly. Sensing may occur if only the root apex is exposed to localised hypoxia which initiates a response along the whole root length (Malik *et al*. 2003). Various mechanisms for sensing hypoxia or anoxia are known to exist, such as plant haemoglobin gene expression linked to increased ADH activity (Silva-Cardenas *et al*. 2003), changes in cellular Ca^{2+} (Pang & Shabala 2010), decreases in pH (Felle 2010) and gaseous ethylene (Bailey-Serres *et al*. 2012).

As oxidative phosphorylation becomes limiting under hypoxia the electron flow through the respiratory pathway is reduced resulting in the generation of less ATP which has a range of diverse consequences for cellular metabolism and developmental processes (Gibbs & Greenway 2003; Greenway & Gibbs 2003; Sairam *et al*. 2008; Visser *et al*. 2003). However, the ability of the plant to tolerate oxygen deficiency depends on the tissue type, developmental stage, genotype, the severity and duration of anoxia.

In the absence of sufficient anatomical modifications to increase internal oxygen transport to the root apex, plant tolerance to oxygen deprivation will depend on the ability to adjust to the resulting energy crisis by utilising a number of possible metabolic mechanisms. These include increasing the activity of enzymes involved in substrate level ATP production from fermentation and glycolysis, increasing the availability of soluble sugars, downregulation of metabolic processes to conserve carbohydrate reserves and increasing the production of antioxidants to neutralise oxidative stress (Colmer & Voesenek 2009; Sairam *et al*. 2008). Since the levels of ATP production under fermentation are much lower, anaerobic respiration can only be sustained so long as there are available carbohydrate reserves. Cell death occurs when there is insufficient ATP to regulate cellular pH (Roberts *et al*. 1984). Thus avoidance of severe energy crisis appears to be related to the capacity of the plant for downregulation of metabolic processes to slow rates of ATP consumption (Gibbs & Greenway 2003; Greenway & Gibbs 2003). Plant survival to oxygen deprivation also depends on their capacity to cope with oxidative stress from the transitions from oxic to anoxic conditions and to the post-anoxic phase (Blokhina *et al*. 2003; Licausi 2011).

6.3.2 Anaerobic respiration and anoxia tolerance

In the absence of oxygen, plant cells show up-regulation of gene expression for induction of enzymes, particularly alcohol dehydrogenase (ADH) and lactic dehydrogenase (LDH) associated with anaerobic respiration for a solely substrate level (instead of oxidative) phosphorylation based generation of ATP by glycolysis and ethanol fermentation. The shift in metabolic processes from aerobic respiration to anaerobic metabolism using ethanolic fermentation as an alternative energy generating pathway that does not require oxygen as a terminal electron acceptor is one of the best characterised responses to oxygen deficiency (Drew *et al*. 1994; Drew 1997). The fermentative pathway involves the carboxylation of pyruvate to acetaldehyde and the subsequent reduction of acetaldehyde to ethanol when catalysed by ADH or lactate by the activity of LDH. However, this metabolic pathway is thermodynamically a less efficient energy generating process involving only the partial break down of glucose and the production of ethanol and carbon dioxide instead of complete oxidation to carbon dioxide and water producing only 2 moles of ATP compared 36 under oxidative phosphorylation. To compensate for the energy deficit glycolysis is accelerated leading to a depletion of stored carbohydrates, the Pasteur Effect. The reduction in availability of ATP leaves less energy for cellular maintenance, transport of ions and cell activities such as cell extension, cell division and nutrient absorption in the root tip decreases or ceases altogether leading to cell and eventual whole plant mortality. There is evidence indicating that variations between species or particular genotypes in their capacity to supply sufficient root sugar levels is likely to be determinant of the degree of tolerance at least in short-term flooding (Gibbs & Greenway 2003).

In poorly adapted plants mortality may be caused by cellular dysfunction resulting from the reduction in ATP which impairs the functioning of vacuolar proton pumps, to regulate pH by removal of protons from the cytoplasm resulting in an increase in acidity, cytoplasmic acidosis, and rapid cell death (Roberts *et al*. 1984). Better adapted species appear to be able to counteract the decrease in pH to a certain extent with the formation of basic amino acids e.g. GABA (Crawford *et al*. 1994; Ratcliffe 1995). The decrease in pH can also function as a signalling mechanism to stimulate a rapid switch to ATP generation using the ethanolic fermentation pathway that also has a regulatory function in maintaining cytoplasmic pH to near neutrality (Roberts *et al*. 1989; Felle 2010).

The plant's response to the limited capacity for fermentation to supply all the energy required to support tolerance to prolonged exposure to oxygen deprivation has been described as active or passive with regard to carbohydrate consumption (Gibbs & Greenway 2003; Greenway & Gibbs 2003). One adaptive strategy involves the rapid consumption of carbohydrate reserves through accelerated anaerobic carbohydrate catabolism in order to fuel developmental changes such as adventitious root growth or aerenchyma formation. The alternative strategy entails the downregulation of energy consumption by entering a relatively quiescent metabolic state by economical use of stored carbohydrates not only to delay the onset of anoxia but also to avoid cellular damage or mortality from the accumulation of toxic metabolites that result from ethanolic fermentation.

6.3.3 Free radicals and post anoxic injury

Reactive oxygen species (ROS), including the singlet oxygen and superoxide radical, hydrogen peroxide and the hydroxyl radical and reactive nitrogen species (RNS) are produced as a normal part of cellular functioning as signal transduction agents that perform important regulatory roles in maintaining cellular homeostasis in response to various biotic and abiotic stresses including oxygen deprivation (Lesser 2006). The activities of these reactive species are normally moderated by the detoxifying effect of various antioxidants (e.g. superoxide dismutase, ascorbate peroxidase, catalase) but many biotic and abiotic stress situations including anoxia can result in excessive ROS formation to unmanageable levels stage (Blokhina *et al*. 2001, 2003; Fukao & Bailey-Serres 2004).

Oxidative stress occurs when waterlogging subsides and tissues are re-exposed to oxygen after prolonged anoxia resulting in the oxidation of accumulated ethanol to form acetaldehyde which is used as a substrate by several enzymes to produce ROS (Blokhina *et al*. 2003). The resulting accumulation of free radicals can cause harmful oxidation of various cellular macromolecules including protein denaturation and lipid peroxidation resulting in the loss of membrane integrity, destruction of cell organelles and ultimately cellular death (Blokhina *et al*. 1999, 2003). Another possible pathway leading to post anoxic injury may result from ethanol accumulation under anoxia. This occurs from the reaction of ethanol with hydrogen peroxide formed by ROS catalysis by the antioxidant superoxide dismutase (SOD) to produce acetaldehyde which itself is highly injurious to cell membranes (Elstner & Osswald 1994). Species that are better adapted to anoxia are likely to avoid excessive ethanol accumulation by diffusive loss through roots and rhizomes or through the shoots via aerenchyma (Studer & Braendle 1987).

The extent to which antioxidants and other ROS scavenging enzymes are able to maintain appropriate levels for ROS modulated regulatory processes to operate while preventing deleterious levels of ROS formation under conditions of oxygen deprivation may be a determining factor in plant tolerance to anoxia. The few published studies of antioxidant responses in anoxic or hypoxic conditions have demonstrated the potential of this metabolic adaptation to confer tolerance in both wetland and non wetland species. Sairam *et al*. (2008) have reviewed the significance of a variety of antioxidants in waterlogging tolerance in crop plants. For example, large post anoxic increases in superoxide dismutase were recorded in *Iris pseudacorus* (Monk *et al*. 1989) during flooding and in the post anoxic phase in *Glycine max* (Van

Toai & Bolles 1991) suggesting increased SOD activity may contribute to anoxia tolerance by increasing the capacity for neutralising superoxide.

In the only published study of antioxidant response to anoxia in a salt marsh species, Alhdad *et al*. (2013), recorded elevated glutathione and polyphenol concentrations in *Suaeda maritima* in experimental waterlogging treatments compared to drained controls. Further investigations of this response in other salt marsh species are needed to confirm if enhanced production of antioxidant molecules is a widespread response and plays an important role in plant tolerance to anoxia stress.

More recently, reactive nitrogen species (RNS), particularly the free radical NO, have emerged as key signal molecules in a range of important physiological and developmental processes in plants (Sairam *et al*. 2008; Hasanuzzaman *et al*. 2013). In a similar manner to ROS, abiotic stresses including oxygen deprivation can induce excessive production of NO both from hypoxia induced enzymes and from the oxidation of exogenously acquired nitrite from the reducing soil environment which can have a deleterious effect on several biochemical processes and in particular has an inhibitory effect in an range of enzymes involved in energy production pathways (Drew 1997; Wink & Mitchell 1998). In addition, NO can react with oxygen or superoxide and the resulting end products NO_2, N_2O_2 and peroxonitrite all have deleterious post-anoxia consequences for biological systems (Wink & Mitchell 1998).

In addition to increasing the antioxidant capacity to neutralise oxidative stress from excessive accumulation of ROS and RNS, anoxia tolerant plants may act as important signalling agents in plants. They are thought to induce metabolic adjustments as adaptive strategies that function to decrease the respiratory capacity in order to avoid complete internal oxygen deprivation and so postpone the onset of ethanolic fermentation and the consequential risks of oxidative stress (Bailey-Serres 2008; Blokhina & Fagerstedt 2010; Igamberdiev & Hill 2009).

6.3.4 Internal transport of oxygen

Longer term survival in anaerobic and reduced sediments with high oxygen demands and high concentrations of phytotoxins therefore presents a particular set of physiological challenges for vascular plants. This usually necessitates the development of specialised anatomical, morphological and adaptations that enables sufficiently high capacity for internal gas transport to support aerobic respiration in roots while, in some species, simultaneously maintaining high fluxes to the rhizosphere (Armstrong 1979; Armstrong *et al*. 1994; Armstrong & Drew 2002; Blom 1999; Vartapetian & Jackson 1997).

The differing abilities of plants to internally transport oxygen and other gases from shoot to roots in anaerobic soils has long been assumed to be a mechanism that determines species zonation or distributions in saltmarshes and other wetland environments (Gleason & Zieman 1981; Maricle & Lee 2007; Laan *et al*. 1989a; Pennings *et al*. 2005; Sorrell *et al*. 2000).

Although roots of all plants contain some intercellular air spaces that serve an important function in unsaturated soils, more efficient transport of gas is made possible through enhanced porosity (per cent volume of inter-cellular air spaces) mainly in the cortical tissues of roots, rhizomes and stems and by the formation of aerenchymatous tissue comprised of enlarged intercellular air spaces. These air spaces (lacunae) can occupy up to 60% of the volume in stem and root cortical tissues of wetland plants (Armstrong 1979) and provide a low resistance internal pathway to facilitate diffusive gas exchange of oxygen from the shoot to distal root regions and the venting of CO_2 and potentially phytotoxic compounds such as ethanol and ethylene in the reverse direction. Internal transport of oxygen is mainly by diffusion but can also occur by convective mass transfer in shoots and extended rhizomes of some species when longer distances have to be traversed (Colmer 2003). The development of extensive aerenchyma also has an additional adaptive function by lowering the metabolic demand for oxygen by decreasing the volume of respiring tissue (Jackson & Armstrong 1999).

Soil anoxia results in the consumption of internally transported oxygen by tissues along the diffusion path in aerenchymatous roots which may decrease the concentration of oxygen available at the root apex, eventually restricting the final length the root attains (Armstrong 1979; Armstrong *et al*. 1991). A number of studies have shown species with higher root porosity tend to form deeper roots and are more tolerant to anoxia (Justin & Armstrong 1987; Laan *et al*. 1989a). Plant roots display considerable variation in morphology with different lengths, thickness and degrees of branching which is likely to be influenced by the interactions between the total oxygen transport of the entire and the external oxygen demand. This interrelationship has been described mathematically where the extent of root penetration in anaerobic media is found to be related to the capacity for internal oxygen diffusion to the root tip which is determined by the fractional root porosity, radial loss of oxygen to the rhizosphere and the oxygen demand in the sub-apical region of the root (Armstrong & Beckett 1987; Armstrong *et al*. 1991).

A consequence of root surface permeability that enables water and nutrient uptake is the loss of some oxygen to the reducing soil environment even among plants highly specialised to thrive in waterlogged soils (Armstrong & Beckett 1987). A degree of radial oxygen loss (ROL) may also be an adaptive advantage in reducing sediments by oxidation of the reduced chemical products of anaerobic microbial respiration and preventing their accumulation in the rhizosphere to phytotoxic concentrations (Lee *et al*. 1999; Mendelssohn & Postek 1982) and facilitating nutrient uptake (Bradley & Morris 1990; Lai *et al*. 2012).

In addition, sediments with low redox potentials act as a competitive and potentially greater sink for the internally transported oxygen compared to sediments that are just anaerobic. This has been confirmed experimentally in several studies that have measured ROL under controlled low redox intensities (*Cyperus involucratus* & *Eleocharis sphacelata*: Sorrell *et al*. 1993; Kludze *et al*. 1994; *Juncus effusus* and *Juncus inflexus* Sorrell 1999; *Typha domingensis* and *Cladium jamaicense*: Chabbi *et al*. 2000; *Myriophyllum spicatum* and *Potamogeton crispus*: Laskov *et al*. 2006; *Salix nigra*: Li *et al*. 2006). Sorrell (1999) reported ROL in *Juncus effusus* and *J. inflexus* was controlled by external oxygen demand at low to moderate reducing intensities created by titanium citrate but remained constant over a wide range of higher external oxygen demand suggesting structural constraints on oxygen diffusion rates prevented increased ROL. A similar result was reported for *Spartina patens* and *Oryza sativa* where, notably, the response to redox capacity was much larger than the response to redox intensity (Kludze & DeLaune 1995).

The limit to ROL is also described in theoretical models of root aeration (Armstrong *et al.* 1991) where ROL increases with increasing external oxygen demand from low redox potentials to a point determined by a given root geometry after which it remains constant due to the internal resistance properties inherent to roots. However, the leakage of too much oxygen along the sub-apical portions of the root may lead to a lethal deficit at the growing apical meristem tissues of the root tip where oxygen demand is likely to be highest. Many better adapted wetland plants demonstrate a capacity to enhance longitudinal diffusion of oxygen to the root apices by the development of a barrier in the form of a suberized exodermis in sub-apical parts of the root to restrict permeability to oxygen leakage into the soil environment and may also function to restrict uptake of phytotoxins in some species (Armstrong & Armstrong 2005; Laan *et al.* 1989b).

Among saltmarsh plants, oxygen transport has been best characterised for *Spartina* spp. Teal and Kanwisher (1966) first suggested that the capacity for internal transport of oxygen in *Spartina alterniflora* might exceed respiratory demand, resulting in leakage across the root surface and oxygenation of the surrounding sediment. Later work indicated that internal transport of oxygen in *S. alterniflora* could be enhanced by the existence of pressurised flow created water vapour gradient across the leaf mesophyll layer sufficient to generate hygrometric pressurisation of the internal gas spaces to facilitate rhizosphere oxidation (Hwang & Morris 1991). Indirect evidence for rhizosphere oxidation also comes from descriptions of the formation of ferric iron plaque precipitates on the root surface (Mendelssohn & Postek 1982). However, this was contradicted by subsequent attempts to quantify ROL that found saturated soils acted as a diffusive barrier to oxygen leakage in *S. alterniflora* and was unable to significantly oxidise surrounding sediments (Howes & Teal 1994). In contrast, the roots of *S. anglica* revealed significant leakage (Lee 2003) and this was also reported by Maricle and Lee (2002) who found substantial differences in the capacity for internal oxygen transport capacity between *S. anglica* and *S. alterniflora* despite both species exhibiting a similar degree of well-developed aerenchyma.

Moreover, the ability of *S. anglica* to colonise bare mudflats and to outcompete native species in the low marsh has been attributed to its high capacity for internal oxygen transport (Maricle & Lee 2007). The extent to which ROL from *S. anglica* is able to alter the oxygen status of the sediment may, however, depend on the severity of the reducing conditions in the sediment. Measurements of oxygen release into sediments by *S. anglica* by in situ microelectrode profiling showed a distinct 2.5mm oxic zone around roots of *S. anglica* (Holmer *et al*. 2002). More recently, planar optode technology has been used for two-dimensional imaging of the oxygen distribution in sediments and has provided a much higher level of detail of spatial distribution and temporal dynamics (Blossfield 2013; Blossfield & Gansert 2012). This technique showed a smaller oxic zone of ~1.5mm which extended 16mm from the root tip and disappeared during periods of darkness and submergence of the aboveground biomass (Koop-Jakobsen & Wenzhöfer 2014).

6.3.5 Aerenchyma development and anoxia avoidance

Well developed aerenchyma is a feature of many wetland plants but is also in some flood intolerant species (Vartapetian & Jackson 1997; Evans 2004). Aerenchyma can either be formed constitutively or induced as a result of hypoxia from waterlogging. Two mechanisms are thought to be responsible for the formation of aerenchyma in roots and shoots: schizogenous which develops by cell separation without cell death and lysigenous that is formed by cell autolysis to create intercellular spaces (Jackson & Armstrong 1999; Evans 2004).

Lysigenous aerenchyma results from the collapse and lysis of cortical cells by programmed cell death, while schizogenous aerenchyma is formed by separation of the cortical cells and the enlargement of existing intercellular spaces through cell division and differential cell enlargement. Although both forms occur in wetland plants, lysigeny is more frequently reported (Smirnoff & Crawford 1987) and is better understood since it occurs in a number of crop species where much of the research has concentrated. Among wetland plants shizogeny has been reported in *Caltha palustris*, *Filipendula ulmaria* (Armstrong 1979; Smirnoff & Crawford 1987) and *Rumex maritimus* (Laan *et al*. 1989). However, distinguishing between the types can be problematic. Seago *et al*. (2005) notes that while lysigeny is often depicted in descriptions of Cyperaceae and *Oryza*, a closer inspection shows that schizogeny always precedes or accompanies lysigenous space formation. Moreover, Longstreth & Borkhsenious (2000) hypothesised that lysigenous aerenchyma in some plants may comprise of collapsed rather than dead cells.

Formation of aerenchyma can either be constitutively in some species while in others it is induced under hypoxic/anoxic conditions. A number of studies have highlighted the role of the gaseous plant hormone, ethylene in inducible aerenchyma formation (Jackson & Armstrong 1999; Evans 2003; Kawase 1976, 1978). This has been confirmed when a chemical inhibitor is used to stop ethylene production, aerenchyma formation also ceases (Drew *et al*. 2000). Low oxygen levels stimulate the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) synthase which is involved in the synthesis of the plant hormone ethylene in aerated parts (Jackson 1994). Ethylene normally diffuses away from plants but this is inhibited in waterlogged conditions (Kawase 1976). The resulting ethylene accumulation leads to cell rupture, cell wall degeneration and an increase in the activity of compounds that degrade cell walls (Vartapetian & Jackson 1997).

Although many wetland plant roots contain large volumes of aerenchyma with porosities up to 55% there can be considerable variation in volume that is generally related to functional type (Smirnoff & Crawford 1983; Justin & Armstrong 1987; Schussler & Longstreth 1996). Moreover, aerenchyma formation is readily induced in many non wetland species in response to waterlogging stress and other abiotic stresses such as drought and nutrient deficiency (Smirnoff & Crawford 1983; Justin & Armstrong 1987).

Smirnoff & Crawford (1983) reported that although waterlogging tolerant plants generally increased root porosity there were exceptions. The flood intolerant species *Senecio jacobaea* produced aerenchyma but did not survive long term flooding. *Filipendula ulmaria* although normally inhabiting waterlogged soils did not produce extensive aerenchyma even when flooded. *Eriophorum angustifolium* and *E. vaginatum* produced extensive aerenchyma under drained conditions that did not further increase on flooding, while in *Nardus stricta* root porosity was increased by low nutrient levels as well as by flooding. It was also found that several flood tolerant species formed aerenchyma at the onset of waterlogging and there was an inverse relationship between porosity and soil redox potential (Smirnoff and Crawford 1983). Plants from fens, bogs and reed swamps varied in porosity from 1.2% - 33.6% after 11 weeks of waterlogging and this increased to 50% in *Eriophorum angustifolium* and

E. vaginatum after 32 weeks. As soil water content increased from 70% to 90%, porosity of *Senecio aquaticus* increased from 10-35%. Other experimental work has found similar relationships with higher volumes of aerenchyma reported from increased soil saturation in *Salicornia virginica* (Seliskar 1987); and with decreasing gradients in redox potential both in the field: *Zostera marina* (Penhale & Wetzl 1983) and experimentally: *Oryza sativa* (Kludze *et al*. 1993), *Spartina patens* (Burdick & Mendelssohn 1990; Kludze & DeLaune 1994), *Cladium jamaicense* and *Typha domigensis* (Kludze & DeLaune 1996).

6.4 Experimental design

To investigate the effects of strong oxygen demand on plant physiology a small number of studies have successfully used titanium(III) citrate (TiCi) as non-toxic, artificial reducing agent in hydroponic systems in order to mimic the low redox potentials that occur naturally in waterlogged soils (Brix & Sorrell 1996 ; Chen 2005; 2008; DeLaune *et al*. 1990; Kludze & DeLaune 1996; Lissner *et al*. 2003a,b; Mendelssohn *et al*. 1981; Sorrell 1999; Sorrell & Armstrong 1994; Wiessner *et al*. 2002). This method represents a significant improvement on experimental soil waterlogging treatments since it allows for predetermined low redox levels to be consistently maintained and minimises the possibility of introducing confounding influences from the accumulation of phytotoxins. It also has the advantage over the method of nitrogen deoxygenated hydroponic culturing because it enables the low and negative redox potentials and the associated conditions of high oxygen demand that are commonly encountered in saltmarsh sediments (Armstrong *et al*. 1985).

The potential of TiCi as a potent but non-toxic reducing agent for culturing obligate anaerobic microbes was first identified by Zehnder & Wuhrmann (1976). Early attempts at using TiCi in plant physiology studies were achieved by manually adding the buffer to the culture solution to investigate the photosynthetic activity in *Spartina patens* (DeLaune *et al*. 1990). A major limitation of this method is a consequence of the sensitivity of the redox potential to small changes in the concentration of TiCi. This means a change in Eh of 50mV results in response to each order of magnitude of change within the concentration range of 10^{-3} to 10^{-7} M TiCi (Zehnder & Wuhrmann 1976). In practical terms, to maintain the redox value within the required range for the experiment would require hourly monitoring and regular additions of TiCi (~15 mins – 6 hours) depending on redox buffering capacity of the hydroponic nutrient solution. While this manual addition method is manageable for short duration experiments it is impractical for long term studies especially where many replicates are involved.

To address the limitation, Lissner *et al*. (2003a) devised an automated system for long-term hydroponic cultivation under controlled redox intensities. This method employs the use of an oxidation-reduction potential (ORP) controller programmed to control the release appropriate volumes of TiCi or compressed air by high and low set point relay switches to maintain a constant pre-determined Eh level.

The current study used a modified version of this basic set-up designed as a larger scale system for simultaneously subjecting twelve plant samples to the same treatment in one tank instead of a single sample necessitating multiple replicates for any one study. A significant problem with the original system came from the difficulty in preventing oxygen contamination in the TiCi reservoir container resulting in increased frequency of dosing events over time as a consequence of the gradual decline in potency from oxidation of the TiCi solution. This was attributed to the gravity fed operation for dosing which required a minute aperture to be made in the top of the reservoir for pressure equalisation that was sufficient to make contamination by oxygen unavoidable thus making it difficult to maintain the intended levels of redox potential level without regular renewals of the TiCi solution necessary every few days. This study presents an improved design for the dosing system that entails no appreciable loss of potency resulting in a much improved level of consistency in maintaining the pre-defined levels of redox potentials over extended periods of time.

The following measurements were undertaken:

1. Weekly above ground growth rates
2. Above and below ground biomass at the end of the experiment.
3. Maximum length of leaves and roots and the end of experiment
4. Root porosity
5. ADH activity (*Triglochin*)
6. Photosynthetic performance based on chlorophyll fluorescence

6.5 Methods

6.5.1 An automated system for hydroponic culture and controlled redox intensities

The completed set up for the experiments are shown in Figures 6.1 & 6.2. For culturing plants hydroponically five 10 L capacity tanks were constructed of dimensions 380 x 220 x 150 mm from 8 mm thick low gas permeable PET sheeting to minimise contamination by oxygen diffusion. A 5 mm PET sheet for the tank lid was cut to size with 12 x ø 23 mm holes drilled into which a 40 mm section of PET tubing (internal ø 20 mm) was welded leaving 10mm to protrude above the surface. The top end of each tube was internally fitted with a 10mm wide collar of 1.5 mm thickness. These tubes were used to support a plant with the base of the stem supported by a sponge neoprene disc of 20 mm diameter and 10 mm thickness. The plants were inserted from the bottom of the tubes and firmly pushed to the base of the collar while still allowing sufficient flexibility in the neoprene sponge to accommodate expansion from growth of the shoot during the course of the experiment. Additional holes were cut for a small vent, an inlet for tubing for nitrogen gas, a calomel reference electrode (Sentek, UK), an inlet for the TiCi delivery tube, a spare inlet used for checking pH and 3 holes for triplicate platinum-tipped measuring electrodes. All tubing and electrodes were held in place by cable glands fitted into holes for a gas tight seal. The tank lid was fitted with a sponge neoprene gasket and clamped to the tank with aluminium frames to the top and bottom connected with threaded rods. The tanks were shrouded black card to eliminate light.

A redox controller (alpha-pH200, Eutech Instruments, USA) was connected to one of the three measuring electrodes and a reference electrode for continuous measurement of the redox potential of the hydroponic culture solution. The controllers were programmed for three different redox treatments (mild +150 mV; moderate 0 mV; severe -150 mV). Since the tendency was for the redox value to gradually increase over time as the TiCi in the culturing solution became oxidised, this was kept in check when the pre-set redox value exceeded a set point and would trigger a relay to turn on a digital timer (B2E, Sestos, UK) connected via a further relay switch to activate a normally-closed miniature solenoid valve (model: 134094, Burkert, UK). The timer was programmed to open the solenoid for a one second interval injecting ~0.1 ml TiCi into the nutrient solution followed by a 5 minute pause to allow sufficient time for mixing by the current generated by the flow of nitrogen gas purging the solution. The dosing is then repeated until the set-point redox value was reached after which the solenoid was turned off until the next relay event is triggered. A 5 mV hysteresis margin was programmed into the controller to avoid unintended "chatter" (rapid on/off actuations) that tends to occur as the redox value in the culturing solution approaches the pre-set level. Of the two remaining tanks, one was subjected to nitrogen purging without addition of TiCi and another was used as control oxygenated using compressed air from an aquarium pump.

Instead of using a bottle reservoir for gravity feeding, 50 ml TiCi was contained in a 100 ml gas-tight syringe (100MR-LL-GT, SGE, Australia) with the plunger attached to a custom made spring-loaded piston to activate a constant flow under low pressure. A blunt 17-gauge dispensing needle was attached to the in-port of the solenoid valve using a 1/8" compression fitting. Another compression fitting was attached to the out-port of the solenoid which to hold a 300 mm long 1/16" stainless steel capillary tube (i.d. Ø 0.010") which served as a feeder tube for delivery of the TiCi. Once the solenoid valve was in place with the delivery tube inserted into the hydroponic tank the luer fitting of the needle was tightly connected via a luer lock connector of the syringe to ensure a gas tight seal. The use of a gas tight syringe as a TiCi reservoir proved to be very effective in preventing oxygen contamination since the potency of the TiCi was not significantly reduced for at least 10 days at which time most of the 50 ml volume was consumed.

The measuring electrodes were of the welded type construction (Mueller *et al.* 1985) and calibrated using quinhydrone redox buffers at pH 4 and pH 7. These were deemed to be acceptable if they were within the range of ±10 mV. Daily readings from the two unused electrodes were compared with the electrode connected to the controller for indications of fouling. Any excessive deviations between measured readings were usually corrected by light polishing with a fibreglass abrasive cleaning pen.

The hydroponic nutrient solution was based on a 20% modified Hoagland's nutrient formulation (Epstein 1972) with salinity adjusted using laboratory grade NaCl to 12‰ to create a salinity concentration ~1/3 seawater. Oxygen-free nitrogen gas was distributed to the tanks at a flow rate of approximately 300 ml min^{-1} using 6 mm PVC tubing terminating with an air stone. The nutrient solution was completely drained and renewed at fortnightly intervals and water levels were monitored daily. Deoxygenated deionised water was added every few days to replace water lost through transpiration. The pH levels tended to increase with additions of TiCi while the treatments without TiCi tended to decrease over time. In order to maintain a target pH range of between pH 5.5 and pH 6.2 daily adjustments by addition of diluted HCl or NaOH solutions were necessary. In order to control for any possibility of adverse effects from the accumulation of oxidised TiCi, all tanks received additions of oxidised TiCi at an equivalent volume to that accumulated in the tank of the severe (-150 mV) treatment.

Each gas tight syringe was filled with 50 ml TiCi solution prepared according to the protocol described by Zehnder & Wuhrmann (1976): 5 ml of 15% titanium (II) chloride in ~10% HCl (Merck) was added to 50 ml of 0.2 M sodium citrate solution made using deionised deoxygenated water and neutralised with sodium carbonate under a N_2 atmosphere in an inflatable glove bag (Atmosbag – Sigma Aldrich).

Seeds were randomly collected from different areas of the Stiffkey general salt marsh community and stored refrigerated at 5 °C for up to 6 month before use. Seeds were surface sterilised with 5% hypochloride for 30 minutes followed by rinsing with RO water. Germination was undertaken in petri dishes using filter paper moistened with RO water and placed in a plant growth cabinet (PL2, LEEC, Nottingham, UK) at 20 °C and 12/12 hr light/dark illumination cycle. Plants were germinated from seeds collected from the Stiffkey general salt marsh community and propagated hydroponically for six weeks initially with 10% Hoagland's nutrient solution increased to 20% after three weeks. After this time maximum plant leaf and root lengths reached ~80 mm and roots ~100 mm respectively and 12 plants were transferred to each experimental hydroponic tank.

The tanks were placed under T5 fluorescent lighting (~650 μmol s^{-1} m^{2}) measured at canopy height under ambient laboratory conditions of (22-24 °C / 40-50% RH). A 12/12 hr cycle light/dark illumination cycle was used for the duration of the experiment over a period of 10 for weeks for *Triglochin* and 4 weeks for *Atriplex*.

Plants were acclimated in the hydroponic tanks for 10 days before commencement of treatments.

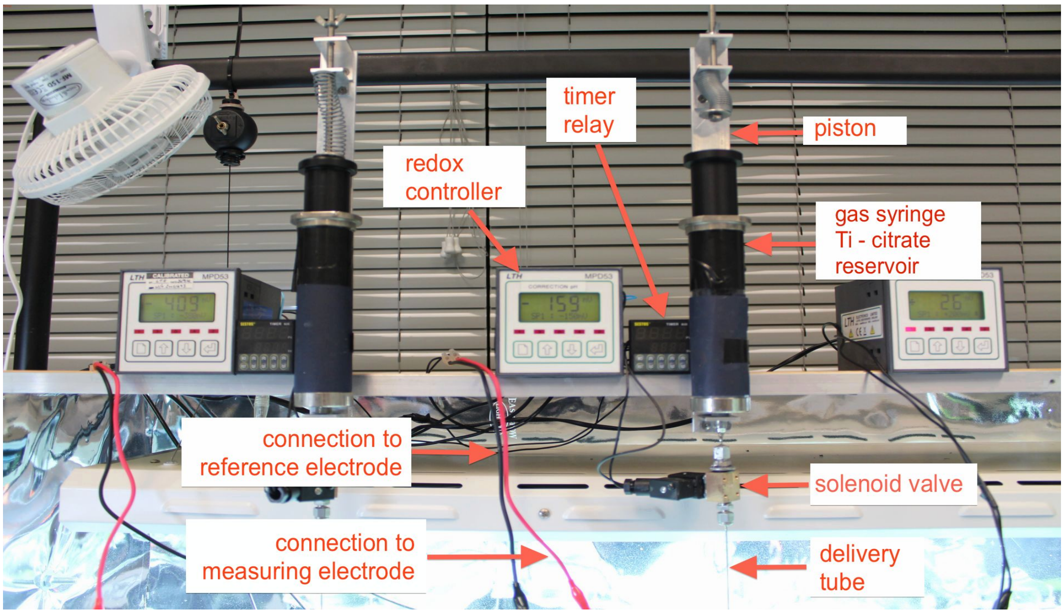


Figure 5.1. The arrangement of equipment for delivery of titanium (III) citrate for controlled redox intensities.

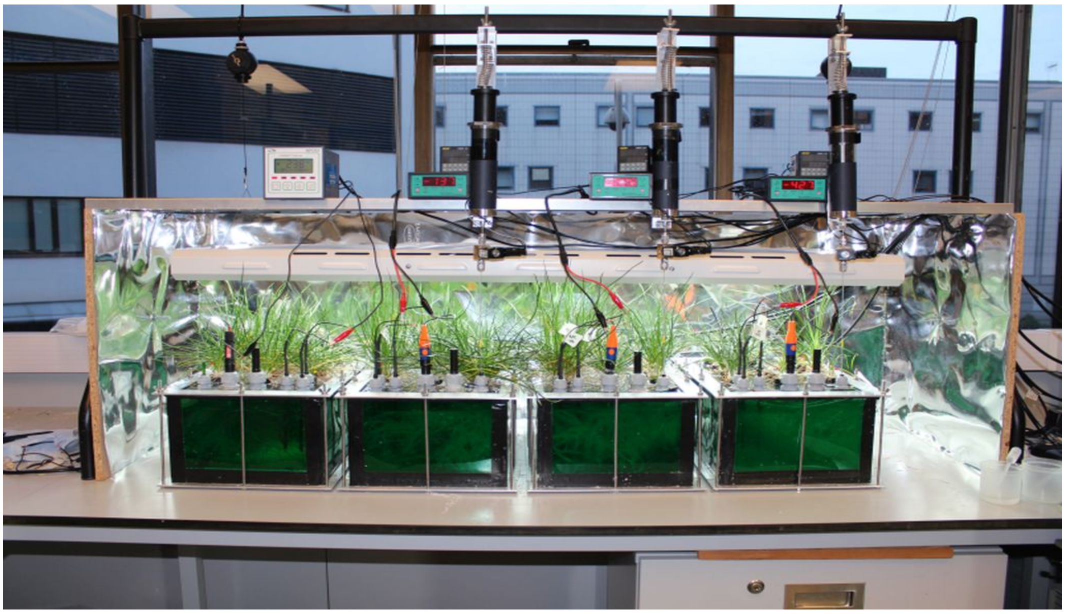


Figure 7. The experimental set up showing the arrangement of anaerobic hydroponic tanks in relation to the system for redox control. From left-right: N2 deoxygenated, +150 mV, 0 mV, **-**150 mV. The control tank (not shown) has an identical lighting arrangement. Photograph taken of *Triglochin maritima* at the end of 10 weeks of anaerobic treatment**.**

6.5.2 Weekly growth rates

Growth rates of *Triglochin* were measured by mean weekly leaf elongation (mm), calculated by selecting the initial ten largest plants in each treatment and the mean change in length of three tagged mid-aged leaves from each plant was recorded at the end of the week. These values were averaged to give the overall mean growth rate for each treatment.

Rates of growth for *Atriplex* were measured as the mean weekly increase in branch length of three longest branches from each of the ten largest plants in each treatment. The mean of these values were taken to give the overall mean growth rate for each treatment.

6.5.3 Root porosity

The method follows the vacuum infiltration procedure described in Visser & Bögemann (2003). This is a simple procedure that enables the calculation of the volume of porosity from measurements of the difference in weight between a root sample before and after it has been subject to water infiltration under near-vacuum conditions. With efficient vacuum infiltration it is assumed that the negative pressure difference causes air contained inside the intercellular spaces of the root sample to be displaced by water and from the weight of the water the volume of the root air spaces can be calculated. Since porosity tends to increase with the distance from the root apex, samples were taken from two locations approximately 20 mm from the apex and 100 mm behind the apex. Segments of 20 mm for each location were taken from 10 plants in each treatment and blotted dry for a few seconds by carefully rolling the sample on tissue paper with a small brush before transferring it to a previously weighed hard gelatin capsule in order to prevent weight loss by evaporation. The weight of the sample contained in the capsule was determined (w1 in mg) using a microbalance before the groups of 5 samples were individually transferred to 10ml beakers half filled with tap water and placed in a small acrylic degassing chamber connected to a small rotary vane vacuum pump. The chamber was sealed and the submerged samples were then subject to a near vacuum pressure of 5 kPa for 5 minutes to induce water infiltration and the removal of air bubbles. Samples were then transferred into a capsule of predetermined weight and reweighed in order to determine the weight of the water-infiltrated sample (w2 in mg). Porosity was calculated according to:

$$Porosity = 100\,.\,(\,w2 - w1)\,.\,\frac{SW}{w2}\ (\%; v\!:\!v)$$

This calculation for porosity requires an estimate for specific weight (SW) of water-infiltrated tissue that was assumed to be 1.036 g ml^{-1}. This figure was based on the finding that this is the average SW of a large number of measurements using the pycnometer method and the differences between various root types and species is small so an average SW provides a good approximation of calculated porosities (Visser & Bögemann 2003).

6.5.4 SEM imagery

Samples were taken from 20 mm and 100 mm from the root apex for qualitative examination at low resolution (200x). Slow air-drying of samples in a desiccator for 24 hours without pre-treatment was used. Samples were sputter coated (SC7640, Quorum Technologies Ltd., Lewes, UK) prior to SEM examination JSM 5900 LV (JEOL Ltd. Japan).

6.5.5 ADH activity

ADH activity was determined using an ADH assay test kit (Abcam Ltd, Cambridge) for spectrophotometric determination of ADH activity based on the reduction of NAD+ to NADH in the ADH catalysed oxidation of alcohol to aldehyde using isopropanol instead of ethanol as a substrate.

Approximately 50 mg of root samples was taken from 5 random plants in each treatment, sealed in 2ml Eppendorf microcentrifuge tubes and immediately preserved by snap-freezing in liquid nitrogen followed by storage at -80 °C until ready for analysis. Prior to analysis, samples were prepared for lysis by re-freezing in liquid nitrogen and grinding samples to a fine powder while still contained in the Eppendorf tube using a micropestle.

An NADH standard curve was derived for colormetric determination by absorbance at λ = 450 nm by adding to a 96 well plate 0 (blank), 2, 4, 6, 8 and 10 μL 0 of a stock solution made by diluting 10 μL of a 10 mM NADH stock solution with 90 μL of ADH assay buffer solution and adding ADH buffer to each well to bring the volume up to 50 μL to give 0, 2, 4, 6, 8 and 10 nmol/well calibration standards.

Ground tissue samples were homogenised in 200 μL and samples centrifuged at 33 k x g for 10 minutes to remove insoluble material from which 50 μL was added to a well to which was added 100 μL of a Reaction Mix comprising 82 μL assay buffer, 8 μL developer and 10 μL of 2 M isopropanol. In order to eliminate any background signal from NADH a sample blank was created by omitting the isopropanol. Measurements of absorbance were taken at 5 minute intervals until the readings stabilised while the well plate was incubated at 37 °C in a temperature controlled UV spectrophotometer at λ = 450 nm. ADH activity was standardised against total protein concentration of the tissue using bovine serum albumin (BSA) standard and reagents provided in a protein assay kit (Bio-Rad, UK).

Measurement of ADH activity was undertaken by comparing absorbance of each sample against the standard curve to determine the amount of NADH generated by the ADH assay over the reaction completion time after correcting for background interferences by subtracting the measurement for the blank NADH standard from the standards. ADH activity determined according to:

$$\text{ADH Activity} = \frac{\text{B x Sample Dilution Factor}}{\text{(Reaction Time) x V}}$$

Where B = amount (nmol) of NADH generated over the reaction time and V is the sample volume (ml) added to the well.

6.5.6 Pulse Amplitude Modulation (PAM) chlorophyll a fluorescence

The measurement of photosynthetic response to waterlogging stress in plants has traditionally been studied directly by gas exchange instrumentation that measures O_2 evolution or CO_2 fixation (Pezeshki 2001). More recently, advances in the knowledge of the biophysical processes that underpin photosynthesis have enabled the development of pulse amplitude modulated (PAM) chlorophyll fluorometry as a technique for gaining insights into the health of photosynthetic systems (Baker 2008; Schreiber 2004). Measurements of the intensity and changes in the level chlorophyll fluorescence emitted from plant leaves have been widely established in ecophysiological studies as a reliable method for non-invasive in vivo assessment of the effects of various abiotic stresses on the efficiency of photosynthesis (Guidi & Degl'Innocenti 2012; Lichtenhaler 1996). Moreover, a particular advantage of this technique is that it provides information on the impact of stresses on the functioning of photosystem II (PSII) which is a component of the photosynthetic apparatus that is known to be highly sensitive to changes in the abiotic environment and under unfavourable or stressful conditions the activity of PSII declines more rapidly than many other physiological activities (Baker 2008; Maxwell & Johnson 2000).

Pulse amplitude modulated (PAM) chlorophyll fluorometry measures the maximum level of photochemical efficiency of a leaf under steady state photosynthesis in the presence of ambient light by the application of a rapid pulsing excitation light to induce a corresponding pulsed fluorescence emission which is selectively recorded while ignoring the ambient light. PAM fluorometry uses the saturation pulse light addition technique to measure partitioning of the absorbed excitation energy between its photochemical utilisation to drive electron transport for photosynthesis and its loss through heat dissipation in order to derive a range parameters widely used for the investigation of plant responses to environmental stresses.

Chlorophyll fluorescence was measured in vivo at a temperature of 20 °C ± 3 °C at weekly intervals during the course of the experiment for the final 6 weeks for *Triglochin* and for a 4 week period for *Atriplex* using a PAM chlorophyll fluorometer (PAM 2000, Heinz-Walz GmbH, Effeltrich, Germany). Procedures for measuring chlorophyll fluorescence were based on standard procedures as described in the PAM 2000 operation manual. Measurements were taken on the same tagged fully-formed leaf for Atriplex and for *Triglochin*, mid-sized leaves were selected. The use of the same leaf was not always possible with all *Triglochin* plants due to normal leaf shedding. Measurements were taken from ten plants from each treatment

Dark-adapted fluorescence measurements were taken after 20 minutes on dark acclimation. The minimal fluorescence (Fo) was measured by the modulated measuring light which is sufficiently low (< 0.1 µmol^{-2} s^{-2}) not to induce any significant variable fluorescence. Maximum fluorescence level (Fm) was measured by a 0.8s saturating pulse at 10000 µmol m^{-2} s^{-2}. Light adapted fluorescence parameters Fo' and Fm' at steady state photosynthesis were measured with leaves continuously illuminated with actinic light at intensity actinic at PPFD of 300 µmol m^{-2} s^{-1}. Fm' was determined by applying a series of saturation pulses of 10 000 µmol quanta m^{-2} s^{-1} white light at 20 s intervals over for a period of 5 minutes until a quasi steady state maximum fluorescence emission Fm' was obtained. Measurements of Fo' were made in the presence of far-red light (*ca*. 2 µmol m^{-2} s^{-1} in order to fully oxidise the PSII acceptor side.

Based on the above minimum and maximum fluorescence yields the following dark and light-adapted parameters were calculated:

- Fv/Fm – the maximum yield of PSII photochemistry which gives an indication of stress induced light damage to the photosynthetic apparatus
- ΔF/ F_m' or Y(II) – the effective yield of PSII chemistry which provides an estimate of the electron transport flow in PSII due to stress induced changes to carbon assimilation rates
- Y(NPQ) – regulation non-photochemical quenching provides information on operation of regulatory processes that dissipate excess absorbed light not used in photosynthesis as harmless thermal emission
- Y(NO) – unregulated non photochemical quenching provides an indication of the stress induced damage to the regulated processes of excess energy dissipation.

Fv/Fm – the maximum yield of PSII photochemistry which gives an indication of stress induced light damage to the photosynthetic apparatus. It is a measure on the maximum potential efficiency of PSII photochemistry based on all reaction centres being in an oxidised state. This was achieved by dark adaptation of the leaf sample 15 minutes. Fv/Fm is the most widely used parameter for a wide range of abiotic stress response studies which are indicated by decreases from the average value for healthy, non-stressed leaves of 0.83 (Maxwell & Johnson 2000). Stress induced decreases in Fv/Fm are used in ecophysiological studies as evidence that the photosynthetic efficiency of leaves under ambient light conditions is compromised. This value conveys important information regarding the extent of photoinhibition induced by an environmental stress and so provides an indication of the underlying health of the complex processes operating in PSII that affects photochemistry (Baker 2008; Maxwell & Johnson 2000). Since Fv/Fm provides a measure of maximal fluorescence as a result of all the PSII reaction centres being in an oxidised state from dark adaptation whereas under conditions of actinic light a portion of the reaction centres are in a reduced state the actual or effective maximum fluorescence yield from photochemistry will always be lower. This difference means that Fv/Fm may not be sensitive to all environmental stresses and it is advantageous to undertake further measurements the fluorescence yield under light adapted conditions.

$\Delta F/ F_m'$ or Y(II) – is the effective PSII quantum yield under steady state photosynthesis after light acclimation and provides an estimate of PSII operating efficiency under prevailing actinic light conditions (Genty *et al.* 1989). The effective yield of PSII chemistry provides an estimate of the electron transport flow in PSII due to stress induced changes to carbon assimilation rates. In a similar way that Fv/Fm is a measurement ratio of photosynthetic efficiency, Y(II) provides an indication of the amount of energy used by photochemistry in PSII but under steady state photosynthetic light conditions and is a relative measure of the number of PSII reaction centres actively involved in photosynthesis. The value of Y(II) varies between 0 and 1, e.g. a value of 0.5 means that 50% of the absorbed quanta are converted to chemically fixed energy and 50% is dissipated as heat or fluorescence. For leaves exposed to actinic light, Y(II) is equivalent to the quantum yield of linear electron flux (LEF) through PSII reaction centres. A linear relationship was generally found to exist, under a variety of conditions, between the quantum yield of CO_2 fixation and the effective quantum yield of PSII photochemistry (Baker & Oxborough 2004). However, for C_3 plants under certain stress situations, where CO_2 assimilation is restricted, this linear relationship can become curvilinear instead if a portion of the

LEF is diverted to other processes such as photorespiration or the Mehler reaction (Baker 2007).

While the effective quantum yield Y(II) corresponds to the fraction of absorbed excitation energy that is photochemically converted in PSII, the saturation pulse method also allows for the simultaneous estimation of the remaining fraction 1-Y(II) that constitutes the total quantum yield of all non photochemical (NPQ) loss processes.

Non photochemical quenching (NPQ) is a protective mechanism involved in the regulation of light harvesting necessary to balance the absorption and utilisation of light energy in order to minimise the potential for photo-oxidative damage. Excess absorbed light energy that is not used in photochemistry stimulates an increase in the pH gradient across the thylakoid membrane of the chloroplast that in turn initiates the xanthophyll cycle which is involved in the dissipation of excess excitation energy as harmless thermal energy (Demmig-Adams & Adams 1992).

The NPQ fraction is divided into two distinct components based on the yield induced by regulated non-photochemical quenching Y(NPQ) and the yield from other, non-regulated, energy dissipation Y(NO) the relative amounts of which provide complimentary assays for valuable insights into photosynthetic processes in plants under abiotic stress (Genty *et al*. 1996; Kramer *et al*. 2004).

The quantum yield of light induced non-photochemical quenching Y(NPQ) corresponds to the fraction of energy dissipated in the form of heat via the down regulation of the light harvesting function of PSII and acts as a photo-protective mechanism against damage from excessive light energy (Kramer *et al*. 2004).

A high Y(NPQ) value relative to Y(NO) indicates that not only is the PPD excessive but also that photosynthetic energy fluxes are well regulated since the leaf has maintained the physiological capacity for dissipating excessive excitation energy as harmless heat. High values of Y(NPQ) are indicative of high photoprotective capacity from efficient heat dissipation which prevents the formation of ROS (Anderson *et al*. 1997: Muller *et al*. 2004) .

Y(NO) is based on the quantum yield of non-regulated quenching based on the 'lake model' and reflects the fraction of energy that is passively dissipated in the form of heat and fluorescence emission mainly due to closed PSII reaction centres (Kramer *et al*. 2004).

A high Y(NO) value relative to Y(NPQ) indicates inefficient functioning in both photochemical energy conversion processes and protective regulatory mechanisms. Under this condition the plant in unable to process the incident radiation and is at risk of photo-damage from the absorption of excessive light energy. High values of Y(NO) reflect the inability of the plant to protect itself against excess illumination.

For a given set of environmental conditions optimal regulation is attained at maximal values of Y(II) and a maximal ratio of Y(NPQ):Y(NO) while at saturation light intensity high values of Y(NO) or low values of Y(NPQ) reflect photoprotective reactions are compromised and will lead to photodamage of the PSII apparatus.

6.5.7 Data analysis

Data was checked for normality using the Kolmogorov-Smirnov test and the Mann-Whitney U test for significant differences at the $p < .05$ level (SPSS, IBM, New York). Analysis and charts were undertaken using R (R Core Team 2013).

6.6 Results

6.6.1 Ability of TiCi experimental design to maintain redox conditions

On commencement of the experiment, initial consumption rates for TiCi were highest for the first few days reaching a maximum of over 1.0 ml hr^{-1} for the -150 mV treatment. Also, the pH increased rapidly to pH 7-7.5 requiring larger volumes of HCl to adjust back to the target pH range of pH 5.5-6.2. These initial adjustments were a consequence of the poorly buffered nature of the hydroponic culture solution at the start of the experiment. Subsequent additions of TiCi decreased over the following days (Table 5.1). While pH tended to increase from the target range this was at a slower than the first few days and required less adjustment with HCl. Conversely, the pH of both the N_2 purged treatment and the oxygenated control tended to decrease over time and require daily adjustments with NaOH

Table 5.1. Average rates of TiCi consumption during week 8 showing large diurnal increase

TiCi Treatment	Day-time (ml/h^{-1})	Night-time (ml/h^{-1})
+150mV	0.20	0.08
0mV	0.42	0.15
-150mV	0.67	0.18

The use of TiCi in this experimental design was successful in consistently maintaining three different pre-defined redox intensity values (+150 mV, 0 mV, -150 mV) over an extended period time demonstrating the suitability of this method for long-term physiological studies for hydroponically cultured plants. The Eh readings over a period of 3 days during weeks 8-9 for the different treatments are shown in Figure 5.3. At this stage the roots of *Triglochin* were sufficiently well developed for oxygen release at a rate to significantly increase the frequency TiCi dosing events during the day compared to the nighttime period. The average rates of TiCi consumption during week 8 are shown in Table 5.1 showing daytime rates can be more than three times higher than nighttime rates.

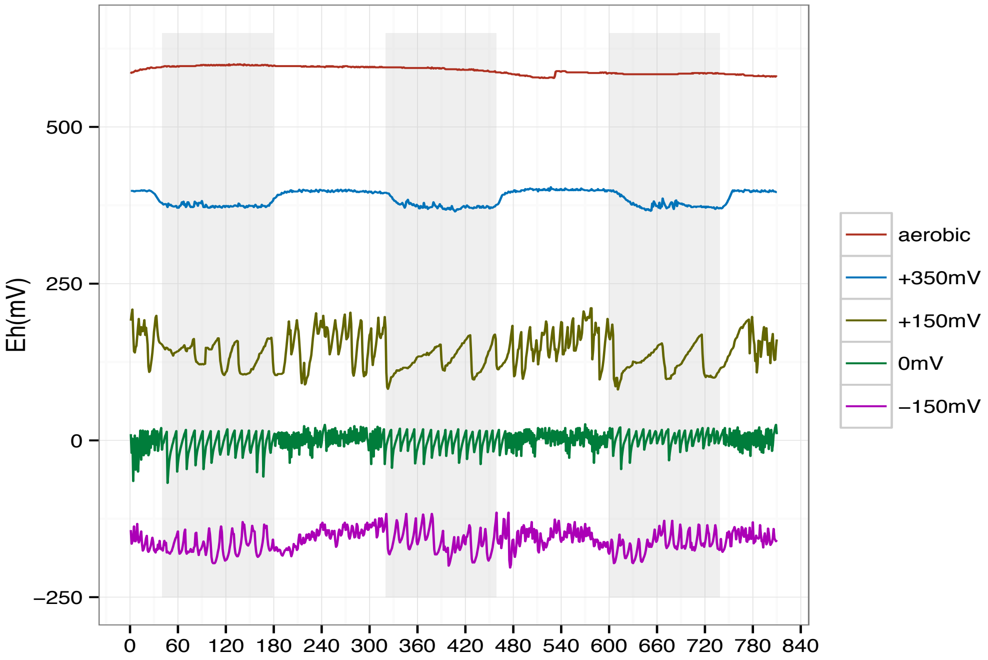


Figure 5.3. Time course of Eh readings recorded at 5 minute intervals over 3 days for three different titanium (III) citrate treatments (-150 mV, 0 mV, +150 mV), deoxygenation by N_2 purging alone (+350 mV) and an oxygenated control (+600 mV). Shaded areas indicate night periods with the slight increase in Eh highlighted during the day period (unshaded area) showing higher TiCi dosing frequency in response to increased photosynthetically enhanced root radial oxygen loss (ROL) during the day – demonstrating the capacity of *Triglochin* for photosynthetic rhizosphere oxygenation.

6.6.2 *Triglochin*

6.6.2.1 Growth rates

In general, the leaf growth rates of *Triglochin* plants in the control and anaerobic treatments were slightly lower in the early weeks of the experiment compared to those in the control (Figure 5.4). Leaf growth rates in the treatment with the highest redox potential (+350 mV) were only significantly different from those in the control in weeks 3 ($p < 0.05$) and 4 ($p < 0.01$). Leaf growth rates in the +150 mV and +0 mV treatments were significantly different from those in the control in weeks 2-5 ($p < 0.05$ and $p < 0.001$ respectively). Growth rates in the most anaerobic treatment (-150 mV) were significantly different from those in the control in weeks 3-5 ($p < 0.01$). However, after week 6 the growth rates of the plants in the anaerobic conditions accelerated until they become statistically indistinguishable from those in the control. There were no significant differences between the anaerobic treatments.

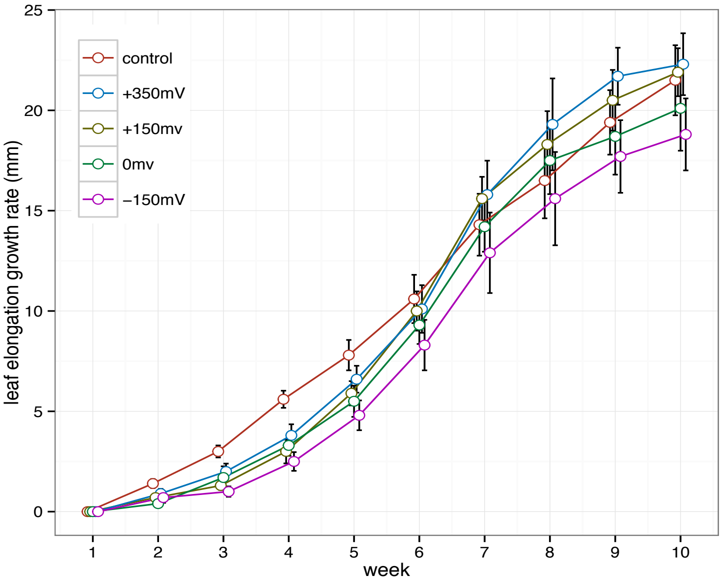


Figure 5.4. Comparison of leaf elongation growth rates for all anaerobic treatments. Significant differences between the aerobic control and all anaerobic treatments were recorded for weeks 2-5 only. No significant differences were recorded between any of the anaerobic treatments.

6.6.2.2 Final leaf and root length

The average maximum leaf and root lengths attained for each treatment are shown in Figure 5.5 and Table 5.2. The mean leaf length was greatest in the N-deoxygenated treatment +350 mV, which was about 10% higher than the mean value for the control, and lowest in the most anaerobic treatment, which was 17% lower than the control. However, there were no significant differences in mean leaf length between the control plants and the anaerobic treatments, or between the different anaerobic treatments.

In contrast, there were significant differences in the mean maximum root lengths between the control plants and the anaerobic plants (all $p < 0.01$), although there were no significant differences between anaerobic treatments.

Table 5.2. Average and maximum leaf and root lengths for *Triglochin* attained after 10 weeks. No significant differences were found for mean leaf lengths between either the aerobic control plants and the anaerobic treatments or between the different reducing treatments. In contrast, a highly significant difference ($p < 0.01$) was recorded between the mean root lengths of the aerobic control and the anaerobic treatments while no differences were found between the different reducing treatments.

	Leaf length mean ± se	Leaf length maximum	Root length mean ± se	Root length maximum	Mean root:shoot
Control	139.7 ±12.2	193	465.1 ±34.0	561	3.26
+350mv	154.4 ±8.5	186	327.6 ±17.2	429	2.12
+150mv	143.9 ±17.2	218	343.5 ±15.0	394	2.38
0mv	134.0 ±15.2	209	340.8 ±21.5	486	2.54
-150mv	115.6 ±12.9	196	291.2 ±22.5	394	2.52

6.6.2.3 Productivity

Measurements of final aboveground and belowground biomass for *Triglochin* are shown in Figure 5.6. There was a high degree of variation within each treatment for both aboveground and belowground biomass (Table 5.3). The above-ground biomass in the most reducing treatment (-150 mV) was most different to the control, with a 32.8% decrease in the mean value and a 31% decrease in the maximum value from the control. However, there were no significant differences in the above-ground biomass between the control and the anaerobic treatments, or between treatments. Interestingly the highest aboveground biomass for an individual plant was recorded for mildly reducing Eh +150 mV treatment at 3.38 g which representing a 24% increase over the control maximum of 2.58 g. Similarly, whilst the mean below ground biomass was generally lower with decreasing redox potential (Table 5.3), there were no significant differences between the control and any treatment

Table 5.3. Mean and maximum (N=10) aboveground and belowground biomass (dry weight g) for *Triglochin*. No significant differences were found in either the above or below ground biomass between the control and any of the anaerobic treatments.

	Aboveground mean ± se	Aboveground maximum	Belowground mean ± se	Belowground maximum	Mean root:shoot
Control	1.25 ±0.24	2.58	0.69 ±0.14	1.43	0.55
+350mv	1.14 ±0.28	2.66	0.56 ±0.10	1.17	0.49
+150mv	1.12 ±0.33	3.38	0.60 ±0.12	1.32	0.53
0mv	1.18 ±0.26	2.95	0.48 ±0.11	1.08	0.40
-150mv	0.84 ±0.49	1.78	0.49 ±0.11	1.12	0.58

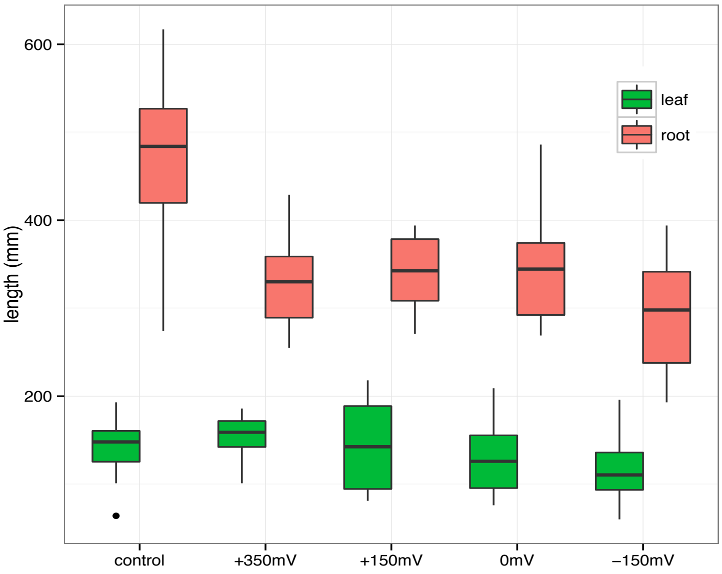


Figure 5.5. The range of values recorded for the maximum leaf and root lengths for *Triglochin* based on the averages values from the three longest leaves and three longest roots from the ten largest plants from each treatment.

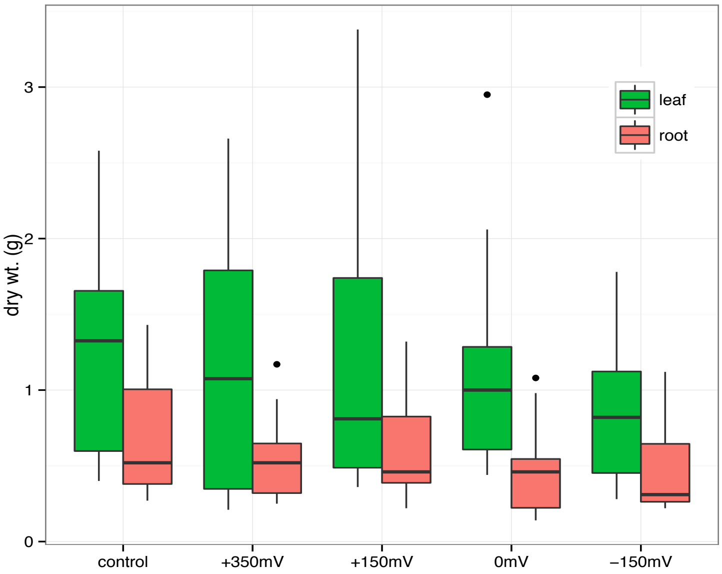


Figure 5.6. The range of values for aboveground and belowground biomass for *Triglochin* based on samples from ten plants from each treatment.

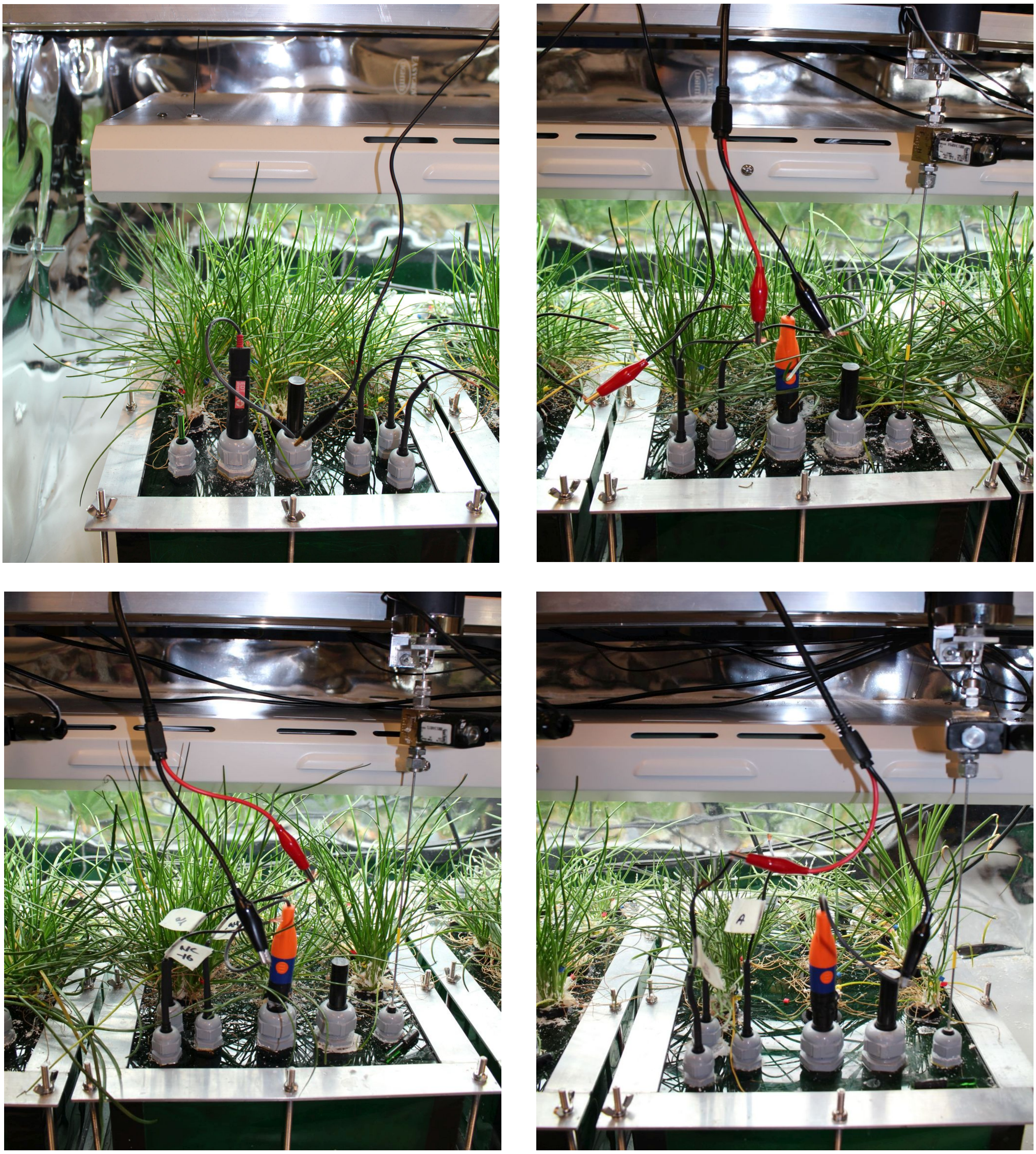


Figure 5.7. Anaerobic treatments at 10 weeks. Top left: N-deoxygenated; top right: mildly reducing +150 mV; bottom left: moderately reducing 0 mV; bottom right: severely reducing -150 mV.

Figure 5.8. The largest individual plants from each treatment based on aboveground biomass from left to right: N_2 deoxygenated, +150 mV, 0 mV, -150 mV.

6.6.2.4 Root porosity

The results for the mean fractional porosity values for *Triglochin* are shown in in Figure 5.9. There was a small but significant difference ($p < 0.001$) between porosities measured from samples at 20 mm and 100 mm distances from the root apex. The highest mean porosities at both the 20 mm and 100 mm distance were recorded for plants at Eh -150 mV represented an average 8.7% and 9.1% increase compared to the equivalent respective values for the control plants. However, there were no differences in porosities between any treatments at both the 20 mm and 100 mm distances from the apex.

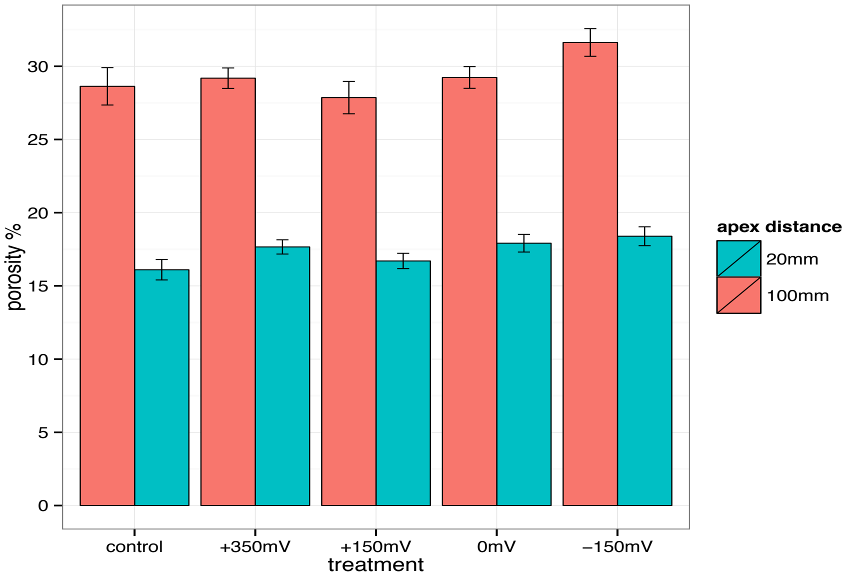


Figure 5.9. Mean porosity (% v/v) measurements for *Triglochin* after 10 weeks of exposure to a range of reducing conditions. Porosities at 100 mm from the apex were significantly higher ($P < 0.001$), but there were no significant differences in porosity between treatments.

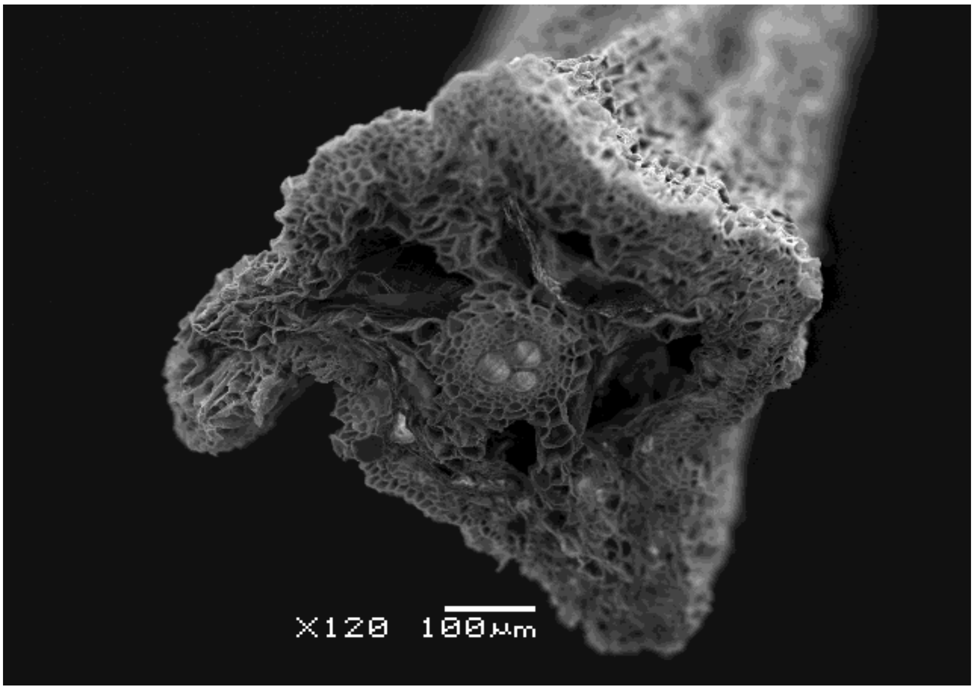
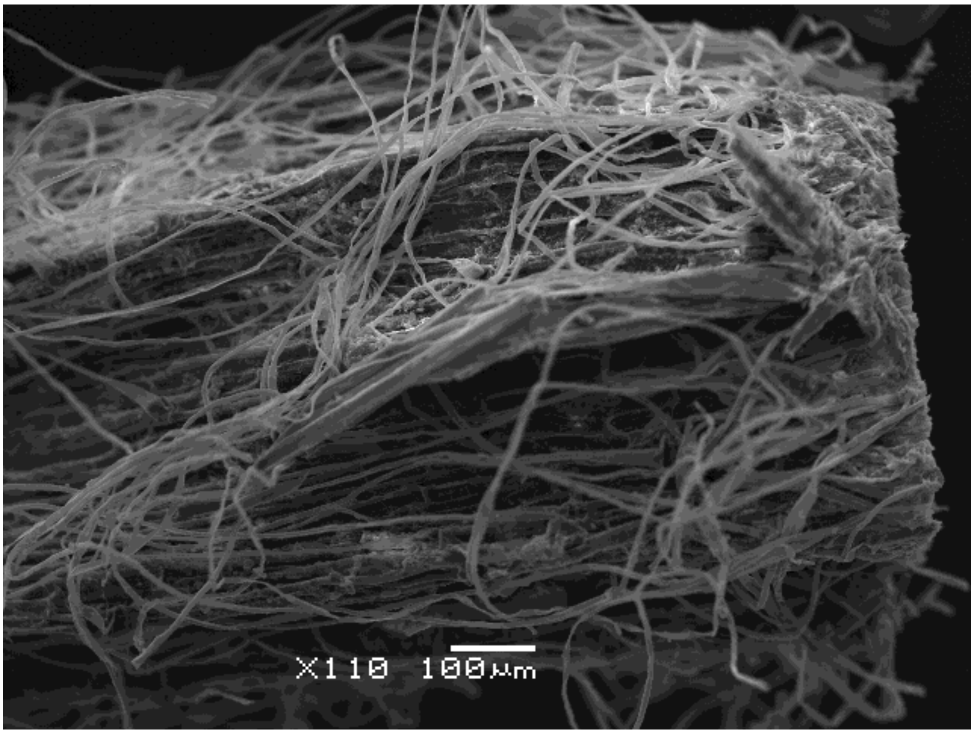


Figure 5.10. SEM of *Triglochin* root section at 20 mm distance from root apex (top). Sample was taken from a plant from the aerobic control showing well developed constitutive aerenchyma that while not found to be statistically significant increased in averaged from 16% v/v in control plants to 18% v/v in Eh -150 mV. Root section 100 mm distance from root apex from the Eh -150 mV treatment (bottom) that again while not found to be statistically significant increased from a mean 28% v/v in control plants to 31% v/v in Eh -150 mV.

Another feature relating to the root anatomy of *Triglochin*, although not investigated in detail, was the appearance of differences in the density of root hair structures between the aerobic and anaerobic treatments. A higher density of root hairs compared to the aerobic control became evident in the anaerobic treatments within 48 hours of the initial dosing with TiCi which was also apparent from examination of SEM images taken at the end of the experiment (Figure 5.11).

In comparison, the root hairs that were observed in plants from the aerobic treatment appeared to be at lower density (Figure 5.11). Furthermore, in a number of the SEM images the root hairs were difficult to discern such that there is a possibility that some of these structures may have been misinterpreted with the possible presence of arbuscular mycorrhizae (AM).



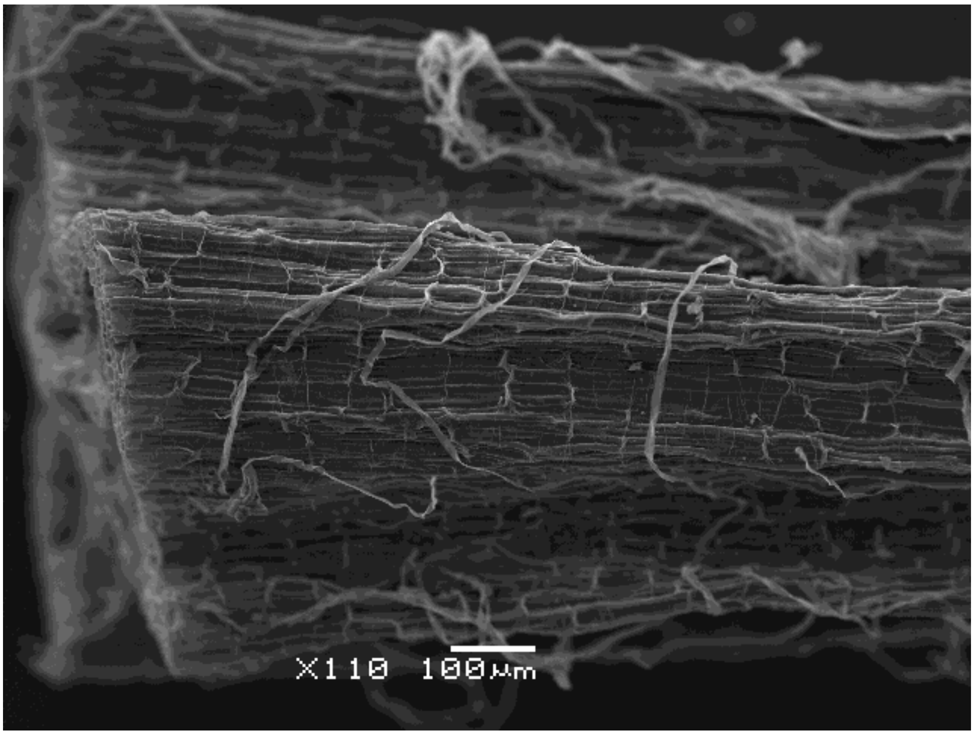


Figure 5.11. Extensive root hair formations were observed on roots examined from anaerobic treatments such as the sample taken from Eh -150 mV (top) but appeared to be less evident on some roots or at a lower density on compared to roots from aerobic control (bottom). Compare also Figure 5.10.

6.6.2.6 ADH analysis

The results for the ADH assay for *Triglochin* are shown in Figure 5.12. As expected, the majority of samples tested produced almost no change in absorbance indicating the absence of any ADH activity. However, an unexpected finding was that 4 samples did produce clear increases in absorbance indicating an ADH catalysed reaction with the isopropanol producing NADH. Four basic patterns emerge from the results (refer to Figure 30): first are the two samples E5 (0 mV) and F6 (-150 mV) with the highest absorbance peaking at O.D. > 0.3; second are two samples F3 (-150 mV) and F4 (-150 mV) with the next highest O.D. > 0.2; third a group of 4 samples with an O.D. > 0.1 composed of F2 (-150 mV), D4 (+150 mV), E2 (0 mV) and E4 (0 mV) and the remaining group consisting of samples almost exclusively from the aerobic control, the nitrogen purged (+350 mV) and the +150 mV treatments and showed very little or no evidence of any increase in ADH activity. The latter group also contains a number of samples with a negative O.D. value that resulted from the subtraction of a single background control (blank) all samples from the same treatment rather than each individual sample having its own blank as not all samples had sufficient volume left over for a blank. The lowest background control data for each set of samples subtracted from the sample A450 data. Since the lowest background since no reaction should take place in the background controls any absorption recorded in these blanks is like to be attributable either to scatter from particulates or something with an alcohol functional group in the sample that ADH is able to turnover.

Only one of the samples (E5) produced a great enough absorption change to make a reliable calculation of the rate using the NADH standard. This gave an ADH activity value of 3.01 mmol.min^{-1} /mL representing the amount of enzyme that is able to convert 1µmol of substrate (NAD^{+}) to product (NADH), and a specific activity (activity/total protein concentration) of 3.05 mmol.min^{-1} /mg.

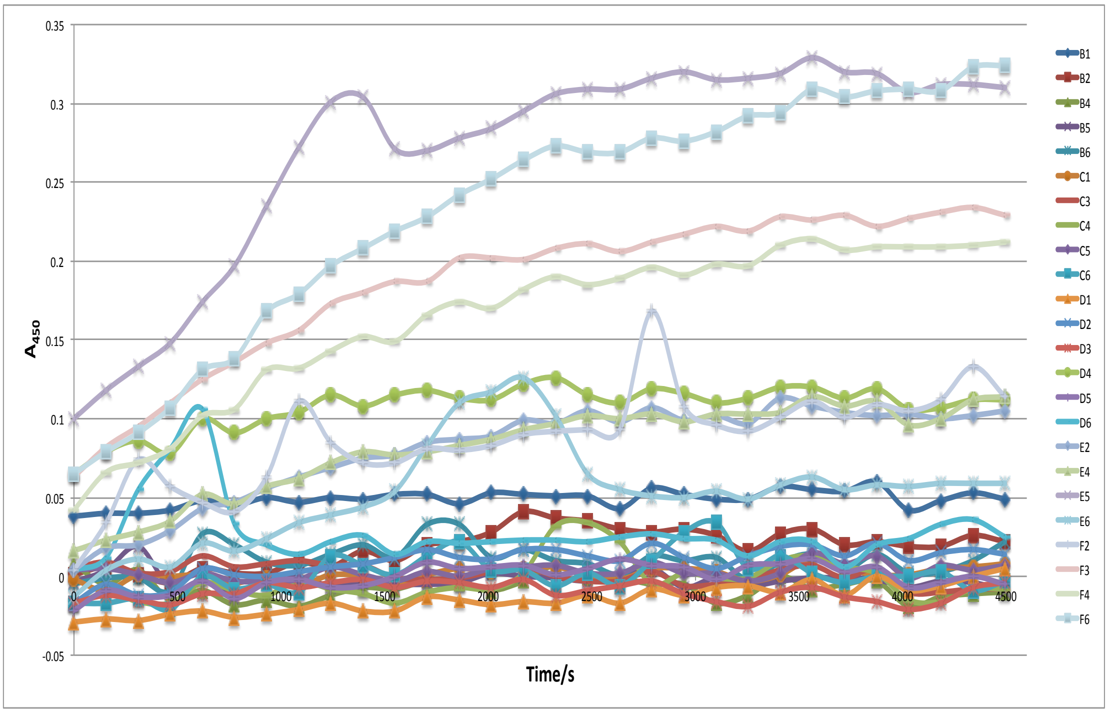


Figure 5.12. Spectrophotometric determination of ADH activity for *Triglochin*. Only samples from the most reducing treatments (Eh -150 mV and 0 mV) registered activities while samples that registered no ADH activity were almost all from the least reducing treatments.

6.6.2.7 Photosynthetic functioning

All stress parameters tested were unable to demonstrate any statistically significant differences between the control and the various redox treatments over the course of the experiment. Furthermore, no significant differences were recorded between the different degrees of redox intensity indicating that *Triglochin* is well adapted for long-term survival in conditions of both oxygen deficiency and high oxygen demand from low redox potentials.

For the duration of this experiment, mean Fv/Fm values for all treatments remained well within the range considered as optimal for healthy physiological functioning (Figure 5.13) and there were no significant differences between the start and the end values for any treatment ($p < 0.05$).

All treatments showed a similar pattern of a gradual increase in mean Y(II) in the first two weeks followed by a slight accelerated increase over the subsequent four weeks

(Figure 5.14). There were significant differences between the values at the start and end of the experiment (Table 5.4) indicating a significant increase in the linear electron flux (LEF), likely reflecting increasing rates of carbon assimilation over this period since it corresponds to a period of accelerated growth (Figure 5.4).

Table 5.4. Mean values for the effective quantum yield Y(II) of PSII photochemistry for *Triglochin* during the final 6 weeks of the experiment. Significant differences between the values at the start and the end of the experiment were detected (* $p < 0.05$; ** $p < 0.001$).

	Y(II) Start	Y(II) End	Difference
Control	0.4287	0.4958	+0.0671**
+350mV	0.4237	0.4673	+0.0436*
+150mV	0.4376	0.4867	+0.0491*
0mV	0.4338	0.4871	+0.0533**
-150mV	0.4282	0.4841	+0.0559**

The non-photochemical quenching parameters Y(NPQ) and Y(NO) (Figures 5.15 & 5.16; Table 5.5) both followed a decrease commensurate with the increase in Y(II) however the decreases were unequal with the bulk of the increase in Y(II) accounted for by the decrease in the regulated non-photochemical quenching parameter Y(NPQ) rather than Y(NO).

Damage to the physiological functioning as a consequence of oxygen deprivation stress would be indicated by a significant increase in Y(NO). No evidence of an increase was measured in any of the anaerobic treatments instead plants in the different treatments demonstrated a remarkable degree of uniformity with no significant differences found in the mean Y(NO) either between the control and the anaerobic treatments and between the different anaerobic treatments.

Table 5.5. Mean values for the non-photochemical quenching parameters Y(NPQ) of PSII and Y(NO) for *Triglochin* during the final 6 weeks of the experiment. There were significant differences in Y(NPQ) between the start and end of the experiment, but no significant differences in Y(NO) (* $p < 0.05$; ** $p < 0.001$).

	Y(NPQ) Start	Y(NPQ) End	Difference	Y(NO) start	Y(NO) End	Difference
Control	0.2924	0.2354	-0.0570*	0.2789	0.2688	-0.0101
+350mV	0.2936	0.2511	-0.0425*	0.2827	0.2816	-0.0011
+150mV	0.2908	0.2569	-0.0339**	0.2716	0.2564	-0.0152
0mV	0.2846	0.2486	-0.0360*	0.2816	0.2643	-0.0173
-150mV	0.2967	0.2525	-0.0442*	0.2751	0.2634	-0.0117

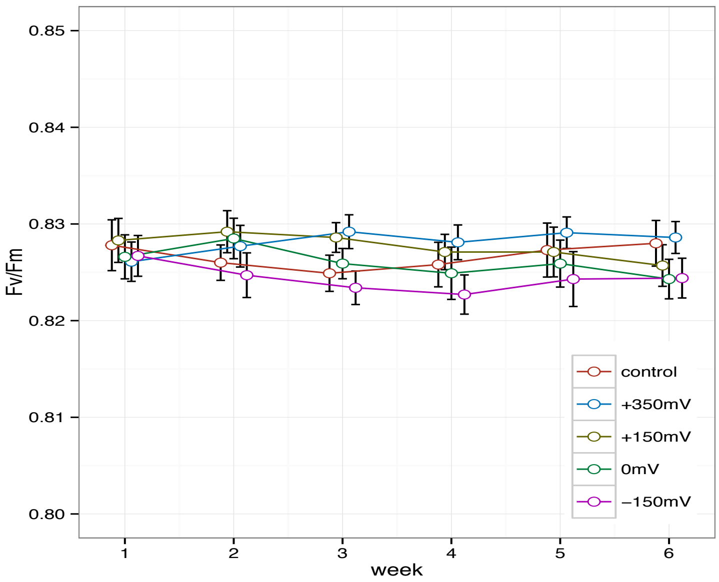


Figure 5.13. Mean (±SE) maximum quantum yield Fv/Fm of PSII photochemistry over the final 6 weeks of anaerobic treatment at different redox intensities (N = 10 plants per treatment). There were no significant differences between any treatments in any week.

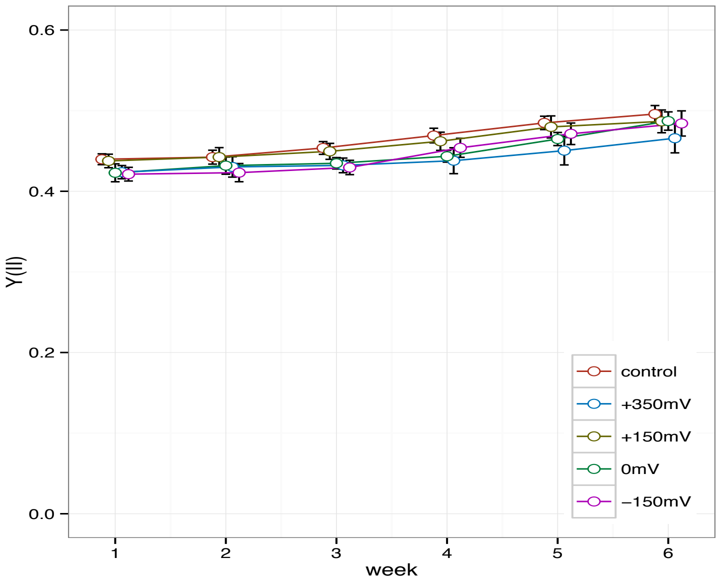


Figure 5.14. Mean (±SE) values for the effective quantum yield Y(II) of PSII photochemistry. No significant differences in Y(II) were recorded either between the control and anaerobic treatments or between the aerobic treatments. Mean values are based on measurements taken from 10 plants from each treatment

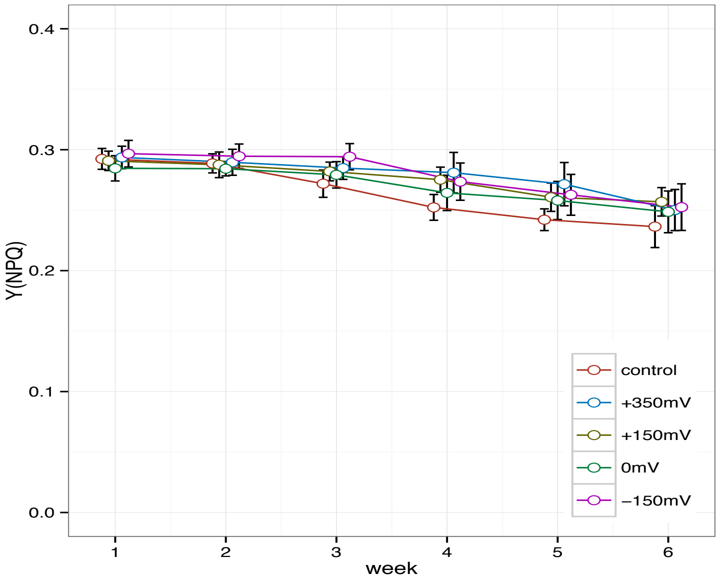


Figure 5.15. Mean (±SE) values for regulated non-photochemical quenching of Y(NPQ). No significant differences in Y(NPQ) were recorded either between the control and anaerobic treatments or between the aerobic treatments. Mean values are based on measurements taken from 10 plants from each treatment

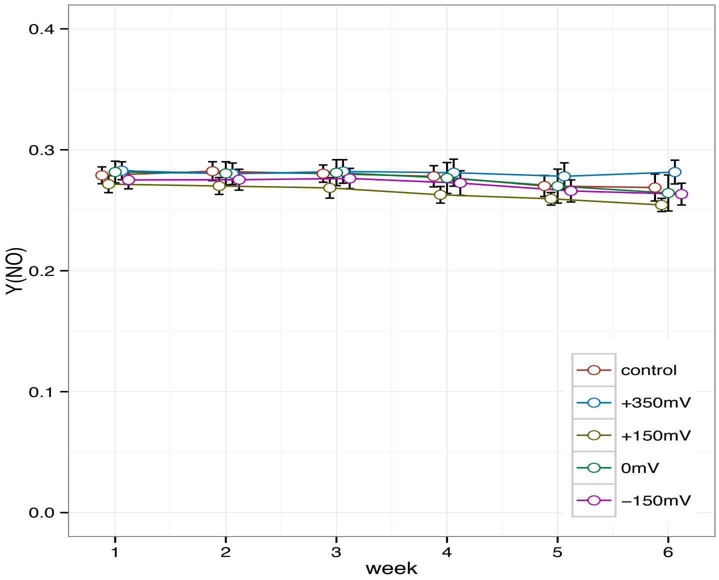


Figure 5.16. Mean (±SE) values for unregulated non-photochemical quenching of Y(NO). The absence of any increase in Y(NO) over this period further corroborates the absence of any loss of photoprotective capacity over this period. No significant differences in Y(NO) were recorded either between the control and anaerobic treatments or between the aerobic treatments. Mean values are based on measurements taken from 10 plants from each treatment.

6.6.3 *Atriplex*

6.6.3.1 Growth rates

Under aerobic control conditions *Atriplex* grew rapidly, with a maximum recorded rate of 34 mm and a mean rate of 18 mm in the first week that accelerated to a maximum rate of 84 mm and a mean of 49 mm by the final week. In contrast to *Triglochin, Atriplex* exhibited a clear and early adverse response to all anaerobic treatments (Figure 5.17), and by week 4 there had been a high degree of plant mortality in each of the anaerobic treatments.

All anaerobic treatments showed a similar trajectory of severely inhibited growth from an early stage with similar mean values throughout the duration of the experiment. In the anaerobic treatments, the mean growth during the first week ranged from 4.2 mm in the N-deoxygenated treatment to 4.7 mm in the Eh -150 mV treatment, representing at least a 74% reduction relative to the mean value for the control of 17.9 mm. The growth rate in all anaerobic treatments was significantly lower than in the control ($p < 0.001$ in all cases); there were no significant differences between anaerobic treatments.

By the end of the second week, the mean rate of growth for all anaerobic treatments decreased further by 62% for 350 mV, 67% for +150 mV and 87% for -150 mV, suggesting the possibility of a small (statistically insignificant) stress effect at different levels of redox intensity. At this stage, the mean rates of growth for all anaerobic treatments was minimal at less than 2 mm and a number of plants in all anaerobic treatments showed the first visible symptoms of stress (Figure 5.18) three plants in the +350 mV and two each in +150 mV and -150 mV treatments). These symptoms took the form of wilting in the growing tips of the stems and restricted growth in youngest leaves at that point indicating impairment of water uptake and/or translocation from anoxia-induced injury to the roots. Furthermore, some of the oldest leaves on several plants in each treatment exhibited the first signs of whole-leaf chlorosis indicating a deficiency of mobile nutrients.

Growth in all plants in the anaerobic treatments had effectively ceased by the end of the third week with rates growth of no more than a few mm in a small number of individuals, which was well within the margins of error during the measurement procedure so cannot reliable be attributed to any net growth at all. The numbers of plants exhibiting stress symptoms including chlorosis, withering of the stem tips, malformation and desiccation of young leaves and leaf abscission increased to seven in the +350 mV, five in each of the +150 mV and -150 mV treatments. At this stage this first mortality was observed for the +350 mV treatment. This pattern of declining health among plants rapidly progressed by the end of the fourth week with approximately one third of the plants experiencing mortality in each of the treatments and the majority of plants showing moderate to severe levels of stress symptoms and consequently registering slight levels of negative mean growth rates from dieback.

6.6.3.2 Recovery from anaerobic stress

To test the capacity for recovery after four weeks of exposure to anoxia, one individual from each treatment was transferred to a propagating tank that was continuously flushed with compressed air from an aquarium pump to restore aerobic conditions. After 4 weeks re-exposure to oxygenated water, none of the plants showed any evidence of recovery. On the contrary, the plants continued along a downward trajectory of decline to eventual mortality. In comparison, recovery appeared evident after 10 days exposure to anoxia in a trial run of the experiment indicated by a resumption of normal rates growth.

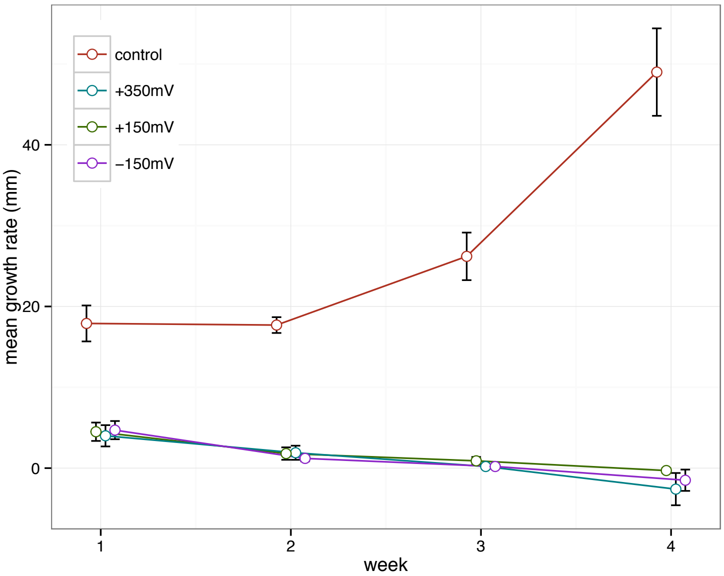


Figure 5.17. Comparison of branch elongation growth rates in *Atriplex* under different redox conditions. Growth in the control was significantly higher in all weeks compared to all anaerobic treatments ($p < 0.001$ in all cases). No significant differences were found between anaerobic treatments.

Figure 5.18. After two weeks some plants in each of the anaerobic treatments developed the first visible signs of stress symptoms in the form of chlorosis to the older leaves, stunted growth of newest leaves and wilting indicating the impairment of root functioning. There was no evidence that these symptoms developed earlier or were more widespread at higher redox intensities. Not all plants developed stress symptoms at the same time and some individual plants did not display symptoms at all until the end of the third week

6.6.3.3 Final branch and root length

The maximum branch and root lengths attained for *Atriplex* in each treatment are shown in Figure 5.19 and Table 5.6. As expected from the growth rate analysis, there were clear and highly significant differences in maximum attained branch lengths between the control plants and all anaerobic treatments ($p < 0.001$ in all cases). However, no significant differences were detected between anaerobic treatments. By the end of the 4 weeks, the final branch lengths of all plants in the control treatment were greater than any maximum branch attained from all plants in the anaerobic treatments.

In contrast, the differences between the maximum root lengths were smaller. Whilst the control plants had the largest mean maximum root length and the longest absolute value for any individual plant (Table 5.6), the overall differences between the control and anaerobic treatments were not significant. The similarity in maximum root lengths in all treatments was an unexpected in the context of the large differences attained in stem lengths between the control and anaerobic plants. However, observation prior to the start of the treatments indicated roots in all treatments were visibly well developed and most of the root elongation in the occurred as rapid initial growth during the two-week acclimation period after plants were transferred from the propagating tanks to the experimental tanks rather than a continuation of growth under the period of anoxia.

Table 5.6. Average and maximum branch and root lengths attained after 4 weeks based on the mean value of the three longest branches and three longest roots from ten plants in each treatment. Branch lengths were significant greater between the control than in all anaerobic treatments ($p < 0.001$ in all cases). There were no significant differences in root length between the control and the anaerobic treatments, or in branch or root lengths between anaerobic treatments.

	Branch length mean ± se	Branch length maximum	Root length mean ± se	Root length maximum	Mean root:shoot
control	236.3 ±11.6	297	335.0 ±37.8	510	1.48
+350mv	115.7 ±7.3	158	278.0 ±34.5	480	2.42
+150mv	118.7 ±8.0	148	296.5 ±37.4	480	2.50
-150mv	120.6 ±6.2	150	280.0 ±31.7	445	2.29

6.6.3.4 Productivity

Measurements of final aboveground and belowground biomass for *Atriplex* are shown in Figure 5.20 and Table 5.7. Again, the results from the growth rate analysis were mirrored in the results for the final overall biomass measurements. The differences between the control plants and all anaerobic plants were found to be highly significant for aboveground biomass ($p < 0.001$) and significant but to a lesser degree for the belowground biomass ($p < 0.05$). There were no significant differences in either above or belowground biomass between anaerobic treatments.

Similar to *Triglochin*, when either species are grown under favourable conditions the growth and productivity of individual plants are unequal and consequently both aboveground and belowground biomass demonstrated a high degree of variability. For *Atriplex* growing in an aerobic control medium, the aboveground biomass ranged from an upper limit of 5.3 g to a lower limit of 2.4 g, and the belowground biomass ranged from 0.783 g to a lower limit of 0.265 g. It is notable that the value for the mean root:shoot ratio for *Atriplex* at 0.15 was markedly smaller than the 0.51 value recorded for *Triglochin* indicating a far greater investment in aboveground productivity in the former.

Table 5.7. maximum and mean (±se) aboveground and belowground biomass (dry weight g). Significant differences in above ($p < 0.001$) and belowground ($p < 0.05$) biomass were found between the control and all anaerobic treatments. No significant differences were recorded for the above or belowground biomass between the different anaerobic treatments.

	Aboveground mean ±se	Aboveground maximum	Belowground mean ±se	Belowground maximum	Mean root:shoot
control	3.79 ±0.37	5.30	0.53 ±0.06	0.78	0.15
+350mv	1.45 ±0.18	2.60	0.29 ±0.04	0.47	0.20
+150mv	1.70 ±0.14	2.30	0.28 ±0.04	0.45	0.17
-150mv	1.67 ±0.18	2.70	0.24 ±0.03	0.35	0.15

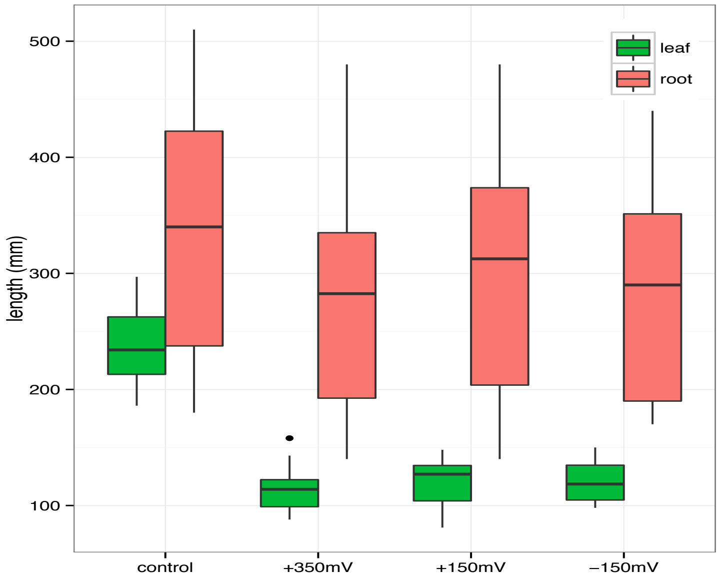


Figure 5.19. The range of values recorded for the maximum branch and root lengths for *Atriplex* based on the averages values from the three longest branches and three longest roots from the ten largest plants from each treatment.

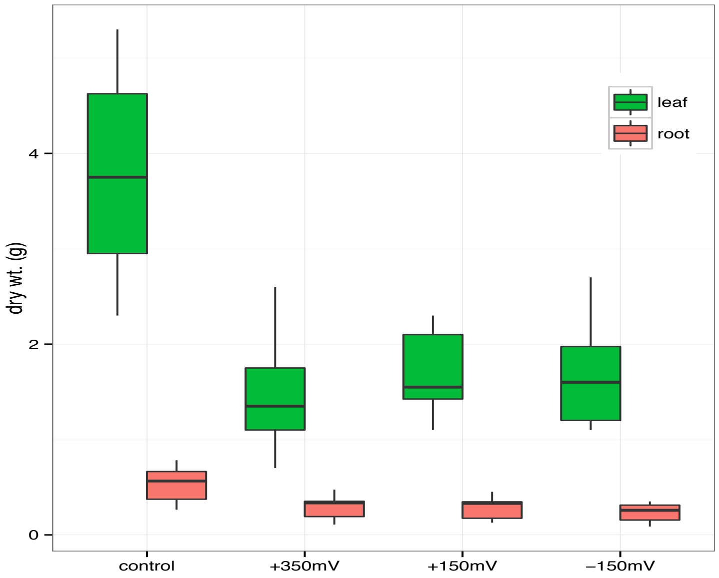


Figure 5.20. The range of values for aboveground and belowground biomass for *Atriplex* based on samples from ten plants from each treatment.

Figure 5.21. *Atriplex* at the end of 4 weeks. Top left: aerobic control; top right: N_2 deoxygenated; bottom left: Eh +150 mV and bottom right: Eh -150 mV. At this stage each anaerobic treatment experienced some 30-40% mortality and almost all plants showed moderate to severe signs of stress. There was no convincing visible evidence to indicate rates of mortality or deterioration was greater in the highest redox intensity treatment.

Figure 5.22. A comparison of *Atriplex* from left-right: aerobic control, N_2 deoxygenated, Eh +150 mV and Eh -150 mV. Root lengths were found to be highly variable in all treatments however the highest mean values for both root length and biomass were recorded for plants from the aerobic control. In all the anaerobic treatments almost all root growth occurred during a two week period of aerated acclimation before the start of the anaerobic conditions.

6.6.3.5 Root porosity

In contrast to *Triglochin*, *Atriplex* did not show any tendency towards developing aerenchyma in response to anaerobic treatments. The results shown in Figure 5.23 clearly illustrate the limited extent of porosity, with mean values for all treatments less than 2% and no significant differences between sample distances or between treatments. It is possible that these low mean values may underestimate the actual extent of porosity since the calculation included a large proportion of zero recorded values in each treatment. There was a relatively high level of variance between samples from the same treatment compared to *Triglochin.* Nevertheless, even if the zero values are omitted from the calculation, the maximum mean values for the 20 mm and 100 mm sampling distances are 3.75% and 3.35% respectively with the majority of recorded values falling well below the respective mean value.

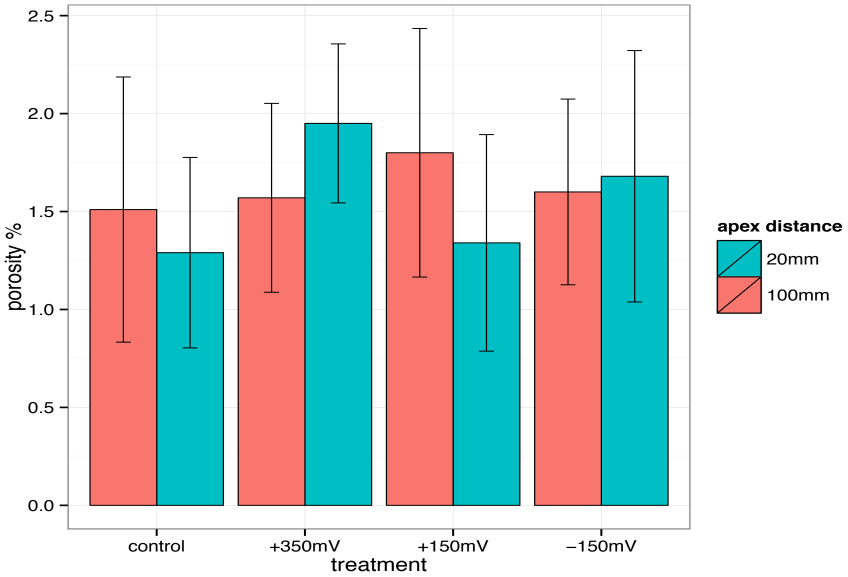


Figure 5.23. Mean porosity (% v/v) measurements for *Atriplex* demonstrating the absence of either constitutive or enhanced porosities after 4 weeks of anaerobic exposure.

6.6.3.6 Photosynthetic performance

The time series measurements for maximum quantum yields Fv/Fm based on the mean values of 10 plants from each treatment are shown in Figure 5.24. Prior to the start of the experiment the plants were permitted a two week period of acclimation for transplant adjustment from the propagation to the experimental hydroponic tanks. The initial recordings (week 1) taken just before commencement of experimental treatments showed a high level of consistency with an overall mean for all plants of Fv/Fm = 0.832 and no significant difference between any treatment. The absence of any significant difference between treatments extended to at least the end of the first week (week 2) although some of the leaves in the anaerobic treatments by this time had begun to exhibit lower values, particularly in the -150 mV treatment where the lowest value of 0.786 was recorded.

Differences between the control Fv/Fm values, which averaged more or less constant (± 0.005) over the four week course of the experiment, and all anaerobic treatments only became significant by the end of the second week: + 350 mV and +150 mV $p < 0.001$; -150 mV $p < 0.01$. Here the decline in Fv/Fm in all the anaerobic treatments was small with mean values of ~ 0.810 (± 0.015) and only 3 values < 0.8. The differences between the anaerobic treatments at this stage were not found to be significant. Although this initial decline suggests the manifestation of a stress effect and while it might be sub-optimal for *Atriplex* these values are still within the optimal range of generally regarded as optimal for most species i.e. 0.79 – 0.84.

After the second week many plants in each anaerobic treatment had leaves showing varying degrees of chlorosis, wilting and abscission. This deterioration in condition was reflected the marked decline in Fv/Fm by the end of week 3 with the majority of values across all anaerobic treatments close to the lower limit of the sub-optimal range with the mean values as: +350 mV = 0.782; +150 mV = 0.792; -150 mV = 0.775. None of the values on leaves from any of the anaerobic treatments exceeded the control mean value (0.828) and the lowest measured value of 0.651 came from the -150 mV treatment.

From the third week of anoxia, the decline in average values Fv/Fm was precipitous as the range of values within each treatment becomes increasingly divergent from the greater frequency of very low values well below sub-optimal levels. At this stage all plants in each anaerobic treatment were exhibiting varying degrees of stress with a mortality rate of 30-40% and the leaves on all surviving plants of showing at least some signs of chlorosis. The final mean values were: +350 mV = 0.629; +150 mV = 0.658; -150 mV = 0.629 and the lowest recorded value 0.33 was from the -150 mV treatment.

Crucially, no significant differences were found ($p < 0.05$) in the decrease in Fv/Fm between anaerobic treatments suggesting that the tolerance of *Atriplex* is not influenced by redox intensity and instead the deficiency of oxygen created by nitrogen deoxygenation alone is sufficient to elicit a similar response regardless of the degree of redox intensity.

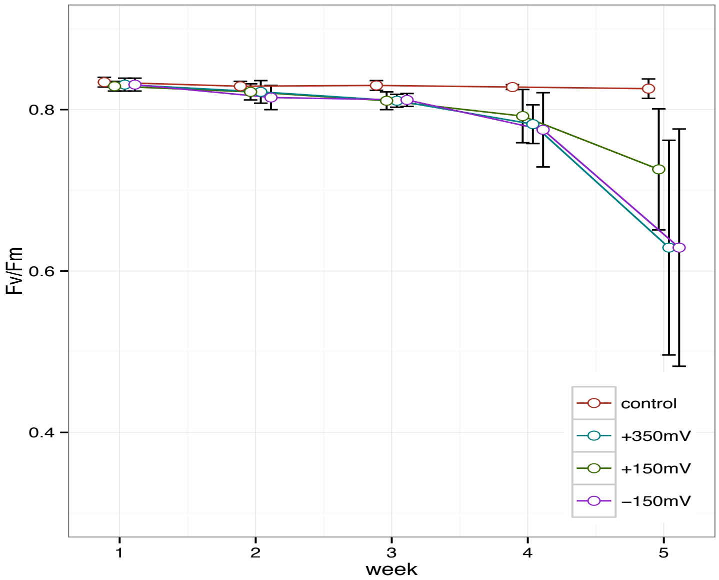


Figure 5.24. Mean (±SE) maximum quantum yield Fv/Fm of PSII photochemistry over 4 weeks of anaerobic treatment at different redox intensities (N=10 per treatment). Decrease in Fv/Fm represents light-induced photo-inactivation of PSII reaction centres.

The decline in photosynthetic efficiency over the course of the experiment was similarly reflected in the measured effective yield parameter Y(II) but with less sensitivity than Fv/Fm (Figure 5.24). While Fv/Fm detected significant deviation in the anaerobic treatments from the control mean value by the end of the second week Y(II) showed significant differences only by the end of the third week: +350 mV and +150 mV ($p < 0.05$); -150 mV ($p < 0.01$). The reduced sensitivity of Y(II) may be attributed to the relatively large variation in values between plants even under non-stressed conditions compared to Fv/Fm which showed a much higher degree of consistency. For example, the Y(II) values of the control at the beginning of the experiment had a mean value of 0.482 and ranged between an upper limit of 0.582 and a lower limit of 0.436 with a standard deviation of 0.046 compared to the equivalent Fv/Fm standard deviation of just 0.06.

Again, no significant differences were found between anaerobic treatments at any stage providing further confirmation that the conditions of oxygen stress by nitrogen deoxygenation alone is enough to cause a detrimental effect and the conditions of increased oxygen demand created by the chemically reduced treatments does not significantly impose any additional stress effect that is detectable by using the two most widely used stress parameters in chlorophyll fluorometry. The most notable difference between the two parameters was the ability of Y(II) to reflect the increase in photosynthetic efficiency in the control plants which resulted in the 174% increase growth rate over the final two weeks relative to the initial two weeks.

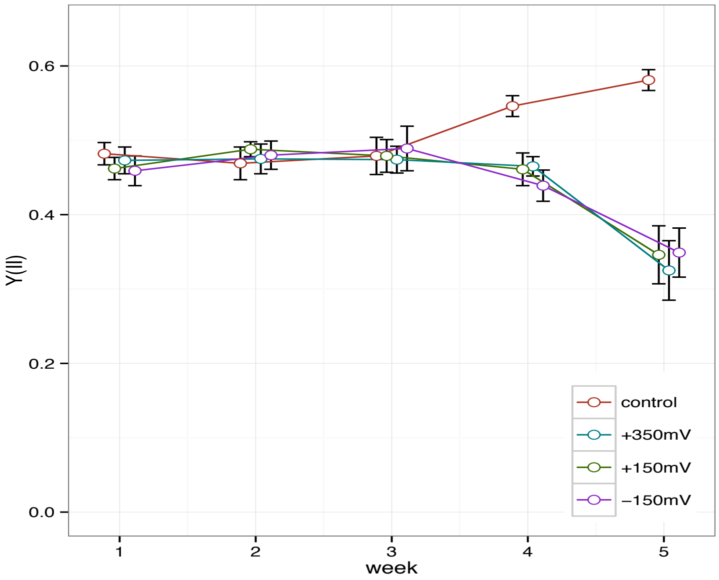


Figure 5.24. Mean (± SE) effective quantum yield Y(II) of PSII photochemistry (N=10 plants per treatment).

The non-photochemical quenching parameters demonstrated a clear partition between regulated and non-regulated energy dissipation over the course of the experiment (Figures 5.25 & 5.26). While in the control plants Y(NPQ) mirrored an inverse relationship with Y(II) which was the expected regulatory response to the increased utilisation of absorbed energy used for photochemistry to fuel the onset of a rapid increase in growth. In contrast, the decrease in the photochemical energy pathway flux, indicated by Y(II) in the anaerobic treatments, was reflected in the increase in Y(NO) from the end of the second week rather than Y(NPQ). The increase in Y(NO) provided a clearer indication of loss of the regulated non-photochemical quenching capacity as a consequence of stress induced irreversible photo-inactivation of PSII complexes leading to an increasing fraction of the energy lost in the form of passive thermal and fluorescence emissions.

The parameter for regulated non photochemical quenching Y(NPQ) showed a decline in all treatments but the decrease in the anaerobic treatments was considerable smaller than the control after the second week. Although the anaerobic treatments appear to follow a different downward trajectory from the control this only became significant at the end of the experiment for two of the three anaerobic treatments: +350 mV (p <

0.05) and -150 mV ($p < 0.01$). In addition, no significant differences were recorded between the anaerobic treatments at any stage.

Since the sum of Y(II) + Y(NPQ) + Y(NO) = 1 changes in Y(II) will be accompanied by a commensurate change in one or both of the non-photochemical quenching parameters. In this experiment, the control and anaerobic treatments produced two very different responses in Y(NPQ) and Y(NO). In the anaerobic treatments the decrease in Y(II) in the third and fourth weeks was compensated by the increase in Y(NO) over the same period. Y(NPQ) in the anaerobic treatments decreased by a smaller degree from the second week which rather than represented a regulated reduction was attributable a gradual replacement from the second week in unregulated thermal dissipation Y(NO). In contrast, the large increase in Y(II) for the control plants in the third and fourth weeks was clearly mirrored in the commensurate decrease in regulated thermal dissipation Y(NPQ) as would be expected in a healthy plant with undamaged PSII reaction centres, while Y(NO) remained largely stable throughout the course of the experiment.

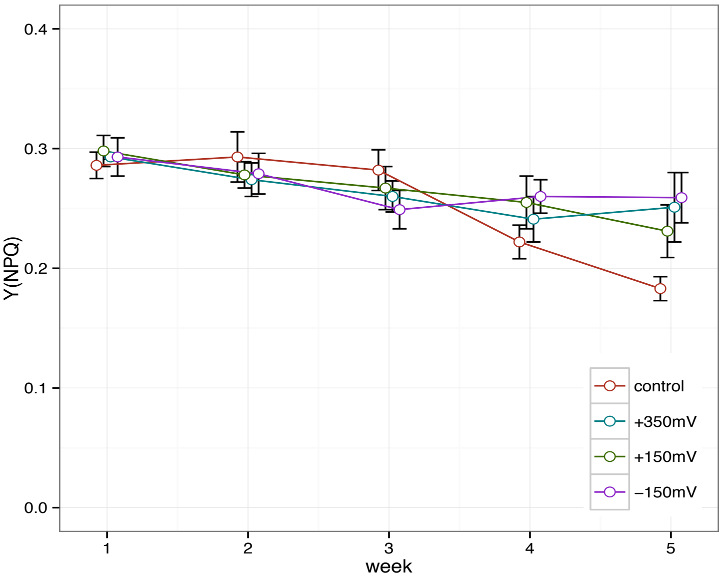


Figure 5.25. Average values for regulated non-photochemical quenching of Y(NPQ) provides an indication of the functioning of photoprotective mechanisms for the conversion and dissipation of excess excitation that is not used to drive photosynthesis.. Mean values are based on measurements taken from 10 plants from each treatment.

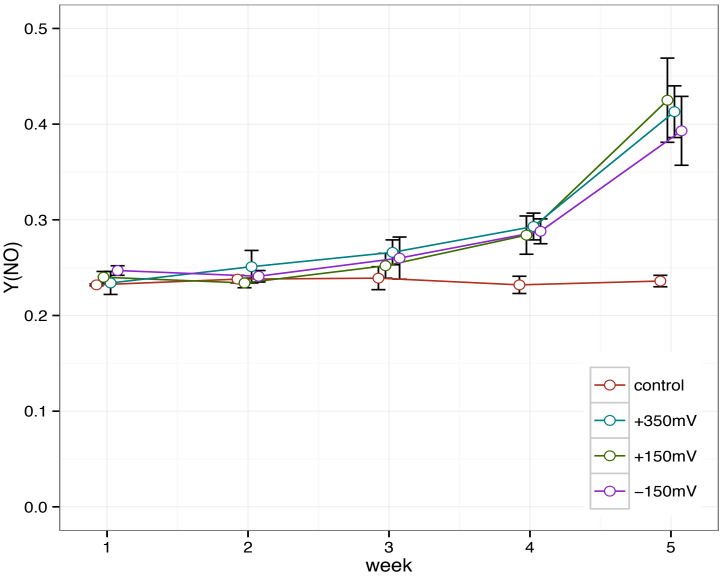


Figure 5.26. Mean (±SE) unregulated non-photochemical quenching of Y(NO). Mean values are based on measurements taken from 10 plants from each treatment.

Y(II) has previously been found to be a more sensitive test for some types of abiotic stresses than Fv/Fm and this is more likely to be the case where the stress response manifests in the rate of increase in carbon assimilation (Genty *et al*. 1989). In the case of *Atriplex*, the effects of oxygen stress effectively inhibited growth at an early stage so a decrease carbon assimilation consumed by growth was expected to result in a decrease in Y(II) at an earlier stage of the experiment as PSII became unable to process the absorbed excitation energy for photochemistry. However, the delayed decrease in Y(II) was not matched by a corresponding delay in the measured increase in energy dissipation through the unregulated non-photochemical pathway Y(NO). The possible reasons for this discrepancy is discussed later but, in the meantime, the absence of a clear early response in Y(II) appears to demonstrate a certain lack of sensitivity of this parameter to detecting oxygen stress in *Atriplex*.

Of the four parameters tested for their capacity to detect in reduction in photosynthetic performance induced by oxygen stress in *Atriplex* both Fv/Fm and Y(NO) proved to be the most sensitive as they were able to demonstrate the manifestation of significant differences between the anaerobic treatments and the control plants earlier than any of the other tests. While significant differences in the mean values between the anaerobic treatments and the control were present by the end of second week for Fv/Fm, they did not become apparent for Y(II) or Y(NO) until the third week and by the fourth week for Y(NPQ). A visual examination of the graphs indicate a consistent and similar pattern of a departure in all anaerobic treatments from the control for Fv/Fm and Y(NO). Both these parameters demonstrated the rapid decline at the start of the third week of anaerobic treatment and signified the onset of widespread loss of photoprotective capacity and large scale irreversible damage the photosynthetic apparatus and this stage likely represents the maximal duration of tolerance to oxygen stress for *Atriplex* after which the chances post anoxic recovery become increasingly curtailed.

Interestingly, these parameters preceded the onset of visible signs of oxygen stress in the measured leaves that became progressively more apparent and widespread from around the middle of the third week. There was no evidence from either the chlorophyll fluorescence parameters or from visible indicators that stress occurred earlier or to a greater degree in the most reducing treatment compared to the least reducing treatment suggesting redox intensity is not an aggravating stress factor during anoxia for *Atriplex*.

6.7 Discussion

This experiment was undertaken to investigate the response of two saltmarsh plant species to prolonged oxygen stress under conditions of controlled redox intensities using an experimental design that represents an improvement on previous versions. This was achieved by modifying the titanium (III) citrate reservoir and delivery system in a way that drastically reduces its rate of contamination by oxidation and therefore maintains its potency for up to two weeks instead of just a few days as in previous systems (e.g. Lissner *et al*. 2003a). The two species, *Atriplex portulacoides* and *Triglochin maritima*, were shown in the current study to occupy areas of saltmarshes with sediments with contrasting redox characteristics. While *Atriplex* favours relatively well drained sites that experience temporary anoxia and low redox potentials over several days in response to tidal flooding, *Triglochin* can survive in sediments that experience continuous, long-term anoxia and low redox potentials over many months. The result from growth analysis and photosynthetic functioning under a range of redox intensities indicate that *Atriplex* is able to tolerate anoxia and low redox potentials for the duration of around two weeks before the onset of irreversible photodamage. In contrast, anoxia and low redox potentials had little statistically significant effect on growth of photosynthetic functioning in *Triglochin* over the 10 week duration of the experiment. Measurements of porosity in *Triglochin* showed that aerenchyma was constitutively well developed and did not show a significant increase induced by low redox potentials. However ADH activity was measured in roots from plants in the low redox treatments suggesting *Triglochin* may not be able to supply all its root system by internal oxygen transport under highly reducing conditions.

To the author's knowledge to date no similar such investigations have been attempted for any species that comprise important components of the halophytic vegetation communities that occur in many British saltmarshes. Any comparable studies are few in number and have been limited to a few *Spartina* species that comprise much of the dominant vegetation of saltmarsh communities in North America. Consequently there are large gaps in our understanding of the capacities of different saltmarsh plant species to survive under conditions of varying degrees of oxygen stress imposed by the high oxygen demand from the low redox potentials that are often a consequence of prolonged waterlogging. An understanding of plant responses to oxygen stress provides important information in aiding to resolve the long-standing debate on the relative importance of the two fundamental abiotic stresses – variations in salinity and oxygen deprivation – that are widely thought to underpin the structuring of vegetation zonation in natural saltmarshes and the differential patterns of colonisation and zonation on recent restoration saltmarshes. The experimental approach used in the current study goes some way in addressing the failure to isolate the effects of oxygen stress from the potentially confounding stresses associated with redox phytotoxicity that has been an under-appreciated constraint in progressing knowledge in this area.

The two species investigated in the present study are representatives of strongly contrasting saltmarsh sediment environments with respect to soil redox potential characteristics. While previous studies indicated that these species differ in their abilities to tolerate experimental waterlogging they provided little information about their relative tolerances to different degrees of redox intensity. The successful design and application of this experiment has enabled further insights on the physiological functioning of these plants under an important but still not fully understood abiotic stress in saltmarsh sediments.

6.7.1 *Triglochin*

The results from *Triglochin* demonstrated that this species is well adapted to survive prolonged periods of oxygen stress without a significant detrimental effect at levels of redox intensity of at least -150 mV which is considered to be highly reducing being well below the level (-100 mV) where sulphide reduction begins to occur in waterlogged soils (Connell & Patrick 1968; Ponnamperuma 1984). None of the parameters measured as performance indicators: leaf elongation rates, aboveground and belowground productivities, maximum height and root lengths, demonstrated any convincing evidence of decline with increasing reducing intensity at least to moderately reducing conditions. Although the relationships between these metrics and the redox conditions, when considered individually, were not found to be statistically significant, a collective examination of the data for these growth parameters shows some evidence of a consistent pattern that emerges indicating the presence of a small degree of growth inhibition induced at the highest levels of redox intensity tested in this experiment particularly at Eh -150 mV.

6.7.1.1 Photosynthetic functioning

A significant part of this experiment was to assess the response of plant photosynthetic efficiency to varying redox intensities using chlorophyll fluorescence parameters as an early indicator of physiological stress in *Triglochin* and *Atriplex*. An absence of any clear negative effect on physiological functioning for *Triglochin* was indicated in measurements obtained from all chlorophyll fluorescence parameters. No evidence was found for the occurrence of significant irreversible photoinhibition or damage to the photosynthetic apparatus at any level of redox intensity. Although there was a slight but consistent reduction for *Triglochin* reflected in the parameter for maximum efficiency of photosynthesis, Fv/Fm, the values were still well within the range of that regarded as optimal for most plants.

The absence of any significant deviation in any of the fluorescence parameters between treatments indicates that the range of processes that influence photosynthesis in *Triglochin* are not significantly impacted at such redox intensities. Instead of representing an indication of stress the small reduction in growth in *Triglochin* possibly reflected a degree of downregulation of photoassimilate consumption directed towards growth and a corresponding upregulation of photoassimilate consumption diverted to fuel a degree of anaerobic respiration as evidenced by the presence of ADH activity in the plants subject to the highest reducing intensity.

It is difficult to compare the outcomes of this study with the relatively small number of previous studies that used a similar approach of experimental manipulation of redox intensities to measure the physiological and developmental responses of wetland plants to oxygen stress. Most of these studies differ with respect to a range of factors most crucially including the developmental stage, redox intensities tested and the length of time the plant under investigation was exposed to the oxygen stress condition. Nevertheless, there is now a sufficient body of convincing data proving that survival times and a range of functions that influence growth and development are impaired to differing degrees by increased levels of redox intensity (Pezeshki & DeLaune 2012).

In the majority of published studies, the species under investigation showed some negative effect but the responses were highly variable reflecting the varying degrees of adaptation to the stress conditions imposed. Much of the work on the effect of redox intensities on saltmarsh plants has been obtained from extensive work on *Spartina patens*: Pezeshki *et al*. (1991) reported severe inhibition of root elongation shortly after the initiation of redox potentials within the range -50 mV to +70 mV and complete cessation of growth at -100 mV (Pezeshki & DeLaune 1990) while overall growth of *S. patens* was reduced at more severe redox intensities resulting in a 25% reduction in aboveground biomass and a 37% reduction in belowground biomass at Eh -200 mv to -300 mV (Kludze & DeLaune 1994).

There are few other studies that have tested the effect of redox intensities on saltmarsh species but work on freshwater wetland species has demonstrated a similar range of negative responses on growth and productivities at low redox intensities. For example, while the rate of photosynthesis and relative growth rates were found to be stable for *Phalaris arundinacea* and *Glyceria maxima* in a deoxygenated treatment by N-purging, the rate of photosynthesis declined within 5 days resulting in negative relative growth rates from loss of mass from plants grown at -250 mV from additions of titanium(III) citrate (Brix & Sorrell 1996); progressively large differences in net photosynthesis and total biomass were reported for *Cladium jamaicense* and *Typha domingensis* between different levels of reducing treatments ranging from +250 mV to -200 mV (Kludze & DeLaune 1996; Pezeshki *et al*. 1996). In contrast, nitrogen uptake CO_2 fixation and growth of *Spartina alterniflora* was not affected by redox intensities from +500 mV to -200 mV (DeLaune *et al*. 1984). The absence of growth inhibition under experimental conditions contrasted with field observations where plant height in *S. alterniflora* was correlated with sediment redox potentials with the shortest forms occurring in the most reducing sediments suggesting other factors such as the presence of free sulphides may be limiting growth instead.

6.7.1.2 Porosity

Many wetland plants, and some non-wetland species, develop aerenchyma either constitutively or in response to waterlogging to increase the diffusion pathway of gases between the atmosphere and submerged organs. In addition, theoretical models have demonstrated a strong positive correlation porosity and root growth under oxygen limiting conditions (Armstrong & Beckett 1987; Armstrong *et al*. 1991).

The occurrence of aerenchyma in the rhizomes and roots of *Triglochin* was first described in detail by Hill (1900) who noted the presence of large lacunae with signs of lysigenous development in the outer parts of the inner cortex of the older regions of the roots while porosity in the inner cortex of the younger regions was comprised of schizogenous intercellular air spaces. In was not until some time later that an attempt was made to quantify the volume of fractional root porosity in *Triglochin* by Justin and Armstrong (1987) in their extensive investigation of a range of wetland and non-wetland species in response to contrasting conditions of drainage and flooding but where redox potentials were largely uncontrolled. Despite the importance of aerenchyma for the survival of many wetland plant species, there have been relatively few studies that have investigated the capacity of saltmarsh plants in general to develop aerenchyma in response to a variable range of experimentally induced redox conditions.

The results from this experiment showed that aerenchyma formation that was found to be constitutively well developed and no significant evidence was found to support the hypothesis that porosity induced can be induced to increase in response to elevated oxygen demand at lower redox potentials. Although mean porosities recorded at 100 mm were roughly 50% higher than those recorded by Justin & Armstrong (1987), they were similar in both flooded and drained treatments providing further confirmation of the absence of an effect from oxygen stress on aerenchyma enhancement for this species.

The lower porosities reported by Justin & Armstrong (1987) may either be attributed to the different technique used which was based on a modified version of the pycnometer method (Jensen *et al*. 1969) where the calculation for porosity was based on the specific gravity of a submerged root sample before and after air was evacuated (rather than using a homogenate). The variation may also be attributed to the very different conditions plants were grown, where redox conditions under flooded soil media were uncontrolled, the length of time under flooding to allow for aerenchyma formation and to the different distances from the apex the measured root samples were taken. Some studies, (e.g. Smirnoff & Crawford 1983), appear to have measured porosity in intact whole root systems which gives only an approximation of the proportion of cortex occupied by air space since aerenchyma is not equally developed along the length of the root and can be considerably reduced near the apex (Armstrong 1971; Visser & Bögemann 2003). Comparisons between results derived from different methodologies are thus highly problematic as they contain potentially significant caveats that need to be taken into consideration.

The absence of a significant inverse relationship between redox intensity and fractional root porosity in *Triglochin* was a somewhat unexpected finding in the context of the lack of any clear differentiation between measurements of root elongation between the anaerobic treatments. Without any compensatory increase in porosity, root elongation was expected to be inhibited if the rate of ROL increased with reducing intensity since root length not only influences the degree of ROL but also the amount of metabolically active root tissue along the oxygen transport pathway thus limiting the availability of oxygen to the growing tissues of the apical meristem, where metabolic activity, and therefore demand for oxygen, is highest (Armstrong *et al*. 1991; Konings & Lambers 1991). The lack of any inhibition in root elongation without a significant increase in porosity in *Triglochin* suggests other mechanisms may be operating to reduce oxygen loss such as an effective barrier to ROL in the sub-apical region of the roots. The existence of such an anatomical feature was alluded to by Hill (1900), who described the presence of lignification and an extremely well marked secondary thickening of the endodermis in the roots and rhizomes of *Triglochin*. The capacity to form both extensive aerenchyma and an effective barrier to ROL indicates *Triglochin* appears well adapted to avoid the stress effects imposed by this experiment by sufficient capacity for internal transport of oxygen to the distal regions of the root system. However, this was contradicted to a certain extent by the finding of presence ADH activity in roots from plants in the treatments with the highest redox intensities.

6.7.1.3 ADH analysis

Increased ADH activity in response to waterlogging has been used in a number of studies as an indicator of oxygen deficiency in plants since it is the main product of anaerobic respiration in both flood tolerant and tolerant intolerant species (Vartapetian & Jackson 1997). In addition to providing an indication of the plants ability to maintain anaerobic respiration ADH may also serve as an indicator of a plant's ability to maintain cytoplasmic pH homeostasis by the replacement of lactic acid with increased ethanol production as the end product of fermentation (Ricard *et al*. 1994; Roberts *et al*. 1989).

However, it is still unclear if the induction of ADH activity contributes to the plant survival or signals the harmful effects of root anoxia. The majority of research on metabolic responses to flood-induced anoxia has been based on flood intolerant cultivated plants and information of the responses of better adapted wetland species is scant. A recent study by Brownstein *et al*. (2013), found a positive correlation between waterlogging tolerances and ADH activity in a number of saltmarsh species and there is evidence to suggest that ADH activity increases at low soil redox potentials even in plants with extensive aerenchyma systems such as *Spartina alterniflora* (Mendelssohn *et al*. 1981; Mendelssohn & McKee 1983) and *S. patens* (Burdick & Mendelssohn 1989; Pezeshki *et al*. 1993). On the other hand, at such low redox potentials the occurrence of sulphide has been found to inhibit root ADH activity (Koch *et al*. 1990).

There is very little existing information about the ADH characteristics of British saltmarsh plants in response to waterlogging and anoxia. In the only published study to date, a relationship with LDH, rather than ADH, and waterlogging was found in *Suaeda maritima* (Wetson *et al*. 2012). Where this species occurred in well-drained sediments that experienced transient oxygen stress this was reflected in the low levels of ADH relative to constitutively high levels of LDH.

Although there were not enough samples for a statistical analysis to be performed, the positive result for measurable ADH activity and the evidence of ADH activity to lesser degree from other samples is potentially an interesting result. It is particularly noteworthy that all the positive samples for ADH activity were derived from plants from the two most reducing treatments, 0 mV and -150 mV. This suggests the possibility exists that under intense reducing conditions extensive formation of root aerenchyma in *Triglochin* may not be sufficient to fully supply the roots with enough oxygen to avert at least a degree of oxygen stress.

The extent to which aerenchyma development in wetland plants is able to supply all the oxygen needed for aeration in the apical meristem tissues is an unresolved question that is neglected in most published work. Crawford (1982) argued that even plants with extensive aerenchyma would not be able to meet all the oxygen demand of the root system and metabolic adaptations may still play a necessary role in tolerating long-term anoxia. The results from ADH measurements in the current study support this hypothesis although the levels of activity were relatively low and only evident under the most reducing treatments. This suggests a degree of oxygen stress was induced but not at levels sufficient to significantly impair the overall development of plants. It would be expected that a severe oxygen deficit in the apical meristem tissues would be reflected in the inhibition of root elongation and there was evidence to indicate a noticeable, but not statistically significant, effect in the final root lengths at the Eh -150 mV (Table 5.2). It is possible that an increase in redox intensity to levels below -150 mV could further increase ADH activity reflecting increasing reliance on anaerobic respiration under very severely reducing conditions at levels of redox intensity that are commonly encountered in waterlogged sediments.

There have been only a relatively small number of studies that have attempted to measure the relationship between redox intensity, aerenchyma formation and ADH activity. However, a similar response was found in these studies suggesting that the phenomenon of concurrent ADH activity with extensive aerenchyma formation may be more widespread than currently appreciated but has been previously overlooked because it may occur only under sufficiently low Eh conditions. For example, Pezeshki *et al.* (1991) reported a three-fold increase in root ADH activity at Eh -50 mV to +70 mV in *Spartina patens* compared to aerobic treatments with concomitant extensive aerenchyma formation and subsequently a four-fold increase at Eh -110 mV (Pezeshki *et al.* 1993). This corroborated an earlier investigation that found an initial dramatic increase in ADH activity in *S. patens* within a few days of flooding was followed by a subsequent decline as root aeration increased but even at maximal aerenchyma development (50% at 29 days) a continuation of ADH activity was recorded. This indicated that an increase in ROL could accompany an increase in root porosity to such a degree that it overwhelms the capacity of a plant to provide roots, even with substantial aerenchyma tissue enhancement, with sufficient oxygen to support aerobic respiration completely under conditions at very low redox potentials (Burdick & Mendelssohn 1990). A similar finding was also made for *S. alterniflora* where even with the detection of oxygen diffusing from the root tips, it was shown that internal transport was not sufficient to sustain aerobic respiration and consequently *S. alterniflora* needs to respond metabolically by increasing ADH activity (Mendelssohn *et al*. 1981).

In the case of *S. patens*, the indication of root oxygen stress given by the elevated ADH activity was also reflected in a significant reduction in the rate of photosynthesis measured by a decline in the rate of carbon assimilation and stomatal conductance thus demonstrating how ROL induced by low redox intensities has the potential to influence plant growth. Increase in ROL in response to low redox intensity has also been associated with reduced root growth in *S. patens* with a consequent reduction in the size of roots as a sink for photoassimilates that was suggested could, in part, be responsible for a negative feedback inhibition of photosynthesis resulting in a reduction in productivity for this species (Pezeshki & DeLaune 1990).

Although the same parameters for photosynthesis were not measured for *Triglochin*, it can be assumed from the results obtained from biomass measurements that the overall gross rate of photosynthesis was largely unaffected with no decrease belowground productivity and only small and statistically insignificant decline in aboveground productivity recorded at Eh -150 mV. There are two possible conclusions that can be drawn from the increase in ADH activity in *Triglochin*: Either the oxygen stress induced at high redox intensities developed at a late stage after an earlier relatively unstressed period when most of the biomass had already accumulated; or that the stress affected only a limited part of the root system. The latter proposition suggests *Triglochin* roots have lower metabolic demands (hence lower rates of oxygen consumption) and/or possesses a more effective barrier to ROL than plants such as *S. patens* that experience a significant decrease in photosynthesis under highly reducing conditions. The possibility exists, however, that if *Triglochin* was tested at a lower reducing intensity of Eh ≤ -200 mV, levels commonly encountered in waterlogged sediments in the current study, this may have resulted in higher rates of ROL and consequently higher levels of ADH activity and a significant reduction in productivity.

6.7.1.4 Root hairs and mycorhizzal fungi

Another feature relating to the root anatomy of *Triglochin*, although not investigated in detail in the current study, was the appearance of differences in the density of root hair structures between the aerobic and anaerobic treatments. A higher density of root hairs compared to the aerobic control became evident in the anaerobic treatments within 48 hours of the initial dosing with TiCi which was also apparent from examination of SEM images taken at the end of the experiment (Figures 5.10 & 5.11). Without a more detailed investigation it is not possible to more than speculative inferences in relation to any adaptive significance. While the presence of root hairs in *Triglochin* has previously been reported in common with a majority of semi-aquatic species examined by Shannon (1953), there is very little available information on the developmental response of root hairs to oxygen deficiency and no published studies that have investigated the affect of low redox intensities on root hair development.

One theory suggests that root hair development induced under anoxic conditions is a consequence of root growth inhibition from the insufficient availability of oxygen to meet the high energy demands involved in cell division at the root tip (Elzenga & van Veen 2010). Such a strategy is based on optimal deployment of limited resources since it entails a low carbon cost per unit of soil exploited. A possible mechanism for root hair formation induced under anoxia may come from the plant hormone ethylene and its precursor ACC, since this was found to enhance root hair formation in *Arabidopsis* (Pitts *et al*. 1988). This may be significant in relation to hypoxic induction of root hair development since ethylene is well known to be a primary signalling molecule that activates a range flood-adapted growth responses in plants as a consequence of its exogenous and endogenous accumulation under waterlogging from its physical properties as a gaseous compound with low aqueous diffusivity (Visser *et al*. 1996; Voesenek & Sasidharan 2013).

In comparison, the root hairs that were observed in plants from the aerobic treatment appeared to be at lower density. Furthermore, in a number of the SEM images the root hairs were difficult to discern and it is possible that some of these structures may have been misinterpreted with the possible presence of arbuscular mycorrhizae (AM). This was noted only on roots of plants in the aerobic treatment but not on those in any of the anaerobic treatments although remains somewhat tenuous as *Triglochin* was germinated from seed so it is unclear how plants could have been inoculated with AM in a laboratory setting.

The ability of AM to survive in conditions of persistent anoxia and low redox potentials and the implications for plant colonisation on saltmarshes is poorly understood. The apparent sensitivity of AM to such conditions was indicated by the negative correlation between AM infection and low Eh described in a number of studies on aquatic plants reviewed by Khan (1995, 2004). There is evidence suggesting that this relationship could also apply to saltmarsh plants but the implications for plant growth and survival remains unclear. For example, the high marsh species *Spartina patens* and *Distichlis spicata* tends to form AM associations (Cooke *et al*. 1993) but AM associations were reported to be generally absent in the low marsh species *S. alterniflora* (Hoefnagels *et al*. 1993). Similarly, a decrease in AM colonisation has also been reported in the saltmarsh species, *Aster tripolium*, in host plants sampled from low marsh compared to plants sampled from higher marsh locations and also under experimental conditions of waterlogging (Yallop 1998). Furthermore, a negative correlation between the occurrence of AM and root hair abundance and length has also been described in several studies where a number of aquatic species devoid of root hairs were found to have AM associations while in other species that possessed root hairs an opposite relationship was observed (Khan 2004). Aspects of root anatomy development and mycorrhizal association under oxygen deprivation and low redox potentials are an understudied area that requires further investigation.

6.7.2 *Atriplex*

In contrast to *Triglochin*, which demonstrated a clear ability to maintain growth in conditions long-term anoxia and low redox potentials, growth in *Atriplex* under similar conditions was severely limited under all anaerobic treatments. The imposition of anoxia and low redox potentials failed to induce an increase in porosity. This supports previous studies which also reported constitutively low levels of porosity in *Atriplex* plants subjected to experimental waterlogging ((Justin & Armstrong 1987; Mohamed 1997). Indications of photosynthetic functioning provided by chlorophyll fluorescence parameters, demonstrated for the first time, the limits of tolerance of *Atriplex* to conditions of continuous anoxia and low redox intensities is confined to a period of no more than 2-3 weeks before the onset of significant irreversible photoinhibition becomes manifest. It was at this stage that a rapid decline commenced in the capacity for photochemical energy conversion accompanied by a corresponding rapid increase in the loss of capacity of protective regulatory mechanisms to process excess excitation energy as a consequence of the progressive closure of PSII reaction centres due to inhibition of electron transport. It does not appear to be long after this time that the damage to the photosynthetic apparatus of a majority of leaves on any individual plant is sufficiently severe and widespread to cause inevitable whole plant mortality since no plant from any of the anaerobic treatments was able to recover on subsequent return to oxygenated conditions. However, evidence from an earlier trial experiment showed that *Atriplex* is able to recover normal levels of photosynthetic functioning on return to oxic conditions after a period of 10 days of root oxygen deprivation and redox intensities as low as Eh -150 mV.

No evidence was found to support the hypothesis that *Atriplex* experienced a greater degree of oxygen stress at lower redox potentials and hence higher oxygen demands since parameters for photosynthetic efficiency and non-photochemical quenching were not significantly different between the nitrogen deoxygenated and the highly reducing TiCi treatments. This suggests prolonged oxygen deprivation alone is sufficient to induce mortality and that increased levels of reducing intensities over this duration do not hasten or exacerbate the induction of detrimental effects. The results from this experiment demonstrated that *Atriplex* has a higher sensitivity to oxygen stress in comparison to that inferred in previous observations of long-term survival with restricted growth under conditions of experimental waterlogging (Mohamed 1997). Survival under waterlogging is not necessarily equivalent to an ability to tolerate long-term anoxia because under such conditions the possibility exists that the plant does not experience a sufficiently severe degree of oxygen deprivation under these conditions. This can occur either because the roots are able to avoid oxygen stress by superficial rooting or are able to exploit aerated microhabitats within the rooting medium.

On the other hand, compared to intolerant non-wetland species that typically exhibit signs of oxygen stress within a relatively short period of hours to a few days of oxygen deprivation (Crawford 1994; Jackson & Drew 1984; Pezeshki 2001), the results also suggests *Atriplex* appears to have the necessary metabolic adaptations that enables medium term tolerance to severe oxygen deprivation. Rather than being categorised as being intolerant of oxygen stress and low redox potentials, *Atriplex* appears to have a degree of anoxia tolerance limited to around 2 weeks. This finding is consistent with the results from extensive field redox measurements collected earlier in the current study where vigorous and dominant growth of *Atriplex* occurred in areas of the saltmarsh that experienced temporary and seasonal anoxia and low redox potentials on sediments that are characterised by efficient drainage. In contrast, *Atriplex* was found to be generally excluded in areas that showed persistent anoxia and low redox potentials. In comparison with *Triglochin*, *Atriplex* displayed a much faster rate of growth and therefore it is reasonable to assume that most of its metabolic resources are invested in a strategy of rapid germination and high rates of growth that enables it to rapidly colonise well-drained sites faster than, and to the exclusion of, relatively slow growing saltmarsh species that invest much of their energy into adaptations that facilitate survival in the areas of the saltmarsh which experience higher levels of oxygen or salinity stress.

6.7.2.1 Fv/Fm and photoinhibition

The gradual decline in Fv/Fm for the initial 2-3 weeks is notable and requires more detailed consideration since it signifies that *Atriplex* does not experience significant irreversible damage to the photosynthetic apparatus in the medium term. Instead, it suggests *Atriplex* is likely to possess the specific metabolic adaptations that allows for a greater degree of tolerance to oxygen stress than intolerant species that, in common with *Atriplex*, lack the necessary anatomical adaptations to avoid oxygen stress but are typically characterised by a drastic reduction in Fv/Fm within the short-term. Rather, the onset of a rapid decline was delayed for a period that is coincident with the duration of temporary anoxia that occurs when well-drained sediments are flooded. Moreover, an absence of variation in responses between redox intensities in this experiment lends support to the likelihood that *Atriplex* is tolerant severe oxygen stress over the medium term.

In order to interpret the pattern of a gradual decline in Fv/Fm during the initial 2-3 week phase of the experiment followed by a subsequent phase of drastic decline in the final week in all anaerobic treatments it is necessary to consider how photoinhibition causes Fv/Fm to decrease. Photoinhibition is a consequence of the susceptibility of the photosynthetic apparatus to damage by excessive light and is described as a state of physiological stress caused by a light induced impairment of the activity of the PSII complex (Osmond *et al.* 1997). In healthy plants photoinhibition occurs continuously as a normal regulatory response to changing light conditions involving rapid cycles of degradation and repair of D1 protein integral to the PSII reaction centre such that equilibrium is maintained and, as a consequence, a steady state concentration of active PSII centres is observed. However, if light-induced damage exceeds the capacity for repair then overall PSII activity decreases causing photoinhibition. Various environmental stresses that cause a reduction in ATP can initiate an imbalance in the homeostasis of the D1 protein as a result of the excessive production of ROS that limits the rate of repair thus lowering the photosynthetic yield (Murata *et al.* 2007). In particular, severe stress situations initiate the formation of the highly reactive singlet oxygen radical that causes oxidative degradation of the protein environment of the electron transport chain leading to the actual inhibition of the PSII electron transport and irreversible photodamage to the PSII reaction centres (Murata *et al.* 2007).

The molecular basis for photodamage is complex but the current consensus has resolved a two-step process. First by donor side photoinactivation by excess light destruction of the oxygen of the oxygen evolving complex of PSII, and second, by acceptor side photoinactivation of the photochemical processes of the PSII reaction centre by light absorbed by chlorophyll (Murata *et al*. 2007; Vass 2012).

The main mechanism is thought to take place due to impairment of the acceptor side of the PSII reaction centre that occurs when a stress limits the capacity of light-independent photosynthetic processes to utilise the electrons generated in the light-dependent reactions. Under unstressed conditions electrons flow from the initial photoreactions to NADP which is reduced to NADPH and subsequently enters the Calvin cycle to reduce the final electron acceptor, CO_2, in the process of carbon assimilation. Thus any stress that impairs carbon assimilation processes in the Calvin cycle and inhibits the regeneration of NADP will restrict the transfer of electrons away from the reaction centre at a fast enough rate leading to an overloading (overreduction) of the electron transport chain from excessive excitation that induces the enhanced production of harmful ROS (Vass 2012).

In parallel with this excitation energy dependent mechanism the donor side photoinhibition is thought to be initiated by the direct effect of light on the oxygen evolving complex of PSII. Photoinactivation here occurs when electron donation from the water oxidising Mn_4Ca cluster is unable to maintain a fast enough rate of electron withdrawal leading to the accumulation of ROS that inhibits the repair of photodamaged PSII by suppressing primarily the synthesis of proteins *de novo* (Murata *et al*. 2007). Thus both donor and acceptor side photoinhibition is a consequence of excessive irradiance exacerbated by the physiological impairments resulting from environmental stress that leads to conformational alterations and possible degradation to the D1 protein and to irreversible photo-oxidative damage to chlorophyll.

The gradual decline in Fv/Fm that commenced from the onset of oxygen stress and continued over the medium term therefore represents a gradual increase in photoinhibition and indicates a small reduction in the ability of *Atriplex* to maintain the equilibrium between rates of D1 protein degradation and repair and hence only a small loss in photoprotective capacity. An indication that the photoinhibition appears to be reversible over the medium term was given in a trial run of this experiment where exposure to 10 days of N-deoxygenated treatment did not cause fatality in

Atriplex. In the actual experiment, it was not until well into the third week that the mean Fv/Fm values for the anaerobic treatments began to fall below the level of 0.8 below which is generally considered to represent sub-optimal PSII functioning in most plants (Baker 2007; Maxwell & Johnson 2000).

Further evidence indicating a degree of tolerance over the medium term was provided by the parameters for both regulated and unregulated non-photochemical quenching which indicated only slight overall loss of PSII photoprotective capacity. It was not until the drastic increase in Y(NO) that occurred in the anaerobic treatments in the third week that capacity of photoprotective mechanisms became exhausted indicating the start of irreversible photodamage to the PSII complexes and consequently the point of maximal duration of sub-lethal oxygen stress *Atriplex* is able to tolerate. The rapid decline in Fv/Fm from the third week is thus assumed to represent the widespread collapse of the D1 protein homeostasis functioning and the start of cumulative large-scale destruction of photosynthetic apparatus that results from the loss of antioxidant capacity and the accumulation of ROS that outstrips the capacity for D1 protein synthesis and repair (Murata *et al*. 2007). At this stage the severe photo-oxidative damage was reflected in visibly widespread signs of stress in form of leaf chlorosis, early senescence and abscission in the majority of *Atriplex* plants in all anaerobic treatments. There was no obvious evidence to suggest that these visible symptoms developed at an earlier stage or to a greater extent in the most reducing treatment compared to the least reducing treatment.

The phase of gradual decline in Fv/Fm in *Atriplex* in the current study is interpreted to be a consequence of root oxygen stress that causes a decrease in photosynthetic capacity by processes that limit CO_2 assimilation leading to inhibition of the repair of D1 and enhanced ROS generation. This can occur by a reduction in leaf CO_2 concentrations from a decrease in stomatal conductance and/or from the metabolic inhibition of photosynthetic dark reactions resulting from a decrease in Rubisco catalysed carboxylation efficiency (Baker 2008; Vass & Aro 2012). Either of these two processes occurs from impairment of the water and nutrient uptake and transport functions of the roots that result from the inability of anaerobic respiration to supply sufficient levels of ATP to the roots (Morales & Warren 2012; Tournaire-Roux *et al*. 2003). The absence of an early rapid decline in Fv/Fm therefore raises the question of how, in the medium term, *Atriplex* is able to maintain a sufficient rate of CO_2 assimilation in the leaves to avoid the inhibition of electron transport that causes enhanced formation of ROS and irreversible photodamage.

The absence of a rapid short-term reduction in Fv/Fm In *Atriplex* indicates a different response compared to that expected when flood sensitive species are exposed to oxygen stress. Less tolerant species commonly exhibit internal water stress that can manifest within hours or a few days that induces stomatal closure, a reduction in leaf CO_2 concentrations and a concomitant drastic decrease in the rate of photosynthesis (Else *et al.* 2001, 2009; Sojka 1992; Sojka *et al.* 2005). It is somewhat ironic that such symptoms of oxygen stress induced by a surplus of water from waterlogging resemble symptoms induced by water stress under drought conditions. However, here the internal water stress is a consequence of reduced water uptake thought to result from the disruption of aquaporin functioning as a consequence cytosolic acidosis induced by insufficient ATP supply from anaerobic respiration leading to a reduction in both root permeability and hydraulic conductance (Tournaire-Roux *et al.* 2003). More tolerant wetland plants show a range of responses, for example, some species show a rapid initial reduction in stomatal conductance and eventual recovery following the development of anatomical adaptations such as the formation of adventitious roots or increased porosity (Pezeshki *et al.* 1996; Pezeshki 2001); while in others, initial stomatal closure occurs in the absence of significant changes to the plant water status as a response to ABA induced regulation of transpiration rates or from a downregulation in photosynthetic capacity induced by restricted growth rates under conditions of prolonged oxygen deficiency (Jackson & Hall 1987; Sojka 1992).

Although clearly visible signs of wilting and leaf dehydration in the newest leaves on some *Atriplex* plants in the anaerobic treatments were apparent by the end of the second week, in the absence of measurements of stomatal conductance it is unknown if a degree of internal water stress may have been present at an early stage. Even if this information was obtained, its contribution to decreased carbon assimilation can be difficult to assess without knowledge of the relative contribution from the non-stomatal metabolic controls on the inhibition of carbon assimilation that can also be induced by oxygen stress. The latter occurs because in addition to the causing water stress, the impairment of root functioning causes major alterations to the nutrient status from reduced uptake and transport processes (Morales & Warren 2012).

The resulting nutrient deficiencies are known to have a key influence on the operation of processes involved in both the light and dark reactions of photosynthesis. In particular, a reduction of nitrogen allocation between Rubisco and other Calvin-cycle enzymes can reduce carboxylation capacity and decrease CO_2 assimilation (Irving *et al*. 2007; Morales & Warren 2012). Nitrogen deficiency is also associated with a reduction in the synthesis of chlorophyll and the pigment-protein molecules leading to increased levels of photoinhibition (Godde 1999; Morales & Warren 2012). In addition, deficiencies in a range of other macronutrients have also been associated with reduced carbon assimilation by impairment of the photosynthetic electron transport chain either by reducing PSII photochemistry and decreasing carboxylation efficiency accompanied by ROS accumulation and oxidative photo-oxidative damage to the photosynthetic apparatus (Morales & Warren 2012; Smethurst *et al*. 2005).

There is evidence indicating control of photosynthesis under a variety of environmental stresses can result from a combination of stomatal and non-stomatal processes (Morales & Warren 2012). With regard to oxygen stress, the relative contribution from each may therefore change as a consequence of variation in severity of the oxygen demand the roots experience at different redox potentials and also the duration of the oxygen stress. In the case of *Atriplex*, it is possible that nutrient deficiency may have made an increasingly significant contribution over time and may have played a key role in hastening mortality rather than the ongoing effects from the decrease in carbon assimilation from stomatal conductance. Without detailed investigations of stomatal conductance and measurements of enzyme activities involved the Calvin cycle such as Rubisco, which were beyond the scope of this study, it is only possible to use Fv/Fm infer that *Atriplex* appears to be able maintain a degree of root functionality sufficient to sustain a rate of electron transport that enables the avoidance of significant photodamage in the medium term.

6.7.2.2 Mechanisms for medium term anoxia tolerance

Further evidence for the maintenance of a sufficient rate of electron flux in the medium term comes from the parameter for the effective quantum yield of PSII photochemistry Y(II). In addition to providing an indication of the proportion of light used in photochemistry Y(II), is also equivalent to the quantum yield of linear electron flux (LEF) through the PSII reaction centres which is related to the quantum yield of CO_2 assimilation (Genty *et al*. 1989; Maxwell & Johnston 2000). This parameter provides an indication of the capacity of the plant to utilise the products of LEF downstream of PSII with regard to ATP and NADPH consumption that prevents the over excitation of the electron transport chain that leads to photoinhibition. Since consumption of these metabolic products is influenced by the supply of atmospheric CO_2 to the site of carboxylation via the stomata any environmental stress that negatively impacts the rate of CO_2 assimilation is likely to be reflected as a decrease in Y(II) (Baker 2008; Vass & Aro 2008). In this context, it is notable that while there were large differences in growth rates in *Atriplex* between the aerobic control and plants in the anaerobic treatments which essentially ceased during the first two weeks of the experiment, this was not accompanied by a proportionate decrease in Y(II). A reduction in growth was expected to lead to a reduction in Y(II) and a corresponding increase in Y(NPQ) as a consequence of the downregulation of photosynthesis as a regulatory response to the loss of sink capacity from much reduced levels of carbon consumption.

This finding contradicts the expected outcome for *Atriplex* since in common with other species that lack the necessary anatomical adaptations that enable the continuation of growth under conditions of anoxia, a rapid decrease in Y(II) within hours to a few days has been reported for a number of intolerant species, for example, *Solanum lycopersicum* (Else *et al*. 2009) and in a waterlogging sensitive variety of *Medicago sativa* (Irving *et al*. 2007). In contrast, no decrease in in Y(II) was observed under anaerobic conditions in better adapted species such as *Triglochin* in the current study and other wetland plants that have been assayed using this parameter including *Phragmites australis* (Li *et al*. 2007). While an absence of a decrease in Y(II) in these better adapted wetland plants is easier to understand the maintenance of high rates of electron transport in the medium term in the absence of growth in *Atriplex* is harder to explain.

Since the extent of photodamage is largely regulated by the capacity of metabolic processes to utilise the electrons produced in the primary photoreactions, the presence of alternative sinks likely explains the absence of rapid decline in LEF as indicated by Y(II) and the upstream electron flow that controls Fv/Fm (Miyake 2010). In the absence of more detailed investigations in the current study, it is possible only to speculate on the nature of the two most probable alternative sinks either of which could operate as metabolic adaptations that enables *Atriplex* to survive oxygen stress in the medium term. The relative significance of these alternative sinks is likely to be dependent on the degree of stomatal conductance suggesting that that the regulation of leaf CO_2 concentrations may be an important factor in surviving oxygen stress for *Atriplex.*

If *Atriplex* is able to maintain sufficiently high rates of stomatal conductance, the rate of carbon assimilation is likely to be only slightly reduced. This may be sufficient to maintain a degree of photosynthetic capacity to supply of photoassimilates necessary to support a range of adaptive metabolic processes required both for maintaining the high energy demands associated with anaerobic respiration and neutralising the negative metabolic side-effects that originate under fermentative pathway. On the other hand, if stomatal conductance is sufficiently low to reduce the leaf CO_2 concentrations in C_3 plants like *Atriplex*, the process of photorespiration may become a more significant factor resulting in a change in the relationship between Y(II) and CO_2 assimilation as the proportion electrons consumed to drive CO_2 assimilation relative to photorespiration decreases (Baker 2007; Maxwell & Johnson 2000).

Depending of the rate of stomatal conductance photorespiration can replace up to 50% of the photosynthetic efficiency but rather than being a wasteful process, by providing an alternative electron sink, photorespiration can, under certain stress situations, provide a possible adaptive function to prevent dangerous levels of photoinhibition causing photodamage to the photosynthetic apparatus (Osmond *et al.* 1997). This photoprotective mechanism occurs since the increase in the rate of oxygen reduction in photorespiration consumes the surplus ATP and NADPH that is no longer consumed in carbon reduction, thereby maintaining the balance in the production and consumption of ATP and NADPH that is necessary to prevent the overreduction of the PSII electron transport chain that would otherwise leads to excessive photoinhibition (Osmond *et al*. 1997).

The results from the current study highlights the potential anomaly between Y(II) and carbon assimilation in C_3 plants experiencing oxygen stress and in the absence of an estimate of the rate of photorespiration makes this chlorophyll fluorescence parameter difficult to interpret. In common with many species that lack the necessary constitutive anatomical structures for avoiding oxygen stress means that it is likely that *Atriplex* does experience a decrease in carbon assimilation from a loss of sink capacity but to what extent is impossible to say since the decreased electron flux to drive photosynthesis is likely, to some extent, to be masked by an increased electron flux diverted for O_2 reduction in photorespiration. Measurements of chlorophyll fluorescence in combination with analysis of photosynthetic gas exchange have previously been used to estimate rates of photorespiration based on partitioning of photosynthetic electrons between CO_2 and O_2 reduction in order to derive a more meaningful interpretation of changes in Y(II) as an indicator of stress induced stomatal control of photosynthesis (Cornic & Briantais 1991). Such dual approach is therefore recommended for future investigations into the physiological responses of C_3 plants to oxygen stress in plants.

In the absence of measurements of rates of stomatal conductance and photorespiration it is possible only to tentatively conclude that maintenance of the electron transport rate in the medium term is an adaptive response to sustain the high level of photosynthetic capacity necessary to support the high energy demands for anaerobic tolerance over the short-medium term. However, the alternative hypothesis that a greater proportion of the electron flow is accounted for by photorespiration and makes a necessary and significant contribution to photoprotection remains a plausible explanation worthy of further examination. Indeed, photorespiration is now appreciated as an important stress response by preventing the accumulation of ROS (Voss *et al*. 2013) and a number of studies have assigned an important photoprotective role for photorespiration based on the relationship between decreased photosynthetic capacities from drought induced water stress (Osmond *et al*. 1997;

Morales *et al*. 2008). Its significance as mechanism for tolerance to oxygen stress remains little reported making this a potentially productive area for future research. In addition, the role played by alternative electron sinks under conditions of suppressed photosynthesis from oxygen stress such as, carbohydrate partitioning in leaves (Godde 1999), the water-water cycle and the cyclic electron flow around PSI in chloroplasts (Miyake 2010) in the alleviation of photoinhibition and preventing photo-oxidation remains to be clarified.

6.8 Conclusion

Most previous studies that have attempted to investigate plant responses to oxygen stress based on experimental soil waterlogging have failed to give rigorous consideration to the contribution of low redox potentials to plant physiological responses. The redox potential in such soil based systems is difficult to control and there is always the possibility of introducing unintended effects from redox phytotoxicity. Nitrogen deoxygenated hydroponic systems are suitable for testing relatively intolerant species such as *Atriplex* which respond negatively to conditions of oxygen deprivation before the onset of low redox potentials. This method may not be sufficient to replicate the degree of oxygen stress better adapted saltmarsh species such as *Triglochin* naturally experience under prolonged exposure to low reducing conditions. This experiment has successfully demonstrated the application of an improved system that addresses this limitation by allowing the possibility of testing plant responses at any levels of redox intensity that are encountered in saltmarsh sediments. In addition, the modification made in this experimental design has demonstrated it is possible to attain a higher level of consistency in maintaining more or less stable redox conditions than previous designs and that it is feasible to scale up experimental treatments to simultaneously test a dozen or more plants at one time.

CHAPTER 7: General discussion

The main aim of this study was to investigate the significance of sediment redox potential (Eh) as an important abiotic influence that could limit colonisation on saltmarshes restored by managed realignment (MR) by a number of halophyte species that are often found to be abundant on natural saltmarshes. This was achieved by using a multi-channel redox datalogger for more detailed spatial and temporal measurements of Eh than previously obtained for saltmarsh sediments and by investigating the responses of some of these plants to experimental manipulations of Eh in field and laboratory experiments. It was anticipated that the current research would contribute to understanding the underlying reasons why plant communities on natural and restoration saltmarshes are frequently found to be different (Wolters *et al*. 2005; Garbutt & Wolters 2008; Mossman *et al.* 2012a,b). An understanding of factors that determine the divergent development of vegetation on artificially created saltmarshes is important for informing interventions that aim to improve their wildlife conservation value and to maximize the range of crucial ecosystem services these diminishing habitats can provide (Roman & Burdick 2012; Zedler 2001).

7.1 Summary of key findings

1) Periods over several months duration of continuous anoxia and low Eh indicative of poorly drained sediments were found to be present in sediments of communities dominated by *Spartina anglica* and *Aster tripolium* that occur low in the tidal frame in both natural and MR marshes. However, similar conditions were also found to be present in the relatively species-rich general saltmarsh communities (GSM) that are common in the upper marshes of the north Norfolk coast. This suggests that plants growing in the GSM communities may be tolerant of extended periods of oxygen deprivation and low Eh.

2) Well drained sediments are characterised by cyclical phases of anoxia and low Eh of several days duration in response to tidal flooding and drainage. Communities occupying these sediments tend to be dominated by *Atriplex portulacoides* and vigorous growth of *Puccinellia maritima* and occur close to drainage channels on the

upper marshes of the north Norfolk coast and are also abundant in better drained areas of MR sites.

3) Physical properties of sediments exert a strong influence on drainage and Eh. This was indicated by the presence of Eh characteristics typical of well-drained sediments of an inner marsh site in the absence of close proximity drainage channels at Freiston MR. Conversely, Eh conditions more representative of poorly drained sediments were present in close proximity to a drainage channel at Brancaster MR. The most oxic sediments were found to occur in the *Atriplex* dominated communities of the natural marsh at Freiston despite receiving more than twice the frequency of inundations as the upper marshes on the north Norfolk coast.

4) Differences in Eh characteristics were evident between MR sites of similar developmental ages and at similar elevations. This suggests differences in the physical properties of sediments are present that could be a consequence of their different reclamation histories.

5) Sediments demonstrated strong seasonal patterns of anoxia and low Eh. Conditions of anoxia and low Eh generally prevailed from summer to late autumn months while oxic conditions prevailed from winter to spring. There is evidence indicating that the seasonal pattern is less pronounced in lower marsh sediments of natural and MR marshes since low Eh conditions were found to be present at an earlier stage of the year.

6) GSM species experimentally planted in MR marshes showed differential survivorship which was correlated with long-term mean Eh. It was hypothesised that while GSM and MR sediments exhibit similar redox intensities as indicated by the measured low Eh values, they differ with respect to the amount of sediment undergoing reduction – the redox capacity. It was proposed that MR sediments of *Spartina-Aster* dominated communities have a higher capacity for reduction which imposes additional stresses on plant functioning. It is further hypothesised that the lower capacity of reduction of GSM sediments could be a consequence of lower frequencies of tidal inundation. Such conditions could result in lower rates of microbial activity either from the rate of oxidant depletion in anaerobic respiration

outstripping the rate of oxidant replenishment by tidal flooding, or microbial inhibition from the generation of hypersaline sediments.

7) Limited colonisation of MR marshes by the GSM species can result from both low and high Eh conditions that occur in MR sediments. Sediments characterised by low Eh and a high capacity for reduction are more likely to impose abiotic constraints from the higher demand for rhizosphere oxygen and the accumulation of reductants, most likely in the form of bioavailable sulphide, to phytotoxic concentrations. Sediments characterised by high Eh are likely to impose biotic constraints by creating favourable conditions for vigorous growth of the more competitive species, *Atriplex portulacoides* and *Puccinellia maritima*.

8) Artificially recreating diverse communities that resemble the GSM community is likely to be impractical due to the particular geochemical conditions associated with GSM sediments. Such conditions limit growth and dominance by any one species and thereby promote higher levels of coexistence. These conditions develop at upper marsh elevations that experience a relatively low frequency of tidal inundations and are unlikely to develop on MR marshes that are usually at a lower elevation in the tidal frame. Of the GSM species investigated, *Limonium vulgare* and *Triglochin maritima* respond well when transplanted to MR marshes, while *Armeria maritima* and *Plantago maritima* respond poorly. There may be scope to enhance the floral diversity of MR marshes to a limited extent by artificially seeding with young plants and by engineering microtopographical variation in a way that ameliorates the stresses imposed by sediments with high redox capacity properties.

9) Laboratory hydroculture of plants under controlled redox intensities was undertaken in order to gain a better understanding about the tolerances of plants to the effects of increased rhizosphere oxygen demand at low Eh. The limit of tolerance to anoxia for *Atriplex* was established to be around two weeks and no additional stress effect was evident at low Eh. Chlorophyll fluorescence measurements indicated *Atriplex* was able to maintain close to normal levels of photosynthetic efficiency during this period. Low levels of root porosity and the absence of stress effect from low Eh suggests that *Atriplex* does not rely on internal transport of oxygen to roots but has the necessary metabolic adaptations to survive this period of anoxia. In contrast,

Triglochin has high volumes of root porosity and was able to survive at least 10 weeks of anoxia and low Eh to -150mV without any decline in photosynthetic efficiency. High root porosity was found to be constitutively well developed and not enhanced by low Eh. Evidence form ADH analysis suggests that internal transport of oxygen to roots might not be sufficient at the lowest Eh treatments and may also need to rely on a degree of anaerobic metabolism for survival.

10) Plant responses under experimental conditions were consistent with field Eh measurements associated with their distributions further supporting the hypothesis that Eh has a significant influence on the composition of plant communities. While a good understanding of plant responses to anoxia and low Eh can be gained using this experimental technique for less tolerant species like *Atriplex*, a fuller understanding for tolerant species like *Triglochin* can only be achieved by investigating their responses to both redox intensity and redox capacity factors.

7.2 Redox potentials on natural and MR saltmarshes

Extensive measurements of Eh over a range of temporal scales represent the first attempt to describe the dynamic nature of this variable in response to tidal flooding in both natural and restoration saltmarshes in order to gain further insights into the influence of this environmental variable on plant distributions. The findings build on earlier work showing that the relationships between frequency and duration of tidal flooding, Eh and species distributions at different topographic elevations on MR sites may not always be straightforward (Davy *et al.* 2011). The current study showed that the imperfect relationship between elevation and Eh could be of greater importance on natural marshes than previously appreciated. This suggests that factors that influence the water retention characteristics of sediments relating as physical properties and microtopography at any given elevation are likely to have stronger influence on the distributions of some species on natural marshes than frequency and duration of flooding. This is in agreement with other studies which have reported the influence of sediment properties and microtopography on Eh in determining community composition and structure (Crooks *et al*. 2002; DeLaune *et al*. 1983; Ewanchuk & Bertness 2004a; Varty & Zedler 2008). Consequently, differences in Eh conditions between MR marshes and natural marshes were shown to be more complex than previously understood. Moreover, the influence of a seasonal effects was revealed for the first time in marshes on the North Norfolk coast and MR marshes at Brancaster and Freiston Shore, indicating the presence of late summer-autumn Eh minima and late winter Eh maxima. These findings potentially have important implications, not only for understanding the relationship of Eh with saltmarsh vegetation, but also for future work on the biogeochemical functions of these ecosystems (Andrews *et al.* 2006; Shepherd *et al*. 2007).

Not unexpectedly, some of the lowest Eh values and the most persistently reducing conditions that demonstrated the least tendency to return to oxic conditions at the end of the flooding cycle, were recorded at the sites sampled lowest in the tidal frame. At Stiffkey, these occurred in the largely undrained *Spartina* dominated sediments of the low marsh where mean Eh values of around –200 mV were recorded at all measured depths which is well within the theoretical zone where sulphate is reduced to potentially toxic concentrations of sulphides (Reddy & DeLaune 2008). Highly reducing sediments that demonstrated a large degree of persistence throughout the tidal cycle were also recorded at the lowest elevations on the MR sites where *Spartina* and *Aster tripolium* are dominant. These communities cover a substantial area of both MR sites and are also a common feature of many other restoration marshes (Garbutt & Wolters 2008; Mossman *et al.* 2012a) underlining the tendency of sediments that may have experienced physical alteration during reclamation to develop conditions of waterlogging (Crooks & Pye 2000; Tempest *et al.* 2015) and low redox potentials (Davy *et al.* 2011; Mossman *et al.* 2012a,b).

However, it was also shown that similarly persistent low Eh conditions could also occur at higher elevations on natural marshes that experience far fewer tidal inundations (Chapter 3). Initial recordings of Eh in the summer of 2012 of a GSM site with co-dominant *Armeria maritima*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima* indicated these sediments can readily attain highly reducing Eh conditions with limited evidence of return to oxic Eh values in the post flooding phase. The greater persistence of low Eh during 2012 might have been attributed to higher levels of waterlogging from an exceptionally wet summer but subsequent measurements made during the exceptionally dry summer of 2013 confirmed a similar pattern of persistent and highly reducing Eh. This finding differed markedly from Eh measurements made at an equivalent time of the year on a GSM site with a similar species composition on a Humber estuary saltmarsh (Armstrong *et al.* 1985). Conditions in these sediments were found to be highly reducing for several days followed by a rapid return to an oxic state indicative of well drained sediments. In contrast conditions more or less continuous anoxia appear to be sustained over several months in the GSM sites sampled on the North Norfolk coast indicating these sediments are poorly drained. This characterisation is consistent with an earlier study demonstrating the high water content of GSM sediments which was ascribed to the low bulk density that results from the high organic matter content of these sediments (Jefferies 1977). These findings support other work where modelling has indicated the important influence of soil physical properties and/surface morphology on the water retention characteristics of saltmarsh sediments areas away from drainage channels (Moffett *et al.* 2012 Li & Lockington 2005; Ursino *et al.* 2004; Xin *et al.* 2010, 2012).

A particularly significant finding was strong evidence of a counter-intuitive seasonal influence on Eh contradicting the assertion that flood induced anoxia is more adverse in the winter (Crawford 2003). Most sites where Eh was measured, both on the natural and the MR marshes, showed a consistent pattern of oxic conditions prevailing in the winter with increasingly anoxic and reducing conditions developing throughout the growing season leading to a low point in late summer-autumn. Measurements of Eh in sediments of the GSM, for example, indicated that moderate-severely reducing Eh could extend over a period of 4-5 months from late summer to well into autumn. Although Armstrong *et al.* (1985) alluded to the existence of a seasonal effect, their assumption was that anoxia was likely to be maximal in the winter and spring when sediments are most saturated. However, this was not supported in the current study. at least in the portion of the sediment profile that appears, from visual examination, to comprise much of the rooting zone. There was no evidence that the bulk of the root biomass was limited to superficial depths. It is during this period that rates of oxygen consumption in the sediment are likely be lowest due to the cessation of plant growth, the reduction in microbial activity and the increased diffusion coefficient and higher solubility of oxygen as a consequence of low sediment temperatures. However, a strict association between Eh and temperature was not always clear in this study supporting the possibility an important additional influence on Eh processes from the seasonal availability of an electron source in the form of labile organic carbon from root exudates (Howarth & Hobbie 1982; Koretsky *et al.* 2005).

The observed seasonal patterns accords with similar patterns found in saltmarshes for seasonal variations in sediment oxygen concentrations that were recorded to be lowest in summer and highest in winter months (Baumann *et al.* 2015) and where iron and sulphate reduction was also found to reach a summer-autumn maxima and winter-spring minima (Abdollahi & Nedwell 1979; Feijtel *et al.* 1988; Koretsky *et al.* 2003; Koska & Luther 1995: Nedwell & Abram 1978). On the other hand, different seasonal patterns in redox biogeochemical cycling have been reported in other saltmarshes where significant plant induced sediment oxidation in the summer months has been reported (Giblin & Howarth 1984; Luther & Church 1988; Sundby *et al.* 2003).

Well-drained sites that are characterised by strongly oscillating Eh between oxic and anoxia levels in response to cyclical phases of flooding and drainage were described previously (Armstrong *et al.* 1985). Similar patterns were observed in the current study where sediments of the creek bank communities dominated by *Puccinellia* and *Atriplex* of the upper marsh elevations on the North Norfolk coast experienced periods of temporary anoxia at all measured depths soon after flooding. However, measurements from early summer to autumn showed that Eh in these sediments underwent a strong seasonal effect presumably reflecting, in part, sub-surface lateral drainage from the low Eh conditions of the GSM. It was shown that with the progression of summer, conditions of low Eh became increasingly pronounced down the sediment profile with the duration of the anoxic phase extending well into the neap tide phase several days after the cessation of tidal flooding. Furthermore, it was shown that by late summer the period of anoxia between flooding tides could be longer than the period of return to an oxic state. It therefore appears that much of the vegetation occupying these creek bank communities is exposed to conditions where sediment oxygen availability may occur in relatively short intervening ‘pulses’ lasting four or five days between extended phases of anoxia. It is possible that the restricted availability of oxygen in this creek bank site limited the dominance by *Atriplex* and enabled a greater abundance of *Puccinellia* which is better adapted to tolerate anoxia (Justin & Armstrong 1987).

Atriplex is dominant in well drained sediments where the period of flood induced anoxia is relatively transient, such as sites close to the edge of a creek bank in the Humber estuary (Armstrong *et al.* 1985). Similar conditions were recorded on the natural saltmarsh at Freiston Shore which were found to be the most oxidised sediments encountered in this study, despite experiencing more than twice as many inundations per annum as the upper marsh sites on the North Norfolk coast; this again illustrated the unreliability of elevation relative to the tidal frame as an exclusive predictor of sediment Eh. Sediments at this site experienced a temporary period of flood-induced anoxia where mean anoxic Eh values at all measured depths persisted for a few days after the final inundation followed by a rapid recovery to oxic Eh values. The current study showed that under suitable edaphic environments such Eh conditions could also occur at a sampling location over at 5-10 m distance from the edge of drainage channel. This contrasted with the anoxic Eh conditions that persisted in the GSM sediments measured at a comparable proximity to a drainage channel. The oxic status of these sediments is clearly reflected in the vegetation dominated by a dense growth of *Atriplex* and vigorous growth of the annual *Suaeda maritima* which are two species that lack the anatomical adaptations to avoid prolonged exposure to anoxia (Justin & Armstrong 1987). Moreover, it is consistent with the experimental work in the current study where it was established that limit to survival of *Atriplex* exposed to continuous anoxia was little more than two weeks.

The current study is the first to compare Eh dynamics in response to tidal flooding and drainage between natural and restoration saltmarshes. Two contrasting MR sites were sampled which differed with respect to their reclamation histories. The *Atriplex-Puccinellia* dominated communities on the Freiston MR were shown to demonstrate Eh responses that were comparable to the well drained sediments recorded on its *Atriplex*-dominated natural counterpart. This suggests it may be possible to restore similar sub-surface hydrological conditions on relatively recently reclaimed sediments that underlie large areas of this site where *Atriplex* is abundant. However, Freiston MR is unrepresentative of most MR sites, since the saltmarsh here has developed on newly accreted marine sediments overlying older saltmarsh sediments that were reclaimed for a relatively short period of 20 years (Nottage & Robertson 2005). Consequently, these sediments may not have experienced the same degree of irreversible changes to their physical structure that lead to the altered sub-surface hydrology associated with older reclaimed sediments Crooks *et al.* 2002; Tempest *et al.* 2015). Saltmarshes on many MR sites that have developed on older sediments that may have undergone periods of more than a century of dewatering and oxidation from historical land claim, can result in their transformation to soils with increased density and reduced porosity (Hazelden & Boorman 2001). In particular, reclamation of clay-rich calcium deficient sediments can result in a breakdown of soil structure and the formation of a dense and impermeable horizon that acts as an aquitard forming an effective impediment to drainage (Crooks & Pye 2000). The influence of such a feature could persist for decades and possibly indefinitely following tidal restoration. Colonisation by a number of perennial species can be inhibited by the resulting waterlogging and low Eh (Crooks *et al.* 2002) and also the adverse conditions arising from the lack of topographic heterogeneity that have been associated with over consolidated sediments (Brooks *et al.* 2015).

Well-drained sediments with abundant *Atriplex* on the Freiston MR were recorded to have similar Eh conditions both in the lower marsh in close proximity to an artificial drainage channel and in a central area of the upper marsh some 80 m distant from the nearest drainage channel. This indicates that well drained sediments indicated by fluctuating Eh in response to flood-drain cycles not only occur close to drainage channels but can also be found in interior areas of the marsh in the presence of suitable sediment microtopography and physical properties. However, the effectiveness of drainage channels on MR sites was not always clear. Some channels have stands of *Atriplex* extending up to 10 m from the bank while others have extensive stands of low marsh vegetation with *Atriplex* restricted to the very edge of the channel. This indicates that drainage channels may not always function to efficiently drain sediments with an over-consolidated horizon or possess other physical properties that confer high water retention properties.

The limited influence of drainage channels in such circumstances was evident at the Brancaster MR, where the existence of a compacted relict soil horizon, possibly as a consequence of the longer period of reclamation, was previously alluded to by Mossman *et al.* (2012b). Measurements of Eh at a mid-elevation site within a few metres from the edge of a drainage channel found that oxic Eh was limited to the upper 5 cm during the growing season, whereas severely reducing Eh persisted at lower depths. The greater prevalence of low Eh in the sediment profile at this site compared to the channel site at the Freiston MR was reflected in the vegetation with *Atriplex* restricted to the edge of the channel and a higher abundance of *Puccinellia*.

In addition to an assessment of Eh conditions in vegetation communities on natural and MR saltmarshes, the measurements at a higher resolution than previously attempted also revealed in greater detail the dynamic nature of Eh in response to tidal flooding. It showed that anoxia development following the onset of tidal flooding could occur over a period of hours, although this rate of change could be obscured by the slow equilibration times demonstrated by the electrodes. It also revealed that the return from a highly reducing to an oxic state can occur over a period hours, following a prolonged anoxic delay of several days. In addition, the occurrence of diel fluctuations in Eh was discovered and this was attributed to the close proximity of the electrode tip to oxidising rhizospheres. There was no clear evidence to support plant-induced oxidation of the bulk sediment in any of the sites, contrary to what has been reported in sediments occupied by *Spartina alterniflora* in north America, for example (Dacey & Howes 1984; Howes *et al.* 1981, 1986).

In regard to the usefulness of using Eh measurements to characterise the intensity of the anaerobic status of saltmarsh sediments, the method of continuous hourly Eh measurements highlighted the potential problem of the long time (up to several days) taken by electrodes to stabilise after their installation. Such long electrode equilibration times have previously been reported for terrestrial soils (e.g. Eshel *et al.* 2002), suggesting that the common method of taking measurements after short equilibration times (often 5-15 minutes) may not always represent the true Eh condition of the substrate, so interpretation of results using this approach needs to be treated with caution. However, a wide variation has been reported with regard with time recommended for electrode stabilisation e.g. 5-10 minutes (Ponnamperuma 1972) to 24 hours (Reddy & DeLaune 2008) suggesting this is likely to vary with the substrate and equipment. The insufficient time given for electrode stabilisation may explain the high variation that is often reported from readings both from electrodes in close proximity to one another and from repeated measurements from the same electrode which is often attributed to environmental heterogeneity (Fiedler *et al.* 2007). Continuous Eh measurements of sediments in the current study showed that, while considerable variation in Eh readings was frequently encountered even after a long equilibration period, on many occasions similar readings were obtained from electrodes both at the same depth and between depths in the sediment profile. This suggests a high degree of uniformity in Eh conditions, particularly where sediment conditions are highly reducing, may be commonly encountered. This demonstrates that despite reservations about using this technique expressed by some researchers (e.g. Lindberg & Runnells 1984), reproducible readings are attainable supporting the validity of this method.

7.3 Assessing the potential role of phytotoxicity in limiting species colonisation from low redox potentials

In addition to potential stresses generated from the increased demand for the oxygen supplied to the rhizosphere, low redox potentials can also potentially impose additional stresses resulting from the accumulation of reduced end products of these reactions: Mn^{2+}, Fe^{2+} and dissolved sulphides to phytotoxic concentrations. Redox potentials well within the theoretical values based on Nernst thermodynamic equilibrium equations for manganese, iron and sulphate reduction were commonly encountered in sediments of both natural and MR marshes. Despite the divergent, non-idealised conditions of sediments that are unlikely to attain redox equilibrium due to continuous additions of oxidants and reductants, measured Eh values are thought to give a reasonable indication of the likely presence of the dominant redox processes in highly reduced wetland soils (Bohn *et al*. 2001; Fiedler *et al*. 2007; Reddy & DeLaune 2008). Differential tolerances to these phytotoxins may potentially have an important influence in determining the extent which species become established on MR marshes. However, estimating the likely significance of phytotoxicity based on the obtained field Eh measurements is problematic since there are few available studies from which inferences can be made.

Only a small number of studies of manganous (Mn^{2+}) and ferrous (Fe^{2+}) ion phytotoxicity have been undertaken for saltmarsh plants and there is little evidence to date to suggest that either plays an important role in determining the establishment of species. Manganous ions can generally occur in higher concentrations than in more terrestrial soils, e.g. up to $35\mu g\ g^{-1}$ (Singer & Havill 1985). Saltmarsh species were shown to exhibit differential responses but most were found to be tolerant of higher concentrations than present in saltmarsh sediments (Cooper 1984; Rozema *et al*. 1985b; Singer & Havill 1985). Iron is usually present in sediments at higher concentrations than Mn and Fe reduction and may play a more important role in carbon mineralization relative to sulphate reduction than previously thought, accounting for 50-100% of anaerobic metabolism in saltmarshes (Tobias & Neubauer 2009). Plants grown in culture solution also showed differential sensitivities to Fe^{2+} and although lower marsh species were found to be generally more resistant, high tolerances were shown by a number of upper marsh species too (Rozema *et al*. 1985b; Van Diggelen 1991).

None of these studies were able to demonstrate a clear relationship between metal tolerance and position in the marsh consequently there is insufficient evidence to suggest that either Mn^{2+} or Fe^{2+} are likely to be important ecological factor for saltmarsh plants. It is noteworthy, however, that *Puccinellia maritima* was reported to show a similar degree of Fe^{2+} tolerance to *Spartina* which was higher than that showed by *Aster* (Rozema *et al*. 1985b). This is potentially significant since *Puccinellia* occurs at low abundances in the *Spartina-Aster* communities of the MR sites, it suggests that sulphide phytotoxicity could be a plausible factor limiting its colonisation in addition to the effects of prolonged anoxia and elevated oxygen demands from low Eh. Indirect support for the possibility that these *Spartina-Aster* communities develop in sulfidic sediments comes from dose-response experimental work which showed *Spartina anglica* has a high degree of tolerance to concentrations of 500 µM Na_2S (Van Diggelen *et al*. 1987). Moreover, other studies have shown *Aster* has a moderate level of tolerance to at least to 100 µM while *Puccinellia* was found to be relatively sensitive, tolerating only short duration exposure to sulphide under hydroculture (Ingold & Havill 1984; Havill *et al*. 1985; Pearson & Havill 1988).

Whether or not sulphide can accumulate to ecologically significant concentrations in sediments that experience relatively transitory low Eh, typically extending 5-7 days in *Atriplex-Puccinellia* communities, or over somewhat longer periods in the surficial *Spartina-Aster* sediments, is uncertain. Even in the more stagnant *Spartina-Aster* sediments, prolonged periods of sulphate reduction may not always result in the accumulation of sulphides in bioavailable form. These occur in dissolved form as "free" sulphides according to pH-dependent dissociation of hydrogen sulphide: $HS^{-}\Leftrightarrow H_2S\Leftrightarrow S^{2}$, each of which is potentially phytotoxic at low concentrations (King 1988; Koch & Mendelssohn 1989; Lamers *et al*. 2013; Otero & Macias 2002). Instead, the major short-term end products of sulphate reduction may accumulate in a less bioavailable state neutralising its potential for phytotoxicity. These occur several forms including: polysulphides, elemental sulphur and a number of precipitated metal sulphides such as iron monosulphide (FeS) and pyrite (FeS_2). The nature of the predominant end products that tend to accumulate largely depends on the reaction kinetics of pyrite formation which varies depending on the particular properties of the sediment (Ponnamperuna 1972; Schoonen 2004).

Without appropriate sampling and analysis, it is not possible to determine the relative contribution of each of the possible end products in any given sediment undergoing sulphate reduction. It is also difficult to make reliable inferences from other studies since there have been so few attempts to measure free sulphides in comparable minerogenic saltmarsh sediments. In the case of British saltmarshes, dissolved sulphide concentrations up to 400 µM were recorded only in *Salicornia* low marsh sediments and at higher mean concentrations in unvegetated creek beds and salt pans (Ingold & Havill 1982). No free sulphide was detected in sediments of other vegetation communities but measurements in this study were limited to surficial (0-5 cm) depths potentially omitting a significant part of the rooting zone. The importance of sampling to greater depths was illustrated by the recording of variable but generally low (< 40 µM) dissolved sulphide concentrations to 15cm which increased up to a maximum of 200 µM at 30 cm in a *Spartina anglica* marsh in the Netherlands (Oenema 1990b). The only other studies based on British saltmarshes, so far as the author is aware, reported the presence of free sulphides at deeper sediment depths (20 cm) but sampling was restricted to salt pans and creek beds rather than vegetated sites (Nedwell & Abram 1978, 1979; Senior *et al*. 1982). Interestingly, these studies noted maximum sulphide concentrations occurred during late summer to autumn months which accords well with the general seasonal Eh trends reported in the current study.

In contrast, the sulphide characteristics of the more organogenic sediments of North American *Spartina alterniflora* saltmarshes have been more intensively studied with contradictory reports of the relative importance of short and long-term end products of sulphate reduction. Several studies have indicated rates of pyrite formation and loss by oxidation are much faster than for sub-tidal marine sediments since they are driven by very different processes with *Spartina alterniflora* playing a key role. Here, the rapid formation of pyrite occurs over a timescale of hours-days and comprises 70-90% of short-term end products of sulphate reduction (Giblin 1988; Howarth 1979; Howarth & Teal 1979; Howarth & Giblin 1983; Howarth & Merkel 1984; Luther *et al.* 1982). High levels of pyrite formation were also reported for *Spartina anglica* sediments (Oenema 1990b). Since the solubility product of pyrite is low, much of the free sulphide fraction in these sediments would therefore be sequestered and maintained below phytotoxic concentrations (Howarth & Teal 1979). However, other studies have reported slower rates of pyrite formation and consequently high concentrations (> 1-5 mM) of bioavailable sulphide (Carlson & Forrest 1982; Howarth *et al.* 1983; Howes *et al.* 1984, 1985; King 1988; King *et al.* 1982; 1985; Skyring *et al.* 1979) suggesting pyrite is not necessarily the major short-term end product of sulphate reduction in saltmarsh sediments. It is unclear if the reported differences for pyrite formation rates could be attributed to inconsistencies in methodology with regard to sampling and analysis, or natural variability depending on the particular properties of sediments undergoing sulphate reduction.

It may also be possible for high concentrations of free sulphide and pyrite to simultaneously occur in the sediment profile at different depths. Such a scenario has been attributed to the rhizosphere oxidising capacity of *S. alterniflora* and *S. anglica* that creates optimal conditions for rapid pyrite formation within a narrow range of depths representing the interface between oxidising and reducing sediments (Giblin 1988). Within this zone it is thought that a fraction of the dissolved sulphide reacts with Fe^{2+} to form iron monosulphide FeS, while another fraction oxidises to form elemental sulphur which together react to form pyrite (Oenema 1990b; Lord & Church 1983). This optimal zone of pyrite formation was found to be restricted in *Spartina alterniflora* sediments at 7-9cm depths (Lord & Church 1983) and in *Spartina anglica* sediments in the Netherlands at a depth of 15-20 cm (Oenema 1990a,b). Clearly, the relationship between low redox potentials and presence of phytotoxins, particularly with regard to sulphide is not straightforward and requires further investigation.

7.4 Prospects and limitations for enhancing vegetation diversity on MR sites

In Chapter 5 it was shown from results of experimental planting of *Armeria*, *Atriplex*, *Plantago*, *Limonium* and *Triglochin* on MR sites that their survival was strongly correlated with mean Eh conditions measured over monthly spring-neap tidal cycles for a period of 18 months. Survival for all species decreased with lower mean Eh, and *Armeria, Atriplex* and *Plantago* showed little ability to survive the lowest mean Eh conditions on these MR sites. In addition, it was shown that survival of all species could be increased by artificially increasing mean Eh conditions to predominantly oxic Eh values by manipulating the microtopography in order to raise the surface elevation in a series of 1 m^2 plots. While the inability of *Atriplex* to survive prolonged periods of low Eh was not unexpected in the context of its association with predominantly oxic Eh sediments in the current study, the finding of interspecific differences in survival between plants all of which appear able to tolerate conditions of prolonged low Eh in the GSM communities was a somewhat contradictory and requires more detailed examination.

Of the target species, *Triglochin* and perhaps to a lesser degree *Limonium,* proved to be tolerant of the low Eh conditions on the MR sites. Both these experimentally planted species were able to survive in the *Spartina-Aster* dominated low marsh sediments of the Brancaster MR that were shown to be continuously reducing at very low mean Eh for much of the year. The possibility that these sediments could be characterised as sulfidic with respect to free sulphides also suggests that these two species may exhibit a certain degree of sulphide tolerance which needs to be tested experimentally. However, since these plants were several weeks old when they were transplanted, the possibility remains that their limited abundances in the low marsh sites could be attributed to establishment inhibition due to higher sensitivities to anoxia and/or the effects of low Eh (with regard to increased oxygen demand or phytotoxicity) during germination or at an early developmental stage. Evidence was presented that indicated sediments of *Spartina-Aster* dominated low marsh communities on MR sites experience low Eh conditions at an earlier stage of the year suggesting such conditions may inhibit seedling establishment in these sites. On the other hand, biotic constraints may play a more relevant role in explaining their limited abundances at mid-upper elevations on MR sites where *Atriplex* and *Puccinellia* tend to be dominant in the relatively oxidised sediments.

While the same biotic constraints may be operating to limit establishment of *Armeria* and *Plantago*, an explanation for their intolerance to conditions of low mean Eh on the MR sites is more elusive. There is little current knowledge about the sensitivity of these species to anoxia or low Eh, although a degree of tolerance is inferred from experimental waterlogging under glasshouse conditions for at least three months albeit with much reduced growth (Cooper 1982; Rozema *et al.* 1985). In seeking an explanation for the low survival of *Armeria* and *Plantago* in the reduced sediments of MR sites a more detailed consideration is required of what Eh actually represents as an operational parameter.

A notable, yet confounding finding in this study was the apparent prevalence of low redox potentials throughout much of the growing season in sediments both of the relatively diverse GSM communities and the generally species-poor communities that develop on MR marshes. In attempting to explain how similar measured Eh values could influence the development of such drastically divergent communities, it is necessary to emphasise the importance of a frequently overlooked characteristic of redox controlled processes is that they are defined by both redox *intensity* and redox *capacity* factors (Bohn *et al.* 2001; Grundl 1994; Reddy & DeLaune 2008). Measurements of Eh with a platinum electrode provide an indication of the redox intensity factor which represents the inherent voltage associated with a chemical species when it undergoes reduction. While this metric provides an indication of the redox status of the sediment based on critical Eh values at which an oxidised compound become unstable as defined by Nernst equilibria, it does not indicate how much of the sediment is undergoing reduction which defines its capacity for reduction (Pezeshki & DeLaune 2012; Reddy & DeLaune 2008). The capacity for reduction is determined by the degree the sediment can support anaerobic microbial respiration, which depends both on the availability of labile organic matter as an electron source and the concentrations oxidants (nitrate, ferric iron, manganese (hydr)oxides and sulphate) to accept electrons and thereby become reduced (DeLaune & Reddy 2005). Thus, while the two sediments of the GSM and the MR sites may provide similar Eh values for redox intensity, they may differ markedly in their relative capacities for reduction. Importantly, what this means is that sediments with a high capacity for reduction can potentially have a much larger volume of reduced chemical species than sediments that exhibit similar Eh values for redox intensities but have lower reduction capacities. Evidence from experimental work, reviewed by Pezeshki & DeLaune (2012), has demonstrated that, at any given level of redox intensity, sediments with a high capacity for reduction can create a more stressful environment for plant functioning since the higher volume of reduced species creates a higher level of rhizosphere oxygen demand and a higher level of potential phytotoxicity.

The possibility therefore exists that sediments of the GSM may have a lower reduction capacity than MR sediments and this may create environments with different levels of redox stresses which limits colonisation by certain species. It has previously been noted that GSM sediments of the North Norfolk coast have a low bulk density which was attributed to their high organic matter content (Jefferies 1977). The high organic matter content of these sediments suggests that, while they are unlikely to be limited in terms of an electron donor source, it also indicates these sediments experience low rates of carbon mineralisation which results in the accumulation of organic matter. Without further investigation, it is possible only to hypothesise that the low Eh GSM sediments are also characterised by a relatively low reduction capacity due to lower rates of organic matter decomposition and this can be attributed to two possible causes.

Firstly, it was estimated that GSM sediments receive fewer than half the number of tidal inundations of the MR marshes because of their higher position in the tidal frame. Such a tidal regime may not be sufficient to replenish the rapid turnover of oxidants involved in anaerobic metabolism. In order to maintain high rates of sulphate reduction, for example, it was suggested that a sufficiently high frequency of inundation would be necessary in order to replace the rapid depletion of sulphate (Howarth & Teal 1979). Secondly, the lower frequency of inundations results in sediment hypersalinity that can reach more than double seawater concentrations during the summer months (Jefferies 1977). While there have been few studies that have investigated the effect of hypersalinity on microbial activity in saltmarshes, there is evidence demonstrating that organic matter decomposition is rate limited at high levels of salinity equivalent to that experienced by GSM sediments (Hemminga *et al*. 1991). Although it possible only to be speculative at this stage, the inhibition of carbon mineralisation by either oxidant depletion or hypersalinity both could have the potential to lower the redox capacity of the GSM sediments and thereby reduce the stresses of anoxia on plant functioning.

The greater prevalence of waterlogging and low Eh on MR sites has been alluded to as possible abiotic constraints for the low abundances of a number of key species that are important components of the GSM communities - notably *Armeria maritima*, *Limonium vulgare*, *Plantago maritima* and *Triglochin maritima* (Garbutt & Wolters 2008; Mossman *et al.* 2012a). The finding that these species occupy sediments of the GSM that are not only experienced predominantly continuous anoxia commencing from late in the growing season and thereafter persisting for several months, but also experienced moderately to severely reducing Eh conditions at depths that encompass much of the rooting zone, raises some interesting questions regarding Eh as an inhibiting factor on MR sites.

Clearly, the measured redox conditions in the current study raise interesting questions about differences in the degree of sediment oxygen demand and nature and extent of phytotoxin accumulation in natural and restoration marshes. If the hypothesis is true and the low redox conditions recorded in the sediments of the GSM also possess a low capacity for reduction, then it follows that both the severity of oxygen demand and the accumulation of these phytotoxins will be attenuated since the fraction of the oxidants that undergo reduction will be relatively small. On the other hand, oxygen demand and phytotoxicity could play a more important role in the MR marshes if the capacity of reduction is less constrained in the absence of high levels of hypersalinity. It would therefore appear that the persistently highly reducing *Aster-Spartina* sediments would, in general, provide a favourable setting for phytotoxin accumulation supporting the possibility that these sediments are sulfidic. It was suggested that the appearance of low Eh in these sediments early in the year could create conditions of high oxygen and phytotoxin accumulations to levels that either could inhibit the germination or establishment of other species at a nascent developmental stage. Further questions also arise about the extent of phytotoxin accumulation in MR sediments that experience relatively transient low Eh conditions, mainly in the *Atriplex-Puccinellia* sediments but also apparent in the surficial *Aster-Spartina* sediments at Freiston MR and how this differs from the more persistently reducing *Aster-Spartina* sediments in general.

An understanding of the differences in sediment properties in terms of redox capacities may therefore assist in illuminating the possible abiotic constraints on the establishment of certain species on MR saltmarshes. The diverse assemblage of species that characterise the GSM communities appear to have developed as a consequence a unique combination of stresses imposed by hypersalinity and low Eh with low redox capacity. Such conditions limits growth and allows a high level of coexistence by ensuring no species becomes dominant. In contrast, different stresses generated by sediments characterised by low Eh and high redox capacity appear to operate in MR marshes. Such conditions are more prevalent in sediments of the *Spartina-Aster* communities since they experience extended periods of anoxia from higher frequencies and durations of tidal flooding. Experimental planting has demonstrated that both *Limonium* and *Triglochin* can tolerate these conditions to a certain extent and it may be possible to enhance their survival within this type of community by ameliorating the conditions associated with high redox capacities from artificially manipulating the microtopography. Stresses associated with high redox capacities are likely to be considerably attenuated in the sediments of the *Atriplex-Puccinellia* communities since they experience relatively transient periods of anoxia. There are indications from casual observation over the period of this study that both *Limonium* and *Triglochin* have naturally colonised and become well established within *Puccinellia* stands in both the Brancaster and Freiston MR marshes. In the case of the former, these species occur at higher abundances, likely due to the proximity of propagules and the greater prevalence of low Eh which appears to restrict the growth of *Puccinellia*. Conversely, the lower abundances of *Limonium* and *Triglochin* that have naturally colonised the *Puccinellia* stands at Freiston MR are probably a consequence of the lower availability of propagules and the greater vigour of *Puccinellia* from a reduced prevalence of low Eh conditions. Individual plants that have become established in the Freiston MR tend to be large and vigorous indicating the abiotic conditions of these sediments are very favourable for the growth of *Limonium* and *Triglochin*. A possible case can therefore be made for management interventions for enhancing the diversity of MR marshes with large *Puccinellia* stands by seeding these communities with transplants of these two species.

In contrast, the low survivorship of *Armeria* and *Plantago* in the MR sites suggests these species may be more sensitive to the increased stresses associated with sediments with higher redox capacity and also appear to be poorer competitors against *Puccinellia*. There is, however, some evidence from the experimental transplants indicating survival rates could be enhanced from microtopographical manipulations and this should be explored further with larger scale interventions. However, artificially raised microtopography, especially when sited at higher elevations on the marsh, often creates favourable conditions for vigorous colonisation by *Atriplex* at the expense of *Armeria* and *Plantago* in particular. Consequently, management interventions that aim to improve the drainage properties of MR sediments may not necessarily provide the requisite conditions for enhancing floral diversity. Ultimately, owing to the difficulty in attempting to artificially replicate the particular conditions in GSM sediments that are responsible for limiting the growth of other species, *Armeria* and *Plantago* are likely to always remain minor components in restoration saltmarshes that develop lower the tidal frame.

7.5 The physiological basis of plant responses to low redox potentials

An understanding of the physiological basis of a plant's capacity to survive low redox potentials can provide important insights into the differential limitations faced by species in establishing on restoration saltmarshes. While this stress factor has been tested on a small number of *Spartina* species (Kludze & DeLaune 1996 Pezeshki & DeLaune *et al*. 1984, 1990; Pezeshki *et al*. 1993), its effect on the functioning of other saltmarsh species remains largely unknown. While both the redox capacity and redox intensity factors can affect plant functioning by influencing oxygen demand in the sediment, their relative importance is often unclear (Pezeshki & DeLaune 2012). In order to address the lack of understanding of the response of saltmarsh plants to redox intensity, a laboratory experiment was devised based on a hydroponic method for long-term cultivation of plants under controlled redox intensities (Lissner *et al.* 2003a).

The results for *Atriplex* grown under experimental treatments of deoxygenated (+300 mv), mildly reducing (+150 mV) and severely reducing (-150 mV) built on what was already understood about the general intolerance of *Atriplex* to anoxic sediments (Atrmstrong *et al.* 1985; Cott *et al.* 2013; Mohamed 1998). Low porosity was previously reported in *Atriplex* (Justin & Armstrong 1987; Mohammed 1998) and this experiment demonstrated that it was not possible to induce enhanced porosity by exposure to increased oxygen demands at low redox potentials. Indications of photosynthetic functioning provided by chlorophyll fluorescence demonstrated for the first time that the limit of tolerance to conditions of continuous anoxia was limited to a period between 2-3 weeks, before the onset of irreversible photoinhibition. No evidence was found to support the hypothesis that *Atriplex* experienced a greater degree of oxygen stress at the treatments of higher oxygen demand, suggesting that prolonged periods of oxygen deprivation alone were sufficient to induce an adverse effect and low Eh did not appear to hasten or exacerbate the detrimental effects. However, chlorophyll fluorescence analysis also revealed that *Atriplex* did not show the typical response associated with anoxia-intolerant species, which tend to exhibit signs of oxygen stress within hours or a few days. In contrast, a significant decline in the Fv/Fm was delayed for over two weeks, agreeing with the field measurement of Eh in the current study, which indicated *Atriplex* was most vigorous and dominant only in areas of the marsh that experience temporary anoxia and low redox potentials for up to a maximum of around 10 days and was largely excluded from areas that were found to experience persistent anoxia.

A notable finding was that anoxia-induced growth inhibition in *Atriplex* was not accompanied by a downregulation of photosynthesis, as indicated by the absence of a decline in the parameter for effective quantum yield Y(II) which had previously been associated with a loss of sink capacity in anoxia-intolerant plants (Else *et al.* 2009; Irving *et al.* 2007). This allowed speculation about the nature of alternative sinks that may enable *Atriplex* to tolerate medium term exposure to anoxia. *Atriplex* may be able to maintain a degree of photosynthetic capacity in order to support the high energy demands associated with root anaerobic respiration that enabled a moderate degree of tolerance to anoxia. Photorespiration may be also be operating as an alternative sink since this is now appreciated as an important stress response by preventing photodamage from the accumulation of reactive oxygen species (ROS) (Voss *et al.* 2013). This experiment indicated that saltmarsh species like *Atriplex* that experience regular periods anoxia but lack the anatomical adaptations for long-term tolerance are likely to have the metabolic adaptations for medium-term anoxia tolerance making them interesting candidate species for future studies in biochemical adaptations to flood-induced anoxia stress.

Not unexpectedly, the response to anoxia and low Eh was found to be markedly different for *Triglochin*. The results confirmed the field observations in the current study of its ability to survive in sediments that experience prolonged and continuous anoxia and low Eh conditions. The findings were also agreement with previous studies of *Triglochin* survival in conditions of experimental waterlogging (Cooper 1982; Rozema *et al.* 1985b). None of the variables measured as performance indicators (leaf elongation rates, aboveground and belowground productivities, maximum height and root lengths) demonstrated a strong evidence of decline with increasing reducing intensity. This was supported by the chlorophyll fluorescence parameters which indicated no decrease in photosynthetic efficiency in any of the treatments over the ten-week duration of the experiment.

Other findings that emerged from this experiment included the lack of evidence of enhanced root porosity in response to low Eh: aerenchyma was found to be constitutively well developed in *Triglochin* and did not significantly increase at high redox intensities as a response to the increased oxygen demand. However, a low but detectable increase in ADH activity was measured at the lowest Eh treatments suggesting *Triglochin* appears to have reached to a threshold where it is unable to supply all the root system with sufficient oxygen. Whether or not the induction of anaerobic metabolism indicated by ADH was a stress response in unclear since there is little evidence to show this was translated into stress symptoms sufficient to impair the overall plant functioning in plants from the low Eh treatments.

This finding is similar to previous reports showing the co-occurrence of anaerobic metabolism with high levels of porosity in *Spartina* spp. exposed to similar levels of redox intensity as the current study (Burdick & Mendelssohn 1990; Mendelssohn *et al.* 1981; Pezeshki *et al.* 1991). Furthermore, this was in agreement with the assertion by Crawford (1982) that even well-adapted plants with extensive aerenchyma may not be able to meet the all the oxygen demand of the root system and metabolic adaptations may still play a necessary role in tolerating long-term anoxia. The possibility remains that while *Triglochin* may not be adversely affected by a high reducing intensity of Eh -150 mV, a high reduction capacity in the hydroponic culture solution can further increase the competition for the internal oxygen of the root and the oxygen of the rhizosphere thus creating a greater stress than induced by reducing intensity alone. Such an effect was demonstrated for *Spartina patens*, where redox capacity was increased by addition of organic matter as an energy source while maintaining a constant level of redox intensity (Kludze & Delaune 1995).

Although the use of titanium(III) citrate can provide useful insights for assessing the tolerance of saltmarsh plants to the effects of redox intensity, a complete evaluation of plant responses to tidal flooding requires both a quantification of both the redox intensity and redox capacity factors (Pezeshki & DeLaune 2012). Further work is needed in this area on a range of saltmarsh species in order to achieve a fuller understanding of the influence of redox potentials on their physiological functioning that can contribute to elucidating reasons for their differential distributions on natural and restoration marshes.

7.4 Concluding remarks and recommendations for future research

Saltmarshes restored by managed realignment frequently result in vegetation compositions that are different from their natural counterparts and there evidence from older reactivated marshes indicating these differences can persist into the long-term (Burd 1992; Garbutt & Wolters 2008; Mossman *et al.* 2012a). Differences in vegetation composition may have significant consequences for saltmarsh ecosystem functioning (Doherty *et al.* 2011). Meeting the requirements of the EU habitats Directive may therefore require additional management interventions that aim to facilitate convergence. However, the effectiveness of these interventions requires a rigorous understanding of the nature of both the biotic and abiotic factors that are likely to influence colonisation and the response of species to them.

The propensity of MR sites to develop sediments that experience excessive waterlogging and low redox potentials is assumed to be a major impediment that limits colonisation for a number of species common at upper elevations on natural marshes (Tempest *et al*. 2015; Crooks *et al*. 2002; Mossman *et al*. 2012a). The development of extensive areas of *Spartina-Aster* dominated communities in sediments characterised by persistently low Eh indicates such conditions are likely to be a major constraint for many potential colonising species in MR marshes that occupy positions low in the tidal frame. On the other hand, restoration marshes can also develop extensive areas dominated by *Atriplex* or vigorous growth of *Puccinellia* where the physical properties of sediments generate better drained conditions where exposure to low redox potentials is relatively limited. Contrary to expectations, it was shown that a number of species that are poor colonists of MR marshes occur at high abundances in sediments that naturally experience extended periods of low Eh that superficially resemble the low Eh conditions measured on MR sites.

This suggests that the assumption that management interventions should aim to generate well-drained, oxic sediments and high Eh as necessary prerequisites for the establishment of diverse vegetation may be misconceived. Measurements of Eh in this study have indicate that where such conditions often promotes vigorous growth by a few dominant species, notably *Atriplex portulacoides* and *Puccinellia maritima*, possibly at the expense of other potential colonists. This is supported by reports of analogous processes that appear to operating in the similarly diverse forb panne communities of North American saltmarshes (Ewanchuk & Bertness 2004a, 2004b). These communities structurally and floristically resemble GSM communities and there is evidence indicating they are maintained by harsh conditions created by the combined effects of waterlogging, low Eh and hypersalinity.

It appears likely that the persistence of GSM communities depends on similar abiotic influences that generate particular conditions of adversity that are fundamentally different in character from the adverse conditions found on MR sites. It is hypothesised that sediments of GSM and MR marshes could differ with respect to their capacity for reduction and this is likely to be higher in MR sediments since they experience higher frequencies of tidal flooding. The higher capacity for reduction has the potential to impose stress by generating conditions of anoxia with a relatively high oxygen demand and phytotoxicity. In contrast, stress in GSM sediments is likely to be characterised by the synergy of hypersalinity and anoxia with a relatively low oxygen demand.

A better understanding of differential species abilities to survive these stress factors is key to informing management interventions that aims to maximise the floral diversity of restoration marshes. Observations of vegetation development in raised microtography experiments at lower elevations on MR sites and natural colonisation of the mid-elevation marsh at Brancaster MR suggests a certain degree of exposure to low Eh may be beneficial in promoting the development of more diverse communities. This implies maintaining or facilitating the development of 'intermediate' conditions of low Eh somewhere between those prevalent in the *Spartina-Aster* and the *Atriplex-Puccinellia* sediments could achieve enhanced diversity. It is therefore proposed that the two stress factors of oxygen demand and phytotoxicity should remain a focus of future research in order to better understand the principal underlying causes for the limited colonisation on restoration marshes. Future research should also investigate the efficacy of larger scale microtopographical manipulations and other techniques for promoting the optimal Eh conditions in sediments for maximising floral diversity.

There is now a sufficient body of data that provides convincing evidence that increased oxygen demand imposed by low redox potentials can impair survival times and a range of physiological functions that influence growth and development (Pezeshki & DeLaune 2012). Conditions of increased oxygen demand are likely to variously impact plants that are otherwise well adapted to survive prolonged oxygen deprivation in sediments with a low oxygen demand and also at different developmental stages. Species that are able to develop extensive aerenchyma, including *Limonium vulgare* and *Plantago maritima* (Justin & Armstrong 1987), appear to be anatomically well equipped to survive anoxia stress, but may not be able to survive beyond a certain threshold of prolonged oxygen demand. The current study has demonstrated the feasibility of an improved experimental design for testing plant responses to controlled low redox potentials under long-term hydroponic cultivation. Further work is therefore needed to test a range of species that are poor colonists of restoration marshes to gain a more complete understanding of the influence of increased oxygen demand as an inhibiting factor.

Little is known about plant responses to redox capacity although there is evidence indicating this factor can exert a greater stress from oxygen demand stress that is additional to the effect from redox intensity (Kludze & DeLaune 1994). For plants like *Triglochin* that display a relatively high level of tolerance to low reducing conditions, this experimental system provides a useful basis for expanding research to test a range of further hypotheses particularly in relation to the effects of redox capacity.

Assessing the effects of phytotoxins remains poorly understood and this should be a further priority for future research. A difficulty presented by previous phytotoxicity-based studies is maintaining consistent concentrations of phytotoxins in their reduced state particular in association with plants that have a high capacity for radial oxygen loss from roots. The experimental system of controlled redox intensities using titanium(III) citrate as an efficient non-toxic oxygen scavenger makes such investigations a much more practical proposition and offers the prospect of opening up productive areas for future research. However, unless the extent of potential phytotoxins in sediments has been reliably determined for natural and restoration marshes, undertaking ecologically meaningful dose-response experimental work will remain problematic.

Another priority for future research should therefore be directed towards establishing the extent that measured Eh represents an indication of the sediment redox chemistry in both MR and natural saltmarsh sediments. Interpretation of measured Eh values from Nernst thermodynamic reaction potentials are based on a number of assumptions that may not be met in natural systems that do not conform to idealised conditions (Bohn *et al.* 2001; McBride 1994). Redox couples in sediments are unlikely to be at equilibrium and instead reactions of several redox couples are likely to be simultaneously operating. Consequently, instead of the measured Eh value representing the activity of one redox couple, the platinum electrode responds to all redox couples in the sediment and provides an average mixed potential instead (Bohn *et al.* 2001; McBride 1994). While field measurements of Eh have been significantly correlated with soil oxygen suggesting Eh measurements provide a reasonable indication of a soil's aeration status (Faulkner *at al.* 1989; Megonigal *et al.* 1993), there is little available information on the correlation with other redox couples in sediments. In anaerobic sediments at neutral pH, the measured Eh value is thought to provide a good indication of the presence of Mn^{2+} and Fe^{2+} since these ions are dominant in minerogenic soils (Reddy & DeLaune 2008; Bohn *et al.* 2001). On the other hand, electrodes do not respond well to other redox couples that do not react in a reversible manner, e.g. NO_3^-/N_2 and SO_4^{2-}/H_2S (Reddy & DeLaune 2008; Bohn *et al.* 2001). Sediments in both the GSM and MR sites readily attain Eh values of < -100 mV indicative of sulphate reduction but the extent this occurs and the degree free sulphides accumulate in either sediments remains unknown. Further investigations are therefore necessary to establish the presence of potential phytotoxins in sediments, particularly the degree to which sulphides accumulate in a bioavailable state.

Despite the intrinsic ecophysiological interest of plants with an unusual combination of adaptations that enable survival under multiple stresses from variable conditions of submergence, hypersalinity, phytotoxicity, oxygen deprivation and oxygen demand, it is somewhat surprising that saltmarsh plants have received relatively little attention in detailed physiological investigations. In addition to providing important knowledge that could potentially inform management interventions which aim to improve the ecological functioning and conservation value of restoration saltmarshes, studies of plant responses to low redox potentials could provide valuable insights for predicting the possible outcomes of saltmarsh vegetation communities to the effects of altered hydroperiods from the projected increases in sea levels. Moreover, the range of adaptations of saltmarsh plants to multiple stresses could be of potential significance to plant geneticists who are charged with improving traits to increase productivity in cultivated plants that are increasingly prone to experience the range of abiotic stresses to which saltmarsh plants are variously yet uniquely adapted.

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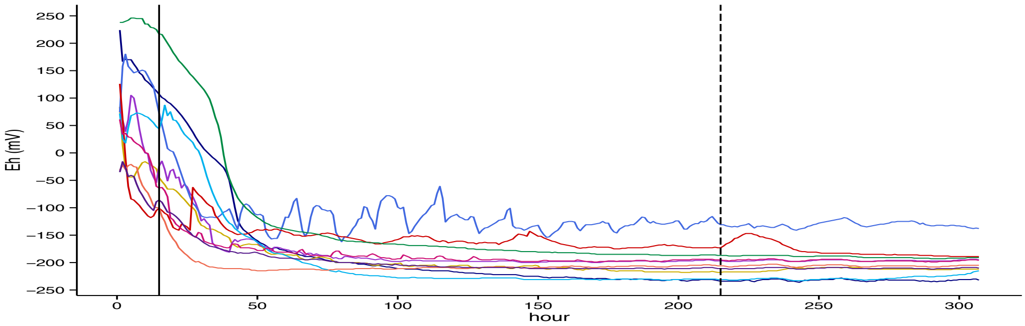
Appendix

a) 2 cm



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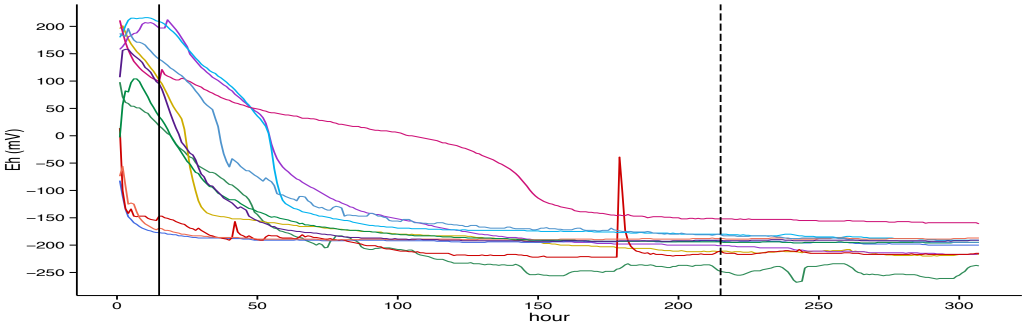

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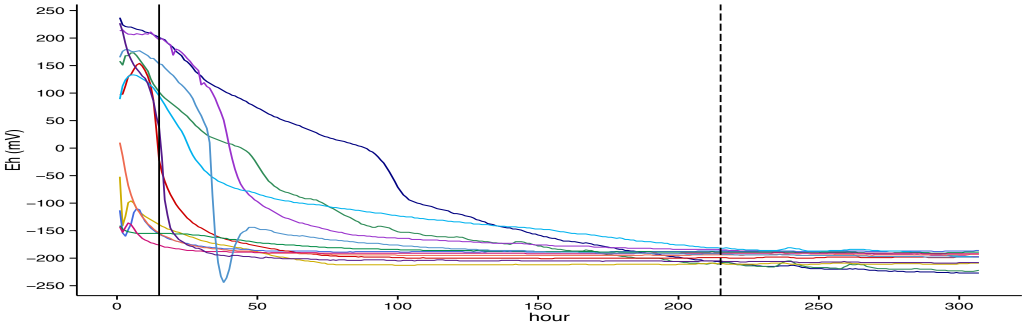

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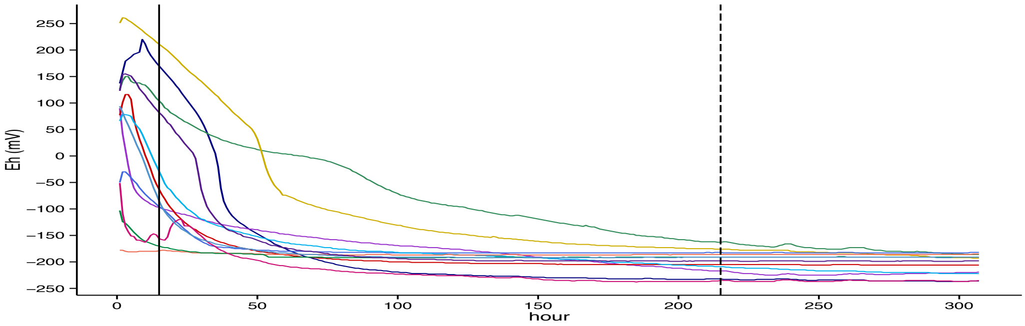
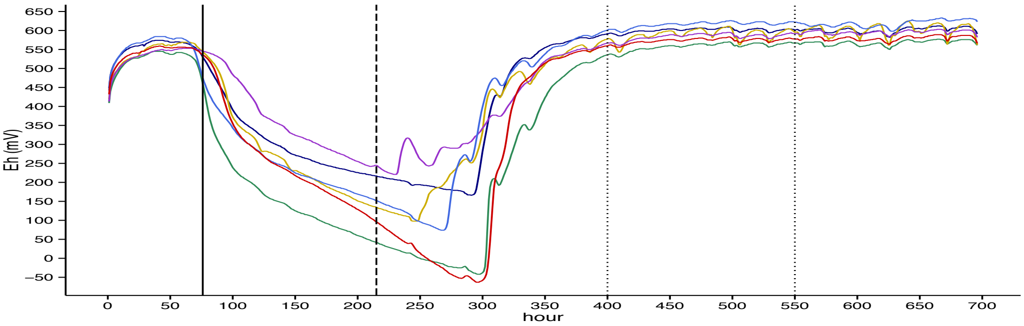


Figure 1. Eh measurements from replicate electrodes at four depths for the period 14 – 27 March 2014 at the Stiffkey low marsh site colonised by *Spartina anglica* at 4 depths in the sediment profile showing consistency of highly reducing Eh readings at all depths.

(a) 2 cm



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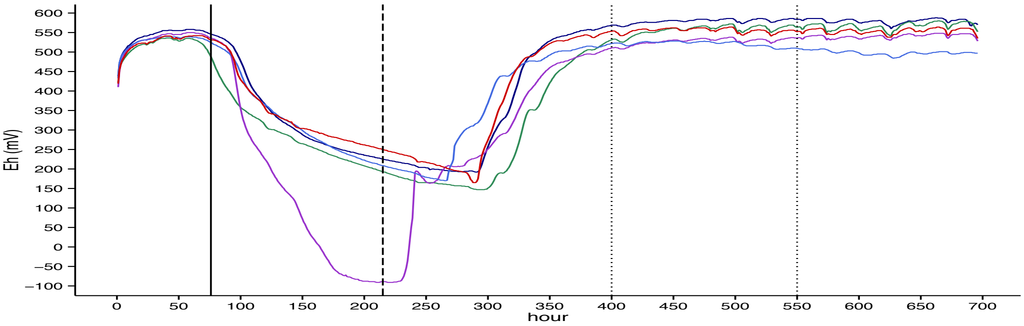

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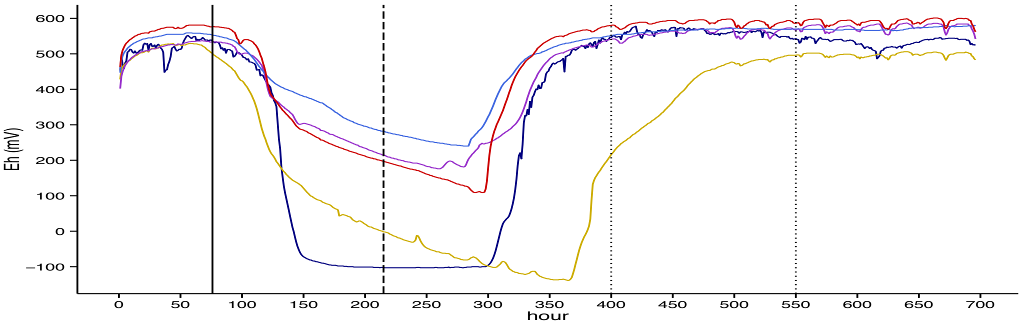


(d) 20 cm

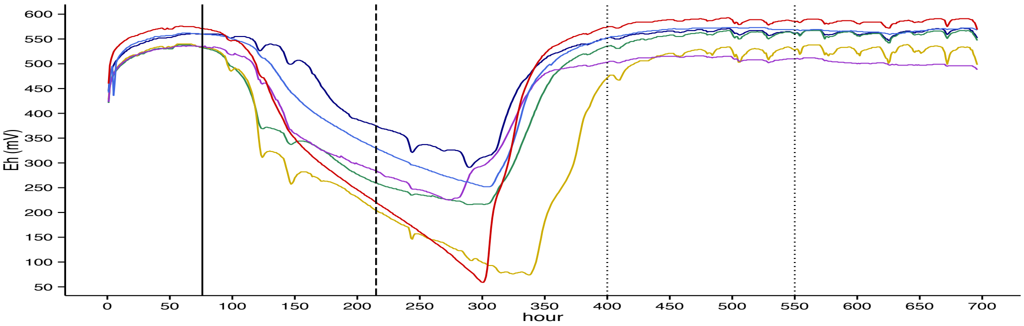
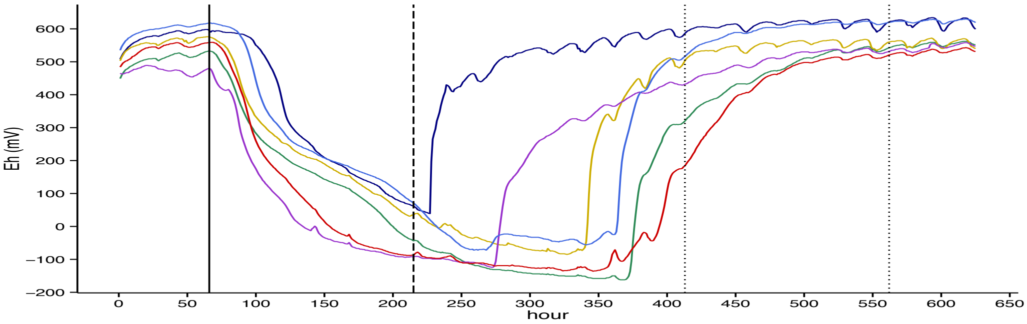
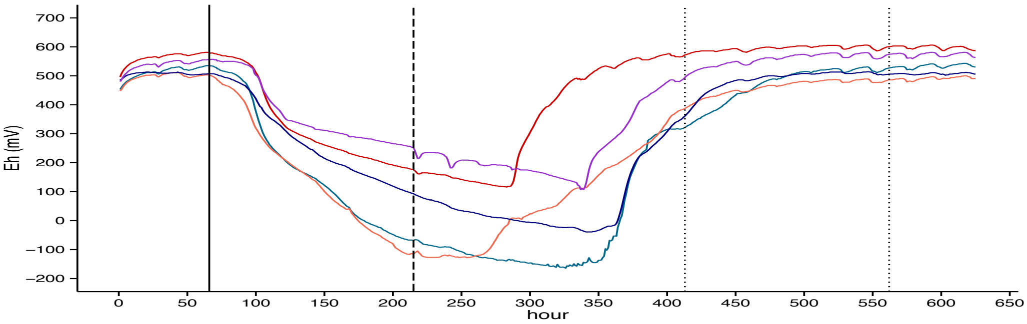


Figure 2. Eh measurements from replicate electrodes at four depths for the period 21 May – 19 June 2013 at the Stiffkey creek site demonstrating the redox characteristics of a well-drained sediment and the high degree of reproducibility of Eh readings at all depths.

(a) 2 cm



(b) 5 cm



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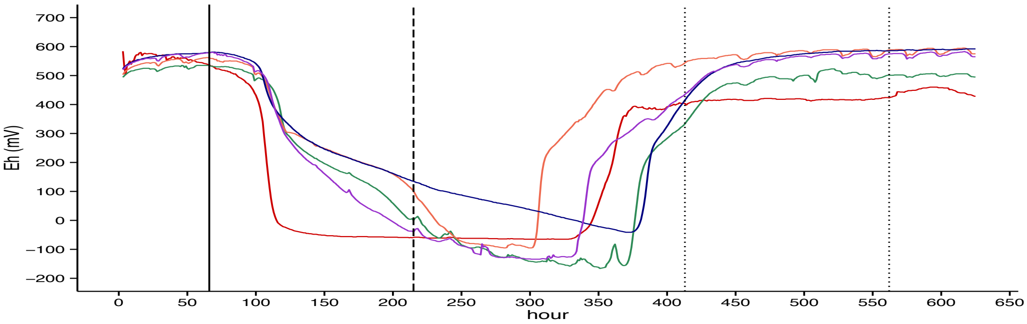

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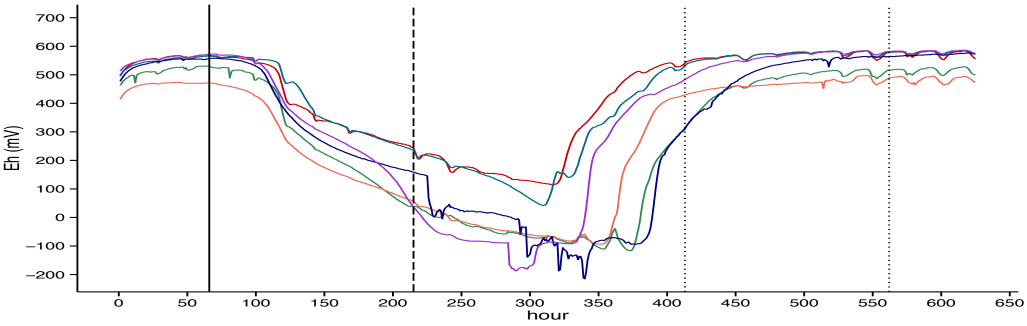
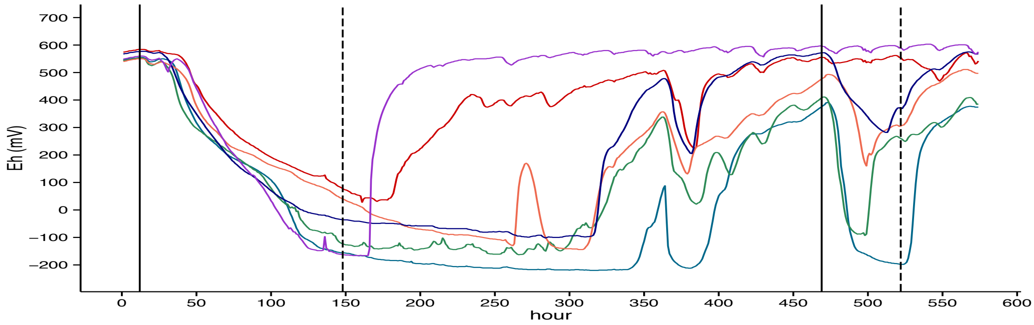
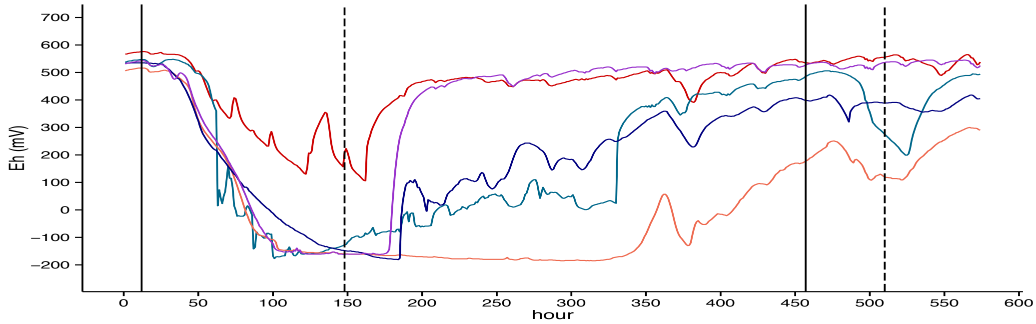


Figure 3. Eh measurements from replicate electrodes at four depths for the period 22 June – 19 July 2013 at the Stiffkey creek site demonstrating the redox characteristics of well-drained sediments and the attainment of a high degree of reproducibility of Eh readings at all depths.

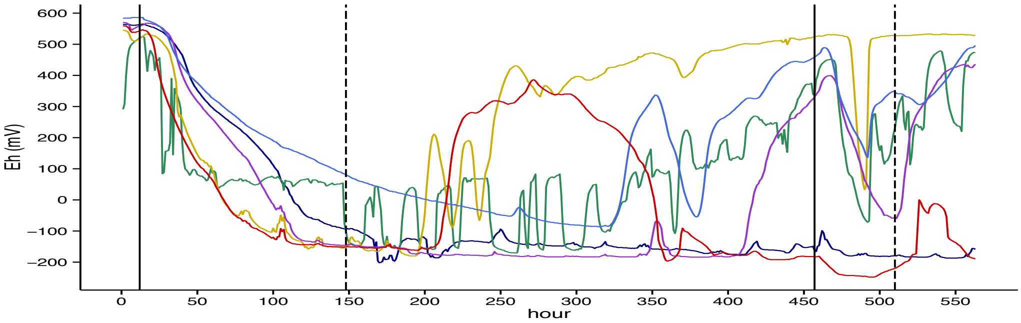
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

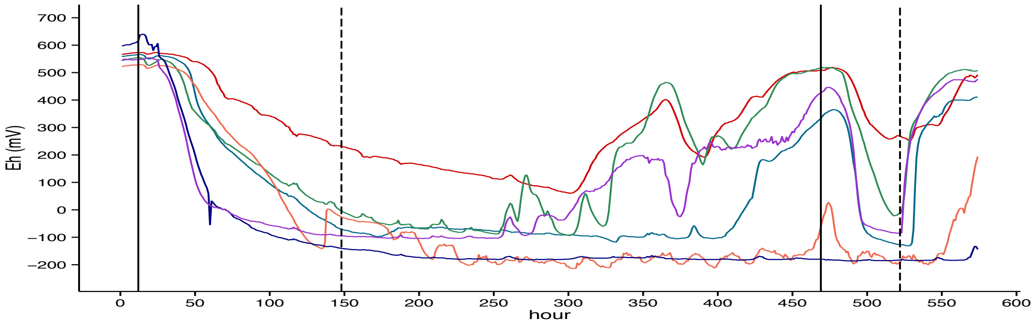
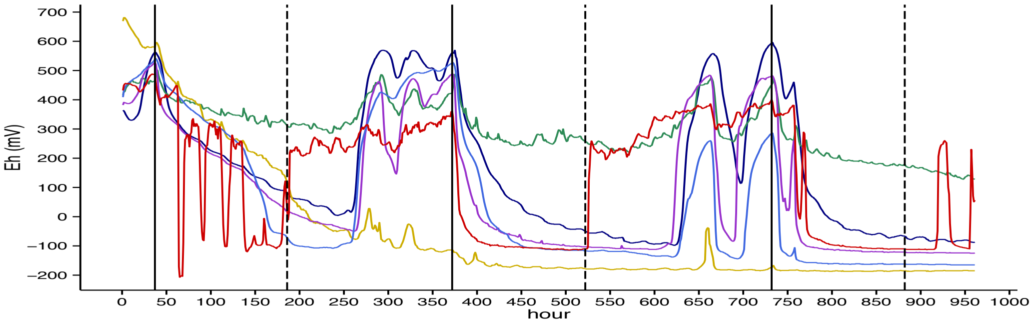
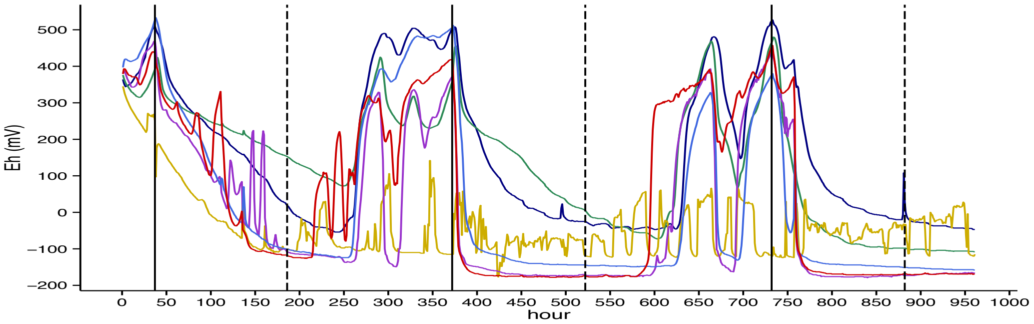


Figure 4. Eh measurements from replicate electrodes at four depths for the period 22 July – 14 August 2013 at the Stiffkey creek site showing high variation in electrode recovery to oxic Eh values and the general depression of values that was particularly noticeable at the greater depths at this late stage of summer with some electrodes failing to show any significant recovery by the following period of tidal flooding.

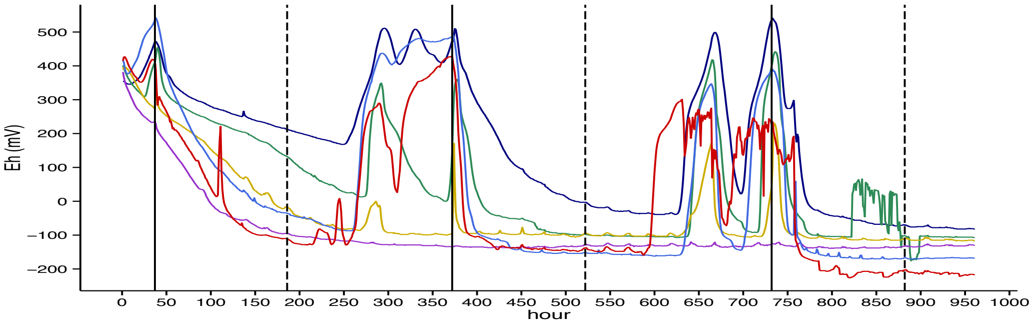
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

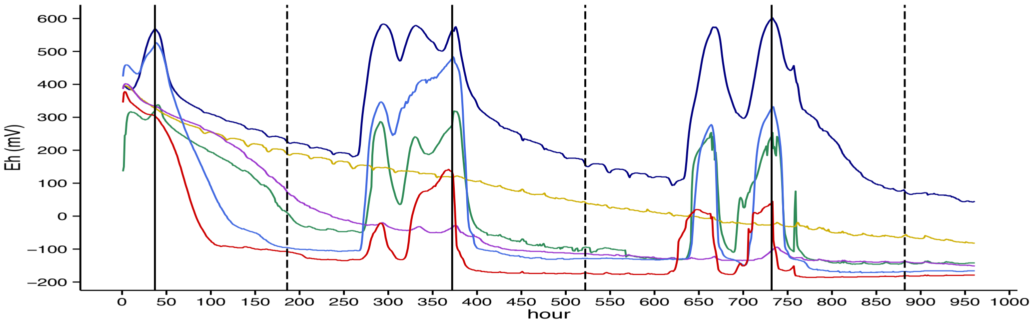
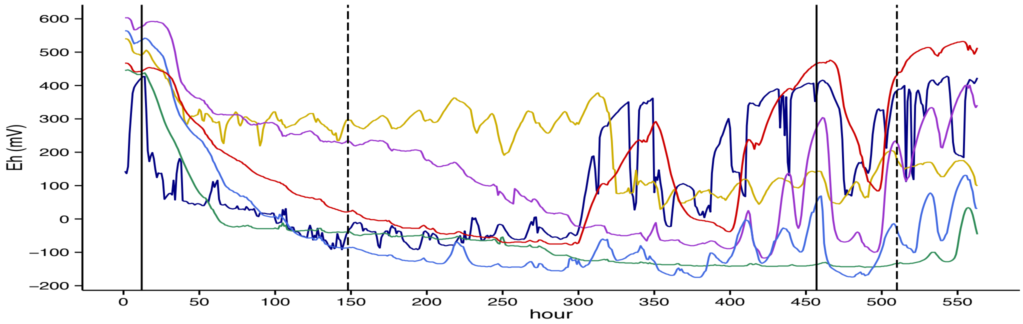


Figure 5. Eh measurements from replicate electrodes at four depths for the period 17 July – 26 August 2012 at the Brancaster creek site showing rapid changes in Eh over flood drain cycles, the effect of heavy precipitation on depressing Eh values during the second neap phase and how the number of electrodes registering oxic Eh values decreases with depth.

(a) 2 cm



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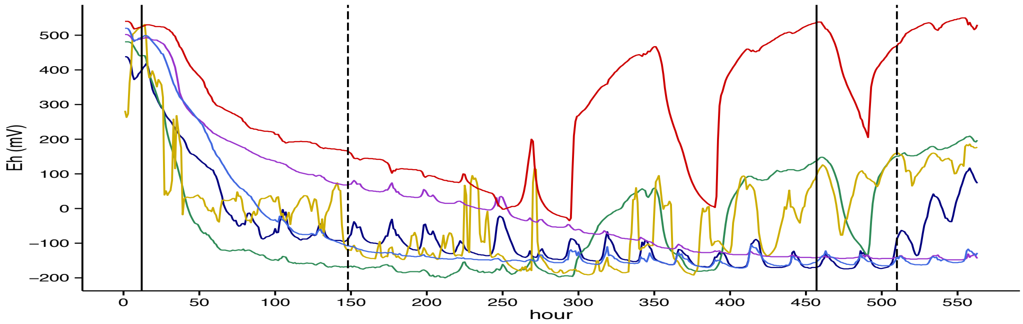

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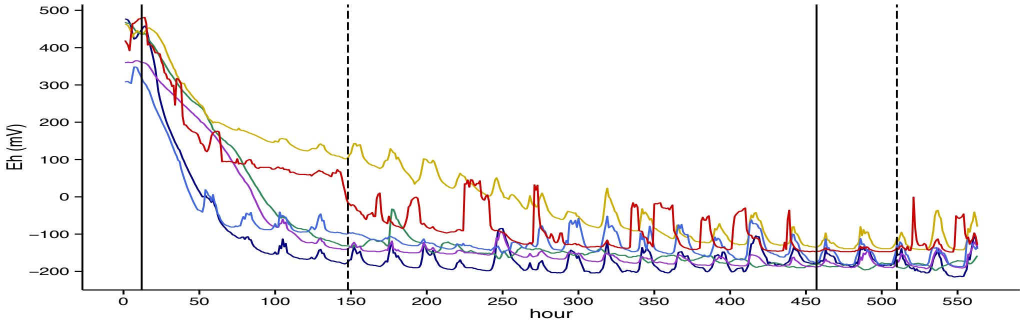

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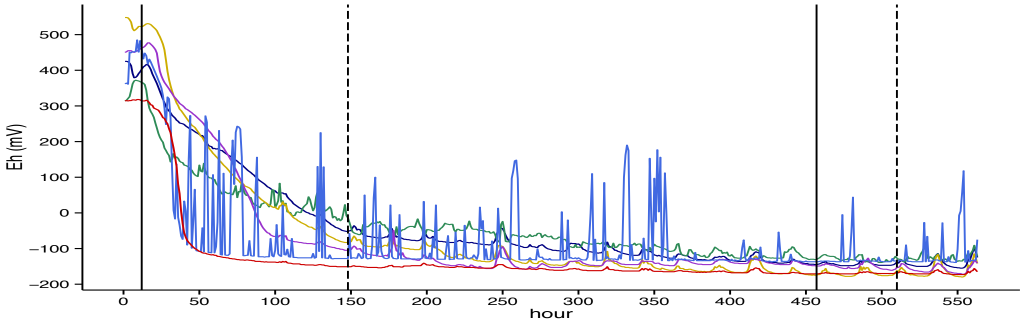
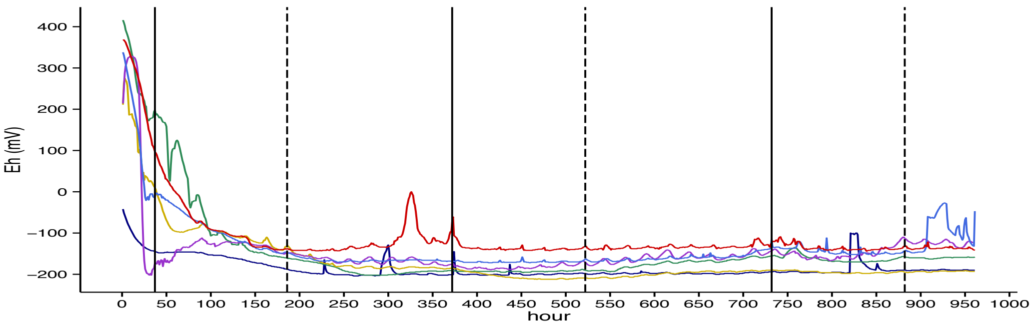
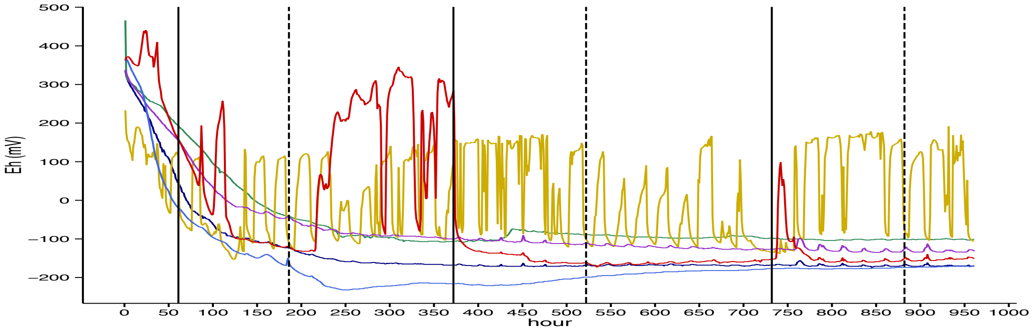


Figure 6. Eh measurements from replicate electrodes at four depths for the period 20 July – 14 August 2013 at the Stiffkey GSM site for the period showing only limited recovery to oxic Eh values in the uppermost depths while deeper sediments remain highly reducing.

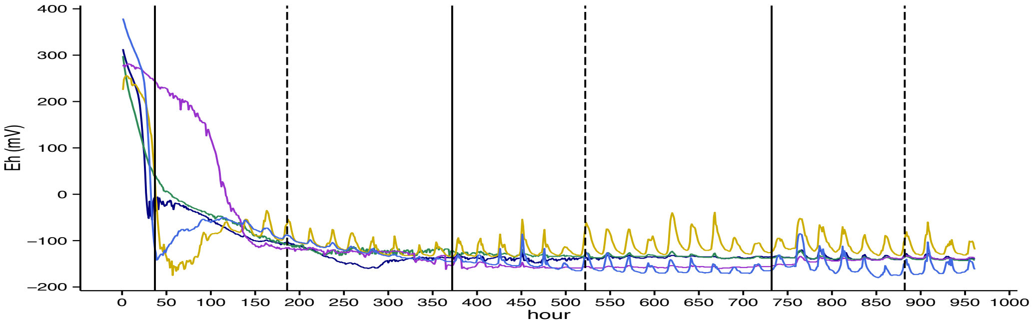
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

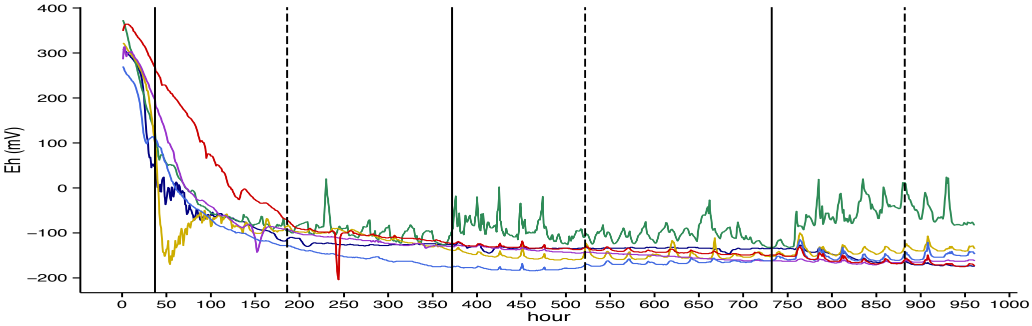
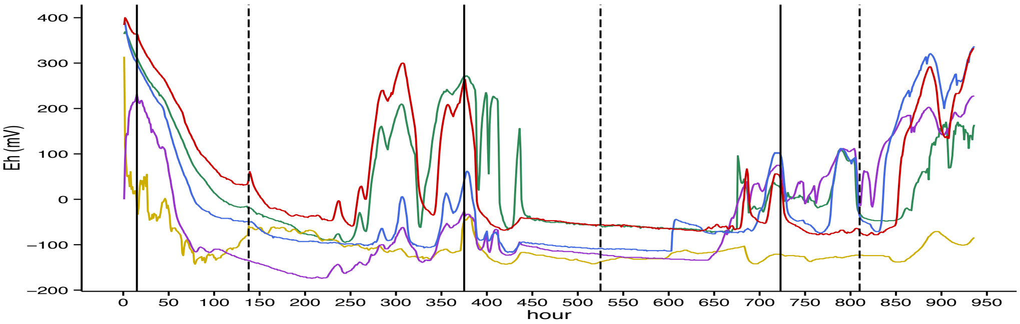
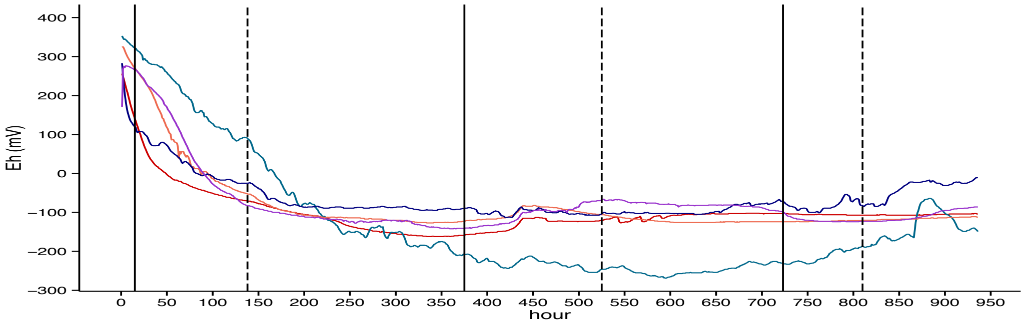


Figure 7. Eh measurements from replicate electrodes at four depths for the period 17 July – 26 2012 August at the Brancaster GSM site showing no evidence of recovery to oxic Eh values even in the uppermost depths.

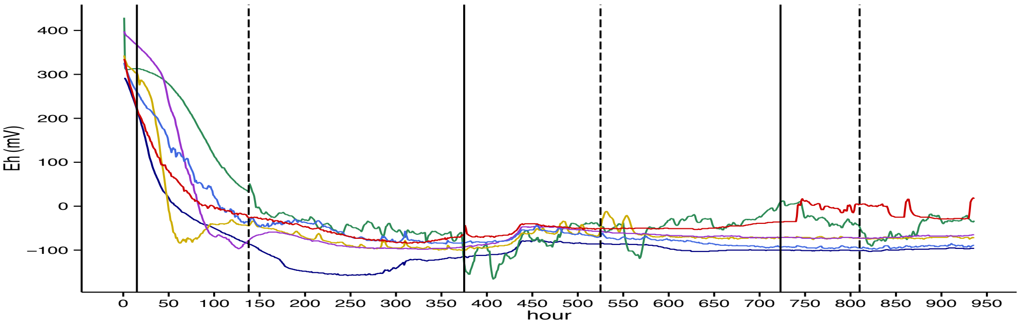
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

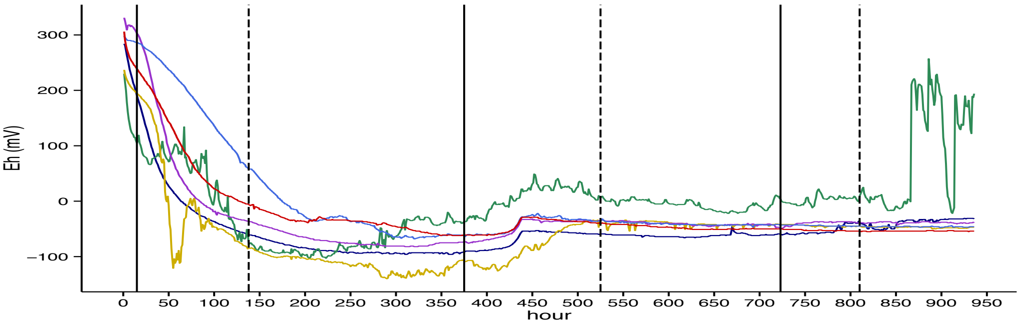
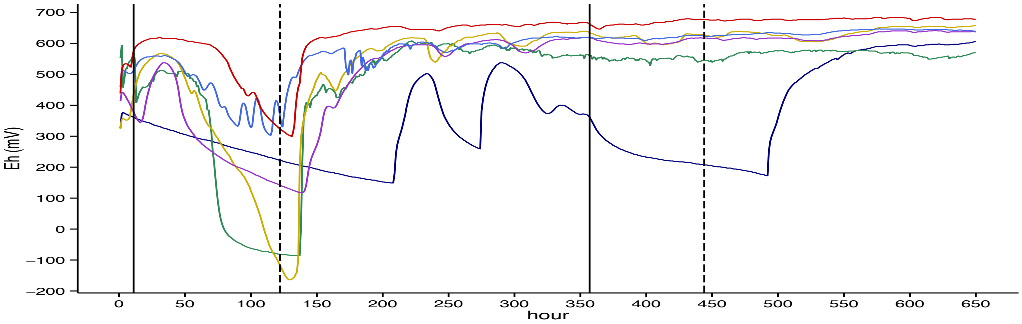
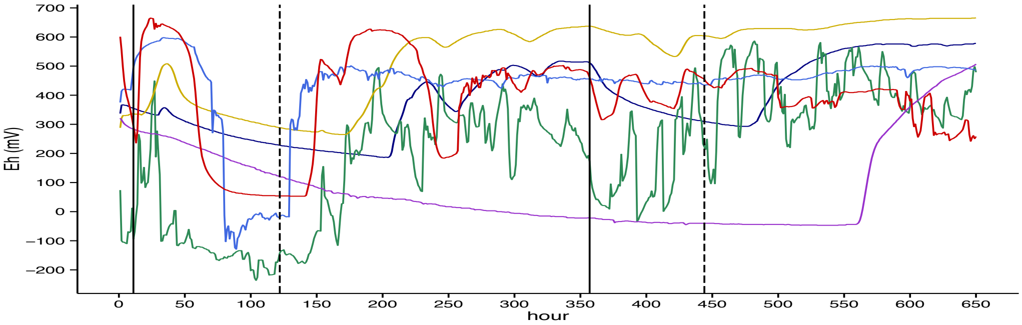


Figure 8. Eh measurements from replicate electrodes at four depths for the period 28 September – 6 November 2012 at the Brancaster GSM site illustrating the persistence of generally anoxic conditions will into the autumn with only limited increase in Eh towards oxic Eh values in the uppermost depth by the end of the recording period.

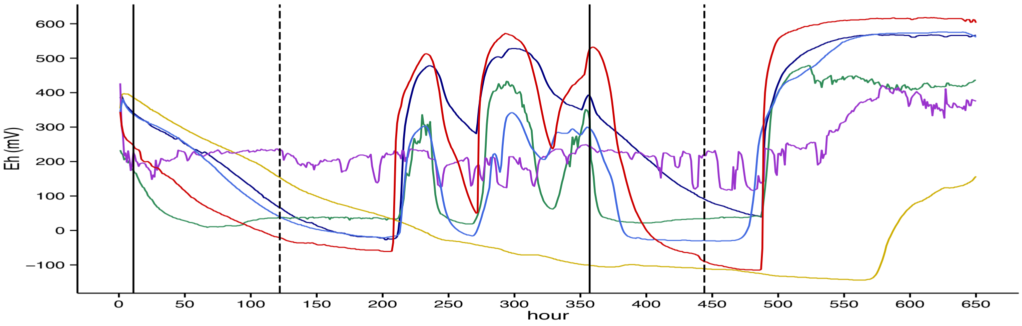
(a) 2 cm



(b) 5 cm



(b) 10 cm



(b) 20 cm

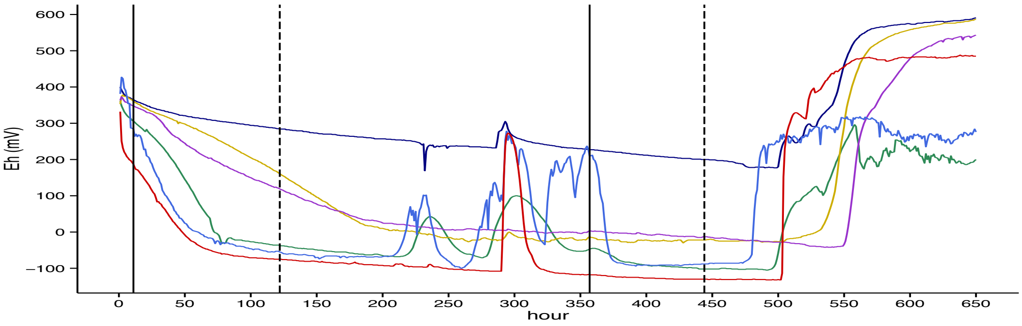
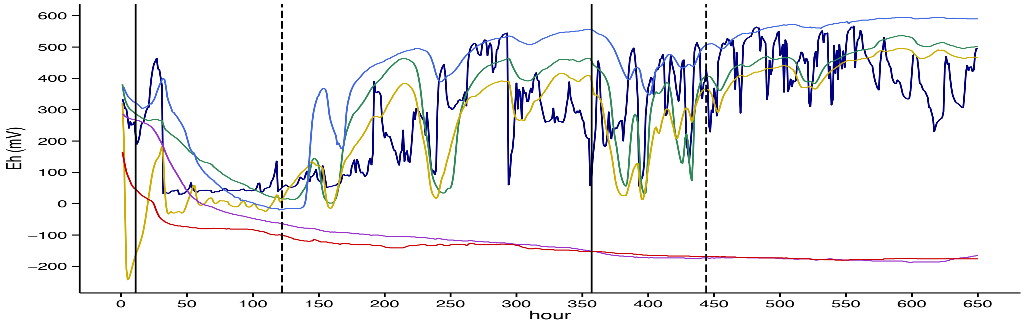
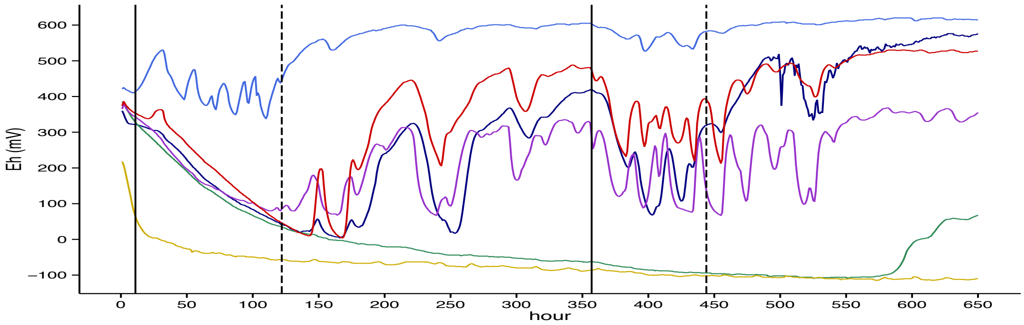


Figure 9. Eh measurements from replicate electrodes at four depths for the period 2 – 29 November 2013 at the Stiffkey creek site showing attenuation of the anoxic phase at uppermost depths of the creek site sediments during the period while a delayed return to oxic conditions is evident at 20 cm.

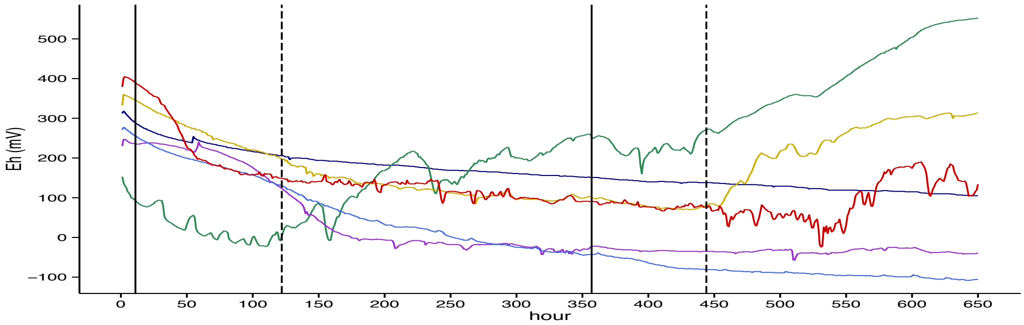
(a) 2 cm



(b) 5 cm



(c) 10 cm



(d) 20 cm

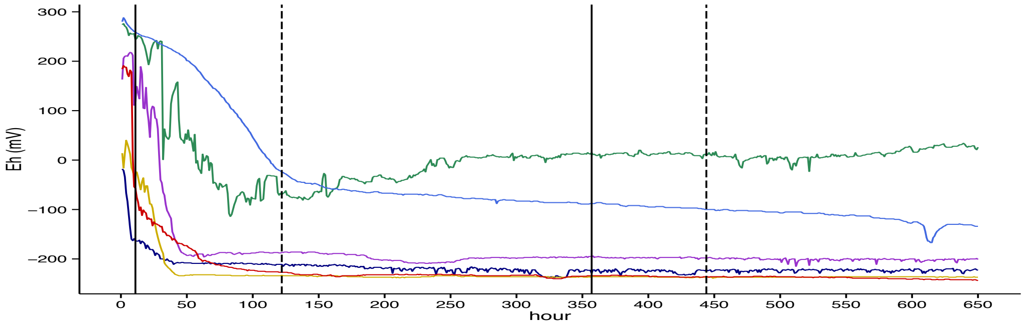


Figure 10. Eh measurements from replicate electrodes at four depths for the period 2 – 29 November 2013 at the Stiffkey GSM site showing evidence of a return to oxic conditions in the uppermost depths of the GSM sediments.

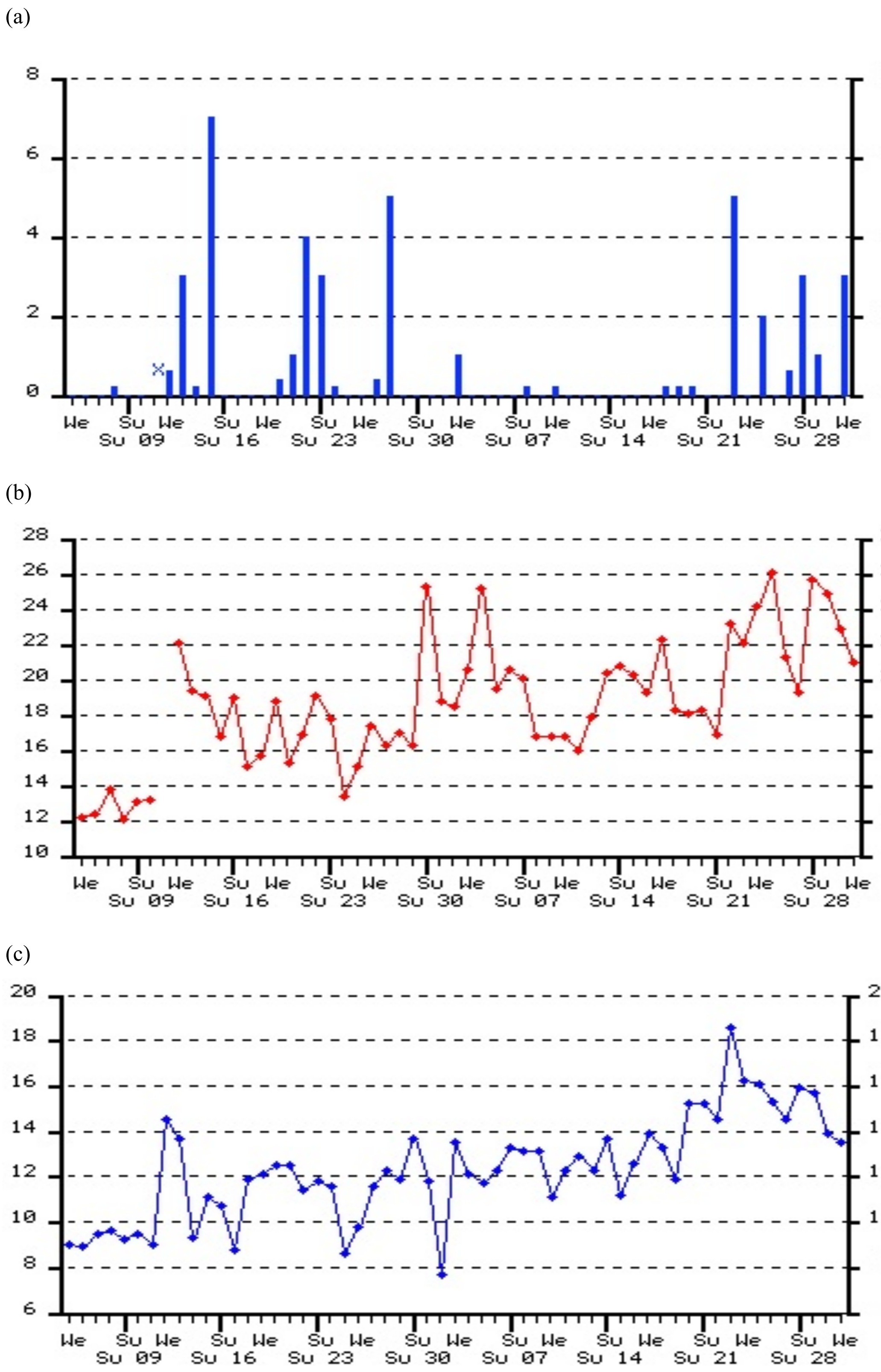


Figure 11. Daily precipitation (mm) (a), maximum temperature (b) and minimum temperature (c) for Weybourne (North Norfolk coast) synoptic weather station for the period 05/06/2013 – 31/07/2013 (Source: Met Office, UK).