The impact of increasing saline penetration upon estuarine and riverine benthic macroinvertebrates

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The impact of increasing saline penetration upon estuarine and riverine benthic macroinvertebrates

by

Sally Little

A doctoral thesis submitted in partial fulfilment
of the requirements for the award of

Doctor of Philosophy of Loughborough University

20th April, 2012

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Coastal and estuarine systems worldwide are under threat from future global climate change, with potential consequences including increased penetration of tidal driven salt water into estuarine surface waters. In coastal climate change research this issue has been neglected, despite increases in salinity potentially detrimentally impacting upper estuarine and riverine ecosystem function worldwide. In this research the first direct attempt is made at predicting the impact of future climate-driven increases in saline penetration upon estuarine and riverine benthic macroinvertebrate communities through the acute salinity tolerances of selected species.

Two study estuary-river systems were selected based upon their perceived susceptibility to future increases in saline penetration. These estuaries exhibited dynamic tide and salinity profiles with large salinity ranges recorded over a tidal cycle and significant differences in saline penetration extents between low (summer) and high (winter) freshwater river discharge conditions. Salinity was shown to be the dominant environmental variable driving benthic macroinvertebrate species distributions in both estuaries; however additional environmental factors were shown to have locally dominant effects (i.e. sediment grain size). Laboratory and field based salinity toxicity experiments suggested that the tolerance of euryhaline-marine and brackish water species to reductions in salinity corresponded well to tolerance values in published literature. In contrast limnic derived species exhibited greater salinity tolerance under laboratory and field tidal cycle conditions than those published. For all test species, actual field distributions did not reflect distributions anticipated by saline tolerances alone, likely due to the effects of additional biotic and abiotic factors experienced under field conditions. The macroinvertebrate species salinity tolerances did not account for actual field distributions with sufficient accuracy to allow for precise prediction of future distribution patterns under projected saline penetration profiles due to the influence of additional environmental factors.

Under the high greenhouse gas emissions climate scenario (SRES A1FI) for the years 2020, 2050 and 2080, projected relative sea level rise was shown to result in an increase in both the upstream extent of saline penetration and gradient of maximum salinity zones in both estuaries. However these increases were moderate even under ‘worst-case’ conditions (0.32 km and 0.15 km) and unlikely to result in large-scale changes to the benthic macroinvertebrate community. However, in addition to relative sea level rise, predicted changes to freshwater river discharge (climatic and anthropogenic induced) and channel morphology could result in significant increases in the upstream extent of saline penetration predicted for projected sea level rise alone. This could result in critical consequences for estuarine and riverine ecology and ecosystem function across all trophic levels. A conceptual model exploring the potential ecological effects of both increases in saline penetration and changes to the estuarine system (anthropogenic and climatic) was developed, and implications for the future management of estuarine and riverine environments were identified.
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Chapter 1. Introduction, background and project aims

1.1 Introduction

Future global climate change, and its associated impacts, arguably pose the greatest threat to coastal and estuarine ecosystems worldwide (Scavia et al., 2002; Vermaat et al., 2005; Harley et al., 2006b; IPCC, 2007; Day et al., 2008; Cheung et al., 2009; Lowe et al., 2009; Dijkstra et al., 2010). Specifically, these systems have been identified as being particularly vulnerable to projected increases in eustatic sea level (e.g. global rise), changes in precipitation patterns and evaporation rates, increased water temperatures, and changes in wind patterns and storminess (Scavia et al., 2002; Nicholls & Klein, 2005; IPCC, 2007; Day et al., 2008; Lowe et al., 2009; Jevrejeva et al., 2010). Whilst much uncertainty still exists concerning exactly how these changes might impact coastal and estuarine systems, likely consequences include loss of habitat through coastal inundation, increased penetration of salt water into estuaries and intrusion into coastal groundwater aquifers, changes in freshwater river discharge regimes and economic impacts associated with (increased) storm surge events (Scavia et al., 2002; Vermaat et al., 2005; Day et al., 2008; Whitehead et al., 2009b; Wilby et al., 2010). Such changes will undoubtedly have significant impacts upon coastal ecosystems, which will in turn affect trophic structure, nutrient cycling and in many cases, pose problems for coastal industries and important human resources (e.g. fisheries and tourism) (Struyf et al., 2004; Cheung et al., 2009; Dijkstra et al., 2010; Howes et al., 2010; Vorosmarty et al., 2010). Global climate, therefore, poses a very real threat to coastal and estuarine ecosystem functioning and urgent research (and knowledge based planning and management strategies) is required for better prediction of how these systems might change in future, to assess their capacity for adaptation to these predicted changes and for successful mitigation and long-term sustainability (Scavia et al., 2002; Walther et al., 2002; Vermaat et al., 2005; Cheung et al., 2009; Dijkstra et al., 2010; Peterson et al., 2010a; Traill et al., 2010).

As the transition between land and sea, estuaries are naturally dynamic, high-energy environments that exhibit rapid spatial and temporal change (McLusky, 1993; Uncles, 2003; McLusky & Elliott, 2004; Savenije, 2005; Prandle, 2009). Physical processes operating on both short (e.g. tidal cycles, freshwater discharge, fluvial input of nutrients and sediment) and long timescales (e.g. climate and sea level change) form the driving forces for many of the biological, sedimentological and physicochemical processes that occur in these systems (McLusky, 1993; McLusky & Elliott, 2004; Prandle, 2009). Estuaries are unique in their steep gradation of
conditions, supporting a niche flora and fauna able to withstand the extremes of constantly changing tidal, salinity and sedimentological regimes (Remane & Schlieper, 1971; Attrill, 2002; Bilton et al., 2002). Species able to inhabit these systems commonly achieve high abundances, making estuaries some of the most productive ecosystems in the world (De Jonge et al., 2002; McLusky & Elliott, 2004; Telesh & Khlebovich, 2010). Many of the world’s estuarine ecosystems are experiencing significant anthropogenic stress, due to dense coastal and inland populations, development pressures and intensely exploited resources (Crain et al., 2009). As a result, issues such as pollution, eutrophication, habitat loss and over-exploitation are extremely common in these systems today (De Jonge et al., 2002; Golubkov et al., 2003; Zhang et al., 2009; Mazik et al., 2010; Jackson & McIlvenny, 2011). Anthropogenic disturbances make it increasingly difficult to understand the already complex, natural, physical and ecological changes operating within estuarine systems (Kappel, 2005; Crain et al., 2009). This is problematic as it affects our ability to accurately predict (with confidence) the impact that future climate change will have on coastal and estuarine systems (Lotze, 2010).

In the UK, lowland coasts are most susceptible to predicted increases in eustatic sea level, especially in regions undergoing post-glacial isostatic land level decline (Shennan et al., 2006; Bradley et al., 2009; Lowe et al., 2009; Teferle et al., 2009). This makes these areas particularly vulnerable to future flooding by tidal inundation, saline penetration into estuaries and saltwater intrusion into ground water aquifers (Day et al., 2008; Mazik et al., 2010; Webb & Howard, 2010). Whilst issues of climate-induced habitat loss by coastal inundation, and degradation of aquifer water quality by saline intrusion, have been well documented (Scavia et al., 2002; Post, 2005; Custodio, 2010; Essink Oude et al., 2010; Schwarzenbach et al., 2010; Werner et al., 2011), the predicted increases in surface water saline penetration upon estuarine ecology has received little attention (Peterson et al., 2010a). Maintenance of these ecosystems, however, is extremely important for obtaining good ecological status as set out by Water Framework Directive (2000/60/EC; European Communities, 2000), and supporting important commercial fisheries, protecting rare species and habitats and preventing invasion of invasive species that are detrimental to ecosystem structure (Walther et al., 2002; Roessig et al., 2004; Hellmann et al., 2008; Dijkstra et al., 2010).

By definition, estuaries are transitional zones between freshwater and marine habitats (McLusky, 1993; Elliot & McLusky, 2002), subject to cyclical (tidal) and occasional (storm surge) saline water input, but also influenced and modulated by freshwater river discharge (flood to low flow) (Savenije, 2005; Prandle, 2009). The spatial extent of saline penetration in estuaries is naturally
variable, but can be particularly sensitive to anthropogenic abstraction of freshwater from above the tidal limits, changing seasonal river discharges and high-magnitude low-frequency episodic events (e.g. storm surges and droughts which can cause short-term, extreme saline penetration pulses, Savenije, 2005). Within estuaries, salinity has been shown to be a dominant environmental factor determining floral and faunal structure and species distributions (De Jonge, 1974; Michaelis et al., 1992; Attrill, 2002; Telesh & Khlebovich, 2010; Telesh et al., 2011). Current knowledge suggests that an individual species’ tolerance to salinity strongly contributes to its estuarine distribution pattern, from marine, salt-tolerant species at the river mouth, through to freshwater, salt-intolerant species in the upper estuary and above the tidal limits (Kinne, 1971; Attrill, 2002; Telesh & Khlebovich, 2010). Benthic macroinvertebrates are considered sensitive indicators of salinity, with variations in the degree of saline penetration into estuaries resulting in shifts in the distribution of macroinvertebrate communities and species (Attrill et al., 1996; Kefford et al., 2007c; Dunlop et al., 2008; Carver et al., 2009; Bessa et al., 2010). Such studies suggest increases in the upstream extent of saline penetration is potentially most detrimental for salt-intolerant freshwater species inhabiting upper estuarine zones and adjoining lower river courses (Attrill et al., 1996; James et al., 2003; Dudgeon et al., 2006). It is therefore likely that future increases in saline penetration, will cause further changes in macroinvertebrate community structure and species distributions in UK estuaries, and that these changes might be predictable, based on the salinity tolerances of benthic macroinvertebrate species (Kefford et al., 2004b; Kefford et al., 2006a; Horrigan et al., 2007; Dunlop et al., 2008). However, this relies on the assumption that the salinity tolerances of benthic macroinvertebrate species (present in UK estuarine systems) are well understood, can be precisely determined and that these tolerances account for species distributions in the field.

This study provides the first direct attempt at predicting the impact of future climate-driven increases in saline penetration upon estuarine benthic macroinvertebrate community structure and species distributions. A multidisciplinary (ecological/biological) approach is employed, including analysis of laboratory and field salinity tolerances of selected species, and measurement of actual species distributions under current salinity profiles for two carefully selected UK estuaries (the River Adur and River Ouse), deemed ‘at risk’ to future increases in saline penetration. Understanding the impacts of increasing saline penetration upon the functioning of estuarine ecosystems is critical for the future conservation and management of these habitats, and has wider implications for other temperate estuarine systems.
This chapter aims to:
1. Briefly introduce the concept of an ‘estuary’, its physiochemical conditions, environmental gradients and ecological importance.
2. Summarise the future projections for global (and UK) climate change and discuss the implications that these changes might have upon coastal and estuarine systems.
3. Introduce the topic of saline penetration in estuaries, its causes, associated problems, predicted future extent and implications for estuarine and riverine ecology.
4. Introduce and summarise current knowledge concerning salinity tolerances of benthic macroinvertebrates, with focus on freshwater species inhabiting the tidal limnetic zone, a group deemed most at risk to future increases in saline penetration.
5. Clearly state the aims and objectives of this research project.

1.2 Background

1.2.1 Estuaries

Estuaries are transitional zones between land and sea, and from freshwater to saltwater (McLusky, 1993; Dyer, 1997; Elliot & McLusky, 2002). They are sites of continuous physiochemical, spatial and temporal change, experiencing chemical (e.g. salinity, dissolved gases, nutrients and trace metals), sedimentary (e.g. turbidity maximum), hydrological (tidal and freshwater flow) and morphological variations, over daily tidal cycles (McLusky, 1993; Dyer, 1997; Uncles, 2003; McLusky & Elliott, 2004; Prandle, 2009). Few flora and fauna can withstand the constantly fluctuating, steep gradation of physiochemical conditions experienced over these transitions, resulting in a reduction in the ecological diversity of estuaries when compared to adjacent marine and river habitats (Remane & Schlieper, 1971; Little, 2000; Attrill, 2002; Dethier, 2010; Telesh et al., 2011). However, the species that can inhabit these systems, often occur in high abundances (Remane & Schlieper, 1971; Barnes, 1989; Little, 2000; Attrill, 2002; McLusky & Elliott, 2004), due to high nutrient supply from both terrestrial (including anthropogenic inputs) and marine sources and high energy (from wave and tidal action) mixing and circulation of food and nutrients, thereby making estuaries some of the most biologically productive ecosystems in the world (Correll, 1978; De Jonge et al., 2002; Prandle, 2009; Antonio et al., 2010).

Determining a universal definition of an estuary has been controversial, with the existence of over 40 varying definitions, based upon the research perspective of the defining author (McLusky, 1993; Elliot & McLusky, 2002; Telesh & Khlebovich, 2010). Principle differences
have revolved around the determination of tidal input and upstream limit (McLusky, 1993; Elliott & McLusky, 2002). The widely used definition by Fairbridge (1980) was deemed most appropriate for this research project, as he emphasizes the importance of tides, defining the upstream extent of an estuary as the limit of tidal penetration (McLusky, 1993; Elliott & McLusky, 2002). Within an estuary, Fairbridge, (1980) identified three ‘sectors’ termed the lower (or marine) estuary, the middle estuary and the upper or fluvial estuary, with each sector being subject to different salinity conditions and variable boundaries, driven by the interaction between tidal action and freshwater river discharge (McLusky, 1993).

Despite the numerous definitions of an estuary described in published literature (see review by Elliot & McLusky, 2002), there is a general consensus that the spatial salinity gradient and its temporal variability is an intrinsic characteristic of estuarine systems (Kinne, 1971; Telesh & Khlebovich, 2010). Sodium and chloride are the dominant ions in seawater, which (in addition to low concentrations of additional inorganic ions and organic substances) make up ~ 3.5% of seawater (the other ~96.5% being water), resulting in a salinity of around 35, although this concentration varies in oceans around the world (33 - 37, Nybakken, 1993). In estuaries, seawater (35) is diluted with freshwater (salinities below 0.5) from riverine inputs and becomes brackish (0.5 - 30, McLusky, 1993). Following the Venice International Symposium in 1958, these brackish waters have been classified according to salinity range, as limnetic (<0.5), oligohaline (0.5 - 5), mesohaline (5 - 18), polyhaline (18 - 30) and euhaline (>30) (Anon, 1958; McLusky, 1993). The structure of estuarine salinity gradients, and upstream extent of saline penetration, depends upon the relationship between the relative strength of tide-driven saline penetration upstream and freshwater discharge downstream, in addition to tidal amplitude, channel topography and local climate (Dyer, 1997; McLusky & Elliott, 2004; Savenije, 2005). In most estuaries, the spatial salinity gradient is never ‘steady-state’, with the balance between tide height and river flow varying over temporal tide cycles, local climate conditions and episodic events (e.g. floods, droughts and storm surges, McLusky & Elliott, 2004; Savenije, 2005; Savenije & Veling, 2005; Wolanski, 2007). In addition to salinity-based division, estuaries have also been divided into stationary zones, based on geographical terms (e.g. river, head, tidal fresh, upper, inner, middle, lower and mouth) and assigned to their associated salinity ranges using the Venice classification system (McLusky & Elliott, 2004). However, unlike the fixed geographical zones, these salinity ranges (based on the Venice classifications) might oscillate depending on the tide height and river flow balance (McLusky, 1993).
In addition to variations in salinity, estuaries experience changes in sediment (e.g. transitions between sediment types and grain sizes), organic matter, turbidity, nutrient loads, oxygen content and temperature along their courses (Little, 2000; Wolanski, 2007; Prandle, 2009). Benthic diversity declines from the outer towards the inner portions of estuaries (Kinne, 1971; Remane & Schlieper, 1971; McLusky, 1993; Attrill, 2002; Telesh & Khlebovich, 2010), but co-variation amongst many estuarine gradients makes it extremely difficult to determine the key forcing processes (Kinne, 1971; Little, 2000; Dethier, 2010). The physical gradients that frequently correlate with estuarine biota are salinity (Deaton & Greenberg, 1986; Attrill, 2002; Telesh & Khlebovich, 2010), sediment grain size and deposition rate (Anderson et al., 2004; Hernandez-Arana et al., 2003; Williams & Hamm, 2002; Hewitt et al., 2005) and oxygen content (Bishop et al., 2006). However, it is generally accepted that salinity is the most dominant environmental variable driving biotic diversity patterns and species distributions in estuaries (Kinne, 1971; Remane & Schlieper, 1971; De Jonge, 1974; Bulger et al., 1993; Attrill, 2002; Telesh, 2004; Dethier, 2010).

1.2.2 Global climate change impacts upon coastal and estuarine systems

Undoubtedly one of the biggest threats to the world’s coastal and estuarine systems is climate change and its associated impacts (Najjar et al., 2000; Scavia et al., 2002; Walther et al., 2002; Roessig et al., 2004; Vermaat et al., 2005; IPCC, 2007; Day et al., 2008; Lowe et al., 2009). Over the last approximately 100 years, global temperatures are believed to have increased by 0.74 ± 0.18 °C (updated 100 year trend for 1906-2005; IPCC, 2007), the largest for any century over the last 1,000 years (Osborn & Briffa, 2006) and global sea level has risen by ~1.7 mm yr^{-1} over the twentieth century (Bindoff et al., 2007). A warming trend is also visible in global average sea surface temperatures (~0.6°C since 1950; Bindoff et al., 2007), whilst precipitation has significantly changed in a number of regions, though this is highly spatially and temporally variable (e.g. increased precipitation in northern Europe, America and central Asia, dryer in the Sahel, the Mediterranean, southern Africa and parts of southern Asia; IPCC, 2007). Many regions have experienced a clear increase in the number and intensity of extreme events, such as heavy rainfall periods, flooding, hurricanes and storm surges (Emanuel, 2005; IPCC, 2007). The impacts of climate change over recent centuries are already being witnessed in many coastal (and oceanic) systems worldwide, with associated impacts including increased saline penetration in estuaries and aquifers (Custodio, 2010; Webb & Howard, 2010; Kingsford et al., 2011), reduction in estuarine and coastal zone habitats (i.e. coastal squeeze; Jackson & McIlvenny, 2011), loss of biodiversity and ecosystem change (Conner et al., 1989; Ross et al., 1994; Scavia et al., 2002; Najjar et al., 2010), increased vulnerability to invasive species (Stachowicz et al.,...
In the UK, average temperatures have also increased, with central England having experienced a 1 °C rise and a lengthening of the growing season (by approximately one month) since the beginning of the twentieth century, whilst summer heat waves have become more frequent (Hulme et al., 2002; Jenkins et al., 2008; Jenkins et al., 2009). Water temperatures have also increased in coastal areas of the UK; for example, average sea surface temperatures in the Seven estuary have increased by between 1.4 - 2.2 °C in winter and 1 - 1.2 °C in summer since 1961 Lowe et al., 2009). UK sea levels have been rising at a rate ~1 mm a year (i.e. ~10 cm rise, when corrected for natural isostatic land movements) throughout the twentieth century, though this has increased over the last two decades (Hulme et al., 2002; Jenkins et al., 2009). All regions of the UK have experienced an increase in heavy winter precipitation events, whilst winters are generally becoming wetter and warmer (and summers hotter and drier) and heavy rainfall events in the winter are becoming more severe (i.e. receiving more rainfall than 50 years ago; Hulme et al., 2002).

Future global climate change is expected to result in further increases in air and water temperatures, increased oceanic acidity, changes in wind patterns and storminess, changes in precipitation and evaporation rates and increases in eustatic sea level rise (Scavia et al., 2002; IPCC, 2007; Day et al., 2008; Lowe et al., 2009; Murphy et al., 2009). The Intergovernmental Panel on Climate Change (IPCC) recently identified coastal and estuarine regions as being extremely vulnerable to a wide range of climate associated impacts over the next ~100 years with high to very high confidence (IPCC, 2007). It is projected (with very high confidence) that many coastal regions will experience greater adverse consequences of hazards associated with climate change (i.e. extreme events such as storm surges) which will be extremely costly both in terms of capital and physical impact (e.g. increased erosion, ecosystem loss and destruction). Furthermore sea level and climate change will put coastal systems at greater risk of coastal erosion and degradation of important ecological habitats such as coral reefs (prone to thermal stress and bleaching) and wetland ecosystems such as salt marshes and mangroves, particularly where they are constrained by their landward margin or starved of sediment (with very high
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confidence; IPCC, 2007). Many of these systems are already at risk from other anthropogenic and natural stressors (e.g. nutrient inputs, pollution, overfishing and exploitation) making them particularly vulnerable to future climate change (De Jonge et al., 2002; Kappel, 2005; Crain et al., 2009; Lotze, 2010). These biologically productive ecosystems provide an essential role in the functioning of coastal and marine systems and also provide a range of important resources and services to human populations (Clark, 1998; McLusky & Elliott, 2004). However, these global climate change impacts are likely to affect both the structure (e.g. flora and faunal composition) and function (e.g. nutrient cycling, primary and secondary production) of these systems, though the exact effects remain largely unknown and under-investigated at present (De Jonge et al., 2002; Walther et al., 2002; Roessig et al., 2004; Struyf et al., 2004; Peterson et al., 2010a). The loss of intertidal habitats due to rising sea levels is also likely to affect populations of sea and migratory birds, which depend on these habitats for feeding (Ens et al., 1995; Najjar et al., 2000, Galbraith et al., 2002; Peterson et al., 2010b).

The IPCC has projected that global air temperatures are likely to increase by 2-4 °C over the next 100 years, with the warming in northern Europe likely to exceed the global mean (IPCC, 2007). Predicted increases in surface water temperatures might influence organism metabolism, resulting in unpredictable future species distributions and interactions, behaviour, life-cycles and assemblages, which in turn might result in mortality, local extinctions and increases in the dominance of invasive species (Hoegh-Guldberg, 1999; Walther et al., 2002; Harley et al., 2006a; Sekercioglu et al., 2008; Dijkstra et al., 2010). Close associations have been recorded between laboratory-based measurements of species responses to temperature and actual field distributions, with many fish and invertebrate species sensitive to temperatures only a few degrees higher than those they experience in nature (Hoegh-Guldberg, 1999; Roessig et al., 2004; Cebrian et al., 2011). Invertebrates that harbour symbionts, such as corals and sponges, might be particularly at risk from warming (Cebrian et al., 2011). For example, extensive mortality in populations of the shallow water sponge *Ircinia fasciculata* (due to a breakdown of the cyanobacteria-sponge symbiosis) have been linked to the higher than average summer temperatures experienced during 2008 and 2009 in the western Mediterranean sea (Cebrian et al., 2011).

Increased oceanic-uptake of atmospheric anthropogenic carbon dioxide, is believed to have caused a 30% increase in ocean acidity (i.e. drop in pH) between 1751 and 1994, and is predicted to cause a further drop in seawater pH of up to 0.4 units by 2100 (Caldeira & Wickett, 2003; Jacobson, 2005; Hall-Spencer et al., 2008). This increase would correspond to a 3-fold rise in
the mean acidity of the oceans, which is an unprecedented decrease in the context of the last 20 million years (Raven, 2005). Research has shown that when exposed to elevated CO$_2$ levels, calcifying organisms (e.g. corals, coccolithophores (calcifying phytoplankton), molluscs, echinoderms and foraminifera (single-celled protists) experience reduced calcification (or enhanced dissolution) (Raven, 2005; Hall-Spencer et al., 2008). This effect has already been detected in a 30 – 35% decline in Antarctic foraminifera shell weights since the end of the 18$^{th}$ century (Moy et al., 2009). Studies investigating the calcification development rates of the thecosome pteropod (shelled pelagic mollusc) *Limacina helicina* and the cold water coral *Lophelia pertusa* recorded a respective 28% and 50% reduction in development rate (compared to current conditions), when maintained in saltwater at the acidity predicted for 2100 (Comeau et al., 2009; Maier et al., 2009). In addition to calcifying effects, increases in ocean acidity might also cause direct reproductive or physiological changes in marine organisms or indirect effects through impacts on food sources (Raven, 2005). Whilst research detailing the effects of elevated oceanic acidity on marine organisms and ecosystems is still in its infancy, it is accepted that these changes are likely to result in extreme future changes to marine and coastal ecosystem processes, trophic interactions and biodiversity (Caldeira & Wickett, 2003; Fabry et al., 2008; Hall-Spencer et al., 2008).

It is predicted that future climate change will result in regional shifts in the timing and magnitude of precipitation in northern Europe, with increases in winter and spring and decreases in summer and autumn (compared to current conditions) (IPCC, 2007; Murphy et al., 2009). This is likely to impact freshwater inputs into, and water quality in, coastal and estuarine systems, through seasonal shifts in river flow including more frequent high and low discharge periods, increased catchment run-off, increased inputs of nutrients and sediment and greater stratification of the water column (Scavia et al., 2002; Struyf et al., 2004; Vermaat et al., 2005; Day et al., 2008). Increases in precipitation and freshwater runoff might also impact estuarine salinity gradients and corresponding salinity tolerance-based species distributions (Savenije, 2005; Cheung et al., 2009; Levinton et al., 2011). For example, predicted increases in precipitation in the northeastern United States might lower salinity to below the threshold required for oyster survival in estuarine regions, potentially disrupting population dynamics and impeding oyster restoration efforts (Levinton et al., 2011). In addition to changes in precipitation, predicted changes in wind patterns and storminess (e.g. coastal storms and storm surges) are likely to increase catchment run-off and estuarine nutrient loading, increasing the vulnerability of coastal systems to eutrophication, hypoxia and anoxia (Conner et al., 1989; De Jonge et al., 2002; Whitehead et al., 2009a; Wilby et al., 2010). For example following hurricane Katrina, storm-
induced fertilizer runoff from the primarily agricultural catchment resulted in a three month increase in nutrient concentrations in Biscayne Bay, Florida (Zhang et al., 2009). Over the past 50 years, a 1 °C increase in sea surface temperatures was accompanied by an 80% increase in total hurricane intensity (Emanuel, 2005), whilst increases in the number of category 4 and 5 storms between 1970-2004 have been directly linked to increases sea surface temperatures, but might alternatively be related to natural decadal storm cycles rather than climate induced changes (Webster et al., 2005; Hoyos et al., 2006). It has been estimated that the recent hurricanes in North America (Katrina and Rita, both in 2005), resulted in a loss of 520 km² of coastal marsh in Los Angeles (Howes et al., 2010; DeLaune & White, 2011). Coastal marsh and wetlands play an important role in the global climate cycle, acting as carbon ‘sinks’ through the sequestration of carbon through vertical accretion (Chmura et al., 2003; Choi & Wang, 2004). The combined loss of coastal wetland from Katrina and Rita was estimated to equal a loss of approximately 15.4 x 10⁶ metric tons of carbon to a depth of 100 cm (DeLaune & White, 2011). Predicted changes in the frequency, intensity, and distributions of wind patterns, strong storms and storm surges will likely alter species composition and biodiversity of coastal and estuarine ecosystems, through erosion and flooding of coastal habitats, short-term saline intrusion, movement or flushing of nutrients, sediment export and the movement and/or resuspension of material (Conner et al., 1989; Najjar et al., 2000; Davis et al., 2004; Day et al., 2008; Najjar et al., 2010; Verspecht & Pattiaratchi, 2010). These impacts are also likely to affect all aspects of coastal and estuarine ecosystem functioning, including nutrient cycling and primary and secondary productivity (Davis et al., 2004; Day et al., 2008).

Eustatic sea level rise (ESLR) over the 20th century is estimated to have been 15 to 20 cm (Miller & Douglas, 2004). In its Fourth Assessment Report, the UN Intergovernmental Panel on Climate Change (IPCC) predicted a rise in eustatic global sea levels of 18 to 59 cm by 2100 under the full range of SRES greenhouse gas emission scenarios (IPCC, 2007). These SRES scenarios (low, medium-low, medium-high and high emissions) reflect different plausible estimates of future social and economic development and resultant greenhouse gas emissions (Nakicenovic et al., 2000; IPCC, 2007). However, recent studies that incorporate polar melting (rapid ice flow changes) into sea level projections, suggest that eustatic global sea levels could in fact increase 3-fold more than current IPCC estimates (IPCC, 2007), with projected sea level rise of more than one metre, possibly up to 1.9 m by 2100 (Vermeer & Rahmstorf, 2009; Jevrejeva et al., 2010). In some areas, the addition of vertical movements of the land (e.g. glacio-isostatic rebound, tectonic uplift and geological subsidence) significantly contribute to increases in sea levels relative to the land (relative sea level rise), and are of much greater concern for coastal and
estuarine areas (Scavia et al., 2002; Nicholls & Klein, 2005). In the UK, postglacial isostatic adjustment from the British Isles Ice sheet has, and is, continuing to produce contrasting relative sea-level changes around the UK coastline (Shennan et al., 2006; Bradley et al., 2009; Teferle et al., 2009). Estimates of land-level changes (negative of relative sea-level change) indicate greatest relative land uplift in central and western Scotland (ca. 1.6 mm yr\(^{-1}\)) and lowest in southwest England (ca. 1.2 mm yr\(^{-1}\)) (Shennan et al., 2006; Bradley et al., 2009; Teferle et al., 2009). This relative sea level rise has led, or in future could lead to significant impacts upon coastal and estuarine ecosystems through erosion of existing shorelines and inundation of low-lying coastal land, increases in coastal flooding, coastal squeeze and increases in the penetration of saltwater into coastal surface waters (e.g. estuaries and lagoons) and ground waters (e.g. aquifers) (Sherif & Singh, 1999; Scavia et al., 2002; Day et al., 2008; Jackson & McIlvenny, 2011; Pool & Carrera, 2011).

1.2.3 Saline penetration into estuaries

The ecological, social and economic impacts of coastline loss (through erosion and inundation) and penetration of salt water into groundwater aquifers has been extensively researched and is commonly included in government coastal climate change risk reports (IPCC, 2007). In contrast, the potential impact of tidal driven increases of salt water into coastal surface waters (e.g. estuaries, rivers and lagoons) under changing climate (and relative sea level rise) has been neglected, despite its potentially deleterious impacts upon estuarine and riverine ecosystem functioning, land use and abstracted water quality (Kingsford et al., 2011; Turak et al., 2011). Episodic decreases in freshwater discharge, resulting from drought episodes and/or increased anthropogenic water abstraction, can result in increases in the upstream extent of saline penetration into estuarine systems, compared to decreases in this extent under high freshwater flood conditions (Savenije, 2005). Large-scale shifts in estuarine salinity structure, particularly under low flow (drought) conditions, can have serious deleterious effects upon estuarine flora and fauna, causing changes to the structure and community compositions of estuarine phytoplankton (Kasai et al., 2010) invertebrates (Andrews, 1977; Attrill et al., 1996; Attrill & Power, 2000; Bessa et al., 2010) and fish (Martinho et al., 2007), particularly in the (under-researched) upper tidal freshwater zones where freshwater fauna is often reduced to those species able to tolerate saline conditions (Attrill et al., 1996; Martinho et al., 2007; Sousa et al., 2007). For example, in south-eastern Australia, the Ramsar-listed coastal wetlands of the Murray-Darling Basin (the Coorong, Lower Lakes and Murray Mouth) have experienced reductions (and even the cessation) of freshwater riverine inputs, through prolonged drought and anthropogenic upstream diversion of river flow (Zampatti et al., 2010; Kingsford et al., 2011).
The resulting increase in salinity had detrimental effects upon faunal structure (species richness and diversity) and recruitment within fish, waterbird, macroinvertebrate and littoral plant communities that inhabit these wetlands (Paton et al., 2009; Zampatti et al., 2010; Kingsford et al., 2011).

A series of studies undertaken in the River Thames estuary investigated the response of benthic macroinvertebrates (animals retained on a 0.5 or 1 mm sieve), to increases in saline penetration and reductions in water quality resulting from drought induced low river flow (Andrews, 1977; Attrill et al., 1996; Attrill, 1998; Attrill & Power, 2000). These salinity increases (and associated reductions in water quality) had a significant impact upon the benthic macroinvertebrate communities, particularly for mobile brackish water species, which exhibited population changes and upstream shifts in species distributions (e.g. Carcinus maenas, Crangon crangon and Gammarus zaddachi). Small increases in salinity recorded in the freshwater upper reaches of the estuary (below Teddington Weir), induced dramatic change in the freshwater macroinvertebrate community composition, with the fauna reduced to those species able to tolerate saline conditions (e.g. the pea mussels, Sphaeriidae). After the recorded saline penetration events, the freshwater macroinvertebrate community of the upper Thames estuary demonstrated a cyclical pattern of disturbance and recovery, with a downstream influx of freshwater species into the upper estuarine zones via invertebrate drift from the non-tidal River Thames, following the return to normal river flows (Brittain & Eikeland, 1988; Attrill et al., 1996). The return to normal freshwater flows was essential for the recovery and recolonisation of freshwater upper estuarine zones, post saline penetration events (Brittain & Eikeland, 1988; Attrill et al., 1996).

Distribution changes of key species due to increasing saline penetration might also alter estuarine food webs through predator and prey interactions. For example, increases in the upstream extent of Carcinus maenas associated with drought events (such as in the Mondego estuary, Portugal; Bessa et al., 2010) could have a significant impact upon populations of juvenile fish species, on which they feed (Ansell et al., 1999; Taylor, 2005). In turn, where estuarine amphipods (e.g. Gammarus sp.) constitute a large proportion of fish species diets, shifts in the distributions and abundances of these amphipods could result in a local decline in fish populations (e.g. the reported decline in juvenile flounder, Platichthys flesus) in the Thames estuary since the mid-1980s; Thomas, 1998). Drought-induced low freshwater flow conditions might also provide ideal microhabitats for the propagation of invasive species, in particular, crustaceans, with increased larval retention due to low flows (preventing them from being washed out into the sea) and increases in salinity, which are critical for larval development (Cohen et al., 1995; Herborg
et al., 2005). For example, the low flows and increased salinities in the Thames estuary as a result of the 1989-1992 drought has been linked to the large upstream increase in distribution of the invasive Chinese mitten crab (*Eriocheir sinensis*) recorded between 1992-1996 (Herborg et al., 2005).

### 1.2.4 Future extents of saline penetration

As indicated above (see Section 7.2.3), climate-change driven projected increases in relative sea levels, seasonal changes in river flow and increased regularity of episodic events (e.g. storm surges) are predicted to result in greater upstream penetration of salt water into estuarine systems (e.g. Scavia et al., 2002; IPCC, 2007; Day et al., 2008; Lowe et al., 2009; Menon et al., 2010). Increases in extreme storm events (including coastal storms and storm surges) is likely to result in short-term salinity pulse events (i.e. causing rapid but not sustained change) into estuarine systems, which might be more detrimental to estuarine ecosystems than typical daily variations in saline penetration experienced over a tidal cycle (e.g. Hurricanes Katrina and Rita; see above), particularly in estuaries that retain salt water following storm surge events (e.g. temporary open/closed estuaries). For example, in 2008 a storm surge breached the closed mouth of the East Kleinmonde estuary in South Africa, resulting in higher than normal salinities in the estuary for the following 15 month closed phase (Riddin & Adams, 2010). These increased salinities had a detrimental impact upon the estuary ecosystem, with reductions recorded in submerged macrophyte cover and diversity, and saltmarsh habitat (Riddin & Adams, 2010). In contrast, studies examining the affects of short term salinity pulses on the growth and survival of freshwater macrophytes have demonstrated that a number of species can tolerate short term periods of exposure to saline conditions (Nielsen et al., 2003; Goodman et al., 2010).

Whilst river flows are an important regulator of the temporal and spatial extent of saline penetration in estuarine systems, it is especially important in cases where freshwater inflow is the ‘major’ factor contributor to wide salinity changes (Gillanders & Kingsford, 2002; Savenije, 2005). Natural river flows into estuarine systems are primarily determined by freshwater catchment runoff, the quantity of which depends on the interaction of climate variables (i.e. precipitation, temperature and evaporation) and the physical characteristics of the drainage basin. In the UK, rivers have a sustained discharge, but with seasonal peaks (e.g. in winter/spring) following heavy precipitation (and occasional snow melt) events (Whitehead et al., 2009b). In UK estuaries, increased saline penetration is generally recorded under summer low flow conditions and reduced penetration compared to under winter high flow conditions. However, future projections for UK climate include warmer, wetter winters and hotter, drier summers,
which are expected to significantly impact river flow regimes, exacerbate seasonal differences in saline penetration and affect groundwater recharge in UK estuaries (Murphy et al., 2009). Under the UKCIP09 medium emissions scenario for 2080, winter precipitation is predicted to increase up to +33% (+9 to +70%; 10 and 90% probability levels), whilst summer precipitation is predicted to decrease down to -40% (-65 to -6%) (Murphy et al., 2009). Increases in the mean daily maximum temperatures are predicted in both summer and winter, with increases in the summer average up to 5.4 °C (2.2 to 9.5 °C) and increases in the winter average up to 1.5 °C (0.7 to 2.7 °C) to 2.5 °C (1.3 to 4.4 °C) (Murphy et al., 2009). River flow and groundwater recharge responses to predicted temperature and precipitation changes, however, will vary depending on catchment location, geology, land-use, soils and model uncertainty (Romanowicz et al., 2006). Modelled changes in UK river flows for the 2020’s (for a range of climate model predictions) have predicted climate induced reductions in flow ranging from 1 to 32% (Romanowicz et al., 2006). In addition, long term mean annual groundwater recharge is predicted to fall by 7% in Paisley (Scotland), 20% in Cotishall (East Anglia) and up to 40% in Gatwick (south-eastern England) by 2080 under the high emissions scenario (SRES:A1FI) for both winter and summer months (despite precipitation increases in winter), as a result of increasing evapotranspiration and soil moisture deficit (Herrera-Pantoja & Hiscock, 2008). Despite these significant predicted climate induced reductions in river flow, it has recently been suggested that future anthropogenic induced changes in river flows through rising demands for freshwater (primarily driven by appropriation of freshwater for human use), might be of more importance than any climate change related impacts on river flow regimes (Vorosmarty et al., 2000; Vorosmarty et al., 2010; Lester et al., 2011).

Most of the world’s major rivers have been anthropogenically modified and regulated, resulting in changes to natural river flow regimes from freshwater abstraction (e.g. agricultural, domestic and industrial), river management strategies (e.g. impoundment, water transfers, channelization, dams and flood control), land use (e.g. urban drainage), waste disposal (e.g. sewage effluent returns) and vegetation removal, all of which have been shown to have detrimental effects upon freshwater ecosystems and biodiversity, regardless of changes in climate (Weatherhead & Knox, 2000; Bunn & Arthington, 2002; Dudgeon et al., 2006; Vorosmarty et al., 2010; Wilby et al., 2010). In some regions of the UK, there is concern that future increases in population will exceed the available freshwater resource (through increased domestic consumption), particularly during droughts (Johnson et al., 2009). For example, in south east England, accommodation for an additional one million people by 2026 is required, despite the fact that surface and groundwater resources in the region already suffer from unsustainable or unacceptable extraction.
regimes and water stress during the summer months (SEP, 2006; EA, 2007; Rodda, 2008). Whilst the abstracted water for domestic consumption is eventually returned to the catchment in the form of treated sewage effluent, much of the freshwater water abstracted for agricultural irrigation is lost through crop evapotranspiration (Johnson et al., 2009). The climate forecasts for hotter, drier summers and the current spate of drought events in the UK (e.g. current droughts in East Anglia; spring-summer 2011) have, and will lead to increased abstractions of freshwater for crop irrigation, with recent forecasts suggesting a 25-180% increase by the 2050s (dependant on socio-economic scenario) (Weatherhead & Knox, 2000; Henriques et al., 2008).

1.2.5 Salinity tolerances

It is well known that increases in saline penetration can force changes in benthic macroinvertebrate structure and species distributions in estuaries (see Section 7.2.3; Attrill et al., 1996; Bessa et al., 2010; Kingsford et al., 2011). Distribution-responses of benthic macroinvertebrates to changing environmental salinities is believed to be dependant upon the tolerances of marine and estuarine derived species to reductions in salinity, and freshwater (limnic) derived species to increases in salinity (Kefford et al., 2004b; Horrigan et al., 2007; Kefford et al., 2007c; Dunlop et al., 2008). Using the salinity tolerance ranges of benthic macroinvertebrate species (particularly those with narrow salinity ranges (i.e. stenohaline), that are well defined) it might be possible to predict changes in the distributions of these species in response to projected increases in saline penetration. The distributions and ecology of estuarine macroinvertebrate species in the UK have been extensively studied, resulting in a large volume of salinity tolerance data on which to base predictions of future species distributions, in relation to future changes in saline penetration (see Kinne, 1971; Lincoln, 1979; Barnes, 1994; Hayward & Ryland, 1995 and references therein). In contrast, there has been limited research undertaken on the salinity tolerances and estuarine field distributions of limnic derived species, due in part to the historic neglect of estuarine tidal limnetic zones by both estuarine and freshwater ecologists (Rundle et al., 1998; Williams & Williams, 1998b; Sousa et al., 2007). It has been shown that increases in saline penetration are most detrimental to freshwater flora and fauna recorded in the upper estuarine and adjoining lower riverine zones and that the susceptibility of these zones to increases in saline penetration might increase under future climate change scenarios (e.g. Attrill et al., 1996; Martinho et al., 2007; Sousa et al., 2007; Kingsford et al., 2011). Determining the responses of limnic macroinvertebrate species to increases in salinity is therefore essential to determine how future increases in saline penetration will affect limnic flora and fauna inhabiting the uppermost estuarine areas.
1.2.6 The salinity tolerances of freshwater macroinvertebrates

Freshwater benthic macroinvertebrates are not able to maintain a body fluid solute concentration below that of the water in which they live and are therefore defined as hyperosmotic regulators (Hart et al., 1991; Brooks & Mills, 2011). By maintaining internal concentrations of major ions above that of the external water (i.e. through active transport), they expend energy (Silva & Davies, 1999; Brooks & Mills, 2011). Where environmental salinity levels are equal to an invertebrates internal osmotic concentration (the isosmotic point), energy consumption for osmoregulation is effectively zero, enabling the energy to be used for growth and reproduction (Potts, 1954; Hart et al., 1991). It has been suggested that this ‘isosmotic point’ might explain peaks in freshwater macroinvertebrate richness at intermediate salinities, recorded in the hyporheic zone (subsurface sediments that exist between surface water and groundwater; Boulton et al., 2007), anthropogenically (secondarily) salinised rivers (Kay et al., 2001), along natural salinity gradients (Piscart et al., 2005) and under laboratory conditions (Kefford et al., 2005a). As the environmental salinity increases to a level that exceeds an invertebrates body fluid solute concentration, ions pass into the cells and water passes out (Potts, 1954). The salinity tolerance of a species is therefore determined by their natural cell solute concentration; the higher the internal ionic concentration, the higher the tolerance to salinity (Hart et al., 1991). This in turn depends on the morphology of the individual taxon, the evolution of the species and the route taken by colonisation (Greenaway, 1986; Carver et al., 2009; Piscart et al., 2010). Hart et al (1991) observed that most freshwater macroinvertebrates have internal ionic concentrations of 1 - 1.5 (psu), though it is generally accepted that solutions containing salts in excess of 9 are deleterious to freshwater invertebrate osmoregulatory mechanisms (Greenaway, 1986). However, salt concentrations of about 0.8 have been observed to effect physical, biochemical and behavioural functions (Hart et al., 1991).

Most freshwater macroinvertebrates are assumed to be intolerant to any increase in environmental salinity. However, where the salinity tolerances of freshwater species have been investigated, many studies report high salinity tolerance values under laboratory (experimental) and/or field conditions (Timms, 1981; Williams & Williams, 1998a; Chadwick & Feminella, 2001; Halse et al., 2003; Williams, 2009; Piscart et al., 2010). These studies have shown that the salinity tolerance of freshwater macroinvertebrate species greatly differs between and within taxonomic groups, with crustaceans widely accepted as the most salt tolerant of the aquatic macroinvertebrate taxa (Hart et al., 1991; Kefford et al., 2003; Kefford et al., 2005a; 2006b; Kefford et al., 2007a; Piscart et al., 2010). For example, Piscart et al (2010), related the high numbers (46%) of non-native freshwater crustaceans in the River Rhine (France), to high salinity
tolerances enabling the survival and translocation of these species in ship ballast water.

It is commonly assumed that aquatic insects (Odonata, Plecoptera, Ephemeroptera and Trichoptera) are the most physiologically intolerant of the benthic macroinvertebrates to increases in salinity (Short et al., 1991; Williams & Williams, 1998a; Williams & Hamm, 2002). For example, Williams and Williams (1998a) classified the Ephemeroptera nymphs *Baetis rhodani* and *Rithrogena semicolorata* as intolerant to salinity as they both exhibited low salinity tolerances under experimental conditions, and field distributions limited to the tidal freshwater zones of the Aber estuary (North Wales). In contrast, freshwater Ephemeroptera nymphs have been recorded in brackish waters (2–10) in Florida (Berner & Sloan, 1954), in saline rivers in Oklahoma (Magdych, 1984) and can survive and grow (at similar rates to those in freshwater systems) in elevated salinities under laboratory conditions (*Hexagenia limbata*; Chadwick & Feminella, 2001). High tolerances to salinity have also been recorded for Trichoptera (Sutcliffe, 1960; Williams & Williams, 1998a; Piscart et al., 2005; Blinn & Ruiter, 2006), Plecoptera (Muller & Mendl, 1979) and Odonata (Butler & Popham, 1958; Kefford et al., 2003), but wide variations in salinity tolerance between species have been recorded within these individual families (Chessman, 2003).

### 1.2.7 Factors affecting salt sensitivity

In addition to variations in salinity tolerance between species (within families), a range of salt sensitivities have also been recorded within individual species. Studies have shown that life stage or age (Brock et al., 2003; Tills et al., 2010) reproductive state (Barnes, 1968), moult cycle stage (Pannikar, 1941; Parry, 1957; Bursey & Lane, 1971a; Hagerman, 1973; Lockwood & Inman, 1973), gender and body size (Lindqvist, 1970; Sornom et al., 2010), nutritional state (Sutcliffe et al., 1981) acclimatisation (Boulton & Brock, 1999; Kay et al., 2001; Halse et al., 2003; Pinder et al., 2005), evolutionary adaptation (Sutcliffe, 1974) and parasitic infection (Williams et al., 2004; Piscart et al., 2007; Brooks & Mills, 2011) all relate to the invertebrate ‘condition’ and thus its ability to regulate cell solute concentrations. For example, Sornom et al (2010) recorded gender differences in saline sensitivity in the freshwater amphipod *Gammarus roeseli*, with females recording significantly higher sensitivities to salt in survival, ventilation and ionoregulation tests. Both males and female however recorded high acute salinity tolerances, with 72 hour LC$_{50}$ values of 8.53 ±0.16 and 12 ±0.19 respectively (Sornom et al., 2010). Tills et al (2010) investigated the developmental phenotypic plasticity of the gastropod *Radix balthica* (from an upper estuarine population) to increasing salinities (2–9). It was hypothesised that developmental phenotypic plasticity might facilitate the persistence of
freshwater species to climate induced increases in saline penetration (Tills et al., 2010). Salinity was recorded to influence both the time of onset of developmental events, duration of developmental stages and the morphology of hatchling snails (shell size and shape of hatchlings). However, the fitness implications that these changes might have on predicted increases in saline penetration was unquantified (Tills et al., 2010).

Piscart et al (2007) investigated the influence of an acanthocephalan parasite, *Polymorphus minutus* (Acanthocephala: Palaeacanthocephala), on the salinity tolerance of the freshwater amphipod *Gammarus roeseli*. A number of studies have shown that acanthocephalan parasites reduce host survival under stressful environmental conditions by exacerbating the toxic effects of a number of compounds, most notably ammonia and aluminium (McCahon & Poulton, 1991; Prenter et al., 2004). However, Piscart et al (2007) recorded increased salinity tolerance in infected gammarids (LC$_{50}$ of 17.3) compared to non-infected gammarids (LC$_{50}$ of 9.7) and related the increased salinity tolerance to parasite-induced physiological changes. Piscart et al (2007) however stressed the need for further work to examine the precise impacts of parasitic infection on *Gammarus roeseli* osmoregulatory mechanisms. Dunn and Hatcher (1997) investigated the impact of salinity on the transovarial transmission of a microspoidian sex ratio disorder in *Gammarus duebeni*. It was observed that exposure of parasitized *G. duebeni* mothers to increased salinity during the gonotrophic cycle impedes parasite transmission to the young. The pattern of parasite growth did not differ between salinities, indicating that salinity only has an effect in the initial parasite burden transmitted to the gamete (Dunn & Hatcher, 1997).

Many freshwater aquatic invertebrates have evolved physiological and morphological adaptations to cope with increased salinity, the degree to which may be dependant on the route of colonisation and ancestral associations with the sea or saline environments (Greenaway, 1986; Williams et al., 1991; Pinder et al., 2005). It can be assumed that ancestral freshwater macroinvertebrates will have less advanced osmoregulatory structures than those with an ancestral history of life in saline environments and will therefore expend more energy maintaining ionic balance (Sutcliffe, 1974). Evolutionary adaptation can also influence the salt tolerance of geographically separated populations of the same species, if the species exist in contrasting saline conditions. These species can become further tolerant of salinity by acclimatisation. Pinder et al (2005) used previous studies (Timms, 1998a, 1998b; Bailey & James, 2000) to conclude that approximately 56% of recorded freshwater macroinvertebrate species occurred at much greater salinities (>20) in south western Australia (the Wheatbelt region) than in eastern Australia. These results suggest that the freshwater invertebrate fauna of
the Wheatbelt region was comparatively more salt-tolerant due to the regions long history of natural salinisation (Boulton & Brock, 1999; Kay et al., 2001; Halse et al., 2003). James et al (2003), however, highlighted the fact that the present rate of salinity increase in Australia is likely to be too fast for most aquatic freshwater biota to adapt, and that current aquatic communities are likely to be the most tolerant remnants of the natural fauna (Williams et al., 1991). Piscart et al (2010) investigated the salinity tolerances non-native macroinvertebrates in France to examine if high salinity tolerance was a physiological characteristic of non-native species, important to the success of translocation to a new area (i.e. through ballast water). Non-native macroinvertebrate species which had originated from outside Eurasia (introduced by ship ballast water) recorded higher salinity tolerances than the native species and non-native species that originated from within Eurasia.

It is important to note that the zonation and distribution of freshwater macroinvertebrate species under field conditions, cannot always be fully understood by (or attributed to) salinity tolerances alone (Dorgelo, 1977). For example, studies by Born (1968) and Dorgello (1977) on the shrimp Syncaris pacifica (Crustacea: Decapoda) and the gammarid Gammarus tigrinus (Crustacea: Amphipoda) respectively, have recorded considerable tolerance to salinity under laboratory conditions, but in nature occur on a very restricted salinity range (Hart et al., 1991).

1.2.8 Freshwater macroinvertebrates in estuaries

In Australia, secondary salinisation of freshwater habitats, has resulted in a large body of research assessing the impact of increasing salinity upon freshwater macroinvertebrates (for reviews see: Hart et al., 1991; Bailey et al., 2002; Clunie et al., 2002; Nielsen et al., 2003), through the development of salinity toxicity test systems and experiment procedures (Kefford et al., 2002; Kefford et al., 2004b; Kefford et al., 2004c; Kefford et al., 2005b) and the resultant acute salinity tolerances of native freshwater macroinvertebrate species (Kefford et al., 2003; Kefford et al., 2004a; Kefford et al., 2004b; Kefford & Nugegoda, 2005; Kefford et al., 2006a; Kefford et al., 2006b; Kefford et al., 2007b; Kefford et al., 2007c). In contrast, very few studies have investigated the community structure, distributions and salinity tolerances of freshwater macroinvertebrates in estuaries (Rundle et al., 1998; Williams & Williams, 1998a, 1998b; Williams & Hamm, 2002; Williams, 2009).

Tidal saline penetration into freshwater ecosystems subjects benthic macroinvertebrates to cyclical and dramatic changes in the osmotic pressure of their environment, with the degree of change dependant upon their longitudinal distribution along the estuarine course (Williams &
Understanding the relationships between tidal driven increases in salt water and freshwater benthic macroinvertebrate species responses is critical for predicting the impacts likely from future increases in saline penetration. Where published studies have investigated the distributions and salinity tolerances of freshwater benthic macroinvertebrate species in estuaries, authors have recorded high tolerances to salinity, both in the laboratory and the field. For example, in the river-dominated stratified Aber Estuary in North Wales, high densities of freshwater insect larvae were present (i.e. Plecoptera, Ephemeroptera, Trichoptera, Coleoptera and Diptera) accounting for 32% of all recorded benthic macroinvertebrates, and inhabited sites inundated by 33.7% and 100% of all incoming tides (32-34) (Williams & Williams, 1998a, 1998b). Similar spatial and temporal patterns were recorded in three coarse grained substratum estuaries in Canada, where freshwater insect larvae made up a 17-54% of all recorded benthic macroinvertebrates, and inhabited estuarine sites inundated by 25% of all incoming tides, experiencing high tide salinities of approximately 24 (Williams & Hamm, 2002).

In the Aber Estuary, it was calculated that each year ca. $31 \times 10^3$ individuals (weighing 62.6 kg) crossed the freshwater-seawater interface through downstream drift, providing an important food source for estuarine fish (Williams & Hamm, 2002). A large number of these freshwater insect species entering the Aber Estuary inhabit benthic sites that experience varying degrees of tidal saline penetration (Williams & Williams, 1998b). Laboratory salinity tolerance experiments undertaken on a number of these freshwater insect species, recorded high tolerances to salinity, particularly within the trichopterans (i.e. Sericostoma personatum, Odontocerum albicorne and Aidicella reducta) which inhabited sites inundated by 80.9–100% of all tides (32-34) (Williams & Williams, 1998a). The comparison of two hydropsychid trichopteran species (Hydropsyche siltalai and Hydropsyche instabilis) from both freshwater and salt water inundated sites on the Aber Estuary suggested that populations of H. siltalai and H. instabilis present in saline inundated sites were functioning normally, through rock attachment, net spinning and food ingestion (Williams, 2009). However, the reduced gut contents of these specimens (when compared to the species from freshwaters), suggested that the downstream (saline inundated) populations of these species were existing in suboptimal conditions (Williams, 2009). In contrast, the downstream drift of populations of the freshwater Plecopteran, Leuctra digitata into north Swedish coastal waters (4.2), produced no noticeable detrimental impacts upon population functioning, with nymphal development continuing till adult emergence (Muller & Mendl, 1979). Further research on the functioning of these populations is required to thoroughly assess the ability of freshwater macroinvertebrate species to inhabit saline inundated estuarine zones (Williams, 2009).
1.2.9 Summary

Estuaries are extremely complex systems, being transitional zones characterised by steep environmental gradients (particularly tidal and freshwater flow driven changes in salinity) and constantly changing physiochemical conditions, operating over a range of timescales (including tidal, monthly, seasonal and yearly variation). Despite this, estuaries can be extremely productive, as the flora and fauna that are able to inhabit these systems often reach extremely high abundances. Global climate change (including rising temperatures, changing precipitation patterns and global sea level rise) and anthropogenic influence (e.g. groundwater abstraction, channel modification) is likely to cause an increase in the upstream extent of saline penetration into estuarine systems over the next century. Likely impacts include changes in ecosystem structure and species distributions, loss of biodiversity, greater susceptibility to invasive species, potential loss and destruction of habitat and potential changes in nutrient cycling, productivity and availability of resources to humans. Whilst an extensive literature is available detailing salinity tolerances for estuarine and brackish water species, one of the faunal groups most at risk to future changes associated with saline penetration is the freshwater macroinvertebrates (inhabiting the tidal limnetic zone), as these taxa are generally considered intolerant to increases in salinity. Greater research is needed assessing salinity tolerances in limnic species under both field and laboratory conditions in order to be able accurately predict how future saline penetration will affect transitional zone ecosystems.

1.3 Aims, Objectives and Hypotheses.

The overall aim of this research project is to determine to what extent saline penetration into estuaries will change under future climate scenarios, and to predict the impact these changes will have upon benthic macroinvertebrate community structure and species distributions over the freshwater to marine transition. This overarching aim incorporates the detailed assessment of current and future (predicted) tide and salinity profiles (Chapter 4), historic (Chapter 3) and contemporary (Chapter 5) benthic macroinvertebrate community structures (and relationships with measured environmental variables) and macroinvertebrate acute salinity tolerances undertaken in the laboratory and field (Chapter 6). It is hypothesised that future changes in climate (i.e. rise in relative sea level and reductions in summer freshwater river flow) will result in an increase in the upstream extent of saline penetration into estuarine and riverine systems, which will produce a shift in the faunal structure and distribution of the resident benthic macroinvertebrate taxa as a function of individual species salinity tolerances. It is hypothesised that the ecological impact of increases in saline penetration upon benthic macroinvertebrates can
be predicted using the quantified salinity tolerances of representative macroinvertebrate species alone.

This project will identify coastal areas of the UK most ‘at-risk’ from future increases in saline penetration, particularly areas vulnerable to significant increases in relative sea levels, climatic and anthropogenically driven changes to the volume of freshwater discharge and episodic events (e.g. storm surges and coastal flooding). Areas that support dense human populations, with current and/or planned large-scale coastal development are also deemed at risk (Chapter 2). The research will utilise comparable estuaries and associated river systems (in the identified ‘at-risk’ area) using a criteria based site selection approach, suitable for the investigation of the potential impacts of future increases in saline penetration upon macroinvertebrate community structure and species distributions (Chapter 2).

This project addresses the following aims, objectives and associated hypotheses.

1. To analyse publically available historic ecological data for the selected estuaries and rivers, in order to investigate historical benthic macroinvertebrate community structure and species distributions (Chapter 4). In addition, the research will critically examine the use of published macroinvertebrate salinity tolerance values and groupings for predicting the upstream extent of saline penetration in the absence of direct salinity measurements (Chapters 4 and 6). It is hypothesised that the influence of salinity will be detected in the distribution and structure of the resident benthic macroinvertebrate communities within the selected estuaries and rivers when sampled longitudinally along the salinity gradient (over the freshwater to marine transition), even in the absence of direct salinity measurements. It is also hypothesised that allocating macroinvertebrate species salinity tolerance values and groupings (from published literature) will enable the determination of the upstream extent of saline penetration in the absence of direct salinity measurements.

2. To determine the current extent and natural variability of saline penetration within the study estuaries through direct measurement of the tidal and salinity cycles, under both high and low freshwater discharge conditions (Chapter 5). In addition, to predict future tide and salinity profiles (and extents of saline penetration) for the study estuaries under projected sea level rise scenarios for both low and high freshwater discharge conditions (Chapter 5). It is hypothesised that the spatial and temporal extent of saline penetration into the study estuaries is naturally dynamic, and reflects the relative opposing strengths of tidal forcing marine water upstream and
freshwater discharge downstream. It is also hypothesised that under projected sea level rise scenarios, the upstream extent of saline penetration into the study estuaries will increase under both high and low freshwater discharge conditions.

3. To investigate the benthic macroinvertebrate community structure and species distributions in the selected study estuaries/rivers under high (winter) and low (summer) freshwater discharge conditions, and explore the relationships between faunal structure, salinity parameters (i.e. maximum, minimum and range) and a range of environmental variables measured in the field (e.g. sediment grain size, dissolved oxygen content and organic content; Chapter 6). It is hypothesised that whilst additional environmental variables will be important in determining macroinvertebrate compositions in estuaries, salinity is the dominant parameter driving faunal structure and species distributions over the freshwater to marine transition. It is therefore hypothesised that spatial variability in the extent of saline penetration (under low and high freshwater discharge conditions) will be associated with shifts in the distributions of benthic macroinvertebrate species relative to their salinity tolerances.

4. To determine the acute salinity tolerances (LC50) of selected benthic macroinvertebrate species under controlled laboratory conditions for summer (18 °C) and winter (7 °C) water temperature regimes (Chapter 7). In addition, to test the survivorship of limnic macroinvertebrate species to actual tidal cycles under field conditions, and to compare the laboratory derived salinity tolerances (LC50) with the field tidal cycle survivorships, recorded field distributions and published salinity tolerance literature (Chapter 5). It is hypothesised that the laboratory acute salinity tolerances (LC50) of benthic macroinvertebrate species will be reduced at high temperatures (18 °C) compared to lower temperatures (7 °C). It is also hypothesised that laboratory acute salinity tolerances (LC50) of individual species will correspond to their recorded survivorship under field tidal cycle conditions, and that these results will reflect the recorded field distributions and salinity tolerances reported in published literature.

5. To answer the overall aim of this research project (using the results and conclusions from chapters 3-6) by predicting how distributions of benthic macroinvertebrate species might change in the study estuaries/rivers in relation to future projected increases in saline penetration. In addition, to assess the predictive accuracy of using salinity tolerances alone in the prediction of future macroinvertebrate distributions in relation to projected increases in saline penetration. It is hypothesised that current and future climate change will increase relative sea levels and reduce freshwater river discharge, resulting in the progressive upstream penetration of saline water into
the River Adur and River Ouse estuaries. It is also hypothesised that the ecological consequences of increased saline penetration would include changes to the distribution and composition of resident benthic macroinvertebrate taxa as a function of a species salinity tolerance. It is further hypothesised that these changes could be predicted using the quantified salinity tolerances of representative macroinvertebrate species.
Chapter 2. Sites

2.1 Introduction

The selection of appropriate study sites is essential to this research project, particularly as the current and future degree of saline penetration into estuarine and riverine systems will (amongst other factors) vary with location and topography. Therefore, a paired catchment approach is adopted for selection of two similar estuaries and rivers in terms of size and character and to enable the impacts of saline penetration in two separate estuarine and river systems to be directly compared.

2.1.1 Aims and Objectives

This chapter aims to identify coastal areas of the UK most ‘at-risk’ from future increases in saline penetration (particularly areas vulnerable to significant increases in relative sea levels, climatic and anthropogenically driven changes to the volume of freshwater discharge and episodic events), and to select two comparable estuary and river systems from within these areas for further study using a criterion based site selection approach. This aim will be achieved through the following objectives:

1. Identify which coastal regions in the UK are potentially most at-risk from future increases in saline penetration.
2. Outline the criteria used for selection of the study estuaries and rivers.
3. Broadly describe the key joint catchment characteristics of the selected estuaries and rivers (i.e. The River Adur and River Ouse, Sussex).
4. Provide a detailed description of the fluvial and estuarine systems of the River Adur and River Ouse.

2.2 Site selection criteria

Postglacial isostatic adjustment from the British Isles ice sheet has, and continues to result in changes in relative land levels across the UK, with central and western Scotland rising and the south of England subsiding (Shennan & Horton, 2002; Shennan et al., 2006; Bradley et al., 2009; Teferle et al., 2009). Relative increases in sea level will therefore have the greatest impact on the south coast of England (Shennan et al., 2006; Bradley et al., 2009; Lowe et al., 2009). Future reductions in summer river flow (from a combination of climate and anthropogenic impacts; Murphy et al., 2009) are likely to be most severe in south east England, where surface
and ground water resources currently suffer from unsustainable extraction regimes, resulting in low river flows in summer months (Herrera-Pantoja & Hiscock, 2008; Rodda, 2008). Projected increases in population for the south east region (up to one million people by 2026; SEP, 2006) may exceed the climate induced (increased summer temperatures and decreased precipitation) reduced freshwater resource (See Chapter 1, Section 1.2.3; Rodda, 2008; Johnson et al., 2009). It is therefore very likely that estuaries on the south coast of England will experience increases in saline penetration under future land/sea level and climate change scenarios (Jenkins et al., 2009; Lowe et al., 2009).

The mainland south coast of England has 30 estuaries situated between Foreness Point on the east coast of Kent and Land’s End in Cornwall (Figure 2.1; Davidson et al., 1991; Buck, 1997). In order to select the most appropriate study sites for this research, all these estuaries (and their associated rivers) were tested for suitability against a carefully designed site selection criteria (Table 2.1). The site selection criteria was produced with the aim of identifying two comparable rivers, susceptible to increasing saline penetration, and likely to be able to accommodate successful field investigations. Only rivers that successfully matched all the specific criteria were deemed suitable for investigation, therefore being able to achieve the aims and objectives of the research project (set out in Chapter 1). Each river was scored on the basis of the site selection criteria outlined below:

1. **Rivers with low gradients and significant estuarine channel length (>10 km to the NTL).**

Rivers with low gradients may be susceptible to the greatest tidal driven penetration of salt water. It is likely that rivers with a significant estuarine channel length (>10 km) will possess long linear gradients of salinity from marine (35) at the river mouth to tidal limnetic (<0.5) at the normal tidal limit (NTL). These linear gradients of salinity should result in the zonations of marine, brackish water and limnic macroinvertebrate communities ideal for this investigation. A significant estuarine channel length may also increase the probability that point sampling would provide a realistic representation of any such zonations.

2. **Rivers with low summer freshwater discharge rates.**

Rivers subject to high abstraction pressures and natural low summer freshwater discharge rates might be less able to oppose the penetration of saline water upstream (Savenije, 2005). Extreme seasonal differences in freshwater discharge may result in seasonal shifts in the degree of saline penetration upstream and the opportunity to observe responsive faunal shifts in the macroinvertebrate community.
3. **Estuaries with a large tidal range (>5 m).**

Macrotidal estuaries with a tidal range of over 5 metres potentially have the greatest and most dynamic tidal saline penetration patterns, as the upstream extent and gradient of saline water will vary daily. This large vertical range will potentially provide the same benefits as outlined in 1 and 2 above (i.e. produce dynamic spatial and temporal saline penetration profiles).

4. **Lack of large intertidal areas (>100 ha).**

Estuaries with large intertidal areas are typically characterised by large bodies of brackish water, which can buffer the effects of tidal amplitude and saline penetration upstream. A lack of intertidal area is often the result of land claim for industrial and commercial usage (e.g. ports) and/or flood and coastal deference management practises. Where estuarine channels have been narrowed and constrained, tidal amplitude is funnelled, resulting in greater penetration of saline water upstream (Dyer, 1997).

5. **Absence of significant water engineering structures.**

The majority of low gradient rivers in southern England are influenced by river engineering structures. These structures can be lateral embankments to prevent flooding or channel obstructions such as locks and weirs that assist navigation and/or manage river levels. These engineering structures have the potential to limit the extent of saline penetration upstream, particularly when located below the normal tidal limit (NTL).

6. **Access for fieldwork and fixed permanent structures.**

Adequate legal and physical access to river margins is necessary to allow for representative sampling of the selected river courses. Fixed structures such as bridges provide midstream access points for sampling, act as GPS coordinate reference points and support fixed instruments (e.g. data-logging sondes, see Chapter 5).

7. **Rivers and estuaries with designated conservation programmes and available third party data sources.**

Rivers and estuaries with active conservation programmes and groups are desirable for local support and assistance. The availability of third party (secondary) data sources particularly of a historical nature, such as river discharge, ecological data or any records of disturbance events (e.g. floods, drought and/or pollution events) would provide baseline data and context for this research.
Figure 2.1. The location and names of the 30 estuaries subjected to the site selection criterion, situated on the mainland south coast of England (adapted from Buck, 1997).
<table>
<thead>
<tr>
<th>Map reference</th>
<th>Site</th>
<th>County</th>
<th>Channel length &gt;10km</th>
<th>Tidal Range &gt;5m</th>
<th>Intertidal area &lt;100 ha</th>
<th>Secondary data available</th>
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<tr>
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Table 2.1. Site selection criteria key differences (Davidson et al., 1991; Buck, 1997).
2.3 Selection of study sites

All 30 estuaries and associated rivers examined exhibited low gradients, engineering structures, access for fieldwork and contained, or were part of, designated conservation areas (Figure 2.1, Table 2.1). A number of differences became apparent when channel length (>10 km), tidal range (>5 m), intertidal area (<100 ha) and availability of secondary data were investigated (Table 2.1). Out of the 30 estuaries and associated rivers on the mainland south coast of England, only the River Ouse in East Sussex and the River Adur in West Sussex fulfilled all the necessary criteria (Table 2.1, Figure 2.2) and were therefore selected as study sites for this research project. These adjacent catchments share the same underlying geology and have similar hydrological regimes (Environment Agency, 2005a). Of particular significance was the availability of secondary data. In 2003, the Sussex Environment Agency implemented a macroinvertebrate sampling programme aimed at investigating the macroinvertebrate communities of the River Arun, River Adur, River Ouse and River Cuckmere estuaries in Sussex (Environment Agency, 2005b). In all four of these estuarine systems, increasing freshwater abstraction demands caused by the 2003 summer drought appeared to be causing greater upstream saline penetration (Environment Agency, 2005a; 2005b). Of particular concern was the large public water supply (PWS) abstraction site located just above the tidal limit at Barcombe on the River Ouse (Environment Agency, 2005a; 2005b). Increased summer freshwater abstraction at Barcombe could potentially lead to increased saline penetration downstream, which could severely impact coarse fisheries and species protected under the Biodiversity Action Plan (BAP), such as the bullhead (**Cottus gobio**) and lamprey (**Lampreta sp**; Environment Agency, 2006).

Macroinvertebrate sampling data for the tidal River Ouse and River Adur was available from 2003 to 2007 (Environment Agency, 2005b). In addition to macroinvertebrate data, water quality and river discharge data were available from Environment Agency routine monitoring stations situated along the course of these two rivers (Environment Agency, 2005a). Both rivers also have active conservation charity groups run by volunteers; the Sussex Ouse Conservation Society (S.O.C.S: access at http://www.sussex-ouse.org.uk) and the River Adur Conservation Society (R.A.C.S: access at http://www.sussex-adur.org.uk). These conservation groups generously provided first-hand historical accounts and data for the River Ouse and River Adur, tours of the rivers and helped obtain land-owners permission for access to large stretches of the water course.
Figure 2.2. The location and river courses of the River Adur and River Ouse.
2.4 The River Adur and River Ouse catchments

The River Adur and River Ouse catchments cover a combined area of 1073 km$^2$ extending into both eastern and western Sussex (Figure 2.2, Environment Agency, 2005a; 2009). The catchment includes 290 km of main river channel and 92 km of tidal banks (Environment Agency, 2005a). Much of the catchment is included within the Sussex Downs and High Weald Areas of Outstanding Natural Beauty (AONBs) and was awarded National Park status in March 2010 (i.e. South Downs National Park Authority, access at http://www.southdowns.gov.uk). The geology of the catchment predominantly consists of Tunbridge Wells Sand and Weald Clay in the North, Gault Clay and Greensand in the centre and Chalk in the South (Figure 2.3, Burrin & Jones, 1991; Environment Agency, 2005a; Waller & Long, 2010). The catchment is bordered by 40.2 km of coastline, most of which is developed, including the large urban city of Brighton and Hove and the large ports of Shoreham and Newhaven (Figure 2.2, Environment Agency, 2010). Inland development mainly consists of small towns and villages, surrounded by rural countryside (Environment Agency, 2006). The main towns include Lewes, Uckfield, Haywards Heath and Burgess Hill. Most employment is associated with tourism, recreation and agriculture with limited industry (Environment Agency, 2006). Surface water quality is assessed through a network of 50 sampling points covering 250 km of river (Environment Agency, 2005a; 2006; 2009). Routine monitoring shows that surface water quality is generally classified as ‘good’ (very good salmonid and cyprinid fisheries and/with ecosystems at or close to natural; Environment Agency, 2005a; 2006).
Figure 2.3. Geology of the River Adur and River Ouse catchment (Environment Agency, 2005).
2.4.1 The River Ouse, East Sussex

The River Ouse has the second largest river catchment in Sussex, comprising of over 225 km of main river and tributaries (Figure 2.4, Environment Agency, 2009). It rises on the Tunbridge Wells Sands in the High Weald and flows predominantly south easterly and then south (west of Uckfield) before cutting through an alluvial valley of the Sussex Downs to enter the English Channel at Newhaven (Figure 2.3, Figure 2.4, Environment Agency, 2005a; 2010; Waller & Long, 2010). The High Weald areas of the upper catchment are comprised of soft sandstones and impermeable Weald and Gault clays (Burrin & Jones, 1991; Waller & Long, 2010). The impermeable Weald clay makes the upper tributaries of the River Ouse highly ‘flashy’ (i.e. responds very quickly to rainfall, with water flow rising rapidly to a high peak before receding) in nature and prone to winter flooding (Figure 2.3, Burrin & Jones, 1991; 2005a), particularly in the River Uck (the largest tributary), but also for the Bevern and Clayhill streams (Environment Agency, 2005a). Due to their ‘flashy’ nature, these tributaries have naturally low summer flow (Burrin & Jones, 1991). The Shell Brook and Cockhaise Brook drain Tunbridge Wells and Ashdown Sands, both minor aquifers, and feed into the rivers upper reaches. These streams are less flashy and have a higher baseflow than the other tributaries (Environment Agency, 2005a; 2009).

The River Ouse has been extensively managed to support a variety of uses including fisheries, limited industry, agriculture, flood defence and water abstraction (Environment Agency, 2005a; 2006; 2009). These activities have resulted in the installation of several engineering structures, such as weirs and sluices, to regulate the flow and levels in the river (Environment Agency, 2005a; 2010). In the eighteenth century the river was modified for navigation, the impact of which can still be seen above the weirs in the upper and middle reaches of the main river, where deep, slow flowing water (typical of canals) is retained (Environment Agency, 2005a; 2010). In contrast, other parts of the upper and middle reaches of the River Ouse are shallow and consist of pool and riffle morphology (Environment Agency, 2005a).

The lower reaches of the river traverse an alluvial floodplain and are typically of uniform depth (Burrin & Jones, 1991; Waller & Long, 2010). A series of weirs at Barcombe Mills mark the normal tidal limit (NTL) and the end of the estuary (Figure 2.4, Buck, 1997; Agency, 2010). From Barcombe Mills, the tidal River Ouse flows for 21.8 km to Newhaven, where it enters the English Channel (Buck, 1997; Environment Agency, 2009; 2010). The tidal river channel is tightly constrained with embankments to prevent flooding and a half-weir at Hamsey moderates
the tidal flow, but is inundated at high tide and therefore does not act as a barrier for tidal water (Environment Agency, 2005a). Due to canalisation and pumping of the floodplain, the estuary channel is very narrow. This has prevented the development of salt marsh and only a small mudflat is exposed at Newhaven at low tide, resulting in an intertidal area of only 6 hectares (Buck, 1997; 2009; 2010). The River Ouse has a Mean High Water Spring (MHWS) tide height of 6.69 m and a Mean Low Water Spring height of 0.77 m at its mouth at Newhaven (National Oceanography Centre; access at http://www.pol.ac.uk/ntslf/tgi/portinfo.php?port=newh.).

Due to the highly ‘flashy’ nature of the river and low summer flows, abstraction of water from the River Ouse must be carefully controlled and monitored. River flow is monitored by four gauging stations on the main river and six stations on its tributaries (Environment Agency 2005a; 2006; 2009). The river system is dominated by a large public water supply abstraction (PWS) operated by South East Water (Environment Agency, 2006). Abstraction takes place upstream of Barcombe Mills, above the tidal limit (Environment Agency, 2006; 2009). Low summer river flows are unable to support high levels of abstraction. The Barcombe abstraction is therefore augmented by releases of freshwater from Ardingly Reservoir at the top of the catchment (Environment Agency, 2006; 2009). The river is used to transport the released water 38 km from the reservoir to the abstraction point (Environment Agency, 2006). The reservoir discharges increase summer river flows well above those that would occur naturally and fundamentally alter the hydrological regime of the river, which has important consequences for water resource availability in the river (Environment Agency, 2006; 2009).

Small areas of the River Ouse estuary lie within biological sites of Special Scientific Interest; Lewes Brooks (333 ha) and Offham Marshes (38 ha) (access at http://www.sssi.naturalengland.org.uk ). Lewes Brooks is a lowland wet grassland site, which occurs on the west bank floodplain of the River Ouse, south of Lewes (Buck, 1997; Environment Agency, 2005a). A large area of the brooks is an RSPB reserve and is also a refuge for rare invertebrates, such as the Lewes water beetle, Laccophilus poecilus. The Offham Marshes are alluvial grazing marshes situated on the west bank of the River Ouse in Hamsey (Buck, 1997; Environment Agency, 2005a). The marshes support large amphibian communities including the common toad (Bufo bufo), the smooth and palmate newt (Triturus vulgaris and Triturus helveticus) and several rare invertebrate species (Buck, 1997; Environment Agency, 2005a).

The ecology of the River Ouse is highly diverse and is routinely monitored by both the Environment Agency and the Sussex Ouse Conservation Society (SOCS). The river and its
tributaries are extensively used for coarse fishing and provide an important spawning ground for sea trout \((Salmo trutta)\) (Environment Agency, 2005a; 2006; 2009). Non-migratory salmonids such as brown trout \((Salmo trutta)\) are distributed throughout the middle and upper reaches of the river and its tributaries. The catchment also supports the bullhead \((Cottus gobio)\), and sea lamprey \((Petromyzon marinus)\), which are Annex II species under the EU Habitats and Species Directive (92/43/EEC) and are also designated Biodiversity Action Plan (BAP) species (Environment Agency, 2005a; 2006; 2009).

Water quality in the River Ouse catchment is generally classified by the Environment Agency as ‘good’ (very good salmonid and cyprinid fisheries and ecosystems at or close to natural) and is tested every month at 18 locations by SOCS (Environment Agency, 2005a; 2009). Water quality in the River Ouse is generally most affected by waste water disposal from waste water treatment works (WWTW) and agricultural run-off. There are five significant WWTW discharges in to the River Ouse catchment, with Scaynes Hill the largest of these accounting for ~30% of all discharges in to the river (Environment Agency, 2005a; 2009). Pollution incidents have also been recorded, for example a quantity of an organophosphate pesticide was released into the River Ouse affecting water quality and causing a notable fish kill event in 2001 (Environment Agency, 2005a).
2.4.2 The River Adur, West Sussex

The River Adur rises in two separate branches, the eastern and western Adur, which join to form the main river west of the town of Henfield (Figure 2.5, Whitehurst & Lindsey, 1990; Environment Agency, 2005a). The western branch of the River Adur rises near Slinfold and flows through Shipley and West Grinstead. The eastern branch of the River Adur rises on Ditchling common and flows north and west passing between Haywards Heath and Burgess Hill (Figure 2.5, Environment Agency, 2005a). The western and eastern branches join near Henfield and flow south across the Henfield Levels, an area of unspoiled wetlands (Environment Agency, 2005a). The lower River Adur flows through the Shoreham Gap Valley, an alluvial valley of the South Downs and an Area of Outstanding Natural Beauty (ANOB) (Buck, 1997; Environment...
Agency, 2005a; 2011). The River Adur enters the English Channel at Shoreham-by-Sea where the mouth has been deflected two miles to the east by a shingle spit (Figure 2.5, Buck, 1997; Environment Agency, 2005a; 2011).

The River Adur headwaters rise in the Low Weald on Gault and Weald clay, which like the River Ouse, makes up more than half of the catchment geology (Figure 2.3, Environment Agency, 2007; Waller & Long, 2010). The lower catchment is characterised by the chalk of the South Downs (Figure 2.3, Waller & Long, 2010). The impermeable clays of the upper catchment make the river highly flashy, responding quickly to rainfall events and having naturally low summer flows (Environment Agency, 2005a; 2007). The River Adur is also fed by perennial springs emanating from the northern scarp slope of the Brighton Chalk, which provides a limited quantity of base flow to some tributaries of the river (Figure 2.5, Environment Agency, 2007; 2009).

The River Adur has a Mean High Water Spring (MHWS) tide height of 6.3 m and a Mean Low Water Spring height (MLWS) of 0.6 m at its mouth at Shoreham (Shoreham Port, pers comms, 2010). The River Adur is tidal for 21 km up to its normal tidal limit (NTL) at Shermanbury on the eastern branch and Bines bridge (18.9 km) on the western branch (Figure 2.5, Buck, 1997; Environment Agency, 2011). The lower estuary at Shoreham is a narrow, winding channel with intertidal mudflats which become sandy towards the mouth (Environment Agency, 2009; 2011). Salt marsh fringes most of the lower estuary, but its development has been restricted by embankment of the river (Buck, 1997; Environment Agency, 2005a; 2011). The deflection of the river mouth by the shingle spit has created a relatively large intertidal area of 46 ha, and it is upon this shingle spit that the town of Shoreham-by-Sea and the Shoreham port has developed (Buck, 1997; Environment Agency, 2011).

The lower reaches of the estuary lie within the River Adur estuary biological Site of Special Scientific Interest (62 ha), which also forms part of a RSPB reserve (access at http://www.sssi.naturalengland.org.uk). The intertidal mudflats support a number of wading birds particularly the redshank, (Tringa tetanus), dunlin (Calidris alpine), and ringed plover (Charadrius hiaticula) (Buck, 1997; Environment Agency, 2005a). The estuarine plant communities are unusual due to the relative scarcity of cord-grass, Spartina spp. The landward margin of the salt marsh supports a variety of herbs and shrubs and the estuary embankment supports a large colony of viviparous lizards (Lacerta vivipara; Buck, 1997; Environment Agency, 2005a).
The ‘flashy’ nature of the River Adur means it has a natural tendency to flood its catchment (Environment Agency, 2007; 2009). The alluvial floodplain has artificial embankments which now prevent inundation of surrounding areas due to high rainfall (and/or tidal events) (Environment Agency, 2007; 2011). River flow is monitored by two gauging stations on the main river, Hatteralls Bridge on the western branch and Sakeham on the eastern branch (Environment Agency, 2005a; 2009). The tidal Adur has two weirs situated on the western and eastern branches, just before the two branches meet, both of which are inundated at high tide and therefore do not act as barriers for the tidal penetration upstream (Environment Agency, 2005a; 2007).

Water quality in the River Adur catchment is generally classified by the Environment Agency as ‘good’ (very good salmonid and cyprinid fisheries and ecosystems at or close to natural (Environment Agency, 2005a; 2006; 2009). There is concern associated with the large waste water treatment works (WWTW) at Goddards Green which discharges in to the eastern branch of the River Adur, which would naturally have low summer river flows. Upstream of the WWTW, urban drainage has reduced water quality (Environment Agency, 2005a; 2006; 2009).
2.5 Summary

- Estuaries and their associated rivers on the south coast of England were identified as the most vulnerable to increasing saline penetration in the UK under future projected land, sea level and climate change scenarios.

- A site selection criterion highlighted two comparable rivers for research, the River Adur in east Sussex and River Ouse in west Sussex. These neighbouring rivers share very similar geological and hydrological characteristics, and are therefore ideal catchments for replication/comparison. The issue of saline penetration in both of the River Adur and River Ouse has been previously highlighted (Environment Agency,
2009), but the extent and potential impacts remain poorly understood and uninvestigated to date.

- Four years of ecological baseline data (available from the Environment Agency) provide the opportunity to examine the historic benthic macroinvertebrate community structure and species distributions of the River Adur and River Ouse estuaries.
Chapter 3. Preliminary Data Analysis

3.1 Introduction

In this chapter historic benthic macroinvertebrate data (2005 & 2006) collected by the Environment Agency (Southern Region) is analysed to explore the spatial and temporal faunal structures and communities of the River Adur and River Ouse estuaries. This faunal data is used to predict the degree of saline penetration into the River Adur and River Ouse estuaries during two years of drought induced low freshwater discharge (2005 & 2006). The results are discussed in relation to the value of historic estuarine macroinvertebrate distribution data in contemporary investigations of saline penetration in the absence of direct salinity measurements. The results and conclusions of this analysis will contribute to the development of methodologies for subsequent research in this project (Chapters 4, 5 & 6).

3.1.1 Aims, Objectives and Hypotheses

Specifically this chapter aims to analyse publically available historic ecological data for the River Ouse and the River Adur estuaries to investigate benthic macroinvertebrate community structure and species distributions through the following objectives:


2. Use multivariate statistical techniques to further explore the major trends in the faunal distributions of the River Adur and River Ouse estuaries, and attempt to identify the main environmental parameters driving the faunal data in the absence of measured environmental variables (e.g. salinity) for the two sample years (i.e. 2005 and 2006).

3. To allocate recorded macroinvertebrate species to salinity tolerance groupings based on published literature, and to use the distributions of these groupings to attempt the determination of the upstream extent of saline penetration in 2005 and 2006 (in the absence of direct salinity measurements).

4. To assess the faunal comparability of the River Adur and River Ouse estuaries and ascertain their suitability for further study of the ecological impact of saline penetration upon benthic macroinvertebrate communities.
It is hypothesised that the similarities between the River Adur and River Ouse estuaries (as determined in Chapter 2) will result in comparable benthic macroinvertebrate community compositions and saline penetration extents, making the two systems suitable for further comparable study. It also is hypothesised that salinity is the main environmental variable driving macroinvertebrate distribution and community composition within the River Adur and River Ouse estuaries, and that the influence of salinity will be detected in the historic data through the zonation of species and communities (over the freshwater to marine transition) based on published salinity tolerances, even in the absence of direct salinity measurements.

3.2 Background to Environment Agency Investigation

In 2003 the UK experienced widespread drought conditions, registering the driest February-October period since 1921 (Marsh, 2004). Stocks in some reservoirs fell to below 20% of the long term average capacity, with Ardingly Reservoir (situated on the River Ouse catchment) being one of those most severely affected (Figure 3.1b). To supplement stocks at Ardingly Reservoir, the Environment Agency (Southern Region) granted drought permits to public water companies (Southern Water and South East Water) to abstract additional quantities of freshwater from the lower reaches of the River Ouse (Marsh, 2004; Marsh et al., 2007). This additional abstraction of freshwater, on top of already reduced freshwater levels caused an increase in the upstream extent of saline penetration in River Ouse estuary (Environment Agency, 2005b). The ecological impact of this increase in saline penetration upon the macroinvertebrates of the River Ouse estuary could not be directly assessed at that time because Environment Agency routine biological monitoring/sampling did not extend below the normal tidal limit (NTL; Environment Agency, 2005b).

The 2003 drought highlighted the shortcomings in ecological data collection for estuarine systems in Sussex (Environment Agency, 2005b). The relatively low rainfall, high population and projected increase in demand for freshwater in south east England, could increase the susceptibility of lowland estuaries in Sussex to future increases in saline penetration. Estuaries (i.e. up to the NTL) are treated as ‘transitional waters’ under the Water Framework Directive (WFD; 2000/60/EC; European Communities, 2000). The purpose of the WFD is to establish a framework for the protection of inland surface waters (rivers and lakes), transitional waters, coastal waters and groundwater and will ensure that all aquatic ecosystems meet 'good status' by 2015, or have measures in place for its future compliance (European Communities, 2000; Environment Agency, 2010). Due to the threat of increasing saline penetration and the necessity to meet the requirements of the WFD, the Environment Agency (Southern Region) initiated a
Transitional Waters Ecology Study, which was undertaken in Sussex during 2005 and 2006, to provide information on the ecology of estuaries in this region (Environment Agency, 2005b). The main focus of the study was to investigate the macroinvertebrate communities of the estuaries (up to the NTL) of the River Cuckmere, River Ouse, River Adur and River Arun in Sussex (Environment Agency, 2005b). Macroinvertebrate sampling took place between spring and autumn (May to November), during the drought years of 2005 & 2006. The faunal data from the River Arun and River Cuckmere were not included in the analysis presented herein because of the limited number of sampling sites (n = 3) on the River Cuckmere estuary and the large size of the River Arun estuary compared to the River Adur and River Ouse estuaries meant that direct comparison was not appropriate.

3.3 Sites and Methods

Macroinvertebrate samples were obtained at five sites along the River Adur estuary and from six sites along the River Ouse estuary using standard three-minute kick-sweep samples within the marginal areas of the channel at low tide (Figure 3.1, Table 3.1; Environment Agency, 1999). Four sites were sampled in the River Ouse estuary in 2005 and two additional sites (O3 and O6) were sampled in 2006 (Figure 3.1b, Table 3.1). Two kick samples were taken from each sampling site between May (spring) and November (autumn) in 2005 and 2006. In total 20 kick samples were collected; 10 from the River Adur estuary and 10 from the River Ouse estuary. All samples were preserved in 10% formaldehyde and identified to species level where possible. Macroinvertebrates that were not identified to species level were excluded from any subsequent analysis. The data received from the Environment Agency had not previously been analysed in detail. Critically, no environmental/physiochemical measurements (e.g. salinity, temperature, nutrient status, water quality parameters, and macrophyte cover) were made at the time of macroinvertebrate sampling.
Figure 3.1. Map of the River Adur and River Ouse, including Environment Agency sampling site locations along each river (for 2005 and 2006).
Macroinvertebrate sampling site locations on the River Adur and River Ouse estuaries with distance from the river mouths.

<table>
<thead>
<tr>
<th>River</th>
<th>Sampling site</th>
<th>Distance from river mouth (km)</th>
<th>N</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adur</td>
<td>A1</td>
<td>8.27</td>
<td>50°52'14.75&quot;</td>
<td>0°18'04.25&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A2</td>
<td>9.85</td>
<td>50°52'57.62&quot;</td>
<td>0°18'24.02&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A3</td>
<td>14.06</td>
<td>50°54'35.73&quot;</td>
<td>0°17'40.79&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A4</td>
<td>16.05</td>
<td>50°55'25.97&quot;</td>
<td>0°18'27.36&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A5</td>
<td>20.45</td>
<td>50°57'03.10&quot;</td>
<td>0°16'42.80&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O1</td>
<td>6.35</td>
<td>50°49'48.09&quot;</td>
<td>0°01'34.03&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O2</td>
<td>12.8</td>
<td>50°52'32.50&quot;</td>
<td>0°00'58.00&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O3</td>
<td>13.44</td>
<td>50°52'45.76&quot;</td>
<td>0°00'34.54&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O4</td>
<td>15.59</td>
<td>50°53'33.71&quot;</td>
<td>0°00'19.16&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O5</td>
<td>17.52</td>
<td>50°54'04.78&quot;</td>
<td>0°01'11.48&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O6</td>
<td>20.39</td>
<td>50°54'55.27&quot;</td>
<td>0°02'05.61&quot;</td>
</tr>
</tbody>
</table>

Table 3.1. Macroinvertebrate sampling site locations on the River Adur and River Ouse estuaries with distance from the river mouths.

### 3.4 Statistical Analysis

Species trends and underlying gradients were explored using the multivariate technique of detrended correspondence analysis (DCA) within the programme CANOCO v.4.54 (ter Braak & Šmilauer, 2006). DCA analysis (unimodal technique) was selected over linear techniques (e.g. principle components analysis), due to each faunal datasets exhibiting a long axis 1 gradient length (>3 std units). This included combining the River Adur and River Ouse faunal datasets to compare differences between estuaries over the two years (i.e. 2005 and 2006), as well as analysing the faunal data independently for each estuary and individual year to fully explore spatial variability. Prior to DCA analysis, all data sets were log transformed and rare species downweighted (ter Braak & Šmilauer, 2006). Unimodal methods are sensitive to rare species that occur in species poor areas, as they have an unduly large influence on the analysis. Downweighting in correspondence analysis reduces the influence of these rare species, by attaching weights to species related to their abundances (ter Braak & Šmilauer, 2006). To investigate the faunal structure of the River Adur and River Ouse estuaries (in both 2005 and 2006), diversity indices including species number, relative abundance (RA), Shannon-Weiner diversity index (H’) and Berger-Parker dominance index (BP) were calculated within the α Species Diversity and Richness software v.3.03 (Henderson & Seaby, 2002) using raw count data. The ecological and palaeoecological data analysis and visualisation program C2 (Juggins, 2007) was used to separately sequence both species distributions (sorted into salinity tolerance groupings) and diversity indices, relative to sample site, year and distance from river mouth.
To enable comparison between years (2005 & 2006), species abundance was transformed into percentage composition of the community. To derive longitudinal zonations along the estuarine courses (based on dissimilarity), the faunal community for both estuaries and years were numerically zoned using optimal splitting by information content as implemented in the programme PsimPoll (Bennett, 1996). Optimal splitting statistically divides a data sequence up into a specified number of groups \( r \), with the aim of reducing the sum of variances (measured using a dissimilarity matrix) in each individual zone, relative to the total variation (Birks & Gordon, 1985; Bennett, 1996). At each stage (i.e. stage 1 \( r=1 \), stage 2, \( r=2 \)...stage \( x, r=x \)) the entire dataset is split directly into the specified number of zones, without any regard for the results from any other stage (i.e. \( r=1 \) zone splits might have no relation to \( r=2 \) etc.). The number of groups/splits \( r \) is generally chosen by measuring the drop in total variance at each stage (i.e. as \( r \) is increased), stopping at the point when the addition of more groups does not substantially reduce the total variation. In this study the total variation was measured using an information content matrix for dissimilarity and a broken stick model (incorporated within the package PsimPoll) was employed for assessment of the significance of generated zone splits at each stage (Bennett, 1996). Optimal splitting was chosen over hierarchical agglomerative zoning techniques such as cluster analyses because the results of these techniques can be difficult to interpret during transitional phases (Gordon & Birks, 1972; Birks & Gordon, 1985).

Determining the historical extent of saline penetration required the allocation of salinity values to the recorded species distribution patterns. The published salinity tolerances of all the recorded macroinvertebrate species from the River Adur and River Ouse estuaries were reviewed and species salinity responses defined in terms of the biotic salinity tolerance groupings of Wolf et al., (2009) (Table 3.2). These groups were adopted as they were developed as part of a classification approach designed specifically to meet the particular circumstances of tidal streams. Where the salinity tolerances of selected species were unknown or unpublished, a biotic salinity tolerance grouping was assigned based on the maximum salinity at which the selected species had been recorded in the field (maximum field distribution - MFD). Where the salinity tolerance of a species has been shown to vary between life stage, the salinity tolerance of the adult life stage was taken as definitive (e.g. Smith, 1964; Ozoh & Jones, 1990).
Salinity tolerance grouping | Salinity range | Description
--- | --- | ---
Holeury-haline (he) | 0 - 35 | Marine derived taxa that tolerate the entire range of salinity from limnic to marine.
Euryhaline-marine (em) | 0.5 - 35 | Marine taxa that tolerate a large range of salinities between 0.5 and 35.
Brackish (b) | 0.5 - 30 | Brackish water taxa that live and reproduce in brackish waters and tolerate varying salinity between 0.5 and 30.
Euryhaline-limnic (el) | 0 - 10 | Freshwater derived taxa that tolerate salinity up to 10 (and higher for a short time).
Limnic, tolerates salt (l(el)) | 0 - <5 | Freshwater taxa that tolerate salinities below 5.
Limnic (l) | <0.5 | Freshwater taxa that do not tolerate even low salinities.

Table 3.2. Salinity tolerance groupings derived from Wolf et al., (2009).

### 3.5 Results

A total of 51 species, representing 5776 individuals, were recorded in the River Adur estuary over the 2005 and 2006 sampling period. Following a review of salinity tolerance literature and field distributions (Table 3.3), the observed macroinvertebrate species were allocated salinity tolerance groupings as follows; 2 holeury-haline (he), 5 euryhaline-marine (em), 7 brackish (b), 4 euryhaline-limnic (el), 23 limnic, salt tolerating (l(el)) and 10 limnic (l) (Table 3.4). A total of 67 macroinvertebrate species representing 2467 individuals were recorded in the River Ouse estuary during the two year sampling period. These macroinvertebrate species were allocated salinity tolerance groupings as follows; 3 holeury-haline (he), 8 euryhaline-marine (em), 6 brackish (b), 6 euryhaline-limnic (el), 28 limnic, salt tolerating (l (el)) and 16 limnic (l) species (Table 3.3, Table 3.5). The River Ouse and River Adur had 36 macroinvertebrate species in common.

Assigning salinity tolerance groupings to the majority of limnic derived macroinvertebrate species recorded in the River Adur and River Ouse estuaries was difficult, due to a lack of published research on the salinity tolerances and estuarine distributions of these limnic species. Salinity tolerance groupings for a number of these species had to be assigned based on maximum field distributions (MFD), a measure unlikely to provide an accurate measure of salinity tolerance (Underwood et al., 2000).
<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
<th>Class</th>
<th>Source</th>
<th>Species</th>
<th>Order</th>
<th>Class</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceratopogon nebulosus</td>
<td>Diptera</td>
<td>l</td>
<td>Smith, 1989</td>
<td>Ceratopogon nebulosus</td>
<td>Diptera</td>
<td>l</td>
<td>Smith, 1989</td>
</tr>
</tbody>
</table>

Note: *em* = marine taxa that tolerate the entire range of salinity from brackish to marine, 0.3–33; *b* = brackish water taxa that live and reproduce in brackish waters and tolerate varying salinity between 0.5 and 30; *f* = freshwater derived taxa that live in freshwater lakes or lakes with limited salinity inputs; *t* = freshwater taxa that do not tolerate even low salinity; <0.5.

Table 3.3. List of all taxa (identified to species level) recorded in the River Adur and River Ouse estuaries in 2005 and 2006. Taxa listed with their allocated salinity tolerance groupings and primary literature sources.
European-marine (0.5 - 35)
- *Crangon crangon* | Decapoda
- *Gammarus salinus* | Amphipoda
- *Helice diversicolor* | Polycheata
- *Heteronius oerstedi* | Malacostraca
- *Telphusa pseudogaster* | Oligochaeta

Brackish (0.5 - 30)
- *Allomelita pennicula* | Amphipoda
- *Corophium multisetosum* | Amphipoda
- *Cynothura carinata* | Isopoda
- *Leptestheria hookeri* | Malacostraca
- *Leptocheirus pilosus* | Amphipoda
- *Neomysis integer* | Mysida
- *Systelloglossus shubnolli* | Polycheata

European-limnic (0 - 10)
- *Ischnura elegans* | Odonata
- *Potamogetrus antipodorum* | Gastropoda
- *Sida cristallina* | Cladocera
- *Sigara fallax* | Hemiptera

**Table 3.4.** All recorded macroinvertebrate species from the River Adur estuary and allocated salinity tolerance groupings based on referenced literature sources presented in Table 3.3.

<table>
<thead>
<tr>
<th>European-haline (0 - 35)</th>
<th>Limnic, salt tolerating (0 - &lt;5)</th>
<th>Limnic (&lt;0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gammarus zaddachi</em></td>
<td><em>Acrorosus luctarius</em></td>
<td><em>Centropilum luteolium</em></td>
</tr>
<tr>
<td><em>Palaemon longirostris</em></td>
<td><em>Anchelopus fluorescens</em></td>
<td><em>Ceratella fulva</em></td>
</tr>
<tr>
<td><em>Palaemonetes varians</em></td>
<td><em>Bibitiana testudinaria</em></td>
<td><em>Ceradactylus fluviatilis</em></td>
</tr>
<tr>
<td><em>Crangon crangon</em></td>
<td><em>Cricotopus hircinula</em></td>
<td><em>Chironomus fasciatus</em></td>
</tr>
<tr>
<td><em>Gammarus salinus</em></td>
<td><em>Cypris cornutus</em></td>
<td><em>Chironomus mexicanus</em></td>
</tr>
<tr>
<td><em>Helice diversicolor</em></td>
<td><em>Daphnia pulex</em></td>
<td><em>Diptera</em></td>
</tr>
<tr>
<td><em>Heteronius oerstedi</em></td>
<td><em>Ephemerella sinuosa</em></td>
<td><em>Ephemerella danica</em></td>
</tr>
<tr>
<td><em>Telphusa pseudogaster</em></td>
<td><em>Ephydra hians</em></td>
<td><em>Ephemeroptera</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>European-limnic (0 - 10)</th>
<th>Limnic, salt tolerating (0 - &lt;5)</th>
<th>Limnic (&lt;0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allomelita pennicula</em></td>
<td><em>Acrorosus luctarius</em></td>
<td><em>Centropilum luteolium</em></td>
</tr>
<tr>
<td><em>Cythera carinata</em></td>
<td><em>Anchelopus fluorescens</em></td>
<td><em>Ceratella fulva</em></td>
</tr>
<tr>
<td><em>Hydrobia ulvae</em></td>
<td><em>Bibitiana testudinaria</em></td>
<td><em>Ceradactylus fluviatilis</em></td>
</tr>
<tr>
<td><em>Leptocheirus pilosus</em></td>
<td><em>Cricotopus hircinula</em></td>
<td><em>Chironomus fasciatus</em></td>
</tr>
<tr>
<td><em>Neomysis integer</em></td>
<td><em>Cypris cornutus</em></td>
<td><em>Chironomus mexicanus</em></td>
</tr>
<tr>
<td><em>Systelloglossus shubnolli</em></td>
<td><em>Daphnia pulex</em></td>
<td><em>Diptera</em></td>
</tr>
<tr>
<td><em>Ischnura elegans</em></td>
<td><em>Ephemerella sinuosa</em></td>
<td><em>Ephydra hians</em></td>
</tr>
<tr>
<td><em>Potamogetrus antipodorum</em></td>
<td><em>Acrorosus luctarius</em></td>
<td><em>Centropilum luteolium</em></td>
</tr>
<tr>
<td><em>Sigara dorsalis</em></td>
<td><em>Anchelopus fluorescens</em></td>
<td><em>Ceratella fulva</em></td>
</tr>
<tr>
<td><em>Sigara fallax</em></td>
<td><em>Bibitiana testudinaria</em></td>
<td><em>Ceradactylus fluviatilis</em></td>
</tr>
<tr>
<td><em>Sigara lateralis</em></td>
<td><em>Cricotopus hircinula</em></td>
<td><em>Chironomus fasciatus</em></td>
</tr>
<tr>
<td><em>Theobrus flavilatis</em></td>
<td><em>Cypris cornutus</em></td>
<td><em>Chironomus mexicanus</em></td>
</tr>
</tbody>
</table>

Detrended Correspondence Analysis (DCA) was undertaken using log transformed faunal data within the programme CANOCO v.4.54 (ter Braak & Šmilauer, 2006) and indicated a comparable faunal community structure along both the River Adur and River Ouse estuaries and...
the presence of a strong environmental gradient within the faunal data. The faunal data showed a longitudinal distribution along axis 1, with downstream sites (marine) on the left through to upstream (tidal limnetic) sites on the right, indicating a strong relationship with salinity (Figure 3.2). Axis 1 accounted for 17.5% of the variance in the faunal data with axis 2 accounting for a further 7.1%. The DCA species biplot provided further evidence of a strong gradient related to salinity on axis 1, with marine species on the left (e.g. *Carcinus maenas, Jaera albifrons, Melita palmata*) through to limnic species on the right (e.g. *Ephemera danica, Caenis horaria, Athripsodes cinereus*; Figure 3.3). Despite this strong salinity signal, the most upstream sampling sites on the River Adur and River Ouse estuaries are more strongly related to axis 2 than axis 1, suggesting that these sites are freshwater and the fauna differ through the presence of additional environmental variable/s (e.g. substrata, flow regime, macrophyte cover etc). Despite the strong salinity signal in the lower to mid estuarine sites, the inclusion of the upper freshwater sites in the River Adur and River Ouse estuaries resulted in an axis 1 variance explained of 17.5% (Figure 3.2).

The results of the combined DCA (River Adur and River Ouse estuaries) correspond to the distribution of macroinvertebrate species along the River Adur and River Ouse estuaries, which appear to be highly correlated with their salinity tolerance grouping (Figure 3.4, Figure 3.6). The species salinity preferences ranged from euryhaline–marine (0.5 – 35) close to the river mouth through to limnic (<0.5) close to the limits of the tide (NTL), indicating that the sampling regime covered the entire salinity transitions of the River Ouse and River Adur estuaries. When analysed separately, the DCA sample scores for each estuary again showed a strong underlying environmental gradient driving the faunal data along axis 1 for both years (2005 & 2006; Figure 3.5, Figure 3.7). The DCA axis 1 sample scores accounted for 28.5% of the variance in the faunal data from the River Ouse estuary and 37.6% of the variance from the River Adur estuary. Axis 2 scores accounted for a further 12.5% and 6.2% respectively. Examination of the DCA species axis 1 scores further supported a strong relationship to salinity in both estuaries. High salinity preference species (euryhaline-marine and brackish water species) such as *Carcinus maenas, Jaera albifrons* and *Palaemonetes varians* had the lowest sample scores and at the other end of the gradient low salinity preference species (limnic species) such as *Limnephilus marmoratus, Valvata cristata* and *Athripsodes cinereus* scored highest (Figure 3.4, Figure 3.7).
Figure 3.2. Detrended correspondence analysis (DCA) faunal data biplot from the River Adur and River Ouse estuaries using CANOCO v.4.54 (ter Braak & Šmilauer, 2006) following log transformation of faunal data.

Figure 3.3. Detrended correspondence analysis (DCA) species biplot from the River Adur and River Ouse estuaries using CANOCO v.4.54 (ter Braak & Šmilauer, 2006) following log transformation of faunal data.
3.5.1 Faunal zones in the River Adur estuary

In both 2005 and 2006, numerical zoning (optimal splitting by information content) of the faunal data from the River Adur estuary, produced 3 statistically significant zone splits (based on dissimilarity and tested using the broken stick model; Bennett, 1996), with boundaries at 9 km and 18.2 km from the river mouth at Shoreham; zone 1 (8.2 – 9 km), zone 2 (9 – 18.2 km) and zone 3 (18.2 – 20.4 km).

Zone 1 (8.2 – 9 km)
In 2005, the faunal community in zone 1 was relatively diverse, consisting of a total of 251 individuals, which were identified as holeury-haline (16.7%), euryhaline-marine (7.1%) and brackish water (75.6%) species (Figure 3.4, Figure 3.5). The most abundant of these were the brackish water amphipod Corophium multisetsosum (35%) and isopod Cyathura carinata (22.7%; Figure 3.4). An individual limnic, salt tolerating gastropod Lymnaea peregra was also recorded in this zone.

In 2006, the faunal community in zone 1 was again relatively diverse, consisting of a total of 264 individuals, identified as holeury-haline (21.9%), euryhaline-marine (8.3%) and brackish water (69.6%) species (Figure 3.4, Figure 3.5). The most abundant species were the brackish amphipod Leptochirus pilosus (29.9%) and the isopod Cyathura carinata (23.1%) (Figure 3.4). In both 2005 and 2006, zone 1 was dominated by brackish water species, with additional records of euryhaline-marine and holeury-haline species, indicating that this zone may have experienced high to mid salinity conditions over the sampling periods (Figure 3.4, Figure 3.5).

Zone 2 (9 – 18.2 km)
In 2005, the faunal community throughout zone 2 was variable. In general, the number of species (18) and relative abundance (1696) decreased throughout the zone, coupled with a rise in dominance and fall in diversity (Figure 3.5). The faunal community in zone 2 consisted of species from all salinity tolerance groupings, with holeury-haline (10.3%), brackish (21.5%) and euryhaline-limnic (66.6%) species recorded in greatest abundance, along with very small numbers of euryhaline-marine (1%), limnic salt tolerating (0.3%) and limnic (0.05%) species (Figure 3.4, Figure 3.5). The faunal community of zone 2 was dominated by the euryhaline-limnic gastropod mollusc Potamopyrgus antipodarum (66.6%), the brackish amphipod Corophium multisetsosum (20.7%) and the holeury-haline amphipod Gammarus zaddachi (10%; Figure 3.4).
In 2006, the faunal community throughout zone 2 was again variable (Figure 3.5). In contrast to 2005, the number of species increased towards the middle of zone 2, followed by a decrease, whilst relative abundance peaked at the beginning of zone 2 and decreased throughout the rest of the zone. This was coupled with an increase in diversity and decrease in species dominance throughout zone 2 (Figure 3.5). The faunal community again consisted of species from all salinity tolerance groupings, but was dominated by the brackish amphipod *Corophium multisetosum* (70.3%) and the holeury-haline amphipod *Gammarus zaddachi* (21.8%; Figure 3.4). The shift from a marine and brackish water dominated fauna in zone 1, to a more mixed fauna of brackish and limnic derived fauna in zone 2, might indicate that in both 2005 and 2006 (over the sampling period), this zone may have experienced mid to low salinity conditions.

**Zone 3 (18.2 – 20.4 km)**

In 2005, the faunal community of zone 3 was diverse (19 species), but lacked particularly high abundances of any species, with only 147 individuals recorded (Figure 3.5). The faunal community mainly consisted of holeury-haline (13.6%) and limnic derived species, including euryhaline-limnic (2%), limnic salt tolerating (81.6%) and limnic (2.7%) species (Figure 3.4, Figure 3.5). The most abundant of these were the limnic salt tolerating ephemeropteran *Caenis luctuosa* (28.5%) and amphipod *Gammarus pulex* (20.4%; Figure 3.4).

In 2006, the faunal community of zone 3 was again diverse (17 species), but overall abundance was relatively low (142 individuals) (Figure 3.5). The faunal community consisted of a mixture of holeury-haline and limnic derived species (euryhaline-limnic (7%), limnic salt tolerating (17.6%) and limnic (4.9%)), but in contrast to 2005, was dominated by the holeury-haline amphipod *Gammarus zaddachi* (70.4%; Figure 3.4, Figure 3.5). The shift from a mixed brackish and limnic derived fauna in zone 2, to a fauna consisting of holeury-haline and limnic derived species in zone 3, suggests that this zone experienced low salinity to tidal limnetic conditions over the sampling period, in both 2005 and 2006.
Figure 3.4. Summary diagram of species distributions in the River Adur estuary in 2005 and 2006, split into significant faunal zones with distance from the river mouth at Shoreham. Species shown occur >2% in one or more samples and are separated into salinity tolerance groupings following a review of published literature (Table 3). DCA axis 1 sample scores depicted for each year (2005 & 2006).
3.5.2 Faunal zones in the River Ouse estuary

In 2005, numerical zoning (optimal splitting by information content) of the River Ouse estuary faunal data produced 2 statistically significant zone splits at 14.19 km from the river mouth at Newhaven; zone 1 (6.35 – 14.19 km) and zone 2 (14.19 – 18.77 km). In 2006, numerical zoning of the faunal data produced 3 statistically significant zone splits (potentially due to the addition of new data or changing environmental conditions). The addition of 3 statistically significant faunal zone splits in 2006 reflects the dynamic nature of the estuary's ecosystem, which is influenced by various factors such as salinity, temperature, and habitat availability.
of 2 sampling sites in 2006) at 9.57 km and 14.51 km; zone 1 (6.35 – 9.57 km), zone 2 (9.57 – 14.51 km) and zone 3 (14.51 – 20.39 km).

**Zone 1**

In 2005, the faunal community of zone 1 (6.35 – 14.19 km), consisted of a relatively low number of species (15), and initially at very low abundances, however abundances increased throughout the zone (from 28 to ~127 individuals). However, this led to a decrease in diversity as individual species (particularly *Leptocheirus pilosus*) became more dominant (Figure 3.7). Overall, this zone was characterised by holeury-haline (9.6%), euryhaline-marine (10.3%) and brackish water (80%) species and was dominated by the brackish isopod *Cyathura carinata* (20.6%) and the amphipod *Leptocheirus pilosus* (57.4%; Figure 3.6, Figure 3.7).

In 2006, the faunal community of zone 1 (6.35 – 9.57 km) consisted of even fewer species (11) and again low overall abundances (136 individuals) (Figure 3.7). Overall, this zone was characterised by holeury-haline (25%), euryhaline-marine (70.5%) and brackish species (4.4%), but was dominated by the euryhaline-marine isopod *Jaera albifrons* (41.9%) and the holeury-haline decapod *Palaemon longirostris* (22.7%; Figure 3.6, Figure 3.7). The faunal communities of zone 1 (euryhaline-marine and brackish water species dominated) in both 2005 and 2006 indicate that this zone experienced high to mid salinity conditions over the sampling periods.

**Zone 2**

In 2005, the faunal community throughout zone 2 (14.19 – 20.39 km), generally showed a pattern of increasing numbers of species, relative abundance and diversity coupled with a slight decrease in species dominance (Figure 3.7). The fauna of zone 2 mainly consisted of holeury-haline (50%) and limnic derived species, including euryhaline-limnic (27.1%), limnic salt tolerating (14.4%) and limnic (7.8%) species (Figure 3.6, Figure 3.7). The fauna was, however dominated by the holeury-haline amphipod *Gammarus zaddachi* (50%) and the euryhaline-limnic gastropod *Potamopyrgus antipodarum* (20.1%; Figure 3.6). A single brackish water isopod *Cyathura carinata* (0.4%) was also recorded in this zone. The shift from a dominant brackish water fauna in zone 1, to a holeury-haline and limnic derived fauna in this zone, might indicate that in 2005 (over the sampling period) zone 2 experienced low salinities through to tidal limnetic conditions.

In 2006, the faunal community of zone 2 (9.57 – 14.51 km) had relatively low diversity and relatively low absolute abundances. Species dominance was high, (Figure 3.7). Overall, this
zone was characterised by holeury-haline (11%), euryhaline-marine (18.8%) and brackish water (68.8%) species, but was dominated by the brackish amphipod *Leptocheirus pilosus* (59.6%; Figure 3.6, Figure 3.7). Three individual limnic derived species were also recorded in this zone, including the euryhaline-limnic gastropod *Potamopyrgus antipodarum* (0.4%), the limnic salt tolerating trichopteran *Limnephilus lunatus* (0.4%) and the limnic odonata *Erythromma najas* (0.4%). The shift from euryhaline-marine species dominance in zone 1, to brackish water species dominance in zone 2, along with the record of a small number of limnic derived species, may indicate that in 2006 this zone experienced mid to low salinity conditions over the sampling period.

**Zone 3**

In 2006, the faunal community of zone 3 (14.51 – 20.39 km) was diverse (high numbers of species and relative abundances), consisting of 1653 individuals. In particular, *Gammarus zaddachi* was abundant throughout, but *Potamopyrgus antipodarum*, *Theodoxus fluviatilis* and *Sphaerium corneum* were more abundant in the lower reaches of this zone. Overall, the zone was characterised by a mixture of holeury-haline (24.7%), euryhaline-limnic (35.6%), limnic salt tolerating (15.9%) and limnic species (23.6%; Figure 3.6, Figure 3.7). The fauna of zone 3 was dominated by the holeury-haline amphipod *Gammarus zaddachi* (24.7%) and the euryhaline-limnic gastropod *Potamopyrgus antipodarum* (25.6%; Figure 3.6). The holeury-haline and mixed limnic fauna recorded in zone 3 in 2006, indicates that this zone experienced low salinity to tidal limnetic conditions over the sampling period.
Figure 3.6. Summary diagram of species distributions in the River Ouse estuary in 2005 and 2006, split into significant faunal zones with distance from the river mouth at Newhaven. Species shown occur >2% in one or more samples and are separated into salinity tolerance groupings following a review of published literature (Table 3). DCA axis 1 sample scores depicted for each year (2005 & 2006).
3.6 Saline penetration

This project employed the 0.5 salt concentration as the upstream indicator of the limits of saline penetration in the River Adur and River Ouse estuaries, in accordance with the Venice system oligohaline/limnetic boundary (McLusky, 1993; see Chapter 4, Section 4.3.1). Determination of the upstream extents of saline penetration into the River Adur and River Ouse estuaries was attempted using the distributions of euryhaline-marine (0.5 - 35) and brackish water species (0.5 – 30) as salinity indicators and limnic derived species (limnic, salt tolerating (0 - <5) and limnic
(<0.5)) as freshwater indicators. Holeury-haline (0 – 35) species such as *Palaemon longirostris* and *Gammarus zaddachi* were excluded as their ability to tolerate the entire range of salinity from freshwater to marine (0 – 35) limited their value as indicators of salinity variability (Table 3.3). The distributions of euryhaline-limnic (0 – 10) species were also excluded as indicators of saline penetration, as their ability to inhabit freshwaters through to mid salinity concentrations could mask the upper extent of a saline penetration profile.

### 3.6.1 The River Adur estuary

In 2005 euryhaline–marine (0.5 - 35) and the majority of brackish water species (0.5 - 30) were recorded up to 9.85 km from the river mouth at Shoreham, with the exception of the brackish water amphipod *Corophium multisetosum*, which was recorded in abundance up to 14.06 km (Figure 3.4, Figure 3.8). The majority of limnic derived species (limnic salt tolerating 0 - <5 and limnic <0.5) were recorded at 20.45 km from the river mouth at Shoreham, with the exception of the limnic trichopteran *Ceraclea fulva* (recorded at 9.85 km) and the limnic salt tolerating isopod *Asellus aquaticus* (recorded at 16.05 km) (Figure 3.4, Figure 3.8). Based on the upstream limit of the brackish water *C. multisetosum* at 14.06 km and the first record of limnic derived species (the limnic, salt tolerating *A. aquaticus*) from 16.05 km upstream, the upstream extent of saline penetration (over the 2005 sampling period) may have been located between 14.06 and 16.05 km from the river mouth at Shoreham. The record of the individual limnic trichopteran *C. fulva* was discounted in this case due to the likelihood that the single specimen was the result of drift from the upper river.

In 2006 only two euryhaline–marine species were recorded in the River Adur estuary, the amphipod *Gammarus salinus* and the polychaete *Hediste diversicolor*. *Gammarus salinus* was recorded the furthest upstream, at 16.05 km from the river mouth at Shoreham, the same distance as the maximum penetration of brackish water species (*C. multisetosum*) and an upstream penetration increase of 6.2 km from 2005 (Figure 3.4, Figure 3.8). The brackish isopod *Cyathura carina* also exhibited an upstream shift in range, from 9.85 km in 2005 to 16.05 km in 2006 (Figure 3.4). In contrast to 2005, the majority of limnic derived fauna of the River Adur estuary were recorded from 14.06 km upstream, a shift downstream in range of 6.3 km from 2005 (Figure 3.4, Figure 3.8). These species included the gastropod mollusc *Lymnaea stagnalis*, the bivalve molluscs *Pisidium* sp., *Sphaerium corneum* and *Unio pictorum*, the isopod *Asellus aquaticus*, the ephemeropterans *Caenis horaria*, *Caenis luctuosa* and *Cloeon dipterum*, the odonata *Calopteryx splendens*, the triclad *Dugesia tigrina* and the hirudinean *Glossiphonia complanata*. Two additional species of limnic salt tolerating amphipods *Gammarus pulex* and
*Crangonyx pseudogracilis* were recorded at 9.85 km from the river mouth, a shift downstream of 10.6 km from 2005. These limnic derived species were recorded overlapping in range (14.06 – 16.05 km) with the euryhaline-marine amphipod *Gammarus salinus*, the brackish amphipod *Corophium multiisetosum* and isopod *Cyathura carinata* (Figure 3.4, Figure 3.8). The overlapping range of euryhaline-marine and brackish water species, with limnic salt tolerating and limnic species, made the determination of the upstream extent of saline penetration difficult. However, the 16.05 km upstream range of euryhaline-marine and brackish water species (*G. salinus* and *C. carinata*) and the abundance of limnic species (limnic, salt tolerating and limnic) recorded at 20.45 km upstream, may indicate that the upstream extent of saline penetration (over the 2006 sampling period) was located between 16.05 and 20.45 km from the river mouth at Shoreham, an upstream shift from the predicted location of the saline front in 2005.

### 3.6.2 The River Ouse estuary

In 2005 euryhaline–marine (0.5 - 35) and the majority of brackish water species (0.5 - 30) were recorded up to 12.8 km from the river mouth at Newhaven, with the exception an individual brackish isopod *Cyathura carinata* which was observed up to 15.59 km (Figure 3.6, Figure 3.8). Limnic species were recorded from 17.52 km upstream, however a number of limnic, salt tolerating species were recorded at 15.59 km (e.g. the gastropod molluscs *Acroloxus lacustris*, *Ancylus fluviatilis* and *Lymnaea truncatula*, the ephemeropteran *Caenis luctuosa*, the hirudineans *Erpobdella testacea* and *Helobdella stagnalis* and the amphipod *Crangonyx pseudogracilis*) (Figure 3.6, Figure 3.8). Based on the upstream limit of the brackish water species *C. carinata* at 15.59 km and the first record of limnic species at 17.52 km, the upstream extent of saline penetration (over the 2005 sampling period) was likely located between 15.59 km and 17.52 km from the river mouth at Newhaven.

In 2006 euryhaline-marine and brackish water species were recorded up to 13.44 km from the river mouth at Newhaven, an increase upstream in range of 0.64 km from 2005 (Figure 3.6, Figure 3.8), although the addition of a new sampling site (O3) in 2006 (at 13.44 km), could account for this apparent increase in upstream penetration range (compared to 2005). As in 2005, the majority of limnic salt tolerating species were recorded from 15.59 km upstream, however a number of limnic species were also recorded at this site, a 1.93 km downstream shift in distribution from 2005. The exceptions to these distributions were a single limnic odonata *Erythromma najas* recorded at 12.8 km and a limnic salt tolerating trichopteran *Limnephilus lunatus* recorded at 13.44 km. (Figure 3.6, Figure 3.8). Based on the upstream limit of euryhaline-marine and brackish water species at 13.44 km and the majority of limnic species
recorded at 15.59 km, the upstream extent of saline penetration (over the 2006 sampling period) appears to have been located somewhere between 13.44 km and 15.59 km from the river mouth at Newhaven, a downstream shift from the predicted location of the saline front in 2005.
Figure 3.8. Silhouette graph showing relative abundance of salinity tolerance groups with distance from river mouth. Significant zones are shown (2005 and 2006).
3.7 Discussion

It was hypothesised within this chapter that the similarities between the River Adur and River Ouse estuaries (as determined in Chapter 2) would result in comparable macroinvertebrate community compositions and saline penetration extents, making the two systems suitable for further comparable study. Analysis of the faunal data from the River Adur and River Ouse estuaries over two years of drought induced low freshwater discharge (2005 & 2006) indicates that these systems are broadly comparable and suitable for the further study of the ecological impact of saline penetration on benthic macroinvertebrate communities. In the absence of quantitative salinity measurements (in conjunction with macroinvertebrate sampling), the allocation of salinity tolerance groupings (i.e. Wolf et al., 2009) to macroinvertebrate species distributions (based published tolerance literature) was the sole basis for the determination of the upstream extent of saline penetration in the River Adur and River Ouse estuaries and the determined extents of saline penetration in both systems were broadly comparable. In the River Ouse estuary, the upstream extent of saline penetration was likely located between 15.59 km and 17.52 km from the river mouth in 2005, which appeared to decrease in extent to between 13.44 km and 15.59 km in 2006. Despite this, euryhaline-marine species recorded an increase in range upstream (from 12.8 km to 13.44 km) between 2005 and 2006, but this was probably best explained by the addition of a sampling site (O3) in 2006, rather than an actual increase in the penetration of these species upstream. In the River Adur estuary, the upstream extent of saline penetration was potentially located between 14.06 km and 16.05 km from the river mouth in 2005, which appeared to increase in extent to between 16.05 km and 20.45 km in 2006. Despite an increase in the upstream range of euryhaline-marine and brackish water species in 2006, the faunal data from the River Adur estuary showed a more complex picture in terms of the overlapping ranges between euryhaline-marine and brackish water species with a number of limnic derived species. Whilst this might be expected to some degree in a dynamic tidal environment, it might also be the result of inaccurate allocation of salinity tolerance groupings (particularly for limnic species), or specimen drift/misidentification.

The degree of saline penetration into estuarine systems is partly determined by freshwater discharge rates, with reductions in freshwater discharge (due to climatic and/or anthropogenic impacts) often resulting in increases in the upstream extent of saline penetration, which should be subsequently reflected in a shift in species distributions (Attrill et al., 1996; Martinho et al., 2007; Sousa et al., 2007; Bessa et al., 2010). The upstream increase in range of euryhaline–marine and brackish species recorded in the River Adur estuary in 2006 (compared to 2005) was
not due to a reduction in freshwater discharge rates, as all gauging stations on the River Adur and River Ouse recorded an increase in freshwater discharge from 2005 to 2006 (over the sampling period). On the River Adur, the freshwater discharge gauges positioned on the eastern (Sakenham) and western (Hatterall Bridge) branches recorded a combined increase in freshwater discharge rate of 0.87 m$^3$s$^{-1}$ from 2005 (1.05 m$^3$s$^{-1}$) to 2006 (1.93 m$^3$s$^{-1}$). The River Ouse gauging station positioned at the normal tidal limit (NTL) (Barcombe mills), recorded an increase of 0.3 m$^3$s$^{-1}$ from 2005 (0.52 m$^3$s$^{-1}$) to 2006 (0.82 m$^3$s$^{-1}$). Despite the fact that 2006 was a third successive drought year, the impermeable upper river catchments of the River Ouse and River Adur (Weald clay) resulted in river flows which were more seasonally depressed in 2005 than in 2006, when low flows/drought impacts were more evident in spring-fed rivers in England (Marsh et al., 2007). The increase in freshwater discharge rate in the River Ouse in 2006 may have resulted in the decrease in the upstream extent of saline penetration from 2005, as recorded in the faunal data. The comparatively large increase in freshwater discharge rate (0.88 m$^3$s$^{-1}$) in the River Adur from 2005 to 2006 (compared to the River Ouse), make it unlikely that the upstream extent of saline penetration in the River Adur estuary significantly increased from 2005 to 2006, as the distributions of euryhaline-marine and brackish water species appear to indicate. It is also unlikely that the small increases in freshwater discharge rates recorded in the River Adur (0.87 m$^3$s$^{-1}$) and River Ouse (0.3 m$^3$s$^{-1}$) in 2006 (compared to 2005), would have significantly decreased the upstream extent of saline penetration. Based on freshwater discharge rates alone, it is likely that the upstream extents of saline penetration into the River Adur and River Ouse estuaries in 2006 were similar to or slightly reduced compared to the extent of saline penetration in 2005. The recorded upstream shift in distribution of euryhaline-marine and brackish water species in 2006 in the River Adur estuary, might therefore be the result of a series of upstream colonisations and range extensions, related to the upstream extension of favourable conditions (increased salinity) over three successive years of drought induced low freshwater flows (Barnes, 1994). This might suggest that even where there is a recorded increase in the upstream extent of saline penetration, it might take some time before benthic euryhaline-marine and brackish water macroinvertebrate species actually respond by extending their upstream distribution.

In contrast to the upstream shift in distributions of euryhaline-marine and brackish water species in 2006, the majority of limnic derived (limnic salt tolerating (l(el)) and limnic (l)) species in the River Adur estuary, recorded a downstream shift in distribution from 2005 to 2006, contradicting evidence of an increase in the upstream extent of saline penetration. Optimal splitting of the faunal data of the River Adur and River Ouse estuaries into significant zones
(based on dissimilarity), also showed no evidence of an increase (River Adur estuary) or decrease (River Ouse estuary) in the upstream extent of saline penetration or saline zones in 2006 compared to 2005. The faunal data from the River Adur estuary recorded no change in significant zone splits between 2005 and 2006, indicating that the overall faunal structure did not significantly change between years. In contrast, the faunal data of the River Ouse estuary did produce significantly different zone divisions in 2006 compared to 2005, which was likely the result of the addition of two sampling sites in 2006 (O3 and O6). The faunal structure and species distributions in the River Adur and River Ouse estuaries appear to be dynamic and ever-changing, likely in response to the environmental conditions experienced in these two systems. The addition of two sampling sites on the River Ouse estuary significantly altered the recorded faunal zones, indicating that due to the dynamic faunal nature of these two estuaries, more sampling sites are required to accurately determine both the faunal structure of these systems and to assess how these faunal communities respond precisely to changes in the degree of saline penetration.

The River Adur and River Ouse estuaries have been heavily managed for flood defence and land reclamation (pumping of the flood plain) with tightly constrained embanked tidal channels and heavily industrialised river mouths (Shoreham and Newhaven ports), resulting in limited intertidal areas at low tide (Environment Agency, 2005a; 2010; 2011). Taken in combination with significant tidal ranges (5.5 m in the River Adur and 6.1 m in the River Ouse), these factors generate energetic and highly dynamic tidal conditions, which propagate upstream (Savenije, 2005; Savenije & Veling, 2005). Consequently, salinity zones, saline penetration and species distributions might be temporally and spatially dynamic over short and long term tidal and seasonal cycles. The long sampling period (May to November) with large time intervals between collection of samples means that short term and/or seasonal shifts in species distributions might not have been detected here, and therefore only broad scale overviews of the faunal structure and species distributions can be extracted for these two estuarine systems. Future investigation of these highly dynamic environments requires a frequent, comprehensive sampling programme that can ensure that data and conclusions are representative of the field realities.

Due to the lack of physiochemical measurements associated with the macroinvertebrate sampling, the reliability of the resulting salinity penetration profiles from historical data is dependent upon how well the relationship between salinity tolerance and field distributions and the precision with which species salinity tolerances are known. In this chapter it was
hypothesised that salinity was the main environmental variable driving macroinvertebrate distribution and community composition within the River Adur and River Ouse estuaries, and that the influence of salinity would be detected in the historic data through the zonation of species and communities based on species salinity tolerances. It is widely accepted that one of the main factors driving species distributions in estuaries is salinity (Remane & Schlieper, 1971; De Jonge, 1974; Bulger et al., 1993; McLusky & Elliott, 2004; Telesh & Khlebovich, 2010; Telesh et al., 2011), however, a number of additional co-occurring biotic and abiotic factors (e.g. inter/intra specific competition, predation, dissolved oxygen content, sediment grain size and deposition rate) have also been shown to impact species distributions in estuarine systems (Williams & Hamm, 2002; Hernandez-Arana et al., 2003; Anderson et al., 2004; Hewitt et al., 2005; Bishop et al., 2006). For example, the upstream range of euryhaline-marine and brackish water species at any point in time could depend on a number of possible factors, such as salinity extremes (e.g. when they were last affected by a period of greatly lowered salinity), sediment composition, competition, predation, timing of breeding and potential rates of faunal dispersal (Barnes, 1994). Whilst the zonation of macroinvertebrate species and communities within the River Adur and River Ouse estuaries revealed a strong salinity signal based on published salinity tolerances (freshwater species at the limits of the tide, through to marine species at the estuary mouths), without appropriate field salinity measurements it is difficult to determine whether recorded shifts in the distributions of macroinvertebrate species in estuaries is directly related to changes in the saline penetration profile or the result of additional biotic or abiotic forcing.

A large amount of research has focussed on the distributions and tolerances to reductions in salinity of euryhaline-marine and brackish water macroinvertebrate species commonly recorded in UK estuaries and coastal systems (see Kinne, 1971; Lincoln, 1979; Barnes, 1994; Hayward & Ryland, 1995, and references therein). In contrast, there is a lack of published research regarding both the salinity tolerances and estuarine distributions for the majority of limnic species recorded in the River Adur and River Ouse estuaries, which made assigning salinity tolerance groupings to these species extremely difficult (Rundle et al., 1998; Williams & Williams, 1998a; Sousa et al., 2007). As a result, salinity tolerance groupings for a number of these species had to be assigned using maximum field distributions (MFD), a measure unlikely to provide an accurate measure of salinity tolerance (Underwood et al., 2000). As the majority of limnic macroinvertebrate species have only ever been recorded in non-tidal limnetic (<0.5) systems, these species have historically been assumed to have no tolerance to any increase in salinity. In this study, a number of limnic derived species (classified as limnic salt tolerating 0 - <5 and limnic <0.5) were recorded in the River Adur and River Ouse estuaries (within the tidal limit;
Table 3.6. Due to the lack of physiochemical measurements undertaken in conjunction with macroinvertebrate sampling, it is not possible to determine whether these limnic species were subjected to saltwater inundation and to what degree, however a number of these species (e.g. the odonata *Erythromma najas*, the gastropod mollusc *Lymnaea peregra* and the trichopterans *Limnephilus lunatus* and *Ceraclea fulva*) were recorded in the high to low salinity estuarine zones (as determined by optimal splitting of the faunal data; Table 3.6). These records could be the result of individual drift or displacement downstream, misidentification or allocation of the incorrect salinity preference grouping due to lack of salinity tolerance or estuarine distribution research/literature (Williams & Williams, 1998b; Williams & Hamm, 2002). A large number of additional limnic species were recorded in the low salinity to tidal limnic zones of both estuaries (Table 3.6). Whilst potentially not experiencing increased salinities, these species would have experienced large shifts in physiochemical conditions over a tidal cycle, including increased turbidity, oscillating water levels, decreased oxygen content and changing current velocities and directions (McLusky, 1993; Schuchardt et al., 1993; Uncles & Stephens, 1993; Rundle et al., 1998). The record of these species in the tidal River Adur and River Ouse estuaries potentially indicates a degree of tolerance and/or adaptability to fluctuating physiochemical tidal conditions and consequently low salinities, which is not currently highlighted in any published literature; an issue which could become prominent in the future under increasing saline penetration scenarios.

<table>
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<tr>
<th>High to low salinity zones</th>
<th>Low salinity to tidal limnic zones</th>
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<tbody>
<tr>
<td><em>Acroneis lacustris</em></td>
<td><em>Trichoptera</em></td>
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<td><em>Ancylopus flavissilis</em></td>
<td><em>Gastropoda</em></td>
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<td><em>Lyce phaeops</em></td>
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<td><em>Cloeon dipterus</em></td>
<td><em>Ephemeroptera</em></td>
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<td><em>Cragonyx pseudogracilis</em></td>
<td><em>Amphipoda</em></td>
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<td><em>Dugesia tigrina</em></td>
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<td><em>Erypoda testacea</em></td>
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<td><em>Erythromma najas</em></td>
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<td><em>Gammarus pulex</em></td>
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<td><em>Glossiphonia complanata</em></td>
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<td><em>Helobdella stagnalis</em></td>
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<td><em>Pisidium subrubescens</em></td>
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<td><em>Sphaerium corneum</em></td>
<td><em>Ephemeroptera</em></td>
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<tr>
<td><em>Unio pictorum</em></td>
<td><em>Ephemeroptera</em></td>
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Table 3.6. The limnic salt tolerating (0 - <5) and limnic (<0.5) macroinvertebrate species recorded in the high to low salinity and low salinity to tidal limnetic zones (determined through optimal splitting of the faunal data) in the River Adur and River Ouse estuaries in 2005 and 2006.
In order to assess the ecological impact of future increases in saline penetration upon the benthic macroinvertebrates of the estuaries and non-tidal limnetic riverine courses of the River Adur and River Ouse, a number of investigative steps need to be undertaken. This study has provided preliminary baseline information on the benthic macroinvertebrate community structure of the River Adur and River Ouse estuaries, but has been limited by a lack of sequential sampling sites, large time intervals between sampling and lack of physicochemical/environmental measurements taken in conjunction with macroinvertebrate sampling (e.g. salinity, water temperature, sediment characteristics). In order to attempt the prediction of the impact of increasing saline penetration, the spatial and temporal faunal structures of the River Adur and River Ouse, both below and above the current limit of tidal penetration (NTL) need to be determined over two seasons of differing saline penetration regimes (e.g. summer and winter). Simultaneous physiochemical (e.g. salinity, sediment parameters, temperature, pH, dissolved oxygen content) and biological (e.g. macrophyte cover and organic content) sampling of the estuarine and riverine courses are required to determine relationships between macroinvertebrate community structure and selected environmental parameters. The dynamic tidal nature of the River Adur and River Ouse estuaries require accurate measurement of tide and salinity profiles to understand the relationship between salinity and current macroinvertebrate community structures, and to progress to predicating future saline penetration profiles under climate scenario projections (Nakicenovic et al., 2000; IPCC, 2007; Lowe et al., 2009; Murphy et al., 2009). Finally, it is necessary to establish salinity tolerance values for selected macroinvertebrate species (both above and below the tidal limit: NTL), in order to attempt the prediction of shifts in species distributions in relation to increasing saline conditions. Establishing accurate salinity tolerances for limnic macroinvertebrate species is a particularly important issue, as this grouping can be expected to be most ‘at risk’ to distribution changes (both in terms of pattern and extent) under future saline penetration scenarios.

3.8 Summary

- Analysis of past faunal data (available for 2005 and 2006) for the River Adur and River Ouse estuaries suggests that these two systems are broadly comparable, confirming that these two systems are ideal for further investigation in this study.

- By allocating salinity tolerance groupings to macroinvertebrate species distributions based on published literature and by division of these systems into salinity zone (in the absence of direct salinity measurement), approximate determinations of the upstream extent of saline penetration for the River Adur and River Ouse estuaries for 2005 and
2006 were made. Using macroinvertebrate species in this way critically depends on the precision with which salinity tolerances are known and assumes a close the relationship between these tolerances and species distributions in the field.

- The tolerances to reductions in salinity and estuarine distributions of marine and brackish water species commonly recorded in the UK coastal zone have been thoroughly researched and studied. In contrast, there is a significant lack of research and published literature on the salinity tolerances and estuarine distributions of limnic derived species, resulting in allocations to salinity tolerance groupings based on species maximum field distributions, a method unlikely to provide an accurate measure of salinity tolerance.

- A sustained increase in the upstream extent of saline penetration might not be immediately reflected in an upstream shift in the distributions of benthic euryhaline-marine and brackish water species, due to the time and method taken for these species to colonise upstream sites with favourable conditions. This could limit the use of these species as indicators of short term shifts in the upstream extent of saline penetration.

- Faunal data from the River Ouse estuary demonstrates the importance of the location and number of macroinvertebrate sampling sites to accurately determine both the faunal structure of these systems and to assess how these faunal communities react in changes in the degree of saline penetration.

- The River Adur and River Ouse faunal data both exhibited a complex picture of overlapping euryhaline-marine, brackish and limnic species on both spatial and temporal scales. This is likely due to dynamic physical conditions inherent in extreme tidal environments such as the River Ouse and River Adur estuaries, subsequently resulting in ever-changing faunal communities. In order to determine the dynamic short and long-term changes in faunal community structure of the River Adur and River Ouse estuaries, a frequent, comprehensive sampling programme is required that can ensure that data and conclusions are representative of the field realities.

- A large number of limnic macroinvertebrate species were recorded within the low salinity to tidal limnetic zones of the River Adur and River Ouse estuaries, potentially indicating a degree of tolerance and/or adaptability to fluctuating physiochemical tidal conditions and consequently low salinities that is not currently highlighted in any published literature. A small number of limnic species were also recorded in the high to low salinity zones of the River Adur and River Ouse estuaries, which could be the result
of drift or displacement down river, misidentification or allocation of the incorrect salinity preference grouping (the latter probably due to lack of salinity tolerance or estuarine distribution research/literature).

- In order to predict the impact of future increases in saline penetration upon the benthic macroinvertebrates of the River Adur and River Ouse, subsequent studies need to:

  1. Investigate the spatial and temporal faunal structures of the River Adur and River Ouse over a minimum of two seasons of differing saline penetration regimes (e.g. summer and winter freshwater flows) in conjunction with detailed physiochemical measurements.
  2. Accurately measure and map the tide and salinity profiles of the River Adur and River Ouse estuaries.
  3. Establish accurate salinity tolerance values for selected macroinvertebrate species, both above and below the current limit of the tide.
Chapter 4. Tide and Salinity profiling

4.1 Introduction

Estuaries experience varying degrees of cyclical and occasional tidal saline water penetration inputs (e.g. storm surges), which are influenced and modulated by riverine freshwater discharge (flood to low flow, Dyer, 1997; Savenije, 2005). The spatial and temporal salinity structure of an estuary depends on the relative strengths of these opposing forces; tide-driven saline forcing upstream against freshwater forcing downstream (Dyer, 1997; Savenije, 2005). Both of these forces depend on the topography of the estuary channel; the tidal amplitude, surface and cross sectional area of the river mouth, which determines the volume of tidal water entering the estuary, and the cross-sectional area of the estuary channel, which in turn determines the efficiency and volume of the freshwater flow to push back the salt water (Savenije, 2005; Savenije & Veling, 2005). The salinity structure of an estuary also depends on the circulation and mixing of water within the channel, which are processes driven by the density difference and the interaction between fresh and salt waters. While the extent of saline penetration may be naturally variable, anthropogenic factors such as freshwater abstraction, flood control engineering and future changes in sea level may be locally significant (IPCC, 2007; Le et al., 2007; Lowe et al., 2009; Murphy et al., 2009; Wilby et al., 2010). All estuaries exhibit unique magnitude and timing responses to input forces (river flow, tidal range), so only by establishing the current tide and salinity profiles of estuaries will it be possible to progress to determining future saline penetration trends (Dyer, 1997).

Under future isostatic land level reductions, eustatic sea level rise, increased storm surge frequency/severity and decreased summer river flows, the upstream extent of saline penetration into the River Adur and River Ouse estuaries is predicted to increase (Scavia et al., 2002; IPCC, 2007; Day et al., 2008; Lowe et al., 2009; Menon et al., 2010), initiating a shift in benthic macroinvertebrate communities (Attrill et al., 1996; Sousa et al., 2007; Bessa et al., 2010). In order to predict shifts in benthic macroinvertebrate distributions, the future tide and salinity profiles likely to have the most impact upon the benthic macroinvertebrate communities of the River Adur and River Ouse were determined.

The tidal River Adur and River Ouse are classified as estuaries, up to their normal tidal limits (NTL) (Chapter 1, Section 1.2.1; McLusky, 1993). Both are coastal plain estuaries, formed during the early Holocene transgression through the flooding of pre-existing valleys (Davidson
et al., 1991; Buck, 1997). The River Adur and River Ouse estuaries have large tidal ranges (macrotidal >4 - <6 m), which result in strong tidal and residual currents that extend far inland (Davidson et al., 1991; Buck, 1997; Dyer, 1997; Savenije, 2005). These strong tidal currents create dynamic environments within the estuaries over a tidal cycle, on which may then be superimposed additional complexities of occasional unpredictable events e.g. storm surges, catchment drought or flood. The River Adur and River Ouse estuaries are therefore never steady-state systems (McLusky & Elliott, 2004). The rapidly changing conditions of these estuaries make single point, one-off measurements of physiochemical factors such as salinity highly unrepresentative. Continuous in-stream monitoring over at least 12-hour tidal cycles, repeated at representative times over the annual tidal cycle, are necessary to profile tide and salinity levels accurately within these dynamic systems. Determining the representative tidal and salinity profiles of the River Adur and River Ouse estuaries as expressed on daily and seasonal cycles was therefore an essential element of this research project.

4.1.1 Aims, Objectives and Hypotheses

This chapter aims to determine the current extent and natural variability of saline penetration within the study estuaries through direct measurement of the tidal and salinity cycles, under both high and low freshwater discharge conditions. In addition, to predict future tide and salinity profiles (and extents of saline penetration) for the study estuaries under projected sea level rise scenarios for both low and high freshwater discharge conditions. These aims will be achieved through the following objectives:

1. Outline the methodology used to determine current and future tide and salinity profiles of the River Adur and River Ouse estuaries under low (summer) and high (winter) freshwater discharge conditions.
2. Determine current tide and salinity profiles of the River Adur and River Ouse estuaries under low and high freshwater discharge conditions.
3. Use these current profiles to describe the spatial and temporal extent of saline penetration in the River Adur and River Ouse estuaries.
4. Determine future predictive tide and salinity profiles of the River Adur and River Ouse estuaries under projected increases in relative sea levels for future high greenhouse gas emissions (SRES: A1F1), for three time periods (2020, 2050 and 2080), under high and low freshwater discharge conditions.
5. Use the predicted tide and salinity profiles of the River Adur and River Ouse estuaries to describe future spatial and temporal patterns of saline penetration under low and high freshwater discharge conditions and to assess the impact of predicted future changes in freshwater discharge...
(climatic and anthropogenic) and episodic events (e.g. storm surges) upon the robustness of the predicted profiles.

It is hypothesised that the spatial and temporal extent of saline penetration into the River Adur and River Ouse estuaries is naturally dynamic, and reflects the relative opposing strengths of tidal forcing marine water upstream and freshwater discharge downstream. It is also hypothesised that under projected sea level rise scenarios, the upstream extent of saline penetration into the study estuaries will increase under both high and low freshwater discharge conditions.

4.2 Sites and Methods

Two types of conductivity meter were deployed during this research project; a hand held point sampling meter and an in-situ data logging sonde which was able to withstand prolonged deployment in the field. A hand-held WinLab®Data-Line Conductivity Meter with automatic calibration and temperature compensation was used to take spot samples along the estuary course during macroinvertebrate sampling (Table 4.1). Two SEBA datalogger type Dipper-TEC meters were used as in-situ sondes in the field. These meters were originally designed for service in water supply boreholes with specifications that allowed for prolonged service in harsh, non-maintenance environments. Suitably anchored, the SEBA units proved to be robust, accurate sondes recording water level (m), temperature (°C) conductivity (μS cm⁻¹), total dissolved solids (g L⁻¹) and salinity (psu) at 2 minute intervals, deployed for up to 24 hours at a time (Table 4.1).
Table 4.1. Full technical specifications of the Winlab®Data-Line conductivity meter and the SEBA datalogger type Dipper-TEC. All conductivity values are cm⁻¹.

Field salinity profiling was undertaken in August 2008, to coincide with low summer freshwater discharge rates, and February 2009, to coincide with high winter freshwater discharge rates. The two SEBA Dipper-TEC sondes were positioned at four stations along the River Adur and River Ouse estuaries in August 2008 and February 2009 (Figure 4.1, Figure 4.2, Table 4.2). A boat-based preliminary tide and salinity survey in August 2008 enabled the approximate mapping of the tide and salinity profiles on both estuaries and acted as a basis for station selection. Station selection was also based on accessibility, security and the availability of structures for sonde attachment.

The SEBA Dipper-TEC sondes were secured in the estuary channels at low tide (Figure 4.2). The sonde data-feed cable was fastened to appropriate available robust structures and camouflaged using surrounding vegetation (Figure 4.2). The sondes were left in situ for 12-hour tidal cycles and were retrieved from the river bed during the following low tide. As only 2 SEBA Dipper-TEC sondes were available for deployment in the field, measurements at different locations along each estuary took place sequentially over 4 days involving slightly different tidal conditions for which a correction factor was introduced (see Section 4.3.1).
<table>
<thead>
<tr>
<th>River</th>
<th>Station</th>
<th>Distance from river mouth (km)</th>
<th>N</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adur</td>
<td>A1</td>
<td>8.27</td>
<td>50°52’14.75&quot;</td>
<td>0°18’04.25&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A2</td>
<td>10.64</td>
<td>50°53’19.46&quot;</td>
<td>0°18’34.79&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A3</td>
<td>14.06</td>
<td>50°54’35.73&quot;</td>
<td>0°17’40.79&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A4</td>
<td>18.01</td>
<td>50°56’17.95&quot;</td>
<td>0°18’03.18&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O1</td>
<td>6.35</td>
<td>50°49’48.09&quot;</td>
<td>0°01’34.03&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O2</td>
<td>13.72</td>
<td>50°52’47.67&quot;</td>
<td>0°00’20.63&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O3</td>
<td>16.1</td>
<td>50°53’45.26&quot;</td>
<td>0°00’37.75&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O4</td>
<td>17.35</td>
<td>50°53’42.32&quot;</td>
<td>0°00’36.58&quot;</td>
</tr>
</tbody>
</table>

Table 4.2. Tide and salinity sampling station locations on the River Adur estuary and River Ouse estuary.

Figure 4.1. Tide and salinity sampling station locations on the River Adur estuary and River Ouse estuary.
4.2.1 Freshwater Discharge

Daily freshwater discharge rates (m$^3$ s$^{-1}$) for the River Adur and River Ouse in August 2008 and February 2009 were provided by the Environment Agency. The River Adur and River Ouse both have gauging stations that constantly record freshwater discharge rates. The River Adur gauges are positioned on the Eastern (Sakenham) and Western (Hatterall Bridge) branches of the river, so data from both gauges were utilised (Figure 4.5). Freshwater discharge data from the River Ouse were collected from the gauging station positioned at the NTL (Barcombe Mills, Figure 4.13). The daily discharge rates (m$^3$ s$^{-1}$) for the four-day salinity sampling periods were averaged to produce a mean discharge rate (m$^3$ s$^{-1}$) for each river and high and low freshwater discharge regimes. The daily discharge rates (m$^3$ s$^{-1}$) were not naturalised and therefore include the anthropogenic effects of abstractions and discharges of freshwater from the river channel upstream of the gauging stations. It is important to note that freshwater discharge values can only be considered accurate for the points immediately downstream of the gauging stations. A range of possible abstractions and discharges (e.g. irrigation, sewage treatment discharge, tributaries and pumped drainage) into the remaining courses of the two rivers mean that freshwater flow volumes become increasing unreliable with distance downstream. By establishing tide and salinity profiles under high and low freshwater discharge conditions, it is
possible to establish the general impact of freshwater discharge rates on the degree of saline penetration. More precise determination of the interactions between specific freshwater discharge rates and saline penetration would require more detailed analysis of freshwater discharge along the estuary lengths which are currently unavailable (e.g. gauging stations on the significant inflow and outflow sources).

4.3 Data Analysis

4.3.1 Current tide and salinity profiles

To develop the current tide and salinity profiles for the River Adur and River Ouse estuaries under low (August 2008) and high (February 2009) freshwater discharge conditions, the tide and salinity profiles recorded at each salinity station were standardised to the Mean High Water Spring (MHWS) and Mean Low Water Spring (MLWS) tide height of each rivers’ respective harbour. The River Adur estuary has a MHWS tide height of 6.3 m and a MLWS of 0.6 m at its mouth at Shoreham (Shoreham Port, pers. comm., 2010). The River Ouse estuary has a MHWS tide height of 6.69 m and a MLWS height of 0.77 m at its mouth at Newhaven (National Oceanography Centre, 2010). Actual tide heights at Newhaven and Shoreham were available through harbour tide gauge records of tide heights logged at 10 minute intervals.

The tide and salinity profiles recorded at each station showed that the tide (m) and salinity profiles were not synchronised (Figure 4.3). Maximum salinity (psu) at each station did not correspond with the highest tide, but was lagged behind it. The lag time between recorded high tide and maximum salinity differed between stations, continuing to rise up to 62 minutes after the high tide (station A1, Figure 3, Table 4.3). To determine the maximum and minimum salinity related to tide height at each station, it was necessary to synchronise the raw tide height level (m) and salinity data, so that high tide level (m) matched maximum salinity and low tide level (m) matched minimum salinity. This produced a corrected salinity and tide level relationship with high tide corresponding to maximum salinity and low tide to the minimum salinity (as shown in Table 4.3).
A ratio of the height (m) of tide at each station and the height (m) of the associated tide at the harbour was determined. The ratio, multiplied by the MHWS or MLWS tide heights, produced a corrected vertical high and low tide height (m) for each station (as shown in Table 4.3). The corrected salinity and estuary level (m) relationships for the rising and falling limbs of the tide profiles at each station and flow regime were plotted and the salinity associated with the corrected vertical height (m) determined. The corrected maximum salinity was determined using the rising limb plot and the minimum salinity determined using the falling limb plot. This required the assumption that the forms of the tide and salinity profiles at each station (Figure 4.3) remain the same irrespective of harbour tide height. This assumption was corroborated by comparison of the shapes of control tidal profiles obtained at each salinity station during the sampling periods (August 2008 and February 2009). These profiles demonstrated consistencies of form over a wide range of vertical harbour tide heights. The range and average salinity at each station was calculated from the corrected maximum and minimum salinities. Using the
maximum and minimum salinities determined at each salinity station, interpolated tide and salinity profiles for the River Adur and River Ouse estuaries were developed for both low (August 2008) and high (February 2009) freshwater discharge conditions (Table 4.3). The resultant tide and salinity profiles were used to determine the current degree of tidal saline penetration of the River Adur estuary and River Ouse estuary under low and high freshwater discharge conditions. This project employed the 0.5 salt concentration as the upstream indicator of the limits of saline penetration. This value corresponds to the established (Venice system) oligohaline/limnetic boundary (McLusky, 1993). Alternative salt concentrations have also been employed in this role, with 1 as a typical agricultural/industrial limits and 2-5 as being biologically significant as the upstream extent of saline penetration in estuarine studies (Deaton & Greenberg, 1986; Kimmerer et al., 1998; Gordon et al., 2004; Telesh et al., 2011).
Table 4.3. Summary table of the tide and salinity profiles of the River Ouse and River Adur estuaries under low (August 2008) and high (February 2009) freshwater discharge conditions (LFD and HFD).

<table>
<thead>
<tr>
<th>Freshwater discharge regime</th>
<th>Site</th>
<th>Distance to site (km)</th>
<th>MHWS tide height (m)</th>
<th>Corrected high tide height (m)</th>
<th>High tide - max salinity time lag</th>
<th>Maximum salinity (psu)</th>
<th>MLWS tide height (m)</th>
<th>Corrected low tide height (m)</th>
<th>Minimum salinity (psu)</th>
<th>Salinity range (psu)</th>
<th>Average salinity (psu)</th>
<th>Tidal range (m)</th>
<th>Temperature range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFD Ouse estuary</td>
<td>O1</td>
<td>6.35</td>
<td>6.69</td>
<td>4.16</td>
<td>00:38:00</td>
<td>35</td>
<td>0.77</td>
<td>0.03</td>
<td>0.3</td>
<td>34.7</td>
<td>17.65</td>
<td>4.13</td>
<td>18 - 18.3</td>
</tr>
<tr>
<td></td>
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<td>13.72</td>
<td>6.69</td>
<td>2.78</td>
<td>00:26:00</td>
<td>6.8</td>
<td>0.77</td>
<td>0.34</td>
<td>0.2</td>
<td>6.6</td>
<td>3.5</td>
<td>2.44</td>
<td>17.2 - 18.5</td>
</tr>
<tr>
<td></td>
<td>O3</td>
<td>16.1</td>
<td>6.69</td>
<td>1.39</td>
<td>00:16:00</td>
<td>0.4</td>
<td>0.77</td>
<td>0.31</td>
<td>0.19</td>
<td>0.21</td>
<td>0.29</td>
<td>1.08</td>
<td>17.9 - 18.3</td>
</tr>
<tr>
<td></td>
<td>O4</td>
<td>17.35</td>
<td>6.69</td>
<td>3.30</td>
<td>00:30:00</td>
<td>0.32</td>
<td>0.77</td>
<td>1.06</td>
<td>0.19</td>
<td>0.13</td>
<td>0.25</td>
<td>2.24</td>
<td>17.4 - 17.6</td>
</tr>
<tr>
<td>HFD</td>
<td>O1</td>
<td>6.35</td>
<td>6.69</td>
<td>4.01</td>
<td>00:30:00</td>
<td>35</td>
<td>0.77</td>
<td>0.19</td>
<td>0.2</td>
<td>34.8</td>
<td>17.6</td>
<td>3.82</td>
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<tr>
<td></td>
<td>O2</td>
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<td>0.49</td>
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<td>7.9 - 8.5</td>
</tr>
<tr>
<td></td>
<td>O3</td>
<td>16.1</td>
<td>6.69</td>
<td>0.82</td>
<td>-</td>
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<td>0.77</td>
<td>0.16</td>
<td>0.18</td>
<td>0.02</td>
<td>0.19</td>
<td>0.66</td>
<td>6.8 - 7.1</td>
</tr>
<tr>
<td></td>
<td>O4</td>
<td>17.35</td>
<td>6.69</td>
<td>2.36</td>
<td>-</td>
<td>0.22</td>
<td>0.77</td>
<td>0.26</td>
<td>0.18</td>
<td>0.04</td>
<td>0.2</td>
<td>2.10</td>
<td>7 - 7.5</td>
</tr>
<tr>
<td>LFD Adur estuary</td>
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<td>6.3</td>
<td>2.92</td>
<td>01:02:00</td>
<td>35</td>
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<td>0.05</td>
<td>0.2</td>
<td>34.8</td>
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<td>17.3 - 18</td>
</tr>
<tr>
<td></td>
<td>A2</td>
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<td>6.3</td>
<td>2.19</td>
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<td>2.11</td>
<td>18.1 - 17.5</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>14.06</td>
<td>6.3</td>
<td>1.98</td>
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<td>0.8</td>
<td>0.6</td>
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<td>0.5</td>
<td>1.89</td>
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<tr>
<td></td>
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<td>6.3</td>
<td>1.18</td>
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<td>0.45</td>
<td>0.2</td>
<td>0.1</td>
<td>0.25</td>
<td>0.73</td>
<td>17.1 - 18.1</td>
</tr>
<tr>
<td>HFD</td>
<td>A1</td>
<td>8.27</td>
<td>6.3</td>
<td>3.05</td>
<td>01:02:00</td>
<td>35</td>
<td>0.6</td>
<td>0.36</td>
<td>0.2</td>
<td>34.8</td>
<td>17.6</td>
<td>2.69</td>
<td>7.8 - 8.2</td>
</tr>
<tr>
<td></td>
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<td>10.64</td>
<td>6.3</td>
<td>2.00</td>
<td>00:38:00</td>
<td>1.14</td>
<td>0.6</td>
<td>0.24</td>
<td>0.2</td>
<td>0.94</td>
<td>0.67</td>
<td>1.76</td>
<td>7.9 - 8.4</td>
</tr>
<tr>
<td></td>
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<td>14.06</td>
<td>6.3</td>
<td>1.70</td>
<td>-</td>
<td>0.3</td>
<td>0.6</td>
<td>0.10</td>
<td>0.2</td>
<td>0.1</td>
<td>0.25</td>
<td>1.61</td>
<td>7.9 - 8.5</td>
</tr>
<tr>
<td></td>
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<td>6.3</td>
<td>1.18</td>
<td>-</td>
<td>0.26</td>
<td>0.6</td>
<td>0.18</td>
<td>0.2</td>
<td>0.06</td>
<td>0.23</td>
<td>0.99</td>
<td>7.2 - 7.9</td>
</tr>
</tbody>
</table>
4.3.2 Predicted future tide and salinity profiles

Predicted future tide and salinity profiles for the River Ouse and River Adur estuaries were determined under the UK climate projection scenario (UKCP09) for high greenhouse gas emissions (SRES: A1FI; Nakicenovic et al., 2000), at three time periods in this century (2020s, 2050s and 2080s) (Table 4.4, Table 4.5, IPCC, 2007; Lowe et al., 2009; Murphy et al., 2009). The high emissions scenario was selected as a predictive base for the tide and salinity profiles as it is a worst case scenario (within the current uncertainty range), based upon current climate modelling and as such will provide the most extreme future saline penetration profiles of the River Adur and River Ouse estuaries (Nakicenovic et al., 2000; IPCC, 2007; Lowe et al., 2009).

High emission scenario relative sea level height projections were selected for 12 km coastal grid squares at Newhaven (grid square ID 25360) and Shoreham (ID 25151) using the UKCP09 User Interface (v1.0) (accessed at: http://ukclimateprojections-ui.defra.gov.uk). The UKCP09 relative sea level projections provide a frequency distribution of projections, which presents an estimate of the range of model results (Lowe et al., 2009). The estimated 5th and 95th percentile range of sea level projections were selected to provide a range of 90% of the modelled results (Lowe et al., 2009). The projected sea level increases (5th and 95th percentiles) for the three time periods (2020, 2050 and 2080), were separately combined with MHWS and MLWS tide heights for the River Adur and River Ouse estuaries and multiplied by the tide height ratios (determined for the current profiles, Section 4.3.1), to produce corrected MHWS and MLWS (5th and 95th percentile) vertical tide heights for each salinity station and freshwater discharge regime (low and high) (Table 4.4, Table 4.5). The maximum and minimum salinities that corresponded to the corrected MHWS and MLWS vertical tide heights (using the corrected salinity and tide level relationship plots of Section 4.3.1) were determined (Table 4.4, Table 4.5). Using the maximum and minimum salinities determined at each salinity station, interpolated predictive tide and salinity profiles for the River Adur and River Ouse estuaries were developed for the high emissions scenario relative increase in sea levels (5th and 95th percentiles), at 2020, 2050 and 2080, under both low (August 2008) and high (February 2009) freshwater discharge conditions.

It was not possible to incorporate storm surge impacts (UKCP09) into the predictive high emissions saline penetration profiles, as storm surge height projections were only available for the medium emissions scenario (SRES:A1B; Nakicenovic et al., 2000; Lowe et al., 2009). However, a preliminary tide and salinity profile for the River Ouse estuary, based on sea level and storm surge height projections for the medium emissions scenario (SRES:A1B), showed that increases in storm surges had minimal impact upon saline penetration profiles, most likely due to
the negligible storm surge projected height increases, when compared to the sea level projections
(1 in 50 yr return level in 2080 of 0.013 – 0.036 m; 5th – 95th percentile range; Lowe et al.,
2009). Due to the complex catchment-based interactions regulating freshwater discharge
regimes, it was also not possible to factor extreme changes in freshwater discharge rates (drought
to flood) into the high emissions climate scenario (Murphy et al., 2009). However, in order to
attempt to model the variability in saline penetration profiles between future summer and winter
freshwater discharge patterns, the tide and salinity profiles were determined under high and low
freshwater discharge regimes (as recorded for the current profiles).
### Table 4.4. Summary table of the predicted tide and salinity profiles of the River Adur estuary, under high greenhouse gas emissions scenario (UKCP09 under SRES:A1F1).

<table>
<thead>
<tr>
<th>Year</th>
<th>Freshwater discharge regime river mouth (km)</th>
<th>Relative sea level rise (% 95% CILE)</th>
<th>MHWS + relative sea level rise (% 95% CILE)</th>
<th>Corrected high tide vertical height (m)</th>
<th>Maximum salinity (% 95% psu)</th>
<th>Difference between current salinity (psu)</th>
<th>MLWS + relative sea level rise (% 95% CILE)</th>
<th>Corrected low tide vertical height (m)</th>
<th>Minimum salinity (% 95% psu)</th>
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### Table 4.5. Summary table of the predicted tide and salinity profiles of the River Ouse estuary, under high greenhouse gas emissions scenario (UKCP09 under SRES:A1F1).

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<th>Year</th>
<th>Freshwater discharge regime</th>
<th>Distance from river mouth (km)</th>
<th>Relative sea level rise (5 - 95 %ile)</th>
<th>MHWS + relative sea level rise (5 - 95 %ile)</th>
<th>Corrected high tide vertical height (m)</th>
<th>Maximum salinity (5 - 95 %ile psu)</th>
<th>Difference between current salinity (psu)</th>
<th>MLWS + relative sea level rise (5 - 95 %ile)</th>
<th>Corrected low tide vertical height (m)</th>
<th>Minimum salinity (5 - 95 %ile psu)</th>
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<td></td>
<td></td>
<td>16.1</td>
<td>0.188 - 0.677</td>
<td>6.878 - 7.367</td>
<td>0.83 - 0.89</td>
<td>0.22 - 0.24</td>
<td>0.02 - 0.04</td>
<td>0.958 - 1.447</td>
<td>0.19 - 0.29</td>
<td>0.18</td>
</tr>
</tbody>
</table>
4.3.3 Limitations

Due to the availability of only two SEBA Dipper-TEC sondes for field deployment, the development of the current and predicted tide and salinity profiles for the River Adur and River Ouse estuaries required an innovative methodology. The robustness and reliability of developing current and predictive tide and salinity profiles using the methodological approach described in this chapter, is critically dependent on the assumption that the shape of the tide and salinity profiles recorded at each salinity station remained the same irrespective of sea level height (Section 4.3.1). This was of particular note where attempting to predict maximum salinities at sites that recorded a low salinity to tidal limnetic profile over the sampling dates (August 2008 and February 2009, see Section 4.3.2).

The determination of the tide height ratios (tide height at station vs tide height at harbour) was critical for the correction of all tide and salinity profiles to a given harbour tide height. The tide gauges at Shoreham and Newhaven harbours record the tide height in 10 minute intervals, which although is more accurate than predicted tide heights, could provide a potential error for both tide height and tide times. In addition, due to the constraints of this study, it was only practical to establish three salinity stations along the main channel of the River Ouse estuary and four along the River Adur estuary. A greater number of salinity stations, more in-situ sondes and longer monitoring periods would be recommended for more robust current and predictive salinity profiles along the estuary channels. The predicted saline penetration profiles were developed under a reference tide height (MHWS, MLWS) and freshwater discharge conditions (moderate low and high) and did not factor in extreme events such as storm surges, significant decreases or increases in freshwater discharge or extreme high tides (highest astronomical tide). The predicted profiles are therefore unlikely to be representative of the full extent of saline penetration under an extreme combination of these conditions.
4.4 Results

4.4.1 The River Adur estuary

The River Adur rises in two separate branches, the eastern and western Adur, which join to form the main river west of the town of Henfield (Figure 4.4; Environment Agency, 2005a). The river flows south across the Henfield Levels, through the Shoreham Gap Valley and enters the English Channel at Shoreham-by-Sea (Figure 4.4, Buck, 1997; Environment Agency, 2005a). In order to provide a longitudinal sequence along the River Adur course, this research study concentrated on the main estuary channel and the Eastern branch of the River Adur (Table 4.2, Figure 4.4).

Figure 4.4. Tide and salinity sampling station locations on the River Adur estuary. Abbreviations: UPS – upstream view, DWS – downstream view.
4.4.1.1 Freshwater discharge

The River Adur freshwater discharge gauges are positioned on the Eastern (Sakenham) and Western (Hatterall Bridge) branches of the river. By combining the mean daily discharge rates from the gauging stations on the Western and Eastern branches of the River Adur it was possible to determine the freshwater discharge in the main channel of the River Adur (Figure 4.5). Over the 4-day August 2008 salinity sampling period, the mean daily discharge was 0.723 m$^3$s$^{-1}$, which was slightly higher than the August 2008 monthly average, at 0.474 m$^3$s$^{-1}$ (Figure 4.5). Over the February 2009 salinity sampling period, the mean daily discharge was 1.237 m$^3$s$^{-1}$, which was significantly lower than the February 2009 monthly average of 4.133 m$^3$s$^{-1}$ (Figure 4.5). Although the February 2009 salinity sampling period did not coincide with very high freshwater discharge episodes, it was still useful for characterising saline penetration profiles under ‘stable’ low and high freshwater discharge conditions.

![Graph showing hydrological regime of the lower River Adur monitored at Sakenham (Eastern branch) Hatterall Bridge (Western branch) for the period July 2008 to July 2009. Dates of salinity sampling periods are identified. Sourced from the Environment Agency.](image)
4.4.1.2 Current tide and salinity profiles of the River Adur estuary

The sequential tide profiles recorded in the River Adur estuary showed a diminishing vertical and horizontal tidal range from the river mouth upstream, a result of tidal damping, where frictional resistance of the bed/banks outweighs the propagating impact of bank convergence (Table 4.3, Figure 4.6, Figure 4.7). Tidal damping is enhanced by the freshwater river discharge, which reduces bank convergence and increases friction (Dyer, 1997). As the vertical and horizontal tidal range decreased, so did the tidal velocity, which can be observed in the slope of the tidal profiles (Figure 4.6, Figure 4.7). The form taken by the tidal profiles and the timing of high, low and slack tide varied along the estuary (Figure 4.6, Figure 4.7). High tide at each station did not correspond with the maximum salinity; with maximum salinity lagging a maximum of 62 minutes behind high tide height (at station A1; see Section 4.3.1, Figure 4.3). This effect may have been due to the influence of the mass of freshwater displaced upstream by the advancing saline tidal waters. As would be anticipated, the maximum observed salinity diminished progressively upstream along the estuary (Figure 4.8, Figure 4.9, Table 4.3).

All salinity stations on the River Adur estuary experienced higher maximum salinities under low freshwater discharge regimes (0.733 m$^3$ s$^{-1}$) compared to high (1.237 m$^3$ s$^{-1}$), with the exception of station A1 which recorded fully marine salinities (35) under both discharge conditions (Figure 4.8, Figure 4.9, Table 4.3). The largest decrease in maximum salinity between discharge regimes was recorded at station A2, with a decrease of 18.86 (20 – 1.14) (Figure 4.8, Table 4.3). Under both low and high freshwater discharge regimes, all salinity stations on the River Adur estuary experienced tidal limnetic (<0.5) minimum salinities (Table 4.3). The majority of salinity stations (A1 – A3) experienced greater tidal ranges (m) under low freshwater discharge conditions compared to high (with the exception of station A4), with an average decrease of 0.27 metres from low to high discharge conditions (Figure 4.6, Figure 4.7, Table 4.3). Over the low freshwater discharge salinity profiling period (August 2008), the water temperature ranged from 17.1 - 18.1 °C, compared to 7.2 – 8.5 °C over the high discharge salinity profiling period (February 2009) (Table 4.3).
Figure 4.6. The tide (m) and salinity profile of the River Adur estuary under low (August 2008) freshwater discharge conditions. Tidal profile standardised to a MHWS tide height of 6.3 m and a MLWS height of 0.6 m at the river mouth at Shoreham.

Figure 4.7. The tide (m) and salinity profile of the River Adur estuary under high (February 2009) freshwater discharge conditions. Tidal profile standardised to a MHWS tide height of 6.3 m and a MLWS height of 0.6 m at the river mouth at Shoreham.
Figure 4.8. Comparative maximum salinity profiles of the River Adur estuary under low (August 2008) and high (February 2009) freshwater discharge conditions. Tidal profile standardised to a MHWS tide height of 6.3 m and a MLWS height of 0.6 m at the river mouth at Shoreham.

4.4.1.3 Saline penetration in the River Adur estuary

At maximum salinity, under low freshwater discharge conditions (0.733 m$^3$ s$^{-1}$), the River Adur estuary was euhaline (>30) from the river mouth at Shoreham to 9.07 km upstream, polyhaline (18 – 30) from 9.07 to 11 km (a range of 1.93 km), mesohaline (5 -18) from 11 to 13.31 km (a range of 2.31 km) and oligohaline (0.5 – 5) from 13.31 to 16.98 km upstream (a range of 3.67 km; Figure 4.9, Figure 4.10). The River Adur estuary was tidal limnetic (<0.5) from 16.98 km to the NTL on the Eastern branch at 21 km (4.02 km) and non-tidal limnetic (<0.5) above the tidal limit (Figure 4.9, Figure 4.10).

At maximum salinity, under high freshwater discharge conditions (1.237 m$^3$ s$^{-1}$), the River Adur estuary was euhaline from the river mouth at Newhaven to 8.62 km upstream, polyhaline from 8.62 to 9.46 km (a range of 0.84 km), mesohaline from to 9.46 to 10.37 km (range 0.91 km) and oligohaline (0.5 – 5) from 10.37 to 13.25 km (range 2.88 km) (Figure 4.9, Figure 4.10). The River Adur estuary was tidal limnetic from 13.25 km to the normal tidal limits (NTL) on the Eastern branch at 21 km (range 7.1 km) (Figure 4.9, Figure 4.10). Non tidal limnetic conditions existed above the NTL (Figure 4.10).

In the River Adur estuary, under low freshwater discharge conditions (0.733 m$^3$ s$^{-1}$) and a MHWS tide height of 6.3 metres, tidal saline water (0.5) penetrated up to 16.98 km from the
river mouth at Shoreham (Figure 4.9). Under high freshwater flow conditions (1.237 m$^3$ s$^{-1}$) saline water penetrated up to 13.25 km, a decrease in penetration range of 3.73 km from low to high discharge conditions (Figure 4.9, Figure 4.10).

Figure 4.9. The maximum salinity profiles of the River Adur estuary under low (August 2008) and high (February 2009) freshwater discharge conditions. Salinity profiles standardised to a MHWS tide height of 6.3 m and a MLWS height of 0.6 m at the river mouth at Shoreham.

Figure 4.10. The maximum salinity saline penetration profile of the River Adur estuary under low (August 2008) and high (February 2009) freshwater discharge conditions. Salinity profiles standardised to a MHWS tide height of 6.3 m.
4.4.2 The River Ouse estuary

The River Ouse is tidal from the river mouth at Newhaven, 21.8 km upstream to Barcombe Mills, where a series of weirs mark the normal tidal limit (NTL) (Figure 4.11, Environment Agency, 2005a; 2010). Historical engineering works have altered the natural course of the River Ouse estuary channel, particularly at Hamsey (15 km from the river mouth), where a cut-through channel (known as “Mighell’s Cut”) by-passes a 2.3 km meandered loop of natural estuary channel (Figure 4.12, Gibbs & Farrant, 1970; Environment Agency, 2005a). This channel was dug in 1790 by the Upper Ouse Navigation Company to cut off the estuary meander and create a navigable canal with locks (Gibbs & Farrant, 1970). The natural course of Hamsey loop is interrupted by a half-weir adjacent to the upstream mouth of the cut-through channel (Figure 4.12). In order to assess the effect of this cut-through channel on the degree of saline penetration upstream, a salinity station (station O4) was established on Hamsey loop, downstream of the half-weir (Table 4.2, Figure 4.12). The half-weir is a modern structure, which presumably occupies the site of an earlier barrier intended to manage river flow so as to maintain a working head of water for the old Hamsey water mill (Gibbs & Farrant, 1970). The water mill was demolished in 1790, after the destruction of the mill race during the construction of the Hamsey canal (cut-through channel, Gibbs & Farrant, 1970). The half-weir acts only as a partial barrier to saline penetration, as is inundated at high tide levels greater than 5.8 metres at the river mouth (Environment Agency, 2005a). Due to its position on Hamsey loop, not the main estuary channel, station O4 was not included in the main River Ouse estuary tide and salinity profile (using stations O1, O2 and O3) (Figure 4.12). A tide and salinity profile of the River Ouse estuary up to Hamsey half-weir (around Hamsey loop) was developed separately to investigate the degree of increasing saline penetration on Hamsey loop alone (using stations 1, 2 and 4) (Figure 4.12).
Figure 4.11. Tide and salinity sampling station locations on the River Ouse estuary. Abbreviations: UPS – upstream view, DWS – downstream view.
Figure 4.12. Tide and salinity sampling station locations on the River Ouse estuary, with particular focus on stations 3 and 4, on Hamsey loop and cut-through channel. Photographs: a - Half-weir at high tide (O4 upstream view), b – Half-weir at low tide (O4 upstream view).

4.4.2.1 Freshwater Discharge

The mean daily freshwater discharge rate recorded at the Barcombe Mills gauging station for the River Ouse estuary was 0.72 m³ s⁻¹ over the August 2008 salinity sampling period, which was slightly higher than the August 2008 mean monthly discharge rate of 0.659 m³ s⁻¹ (Figure 4.13). Over the February 2009 salinity sampling period, the mean daily discharge was 1.43 m³ s⁻¹, which was significantly lower than the February 2009 mean monthly discharge rate of 2.904 m³ s⁻¹ (Figure 4.13). As with the River Adur estuary, the February 2009 salinity sampling period did
not coincide with very high freshwater discharge episodes, but was still useful for characterising saline penetration profiles under ‘stable’ low and high freshwater discharge conditions.

Figure 4.13. Hydrological regime for the tidal River Ouse, monitored at Barcombe Mills for the period July 2008 to July 2009. Dates of salinity sampling periods are identified.

4.4.2.2 Current tide and salinity profiles of the River Ouse estuary

The sequential tide profiles recorded in the River Ouse estuary share similar characteristics to those recorded in the River Adur estuary, with diminishing vertical and horizontal tidal ranges and velocities from the river mouth upstream (Figure 4.14, Figure 4.15). This diminishing tidal influence can be attributed to the same effects of tidal damping. Similarly high tide at each station did not correspond with the maximum salinity and the maximum salinity concentrations diminished upstream along the estuary.

The majority of salinity stations on the River Ouse estuary (O2 - O4) experienced higher maximum salinities under low freshwater discharge regimes (0.733 m$^3$ s$^{-1}$) compared to high (1.237 m$^3$ s$^{-1}$), with the exception of station O1, which experienced fully marine conditions (35) under both discharge regimes (Table 4.3, Figure 4.16). The largest decrease in maximum salinity between discharge regimes was recorded at station O2, with a decrease of 6 (from 6.8 –
0.8) (Table 4.3). Under both low and high freshwater discharge conditions stations O3 and O4 experienced tidal limnetic (<0.5) maximum salinities (Table 4.3, Figure 4.16). However, under low freshwater discharge conditions, station O3 experienced a greater maximum salinity (0.4) than station O4 (0.32), demonstrating that the Hamsey cut-through channel acts as a ‘short-cut’ for tidal driven saline penetration upstream (Table 4.3, Figure 4.16). Under both low and high freshwater discharge regimes, all salinity stations on the River Ouse estuary experienced tidal limnetic (<0.5) minimum salinities (Table 4.3). All salinity stations (O1 – O4) experienced greater tidal ranges (m) under low freshwater discharge conditions compared to high, with an average decrease of 0.29 metres from low to high discharge conditions (Table 4.3, Figure 4.14, Figure 4.15). Over the low freshwater discharge salinity profiling period (August 2008), the water temperature ranged from 17.2 – 18.5 °C, compared to 6.5 – 8.5 °C over the high discharge salinity profiling period (February 2009; Table 4.3).
Figure 4.14. The tide (m) and salinity profile of the main River Ouse estuary channel (a.) and Hamsey loop (b.) under low (August 2008) freshwater discharge conditions. Tidal profile standardised to a MHWS tide height of 6.69 m and a MLWS height of 0.77 m at the river mouth at Newhaven.
Figure 4.15. The tide (m) and salinity profile of the main River Ouse estuary channel (a.) and Hamsey loop (b.) under high (February 2009) freshwater discharge conditions. Tidal profile standardised to a MHWS tide height of 6.69 m and a MLWS height of 0.77 m at the river mouth at Newhaven.
Figure 4.16. Comparative maximum salinity profiles of the main River Ouse estuary channel (a.) and Hamsey loop (b.) under low (August 2008) and high (February 2009) freshwater discharge conditions. Salinity profiles standardised to a MHWS tide height of 6.69 m and a MLWS height of 0.77 m at the river mouth at Newhaven.
4.4.2.3 Saline penetration of the River Ouse estuary

At maximum salinity, under low freshwater discharge conditions, the main River Ouse estuary channel (O1-O3) was euhaline (>30) from the river mouth at Newhaven to 7.67 km upstream, polyhaline (18 – 30) from 7.67 to 10.8 km (a range of 3.13 km), mesohaline (5 – 18) from 10.8 to 14.4 km (range 3.6 km) and oligohaline (0.5 – 5) from 14.4 to 16.06 km upstream (range 1.66 km) (Figure 4.17a, Figure 4.18a). The River Ouse estuary was tidal limnetic (<0.5) from 16.06 km to the normal tidal limit at 21.8 km (range 5.74 km) and non tidal limnetic (<0.5) above the NTL (Figure 4.17a, Figure 4.18a). Around Hamsey loop, the maximum salinity was mesohaline (5 – 18) from 10.8 to 14.73 km (range 3.93 km), oligohaline (0.5 – 5) from 14.73 to 17.25 km (range 2.52 km) and tidal limnetic from 17.25 km to the half-weir at 17.35 km (range 0.1 km) (Figure 4.17b, Figure 4.18b).

At maximum salinity, under high freshwater discharge conditions, the main River Ouse estuary channel (O1-O3) was euhaline from the river mouth at Newhaven to 7.43 km upstream, polyhaline from 7.43 to 10.01 km (range 2.58 km), mesohaline from 10.01 to 12.82 km (range 2.81 km) and oligohaline from 12.82 to 14.91 km (range 2.09 km) (Figure 4.17a, Figure 4.18a). The River Ouse estuary was tidal limnetic from 14.91 km to the NTL at 21.8 km (range 6.89 km) and non tidal limnetic above the NTL (Figure 4.17a, Figure 4.18a). Around Hamsey loop, the maximum salinity was oligohaline from 12.82 to 15.6 km (range 2.78 km) and tidal limnetic from 15.6 km to the half-weir at 17.35 km (range 1.75 km) (Figure 4.17b, Figure 4.18b).

In the main River Ouse estuary channel (O1-O3), under low freshwater discharge conditions (0.72 m$^3$ s$^{-1}$) and a MHWS tide height of 6.69 metres, tidal saline water (0.5) penetrated up to 16.06 km from the river mouth at Newhaven (Figure 4.17a, Figure 4.18a). Around Hamsey loop (O1, O2 & O4), saline water penetrated up to 17.25 km from the river mouth at Newhaven (Figure 4.17b, Figure 4.18b). Under high freshwater discharge conditions (1.43 m$^3$ s$^{-1}$), in the main River Ouse estuary channel (O1-O3), saline water penetrated up to 14.91 km from the river mouth, a decrease in penetration range of 1.15 km from low discharge conditions (Figure 4.17a, Figure 4.18a). Under high discharge conditions, saline water penetrated up to 15.6 km around Hamsey loop (O1, O2 & O4), a decrease in penetration range of 1.65 km from low to high discharge conditions (Figure 4.17b, Figure 4.18b).

The two tide and salinity profiles developed for the River Ouse estuary (main River Ouse estuary channel and Hamsey loop) show that tidal saline water penetrates a greater distance around Hamsey loop than the along the main estuary channel (through the cut-through channel) (Figure 4.17, Figure 4.18). Under low freshwater discharge conditions (0.72 m$^3$ s$^{-1}$) and a MHWS tide height of 6.69 meters, tidal saline water penetrated up to 17.25 km from the river mouth to the
Hamsey loop (Figure 4.18b). Under the same conditions, saline water only penetrated up to 16.06 km along the main estuary channel (through the Hamsey cut-through channel), a difference of 1.19 km (Figure 4.18a). The recorded differences in saline penetration distances can be accounted for by the relative lengths and gradients of the cut-through channel and Hamsey loop. The Hamsey loop (2.28 km) is the natural low gradient course of the River Ouse estuary and as such is no obstacle to saline penetration up to the physical vertical barrier of the half-weir (Figure 4.12). The half-weir acts as a barrier to the progress of saline waters, corresponding to tidal ranges up to approximately 5.8 metres at the river mouth. The cut-through channel (main River Ouse channel) breaches the five metre contour in the chalk ridge occupied by Hamsey village (Figure 4.12). It extends for 0.93 km and has a correspondingly steeper bed gradient than the Hamsey loop. It was this steep fall that allowed for the original water mill development and the subsequent requirement for locks to enable navigation (circa 1790). It should be noted that the natural course of the River Ouse estuary does not exceed the 5 metre contour until Barcombe Mills, which is 4.27 km upstream (from the end of Hamsey loop) (Figure 4.12). The observed difference in upstream extent of saline penetration in the River Ouse estuary is therefore a consequence of the distances to and gradients of the two obstacles, one being vertical (half-weir) and the other a progressive incline (cut through channel).
Figure 4.17. The maximum salinity profiles of the main River Ouse estuary (a.) and Hamsey loop (b.) under low (August 2008) and high (February 2009) freshwater discharge conditions. Salinity profiles standardised to a MHWS tide height of 6.69 m and a MLWS height of 0.77 m at the river mouth at Newhaven.
4.4.3 Predicted tide and salinity profiles - high emission sea level scenarios

Under the high emissions scenario (SRES: A1FI), relative sea levels (in the Newhaven and Shoreham 12 km grid squares; UKCP09 UI) are projected to increase by 0.053 m to 0.176 m (5th to 95th percentile range) in 2002, 0.115 m (0.116 m for Shoreham) to 0.402 m in 2050 and by 0.188 m to 0.677 m in 2080 (Table 4.4, Table 4.5). In both the River Ouse (main channel) and River Adur estuaries, the projected increases in relative sea level height resulted in an increase in both the gradient of maximum salinity zones and the upstream extent of saline penetration when compared to the current tide and salinity profiles (August 2008 and February 2009) (Figure 4.19, Figure 4.20, Figure 4.21). The upstream extent of saline penetration and gradient of salinity zones differed between year, freshwater discharge regime and estuary (Figure 4.20, Figure 4.21). However, the predicted increases in saline penetration in both the River Adur and River Ouse estuaries were modest, even under the ‘worst-case’ predicted profiles of high emissions scenario.
increases in relative sea level (95th percentile), for the year 2080, under low freshwater discharge conditions (Figure 4.19, Figure 4.20, Figure 4.21).

As recorded in the current profiles, the predictive profiles highlight a clear relationship between freshwater discharge regime (low and high) and the predicted degree of saline penetration, with low freshwater discharge regimes resulting in an increase in both the predicted upstream extent of saline penetration and maximum salinity zones (Figure 4.19, Figure 4.20, Figure 4.21). In the River Adur estuary, under high emissions projected sea levels for 2080 (0.677 m; 95th percentile), the upstream extent of saline penetration increased from 16.98 to 17.3 km (0.32 km) under low freshwater discharge conditions (0.73 m$^3$ s$^{-1}$) and from 13.25 to 14.03 km (0.78 km) under high freshwater discharge conditions (1.237 m$^3$ s$^{-1}$), compared to the current profiles (Figure 4.19, Figure 4.20). Therefore, a 0.51 m$^3$ s$^{-1}$ reduction in freshwater discharge in the River Adur resulted in a 3.27 km upstream shift in saline penetration in the River Adur estuary (high emissions for 2080; 95th percentile). In the River Ouse estuary, the high emissions projected sea levels for 2080 (0.677 m; 95th percentile), produced only small increases in the upstream extent of saline penetration, from 16.06 to 16.21 km (0.15 km) under low freshwater discharge conditions (0.72 m$^3$ s$^{-1}$) and from 14.91 to 15.8 km (0.89 km) under high discharge conditions (1.43 m$^3$ s$^{-1}$) compared to the current profiles (Figure 4.19, Figure 4.21). Therefore a 0.71 m$^3$ s$^{-1}$ reduction in freshwater discharge in the River Ouse estuary, resulted in a small 0.41 km increase in the upstream extent of saline penetration (high emissions for 2080; 95th percentile).

The predictive saline penetration profiles determined for the River Adur and River Ouse estuaries appear to strongly contrast in form, which may in part be explained by the number and location of salinity stations on the River Ouse estuary (Figure 4.19). The River Adur estuary predictive profiles display an approximately sigmoid pattern for the relationship between maximum salinities and distance from the river mouth (Figure 4.19). These predictive profiles are interpolated from tide and salinity profiles derived from four field salinity stations (see Section 4.3), which although not equidistant in distribution, would appear to reasonably represent the future saline penetration profiles without taking liberties with interpolations. In the River Adur estuary predictive profiles, the effect of increasing relative sea levels is initially apparent in the upstream extension of fully marine saline conditions (35) by up to 2.37 km by 2050 and 2080 (under low freshwater discharge conditions). This effect is not recorded in the River Ouse estuary predictive salinity profiles, which are based upon tide and salinity profiles.
from only three field salinity stations. Critically, the 7.3 km sampling gap between the first and second field salinity station locations (O1 and O2) includes those areas in which the future upstream extension of fully marine conditions could be reasonably expected to take place (Figure 4.19).

Under low freshwater discharge conditions, the River Adur estuary saline penetration profiles indicate that between 8.2 and 14.06 km from the river mouth, the years 2020, 2050 and 2080 (under high emission scenarios) produce an increase in saline penetrations most pronounced at 10.64 km and decreasing proportionally to 14.06 km (Figure 4.19). The contributions of the 2020 and 2050 scenarios to these salinity shifts are proportionate and progressive. The 2080 scenario demonstrates only limited influence restricted to the lower salinity ranges (Figure 4.19). The reason for this is unclear and is in contrast to the River Ouse estuary predictive profile in which the 2080 scenario is well represented (Figure 4.19). The River Adur estuary predictive profiles indicate an extensive upstream ‘tail’ of increased penetration at low salinities (0.2 – 1.28) over 3.95 km upstream. The effect of the 2080 scenario is more distinct in this portion of the graph than in the preceding zone (Figure 4.19). The absence of an extensive low salinity ‘tail’ of saline penetration on the River Ouse estuary predictive profiles is due to both the absence of an upstream salinity station, and the influence of the cut-through channel and half weir features at Hamsey (as detailed in Section 4.4.2). In the absence of the Hamsey structures, it could be assumed (based on the predicted profiles) that saline waters would penetrate further into the River Ouse than the River Adur estuary (Figure 4.9). The form of the predictive relationships between maximum salinity and distance from the river mouth is best depicted in the River Adur estuary saline penetration profiles, which benefited from more representative distribution of salinity stations and the absence of significant engineering structures compared to the River Ouse estuary.
Figure 4.19. Predictive maximum MHWS salinity profiles for the River Adur (a.) and River Ouse (b.) estuaries under projected high emission scenario relative sea level increases for the years 2020, 2050 and 2080. Maximum salinity profiles depicted as a range of model scenarios (5th to 95th percentile) under low and high freshwater discharge conditions.
Figure 4.20. The maximum MHWS salinity profiles of the River Adur estuary under predicted high emission scenario relative sea level increases (95th percentile) for the years 2020, 2050 and 2080, under low and high freshwater discharge conditions.
Figure 4.21. The maximum MHWS salinity profiles of the River Ouse estuary under predicted high emission scenario relative sea level increases (95th percentile) for the years 2020, 2050 and 2080, under low and high freshwater discharge conditions.

4.5 Discussion

In this chapter it was hypothesised that the spatial and temporal extent of saline penetration into the River Adur and River Ouse estuaries is naturally dynamic, and reflective of the relative opposing strengths of tidal forcing marine water upstream and freshwater discharge upstream. The current tide and salinity profiles described in this chapter demonstrate that the River Adur and River Ouse estuaries are highly dynamic, with some stations experiencing a 34.6 range between maximum and minimum salinity over as short a period as 3 hours 42 minutes. These
profiles are in part related to the opposing strengths of tidal forcing upstream and freshwater discharge downstream, with the River Ouse and River Adur estuaries having the second and third largest mean spring tidal ranges in Southern England at 6.1 and 5.5 m (Buck, 1997). However in addition to large tidal ranges and freshwater discharge rates, the River Ouse and River Adur estuaries have heavily managed and modified courses, resulting in narrow, constrained, parallel-banked estuarine channels from the river mouths through to the limits of the tide (Environment Agency, 2005a; 2009). Pumping of the floodplain and canalisation of the estuary channel has prevented any development of salt marsh on the lower River Ouse estuary and only a small mudflat is exposed at Newhaven at low tide, resulting in an intertidal area of only 6 hectares (Buck, 1997; Environment Agency, 2005a). The lower River Adur estuary is also heavily embanked, although to a lesser extent than the River Ouse and does have salt marsh fringe development (Buck, 1997; Environment, 2005). The deflection of the river mouth by a shingle spit at Shoreham has created a relatively large intertidal area of 46 hectares (Buck, 1997; Environment Agency, 2005a; 2011). The narrow constrained estuarine channels and limited intertidal area provide no real buffer to moderate the inland propagation of the tide (Savenije, 2005; Savenije & Veling, 2005). It is likely that the combination of large tidal ranges, varying freshwater discharge rates and narrow constrained estuarine channels contribute to the highly dynamic tide and salinity profiles recorded in the River Adur and River Ouse estuaries.

While no evidence of saline wedge effects were recorded or observed during the tide and salinity profiling, the experimental methods employed in this research were not designed to detect vertical stratification of the incoming tide. Vertical salinity profiles measured from mid-stream bridge stations on the incoming tide on several separate occasions showed no saline/freshwater density stratifications. This could indicate very active vertical mixing and the absence of saline wedge effects, although rigorous vertical interval salinity profiling of the incoming tide would be required to confirm this. Savenije, (1992) observed that in tidal estuaries, despite popular opinion, salt wedge saline intrusion did not occur, or only occurred during high river floods.

In this chapter it was hypothesised that under projected sea level rise scenarios, the upstream extent of saline penetration in to the study estuaries would increase under both high and low freshwater discharge conditions. In both the River Adur and River Ouse estuaries, increases in relative sea levels predicted under the high emissions scenario (SRES:A1FI), resulted in an increase in both the upstream extent of saline penetration and gradient of maximum salinity zones, which differed according to predicted year, freshwater flow regime (low and high) and
estuary. However, in both estuaries the predicted upstream increase in saline penetration was
moderate, even under the ‘worst-case’ conditions. In the River Ouse estuary, the predicted
upstream extent of saline penetration under ‘worst-case’ conditions increased by only 0.15 km
from the current profile extent (August 2008), compared to a 0.32 km increase in the River Adur
estuary. The reduced upstream extent of saline penetration recorded in both the current and
predicted profiles of the River Ouse estuary (when compared to the River Adur estuary), is likely
the result of the increase in effective river bed gradient at the Hamsey cut-through channel,
acting as a partial in-stream barrier to the upstream ingress of saline water. This suggests that
the upstream extent of saline penetration into estuarine systems can be reduced or moderated by
natural or artificial in-stream barriers that partially impede tidal flow (Le et al., 2007). In
contrast, the River Adur estuary has no comparable historic engineering barriers and flows
uninterrupted 2.98 km further than the River Ouse estuary before encountering contemporary
flood control weirs (on the Eastern and Western branches). These weirs do not represent NTL,
which occur beyond the two branches. The more natural course of the River Adur estuary (at
least compared to the River Ouse estuary) and the more representative distribution of salinity
stations suggests that the patterns of current and predicted tidal saline penetration under low and
high freshwater discharge conditions is a more realistic representation of the hydrodynamics of
low-lying coastal river valleys with essentially consistent and gradual gradients.

The salinity profiles developed in this chapter demonstrate the impact of freshwater discharge
regime on both the current and predicted upstream extent of saline penetration. The high and
low freshwater discharge regimes recorded during the salinity sampling periods on both the
River Adur and River Ouse estuaries, did not coincide with any extreme freshwater discharge
events (drought or flood). Instead, the salinity sampling periods coincided with two periods of
relatively stable summer and winter freshwater discharge regimes. Despite a difference of only
0.71 m³ s⁻¹ (Ouse) and 0.51 m³ s⁻¹ (Adur) between high and low freshwater discharge conditions,
both the River Adur and River Ouse estuaries exhibited increased saline penetration under low
freshwater discharge conditions when compared to high, in both the current and predicted
profiles. The impact of freshwater discharge regime on saline penetration was more notable in
the River Adur estuary than the River Ouse estuary, with a difference between discharge
conditions of 3.73 km compared to 1.15 km on the River Ouse (based on current profiles).
Freshwater discharge regime also appeared to impact the tidal range experienced at each station,
with an average increase of 0.2 m under low discharge conditions compared to high.
The extent of saline penetration is in part dependant on the balance between downstream freshwater river flow and upstream tidal influences (Savenije, 2005; Nguyen et al., 2008). Greater freshwater discharge results in higher resistance to tidal penetration, and subsequent lower tide heights and reduced saline penetration (Nguyen et al., 2008). Under low flow conditions, the tide experiences less resistance and so salt water can penetrate further upstream (Peirson et al., 2001; Nguyen et al., 2008). The impact of freshwater flow on saline penetration can be observed most acutely in estuaries in tropical climates, which have pronounced wet and dry seasons (Amphlett & Brabben, 1998). In the Gambia River, freshwater discharge can vary between 2 and 200 m$^3$s$^{-1}$ from the dry to wet season and at low flows, saline water can penetrate from 200 to 250 km of its 526 km tidal reach (Sanmuganathan & Waite, 1975). In contrast, in the Amazon River, the dry season freshwater discharge is of such significant volume that at present no salt water penetration occurs (Gibbs, 1970).

Although freshwater discharge is seasonably variable, it can also be exacerbated by freshwater abstractions above the tidal limit. Increased abstraction of freshwater from above tidal reaches has been observed to increase saline penetration in estuarine systems with detrimental effects upon freshwater biota (Andrews, 1977; Attrill et al., 1996; Peirson et al., 2001). Although not factored into the predictive tide and salinity profiles for the River Adur and River Ouse estuaries, predicted climate (reduced summer precipitation, increased temperatures) and anthropogenic (e.g. increased abstraction) induced reductions in summer river flows are likely to exacerbate saline penetration events. It was beyond the scope of this project to model changes in freshwater discharge in the River Adur and River Ouse under future climate scenarios. However, where modelled changes in river flows have been undertaken, an average reduction of 11% has been projected for the 2020s under different climate model predictions (Romanowicz et al., 2006). Depending on catchment location, geology, land-use, soils and model uncertainty, this reduction in river flows could range from 1 to 32% (Romanowicz et al., 2006). Under high emissions (SRES: A1FI) projected increases in relative sea levels for the year 2080 (95th percentile), a reduction in freshwater discharge of 0.51 m$^3$s$^{-1}$ (between high summer and low winter discharge conditions) resulted in a 3.27 km increase in the upstream extent of saline penetration in the River Adur estuary. Assuming a constant relationship between freshwater discharge and the upstream extent of saline penetration, a 32% decrease in summer River Adur flow (from 0.73 m$^3$s$^{-1}$ to 0.5 m$^3$s$^{-1}$) would result in a ~1.46 km increase in the upstream extent of saline penetration currently predicted for 2080 (from 17.3 km to 18.76 km; high emissions scenario, 95th percentile). This modelled reduction (32%) in summer river flow would increase the upstream extent of saline penetration in the River Adur over five times as much as projected increases in
sea level (0.677 m; 95th percentile) based on current low freshwater discharge (0.73 m$^3$ s$^{-1}$) alone (for high emissions, 2080 (95th percentile) predictions). In contrast, predicted increases in winter (high) river flows (from increased temperatures and precipitation; Murphy et al., 2009), are highly likely to result in a reduction in the predicted extents of saline penetration in the River Adur estuary, based on current high freshwater discharge conditions (1.237 m$^3$ s$^{-1}$). This highlights the critical importance of future reductions in summer river discharge on the upstream extent of saline penetration in estuarine systems. Recent studies have suggested that the impact of future anthropogenic freshwater abstraction for human use, may exceed any affects of climate change on river flow regimes, potentially significantly increasing the five-fold increase in upstream extent of saline penetration (in addition to high emission relative sea levels for 2080), predicted in the River Adur (Vorosmarty et al., 2000; Vorosmarty et al., 2010; Lester et al., 2011). As a catchment variable which can to some degree be influenced (e.g. abstraction regulation and balancing reservoir discharges), these freshwater discharge effects may represent a river management feature that could be used to ameliorate a degree of future saline penetration.

In addition to the impact of climate-driven changes on estuaries, anthropogenic estuarine and riverine management strategies can have significant impact upon the upstream extent of saline penetration. As shown in the River Adur and River Ouse tide and salinity profiles, the management of estuarine channels by channel constriction, bank stabilisation and dredging can have a large impact on estuarine tidal hydraulics, potentially leading to increased tidal amplification and saline intrusion upstream.

It has been predicted that climate impacts, such as rising sea levels, increasing storm surge frequency/intensity and modified river discharge regimes may significantly increase the upstream extent of saline penetration (Dyer, 1997; Savenije, 2005). This is predicted to result in considerable detrimental effects upon estuarine and riverine ecology, land use and abstracted water quality, particularly during drought years (Attrill et al., 1996; Martinho et al., 2007; Bessa et al., 2010; Kingsford et al., 2011). In the UK, considerable research has been undertaken on the prediction and prevention of saline water intrusion into groundwater aquifers in relation to increases in relative sea level, but very little consideration has been given to the related issue of increasing saline penetration into coastal surface waters (e.g. estuaries and lagoons) (Custodio, 2010; Webb & Howard, 2010; Werner et al., 2011). This is highlighted in Environment Agency climate-driven coastal management reports, where the issues of increases in saline penetration are not acknowledged (Environment Agency, 2005a; 2006). Predictive models linking projected
relative sea level increases and additional climate change impacts (e.g. storm surges, freshwater discharge variations) to the degree of saline penetration in estuarine systems is lacking, and where available, tends to be focussed on broad scale, global inundation impacts (Xingong et al., 2009). In contrast to the UK, the issue of increasing saline penetration appears to be of much greater prominence in tropical climates and developing countries with high coastal based populations, especially where the available freshwater is in high demand and land is often very low-lying (Sestini, 1992; Alam, 1996; Hettiarachchi, 1997; Broadus, 2001; Kasperson & Kasperson, 2001; Lewsey et al., 2004; Dasgupta et al., 2009).

The River Adur and River Ouse estuaries were selected as study sites for this research project based on their perceived susceptibility to future increases in saline penetration (e.g. long, low gradient, macrotidal and anthropogenically constrained; Chapter 3). However, whilst saline penetration was predicted to increase in relation to projected relative sea levels, these increases were minimal, even under ‘worst-case’ conditions. It could therefore be speculated that although the degree of saline penetration would vary with estuary morphology and location, the impact of current predicted increases in relative sea levels (based on current low and high river discharge) may only result in small scale increases in saline penetration, when compared to current profiles and natural variability. However, the additional impact of predicted changes in river discharge might be a critical component of future increases in saline penetration, with modelled decreases in summer flow (32%) resulting in over five times the increase in upstream extent of saline penetration predicted for projected increases in relative sea level based on current low discharge alone (for high emissions, 2080; 95th percentile). In addition, the combination of low summer river flows and high tides (highest astronomical tides), with extreme storm surge events are unknown, but could result in short-term saline penetration pulse events, which could have significant impact upon estuarine ecology and benthic macroinvertebrate communities. For example, in late summer 2005, the hurricanes Katrina and Rita pushed salt water from the Gulf of Mexico into fresh wetland marsh communities in coastal Louisiana, resulting in long-term loss of biodiversity (Steyer et al., 2007; Howes et al., 2010; DeLaune & White, 2011).

In order to progress to determining the impact of future increases in saline penetration (predicted in this chapter) upon benthic macroinvertebrates, the current temporal and spatial structure and distributions of the macroinvertebrates of the River Adur and River Ouse estuaries was investigated. In addition, the relationships between macroinvertebrate community structure, salinity and additional environmental variables were explored (Chapter 6)
4.6 Summary

- The River Adur and River Ouse estuaries are very dynamic tidal environments, due to large tidal ranges, variable freshwater discharge conditions, low gradients and anthropogenically-constrained channels.

- Both estuaries exhibit dramatic fluctuations between saline and freshwater conditions over a tidal cycle, with 34.6 variations being recorded over periods as short as 3 hours 42 minutes.

- The profile of the tidal ‘wave’ as recorded at intervals along the estuaries was consistent at each location but altered in amplitude under different harbour mouth tidal heights and downstream river flows.

- High emission scenario projected increases in relative sea levels (for the years 2020, 2050 and 2080) resulted in an increase in both the upstream extent of saline penetration and gradient of maximum salinity zones in the River Adur and River Ouse estuaries, however this increase was moderate, even under ‘worst-case’ conditions.

- Freshwater discharge regime has a significant impact upon the current and predicted degree of saline penetration into the River Adur and River Ouse estuaries, with an increase in upstream extent under low freshwater discharge conditions compared to high. The difference in upstream extent between discharge regimes was more notable in the River Adur estuary than the River Ouse estuary.

- Future climate and anthropogenically induced changes in summer (reduced) and winter (increased) river flows are likely to have a significant impact upon the upstream extent of saline penetration, with modelled reductions in summer flows likely to significantly increase the upstream extent of saline penetration predicted for projected increases in relative sea levels under current low discharge regimes (in this study).

- The current and predicted upstream extent of saline penetration in the River Ouse estuary is moderated by a river bed gradient change at Hamsey cut-through channel and half-weir.

- Under MHWS tide height of 6.69 metres, the River Ouse estuary exhibited a current saline penetration upstream extent of 16.06 km under low freshwater discharge conditions and 14.91 km under high freshwater discharge conditions. Under high
emissions projected sea levels for 2080 (0.677 m; 95\textsuperscript{th} percentile), these extents increased by 0.15 km under low freshwater discharge conditions (from 16.06 to 16.21 km) and 0.89 km under high freshwater discharge conditions (from 14.91 to 15.8 km).

- Under MHWS tide height of 6.3 metres, the River Adur estuary exhibited a current saline penetration upstream extent of 16.98 km under low freshwater discharge conditions and 13.25 km under high discharge conditions. Under high emissions projected sea levels for 2080 (0.677 m; 95\textsuperscript{th} percentile), these extents increased by 0.32 km under low freshwater discharge conditions (from 16.98 to 17.3 km) and 0.78 km under high freshwater discharge conditions (from 13.25 to 14.03 km).

- The more ‘natural’ course of the River Adur estuary (compared with the River Ouse) and more representative distribution of salinity stations, suggests that the tide and salinity profiles determined for the River Adur estuary, are more realistic representations of the current and predicted tidal hydrodynamics of low-lying coastal river valleys.

- The current and predicted tide and salinity profiles for the River Adur and River Ouse estuaries, have been determined under reference conditions (MHWS/MLWS tide heights and moderate low and high freshwater discharge regimes) and as such do not factor in extreme events (e.g. highest tides, storm surges, freshwater drought and flood). These profiles are therefore unlikely to be representative of the full extent of saline penetration under an extreme combination of these conditions.
Chapter 5. The spatial and temporal variation in the macroinvertebrate fauna of the River Adur and River Ouse

5.1 Introduction

In order to predict the impacts of increasing saline penetration on the benthic macroinvertebrates of estuaries and rivers, it is first necessary to establish the existing longitudinal field distributions and compositions of benthic macroinvertebrate communities of the River Adur and River Ouse. By examining the current faunal patterns, distributions and abundances of macroinvertebrate taxa and understanding the mechanisms that determine them, it may be possible to predict the potential future impact of increasing saline penetration associated with net changes in sea level, land level and reduced summer river flows (IPCC, 2007; Lowe et al., 2009; Murphy et al., 2009).

5.1.1 Aims, Objectives and Hypotheses

This chapter aims to investigate the benthic macroinvertebrate community structure and species distributions in the River Adur and River Ouse under high (winter) and low (summer) freshwater discharge conditions, and explore the relationships between faunal structure, salinity parameters (i.e. maximum, minimum and range; established in Chapter 4) and a range of environmental variables measured in the field (e.g. sediment grain size, dissolved oxygen content, organic content etc). This chapter will also critically examine the use of published macroinvertebrate salinity tolerance groupings for prediction of the upstream extent of saline penetration, using the River Adur and River Ouse estuary tide and salinity profiles (Chapter 4). These aims will be achieved through the following objectives:

1. Outline the methodological approaches used for the collection and analysis of benthic macroinvertebrate samples and associated environmental data for the River Adur and River Ouse over the two field study periods: August 2008 (low freshwater discharge) and February 2009 (high freshwater discharge).

2. Allocate recorded benthic macroinvertebrate species to salinity tolerance groupings based on published literature, and using these tolerance groupings, critically assess the use of present (and past, produced in Chapter 3) species distributions in determining the upstream extent of saline penetration, against measured tide and salinity profiles (determined in Chapter 4).

3. Use multivariate statistics on the combined River Adur and River Ouse faunal and environmental datasets (August 2008 & February 2009) to compare faunal structure and species
distributions between rivers and assess which common environmental variables are driving the faunal communities in these systems.

4. Analyse the benthic macroinvertebrate data (both qualitatively and using statistical techniques) and describe/discuss the spatial and temporal faunal structure and species distributions independently for each river under low (August 2008) and high (February 2009) freshwater conditions.

5. To assess species-environment relationships (through multivariate techniques) operating within the River Adur and River Ouse (independently), with particular focus placed upon the role of the salinity parameters in driving faunal structure and determining species distributions.

6. Discuss the impact of freshwater discharge induced (low August 2008 to high February 2009) changes in the upstream extent of saline penetration on benthic macroinvertebrate community structure and species distributions in the River Adur and River Ouse estuaries, with particular reference to salinity as a driver of estuarine benthic macroinvertebrate distributions.

It is hypothesised that whilst additional environmental variables will be important in determining macroinvertebrate compositions in estuaries, salinity is the dominant parameter driving faunal structure and species distributions over the freshwater to marine transitions. It is therefore hypothesised that spatial variability in the extent of saline penetration (under low and high freshwater discharge conditions) will be associated with shifts in the distributions of benthic macroinvertebrate species relative to their salinity tolerance. It is also hypothesised that determination of the upstream extent of saline penetration using macroinvertebrate salinity tolerance groupings will closely correspond to measured extents of saline penetration recorded in chapter 4.

5.2 Sites and Methods

Macroinvertebrate sample sites on the River Adur and River Ouse were selected to cover the transition from fully marine (river mouth) through to non-tidal freshwater (above NTL) (Figure 5.1, Figure 5.2). The mouths of the River Adur and River Ouse are both large industrialised ports (Shoreham and Newhaven) and establishing sample sites was difficult, especially at Newhaven (see Chapter 2). Marine sampling sites were established as close to the full marine conditions at the river mouth as possible. The majority of sampling sites were established along the River Adur and River Ouse estuaries (up to the NTL) (Figure 5.1, Figure 5.2). To enable direct comparisons with the saline penetration determinations of chapter 3 and the salinity profiles of chapter 4, some sample sites were established at pre-existing Environment Agency
sampling sites (Chapter 3) and salinity station sites (Chapter 4) (Environment Agency, 2005b). Initial surveys indicated that due to the dynamic tidal conditions of the River Adur and River Ouse estuaries and numerous bank and bed obstacles, boat based survey and sampling was unreliable and hazardous. Site selection was therefore constrained by the need for safe, 24 hour low tide access for channel sampling. Where possible, sites were selected that had or were close to fixed, permanent structures such as bridges, to provide midstream access for sampling and instrument attachment (Chapter 4) and to act as reference points (with GPS coordinate recorded) for any future follow up studies.

Macroinvertebrate sampling was undertaken during August 2008 and February 2009 to coincide with low summer freshwater discharge, indicative of a high degree of tidal saline penetration (August 2008) and high winter freshwater discharge, indicative of a low degree of tidal saline penetration (February 2009) (Chapter 4). Macroinvertebrate sampling was completed in conjunction with the tide and salinity profiling (Chapter 4). Twelve sampling sites were established on the River Adur and 14 on the River Ouse (Figure 5.1, Figure 5.2, Table 5.1, Table 5.2). Two additional sampling sites were established on the River Ouse under high freshwater flow conditions (February 2009) to fill a sampling gap (O4 & O5) (Figure 5.2, Table 5.2). One standard three-minute kick-sweep sample was taken in the marginal area of the channel at low tide (Environment Agency, 1999). In total, 54 kick samples were collected, 26 under low freshwater discharge conditions (August 2008) and 28 under high freshwater flow conditions (February 2009). All samples were preserved in 10% formaldehyde and identified to species level where possible. After identification, macroinvertebrate samples were catalogued and stored in 70% industrial mentholated spirit (IMS). Midstream sampling was attempted during August 2008 using a standard ekman grab; however this method proved unreliable due to solid and/or rocky substrate of the river channel (especially around bridges) and strong tidal currents. Spot samples of river water temperature (°C), pH, dissolved oxygen content (DO %), conductivity (µS cm⁻¹) and salinity (psu) were recorded at each sample site in conjunction with macroinvertebrate sampling. Due to equipment malfunction, spot samples of dissolved oxygen content at each sample site were only recorded in February 2009 (high freshwater discharge conditions).
Figure 5.1. Map of sampling site locations on the River Adur.

<table>
<thead>
<tr>
<th>River</th>
<th>Sampling site</th>
<th>Distance from river mouth (km)</th>
<th>N</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adur</td>
<td>A1</td>
<td>0.32</td>
<td>50°49'44.99&quot;</td>
<td>0°14'58.47&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A2</td>
<td>3.76</td>
<td>50°50'26.19&quot;</td>
<td>0°17'16.21&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A3</td>
<td>8.27</td>
<td>50°52'14.75&quot;</td>
<td>0°18'04.25&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A4</td>
<td>10.64</td>
<td>50°53'19.46&quot;</td>
<td>0°18'34.79&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A5</td>
<td>14.06</td>
<td>50°54'35.73&quot;</td>
<td>0°17'40.79&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A6</td>
<td>16.05</td>
<td>50°55'25.97&quot;</td>
<td>0°18'27.36&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A7</td>
<td>17.88</td>
<td>50°56'14.09&quot;</td>
<td>0°18'03.38&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A8</td>
<td>18.01</td>
<td>50°56'17.95&quot;</td>
<td>0°18'03.18&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A9</td>
<td>18.9</td>
<td>50°56'42.64&quot;</td>
<td>0°18'28.88&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A10</td>
<td>20.45</td>
<td>50°57'03.10&quot;</td>
<td>0°16'42.80&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A11</td>
<td>23.99</td>
<td>50°57'43.97&quot;</td>
<td>0°14'28.03&quot;</td>
</tr>
<tr>
<td>Adur</td>
<td>A12</td>
<td>26.46</td>
<td>50°58'07.22&quot;</td>
<td>0°12'46.68&quot;</td>
</tr>
</tbody>
</table>

Table 5.1. Sampling site locations on the River Adur.
Figure 5.2. Map of sampling site locations on the River Ouse.

<table>
<thead>
<tr>
<th>River</th>
<th>Sampling site</th>
<th>Distance from river mouth (km)</th>
<th>N</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouse</td>
<td>O1</td>
<td>1.67</td>
<td>50°47'44.18&quot;</td>
<td>0°03'04.25&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O2</td>
<td>3.68</td>
<td>50°48'35.31&quot;</td>
<td>0°02'11.79&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O3</td>
<td>6.35</td>
<td>50°49'48.09&quot;</td>
<td>0°01'34.03&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O4</td>
<td>8.7</td>
<td>50°50'47.93&quot;</td>
<td>0°02'17.30&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O5</td>
<td>9.18</td>
<td>50°51'00.89&quot;</td>
<td>0°02'04.44&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O6</td>
<td>11.31</td>
<td>50°51'55.61&quot;</td>
<td>0°01'21.76&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O7</td>
<td>12.8</td>
<td>50°52'32.50&quot;</td>
<td>0°00'58.00&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O8</td>
<td>13.44</td>
<td>50°52'45.76&quot;</td>
<td>0°00'34.54&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O9</td>
<td>15.03</td>
<td>50°53'21.84&quot;</td>
<td>0°00'01.17&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O10</td>
<td>17.35</td>
<td>50°53'42.32&quot;</td>
<td>0°00'36.58</td>
</tr>
<tr>
<td>Ouse</td>
<td>O11</td>
<td>16.1</td>
<td>50°53'45.26&quot;</td>
<td>0°00'37.75&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O12</td>
<td>17.52</td>
<td>50°54'04.78&quot;</td>
<td>0°01'11.48&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O13</td>
<td>20.39</td>
<td>50°54'55.27&quot;</td>
<td>0°02'05.61&quot;</td>
</tr>
<tr>
<td></td>
<td>NTL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouse</td>
<td>O14</td>
<td>22.37</td>
<td>50°55'30.04&quot;</td>
<td>0°03'02.70&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O15</td>
<td>28.22</td>
<td>50°58'04.20&quot;</td>
<td>0°02'55.14&quot;</td>
</tr>
<tr>
<td>Ouse</td>
<td>O16</td>
<td>47.16</td>
<td>51°02'23.73&quot;</td>
<td>0°05'45.89&quot;</td>
</tr>
</tbody>
</table>

Table 5.2. Sampling site locations on the River Ouse.
5.2.1 Environmental Variables

For each sample site, a number of environmental parameters were recorded, including channel morphology (channel width, bank angle), vegetation parameters (macroalgae and macrophyte speciation and cover), physiochemical parameters (pH, dissolved oxygen content and temperature), salinity parameters (maximum, minimum, average and range) and sediment characteristics (grain size (fine and coarse), organic, calcium carbonate, minerogenic and water content). In August 2008 (low freshwater discharge conditions), the key emergent macrophytes/macroalgae were identified to species level and recorded as percentage cover. In February 2009 (high flow conditions), many emergent macrophyte species had died back to their over-wintering condition, so the August 2008 (low flow conditions) key species lists were taken as representative. The spot samples of physiochemical parameters (temperature, pH, dissolved oxygen content) were recorded in conjunction with macroinvertebrate sampling (see Section 5.2).

The tide and salinity profiles developed in chapter 4 enabled the interpolation of the maximum, minimum, average and range of salinity (psu) experienced at each macroinvertebrate sampling site (for MHWS and MLWS tide heights) along the River Adur and River Ouse under low freshwater discharge conditions and high freshwater discharge conditions (see Chapter 4).

Brief sediment descriptions were performed at each site in the field, with sediments subjectively characterised by their colour (using a Munsell colour chart), relative sand, silt and clay contents and the relative proportions (%) of gravel, pebbles or boulders present. To ensure representative sample sizes, surface sediment was collected from three 0.2 x 0.3 metre grids at each sample site using a trowel. Care was taken to collect only the top 5 cm of sediment, to best represent the type of sediment disturbed during kick/sweep sampling. The sediment was transported back to the laboratory for grain size analysis, sediment classification (i.e. Wentworth class, particle roundness, sphericity (Krumbein, 1941, Gordon et al. 1994) and measurement of sedimentary parameters such as organic, calcium carbonate and minerogenic content (via loss-on-ignition; Dean, 1974).

For fine grain size analysis, bulk sediment samples (of known weight) were dry sieved through a range of mesh sizes (2 and 1 mm, 500, 525, 125, 90 and 63 µm) using a sieve shaker (Endecotts Ltd, London, UK). Prior to sieving, samples were thoroughly dried and gently ground using a pestle and mortar to avoid clumping, but carefully enough not to alter the particle size of the
sample. To reduce the influence of atypically large particles on the calculated percentage of fine sediment, particles greater than >60 mm were removed prior to the dry sieving process. The 60 mm truncation size is an arbitrary value/threshold, but adopting a consistent approach enabled comparison between samples. After being passed through a 60 mm sieve for removal of overlarge particles, each sample was weighed and the initial weights were recorded. For the sieving process, the dried samples were placed into the uppermost sieve and subsequently left for 15 minutes in the sieve shaker on a medium setting, to enable the particles to separate out. After a final gentle brushing, each fraction was weighed individually (in foil trays of known weight) and converted into percentages relevant to the total sediment size. Comparison of the initial sediment weight and final sediment weight suggest less than 1% of sediment was lost during the sieving process for each sample. The modal class, median (50%) and quartile (25% and 75%) grain sizes were subsequently calculated (in millimetres applying a semi-log scale), with the average grain size being taken as the mean interquartile range (i.e. = (75-25)/2).

For samples containing visible coarse material (i.e. >60 mm: gravel, cobbles and boulders), further analysis was undertaken to determine particle characteristics such as average coarse grain size, roundness and shape/sphericity. Particles were selected randomly from a sediment matrix for each site and the a, b and c axis measured and recorded for determination of standard sedimentological parameters. The number of particles measured per site varied, being largely determined by the number required to stabilise the averages for the a, b and c axes (generally 34-40), unless there were insufficient clasts within the sediment sample collected. The length of the b-axis was used to allocate each particle into a Wentworth size class category and the ratio of the b/a and c/b axes was used to determine particle sphericity (after Gordon et al. 1994). Each measured particle was also compared to the Krumbein chart (Krumbein, 1941) for estimation of sediment roundness (0.1 = angular, 0.9 = rounded), characterised by the average of all measured particles for each site.

For calculation of the sedimentary parameters, weighed aliquots (~1-5 g) of sediments were placed in a laboratory oven at 105 °C until dry and then cooled in a dessicator at room temperature. After being re-weighed (enabling the calculation of percentage water content), samples were heated to 550 °C for two hours, cooled in a dessicator and re-weighed for calculation of percentage organic matter. Finally, samples were ignited to 925 °C, at which temperature they were held for four hours for calculation of carbonate (CaCO₃) content, determined by the percentage weight loss after cooling. The percentage weight of the remaining
residue after the organic and carbonate ignition processes makes up the minerogenic component of the sediment.

5.3 Data Analysis

5.3.1 Trends in the faunal data

All data analysis was undertaken on the full faunal dataset (unless otherwise indicated). In order to assess the longitudinal sequences of faunal data along the River Adur and River Ouse, sites not located on the main River Adur and River Ouse course were removed from all subsequent analysis. These sites included site A9 on the eastern branch of the River Adur and site O10 (downstream weir, Hamsey loop) on the River Ouse.

The published salinity tolerances of all macroinvertebrate species recorded during sampling were reviewed and species salinity responses defined in terms of the biotic salinity tolerance groupings of Wolf et al., (2005) (Table 5.3). These groupings were adopted due to their development as part of a classification approach to meet the dynamic salinity conditions experienced by macroinvertebrates in tidal streams, where salinity at some sites can shift from freshwater to marine depending on the height of the tide (Wolf et al., 2009, as in Chapter 3). Where the salinity tolerances of selected species were unknown or unpublished, a biotic salinity tolerance grouping was assigned based on a species maximum field distribution (MFD). Where salinity tolerance has been shown to vary between life stage, the salinity tolerance of the adult was taken as definitive. These groupings were initially used (in Chapter 3) to assess the value of species distributions alone in determining the upstream extents of saline penetration in the River Adur and River Ouse estuaries. In this chapter, this approach will be validated against the actual current tide and salinity profiles determined in chapter 4.

<table>
<thead>
<tr>
<th>Salinity tolerance grouping</th>
<th>Salinity range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holarctic-haline (he)</td>
<td>0 - 35</td>
<td>Marine derived taxa that tolerate the entire range of salinity from limnic to marine.</td>
</tr>
<tr>
<td>Euryhaline-marine (em)</td>
<td>0.5 - 35</td>
<td>Marine taxa that tolerate a large range of salinities between 0.5 and 35.</td>
</tr>
<tr>
<td>Brackish (b)</td>
<td>0.5 - 30</td>
<td>Brackish water taxa that live and reproduce in brackish waters and tolerate varying salinity between 0.5 and 30.</td>
</tr>
<tr>
<td>Euryhaline-limnic (el)</td>
<td>0 - 10</td>
<td>Freshwater derived taxa that tolerate salinity up to 10 (and higher for a short time).</td>
</tr>
<tr>
<td>Limnic, tolerates salt (Kel)</td>
<td>0 - &lt;5</td>
<td>Freshwater taxa that tolerate salinities below 5.</td>
</tr>
<tr>
<td>Limnic (l)</td>
<td>&lt;0.5</td>
<td>Freshwater taxa that do not tolerate even low salinities.</td>
</tr>
</tbody>
</table>

Table 5.3. Summary table of salinity tolerance groupings derived from Wolf et al., (2009).
Major trends in the faunal dataset were explored using detrended correspondence analysis (DCA) within the programme Canoco, v4.5 (ter Braak & Šmilauer, 2006). Rare species were downweighted prior to analysis. Diversity indices including species number (only fauna identified to species level), relative abundance (RA), Shannon-Weiner diversity index (H) and Berger-Parker dominance index (BP) were calculated within the α Species Diversity and Richness software v.3.03 (Henderson & Seaby, 2002). Faunal abundance data were transformed (log e + 1) during analysis.

To derive longitudinal zonations along the river courses, the faunal community for both rivers and flow regimes were numerically zoned using optimal splitting by information content as implemented in the programme PsimPoll (Bennett, 1996). Optimal splitting was chosen over hierarchical agglomerative techniques such as cluster analysis because the results of these techniques can be difficult to interpret during transitional phases (Gordon & Birks, 1972; Birks & Gordon, 1985). The significant number of longitudinal zones was assessed using the broken stick model (Bennett, 1996). The ecological and palaeoecological data analysis and visualisation program C2 V.1.6.3 (Juggins, 2007) was used to sequence species distributions into salinity tolerance groupings relative to sampling point, distances from river mouths, freshwater flow regime and species abundance.

5.3.2 Environmental – species relationships

To investigate the environmental-species relationships within and between the River Adur and River Ouse, the faunal and environmental data were combined and analysed using detrended canonical correspondence analysis (DCCA) within the programme CANOCO v.4.54 (ter Braak & Šmilauer, 2006). Species-environmental relationships were explored using the detrended form of canonical correspondence analysis (DCCA) due to the strongly unimodal distribution of species for all sites as suggested by the DCA axis 1 gradient lengths (Table 5.6). Species data were expressed as percentages and downweighting of rare species was applied.

Due to inherent tolerance differences within taxonomic family groups, family data (not identified to species level) were removed from the faunal dataset. The influence of environmental variables on faunal structure (e.g. diversity indices; species number (SPN), relative abundance (RA), Shannon-Weiner Diversity Index (H), Berger-Parker Dominance Index (BP)) was examined using correlation and regression analysis, within the programme SPSS V.18.
5.4 Results

5.4.1 Macroinvertebrate salinity tolerance groupings

A total of 91 macroinvertebrate taxa (identified to species level) from 18 orders were recorded in the River Ouse and River Adur over the sampling period. Of these 91, the River Ouse and River Adur had 47 species in common. Despite the higher number of sampling sites on the River Ouse, the River Adur had the larger number of species at 73, compared to 61 in the River Ouse.

The 91 recorded macroinvertebrate species were assigned a salinity tolerance grouping (Wolf et al., 2009) based on the analysis of published literature sources (Table 5.5). Four species were classified as holeury-haline (marine-derived taxa that tolerate the entire range of salinity from limnic to marine, 0.5 – 35), 16 species classified as euryhaline-marine (marine taxa that tolerate a large range of salinities between 0.5 and 35), 5 as brackish (brackish water taxa that live and reproduce in brackish waters and tolerate varying salinity between 0.5 and 30), 9 as euryhaline-limnic (freshwater derived taxa that tolerate salinity up to 10), 33 as limnic, salt tolerating (freshwater derived taxa that tolerate salinities below 5) and 24 as limnic (<0.5; Table 5.5). A significant amount of historic and contemporary published literature was available on the distributions and salinity tolerances of marine and brackish water species recorded in estuarine systems (see Hayward & Ryland, 1995; Kinne, 1971; Lincoln, 1979; Barnes, 1994 and references therein). However, only a small amount of literature was available on the salinity tolerances of limnic derived species, with many of the records based on observations in the field (Rundle et al., 1998; Williams & Williams, 1998a; Sousa et al., 2007).

By allocating recorded macroinvertebrate species to salinity tolerance groupings, it was possible to attempt the prediction the degree of saline penetration into the River Adur and River Ouse estuaries, (under both low and high freshwater discharge regimes) using species distributions alone (Figure 5.3, Table 5.4). The upstream extents and distributions of euryhaline-marine (0.5 – 35) and brackish water species (0.5 – 30) and the downstream extents of limnic, salt tolerating (0 - <5) and limnic (<0.5) species, were used to attempt to identify the upstream extents of saline penetration in the River Adur and River Ouse estuaries (as in Chapter 3) (Table 5.4). These predictions were validated against the actual upstream limits of saline penetration determined from the River Adur and River Ouse estuary tide and salinity profiles (Figure 5.3, Table 5.4). Despite the broad salinity ranges of the tolerance groupings (particularly for the euryhaline-
marine and brackish species), this approach produced reasonable approximations of the actual saline penetration limits in the River Adur and River Ouse estuaries under both low and high freshwater discharge conditions (Figure 5.3, Table 5.4). However, the predicative ranges (based on faunal distributions) were a more accurate representation of actual saline penetration limits in the River Ouse estuary than the River Adur, potentially due to a greater number of sampling sites enabling greater precision in determining species distributions (Table 5.4, Figure 5.3).

<table>
<thead>
<tr>
<th>Estuary</th>
<th>Freshwater discharge rate (m$^3$ s$^{-1}$)</th>
<th>Saline penetration extent (measured) (km)</th>
<th>Saline penetration extent (predicted from fauna) (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ouse estuary</strong></td>
<td>Low freshwater discharge regime (Aug 2008) 0.72</td>
<td>16.2</td>
<td>16.1 - 17.52</td>
</tr>
<tr>
<td></td>
<td>High freshwater discharge regime (Feb 2009) 1.43</td>
<td>15.5</td>
<td>15.03 - 16.1</td>
</tr>
<tr>
<td><strong>Adur estuary</strong></td>
<td>Low freshwater discharge regime (Aug 2008) 0.73</td>
<td>17.55</td>
<td>17.88 - 18.01</td>
</tr>
<tr>
<td></td>
<td>High freshwater discharge regime (Feb 2009) 1.237</td>
<td>13.9</td>
<td>14.06 - 16.05</td>
</tr>
</tbody>
</table>

Table 5.4. The upstream extent of saline penetration (0.5) determined from measured tide and salinity profiles (corrected for MHWS) and predicted from the distributions of species salinity tolerance groupings, for the River Ouse and River Adur estuaries under low and high freshwater discharge conditions.
### Table 5.5

<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
<th>Class</th>
<th>Source</th>
<th>Order</th>
<th>Class</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancyron auritum</td>
<td>Coleoptera</td>
<td></td>
<td>Friday, 1988.</td>
<td>Ichneumia elegans</td>
<td>Odontotaelida</td>
<td>Ward, 1992; Barnes, 1994; Mertitt et al., 1996.</td>
</tr>
<tr>
<td>Danio rerio</td>
<td>Cyprinidae</td>
<td></td>
<td>Macan, 1979; Gairdner-Mayence, 1994; Williams &amp; Williams 1988b; Rundle et al., 1998.</td>
<td>Polychaeta marina</td>
<td>Trichoptera</td>
<td>Williams &amp; Williams, 1998b; Wolf et al., 2009; Bäthe &amp; Corning, 2010.</td>
</tr>
<tr>
<td>Daphnia pulex</td>
<td>Cladocera</td>
<td></td>
<td>Macan, 1979; Gairdner-Mayence, 1994; Williams &amp; Williams 1988b; Rundle et al., 1998.</td>
<td>Prosoplisthaca marina</td>
<td>Trichoptera</td>
<td>Williams &amp; Williams, 1998b; Wolf et al., 2009; Bäthe &amp; Corning, 2010.</td>
</tr>
<tr>
<td>Daphnia longispina</td>
<td>Cladocera</td>
<td></td>
<td>Macan, 1979; Gairdner-Mayence, 1994; Williams &amp; Williams 1988b; Rundle et al., 1998.</td>
<td>Phyllophora inaequalis</td>
<td>Trichoptera</td>
<td>Williams &amp; Williams, 1998b; Wolf et al., 2009; Bäthe &amp; Corning, 2010.</td>
</tr>
</tbody>
</table>

*he = marine derived taxa that tolerate the entire range of salinity from brackish to marine, 0.5-35, em = marine taxa that tolerate a large range of salinities between 0.5 and 35, b = brackish water taxa that live and reproduce in brackish waters and tolerate varying salinity between 0.5 and 20, el = freshwater derived taxa that tolerate salinities up to 10, el(e) = freshwater derived taxa that tolerate salinities below 5, I = freshwater taxa that do not tolerate even low salinity, <0.5.*
Figure 5.3. Summary diagram of the upstream extent of saline penetration (LSP 0.5) determined from current tide and salinity profiles and predicted range based on the distributions of species salinity tolerance groupings, for the River Adur (a.) and River Ouse (b.) estuaries under low (1) and high (2) freshwater discharge conditions.
5.4.2 The River Adur and River Ouse

The River Adur and River Ouse were selected as study sites for this research project due to their perceived susceptibility to increases in saline penetration, and their comparable geological and hydrological characteristics (Chapter 2). Preliminary analysis of historic faunal data, suggested that these two systems were comparable and ideal for further comparison and study (Chapter 3). In this chapter, the faunal data from the River Adur and River Ouse (under both low and high discharge conditions) was analysed separately in detail, with key findings and comparisons highlighted and reviewed in the discussion. However, to investigate the comparability of the benthic macroinvertebrate communities of the River Adur and River Ouse, the faunal data was first combined and analysed with reference to faunal structure and relationships with environmental parameters.

Detrended correspondance analysis (DCA) of the whole faunal dataset, under both high and low freshwater discharge conditions, suggested that species distributions along the River Adur and River Ouse were strongly associated with DCA axis 1 (explaining 11.9% of the variation in the dataset; Table 5.6). This axis was likely driven by salinity, with higher salinity downstream sites plotting to the left of the samples biplot and freshwater upstream sites on the far right (Figure 5.4a).

The distinct clustering of samples within the DCA biplot, clearly indicated sites with similar faunal assemblages, and the location of major faunal changes between and within the River Adur and River Ouse (Figure 5.4). The general pattern of faunal distributions, suggested that these two rivers were broadly comparable in terms of resident macroinvertebrate species and their locations along the salinity gradient. An exception to this, however, occurred in the lower to mid estuary (8.27-14.06 km on Figure 5.4a), where sites A4 and A5 on the River Adur estuary, exhibited a quite different macroinvertebrate fauna to the corresponding sites on the River Ouse estuary (O4-O8). This was likely due to the dominance (76 and 81%) of the amphipod Corophium multisetosum in the River Adur estuary sites (A4 – A5), compared to the more diverse fauna in the corresponding River Ouse estuary sites (O4-O8) (e.g. Cyathura carinata, Leptochirus pilosus, Melita palmata and Hediste diversicolor) (Figure 5.4b).
Under both high and low freshwater discharge conditions, macroinvertebrate distributions appeared to be most strongly associated with salinity (particularly average, maximum and minimum salinity), although there was a degree of co-variation with sediment grain size, macrophyte cover and organic content (Figure 5.5a). Substantial faunal shifts were clearly identifiable in response to salinity changes along the River Adur and River Ouse estuaries, although these were most pronounced in the lower estuarine zones, where the largest changes in average, maximum and minimum salinity occurred (Figure 5.5). As the lower catchment area for each river is situated on chalk, it is unremarkable that calcium carbonate exhibited some co-variation with these salinity parameters. Whilst this might be largely coincidental, sites O2 and O3 (in both seasons) appeared more strongly associated with calcium carbonate content, which may represent significant shell deposits and/or human modification of the channel at these sites.

Figure 5.4. Detrended Correspondence Analysis (DCA) biplot for the full faunal dataset of the River Adur and River Ouse, under low and high freshwater discharge conditions, with samples (a.) and most abundant species (b.)
(i.e. addition of chalk rubble for pole-wharfing; see Section 5.4.4.3 for more discussion).

Exhibiting an inverse relationship with the salinity parameters, organic content and macrophyte cover were also important in the upper estuary and above the normal tidal limits (NTL), where the majority of sites in this region (i.e. sites above 14 km on the River Adur and 13.4 km on the River Ouse) contained substantial emergent macrophyte cover and high organic content (for River Adur see Section 5.4.3.3, for River Ouse see Section 5.4.4.3).

Figure 5.5. Detrended Canonical Correspondence Analysis (DCCA) triplot for the full faunal dataset of the River Adur and River Ouse, under low and high freshwater discharge conditions, indicating selected environmental variables with samples (a.) and most abundant species (b.)
5.4.3 The River Adur

5.4.3.1 Faunal data under low freshwater discharge conditions (0.733 m³ s⁻¹, August 2008)

Under low freshwater discharge conditions, a total of 66 taxa (consisting of 4,869 individuals) were recorded in the River Adur, with a total of 51 identified to species level. Within the faunal dataset, the first DCA axis explained 19.8% of the variation, with axis 2 explaining a further 8.9% (Table 5.7). The majority of species appeared to be strongly associated with axis 1, which appeared to be driven by salinity, with limnic species (<0.5) exhibiting the highest scores on axis 1 and euryhaline-marine species (0.5 – 35) exhibiting the lowest (Figure 5.7, Figure 5.8).

Numerical zoning (optimal splitting by information content) of the faunal data produced 4 statistically significant zone splits (based on dissimilarity) at 9.45, 15.05 and 22.22 km from the river mouth at Shoreham.

### Table 5.7. Summary of Detrended Correspondence Analysis (DCA) results for the River Adur faunal dataset, under low freshwater discharge conditions (0.733 m³ s⁻¹).

<table>
<thead>
<tr>
<th>Dataset</th>
<th>No. of samples</th>
<th>No. of species</th>
<th>Total inertia</th>
<th>Axis</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low freshwater discharge</td>
<td>11</td>
<td>49</td>
<td>4.978</td>
<td>Eigenvalues</td>
<td>0.984</td>
<td>0.447</td>
<td>0.072</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lengths of gradient</td>
<td>10.793</td>
<td>3.376</td>
<td>1.238</td>
<td>3.034</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% variance explained</td>
<td>19.8</td>
<td>8.9</td>
<td>1.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>
(16%) and the amphipod *Corophium arenarium* (26.3%) (Figure 5.6, Figure 5.7). Zone 1 was characterised by fully marine maximum salinities (35), a large range in minimum salinities (33.7 – 0.2), high dissolved oxygen content (76 – 89%), low organic content (2.6 – 4.7%), macroalgal cover (e.g. *Enteromorpha intestinalis*, *Cladophora rupestris*, *Fucus spiralis*, *Fucus ceranoides*) and saltmarsh vegetation growth on the upper banks (e.g. *Puccinellia maritima*, *Agropyron pungens*, *Aster tripolium*, *Salicornia perennis*) (Figure 5.9). The sediment consisted of a surface layer of poorly sorted gravel to small cobbles (covered with a thick algal (diatom) film); with a sandy anoxic layer approximately 1 cm below the surface (Figure 5.9).

9.45 – 15.05 km (Zone 2) – The faunal community in zone 2 was abundant, but not diverse, consisting of a total of 946 individuals, dominated by a small number of brackish (0.5 – 30) and euryhaline-limnic (0 – <30) species (Figure 5.6, Figure 5.7, Figure 5.8). These species included the amphipod *Corophium multisetosum* (81.4%) and gastropod mollusc *Potamopyrgus antipodarum* (11.1%) (Figure 5.6, Figure 5.7). Zone 2 was characterised by a large range in maximum salinities (20 – 0.8), a tidal limnetic minimum salinity (0.2), a shift from coarse (>60 mm) (site A4) to fine (<60 mm) (site A5) grained sediment and a shift from bank saltmarsh vegetation (e.g *Aster tripoleum*, *Parapholis strigosa*), to emergent macrophyte species more representative of freshwater bodies (e.g. *Phalaris arundinacea*, *Schoenoplectus lacustris*, *Impatiens glandulifera*, *Sparganium erectum*) (Figure 5.9).

15.05 – 22.22 km (Zone 3) – The faunal community throughout zone 3 was variable. In general, the number of identified species (17) and macroinvertebrate abundance (1346) rose upstream throughout zone 3, coupled with a rise in diversity and fall in species dominance towards the head of the estuary (NTL) (Figure 5.8). The faunal community consisted of holeury-haline (0 – 35), brackish (0.5 – 30) and limnic derived species, but was dominated by the holeury-haline amphipod *Gammarus zaddachi* (73.7%) and the limnic, salt tolerating isopod *Asellus aquaticus* (5.1%) and gastropod mollusc *Bithynia tentaculata* (4.5%) (Figure 5.6, Figure 5.7). Zone 3 was characterised by low maximum salinities (0.59 – 0.3), low dissolved oxygen content (53 – 63%), fine grained (<60 mm) sediment with high organic content (7 – 13%) and dense marginal macrophyte cover (e.g. *Phalaris arundinacea*, *Schoenoplectus lacustris*, *Sparganium erectum*, *Lythrum salicaria* and *Phragmites australis*) (Figure 5.9).

22.22 – 26.46 km (Zone 4) – The faunal community in zone 4 was abundant and diverse, consisting of a total of 1745 individuals, which were identified as euryhaline-limnic (0 - <30), limnic, salt tolerating (0 - <10) and limnic (<0.5) species (Figure 5.6, Figure 5.7, Figure 5.8).
The most abundant of these was the amphipod *Gammarus pulex* (68.6%) and isopod *Asellus aquaticus* (17.8%) (Figure 5.6, Figure 5.7). Zone 4 was characterised by non-tidal freshwater conditions (<0.5), from lowland slow flowing (DO 57%) waters with fine grained (<60 mm) sediments (site A11) and high organic matter (1%), to fast flowing (DO 75%) waters with coarse grained (>60 mm) substrate (A12) and lower organics (7%) (Figure 5.9). The channel banks in this zone were densely vegetated with the macrophyte species *Phalaris arundinacea*, *Schoenoplectus lacustris*, *Salix* sp., *Glyceria maxima* and *Impatiens glandulifera*.

5.4.3.2 Faunal data under high freshwater discharge conditions (1.237 m$^3$s$^{-1}$, February 2009)

Under high freshwater discharge conditions a total of 69 genera (consisting of 6333 individuals) were recorded in the River Adur, with a total of 57 identified to species level (including site A9). Within the faunal dataset, the first DCA axis was responsible for explaining 26% of the variation, with axis 2 explaining a further 6.3% (Table 5.8). As under low freshwater discharge conditions, DCA axis 1 appeared to be driven by salinity, with limnic species scoring highest on axis 1 and euryhaline-marine species scoring lowest (Figure 5.7). Numerical zoning of the faunal data produced 3 statistically significant zone splits (based on dissimilarity) at 9.45 and 17.94 km from the river mouth at Shoreham.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>No. of samples</th>
<th>No. of species</th>
<th>Total inertia</th>
<th>Axis</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>High freshwater discharge (Feb 09)</td>
<td>11</td>
<td>55</td>
<td>3.643</td>
<td>Eigenvalues</td>
<td>0.949</td>
<td>0.229</td>
<td>0.059</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lengths of gradient</td>
<td>7.861</td>
<td>1.519</td>
<td>1.495</td>
<td>1.873</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% variance explained</td>
<td>26</td>
<td>6.3</td>
<td>1.7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 5.8. Summary of detrended correspondence analysis results for the River Adur faunal dataset, under high freshwater discharge conditions (1.237 m$^3$s$^{-1}$).

0.32 – 9.45 km (Zone 1) – The faunal community in zone 1 was diverse, consisting of a mixture of holeury-haline (0 – 35), euryhaline-marine (0.5 – 35), brackish water (0.5 – <30) and limnic derived species (Figure 5.6). Despite a diverse fauna, the abundances observed were generally low (356 individuals), with the most abundant species were the brackish water isopod *Cyathura carinata* (28.6%), the errant polychaete *Hediste diversicolor* (19.6%) and the euryhaline-marine sessile barnacle *Elminius modestus* (8.5%) (Figure 5.6, Figure 5.7, Figure 5.8). A single limnic (<0.5) trichopteran *Limnephilus flavicornis* and limnic, salt tolerating (0 - <5) isopod *Asellus aquaticus* were also recorded, but this specimen may have been washed down from the upper river (Figure 5.6). Zone 1 was characterised by fully marine maximum salinities (35), a large range in minimum salinities (33.7 – 0.2), high dissolved oxygen content (76 – 89%), low organic
content (2.6 – 4.7%), macroalgal cover (Enteromorpha intestinalis, Cladophora rupestris, Fucus spiralis, Fucus ceranoides) and saltmarsh vegetation growth on the upper banks (e.g. Puccinellia maritima, Agropyron pungens, Aster tripolium, Salicornia perennis) (Figure 5.9). The sediment consisted of a surface layer of poorly sorted gravel to small cobbles (often covered with a thick algal (diatom) film); with a sandy anoxic layer approximately 1 cm below the surface.

**9.45 – 17.94 km (Zone 2)** – The faunal abundance and number of species throughout zone 2 was variable, but in general followed a downward trend (Figure 5.8). This was coupled with a decrease in diversity and an increase in species dominance (Figure 5.8). The faunal community consisted of holeury-haline (0 – 35), euryhaline-marine (0.5 – 35), brackish (0.5 - <30) and limnic derived species and was dominated by the brackish water amphipod Corophium multisetosum (68.9%), euryhaline-limnic gastropod mollusc Potamopyrgus antipodarum (13.3%) and the holeury-haline amphipod Gammarus zaddachi (12.7%) (Figure 5.6, Figure 5.7). Zone 2 was characterised by low maximum salinities (1.14 – 0.27), low dissolved oxygen content (62 – 63%) and both coarse (site A4) and fine (A5-A7) grained sediments with medium organic content (4 – 8%) (Figure 5.9). Zone 2 was also characterised by a shift from macroalgae (Cladophora rupestris) and saltmarsh related vegetation (e.g Aster tripoleum, Parapholis strigosa) (A4), to macrophyte species more representative of freshwater bodies (e.g. Phalaris arundinacea, Schoenoplectus lacustris, Impatiens glandulifera, Sparganium erectum) (A5-A7).

**17.94 – 26.46 km (Zone 3)** – The faunal community (in terms of abundance and number of species) throughout zone 3 was variable, but in general followed a strong upwards trend towards the head of the estuary (NTL) into the non-tidal freshwaters (Figure 5.8). This was coupled with an increase in diversity and a decrease in species dominance (Figure 5.8). The faunal community mainly consisted of limnic derived species, including the limnic, salt tolerating amphipod Gammarus pulex (43.2%), the isopod Asellus aquaticus (29.9%) and the bivalve mollusc Sphaerium corneum (9.9%) (Figure 5.6, Figure 5.7). This zone was characterised by densely vegetated (e.g. Nuphar lutea, Glyceria maxima, Phalaris arundinacea, Sparganium erectum, Impatiens glandulifera, Lythrum salicaria and Salix sp.) tidal and non-tidal freshwaters (<0.5), from lowland slow flowing (DO 57%) waters with fine grained (<60 mm) sediments and high organic content (9-13%) (A8, A10, A11) to fast flowing (DO 75%) waters with coarse grained (>60 mm) sediments and low organic content (6%) (A12) (Figure 5.9).
<table>
<thead>
<tr>
<th>Low freshwater discharge conditions (0.733 m³/s⁻¹)</th>
<th>High freshwater discharge conditions (1.237 m³/s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zone 1 (0.32 - 9.45 km)</strong></td>
<td><strong>Zone 1 (0.32 - 9.45 km)</strong></td>
</tr>
<tr>
<td><strong>Euryheline-marine (0.5 - 35)</strong></td>
<td><strong>Euryheline-marine (0.5 - 30)</strong></td>
</tr>
<tr>
<td>Hediste diversicolor (42%), Corophium arenarium (26.3%), Gammarus salinus (2.9%), Carcinus maenas (1.1%), Palaemon serratus (0.9%), Arthys vedelensis (0.7%), Crangon crangon (0.7%), Melita palmaria (0.4%), Mytilus edulis (0.2%)</td>
<td>Hediste diversicolor (19.6%), Elminius modestus (8.5%), Melita palmaria (7.6%), Gammarus salinus (7.1%), Carcinus maenas (4.9%), Heterotrocha oestremi (2.7%), Crangon crangon (2.2%), Mytilus edulis (2.2%), Corophium arenarium (1.8%), Cerasoderma edule (0.9%)</td>
</tr>
<tr>
<td><strong>Brackish water (0.5 - 30)</strong></td>
<td><strong>Brackish water (0.5 - 30)</strong></td>
</tr>
<tr>
<td>Cyathura carinata (16 %), Hydrois ulvae (6.3%), Corophium multisetosum (0.9%), Neomysis integer (0.9%), Leptocheirus pilosus (0.7%)</td>
<td>Cyathura carinata (28.6%), Corophium multisetosum (8%), Leptocheirus pilosus (2.7%), Neomysis integer (0.9%)</td>
</tr>
<tr>
<td><strong>Zone 2 (9.45 - 15.05 km)</strong></td>
<td><strong>Lineic, salt tolerating (0 - &lt;5)</strong></td>
</tr>
<tr>
<td><strong>Holleury-haline (0 - 35)</strong></td>
<td><strong>Holleury-haline (0 - 35)</strong></td>
</tr>
<tr>
<td>Gammarus zadachi (3.3%), Palaemonetes varia (0.1%)</td>
<td>Gammarus salinus (12.7%)</td>
</tr>
<tr>
<td><strong>Euryheline-marine (0.5 - 35)</strong></td>
<td><strong>Euryheline-marine (0.5 - 30)</strong></td>
</tr>
<tr>
<td>Heterotrocha oestremi (3.6%)</td>
<td>Heterotrocha oestremi (2.7%)</td>
</tr>
<tr>
<td><strong>Brackish water (0.5 - 30)</strong></td>
<td><strong>Brackish water (0.5 - 30)</strong></td>
</tr>
<tr>
<td>Corophium multisetosum (81.4%), Cyathura carinata (0.5%)</td>
<td>Corophium multisetosum (68.9%), Cyathura carinata (0.4%)</td>
</tr>
<tr>
<td><strong>Euryheline-limnic (0 - 10)</strong></td>
<td><strong>Euryheline-limnic (0 - 10)</strong></td>
</tr>
<tr>
<td>Potamopyrgus antipodarum (11.1%)</td>
<td>Potamopyrgus antipodarum (13.3%), Rhantus frontalis (0.2%)</td>
</tr>
<tr>
<td><strong>Zone 3 (15.05 - 22.2 km)</strong></td>
<td><strong>Lineic, salt tolerating (0 - &lt;5)</strong></td>
</tr>
<tr>
<td><strong>Holleury-haline (0 - 35)</strong></td>
<td><strong>Holleury-haline (0 - 35)</strong></td>
</tr>
<tr>
<td>Gammarus zadachi (73.3%)</td>
<td>Gammarus salinus (12.7%)</td>
</tr>
<tr>
<td><strong>Brackish water (0.5 - 30)</strong></td>
<td><strong>Brackish water (0.5 - 30)</strong></td>
</tr>
<tr>
<td>Corophium multisetosum (8.8 %)</td>
<td>Corophium multisetosum (68.9 %), Cyathura carinata (0.4%)</td>
</tr>
<tr>
<td><strong>Euryheline-limnic (0 - 10)</strong></td>
<td><strong>Euryheline-limnic (0 - 10)</strong></td>
</tr>
<tr>
<td>Ischnura elegans (5.1%), Sigara falleri (3%), Potamopyrgus antipodarum (2.6), Sida crystallina (0.2%)</td>
<td>Potamopyrgus antipodarum (13.3%), Rhantus frontalis (0.2%)</td>
</tr>
<tr>
<td><strong>Lineic, salt tolerating (0 - &lt;5)</strong></td>
<td><strong>Lineic, salt tolerating (0 - &lt;5)</strong></td>
</tr>
<tr>
<td>Asellus aquaticus (5.1%), Bithynia tentacularia (4.5%), Physa fontinalis (1.2%), Euproctella octoculata (0.6%), Stiols lutaria (0.6%), Piscicola aquatica (0.4%), Piscicola geometra (0.4%), Catopryx splendens (0.2%), Oulinina tuberculata (0.2%)</td>
<td>Asellus aquaticus (1.6%), Piscicola geometra (0.6%), Hesperocorixa salbigei (0.4%), Euproctella octoculata (0.2%), Glossiphipia complanata (0.2%), Stiols lutaria (0.2%)</td>
</tr>
<tr>
<td><strong>Lineic (&lt;0.5)</strong></td>
<td><strong>Lineic (&lt;0.5)</strong></td>
</tr>
<tr>
<td>Pleuroxus bicornus (0.6%), Libella fulva (0.4%), Limnéphilus flavicornis (0.4%)</td>
<td>Limnéphilus flavicornis (0.8%)</td>
</tr>
<tr>
<td><strong>Zone 4 (22.22 - 26.46 km)</strong></td>
<td><strong>Lineic (&lt;0.5)</strong></td>
</tr>
<tr>
<td><strong>Euryheline-limnic (0 - 10)</strong></td>
<td><strong>Lineic (&lt;0.5)</strong></td>
</tr>
<tr>
<td>Sigara dorsalis (0.7%), Ischnura elegans (0.1%)</td>
<td><strong>Lineic (&lt;0.5)</strong></td>
</tr>
<tr>
<td><strong>Lineic, salt tolerating (0 - &lt;5)</strong></td>
<td><strong>Lineic, salt tolerating (0 - &lt;5)</strong></td>
</tr>
<tr>
<td>Gammarus pulex (68.6%), Asellus aquaticus (17.8%), Bithynia tentacularia (4.2%), Euproctella octoculata (1.7%), Cloeon dipterum (0.9%), Physa fontinalis (0.5%), Catopryx splendens (0.4%), Polyconotus flavomaculatus (0.4%), Halisicus lividus (0.2%), Helobdella stagnalis (0.2%), Caenis horaria (0.2%), Caenis luctuosa (0.2%), Lymnaea peregra (0.2%), Piscicola geometra (0.2%), Acrolepis lacustris (0.1%), Elmis aenea (0.1%), Hesperocorixa salbigei (0.1%), Notonecta glauca (0.1%), Stiols lutaria (0.1%)</td>
<td>Asellus aquaticus (1.6%), Piscicola geometra (0.6%), Hesperocorixa salbigei (0.4%), Euproctella octoculata (0.2%), Glossiphipia complanata (0.2%), Lymnaea peregra (0.2%), Planoberis planorbis (0.2%), Stiols lutaria (0.2%), Acrolepis lacustris (0.1%), Caenis horaria (0.1%), Helobdella stagnalis (0.1%), Notonecta glauca (0.1%)</td>
</tr>
<tr>
<td><strong>Lineic (&lt;0.5)</strong></td>
<td><strong>Lineic (&lt;0.5)</strong></td>
</tr>
<tr>
<td>Sphaerium corneum (1.5%), Planorbis carinatus (0.7%), Bithynia leachi (0.3%), Limnéphilus flavicornis (0.2%), Pleuroxus bicornus (0.2%)</td>
<td>Sphaerium corneum (9.9%), Planoberis planorbis (0.4%), Chaoborus flavicans (0.2%), Limnéphilus flavicornis (0.2%), Lymnaea militaris (0.2%), Lymnaea stagnalis (0.2%), Hesperocorixa salbigei (0.2%), Notonecta glauca (0.1%)</td>
</tr>
</tbody>
</table>

**Figure 5.6.** Macroinvertebrates species and relative abundances recorded in the significant faunal zones of the River Adur under high and low freshwater discharge regimes.
Figure 5.7. Summary diagram of species distributions, split into significant faunal zones with distance from the river mouth, under high and low freshwater discharge regimes. Species shown occur >2% in one or more samples and are separated into salinity tolerance groupings following a review of published literature (Table 5.5). DCA axis 1 and axis 2 sample scores depicted for each river and freshwater discharge regime.
5.4.3.3 Faunal data and environmental variables

Under both discharge conditions, the environmental variables recorded in the River Adur estuary (up to the NTL), showed a distinctive division into lower (0.32 – 10.64 km) and upper (14.06 – 21 km) estuarine zones (Figure 5.9). The lower River Adur estuary course has been heavily managed and embanked, resulting in a narrow winding channel, which experiences dynamic salinity conditions ranging from fully marine (35) through to freshwater (<0.5) over a tidal cycle (see chapter 4) (Figure 5.9). The lower estuary was characterised by extensive algal cover (A1), high dissolved oxygen content (63 - 89%) and coarse grained (>60 mm), poorly sorted sediment
with high calcium carbonate (9 – 31%) and low organic content (2 – 4%) (Figure 5.9). The upper River Adur estuary was characterised by tidal freshwater (<0.5) conditions with dense emergent macrophyte cover (100%), relatively low oxygen content (53 – 67%) and fine grained (<60 mm) sediment with decreasing calcium carbonate content (17 – 3%) and increasing organic content (6 – 13%) (Figure 5.9). The non-tidal freshwater River Adur (above the NTL, 21 – 26.46 km) can be divided unto two sections; mid to lower reaches and headwaters. The mid to lower reaches were characterised by low flow (low DO (57%)), fine grained sediments with high organic content (11%), to fast flow (DO 75%), narrow, steep banked channels with coarse grained sediments and low organic content (6%) in the headwater reaches (Figure 5.9).
Figure 5.9. Summary diagram of the recorded environmental variables from the River Adur split into significant faunal zones under high and low freshwater discharge conditions.
5.4.3.4 Environment-species relationships under low freshwater discharge conditions (0.733 m$^3$s$^{-1}$, August 2008)

A DCCA performed on the River Adur dataset (i.e. sampling sites A1-A12) under low freshwater discharge conditions, suggested that axis 1 (19.8% variation) was heavily driven by salinity, due to the large gradient from fully marine in the lower estuary through to non-tidal freshwater above the NTL (35 – 0.1) (Figure 5.10). Within the tidal limit, sites A1-A4 exhibited much higher average and maximum salinities than the more upstream sites (A5-A10). It is likely that some of the variation exhibited between sites A1, A2 and A3 was explained by minimum salinity, which dropped from a minimum of 33.7 to 19.2 between sites A1 and A2, down to a tidal limnetic 0.2 at site A3. In the faunal dataset this was represented by the presence of euryhaline-marine species (e.g. *Carcinus maenas*, *Gammarus salinus*, *Corophium arenarium*) abundant in sites A1 and A2 compared to the dominance of brackish water species (e.g. *Cyathura carinata*, *Leptocheirus pilosus*) in site A3 (Figure 5.10). Minimum salinity at sites A3 - A10 were very similar, at a tidal limnetic 0.2 – 0.1, but maximum salinity dropped from 35 to 20 between sites A3 and A4, and down to 0.8 in site A5. The large drop in maximum salinity between sites A4 and A5 minimally impacted the faunal community, due to low overall diversity and dominance of the brackish water amphipod *Corophium multisetosum* (70 and 60% respectively) at both sites (Figure 5.10). Brackish (*Cyathura carinata*, *Corophium multisetosum*) and euryhaline-limnic (*Potamopyrgus antipodarum*) species strongly associated with sites A4 and A5 were replaced by holeury-haline (*Gammarus zaddachi*) and limnic derived species (*Erpobdella testacea*, *Sigara falleni*) upstream (A6 – A10) (Figure 5.10). A strongly significant (negative) relationship existed for all salinity parameters (maximum, minimum, average and range) when plotted against DCA axis 1 sample scores (Table 5.9) further supporting the importance of salinity in driving the recorded changes in species distributions.

The non-tidal freshwater sites (A11, A12) were represented by limnic fauna (e.g. *Polycentropus flavomaculatus*, *Caenis horaria*, *Claoeon dipterum*) (Figure 5.10). These sites also appeared to be influenced by other environmental variables, with site A11 showing close association with organic content, which possibly explained the high abundance of species that feed on organic detritus (e.g. *Gammarus pulex* and *Asellus aquaticus*) (Figure 5.10). Site A12 exhibited little association with any of the other measured environmental parameters (Figure 5.10), but it is likely that the characteristic high freshwater flow velocities at this site were important in explaining some of the variation in the faunal data set. For example the net spinning caddisfly *Polycentropus flavomaculatus* is indicative of a high flow environment and was abundant at site A12 (Killeen *et al*., 2004; Edington & Hildrew, 2005).
Calcium carbonate content exhibited some colinearity with the salinity parameters, due to the chalk geology in the lower River Adur catchment (Figure 5.10). Macrophyte cover exhibited an inverse relationship with salinity, with most emergent macrophyte species present (with the exception of *Phragmites* sp.) in limnic, slow-flow, fine sediment sites (Figure 5.10). Organic content was also highest in the more upstream lower salinity sites (A7-A11) and therefore exhibited a significant negative relationship with the salinity parameters and subsequently a relatively strong positive relationship (r = 0.728, p>0.05) with DCA axis 1 scores under correlation and regression analysis (Table 5.9). DCA axis 2 sample scores did not correlate with any recorded environmental variables (Table 5.9).

![Figure 5.10. Detrended Canonical Correspondence Analysis (DCCA) triplot of the River Adur under low freshwater discharge conditions, indicating selected environmental variables and selected most abundant macroinvertebrate species.](image)

A negative relationship was recorded between species number (SPN) and calcium carbonate content (r = -0.838, p<0.05), but a positive relationship existed with organic content (r = 0.611, p<0.05; Table 5.9). Calcium carbonate also exhibited a negative relationship (r = -0.653, p<0.05) with the Shannon-Wiener diversity index (H'), as the sites with the lowest diversity (A4-A6) contained the highest calcium carbonate content (17 – 31%; Table 5.9). The mid estuarine zone typically exhibited low faunal diversity as it was typically dominated by a small number of holeuryhaline species (Attrill, 2002; Rutger & Wing, 2006). Despite the significant relationship, it was therefore unlikely that the low faunal diversity was a consequence of high calcium carbonate content. No significant relationships were recorded between the diversity variables
relative abundance (RA) and Berger Parker dominance index (BP) with any of the recorded environmental variables (Table 5.9).

5.4.3.5 Environment-species relationships under high freshwater discharge conditions (1.237 m³ s⁻¹, February 2009)

Under high freshwater discharge conditions, the salinity parameters again appeared to be driving axis 1 (26% variation) (Figure 5.11). Sites A1- A3 exhibited much higher maximum salinities (35) than the more upstream sites within the tidal limit (A4-A10) (1.14 – 0.2). As under low freshwater discharge conditions, it is likely that some of the variation exhibited between sites A1, A2 and A3 was explained by minimum salinity, which dropped from a minimum of 33.7 to 19.2 between sites A1 and A2 and down to a tidal limnetic 0.2 at site A3 (Figure 5.11). A shift from euryhaline-marine species (e.g. *Mytilus edulis*, *Crangon crangon*, *Gammarus salinus*) present in sites A1 and A2 to brackish water species (e.g. *Cyathura carinata*, *Leptocheirus pilosus*) occurred in site A3 (Figure 5.11). It is likely that the variation between sites A3 and A4 was largely explained by maximum salinity, which dropped from 35 in A3, to 1.14 in A4 (Figure 5.11). Euryhaline-marine and brackish water species recorded in site A3 were replaced with a low diversity, holohaline, brackish and euryhaline-limnic dominated fauna in sites A4 and A5 (e.g. *Gammarus zaddachi*, *Corophium multisetosum* and *Potamopyrgus antipodarum*) (Figure 5.11). This fauna was then replaced by limnic derived species upstream (e.g. *Hesperocorixa sahlbergi*, *Piscicola geometra*, *Sialis lutaria*) (A6 – A12) (Figure 5.11). Under correlation and regression analysis DCA axis 1 sample scores exhibited a strong negative relationship with the salinity parameters (maximum, minimum, average and range) and dissolved oxygen content (Table 5.9). Site A7 and to a lesser extent sites A10 and A11 appeared to be associated with organic content, with an abundance of detritus-feeding species recorded in these sites (particularly *Potamopyrgus antipodarum* in sites A7 and A10 and *Gammarus pulex* and *Asellus aquaticus* in sites A10 and A11) (Figure 5.11). Furthermore a positive relationship (r = 0.829, p>0.01) was recorded between DCA axis 1 sample scores and organic content (Table 5.9). Under high freshwater discharge conditions, colinearity again existed between calcium carbonate content and the recorded salinity parameters, whilst macrophyte cover was again inversely related to salinity (Figure 5.11).
A significant negative relationship was recorded between species abundance with maximum (r = -0.617, p<0.05) and average (r = -0.623, p<0.05) salinity. Conversely organic content, which was highest in the lower salinity more upstream sites exhibited a positive relationship with species abundance (r = 0.704, p<0.05) (Table 5.9). A negative relationship was recorded between species number and calcium carbonate content (r = -0.829, p<0.01), which again was likely due to the sites with the lowest number of species (A4 and A5) also containing the highest calcium carbonate content (20 – 31%) (Table 5.10). The grain size parameters (average fine grain size, average coarse grain size and standard deviation of grain size) and dissolved oxygen content had a positive relationship with the Shannon-Wiener diversity index (H) (Table 5.9) and as expected exhibited a (significant) negative relationship with the Berger-Parker dominance index (BP) (Table 5.9). A significant negative relationship was recorded between DCA axis 2 sample scores and organic content (r = -0.704, p<0.05) (Table 5.10).
<table>
<thead>
<tr>
<th>Diversity indices</th>
<th>Low freshwater discharge conditions (Aug 08)</th>
<th>High freshwater discharge conditions (Feb 09)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative abundance (RA)</td>
<td>Maximum salinity ($r = -0.617, p&lt;0.05$)</td>
<td>Average salinity ($r = -0.623, p&lt;0.05$)</td>
</tr>
<tr>
<td>Species number (SPN)</td>
<td>Calcium carbonate ($r = -0.838, p&lt;0.01$)</td>
<td>Calcium carbonate ($r = -0.829, p&lt;0.01$)</td>
</tr>
<tr>
<td>Shannon-Wiener diversity index (H')</td>
<td>Calcium carbonate ($r = -0.653, p&lt;0.05$)</td>
<td>Average grain size (fine) ($r = 0.794, p&lt;0.01$)</td>
</tr>
<tr>
<td>Berger-Parker dominance index (BP)</td>
<td>Average grain size (fine) ($r = -0.837, p&lt;0.01$)</td>
<td>Dissolved oxygen ($r = -0.698, p&lt;0.05$)</td>
</tr>
<tr>
<td>DCA Axis 1</td>
<td>Maximum salinity ($r = -0.867, p&lt;0.01$)</td>
<td>Minimum salinity ($r = -0.901, p&lt;0.01$)</td>
</tr>
<tr>
<td>DCA Axis 2</td>
<td>Organic ($r = 0.704, p&lt;0.05$)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.9. Summary table of significant correlations ($p<0.05$) recorded between faunal diversity parameters and environmental variables in the River Adur with distance from the river mouth at Shoreham, under high and low freshwater discharge regimes.

5.4.3.6 Species distributions and salinity under low freshwater discharge conditions (0.733 m$^3$ s$^{-1}$, August 2008)

Under low freshwater discharge conditions, the holeury-haline (0–35) amphipod *Gammarus zaddachi* was recorded from 10.64 km (site A4) from the river mouth to just below the tidal limit at 20.45 km (A10; Figure 5.12). Over this transition *G. zaddachi* experienced a maximum salinity range of 20 to 0.3, but was recorded in greatest abundance in the tidal limnetic (<0.5) reaches (sites A8 and A10), where it dominated the fauna (69 and 77% respectively; Figure 5.12).

The majority of euryhaline-marine (0.5–35) species were recorded at site A1, 0.32 km from the river mouth at Shoreham (Figure 5.12, Figure 5.13a, Figure 5.14a). At this site the salinity was relatively stable at a euhaline maximum of 35 and a minimum of 33.7. Large numbers of the amphipod *Corophium arenarium* (116 individuals) and errant polychaete *Hediste diversicolor* (172; mainly juveniles) were observed at site A2 (3.76 km from the river mouth), where they experienced a euhaline maximum salinity of 35 and a polyhaline minimum salinity of 19.2 (Figure 5.12, Figure 5.13a). Low numbers of the decapod *Crangon crangon* (3), the amphipod *Gammarus salinus* (2) and polychaete *Hediste diversicolor* (4) were recorded at site A3 (8.27 km), where they experienced a large range of salinity over a tidal cycle, from an euhaline
maximum salinity of 35 down to a tidal limnetic minimum salinity of 0.2 (Figure 5.12, Figure 5.13a). Only the malacostracan *Heterotaniais oerstedti* was observed further upstream (10.64 km), possibly due to its tolerance of low and varying salinity, with this site experiencing a polyhaline maximum salinity of 20 and a tidal limnetic minimum salinity of 0.2 (Figure 5.12; Hayward & Ryland, 1995)

The majority of brackish water species (0.5–30), were recorded between 3.76 and 8.27 km from the river mouth (sites A2 and A3) (Figure 5.12, Figure 5.14a). These sites experienced euhaline maximum salinities of 35 and minimum salinities of 19.2 and 0.2 respectively, with the most abundant species the isopod *Cyathura carinata* and gastropod mollusc *Hydrobia ulvae* (Figure 5.12, Figure 5.13a). In contrast to the majority of brackish water species, the amphipod *Corophium multisetosum* was recorded from 8.27 to 17.88 km from the river mouth (A3 – A7), where it experienced the full range of maximum and minimum salinities, from euhaline to tidal limnetic (35 – 0.2). This species, however, was recorded in greatest abundance at sites A4 and A5 (10.64 – 14.06 km), where it experienced maximum salinities of 20 and 0.8 and tidal limnetic minimum salinities of 0.2 (Figure 5.12).

Most of the euryhaline-limnic species (0 – 10) were recorded from 17.88 (A7) to 26.46 km (A12) from the river mouth, in tidal and non-tidal limnetic conditions (<0.5), with the exception of the gastropod mollusc *Potamopyrgus antipodarum*, which was observed further downstream at site A4 (10.64 km from the river mouth) (Figure 5.12). The downstream distribution of *P. antipodarum* reflects the difficulties in assigning a salinity tolerance grouping to this species, as in the UK it is predominantly a limnic species, but can withstand short term exposure to salinities of 32 and can feed, grow and reproduce in salinities of up to 20 (Barnes, 1994; Jacobsen & Forbes, 1997; Gérard et al., 2003; Alonso & Castro-Díez, 2008). At site A4, *P. antipodarum* experienced a maximum salinity of 20 and a tidal limnetic minimum salinity of 0.2. Despite the euryhaline-limnic species capacity to withstand brackish water (0 – 10), only *P. antipodarum* was recorded in sites with elevated salinities. The odonata *Ischnura elegans*, hemipteran *Sigara falleni* and cladoceran *Sida crystallina* were recorded in tidal limnetic (<0.5) conditions at site A10, however the hemipteran *Sida dorsalis* was only recorded in limnic conditions above the tidal limit (Figure 5.12).

The majority and highest abundances of limnic, salt tolerating (0 - <5) species were recorded above the limit of the tide (NTL) in the non-tidal limnetic sites A11 (23.99 km) and A12 (26.46 km) (Figure 5.12, Figure 5.13a, Figure 5.14a.). A number of species were however recorded
below the limit of the tide, within the tidal limnetic (<0.5) zone, including the isopod *Asellus aquaticus*, the gastropods *Bithynia tentaculata* and *Physa fontinalis*, the odonata *Calopteryx splendens*, the hirudineans *Erpobdella octoculata* and *E. testacea* and the megalopteran *Sialis lutaria* (Figure 5.12). A single *Asellus aquaticus* was recorded at site A6, where it experienced a maximum salinity of 0.59 and a tidal limnetic minimum salinity of 0.2 (Figure 5.12, Figure 5.13a).

As expected, the majority and highest abundances of limnic (<0.5) species were observed above the limits of the tide, in sites A11 (23.99 km) and A12 (26.46 km) (Figure 5.12, Figure 5.14 b). Surprisingly, a number of limnic species including the odonata *Libella fulva* and the trichopterans *Limnephilus flavicornis* and *Phryganea bipunctata* were recorded below the limits of the tide at site A10, where they experienced tidal freshwater conditions (0.3) (Figure 5.12, Figure 5.13a). A single *Phryganea bipunctata* was also recorded at site A8 (18.01 km), which experienced tidal limnetic conditions (0.39) (Figure 5.12).

### 5.4.3.7 Species distributions and salinity under high freshwater discharge conditions (1.237 m³ s⁻¹, February 2009)

Under high freshwater discharge conditions, the holeury-haline (0 - 35) amphipod *Gammarus zaddachi* was recorded from 8.27 (A3) to 17.88 km (A7) from the river mouth at Shoreham, covering a maximum salinity range of 35 to 0.27 (Figure 5.12). *G. zaddachi* was recorded in greatest abundance in low salinity (A4) and tidal limnetic sites (A5 and A6) (1.14 – 0.27), where it dominated the faunal community (46% and 33%) (Figure 5.12).

The majority of euryhaline–marine (0.5 – 35) species were recorded up to 3.76 km from the river mouth (A1 and A2), where they experienced fully marine maximum salinities of 35 and a minimum salinity range of 33.7 and 19.2 respectively (Figure 5.12, Figure 5.13b, Figure 5.14b). Low numbers of the decapod *Carcinus maenas* (1), polychaete *Hediste diversicolor* (4) and malacostracan *Heterotanais oerstedi* (6) were recorded at site A3 (8.27 km from the river mouth) and experienced a euhaline maximum salinity of 35 and tidal limnetic minimum salinity of 0.2 (Figure 5.12, Figure 5.13b). A single *Hediste diversicolor* and *Sphaeroma serratum* were observed at site A4 (10.64 km), where they experienced an oligohaline maximum salinity of 1.14 and tidal limnetic minimum salinity of 0.2 (Figure 5.12, Figure 5.13b).

The majority of brackish water species (0.5 – 30) were recorded from 3.76 to 10.64 km from the river mouth (A2 - A4) over a maximum salinity range of 35 to 1.14 and minimum salinity range
of 19.2 to 0.2 (Figure 5.12, Figure 5.13b, Figure 5.14b). As under low freshwater discharge conditions, the amphipod *Corophium multisetosum* was recorded much higher upstream than its fellow brackish water species, from 8.27 (A3) to 18 km (A8) from the river mouth, where it the full range of maximum and minimum salinities, from 35 to 0.2 (although at low abundances) (Figure 5.12, Figure 5.13b). *C. multisetosum* was recorded in highest abundances at site A4 (10.64 km), at a maximum oligohaline salinity of 1.14 and a tidal limnetic minimum salinity (0.2), where it dominated the faunal community (77.6%) (Figure 5.12).

The majority of the euryhaline-limnic species were observed from 16.05 (A6) to 26.46 km (A12) from the river mouth, in tidal and non-tidal limnetic conditions (<0.5) (Figure 5.12, Figure 5.14b). The gastropod mollusc *Potamopyrgus antipodarum* was recorded from 10.64 (A4) to 26.46 km (A12) from the river mouth, but was most abundant at site A4, with a maximum salinity of 1.14 and tidal limnetic minimum salinity (0.2) (Figure 5.12). As under low discharge conditions, *P. antipodarum* was the only euryhaline-limnic species observed in brackish water (Figure 5.12).

The majority and highest abundances of limnic, salt tolerating (0 - <5) species were recorded above the limit of the tide (NTL 21 km) (A11 and A12) (Figure 5.12, Figure 5.14b). A large number of species were observed below the tidal limit in the tidal limnetic zone, from 14.06 (A5) to 20.45 km (A10), including the isopod *Asellus aquaticus*, the amphipod *Gammarus pulex*, the hirudineans *Piscicola geometra* and *Glossiphonia complanata*, the gastropod molluscs *Bithynia tentaculata* and *Planorbis planorbis*, the megalopteran *Sialis lutaria* and the trichopteran *Cyrnus trimaculatus* (Figure 5.12, Figure 5.13b). A single *Asellus aquaticus* was recorded at site A3 (8.27 km), where it experienced a euhaline maximum salinity of 35 and a tidal limnetic 0.2 (Figure 5.12).

The majority and highest abundances of limnic (<0.5) species were recorded in freshwaters (<0.5) above the limits of the tide, in sites A11 and A12 (Figure 5.12, Figure 5.13b, Figure 5.14b). However a number of limnic species were observed below the tidal limits, in the tidal limnetic zone (<0.5). These species included the trichopterans *Limnephilus flavicornis*, *Lype reducta*, and *Plectrocnemia conspersa* and the coleopteran *Limnius volckmari* (Figure 5.12). A single trichopteran *Limnephilus flavicornis* was recorded at site A2 (3.76 km from the river mouth), with a euhaline maximum salinity of 35 and polyhaline minimum salinity of 19.2 (Figure 5.12).
Figure 5.12. River Adur summary diagram of species distributions, maximum salinity and maximum salinity zones under high and low freshwater discharge regimes with distance from the river mouth. Species shown occur >2% in one or more samples and are separated into salinity tolerance groupings following a review of published literature (Table 5.5).
5.4.3.8 Faunal zones and salinity

Under both high and low freshwater discharge regimes, euryhaline-marine (e.g. *Corophium arenarium*, *Hediste diversicolor*) and brackish water species (e.g. *Cyathura carinata*, *Hydrobia ulvae*) dominated the faunal community from 0.32 (A1) to 8.27 km (A3) from the river mouth at Shoreham, over fully marine maximum salinities of 35 under high and low freshwater discharge conditions (Figure 5.13, Figure 5.14). At 10.64 km (A4), the faunal community became more diverse, with the introduction of holeury-haline and euryhaline-limnic species; a transition highlighted by a significant faunal zone split (based on dissimilarity) at 9.45 km from the river mouth (under both discharge conditions; Figure 5.14).

Under low discharge conditions, the brackish water amphipod *Corophium multisetosum* dominated the faunal community from 10.64 (A4) to 14.06 km (A5) (0.8 - 20), and was replaced by the holeury-haline amphipod *Gammarus zaddachi* at 16.05 km (A6) (0.59), a shift
represented by a significant zone split at 15.05 km (zone 2; Figure 5.12, Figure 5.14a). Zone 2 covered a maximum salinity range of 20 to 0.8. The holeury-haline *Gammarus zaddachi* dominated the faunal community from 16.05 (A6) to 20.45 km (A10), over a salinity gradient of 0.59 to 0.3, but was replaced by limnic derived species above the limit of the tide (23.99 to 26.46 km), represented by a significant zone split at 22.22 km from the river mouth (zone 3; Figure 5.12, Figure 5.14a). Zone 3 covered a maximum salinity gradient of 0.59 to 0.3. Zone 4 (22.12 – 26.46 km) consisted of limnic derived species in non-tidal limnetic conditions (0.1 - 0.2; Figure 5.14a).

Under high freshwater discharge conditions, a diverse community of holeury-haline, marine, brackish and limnic derived species were represented from 10.64 (A4) to 17.88 km (A7) from the river mouth (0.27 - 1.14), which shifted to a faunal community dominated by limnic species at 18.01 km (A8), resulting in a significant zone split at 17.94 km (zone 2) (Figure 5.14b). Zone 2 covered a maximum salinity gradient of 0.27 to 1.14. Zone 3 (17.94 -26.46 km) consisted of limnic derived species over tidal and non-tidal limnetic maximum salinities of 0.2 to 0.1 (Figure 5.13b, Figure 5.14b).
Figure 5.14. River Adur summary diagram of salinity parameters (maximum, minimum and range) and associated maximum salinity zones, relative abundance of salinity tolerance groups (silhouette graph) and significant faunal data zone splits with distance from the river mouth at Shoreham, under low (a) and high (b) freshwater discharge conditions.
5.4.3.9 Diversity indices and salinity

Under both low and high discharge conditions, faunal diversity (Shannon-Weiner H’ index), decreased from the head of the estuary (freshwater) downstream and from the river mouth (marine) upstream, with the lowest diversity recorded in the mid to upper estuary (Figure 5.15). This was coupled with an increase in species dominance (Berger-Parker index), peaking in the mid to upper estuary (Figure 5.15). Under low discharge conditions, the lowest diversity and consequently the highest dominance was recorded from 14.06 (A5) to 17.88 km (A7) from the river mouth at Shoreham, over an oligohaline maximum salinity gradient of 0.41 to 0.8 and tidal limnetic minimum salinities of 0.2 (Figure 5.15a). Under high discharge conditions, the lowest diversity and highest dominance in the faunal data set was also recorded from 14.06 to 17.88 km from the river mouth, over a tidal limnetic maximum salinity range of 0.27 to 0.3 and minimum salinity of 0.2 (Figure 5.15b). Within this low diversity/high dominance zone, a peak in diversity and drop in dominance was recorded at 16.05 km (A6) (Figure 5.15b). Unlike number of species (which is closely related to the diversity index: H’), relative abundance was generally high throughout the estuary, with the exception of site A7 (17.88 km), where there was a marked drop in abundance under both discharge conditions (Figure 5.15).
Figure 5.15. Summary diagram of salinity parameters (maximum, minimum and range), maximum salinity zones, diversity indices and relative abundance of salinity tolerance groups (silhouette graph) with distance from the river mouth at Shoreham, under low (a) and high (b) freshwater discharge conditions.

### 5.4.3.10 Faunal data and salinity summary

Under both high and low discharge conditions, a strong correlation was observed between species distributions, their allocated salinity tolerance groupings and the recorded maximum salinity gradient (Figure 5.12, Figure 5.14). A number of species were however, recorded
outside of their allocated salinity zones, including the amphipod *Corophium multisetosum* and the gastropod mollusc *Potamopyrgus antipodarum* (Figure 5.12). *C. multisetosum* was classified as a brackish water species (0.5 - 30) and yet was recorded at sites that experienced tidal limnetic maximum salinities (<0.5). *P. antipodarum* was classified as a euryhaline-limnic (0 – 10) species, but was recorded in sites with salinities up to 20. Both of these species were difficult to assign to the salinity tolerance groupings, due to their wide salinity tolerances and contradictory tolerance literature (Alonso & Castro-Díez, 2008). Despite strong correlation with salinity tolerance groupings and recorded maximum salinities, a poor correlation was observed between distributions, tolerance groupings and minimum salinities (Figure 5.12, Figure 5.14). The dynamic tide and salinity regimes of the River Adur estuary (see Chapter 4) result in sections of the estuarine course experiencing both high maximum salinities (corresponding to high tide) and tidal limnetic minimum salinities (corresponding with low tide) over a tidal cycle. Both euryhaline-marine (0.5 - 35) and brackish water species (0.5 - 30) were recorded in these sites at low tide (e.g. *Gammarus salinus*, *Hediste diversicolor*, *Cyathura carinata*, *Leptocheirus pilosus*) (Figure 5.12, Figure 5.14). This could indicate a degree of tolerance (and/or adaptability) to tidal limnetic salinities (<0.5) over short periods of time (e.g. at low tide), or the ability of these species to seek refuge at low tide in the sediment (under stones, cobbles or buried into the sediment), where the interstitial salinity may be higher and less variable than the tidal limnetic surface waters (Chapman & Brinkhurst, 1981; McLusky, 1968; Sanders *et al.*, 1965).

Although not recorded outside of their allocated salinity tolerance grouping, a number of species classified as limnic (<0.5) were recorded below the tidal limit, in tidal limnetic conditions, where they were subject to significant physiochemical shifts over a tidal cycle. These species included the trichopterans *Limnephilus flavicornis*, *Phryganea bipunctata*, *Plectrocnemia conspersa*, the coleopteran *Limnius volckmari* and the odonata *Libella fulva* (Figure 5.12).

Under low freshwater discharge conditions tidal saline water penetrated up to 16.98 km from the river mouth at Shoreham, compared to 13.25 km under high freshwater discharge conditions (February 2009) (See Chapter 4). The increased degree of saline penetration under low freshwater discharge conditions compared to high, appeared to have little effect on the distributions of euryhaline-marine and brackish water species in the River Adur estuary, with both groups recorded up to 10.64 km (A4) from the river mouth under both discharge conditions (after the removal of non-conforming *C. multisetosum*) (Figure 5.14). This static distribution was recorded in the faunal data, with the zone 1 split at 9.45 km under both discharge regimes (Figure 5.14). A shift was however recorded in the distributions of limnic derived species, with
euryhaline-limnic species extending their range downstream by 2.44 km (after the removal of *P. antipodarum*) and limnic salt tolerating species by 1.96 km under high discharge conditions when compared to low (Figure 5.12, Figure 5.14). The downstream distribution of limnic classified species remained static at 18.01 km. The downstream shifts in distributions of limnic derived species was significantly recorded in the faunal data with a reduction in the number of significant zone splits under high discharge conditions (from 4 zones to 3) and the extension of the final zone to below the tidal limit (17.94 km) (Figure 5.14). A large downstream shift was recorded in the distribution of the holeury-haline amphipod *Gammarus zaddachi* from high (10.64 to 20.45 km) to low freshwater discharge conditions (8.27 to 17.88 km) (Figure 5.12). Due to the holeury-haline (0 – 35) salinity tolerance of *G. zaddachi*, the recorded downstream distribution shift may have been the result of interspecific competition, forced by the downstream salinity related shifts of competing sibling species, e.g. the downstream shifts in distributions of the limnic salt tolerating *Gammarus pulex* (from 23.99 to 18.01 km under high discharge conditions) and the euryhaline-marine *Gammarus salinus* (from 8.27 to 3.76 km; Figure 5.12) (as recorded in published literature; Lincoln, 1979; Kolding, 1986; Korpinen & Westerbom, 2009).

Under both discharge conditions, the zone of lowest diversity and highest dominance in the faunal community of the River Adur estuary was recorded from 14.06 to 17.88 km from the river mouth, at oligohaline (0.41 – 0.8, low discharge conditions) to tidal limnetic maximum salinities (0.27 - 0.3, high discharge conditions; Figure 5.15).

### 5.4.4 The River Ouse

#### 5.4.4.1 Faunal data under low freshwater discharge conditions (0.72 m$^3$s$^{-1}$, August 2008).

Under low freshwater discharge conditions a total of 71 genera (consisting of 9234 individuals) were recorded in the River Ouse, with a total of 56 identifiable to species level. Within the faunal dataset the first DCA axis was responsible for explaining 16.3% of the variation in the faunal dataset, with axis 2 explaining a further 7.7% (Table 5.10). As in the River Adur, the majority of species appeared to be strongly associated with axis 1, which appeared to be driven by salinity, with limnic species exhibiting the highest scores on axis 1 and euryhaline-marine species exhibiting the lowest scores (Figure 5.17). Numerical zoning of the faunal data produced 3 statistically significant zone splits (based on dissimilarity) at 13.12 and 21.38 km from the river mouth at Newhaven.
1.67 – 13.12 km (Zone 1) – The faunal community in zone 1 was diverse and abundant, consisting of 1616 individuals, which were identified as holeury-haline (0 – 35) euryhaline-marine (0 – 35) and brackish water (0.5 – 30) species (Figure 5.16, Figure 5.17, Figure 5.18). The most abundant of which were the euryhaline-marine amphipod *Melita palmata* (22.3%) and the brackish water isopod *Cyathura carinata* (15%) (Figure 5.16, Figure 5.17). Zone 1 was characterised by a large maximum salinity range (10.3 – 35), high dissolved oxygen content (76 – 91%), low organic content (2 – 4%), algal cover (e.g. *Cladophora rupestris*, *Fucus spiralis*, *Fucus ceranoides*) and saltmarsh vegetation on the upper banks (e.g. *Atriplex portulacoides*, *Agropyron pungens*, *Seriphidium maritimum*, *Beta vulgaris*) (Figure 5.19). The surface sediment consisted of poorly sorted chalk gravel to small cobbles (often covered in a thick algal (diatom) film), with a sandy anoxic layer approximately 1 cm below the surface (Figure 5.19).

13.12 – 21.38 km (Zone 2) – The faunal community in zone 2 was abundant, but not diverse, with a total of 1653 individuals and just 12 identified species (Figure 5.18). The faunal community consisted of a mixture of holeury-haline (0 – 35), brackish (0.5 – 30) and limnic derived species, but was dominated by the holeury-haline amphipod *Gammarus zaddachi* (63.7%) and the euryhaline-limnic gastropod mollusc *Potamopyrgus antipodarum* (24.6%) (Figure 5.16, Figure 5.17). Zone 2 was characterised by medium to low maximum salinities (0.1 – 7.9), high dissolved oxygen content (64 – 91%) and fine grained sediment with a relatively high organic content (7 – 11%) (Figure 5.19). Within this zone, the vegetation shifted from saltmarsh species (e.g. *Aster tripolium*, *Parapholis strigosa*) to emergent macrophytes indicative of freshwater conditions (e.g. *Phalaris arundinacea*, *Sparganium erectum*, *Phragmites australis*).

21.38 – 47.16 km (Zone 3) – The faunal community in zone 3 was abundant and diverse, consisting of a total of 5874 individuals, identified as euryhaline-limnic (0 – 10), limnic salt tolerating (0 - <5) and limnic (<0.5) species (Figure 5.16, Figure 5.17, Figure 5.18). The most abundant were the trichopterans *Neureclipsis bimaculata* (32.5%) and *Hydropsyche*.

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Table 5.10. Summary of Detrended Correspondence Analysis results for the River Ouse faunal dataset, under low freshwater discharge conditions (0.72 m$^3$s$^{-1}$).
angustipennis (17.5%), along with the bivalve mollusc Sphaerium corneum (26.1%) (Figure 5.16, Figure 5.17). Zone 3 was characterised by non-tidal freshwaters, from a lowland low-flow (DO 65%), sluiced site with fine grained sediment (O14), to headwater fast-flow sites (DO 97 - 73%), with densely vegetated banks (e.g. Sparganium erectum, Rumex hydrolapathum, Phalaris arundinacea, Phragmites australis), but a lack of marginal vegetation within the river channel (O14, O15) and coarse grained (>60 mm) sediment (O15) (Figure 5.19).

5.4.4.2 Faunal data under high freshwater discharge conditions (1.43 m³s⁻¹, February 2009).

Under high freshwater discharge conditions a total of 59 genera (consisting of 5503 individuals) were recorded in the River Ouse, with a total of 46 identifiable to species level. Within the faunal dataset the first DCA axis was responsible for 19.7% of the variation in the faunal dataset, with axis 2 explaining a further 7.5% (Table 5.11). As under low freshwater discharge conditions, the majority of species appeared to be strongly associated with axis 1, which appeared to be driven by salinity, with limnic species exhibiting the highest scores on axis 1 and euryhaline-marine species exhibiting the lowest scores (Figure 5.17). Numerical zoning of the faunal data produced three statistically significant zone splits (based on dissimilarity) at 7.52 and 18.95 km from the river mouth at Newhaven.

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<th>No. of species</th>
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Table 5.11. Summary of detrended correspondence analysis results for the River Ouse faunal dataset, under high freshwater discharge conditions.

1.67 – 7.52 (Zone 1) – The faunal community in zone 1 was abundant and diverse, consisting of 972 individuals, identified as holarctic-haline (0 – 35), euryhaline-marine (>0.5 – 35) and brackish water (0.5 - 30) species (Figure 5.16, Figure 5.18). The most abundant species were the euryhaline-marine amphipod Melita palmata (30.6%) and the sessile barnacles Semibalanus balanoides (17.2%) and Elminius modestus (9.8%) (Figure 5.16, Figure 5.17). Zone 1 was characterised by high maximum salinities (35), a large range of minimum salinities (0.2 - 25.8), high dissolved oxygen content (91 – 85%) low organic content (2 – 4%), algal cover (e.g. Cladophora rupestris, Fucus spiralis, Fucus ceranoides), and saltmarsh vegetation on the upper banks (e.g. Atriplex portulacoides, Agropyron pungens, Seriphidium maritimum, Beta vulgaris) (Figure 5.19). The sediment consisted of poorly sorted gravel to small cobbles (frequently
covered with a thick algal (diatom) film) with an anoxic sand layer approximately 1 cm below the surface (Figure 5.19).

7.52 – 18.95 (Zone 2) – The faunal community in zone 2 was variable (species number and abundance), but generally followed a ‘U’ shaped pattern, with diversity decreasing towards the middle of the zone, then increasing, with dominance peaking in the middle of the zone (Figure 5.18). The faunal community consisted of a mixture of holeury-haline (0 – 35), euryhaline-marine (0.5 – 35), brackish (0.5 – 30) and limnic derived species (Figure 5.16, Figure 5.17) The most dominant of these were the holeury-haline amphipod *Gammarus zaddachi* (62%), the brackish isopod *Cyathura carinata* (24%) and the amphipod *Leptocheirus pilosus* (6%) (Figure 5.16, Figure 5.17, Figure 5.18). Zone 2 was characterised by a dramatic shift in environmental variables, from high to mid salinity (5.1 – 24.1) sites with coarse grained (>60 mm) sediment (pole-wharfing sites), consisting of high calcium carbonate (44 – 57%) and low organic content (2 – 5%), to low salinity sites (0.2 – 2.1), with fine grained sediment, consisting of low calcium carbonate (9 – 21%) and high organic content (7 – 9%) (O8 – O11) (Figure 5.19). This shift was also observed in vegetation cover, from macroalgal (*Fucus ceranoides*), saltmarsh (*Atriplex portulacoides Agroyiran pungens, Aster tripolium and Parapholis strigosa*) species to vegetation indicative of more typically freshwater conditions (e.g. *Phalaris arundinacea, Phragmites australis, Sparganium erectum*).

18.95 – 47.16 (Zone 3) – The faunal community in zone 3 was diverse (high species numbers and abundance), consisting of 2143 individuals, identified as euryhaline-limnic (0 - 10), limnic, salt tolerating (0 - <5) and limnic (<0.5) species (Figure 5.16, Figure 5.17, Figure 5.18). The most abundant of which included the limnic, salt tolerating amphipod *Gammarus pulex* (38.7%) and the limnic trichopteran *Hydropsyche angustipennis* (20.2%) (Figure 5.16, Figure 5.17). Despite a general pattern of high diversity in this zone, site O15 was dominated by a small number of species, including the limnic salt tolerating isopod *Asellus aquaticus* (30%) and limnic trichopteran *Brachycentrus subnubilis* (24%; Figure 5.17). Zone 3 was characterised by tidal and non-tidal freshwater sites, with densely vegetated banks (*Sparganium erectum, Rumex hydrolapathum, Phalaris arundinacea, Phragmites australis*), from lowland sites with fine grained sediment and high organic content (O13, O14), to fast-flowing coarse grained (>60 mm) sediment sites at the river source (O15, O16; Figure 5.19).
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<th>Low freshwater discharge conditions (0.72 m$^3$s$^{-1}$)</th>
<th>High freshwater discharge conditions (1.43 m$^3$s$^{-1}$)</th>
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<td><strong>Zone 1 (1.67 - 7.62 km)</strong></td>
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<td><strong>Holocery-haline (0 - 35)</strong></td>
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<td>Palaeon longirostris (5.5%)</td>
</tr>
<tr>
<td><strong>Euryhaline-marine (0.5-35)</strong></td>
<td><strong>Euryhaline-marine (0.5 - 35)</strong></td>
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<td>Melita palmata (22.2%), Hediste diversicolor (10.6%), Semibalanus balanoides (8%), Elminius modestus (5.5%), Carcinus maenas (3.9%), Heterothais oerstedii (3.1%), Gammaurus salinus (2%), Jaera albifrons (1.9%), Corophium arenarium (1%), Mytilus edulis (1.8%), Palaeon elegans (1.6%), Crangon crangon (1%), Ceratoderma edule (0.1%)</td>
<td>Melita palmata (30.6%), Semibalanus balanoides (17.2%), Elminius modestus (9.8%), Hediste diversicolor (7.8%), Jaera albifrons (6.2%), Carcinus maenas (1.5%), Cerasodera edule (1.3%), Corophium arenarium (1%), Mytilus edulis (1%), Gammaurus salinus (0.9%), Sphaeroma serratum (0.6%)</td>
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<td><strong>Brackish water (0.5 - 30)</strong></td>
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<td>Cyathura carinata (15%), Leptochirus pilosus (13.9%)</td>
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<td><strong>Zone 2 (13.12 - 21.38 km)</strong></td>
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<td><strong>Limmic (&lt;0.5)</strong></td>
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<tr>
<td>Hydropsyche angustipennis (17.5%), Gammarus pulex (14.4%), Asellus aquaticus (2.8%), Cyprinus carpio (0.8%), Bithynia tentaculata (0.4%), Polycentropus flavicollis (0.2%), Calopteryx splendens (0.1%), Erpobdella octoculata (0.1%), Glossiphonia complanata (0.1%), Helobdella stagnalis (0.1%), Rhyothemus dorsalis (0.1%), Planorbus planorbus (0.02%)</td>
<td>Gammarus pulex (38.7%), Hydropsyche angustipennis (20.2%), Asellus aquaticus (3.3%), Baetis rhodani (2.7%), Anabola nervosa (0.8%), Sericostoma personatum (0.8%), Caenis horaria (0.2%), Glossiphonia complanata (0.2%), Rhyothemus dorsalis (0.2%), Erpobdella octoculata (0.1%), Sialis lutaria (0.1%)</td>
</tr>
<tr>
<td><strong>Limmic (&lt;0.5)</strong></td>
<td></td>
</tr>
<tr>
<td>Neurephlea bimaculata (32.5%), Sphaerium cornutum (26.1%), Limnephilus flavicornis (1.7%), Bithynia leachi (1.4%), Althinodes cinctus (0.7%), Lypae reducta (0.2%), Limnias volckmari (0.2%), Planorbus carinatus (0.1%), Phryganea bipunctata (0.04%), Ephemerella danica (0.5%)</td>
<td>Neurephlea bimaculata (19.9%), Sphaerium cornutum (7.4%), Ephemerula danica (3.1%), Brachycentrus subnubilis (0.8%), Plecoptera conspersa (0.4%), Limnephilus flavicornis (0.3%), Limnias volckmari (0.3%), Halesus radiatus (0.3%), Bithynia leachi (0.1%)</td>
</tr>
</tbody>
</table>

Figure 5.16. Macrinovertebrate species and relative abundances recorded in the significant faunal zones of the River Ouse under high and low freshwater discharge regimes.
Figure 5.17. Summary diagram of species distributions, split into significant faunal zones with distance from the river mouth, under high and low freshwater discharge regimes. Species shown occur >2% in one or more samples and are separated into salinity tolerance groupings following a review of published literature (Table 5.5). DCA axis 1 and axis 2 sample scores depicted for each river and freshwater discharge regime.
5.4.4.3 Faunal data and environmental variables

As in the River Adur, the environmental variables recorded in the River Ouse estuary (up to the NTL), exhibit a distinctive division into a lower (1.6 – 13.44 km) and upper (13.44 – 21.8 km) estuarine zone (Figure 5.19). The lower River Ouse estuary is characterised by a narrow, tightly constrained channel, experiencing high salinities (maximum and average) and dynamic salinity ranges (35 - <0.5), algal cover (O1-O5), high dissolved oxygen content (76 - 91%) and poorly sorted, coarse grained (>60 mm) sediment with a high calcium carbonate (16 – 72%) and low
organic content (2 – 5%) (Figure 5.19). The coarse grained sediment is mainly artificial, placed on the estuary banks to promote stabilisation by reducing erosion processes; a management strategy known as ‘pole-wharfing’ (sites O2 – O5). Pole-wharfing is the placement of a surface layer of chalk and/or limestone riprap held in place by woven horizontal beech poles secured into the estuary banks between high and low tide level (Environment Agency, 2010). The high calcium carbonate content recorded at these sites may also be indicative of pole-wharfing, although care must be taken as this may be due to the chalk geology of the lower River Ouse catchment (Figure 5.19). This is further supported by the lower calcium carbonate content recorded at the lower River Adur estuary sites that are also situated in the chalk catchment, but not subjected to pole-wharfing (Figure 5.9).

The upper River Ouse estuary is characterised by relatively stable environmental conditions. Under both high and low freshwater discharge conditions the upper estuary experiences low salinity (3.3) to tidal limnic (<0.5) conditions, which is reflected in a shift from saltmarsh vegetation to emergent macrophytes associated with more freshwater conditions (Figure 5.19). Fine grained sediments with high organic (7 – 11%) and low calcium carbonate content (2 – 21%) dominate this zone (Figure 5.19).

The freshwater River Ouse (above the NTL) can be divided into mid to lower reaches and headwaters. The mid to lower reaches of the River Ouse are characterised by low flow (low DO content; 65%), wide channels with fine grained sediment, to fast flow (high DO content; 73 – 97%) headwater sites with narrow, steep banked channels and coarse grained gravel sediments (Figure 5.19).
Figure 5.19. Summary diagram of the recorded environmental variables from the River Ouse split into significant faunal zones under high and low freshwater discharge conditions.
5.4.4.4 Environment-species relationships under low freshwater discharge conditions (0.73 m³ s⁻¹, August 2008)

A DCCA performed on the River Ouse dataset (i.e. samples O1-O16) under low freshwater discharge conditions, suggested that DCA axis 1 (16.3% variation) for the River Ouse was also predominantly driven by salinity (Figure 5.20a). This was also supported by the strong negative relationship between the DCA axis 1 sample scores and all four salinity parameters (Table 5.12). Sites O1 – O3 exhibited fully marine maximum salinities (35) and much higher average salinities than the more upstream sites within the tidal limit (O6-O13, NB. O4 and O5 not sampled under low freshwater flow conditions) (Figure 5.20a). This can be observed in the macroinvertebrate species distributions, with euryhaline-marine species (e.g. *Gammarus salinus*, *Melita palmata*, *Carcinus maenas*) strongly associated with sites O1-O3, replaced by brackish water species (e.g. *Leptocheirus pilosus*, *Cyathura carinata*) associated with sites O6-O8, which are in turn replaced by holeury-haline (*Gammarus zaddachi*) and euryhaline-limnic (*Potamopyrgus antipodarum*) species towards the normal tidal limits (sites O9-O13) (Figure 5.20a). The sites above the limit of tide (O14-O16) were situated at the far freshwater end of the salinity gradient and were characterised by an entirely limnic fauna (e.g. *Asellus aquaticus*, *Bithynia tentaculata*, *Gammarus pulex*) (Figure 5.20a). For minimum salinity, a large decrease occurred between sites O1 (25.9) and O2 (15.9), possibly explaining some of the variation between these sites (Figure 5.20a). A further decrease in minimum salinity occurred at site O3, where it dropped to a tidal limnetic 0.3.

As the higher salinity sampling sites (i.e. sites O1-O11) were situated on the lower River Ouse geological chalk catchment, calcium carbonate content again exhibited a close (positive) relationship with salinity (Figure 5.20a). The freshwater sites above the tidal limit were situated on the sand and clay formations of the upper River Ouse catchment, and therefore the river bed sediments subsequently contained less calcium carbonate. However, it is important to note that superimposed upon this trend are several fluctuations in carbonate content within the River Ouse estuary (Figure 5.19), which might account for some of the variation in the faunal data (Figure 5.20). For example, calcium carbonate content increased from 16% at site O1 to 43 and 73% at sites O2 and O3 respectively. Further fluctuations were apparent between sites O6-O8 before gradually decreasing in the upper part of the catchment (Figure 5.19).

To further explore the trends in the estuarine section of the river, a DCCA was performed on sites O1-O13 (Figure 5.20b). In this reduced dataset, salinity (particularly range, average and maximum salinity) was still the most important variable explaining the variation in the faunal...
dataset, but the relationship between the salinity parameters and calcium carbonate content appeared to be slightly weaker (Figure 5.20b). Calcium carbonate content appeared closely related to grain size parameters, for example in addition to an increase in calcium carbonate between sites O1 (16%), O2 (43%) and O3 (72%), there was also an increase in the average coarse grain size (i.e. 10 mm in O1 to 14 and 15 mm in O2 and O3 respectively) and standard deviation of coarse grain size (3 mm in O1 to 12 and 11 mm in O2 and O3 respectively) (Figure 5.19). A significant negative relationship was observed between DCA axis 1 scores with both calcium carbonate content and the grain size parameters (fine, std dev of coarse) (Table 5.12), further suggesting that changes associated with these parameters are likely to explain some of the variation in the faunal dataset.

Sites characterised by the highest calcium carbonate content and largest average coarse grain sizes (and std dev of coarse grain size) had large amounts of chalk rubble on the estuary banks. This could be explained by bank stabilisation by the Environment Agency. Pole-wharfing has been undertaken on 3 km of the lower River Ouse estuary, at selected locations from above Newhaven to below the town of Lewes, including sites O2, O3, O4 and O5. The addition of riprap significantly changes the sediment characteristics of a site, which can subsequently influence macroinvertebrate biomass and density (Fischenich, 2003). It is therefore plausible that these environmental variables (i.e. calcium carbonate content and grain size parameters) may to some degree act as predictors for human influence on the river bed, though as indicated previously care must be taken particularly with regards to calcium carbon content as the geology of the lower River Ouse catchment is predominantly chalk and marine shell fragments in the sediment could also account for high calcium carbonate content at selected sites.

Macrophyte cover and organics showed an inverse relationship with the salinity parameters (and subsequently grain size, temperature and calcium carbonate content) with macrophytes only present in low salinity, fine grained sediment environments (Table 5.12). A significant negative relationship existed between macrophyte cover with both species number \(r = 0.745, p<0.01\) and the Shannon-Weiner diversity index \(H'\) \(r = -0.827, p<0.01\) and subsequently a significant positive relationship existed between macrophyte cover and the Berger Parker dominance index \(BP\) \(r = 0.745, p<0.01\) (Table 5.12).
5.4.4.5 Environment-species relationships under high freshwater discharge conditions (1.43 m$^3$s$^{-1}$, February 2009)

Under high freshwater flow conditions, DCCA axis 1 was again largely explained by the salinity parameters, with maximum salinity ranging from fully marine conditions in the downstream sites (35 in sites O1 to O3), through to tidal freshwater conditions in upstream sites (O11 to O16) (Figure 5.21a). The minimum salinity dropped sharply between sites O1 (25.8), O2 (15.8) and
O3 (0.2), whereas the maximum salinity was fully marine and stable at 35. Average salinity dropped gradually from 30.4 at site O1 to a tidal limnetic 0.32 at site O9. It is therefore likely that minimum and average salinity was important in explaining some of the variation between sites O1, O2 and O3 (Figure 5.21). Additionally, significant negative relationships were recorded between DCA axis 1 sample scores with all salinity parameters (maximum, minimum, average and range, Table 5.12).

Whilst salinity was the strongest gradient, some of the variation in the faunal dataset appeared to be explained by sediment characteristics, organic content and macrophyte cover (Figure 5.21a). For example carbonate content and grain size parameters showed some co-linearity with the salinity parameters and strong co-linearity when the non-tidal freshwater sites were removed (DCCA O1-O13, Figure 5.21b). This was due to the most downstream, higher salinity sites containing coarse sediments (O1 – O7 contain gravels and cobbles) and high calcium carbonate content (fluctuating between 16-72% in sites O1 to O9; Figure 5.19). As the coarsest grain sizes and highest carbon calcium contents occurred at the sites affected by pole-wharfing, it is plausible that this activity accounted for some of the observed variance in the high freshwater flow faunal dataset. Regression and correlation analysis further supported this, as both calcium carbonate content and the average grain size parameters (fine, coarse, std dev of coarse) had a significant (p<0.05) negative relationship with DCA 1 axis score (Table 5.12). However high calcium carbonate content could also be the result of marine shell fragments in the sediment. Organic content (and macrophyte cover) appeared to be important in sites O4, O8, O12 and O15 (Figure 5.21a). This was likely due to the dominance of the detritus feeding amphipods Gammarus zaddachi (91% at site O4 and 94% at O8) and Gammarus pulex (85% at O12 and 37% at O15) at these sites (Figure 5.17). Organic content and macrophyte cover had an inverse relationship with salinity, as many emergent macrophyte species inhabit low salinity to limnic reaches (Figure 5.21a). This has subsequently resulted in a positive relationship between DCA axis 1 sample scores and organic content under regression analysis (Table 5.12).
Figure 5.21. Detrended Canonical Correspondence Analysis (DCCA) triplots of the River Ouse under high freshwater discharge conditions, indicating environmental variables and selected most abundant macroinvertebrate species: a. DCCA of sites O1-O16, b. DCCA of sites O1-O13.

A significant negative relationship was recorded between relative abundance (RA) with both macrophyte cover ($r = -0.673$, $p<0.01$) and organic content ($r = -0.525$, $p<0.05$), whilst a positive relationship existed with average coarse grain size ($r = 0.538$, $p<0.05$) (Table 5.12). No significant relationships existed between the measured environmental variables with species number, Shannon-Weiner index or the Berger Parker index (Table 5.12).
### Table 5.12. Summary table of significant correlations (p<0.05) recorded between faunal diversity parameters and environmental variables with distance from the river mouth at Newhaven, under high and low freshwater discharge regimes.

<table>
<thead>
<tr>
<th>Diversity indices</th>
<th>Low freshwater discharge conditions (Aug 08)</th>
<th>High freshwater discharge conditions (Feb 09)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative abundance (A)</td>
<td>Macrophyte cover (r = -0.673, p&lt;0.01)</td>
<td>Organic (r = -0.525, p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average grain size (coarse) (r = 0.538, p&lt;0.05)</td>
</tr>
<tr>
<td>Species number (SPN)</td>
<td>Macrophyte cover (r = -0.848, p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Shannon-Wiener diversity index (H)</td>
<td>Macrophyte cover (r = -0.827, p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Berger-Parker dominance index (BP)</td>
<td>Macrophyte cover (r = 0.745, p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA Axis 1</strong></td>
<td>Maximum salinity (r = -0.898, p&lt;0.01)</td>
<td>Maximum salinity (r = -0.863, p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>Minimum salinity (r = -0.574, p&lt;0.05)</td>
<td>Minimum salinity (r = -0.767, p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>Average salinity (r = -0.889, p&lt;0.01)</td>
<td>Average salinity (r = -0.890, p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>Range salinity (r = -0.870, p&lt;0.01)</td>
<td>Range salinity (r = -0.774, p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>Calcium carbonate (r = -0.916, p&lt;0.01)</td>
<td>Organic (r = 0.528, p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Average grain size (fine) (r = -0.783, p&lt;0.05)</td>
<td>Calcium carbonate (r = -0.774, p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>Average grain size (coarse) (r = -0.690, p&lt;0.05)</td>
<td>Average grain size (fine) (r = -0.676, p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>Std dev average grain size (coarse) (r = -0.639, p&lt;0.05)</td>
<td>Std dev average grain size (coarse) (r = -0.600, p&lt;0.05)</td>
</tr>
</tbody>
</table>

#### 5.4.4.6 Species distributions and salinity under low freshwater discharge conditions (0.73 m³ s⁻¹, August 2008)

Under low freshwater discharge conditions, holeury-haline (0 – 35) species were recorded from 6.35 (O3) to 20.39 km (O13) from the river mouth at Newhaven, covering a maximum salinity gradient of 0.1 to 35 (Figure 5.22, Figure 5.23a, Figure 5.24a). The amphipod *Gammarus zaddachi* was the most abundant holeury-haline species in the faunal data set and was recorded from 12.8 (O7) to 20.39 km (O13) from the river mouth, experiencing a maximum salinity gradient of 0.1 to 10.3 with tidal limnetic minimum salinities (<0.5) (Figure 5.22). *G. zaddachi* was most abundant at sites O11 (16.1 km) and O12 (17.52 km), at maximum salinities of 0.2 and 0.4, where it dominated the faunal community (74.3 and 98.5% respectively) (Figure 5.22). The decapod *Palaemon longirostris* was recorded at 6.35 km (O3) and in abundance at 11.31 km (O6), at a maximum mesohaline salinity of 16 and minimum tidal limnetic salinity of 0.2 (Figure 5.22).

The majority of euryhaline-marine species (0.5 – 35) were recorded up to 6.35 km (O3) from the river mouth at Newhaven, at a euhaline maximum salinity of 35 and tidal limnetic minimum salinity of 0.2 (Figure 5.22, Figure 5.23a, Figure 5.24a). The amphipod *Corophium arenarium* along with the polychaete *Hediste diversicolor* were recorded up to 11.31 km (O6) from the river.
mouth, at a mesohaline maximum salinity of 16 and tidal limnetic minimum salinity of 0.2 (Figure 5.22). The malacostracan *Heterotanais oerstedti* and the decapods *Crangon crangon* and *Palaemon elegans* were recorded furthest upstream at 12.8 km from the river mouth (O7) and experienced a mesohaline maximum salinity of 10.3 with a tidal limnetic minimum salinity of 0.2 (Figure 5.22).

Under low freshwater discharge conditions, only three brackish water species were recorded in the River Ouse, the amphipod *Leptocheirus pilosus*, the isopod *Cyathura carinata* and the mysid shrimp *Neomysis integer*. The isopod *Cyathura carinata* was recorded over the largest transition, from 1.67 (O1) to 13.44 km (O8) from the river mouth, experiencing a maximum salinity gradient of 7.9 to 35 and minimum salinity gradient of 0.2 to 25.9 (Figure 5.22). The mysid shrimp *Neomysis integer* was only recorded at site O11, 16.1 km from the river mouth, at a tidal limnetic maximum salinity of 0.4 and minimum salinity of 0.2 (Figure 5.22).

Only two euryhaline-limnic (0 – 10) species were recorded in the River Ouse under low freshwater discharge conditions, the gastropod mollusc *Potamopyrgus antipodarum* and the odonata *Ischnura elegans*. *P. antipodarum* was recorded from 13.44 (O8) to 16.1 km (O11) from the river mouth, experiencing a maximum salinity range of 0.4 to 7.9 and tidal limnetic (<0.5) minimum salinities (Figure 5.22). The high abundance of *P. antipodarum* at site O9 (15.03 km) dominated the faunal community (93.6%). *Ischnura elegans* was only recorded above the tidal limit, at the non-tidal limnetic (<0.5) site O14 (22.37 km; Figure 5.22).

The majority of limnic, salt tolerating species (0 - <5) were recorded in the non-tidal limnetic sites (O14 – O16), 22.37 to 47.16 km from the river mouth at Newhaven (Figure 5.22, Figure 5.23a, Figure 5.24 a). The hirudineans *Erpobdella octoculata* and *E. testacea* were recorded furthest downstream. *Erpobdella octoculata* was recorded at 15.03 km (O9) from the river mouth, at a maximum oligohaline salinity of 3.3 and a tidal limnetic minimum salinity of 0.2 (Figure 5.22). *Erpobdella testacea* was recorded at 16.1 km (O11) from the river mouth, at a tidal limnetic maximum salinity of 0.4 and minimum salinity of 0.19 (Figure 5.22). The isopod *Asellus aquaticus*, odonata *Calopteryx splendens* and trichopteran *Cyrnus trimaculatus* were recorded in the tidal limnetic site O13 (0.1), 20.39 km from the river mouth at Newhaven (Figure 5.22).

The majority of limnic species in the River Ouse were recorded in the non-tidal limnetic sites O14 to O16, 22.37 – 47.16 km from the river mouth at Newhaven (Figure 5.22, Figure 5.23a,
The ephemeropterans *Centroptilum luteolum* and *Ephemera vulgata* were the exceptions to this rule and were only recorded in the tidal limnetic (0.17) site O13, 20.39 km from the river mouth (Figure 5.22).

### 5.4.4.7 Species distributions and salinity under high freshwater discharge conditions (1.43 m$^3$s$^{-1}$, February 2009)

Under high freshwater discharge conditions, holeury-haline species (0 – 35) were recorded from 1.67 (O1) to 17.52 km (O12) from the river mouth at Newhaven, covering a maximum salinity range of 0.2 to 35 (Figure 5.22, Figure 5.23b, Figure 5.24b). The decapod *Palaemon longirostris* was recorded from 1.67 (O1) to 6.35 km (O3) from the river mouth, at a stable maximum salinity of 35 and a minimum salinity gradient of 0.2 to 25.8 (Figure 5.22). The amphipod *Gammarus zaddachi* was recorded in high abundances from 8.7 (O4) to 17.52 km (O12) from the river mouth at Newhaven, experiencing a maximum salinity gradient of 0.2 to 24.1 and tidal limnetic (<0.5) minimum salinities (Figure 5.22). *G. zaddachi* dominated the faunal community at a number of sites, including O4 (91.3%), O8 (94.2%) and O12 (56.3%) (Figure 5.22).

The majority of euryhaline-marine species (0.5 – 35) were recorded up to 6.35 km (O3) from the river mouth at Newhaven, at a euhaline maximum salinity of 35 and tidal limnetic minimum salinity of 0.2 (Figure 5.22, Figure 5.23b, Figure 5.24b). The amphipod *Corophium arenarium* was recorded furthest upstream, from 3.68 (O2) to 13.44 km (O8) from the river mouth, at an oligohaline maximum salinity of 2.1 and a tidal limnetic minimum salinity (0.19; Figure 5.22). The errant polychaete *Hediste diversicolor* was recorded up to 11.31 km (O6), to a maximum salinity of 12 and a tidal limnetic minimum salinity of 0.2 (Figure 5.22).

Under high freshwater discharge conditions, only two brackish water species were recorded in the River Ouse, the isopod *Cyathura carinata* and the amphipod *Leptocheirus pilosus*. *L. pilosus* was recorded from 1.67 (O1) to 9.18 km (O5) from the river mouth, a maximum salinity gradient of 21.9 to 35 and minimum salinity gradient of 0.2 to 25.8 (Figure 5.22). *C. carinata* was recorded from 3.68 (O2) to 12.8 km (O7) from the river mouth, a maximum salinity gradient of 5.1 to 35 and minimum salinity gradient of 0.2 to 35 (Figure 5.22). *C. carinata* was most abundant and dominated the faunal community at sites O6 (11.31 km) (71.9%) and O7 (12.8 km) (46.4%), at maximum salinities of 12 and 5.1 and tidal limnetic minimum salinities of 0.2 (Figure 5.22).
The gastropod mollusc *Potamopyrgus antipodarum* had the greatest downstream distribution of all the recorded euryhaline-limnic species (Figure 5.22). *P. antipodarum* was recorded from 17.52 (O12) down to 11.31 km (O6) from the river mouth, experiencing a maximum salinity gradient of 0.2 to 12 and tidal limnetic (<0.5) minimum salinities (Figure 5.22). A single odonata *Pyrrhosoma nympha* was recorded at the tidal limnetic (0.2) site O12 (17.52 km) and a single *Ischnura elegans* was recorded in the non-tidal limnetic (0.1) site O14 (22.37 km) (Figure 5.22).

Under high freshwater discharge conditions, the isopod *Asellus aquaticus* had the greatest downstream range of all the recorded limnic, salt-tolerating species, being recorded at 13.44 km (O8) from the river mouth (Figure 5.22). At this site *A. aquaticus* experienced an oligohaline maximum salinity of 2.1 and a tidal limnetic minimum salinity of 0.19. As under low freshwater discharge conditions, the hirudinean *Erpobdella octoculata* was recorded 15.03 km (O9) from the river mouth, at a tidal limnetic maximum salinity of 0.45 and minimum salinity of 0.19 (Figure 5.22). A single trichopteran *Polycentropus flavomaculatus* was also recorded at this site. The remaining limnic salt tolerating species were all recorded in the tidal (O11 – O13) and non-tidal (O14 – O16) limnetic sites (<0.5), but were recorded in greatest abundance in the non-tidal limnetic sites above the tidal limits (Figure 5.22). Under high freshwater discharge conditions, all limnic species (with the exception of the trichopteran *Limnephilus flavicornis*) were recorded in the non-tidal limnetic sites O15 and O16, 28.22 to 47.16 km from the river mouth at Newhaven (Figure 5.22, Figure 5.23b, Figure 5.24b). A single *Limnephilus flavicornis* was recorded at site O11, 16.1 km from the river mouth, in tidal limnetic maximum (0.2) and minimum (0.18) salinities.
Figure 5.22. Summary diagram of species distributions, maximum salinity and maximum salinity zones under high and low freshwater discharge regimes with distance from the river mouth at Newhaven. Species shown occur >2% in one or more samples and are separated into salinity tolerance groupings following a review of published literature (Table 5.5).
5.4.4.8 Faunal zones and salinity

Under low freshwater discharge regimes, euryhaline-marine species (e.g. *Carcinus maenas*, *Gammarus salinus*, *Melita palmata*) were recorded up to 12.8 km (O7) from the river mouth at Newhaven, and together with brackish water species (e.g. *Cyathura carinata*, *Leptocheirus pilosus*), dominated the faunal community, covering a maximum salinity gradient of 10.3 to 35 (Figure 5.24, Figure 5.23a). At 13.44 km (O8) the faunal community shifted to a holeury-haline (*Gammarus zaddachi*) and brackish water (*Cyathura carinata*) dominant fauna, with the addition of the euryhaline-limnic gastropod mollusc *Potamopyrgus antipodarum* (Figure 5.22, Figure 5.24a). This shift from marine derived species to those more tolerant of brackish and
freshwaters, was highlighted with a significant zone split at 13.12 km from the river mouth (zone 1) (Figure 5.24a). Zone 1 covered a maximum salinity gradient of 10.3 to 35 (Figure 5.24a). From 13.44 (O8) to 20.39 km (O13) (0.1 – 7.9), the faunal community consisted of holeuryhaline, brackish and limnic derived species, but was dominated by the holeury-haline amphipod *Gammarus zaddachi* and the euryhaline limnic *Potamopyrgus antipodarum* (Figure 5.22, Figure 5.24a). This community shifted to a limnic fauna above the tidal limit (21.8 NTL), represented by a significant zone split at 21.38 km (0.1) (zone 2) (Figure 5.24a). Zone 2 covered a maximum salinity gradient of 0.1 to 7.9. Zone 3 (21.38 – 47.16 km) consisted of entirely limnic fauna over a tidal limnetic maximum salinity of 0.1 (Figure 5.24a).

Under high freshwater discharge conditions, euryhaline-marine and brackish water species dominated the faunal community up to 6.35 km (O3) from the river mouth, over a stable maximum salinity of 35 and a minimum salinity gradient of 0.2 to 25.8 (Figure 5.24b, Figure 5.23b). At 8.7 km (O4) (24.1) the faunal community shifted from mainly marine and brackish water species, to a community dominated by the holeury-haline amphipod *Gammarus zaddachi*, represented by a significant zone split at 7.52 km from the river mouth (zone 1; Figure 5.24b). Zone 1 covered a maximum salinity gradient of 25 to 35 (Figure 5.24b). *Gammarus zaddachi* dominance continued from 8.7 km (O4) to 13.44 km (O8) from the river mouth, over a maximum salinity gradient of 2.1 to 24.1 (Figure 5.22, Figure 5.24b). At 13.44 km (O8) euryhaline-limnic and limnic salt tolerating species were recorded in the faunal community (in addition to *Gammarus zaddachi*) and increased in abundance and dominance up to 17.52 km (O12) from the river mouth, covering a maximum salinity gradient of 0.1 to 2.1 (Figure 5.22, Figure 5.24b). At 20.39 km (O13) the faunal community consisted entirely of limnic salt tolerating species (100%) marked by a significant zone split at 18.95 km (zone 2; Figure 5.22, Figure 5.24b). Zone 2 (7.52 – 18.95 km) covered a maximum salinity gradient of 0.2 to 24.1 (Figure 5.24). The faunal community in zone 3 (18.95 – 47.16 km) consisted of limnic derived species, at a stable tidal and non-tidal limnetic salinity of 0.1 (Figure 5.22, Figure 5.24b, Figure 5.23b).
Figure 5.24. Summary diagram of salinity parameters (maximum, minimum and range) and associated maximum salinity zones, relative abundance of salinity tolerance groups (silhouette graph) and significant faunal data zone splits with distance from the river mouth at Newhaven, under low (a) and high (b) freshwater discharge conditions.
5.4.4.9 Diversity indices and salinity

Under both low and high discharge conditions, faunal diversity (Shannon-Weiner H’ index), decreased from the head of the estuary (freshwater) downstream and from the river mouth (marine) upstream, with the lowest diversity recorded in the mid to upper estuary (Figure 5.25). This was coupled with an increase in species dominance (Berger-Parker index), peaking in the mid to upper estuary (Figure 5.25). Under high discharge conditions, this pattern was altered by site O4 (8.7 km), where extremely large abundances of *Gammarus zaddachi* were recorded (520 individuals) during sampling, significantly skewing the diversity and dominance indices (Figure 5.25b).

Under low discharge conditions, the lowest diversity and consequently the highest dominance was recorded from 15.03 (O9) to 17.51 km (O12) from the river mouth at Newhaven, over a oligohaline maximum salinity gradient of 0.2 to 3.3 and tidal limnetic minimum salinities of 0.19 to 0.2 (Figure 5.25a). In this low diversity/high dominance zone, the number of recorded species was low, but relative abundance was high (Figure 5.25a). Under high discharge conditions, (after the removal of site O4), the lowest diversity and highest dominance in the faunal data set was recorded from 12.8 (O7) to 15.03 km (O9) from the river mouth, over a maximum salinity range of 3.3 to 10.3 and minimum salinity of 0.2 (Figure 5.25a). Under both discharge conditions, relative abundance of macroinvertebrates was generally high along the estuary course, but decreased in the tidal limnetic zones (Figure 5.25).
5.4.4.10 Faunal data and salinity summary

Under both high and low discharge conditions, a strong correlation was observed between species distributions, their allocated salinity tolerance groupings and the recorded maximum salinity (Figure 5.22, Figure 5.23). However, as in the River Adur, poor correlation was observed between distributions, tolerance groupings and minimum salinities, with euryhaline-marine and brackish water species recorded in sites which ran tidal limnetic at low tide (<0.5)
(e.g. *Carcinus maenas*, *Gammarus salinus*, *Melita palmata*, *Leptocheirus pilosus*), again potentially indicating tolerance to tidal limnetic conditions and/or the ability to seek refuge (Figure 5.22). In contrast to the River Adur, the brackish water (0.5 - 30) mysid shrimp *Neomysis integer* was the only species in the River Ouse recorded outside of its allocated salinity zone (e.g. site O11, at a maximum salinity of 0.4) (Figure 5.22). Although not observed at the limits of their salinity tolerance, the limnic ephemeropterans *Centroptilum luteolum* and *Ephemera danica* and trichopteran *Limnephilus flavicornis* were recorded in tidal limnetic conditions below the limits of tide (O13), where they were subject to significant physiochemical shifts over a tidal cycle, which could indicate an adaptability and/or tolerance to tidal conditions (Figure 5.22).

Under low freshwater discharge conditions (August 2008) saline water penetrated up to 16.06 km from the river mouth at Newhaven, compared to 14.91 km under high freshwater discharge conditions (February 2009) (see Chapter 4). The macroinvertebrate community structure of the River Ouse estuary appeared to change in relation to the degree of saline penetration, with most salinity groupings recording a downstream shift in distribution under high freshwater discharge conditions when compared to low flows. This was represented in the faunal data with downstream shifts in significant faunal zones under high discharge conditions, compared to low flows (Figure 5.22, Figure 5.24). Under low discharge conditions, euryhaline-marine species were recorded up to 12.8 km from the river mouth at Newhaven, compared to 11.31 km under high discharge conditions, a downstream shift of 1.49 km (Figure 5.24 a). A single euryhaline-marine amphipod *Corophium arenarium* was however recorded at 13.44 km (O8), but was an outlier from the general trend. The majority of brackish water species were recorded up to 13.44 km (O8) from the river mouth under low discharge conditions, with the motile mysid shrimp *Neomysis integer* observed a further 2.66 km upstream (16.1 km, O11) (Figure 5.22, Figure 5.24 a). Under high discharge conditions, the distribution of brackish species had shifted downstream by 0.64 km to 12.8 km from the river mouth at Newhaven (Figure 5.22, Figure 5.24 b). A shift was also observed in the distributions of limnic derived species, with euryhaline-limnic species extending their range downstream by 4.85 km (after the removal of *P. antipodarum*) and limnic salt tolerating species by 1.59 km under high discharge conditions when compared to low (Figure 5.24). This downstream shift in limnic-derived faunal data was represented with a significant zone 3 split at 18.95 km compared to 21.38 km under low discharge conditions (Figure 5.24). The majority of limnic species did not exhibit a downstream shift in distribution between discharge regimes, with the exception of a single trichopteran *Limnephilus flavicornis* recorded at 16.1 km (O11) under high discharge conditions compared to 22.37 km (O14) under
low flows (Figure 5.22, Figure 5.24). As recorded in the River Adur estuary, a downstream shift was recorded in the distribution of the holeury-haline amphipod *Gammarus zaddachi* under high freshwater discharge conditions, from a range of 12.8 to 20.39 km under low discharge conditions to 8.7 to 17.52 km under high discharge conditions (Figure 5.22). It is again likely that this downstream distribution shift was forced by the downstream salinity-related shifts of competing sibling species, e.g. the downstream shifts in distributions of the limnic salt tolerating *Gammarus pulex* (from 22.37 to 20.39 km under high discharge conditions) (Figure 5.22).

Under low discharge conditions the lowest diversity and highest dominance in the faunal community of the River Ouse estuary was recorded from 15.03 to 17.52 km from the river mouth, over a maximum salinity gradient of 0.2 to 3.3, compared to 12.8 to 15.03 km and a maximum salinity gradient of 0.45 to 5.1 under high discharge conditions (Figure 5.25).

### 5.5 Discussion

Salinity has been shown to be one of the main environmental variables driving species distributions in estuaries (Bulger *et al.*, 1993; Remane & Schlieper, 1971; Attrill, 2002). Where variations in the degree of saline penetration have been recorded as a result of changes in freshwater riverine output, shifts in the distributions of macroinvertebrate species have been observed (Attrill, 2002; Herborg *et al.*, 2005; Bessa *et al.*, 2010; Kingsford *et al.*, 2011). By allocating salinity tolerance groupings to recorded macroinvertebrate species in the River Adur and River Ouse over both low and high freshwater discharge regimes, it has been possible to relate both salinity tolerance group and individual species distributions and diversity to changes in salinity and other recorded environmental parameters. These salinity groupings were also used to assess the value of species distributions alone in determining the upstream extents of saline penetration into the River Adur and River Ouse estuaries in the absence of salinity measurements (as in Chapter 3). It was hypothesised that determination of the upstream extent of saline penetration using macroinvertebrate salinity tolerance groupings would closely correspond to the measured extents of saline penetration recorded in chapter 4. Based on the faunal data from the River Adur and River Ouse estuaries and compared with the actual upstream extents of saline penetration (Chapter 4), this approach produced reasonably accurate results. This approach could enable researchers to make approximate determinations of past saline penetration profiles, based on historic macroinvertebrate data in the absence of salinity measurements. However, care should be taken, as this approach was shown to be dependant
upon the number and distribution of sampling sites, particularly at the upstream extent of saline penetration.

The benthic macroinvertebrates of the River Adur and River Ouse were shown to be broadly comparable in terms of recorded species, community structure and response to a range of environmental variables. In this chapter it was hypothesised that whilst additional environmental variables will be important in determining macroinvertebrate compositions in estuaries, salinity will be the dominant parameter driving faunal structure and species distributions over the freshwater to marine transition. It is also hypothesised that spatial variability in the extent of saline penetration (under low and high freshwater discharge conditions) will be associated with shifts in the distributions of benthic macroinvertebrate species relative to their salinity tolerance. In this study, salinity has been shown to be the dominant environmental variable driving faunal structure and species distributions in the both the River Adur and River Ouse estuaries, with the recorded decrease in saline penetration between low and high freshwater discharge conditions resulting in a shift in the distribution and structure of the macroinvertebrate community.

However, in contrast to this general pattern, the distributions of euryhaline-marine (0.5 – 35) and brackish water (0.5 – 30) species in the River Adur estuary, did not change in relation to the degree of saline penetration, despite a decrease in saline penetration range of 3.73 km and drop in maximum salinity of 18.86 (at 10.64 km) from low to high freshwater discharge conditions. Under both discharge conditions, euryhaline-marine and brackish water species (with the exception of Corophium multisetosum) were recorded up to 10.64 km from the river mouth at Shoreham, despite the ability of these species to tolerate maximum salinities up to 6.34 km (under low discharge conditions) further upstream. Beyond 10.64 km from the river mouth, the sediment of the estuary channel changes from coarse grain size (>60 mm) to fine (<60 mm), which may have acted as a barrier to further penetration of euryhaline-marine and brackish water species upstream. The amphipod C. multisetosum was the only brackish water species recorded beyond the 10.64 km limit, in fine grained sediment sites, which may be due to its ability to inhabit both fine and coarse grained sediments (Barnes, 1994; Hayward & Ryland, 1995). As no shift in distribution was recorded between high and low discharge conditions, it can be assumed that the euryhaline-marine and brackish species recorded up to 10.64 km were not at their maximum upstream penetration range based on salinity. It therefore appears that the sediment characteristics (coarse grain size) of the lower River Adur estuary are of more local importance than maximum salinity in determining the distributions of euryhaline-marine and brackish water species.
In the River Ouse estuary, the decrease in saline penetration under high discharge conditions compared to low was recorded in a downstream shift of euryhaline-marine and brackish water species. However in addition to salinity, the distribution of euryhaline-marine and brackish water species may also have been partially dependant on the sediment characteristics of the estuary channel, with euryhaline-marine and the majority of brackish water species only recorded at sites with coarse grained sediment (>60 mm) (with the exception of the mud dwelling brackish isopod *Cyathura carinata* and mysid shrimp *Neomysis integer*). Due to human modification of the lower River Ouse estuary channel (pole-wharfing), coarse sediment extends up to 12.8 km from the river mouth at Newhaven. As observed in the River Adur estuary, coarse sediment (>60 mm) may provide preferential habitat for euryhaline-marine and brackish water species, enabling the migration and colonisation of these species upstream during high salinity/low freshwater discharge conditions and downstream during low salinity/high freshwater discharge conditions. In contrast to the River Adur, the (human-influenced) extension of coarse grained sediment in the River Ouse estuary, made it possible to record the downstream shift in distribution of euryhaline-marine and brackish water species with relation to decreases in the degree of saline penetration (under high freshwater discharge conditions). Under low discharge conditions, however, euryhaline-marine and the majority of brackish water species were recorded up to the limit of coarse grained sediment (12.8 km). Due to the effective discontinuity in habitat at 12.8 km (from coarse (>60 mm) to fine (<60 mm) grained sediment), it is not possible to relate the upstream penetration distance of euryhaline-marine species under low discharge conditions to salinity.

The strong association between coarse sediment grain size (>60 mm) and the distribution of euryhaline-marine and brackish water species in the River Ouse and River Adur estuaries is likely due to the capacity of the sediment to act as a refuge for benthic dwelling macroinvertebrates from tidal limnetic minimum salinities (<0.5). This would explain the penetration of these species, which are not tolerant of limnetic salinities, into sections of the estuary which ran tidal limnetic at low tide. In tidal estuaries, where the proportions of sea water and freshwater inflow vary considerably depending on the state of the tide, the interstitial salinity of the underlying sediment has been shown to be far less variable than that of the overlying water, with the high tide (maximum salinity) water more effective in determining the sediment salinity than the low tide (minimum salinity) water (McLusky, 1968a; Wolff, 1973, Sanders et al, 1965; Chapman 1981). The large tidal ranges in the lower River Adur and River Ouse estuaries (5.5 and 6.1 m), expose the intertidal estuary banks at low tide, where surface dwelling euryhaline-marine and brackish water species have been observed (personal observation).
partially buried in the sediment under stones and cobbles (e.g. *Gammarus salinus, Melita palmata, Carcinus maenas* and *Leptocheirus pilosus*). It is therefore likely that the coarse grained sediment acts as a refuge for these species in the intertidal zone during low tide, potentially providing access to high interstitial salinities within the sediment and protection from aerial exposure, desiccation and potential predators (e.g. wading birds) (Fischenich, 2003). In addition to the refuge potential of coarse grain sizes, these sediments yielded a high calcium carbonate content (from both the natural chalk geology, the addition of chalk riprap and potentially marine shell fragments), which could also help to explain the penetration of these species into sites with low and tidal limnic minimum salinities. Studies have shown that in low total salt concentrations, high proportions of calcium may exert a stabilising effect on protein structures and metabolic processes and may even counteract tissue effects of low salinities, resulting in an overall increase in salinity tolerance (Kinne, 1971). In the River Adur estuary, the euryhaline-marine and brackish water species distributions cover a sediment calcium carbonate content of 9 to 31% compared to 16 to 72% in the pole-wharfed River Ouse estuary. It is not known if the high sediment calcium carbonate contents at these sites affect the salinity tolerances of the recorded benthic macroinvertebrates in this way and is an issue that warrants further study. It is likely that due to the refuge potential of coarse (>60 mm) sediment (and potentially the high calcium carbonate content), euryhaline-marine and brackish species can inhabit sections of estuary that would otherwise be outside their tolerance zone (tidal limnetic at low tide). The interstitial salinity of intertidal sediments has been shown to vary with season and large-scale changes in river flow (Wolff, 1973; Chapman & Brinkhurst, 1981). It could therefore be assumed that the downstream shift in euryhaline-marine and brackish water species recorded in the River Ouse under high discharge conditions, could be indicative of both a reduction in the maximum salinity of the overlying water (at high tide), but also a reduction in the interstitial salinity of the sediment used as refuge.

Under both low and high freshwater discharge conditions, the upper to tidal fresh sections of both the River Ouse and River Adur estuaries were the least diverse, dominated by large abundances of a small number of ‘hardy’ species, including the holeury-haline amphipod *Gammarus zaddachi*, the brackish water amphipod *Corophium multisetosum*, the isopod *Cyathura carinata* and the gastropod mollusc *Potamopyrgus antipodarum*. The brackish water (0.5 - 30) amphipod *C. multisetosum* and euryhaline-limnic (0 – 10) gastropod mollusc *P. antipodarum* were recorded in salinities outside of their salinity tolerance grouping, with *C. multisetosum* recorded in tidal limnetic (<0.5) and *P. antipodarum* recorded in polyhaline maximum salinities (20). In addition to wide salinity tolerances, these species can inhabit both
coarse (>60 mm) and fine (<60 mm) grained sediment sites, and as such transcend the sediment habitat boundaries that limit other species distributions (e.g. euryhaline-marine and brackish water species). This appears to be the case for the brackish isopod *Cyathura carinata*, whilst not recorded outside of its respective salinity tolerance grouping (0.5 – 30), its ability to inhabit both coarse and fine grained sediment sites may enable this species to penetrate beyond the 12.8 km coarse sediment range limit (in the River Ouse estuary), in contrast to its fellow brackish water and euryhaline-marine species. It therefore appears that the ability to withstand large changes in salinity (from marine to fresh) and/or having the ability to inhabit sites with markedly different sediment characteristics (coarse to fine) are the attributes necessary to inhabit the upper to tidal fresh estuarine zones in the River Adur and River Ouse.

In the River Adur estuary, the zone of lowest faunal diversity and highest dominance (14.06 to 17.88 km) did not shift in location in relation to the degree of saline penetration and was recorded over a maximum salinity range of 0.41 to 0.8 under low discharge conditions and from 0.27 – 0.3 under high. This zone was situated on the environmental transition between the lower and upper estuary, from the salt marsh vegetation, macroalgal cover, coarse grained sediment (>60 mm), high calcium carbonate and low organic content of the lower estuary (0.32 to 10.64 km), to freshwater emergent macrophytes, fine grained sediment, reduced calcium carbonate and high organic content typical of the upper estuary (14.06 to 21 km). As no shift in the distribution (or location) of this zone was recorded, despite a large shift in saline penetration range between discharge regimes (4.75 km), it can be hypothesised that the change in other environmental parameters than salinity between the upper and lower River Adur estuary were of greater importance in determining the diversity and dominance of the mid to upper estuarine faunal community. As previously highlighted, the shift in sediment grain size (from coarse to fine) over this transition appears to exert a strong influence on the distribution limits of euryhaline-marine and brackish water species upstream. It is therefore likely that the change in sediment characteristics (potentially with the addition of other environmental parameters) has a large impact on the diversity and dominance of the faunal community of the mid to upper River Adur estuary. This is highlighted by the significant correlation between the diversity (H’) and dominance (BP) indices with sediment grain size parameters (under high discharge conditions) (Table 1.16).

In the River Ouse estuary, the zone of lowest faunal diversity and highest dominance did appear to shift downstream in relation to the degree of saline penetration, from 15.03 to 17.52 km under low discharge conditions to 12.8 to 15.03 km under high discharge conditions. The low
diversity, high dominance zones under both discharge regimes were located around the freshwater seawater interface (FSI), over a maximum salinity gradient of 0.2 to 3.3 under high discharge conditions, and 0.45 to 5.1 under low discharge conditions. The FSI has been highlighted as a key site for physical, chemical and biological interactions within the water column, but is not typically associated with zones of lowest diversity (Uncles, 2003; McLusky & Elliott, 2004, Deaton & Greenberg, 1986; Rundle et al., 1998). These zones have been predicted to exist from 5 – 8 (often termed the area of “critical salinity” or horohalinicum), which is believed to represent an ecophysiological boundary at the point at which limnic species become intolerant to increases in salinity and only a few euryhaline-marine and brackish species can survive (Remane & Schlieper, 1971; Wolff, 1973; Deaton & Greenberg, 1986; McLusky & Elliott, 2004; Telesh & Khlebovich, 2010). With the exception of P. antipodarum, no euryhaline-limnic (0 – 10) and only small numbers of limnic salt tolerating species (0 - <5) were recorded in the low diversity/FSI zones, despite being within these species salinity tolerance capacity. This may be due to the fluctuating physiochemical conditions experienced at the FSI over a tidal cycle, compared to the relatively stable tidal limnetic sites, indicative of larger numbers of these species, further up the estuary course (Uncles & Stephens, 1993). Studies have shown that in addition to varying salinities, increased turbidity, decreased oxygen content and changes in sediment type, the biogeochemical processes that occur at the FSI (from the meeting of freshwater and seawater), include changes in the compositional ratios of the major ions (sodium, chloride, calcium) at salinities of around 1 - 2 (Uncles, 2003; McLusky, 1993). It appears likely that a combination of these fluctuating physiochemical conditions at the FSI results in the low diversity zones recorded in the River Adur and River Ouse estuaries.

A downstream shift in the distribution of euryhaline-limnic (0 – 10) and limnic, salt-tolerating (0 - <5) species was recorded in both the River Adur and River Ouse estuaries under high discharge conditions compared to low, indicating a distribution response of freshwater derived species to salinity, which has been reported in many estuarine studies (Attrill, 1998; Rundle et al., 1998; Attrill, 2002; Martinho et al., 2007; Sousa et al., 2007). The limnic, salt tolerating species that were recorded in these FSI zones, and appeared to tolerate tidal conditions (in both the River Adur and River Ouse estuaries), included the isopod Asellus aquaticus, the hirudineans Erpobdella octoculata and Erpobdella testacea, the megalopteran Sialis lutaria and the trichopteran Polycentropus flavomaculatus. It may therefore be possible to use the downstream distributions of these species to help determine the location of the FSI zone and the upstream limit of saline penetration in the absence of salinity measurements or profiles. Due to the role of sediment type as a determinant of the distribution of euryhaline marine and brackish water
species in the River Adur, and to some degree the River Ouse, the downstream distributions of euryhaline limnic and limnic salt tolerating species may be the only way to determine the degree of saline penetration using macroinvertebrates alone in rivers where substrate varies.

The downstream shift of the limnic salt tolerating amphipod *Gammarus pulex* in relation to the decrease in saline penetration may have caused a downstream shift in distribution of the dominant holeury-haline *Gammarus zaddachi*, due to competition between these sibling species (Gaston, 2001). This would demonstrate a biological secondary distribution-response relationship to salinity, with the holeury-haline *G. zaddachi* pushed downstream due to the extension of the habitable tidal freshwater zone by *G. pulex*. The downstream shift in distribution of *G. zaddachi* may also have resulted in a reduction in upstream range of the competing euryhaline-marine *G. salinus*, although the reduction in range of this species could also have been caused by the reduction in maximum salinities.

The majority of limnic (<0.5) species in the River Adur and River Ouse were recorded above the limit of the tide, in non-tidal limnetic conditions. A small number of limnic species were also recorded in the tidal-limnetic zones of the upper River Adur and River Ouse estuaries, where they experienced large shifts in physicochemical conditions over a tidal cycle, including increased turbidity, oscillating water levels, decreased oxygen content and changing current velocities and directions (Schuchardt *et al.*, 1993; Rundle *et al.*, 1998). These species included the trichopterans *Limnephilus flavicornis*, *Phryganea bipunctata* and *Plectrocnemia conspersa*, the coleopteran *Limnius volckmari*, the ephemeropterans *Centroptilum luteolum* and *Ephemera vulgata* and the odonata *Libella fulva*. The ability of these species to inhabit these tidally-influenced limnetic zones may potentially indicate a degree of tolerance and/or adaptability to fluctuating physicochemical tidal conditions, and consequently low salinities that is not currently highlighted in any published literature. This could be of consequence in relation to future predicted increases in saline penetration, when these limnic derived species could experience both higher maximum salinities and greater salinity ranges.

### 5.6 Summary

- The macroinvertebrate species and communities of the River Adur and River Ouse were broadly comparable, with the exception of the lower mid estuarine zones, where sites on the River Adur estuary were dominated by the amphipod *Corophium multisetosum.*
Assigning salinity tolerance groupings to macroinvertebrate species based on published literature, enabled the approximate determination of the upstream extent of saline penetration in the absence of salinity measurements.

Compared to the well studied marine and brackish water macroinvertebrate species that inhabit the estuarine zone, there is only a limited amount of literature available on the salinity tolerances of limnic derived species recorded both above and below the tidal limit.

Whilst salinity has been shown to be the overriding environmental variable driving species distributions in the both the River Adur and River Ouse estuaries, sediment characteristics (coarse grain size >60 mm) may be of greater importance locally than salinity in determining both the distributions of euryhaline-marine and brackish water species and the zone of lowest faunal diversity in the River Adur estuary.

The coarse grained sediment (>60 mm) of the lower River Adur and River Ouse estuaries (and potentially the high sediment calcium carbonate content) may enable benthic euryhaline-marine and brackish species to inhabit sections of estuary that would otherwise be outside their tolerance zone (tidal limnetic at low tide).

The extension of coarse grained sediment in the River Ouse estuary by pole-wharving may have significantly altered the faunal composition of the lower estuary, by increasing the penetration of surface-dwelling euryhaline-marine and brackish species upstream and likely increasing the abundance and diversity of these sites compared to under natural conditions.

The extended range of coarse sediment in the River Ouse estuary may enable the movement of euryhaline-marine and brackish water species downstream in relation to decreases in saline penetration range. This group, however, is blocked from significant upstream movement (in relation to increases in saline penetration) due to the upstream limit of coarse grained sediment.

The upper to tidal fresh zones of both the River Adur and River Ouse estuaries were dominated by a small number of ‘hardy’ species. It appears that the ability to withstand large ranges of salinity (marine to fresh) and/or having the ability to inhabit sites of varying sediment characteristics (coarse to fine) are the faunal traits necessary to populate the low diversity upper to tidal freshwater zones in the River Adur and River Ouse estuaries.
A distribution-response relationship was observed between euryhaline-limnic and limnic salt tolerating species and salinity, with a downstream shift of these species recorded in response to a decrease in saline penetration in both the River Adur and River Ouse.

In addition to the primary physiological impacts of salinity shifts in relation to species distributions, secondary biological shifts of holohaline-haline species were observed in relation to the increase or decrease in competitive species upstream or downstream ranges.

In the River Ouse estuary, the zone of lowest faunal diversity was located around the fluctuating physiochemical conditions of the freshwater seawater interface (FSI). A number of limnic derived species recorded in the FSI zones from both the River Adur and River Ouse estuaries are highlighted as potential indicators of the FSI zone (e.g. *Asellus aquaticus, Erpobdella octoculata, Erpobdella testacea, Sialis lutaria* and *Polycentropus flavomaculatus*).

The majority of limnic species were recorded above the tidal limits in both the River Adur and River Ouse; however a small number of these species were recorded in the fluctuating physiochemical conditions of the tidal limnetic zone, potentially indicating a degree of tolerance and/or adaptability to fluctuating tidal conditions and consequently low salinities that is not currently considered in published literature.
Chapter 6. Salinity tolerance determination

6.1 Introduction

A review of the salinity tolerances of benthic macroinvertebrate species recorded in this study (see Chapter 4 and 6), has shown that whilst a wealth of literature is available on euryhaline-marine and brackish water species commonly recorded in the estuarine zone (e.g. Kinne, 1971; Lincoln, 1979; Barnes, 1994; Hayward & Ryland, 1995), only a limited amount of literature is available for limnic derived species both above and below the tidal limit (Rundle et al., 1998; Williams & Williams, 1998a; Sousa et al., 2007). Even where a wealth of such information exists (e.g. for euryhaline-marine and brackish water species), the determination of salinity tolerance often differs between studies and authors, making comparisons of the results between investigations difficult. For example, a number of studies report the maximum field salinity at which species were collected in the field (Ettershank et al., 1966; Scudder & Mann, 1968; Knowles & Williams, 1973; Aladin & Potts, 1995; Williams & Williams, 1998b; Bailey & James, 2000; Bailey et al., 2002; Greenwood & Wood, 2003), a method unlikely to provide a robust measure of salinity tolerance (Underwood et al., 2000; Williams, 2009), whilst other investigations have been experimentally based, but undertaken using a range of procedures (e.g. differing salt sources, flow conditions, time periods, life stage, gender and species), which makes the extrapolation and comparison of results between studies difficult and error prone (Williams & Williams, 1998a; Radke et al., 2002; Kefford et al., 2004b; Kefford et al., 2005b; Kefford et al., 2006a; Sornom et al., 2010). It seems likely that in order to attempt any prediction of the impact of increasing saline penetration on estuarine and riverine macroinvertebrate species, a standardised set of experimental procedures need to be developed that will produce directly comparable salinity tolerance results for a range of marine, brackish and limnic derived species.

The effects of salinity upon the physiology of macroinvertebrate species have been investigated using a number of methodologies (K Kapoor, 1979; Colburn, 1983; Stern et al., 1984; Kefford et al., 2007c; Sornom et al., 2010). In the context of this study, the cause and effect relationships between salinity and macroinvertebrate mortality was selected as the most appropriate experimental approach. This selection was guided by the need to establish definitive survival values regardless of the precise physiological mechanisms which determine salinity tolerance or susceptibility. The most common way to quantify a link between salinity and mortality is the concentration of salts that is lethal to 50% of individuals (LC\textsubscript{50} value) over an arbitrary time period of usually 48-96 hours (Kefford et al., 2005b). Whilst providing a link between salinity...
and mortality, these experimental values do not take into account any other environmental and/or biological factors (e.g. sediment dynamics, physiochemical changes, predation, competition etc.) that could co-occur with changes in salinity over a tidal cycle, and so may not therefore be comparable to salinity tolerances under field conditions (Kefferd et al., 2004b; Kefferd et al., 2007c; Mann et al., 2010). It was therefore considered critical that confirmatory studies were conducted under field conditions to corroborate laboratory salinity tolerance results (Mann et al., 2010).

6.1.1 Aims, Objectives and Hypotheses

This chapter aims to determine the acute salinity tolerances (LC$_{50}$) of selected benthic macroinvertebrate species under controlled laboratory conditions for summer (18 °C) and winter (7 °C) water temperature regimes. In addition, to test the survivorship of limnic macroinvertebrate species to actual tidal cycles under field conditions, and to compare the laboratory derived salinity tolerances (LC$_{50}$) with the field tidal cycle survivorships, recorded field distributions and published salinity tolerance literature (Chapter 5). The resultant salinity tolerances will be used to form the basis for predictions of the impact of increasing saline penetration upon the benthic macroinvertebrates of estuaries and rivers. These aims will be achieved through the following objectives:

1. Outline the procedure employed for the selection of suitable benthic macroinvertebrate species for salinity toxicity testing.

2. Outline the salinity toxicity test methodology including the design and construction of the laboratory test facility and the procedure used to determine the salinity tolerances of the selected test species (under both summer and winter temperature regimes: 18 °C and 7 °C respectively).

3. Describe the results of the salinity toxicity tests (after determination of salinity tolerance values: LC$_{X}$) of the test species and compare with both the allocated salinity tolerance groupings (based on published literature; Chapter 5) and the salinities at which these species were recorded in the River Adur and River Ouse (August 2008 and February 2009; Chapter 5).

4. Outline the methodology used to test survival rates of selected limnic macroinvertebrate species to a range of salinities under natural tidal cycle conditions in the River Ouse estuary (including design and construction of the field test cells).

5. Describe the results of the field tidal cycle tests and compare/contrast these results with salinity tolerance values derived under laboratory conditions (LC$_{X}$).

6. Compare the results of the laboratory and field tests with published literature for benthic macroinvertebrate species, discuss relationships between salinity tolerances and recorded field
distributions, and assess the use of benthic macroinvertebrate salinity tolerances as predictors of ecological change to predicted future increases in saline penetration.

It is hypothesised that the laboratory acute salinity tolerances (LC₅₀) of benthic macroinvertebrate species will be reduced at high temperatures (18 °C) compared to lower temperatures (7 °C). It is also hypothesised that laboratory acute salinity tolerances (LC₅₀) of a species will correspond to its recorded survivorship under field tidal cycle conditions, and that these results will reflect the recorded field distributions and salinity tolerances reported in published literature (Chapter 5).

6.2 Test species

In order to predict community level responses of estuarine and riverine macroinvertebrates to increasing saline penetration, it was firstly necessary to investigate the salinity tolerances of individual selected macroinvertebrate species. Whilst increases in saline penetration are likely to have the biggest impact on the freshwater macroinvertebrates of upper tidal, to non tidal freshwaters, shifts in the macroinvertebrates of the whole estuarine transition might be expected (Andrews, 1977; Attrill et al., 1996; Attrill & Power, 2000; Bessa et al., 2010; Tills et al., 2010).

It was therefore necessary to test the acute salinity tolerances of a range of macroinvertebrate species, classified as euryhaline-marine (0.5 – 35) through to limnic (<0.5) (see Chapter 5).

Macroinvertebrate species were selected that were (1) common, (2) widespread, (3) frequently occurring at high densities, (4) easy to collect, (5) frequently used as salinity or fresh water indicators, (6) easy to identify to species level, (7) considered important in ecosystem structure/function and (8) considered as an important food resource for fish, bird and/or amphibian species.

1. Gammarus salinus and 2. Gammarus zaddachi (Crustacea: Amphipoda)

Gammarus zaddachi and Gammarus salinus are sibling species of amphipod of the genus Gammaridae (Sexton, 1942). Both species are common and abundant in estuarine environments, although they are seldom recorded together, often partitioning to a given habitat along a salinity gradient (Lincoln, 1979). Gammarus zaddachi is more tolerant of fresh water and less sensitive to low oxygen concentrations and aerial exposure than Gammarus salinus, therefore G. salinus is most frequently recorded in brackish and marine habitats (2 - 35) whereas G. zaddachi is often observed in fresh water at or near high tide spring tide level (0 - 35) (Hartog, 1964; Lincoln, 1979; Bulnheim, 1984; Kolding, 1985; Gaston & Spicer, 2001; Korpinen & Westerbom, 2009). This habitat partitioning is also suggested by a number of authors to avoid abortive hybridization between these sibling species (Kolding, 1981; Kolding & Fenchel, 1981; Gledhill et al., 1993).
In the stable salinity of the Baltic Sea, both species often occur together in coastal habitats and the separation of breeding populations is achieved by migrating between zones of algae from the genus *Ceramium, Cladophora* and *Fucus* (Bulnheim & Scholl, 1981; Kolding, 1981). In estuarine environments, *G. salinus* and *G. zaddachi* experience regular periods of immersion and emersion (Hayward & Ryland, 1995). At low tide, in order to avoid the effects of desiccation, these species have been observed seeking refuge amongst vegetation, under pebbles/rocks or loosely burrowed into the surface of the substratum (Lincoln, 1979; Hayward & Ryland, 1995). In Britain both *G. zaddachi* and *G. salinus* reproduce throughout the year, with two main generations in summer and winter, each producing 2 – 5 broods. Life spans vary between 8 and 15 months (Lincoln, 1979; Gledhill *et al.*, 1993). Both of these species are omnivorous and play an important role in the breakdown of algal material. They are also important prey items for a number of fish and waterfowl (Moore & Moore, 1976).

3. *Gammarus pulex* (Crustacea: Amphipoda)

*Gammarus pulex* is a limnic amphipod of the genus Gammaridae (Gledhill *et al.*, 1993). It is a common, widespread and a locally abundant inhabitant of British inland waters, occurring in both standing and running water habitats (Gledhill *et al.*, 1993). In these habitats, this species often becomes the dominant macroinvertebrate in terms of biomass (Macneil *et al.*, 1997).

*G. pulex* is sensitive to a wide range of pollutants, but is normally absent from acidic waters where the pH is below 5.7 (Sutcliffe & Carrick, 1973a, 1973b; Scullion & Edwards, 1980). This species can tolerate low oxygen concentrations when coupled with low water temperatures, but is more often observed in well-oxygenated localities and temperatures below 20 °C (Grant & Hawkes, 1982; Sutcliffe, 1984). In this study, *G. pulex* is classified as a limnic salt tolerating species (0 - <5), as it has occasionally been observed in dilute estuarine or brackish waters, at salinities less than 6 (Barnes, 1994). A recent study into the acute 72-hour toxicity of *G. pulex* to sea salt determined an LC$_{50}$ of 12.8 ± 1.3, a tolerance much higher than expected for a limnic amphipod (Piscart *et al.*, 2010).

*Gammarus pulex* can reproduce throughout the year, but has a pronounced peak of activity in spring and early summer which is reduced or paused from October to December. The life-span is 1-2 years, with females producing up to 6 broods (Gledhill *et al.*, 1993). *G. pulex* has an important ecological role in limnic aquatic systems as shredders of leaf material, which they consume as detritivores, although they are in fact omnivorous and have strong carnivorous tendencies (Willoughby & Sutcliffe, 1976; Sutcliffe *et al.*, 1981; Little *et al.*, 2006). This wide
diet or ‘foraging plasticity’ is linked to the success of *G. pulex* in colonizing and invading disturbance-prone ecosystems (Macneil *et al.*, 1997). *G. pulex* is also an important prey item for many carnivorous invertebrates, fish and waterfowl (Armitage & Young, 1990; Sutcliffe, 1991).


*Melita palmata* is a gammaridean amphipod of the family Melitidae (Lincoln, 1979). It is a common and abundant inhabitant of lagoons, estuaries and brackish environments along the European coasts of the Atlantic, the Baltic, the Mediterranean and the Black Sea and has been observed from the intertidal down to a depth of 50 metres (Lincoln, 1979; Marchini *et al.*, 2008). *M. palmata* is tolerant of a wide range of salinity, with some authors classifying the species as euryhaline (Hartog, 1964). In estuarine environments it has been observed at salinities as low as 5 - 6, whereas in brackish inland waters and the stable non-tidal salinities of the Baltic Sea, *M. palmata* has been observed at salinities as low as 3.8 (Goodhart, 1941; Jones, 1948; Hartog, 1964; Barnes, 1994). Marchini *et al* (2008) noted that *M. palmata* is often observed where the influence of freshwater is stronger, for example in lagoons or near river mouths.

*M. palmata* lives on sandy and muddy sediments, under stones or cobbles and among algae on which it feeds (Lincoln, 1979). It can reproduce all year, but the population generally declines in winter, displaying peaks of reproduction in the spring and autumn (Cunha *et al.*, 1999). *M. palmata* is an important prey item for some species of estuarine and marine fish species and waterfowl (Lincoln, 1979).

5. *Asellus aquaticus* (Crustacea: Isopoda)

*Asellus aquaticus* is an isopod of the genus Assellidae (Gledhill *et al.*, 1993). It is widely distributed throughout the British Isles and is found in habitats ranging from clear streams to stagnant, polluted ponds (Moon, 1957; Williams, 1962a, , 1962b, , 1962c; Moon, 1968; Holland, 1976; Ham, 1982; Moon & Harding, 1982). *Asellus aquaticus* is tolerant of organic pollution, high salinities, low pH and high metal concentrations (Lockwood, 1959b; Lagerspetz & Mattila, 1961; Brown, 1977, , 1978; Boet, 1984). These conditions often exclude other crustaceans, and *A. aquaticus* often replace *Gammarus pulex* in streams polluted by organic nutrients (Hawkes & Davies, 1971; Whitehurst, 1991b, 1991a).

In Britain *Asellus aquaticus* has only been observed in non-tidal freshwaters (<0.5) and is therefore classified as a limnic species in identification keys, despite the fact that it has been observed in the brackish waters of the Baltic, on the coast of Germany and the tidal freshwaters
of the River Meuse in the Netherlands (Wolff, 1973; Gledhill et al., 1993). Lagerspetz and Mattila (1961), examined the fresh and brackish *Asellus aquaticus* communities of the Baltic and observed that both communities inhabited brackish water of up to 6 without demonstrating any behavioural adaptations. Lockwood (1959a), experimentally determined that *A. aquaticus* was able to adapt to salinities of around 14. In German coastal sites, Gruner (1965) recorded *A. aquaticus* living alongside the brackish water isopods *Cyathura carinata* and *Idotea chelipes* and thus determined that the species was euryhaline.

*Asellus aquaticus* has two main breeding periods (spring and late summer) with a reproductive diapause in over-wintering animals (Gledhill et al., 1993). Depending on the birth cohort, lifespans range from three months to one year. *Asellus aquaticus* is omnivorous, feeding on algae, organic detritus and bacteria. It is an important prey item for some species of freshwater fish, waterfowl and flatworms (triclads) (Young et al., 1964; Macan & DeSilva, 1979; Petridis, 1990b).

### 6. *Ephemera danica* (Insecta: Ephemeroptera)

*Ephemera danica* is a burrowing Ephemeroptera nymph of the genus Ephemeridae and is widely distributed in Britain (Macan, 1979). The nymphs inhabit lakes and rivers with sandy or gravelly substrate within which they borrow, forming U-shaped tunnels (Macan, 1979; Tokeshi, 1985; Wright & Symes, 1999). *Ephemera danica* possesses modified mouthparts and particular processes on the head and prothorax that facilitate burrowing and use their gills to create water currents within their tunnels (Ladle & Radke, 1990).

The life cycle pattern cited by several authors is variable and ranges from one to three years, but is believed to consist of a short hatching period (one to two months) followed by a long larval development (more than 20 months) (Landa, 1968; Sowa, 1975; Otto & Svensson, 1981; Tokeshi, 1985; Elliott et al., 1988). *Ephemera danica* has been shown to feed predominantly on detritus, but may also have an important role as shredders of leaves obtained from substrate outside the tunnel (Otto & Svensson, 1981; Elliott et al., 1988; Ladle & Radke, 1990; Merritt & Cummins, 2006; Lopez-Rodriguez et al., 2009). *Ephemera danica* nymphs are an important food source for freshwater fish and birds (Macan, 1979).

### 7. *Hydropsyche angustipennis* (Trichoptera: Hydropsychidae)

*Hydropsyche angustipennis* is a caseless, net-spinning Trichopteran of the family Hydropsychidae (Edington & Hildrew, 2005). *H. angustipennis* larvae are sometimes found in
the lower reaches of large rivers, but are more often located in eutrophic streams, especially those which drain large water bodies such as lakes and reservoirs (Greve et al., 1998; Edington & Hildrew, 2005). The larvae have been found to be tolerant of high water temperatures, low oxygen concentrations, organic pollution, metals and acidity (Philipson & Moorhouse, 1974; Philipson & Moorhouse, 1976; Jacob et al., 1984; Vuori, 1995). The larvae have a fairly low-flow preference, with some larvae spinning nets at velocities as low as 5 cm s\(^{-1}\) (Tachet et al., 1992). They are omnivorous, eating algae, detritus and small invertebrates caught in their nets and they have also occasionally been observed scraping periphyton off substrate (Greve et al., 1998). Due to this, the larvae have an important ecological role as decomposers of organic material and also as a food source for fish and birds (Greve et al., 1998).

8. *Neureclipsis bimaculata* (Trichoptera: Polycentropodidae)

*Neureclipsis bimaculata* is a caseless net-spinning trichopteran of the genus polycentropodidae (Edington & Hildrew, 2005). In Britain, *N. bimaculata* larvae are localised in distribution and are usually found in lake outflow streams (Richardson, 1984; Edington & Hildrew, 2005; Hunken & Mutz, 2007). Their nets are trumpet shaped with a narrow tail and are adapted to slow flow velocities in the range of \(<0.04-0.4\) m s\(^{-1}\) (Petersen & Petersen, 1984). The larvae are able to tolerate a pH of less than 4.7 and have been observed in abundance in an acidic and iron-rich post-mining stream in Germany (pH 2.5-3.6) (Fjellheim & Raddum, 1990; Larsen et al., 1996; Hunken & Mutz, 2007).

The life cycle of *N. bimaculata* takes place takes place over one to two years. Eggs are laid beneath the water by females in May to early August and hatch in 10-16 days (Edington & Hildrew, 2005). Fast growing larvae pupate the following March to May and emerge in June or July (year 1) and slow growing larvae pupate the following year (year 2), hence instars 2 to 5 are present for most of the year (Rossiter, 1989). *N. bimaculata* larvae are omnivorous, although the food ingested primarily consists of zooplankton (Richardson, 1984b; Otto, 1985).


*Pyrrhosoma nymphula* is a zygopteran of the family Coenagrionidae (Hammond, 1985). The larvae are relatively easy to identify owing to extended dorsal wing sheaths, which give the species a short and squat appearance and broad, pointed lamellae with characteristic dark markings (Hammond, 1985; Cham, 2009).
*Pyrrhosoma nymphula* takes two years to complete larval development. Eggs are laid in May to June in slow moving water bodies, such as streams, canals, lakes and marshes (Hammond, 1985). The eggs hatch in July and the larvae usually reach the fifth instar development by November, pause over-winter and resume growth in April. The larvae enter the final instar (instar 12) the following October. A dispause in the final instar serves to synchronise emergence (Corbet, 1952; Gribbin & Thompson, 1991). Metamorphosis from larva to adult and emergence takes place in March and April. The two-year development period leads to two generations being present in any one year (Lawton, 1970).

*Pyrrhosoma nymphula* larvae have been observed to reach their highest densities in standing waters with dense vegetation and debris for refuge and foraging (Lawton, 1970). The larvae are believed to be tolerant of acidic habitats, having been observed in iron-rich waters and acidic *Sphagnum* bogs (Cham, 2009). Although mainly recorded in limnic systems, the larvae are commonly cited as being able to inhabit brackish water, as stated in the key by Hammond (1985). This brackish water tolerance appears to stem from a study by Butler and Popham (1958), who recorded two *P. nymphula* in brackish (24) waters on the Spurn Peninsula in Yorkshire following heavy flooding by the North Sea. Despite extensive research, it had not been possible to locate any subsequent published records of this species in brackish waters.

### 6.3 Laboratory salinity toxicity studies

In order to achieve the aims of this research project, the design and selection of appropriate salinity toxicity testing methodologies was essential. The original nature of this research required an innovative approach to the methodology, which included the design and construction of a dedicated laboratory test facility and bespoke equipment for use in both the field and laboratory.

#### 6.3.1 Laboratory saline toxicity test facility – Design and construction

To ensure the reliability and accuracy of the acute salinity tolerance determinations (LC$_{50}$), a dedicated facility was designed and constructed (Figure 6.1, Figure 6.2). The facility was designed to enable the precise control of all relevant experimental factors. The facility consisted of a large glass test aquarium (122 x 31 x 38 cm) (Figure 6.1, K), which held the organism test cells, a heater with external thermostat, two temperature probes, two LED daylight lighting strips, a number of air pumps, insulating foam surrounds and a transparent polypropylene lid. The aquarium contained 70 litres of water, temperature controlled using an Aqua Medic Titan 250 aquarium chiller unit (± 1 ºC) and a Deltatherm aquarium heater (± 1 ºC) with external
thermostat control (Figure 6.1, A, H). The mass of temperature controlled water was circulated by a submersible water pump in the aquarium chiller circuit. Water temperature was monitored using two temperature probes, attached to the inside of the aquarium (Figure 6.1, I). The aquarium was surrounded by a dense foam sheet, open-topped box with a polypropylene lid to reduce heat exchange, evaporation and permit illumination (Figure 6.1, G). Two LED AquaBeam 500 natural daylight lighting strips were attached to a lighting rack positioned above the aquarium and time controlled to provide a simulated diurnal light cycle (12 hours on 12 hours off) (Figure 6.1, C, F). Non-slip rubber matting was placed on the inside floor of the aquarium to provide a base for the experimental test cells. Two types of experimental test cell (flow and non-flow) were designed and constructed to fit inside the aquarium in good contact with the circulating temperature controlled water (Figure 6.3, Figure 6.4). These test cells held the macroinvertebrate specimens in the test solutions employed during the experimental periods (Figure 6.1, J). Three HAILEA ACO-2206 air pumps positioned in the overhead lighting rack provided aeration to the test cells solutions through pipe manifolds (Figure 6.1, D, E).

Rheophilic macroinvertebrate species (those that prefer fast-flowing water) were tested in the flow test cells. Low-flow, still water (non-rheophilic) macroinvertebrate species were tested in the non-flow test cells. The test cells were designed to be produced in volume, to accommodate the large number of individual specimens used in each experiment. Comparison tests have shown that salinity tolerances are not affected by flow regimes (Kefford et al, 2004).
Figure 6.1. Diagram of the laboratory saline tolerance facility. Abbreviations: A - AQUA MEDIC TITAN 250 chiller unit, B - Chiller plumbing circuit, C - AquaBeam 500 Natural daylight lighting strip, D - HAILEA ACO-2206 air pump, E - Silicone air pipe, F - Overhead lighting rack, G - Dense foam sheet, H - Deltatherm 300w heater, I - Temperature probe, J - Flow test cell, K - Glass aquarium.
6.3.1.1 Flow test cell

The flow test cell was designed to provide a constant flow of aerated water through an experimental chamber using an air lift combined aeration and circulation system (Figure 6.3B). The final design of flow test cell comprised 10 pieces of 4 mm un-toughened glass, cut to size and bonded with high modulus silicone sealant. A foam mesh block downstream of the test chamber ensured that the organism was constrained within the chamber and could not escape to the bottom of the test cell (Figure 6.3D). The large size of the flow test cell chambers (31 x 17 cm) meant that up to 10 macroinvertebrates could be tested in each cell at once. The flow test cell was subject to a number of tests prior to its inclusion in the salinity tolerance experiments. The air bubble induced flow was tested by ink injection and followed the pattern indicated in Figure 6.3B. Salinity, pH and dissolved oxygen levels were monitored over a 72 h test period and were shown to remain stable. To ensure good heat exchange with the temperature controlled aquarium circulation, the test cells were produced with high surface area to volume ratios and packed into the aquarium with water contact on all sides. Test runs confirmed that water temperature inside the test cells was kept at near constant over the 72 h test period. A total of 22 flow test cells were constructed, with 11 in use at any one time.
6.3.1.2 Non-Flow test cell

The non-flow test cells were designed around standard plastic medical sample tubes (Figure 6.4). Two 4 mm holes were drilled in the tube screw cap and one straight and one ‘L’ shaped rigid pipe connectors were force fitted through the cap (Figure 6.4B, C). A 10 cm section of 4 mm silicone air pipe was fitted inside the test cell connected to the down leg on the ‘L’ connector as an aeration delivery tube (Figure 6.4F). The end of the tube was heat sealed and perforated with a single pin hole so as to deliver a continuous fine stream of air bubbles when pressurised through the compressed air delivery manifold connections. The straight air connector allowed air to equalise pressure by escaping from the test cell (Figure 6.4C). One hundred non-flow test cells were constructed; each designed to hold an individual macroinvertebrate. To maintain a precise water temperature the non-flow test cells were also designed to fit inside the large glass aquarium and were held in place by force fitting into the perforated rubber matting on the floor.
of the aquarium. Test runs confirmed that water temperature inside the test cells was kept effectively constant over the 72 h test period. Air flow, salinity (psu), pH and dissolved oxygen concentration (%) was monitored over a 72 h test run and were also shown to remain at near constant values.

Figure 6.4. Diagram and photograph of the non-flow test cell. Abbreviations: A – Silicone air tube, B – ‘L’ shaped connector, C – Straight connector, D – Screw cap, E – Sample tube, F – Heat sealed silicone air pipe and pin hole, G. Air flow.
6.4 Methods

6.4.1 Field collection

Macroinvertebrate species were collected from the River Ouse in the summer and autumn months of 2009 and the winter and spring months of 2009/10. Acute salinity toxicity testing was conducted under two temperature regimes, 7 ºC and 18 ºC, designed to mimic summer and winter field temperatures in the River Adur and River Ouse. Collection of macroinvertebrates was undertaken when field river water temperatures were similar to that of the experimental cells. This temperature matching ensured that species did not need to be acclimated to the selected temperature prior to salinity tolerance experimentation. Species collected in the summer and autumn months of 2009 were subject to acute salinity toxicity tests at 18 ºC. The same species (where possible) were collected in the winter and spring months of 2009-10 and subject to acute salinity toxicity tests at 7 ºC.

The selected macroinvertebrate species were collected at sample sites established for the field sampling and known to contain an abundance of the selected species (see Chapter 5). The species were collected using standard three-minute kick-sweep samples within marginal areas of the channel, with the target of obtaining >80 individuals (where possible; Table 6.1). Spot samples of river water temperature (ºC), pH, dissolved oxygen and salinity (psu) were recorded at each collection. The selected species were placed in clip-top plastic buckets, filled with river water from the sample site. The buckets were sealed with perforated lids and temperature changes minimised on the return journey to the laboratory (4-5 h) by wrapping in thermal insulating blankets. Spot checks of dissolved oxygen and water temperature were conducted on the journey to ensure that the temperature did not rise significantly above that at which the samples were collected and that dissolved oxygen remained at >90% saturation.

6.4.2 Acute salinity toxicity tests

Acute salinity tolerances (LC$_x$) of selected macroinvertebrate species were determined through lethal toxicity tests following the rapid tolerance testing protocol proposed by Kefford et al. (2005b). Rather than extensive repetitive tolerance toxicity tests on a single species, this protocol is less rigorous and enables the testing of a large number of macroinvertebrate species within a limited time frame (Kefford et al., 2003; Kefford et al., 2005b; Kefford et al., 2006a). This method is of particular relevance where collection of significant numbers (>80) of individual macroinvertebrate species in the field is problematic (Kefford et al., 2005). Salinity
tolerances were expressed in terms of the saline concentration lethal to a given percentage of individuals over a 72 hour period (LC$_{x}$). The euryhaline marine species tested in this study (Melita palmata and Gammarus salinus) were tested on their tolerance to reductions in salinity.

Acute salinity toxicity tests were undertaken over 72 hours, under two temperature regimes, 7 ºC and 18 ºC, which were maintained by an aquarium chiller unit (± 1ºC) and heater (± 1 ºC) controlled by an external thermostat (Figure 6.1, A, H). A 12 hour diurnal cycle was maintained using daylight simulated illumination (Figure 6.1, C). Ten salinity treatments, from freshwater to fully marine equivalence, were selected for the toxicity tests; 0.5, 4, 8, 12, 16, 20, 24, 28, 32 and 35. Water of the required salinity was prepared by dissolving Instant Ocean sea salt in distilled water. Instant Ocean sea salt produces a saline solution with ionic proportions similar to seawater, which is sodium chloride dominated (Atkinson & Bingham, 1999). Each set of 10 saline test solutions was accompanied by a control solution, consisting of water obtained from the field sampling site during macroinvertebrate collection. Experimental solutions were aerated to saturation prior to the start of the experiment and constantly aerated throughout the experiment.

Each macroinvertebrate species was allocated an experimental test cell type (flow, non-flow) dependent upon the individual species habitat preferences (Table 6.1). The number of individual macroinvertebrates tested was dependent upon their availability and ease of collection. The preference was for >80 individuals (Table 6.1). Where the flow cells were used, up to 10 individuals were placed in each cell chamber. Ten of the flow cells contained one of the saline solutions and the final cell held the control. Each non-flow test cell held one individual macroinvertebrate specimen, with ~10 cells allocated to each of the 11 test solutions (including the control).

Evaporation was monitored by direct level measurements marked on the cells and the levels adjusted as appropriated by the addition of distilled water. In practise the enclosed structure of the chambers test cells and aquarium meant that evaporative losses were minimal and test solution top up infrequent. Salinity was constantly monitored by direct reading sensors and expressed in psu with an WinLab®Data-Line Conductivity Meter (Windaus, Labortechnik) employing automatic calibration and temperature compensation (accuracy of 0.5%). Dissolved oxygen concentration and pH of the test solutions within each test cell was tested in conjunction with survival checks.
Survival was checked at 1, 12, 24, 48 and 72 h after the start of the experiment. Individuals were considered dead if they failed to respond by moving in response to gentle probing. Dead individuals were removed, classified, preserved in IMS and stored. In the few instances where living individuals were mistakenly removed, they were excluded from the analysis. In the few instances where an individual moulted, they were excluded from the analysis, due to changes in body fluid ion content recorded throughout the moulting cycle (Robertson, 1960; Bursey & Lane, 1971b; Lockwood & Inman, 1973; Dorgelo, 1974; Hagerman, 1980). Specimens were sorted, labelled and preserved in 80% IMS. Post experimental confirmation of species identification was undertaken by microscope.

<table>
<thead>
<tr>
<th>Species</th>
<th>Salinity preference</th>
<th>Test cell type</th>
<th>Temp (°C)</th>
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<tr>
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<td>Crustacea: Amphipoda</td>
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<td>Non-flow</td>
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<tr>
<td><em>Melita palmata</em></td>
<td>Crustacea: Amphipoda</td>
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<td>Non-flow</td>
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<td><em>Gammarus zaddachi</em></td>
<td>Crustacea: Amphipoda</td>
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<td>Non-flow</td>
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<tr>
<td><em>Gammarus pulex</em></td>
<td>Crustacea: Amphipoda</td>
<td>l(el)</td>
<td>Non-flow</td>
<td>7</td>
</tr>
<tr>
<td><em>Asellus aquaticus</em></td>
<td>Crustacea: Isopoda</td>
<td>l(el)</td>
<td>Non-flow</td>
<td>18</td>
</tr>
<tr>
<td><em>Hydropsyche angustipennis</em></td>
<td>Trichoptera: Hydropsychidae</td>
<td>l(el)</td>
<td>Flow</td>
<td>7</td>
</tr>
<tr>
<td><em>Neureclipsis bimaculata</em></td>
<td>Trichoptera: Polycentropodidae</td>
<td>l</td>
<td>Flow</td>
<td>18</td>
</tr>
<tr>
<td><em>Pyrrhosoma nymphula</em></td>
<td>Odonata: Zygoptera</td>
<td>el</td>
<td>Flow</td>
<td>7</td>
</tr>
<tr>
<td><em>Ephemera danica</em></td>
<td>Insecta: Ephemeroptera</td>
<td>l</td>
<td>Flow</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 6.1. Summary table of acute salinity toxicity experiments. Selected species, salinity tolerance group allocation (chapter 5), experimental test cell type (flow, non-flow), temperature (7, 18 °C) and number of individuals tested.

6.5 Data Analysis

Standard logistic regression (Agresti, 2007) was fitted to the dose-response curve of each of the study species, with the dependant variable classified into two groups (dead, alive) and the independent variable the salinity (psu). The logistic regression model was used to predict the probability of death by fitting the experimental data to a logit function logistic curve. Where the
logistic regression model was significant (chi squared p < 0.05), the lethal concentration of salt (and 95% confidence intervals) to a given percentage of individuals (LC$_x$) for each species and temperature regime were calculated (LC$_{99}$, LC$_{95}$, LC$_{50}$, LC$_{5}$ and LC$_{1}$). Statistical differences (p < 0.05) between the temperature regime LC$_{50}$ estimates were compared by the 95% confidence limits overlap test (Wheeler et al., 2006). Where LC$_{50}$ confidence intervals did not overlap between the corresponding pairs, differences were considered significant (p < 0.05). If control mortality exceeded 20%, the toxicity test was considered invalid (Greve et al., 1998).

6.6 Results

All nine selected experimental species were collected in sufficient numbers to be exposed to the full range of test salinities (0.5 – 35) under at least one temperature regime (7 and 18 ºC). The dynamic nature of species distributions in the River Ouse estuary (see Chapter 5) meant that the selected test estuarine species were only located and collected once during numerous sampling/collection trips, and as such were only tested under one temperature regime (e.g. Gammarus zaddachi, Gammarus salinus, Melita palmata; Table 6.1). Survivorship in the controls was generally high (90 – 100%), except for Ephemera danica and Hydropsyche angustipennis tested at 18 ºC with 85 and 80% survivorship respectively.

1. Asellus aquaticus

Under both temperature regimes (7 and 18 ºC), survivorship of A. aquaticus in the control treatment (0.1) was 100% (Figure 6.5b, c). At 7 ºC, survival was generally high in all salinity treatments, with the exception of 28, 32 and 35, which produced a significant drop in survivorship over the first 24 hours (Figure 6.5a, b, d). The 72 hr concentration of salt lethal to 50% of individuals (LC$_{50}$) was 30.5 with a 95% confidence interval range of 30.2 to 31.3 (Figure 6.5a).

At 18 ºC, there was a decrease in survival in nearly all salinity treatments (Figure 6.5 a, c, e). Complete mortality (100%) was recorded in the highest salinity treatments (28, 32 and 35) within the first 24 hours (Figure 6.5c, e). In the lower salinity treatments, mortality continued to increase for up to 72 hours (Figure 6.5c). The 72 hr LC$_{50}$ value was 20.2 (CI range of 19.4 to 21.1), significantly lower (no 95% CI overlap) than the LC$_{50}$ value recorded at 7 ºC (Figure 6.5a).
The high salinity tolerances determined in this study do not correspond with the limnic, salt tolerating (l(el)) salinity preference grouping allocated to *A. aquaticus* in chapter 5 (based on published literature) or the maximum salinities at which *A. aquaticus* was recorded in the River Adur and River Ouse (Table 6.2, Table 6.3). Under winter temperatures (7.4 – 7.5 °C), *A. aquaticus* was recorded up to a maximum salinity of 2.1 in the River Ouse and 0.28 in the River Adur. Although one specimen was recorded in the River Adur at a maximum salinity of 35, it had been assumed that this was the result of drift or displacement downstream by river flow from the upper river (Table 6.3). Under summer temperatures (17.7 – 17.8 °C), *A. aquaticus* was recorded up to a maximum salinity of 0.59 in the River Adur and 0.2 in the River Ouse (Table 6.3).

![Figure 6.5. The 72 hr toxicity of salinity on *Asellus aquaticus* at 7 °C and 18 °C, with the LC50 and associated 95% confidence intervals (a), the rate of mortality of *A. aquaticus* to salinity at 7 °C (b) and 18 °C (c) and the response-surface estimation of percent survival of *A. aquaticus* at 7 °C (d) and 18 °C (e).]
2. *Ephemera danica*

At 7 °C, the control treatment (0.1) survivorship of *Ephemera danica* was 100% (Figure 6.6b). Survivorship in the low salinity treatments (0.5 – 16) was generally high (100%), compared to low survivorship (<42%) in the high salinity treatments (20 - 35) (Figure 6.6a, b, d). Complete mortality (100%) was recorded in the highest salinity treatments 32 and 35 within the first 12 hours (Figure 6.6b, d). The 72 hr concentration of salt lethal to 50% of individuals (LC$_{50}$) was 23.1, with a 95% confidence interval range of 22.8 - 23.9 (Figure 6.6a).

At 18 °C, survivorship in the control treatment (0.1) was 85% (Figure 6.6c). Survivorship in nearly all salinity treatments was low, with complete mortality (100%) recorded in the highest salinity treatments (28 – 35) after 24 hours (Figure 6.6a, c, e). The 72 hr LC$_{50}$ was 8.6 (CI range of 8 - 9.4), which was significantly lower (no 95% CI overlap) than the recorded salinity tolerance at 7 °C (Figure 6.6a).

The high salinity tolerances determined in this study do not correspond the salinity preference grouping (l) allocated to *E. danica* in chapter 5 (based on published literature) or the maximum salinities at which this species was recorded in the field (Table 6.2, Table 6.3). Under both winter (6.7 – 9.3 °C) and summer (18.1 °C) temperatures, *E. danica* was recorded in the non-tidal limnetic (<0.5) sections of the River Ouse (0.1) (Table 6.3).
3. *Gammarus pulex*

Under both temperature regimes (7 and 18 °C), survivorship in the control treatment (0.1) was high at 100% and 90% respectively (Figure 6.7b, c). At 7 °C, survivorship in the high salinity treatments (20 – 35) was low, with complete mortality (100%) recorded in 28, 32 and 35 treatments within the first 12 hours (Figure 6.7a, b, d). The lower salinity treatments (0.5 – 16) recorded 90% to 100% survivorship over 72 hours (Figure 6.7a, b, d). The 72 hr concentration of salt lethal to 50% of individuals (LC₅₀) was 21.9, with a 95% confidence interval range of 21.5 to 22.6 (Figure 6.7a).

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**Figure 6.6.** The 72 hr toxicity of salinity on *Ephemera danica* at 7 and 18 °C, with the LC₅₀ and associated 95% confidence intervals (a), the rate of mortality of *E. danica* to salinity at 7 °C (b) and 18 °C (c) and the response-surface estimation of percent survival of *E. danica* at 7 °C (d) and 18 °C (e).
At 18 °C, survivorship was low in the high salinity treatments (16 - 35), with the treatments 24, 28, 32 and 35 resulting in maximum mortality (100%) within the first 12 hours (Figure 6.7a, c, e). Low salinity treatments (0.5 - 12) recorded >80% survivorship over 72 hours (Figure 6.7a, c, e). The 72 hr LC50 was 16.6 (CI range of 16 – 17.4), significantly lower (no 95% CI overlap) than the salinity tolerance recorded at 7 °C (Figure 6.7a).

The high salinity tolerances determined in this study do not correspond with the salinity preference grouping (l(el)) allocated to *G. pulex* in chapter 5 (based on published literature), or the maximum salinities at which this species was recorded in the River Ouse and River Adur (Table 6.2, Table 6.3). Under both winter (7.5 – 8.6 °C) and summer (17.8 °C) temperature regimes, *G. pulex* was recorded in the limnetic (<0.5) sections of the River Ouse (0.1) and River Adur (0.2) (Table 6.3).
Figure 6.7. The 72 hr toxicity of salinity on *Gammarus pulex* at 7 °C and 18 °C with the LC$_{50}$ and associated 95% confidence intervals (a), the rate of mortality of *G. pulex* to salinity at 7 °C (b) and 18 °C (c) and the response-surface estimation of percent survival of *G. pulex* at 7 °C (d) and 18 °C (e).

4. *Gammarus zaddachi*

Due to collection difficulties in the River Ouse estuary, the acute salinity tolerance of *Gammarus zaddachi* could only be tested at 7 °C. After 72 hrs survivorship was high (>80%) in all salinity treatments, including the control (0.5) (Figure 6.8a, b), therefore suggesting that this species can tolerate limnic (<0.5) through to fully marine (35) conditions. This holarctic-haline tolerance corresponds both with published literature (see chapter 5) and the maximum and minimum
salinity range which this species was recorded in the River Adur (0.2 – 35) and River Ouse (0.1 – 35) (Table 6.3).

Figure 6.8. The 72 hr toxicity of salinity on *Gammarus zaddachi* at 7 °C (a) and the rate of mortality of *G. zaddachi* to salinity at 7 °C (b).

5. *Gammarus salinus*

Due to collection problems, the tolerance of *Gammarus salinus* to reduced salinity and limnic conditions could only be tested at 18 °C. Survivorship in the control treatment (8.2) was 88% (Figure 6.9b). Survivorship in most salinity treatments (8 – 35) was high (100%), with the exception 0.5 and 4, which produced a significant drop in survivorship over the first 12 hours (Figure 6.9a, b, c). Complete mortality (100%) was recorded at 0.5 after 24 hours (Figure 6.9b). The 72 hr LC$_{50}$ was 4.1 with a 95% confidence interval range of 2.9 to 7.6 (Figure 6.9a).

The tolerance of *Gammarus salinus* to reduced salinities in this study corresponds to the tolerances and field distributions of *G. salinus* in published literature, and the euryhaline-marine (em) salinity preference grouping allocated to this species in chapter 5 (Table 6.2, Table 6.3). It does not however correspond to the distribution of this species in the River Adur and River Ouse estuaries, where it was recorded at sites which ran tidal limnetic (<0.5) at low tide.
Figure 6.9. The 72 hr toxicity of reduced and limnetic salinities on Gammarus salinus at 18 °C with the LC$_{50}$ and associated 95% confidence intervals (a) and the rate of mortality of G. salinus to reduced and limnetic salinities at 18 °C (b).

6. Hydropsyche angustipennis

At 7 °C, the control treatment (0.1) survivorship for Hydropsyche angustipennis was 100% (Figure 6.10b). In the majority of salinity treatments (0.5 – 24) survivorship was high (>80%) (Figure 6.10a, b, d). Complete mortality (100%) was recorded in the salinity treatments 32 and 35 within 48 hours (Figure 6.10a, b, d). The 72 hr LC$_{50}$ was 30.6, with a 95% confidence interval range of 30 to 31.5 (Figure 6.10a).

At 18 °C, survivorship in the control treatment (0.1), was 80% (Figure 6.10c). A decrease in survival was recorded in all salinity treatments, with the highest treatments (28 - 35) recording complete mortality (100%) after 24 hours (Figure 6.10a, c, e). The 72 hr concentration of salt lethal to 50% of individuals (LC$_{50}$) was 17.6 (CI range of 16.9 to 18.5), which was significantly lower (no 95% CI overlap) than the recorded salinity tolerance at 7 °C (Figure 6.10a).
The high salinity tolerance values determined in this toxicity study, do not correspond with the limnic, salt tolerating (l(el)) salinity preference grouping allocated to *H. angustipennis* in chapter 5 (based on published literature) or the maximum salinities at which this species was recorded in the field (Table 6.2, Table 6.3). Under both winter (7.2 – 9.3 °C) and summer (18. °C) temperatures, *H. angustipennis* was only recorded in the non-tidal limnetic (<0.5) sections of the River Ouse (0.1) and River Adur (0.2) (Table 6.3).

Figure 6.10. The 72 hr toxicity of salinity on *Hydropsyche angustipennis* at 7 °C and 18 °C, with the LC50 and associated 95% confidence intervals (a), the rate of mortality of *H. angustipennis* to salinity at 7 °C (b) and 18 °C (c) and the response-surface estimation of percent survival of *H. angustipennis* at 7 °C (d) and 18 °C (e).
7. *Melita palmata*

Due to collection problems in the River Ouse estuary, the tolerance of *Melita palmata* to reduced and limnetic salinities could only be tested at 18 °C. Survivorship in the control treatment (8.2) was 90% (Figure 6.11a, b). Survivorship in most salinity treatments (12 - 35) was high (>90%), with the exception of 0.5, 4 and 8 (Figure 6.11a, b, c). Only the 0.5 salinity treatment produced complete mortality (100%) after 48 hours (Figure 6.11b, c). The 72 hr LC<sub>50</sub> was 4 with a 95% confidence interval range of 2.8 – 5.6 (Figure 6.11a).

The tolerance of *Melita palmata* to reduced salinities determined in this study, corresponds to published tolerance and field distribution studies and the salinity preference grouping (em) allocated to this species in chapter 5 (Table 6.3). The tolerance does not however correspond to the distribution of this species in the River Ouse estuary, where it was recorded at sites which ran tidal limnetic (<0.5) at low tide.

![Graphs showing the 72 hr toxicity of reduced and limnetic salinities on *Melita palmata* at 18 °C, with the LC<sub>50</sub> and associated 95% confidence intervals (a), the rate of mortality of *M. palmata* to reduced and limnetic salinities at 18 °C (b) and the response-surface estimation of percent survival of *M. palmata* at 18 °C (c).]

8. *Neureclipsis bimaculata*
At 7 °C, the control treatment (0.1) survivorship for Neureclipsis bimaculata was 100% (Figure 6.11b). Survivorship was high (>80%) in the low to mid salinity treatments (0.5 – 20) and low (%) in the high salinity treatments (24 – 35; Figure 6.12a, b, d). Complete mortality (100%) was recorded in the salinity treatments 28, 32 and 35 after 24 and 48 hours (Figure 6.12a, b, d). The 72 hr concentration of salt lethal to 50% of individuals (LC$_{50}$) was 26.6, with a 95% confidence interval range of 26.1 to 27.5 (Figure 6.12a).

At 18 °C, survivorship in the control treatment (0.1) was 91% (Figure 6.12c). A decrease in survival was recorded in nearly all salinity treatments, with the highest treatments (28 to 35) recording complete mortality (100%) after 24 and 48 hours (Figure 6.12a, c, e). The 72 hr LC$_{50}$ was 23.1 (CI range of 22.4 to 24), which was significantly lower (no 95% CI overlap) than the recorded salinity tolerance at 7 °C (Figure 6.12a).

The high salinity tolerance values determined in this study, do not correspond with the limnic (l) salinity preference grouping allocated to N. bimaculata in chapter 5 (based on published literature) or the maximum salinities at which this species was recorded in the field (Table 6.2, Table 6.3). Under both winter (6.7 °C) and summer (18.1 °C) temperatures, N. bimaculata was only recorded in the non-tidal limnetic (<0.5) River Ouse (0.1) (Table 6.3) (Chapter 5).
Figure 6.12. The 72 hr toxicity of salinity on Neureclipsis bimaculata at 7 °C and 18 °C, with the LC$_{50}$ and associated 95% confidence intervals (a), the rate of mortality of N. bimaculata to salinity at 7 °C (b) and 18 °C (c) and the response-surface estimation of percent survival of N. bimaculata at 7 °C (d) and 18 °C (e).

9. *Pyrrhosoma nymphula*

Under both temperature regimes (7 and 18 °C) the survivorship of *Pyrrhosoma nymphula* in the control treatments (0.1) was 100% (Figure 6.13b, c). Survivorship was high (<75%) in all salinity treatments (0.5 – 35) under both salinity regimes (7 and 18 °C), indicating that this species can tolerate limnic (<0.5) through to fully marine (35) conditions (Figure 6.13a, b, c.). This holeury-haline tolerance does not correspond with the maximum salinity range which this species was recorded in the River Ouse (0.1), or the euryhaline-limnic salinity.
tolerance/preference grouping allocated in Chapter 5. This high salinity tolerance does however correspond with the one report of this species in brackish water (24) (Butler & Popham, 1958; Table 6.3).

Figure 6.13. The 72 hr toxicity of salinity on Pyrrhosoma nymphula at 7 ºC and 18 ºC, the rate of mortality of P. nymphula to salinity at 7 ºC (b) and 18 ºC (c).
<table>
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<tr>
<th>Species</th>
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<th>Control mortality (%)</th>
<th>Regression model sig.</th>
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<th>LC95</th>
<th>LC50</th>
<th>LC5</th>
<th>LC1</th>
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<td>0</td>
<td>0.005*</td>
<td>31.2 (30.9-32)</td>
<td>31.1 (30.9-31.9)</td>
<td>30.5 (30.2-31.3)</td>
<td>28.2 (27.9-29.0)</td>
<td>26.6 (26.3-27.4)</td>
</tr>
<tr>
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<td>18</td>
<td>0</td>
<td>0.000*</td>
<td>20.9 (20.1-21.8)</td>
<td>20.9 (20.1-21.7)</td>
<td>20.2 (19.4-21.1)</td>
<td>17.9 (17.1-18.8)</td>
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<td>20.8 (20.5-21.6)</td>
<td>19.2 (18.9-20.0)</td>
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<td>9.2 (8.6-10.1)</td>
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Table 6.2. Summary table of 72 hr acute salinity toxicity test results, including test temperature, control mortality and regression model significance (*model significant at p<0.01). Salinity tolerances classified as the lethal concentration of salt to a given percentage of the test population, with associated 95% confidence interval ranges (psu). *Note – for the marine derived Gammarus salinus and Melita palmata, the lethal concentration of reduced salinities were tested.
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<th>Species</th>
<th>Salinity tolerance grouping</th>
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<th>Laboratory salinity tolerance (72 hr)</th>
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<td>River Adur</td>
</tr>
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<td>Average field temp (°C)</td>
<td>Maximum salinity range (psu)</td>
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<td>0.1 - 2.1</td>
</tr>
<tr>
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<td></td>
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<td>0.1 - 0.2</td>
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</tr>
<tr>
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<td></td>
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<td>0.1</td>
</tr>
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</tr>
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<td>0.2 - 24.1</td>
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<tr>
<td></td>
<td></td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td><em>Melita palmata</em></td>
<td>em 0.5 - 35</td>
<td>8.1</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>35</td>
</tr>
</tbody>
</table>

he = marine derived taxa that tolerate the entire range of salinity from limnic to marine, 0 - 35.
em = marine taxa that tolerate a large range of salinities between 0.5 and 35.
el = freshwater derived taxa that tolerate salinity up to 10.
I(el) = freshwater derived taxa that tolerate salinities below 5.
I = freshwater taxa that do not tolerate even low salinity, <0.5.

Table 6.3. Summary table displaying species salinity preference grouping (based on published literature; chapter 5) maximum field salinity range (as recorded in the River Ouse and River Adur) and laboratory salinity tolerance results (LC₅₀) for test species.
6.6.1 Summary

- Temperature regime has been shown to have a significant impact on the toxicity of salinity on macroinvertebrate taxa, with all species (tested under both temperature regimes) exhibiting a significantly higher salinity tolerance ($LC_{50}$ and associated CI) at 7 °C compared to 18 °C.

- The tolerance of species commonly recorded in the estuarine zone (*Gammarus salinus*, *Melita palmata* and *Gammarus zaddachi*) to reductions in salinity, matches the field distributions and tolerances of these species recorded in published literature. However, the tolerances of *G. salinus* and *M. palmata* to reductions in salinity did not correspond to their distributions in the River Ouse and River Adur estuaries, where they were recorded with tidal limnetic minimum salinities (<0.5) at low tide. *Gammarus salinus* and *Melita palmata* recorded a 72 hr tolerance to reduced salinities of 4.1 (2.9 – 7.6) and 4.0 (2.8 – 5.6) respectively (at 18 °C). *Gammarus zaddachi* exhibited a holarctic (0 – 35) tolerance to salinity, with high survivorship in all treatments (at 7 °C), corresponding to the ubiquitous distributions of this species in the River Adur and River Ouse estuaries.

- The 72 hr acute salinity tolerances of all limnic test species (*Asellus aquaticus*, *Gammarus pulex*, *Ephemera danica*, *Hydropsyche angustipennis*, *Neureclipsis bimaculata* and *Pyrrhosoma nymphula*) were significantly higher than published field distributions would indicate. At 7 °C, the 72 hr salinity tolerance ($LC_{50}$) of these species ranged from 30.6 – 21.9, and from 23.1 - 8.6 at 18 °C. *Pyrrhosoma nymphula* demonstrated a holarctic (0 – 35) tolerance to salinity, with high survivorship in all treatments. These surprisingly high salinity tolerances do not correspond to the salinity preference/tolerance groupings allocated to these species in Chapter 5 (mainly based on published habitat preferences), or the field distributions of these species in the River Adur and River Ouse.
6.7 Field tidal cycle salinity toxicity determination

6.7.1 Introduction

In order to assess the acute laboratory salinity toxicity results and attempt to explain the differences between the laboratory derived salinity tolerance values \( (LC_x) \) and recorded field distributions, macroinvertebrate species responses to actual field tidal cycle conditions were assessed.

6.7.2 In-situ river test cell design

The in-situ test cells were designed to produce salinity tolerance measurements under the extreme tidal environment of the River Ouse. This required robust design and materials and reliable anchorage into varying substrates, such as would withstand the high velocities of tidal flood and river ebb (Figure 6.14).

The test cells consisted of 300 mm lengths of 40 mm diameter UPVC wastewater tubing, incorporating 45° exit and entry elbows. The elbow fittings were push-fit and incorporated elastomeric O-ring seals (Figure 6.14). Woven stainless steel mesh (0.5 mm aperture) discs covered the inlets and exits and were fitted within the elbow outer O-ring seals. When deployed, the tubes were orientated to the river and tide flows, with the 45° elbows angled uppermost. The angled elbows were intended to reduce the velocity of the currents within the tubes and the effect of these upon the test specimens. The angled meshes provided predator protection and were intended to be to a degree self cleaning (by deflection) of debris carried in the river and tidal flows. The 45° elbows ensured that in the event that tube placement and/or late collection led to tube exposure at low tide, the tube would remain filled with water as a refuge reservoir for the specimens. The tubes were attached with cable ties to steel frames cut from commercial mild steel, 4 mm welded-rod concrete reinforcing mesh, with the free rod ends turned down 90° to provide anchor legs for driving in to the substrate (Figure 6.14). Each tube was engraved with an identification code and the frame was provided with a secondary security anchorage using 4 mm non-floating synthetic rope attached to a convenient fixed structure (tree, bridge etc). A SEBA datalogger type Dipper-TEC sonde was additionally attached to the frame using cable ties and the data cable attached to the rope. The SEBA Dipper-TEC sonde recorded the conductivity \( (\mu S \ cm^{-1}) \), total dissolved solids \( (g \ L^{-1}) \), salinity (psu), temperature \( (^{\circ}C) \) and water level (m) over the experimental time periods (Chapter 5, Section 5.2). Each test cell was designed to hold one
macroinvertebrate species. The size of the cells meant that up to 50 individual specimens could be tested in the same cell.
6.7.3 Sites and Methods

During March 2010 four sets of field test cells were deployed at four sites (E1 – E4) along the River Ouse estuary (Figure 6.15, Table 6.4). Site selection was based on tide and salinity mapping of the River Ouse estuary using the SEBA Dipper-TEC probes on proceeding days. The sites were secondarily selected to include areas where the selected experimental species had not been recorded, but laboratory tolerances indicated that they should be able to survive. Four macroinvertebrate species (i.e. *Gammarus pulex*, *Hydropsyche angustipennis*, *Neureclipsis bimaculata* and *Ephemera danica*) were selected for experimentation on the basis of their known current field distributions conflicting with their laboratory salinity tolerance assessments (Table 6.3). Over the field experimental period (March 2010), the water temperature of the River Ouse estuary (6.8 – 14.8 °C) complimented the winter temperature regime (7 °C) undertaken during the laboratory salinity tolerance study, enabling direct comparison between studies (Table 6.5).

Each set of field test cells consisted of 4 test cell tubes attached to a common anchoring frame (Figure 6.14). Fifty individuals of each macroinvertebrate species was allocated to 1 tube of each 4 tube set. A SEBA Dipper-TEC probe was attached to the experimental tube set to record the temperature (°C), conductivity (µS cm⁻¹), total dissolved solids (g L⁻¹), level (m) and salinity (psu) every 2 minutes over the 24 hour experimental period.

The selected macroinvertebrate species were collected from one site on the River Ouse (O16) (where the selected species were known to occur in abundance) using a standard kick sample (Figure 6.15, Table 6.3). Specimens were screened in white trays and 100 large individuals of the required species were selected. A control test set was left at the collection site (C) and the remaining specimens transported in insulated buckets to test sites (50 individuals in each). The test cells were immersed in the River Ouse estuary for 24 hours. After 12 hours tubes were opened and washed out into inspection trays. Mortality was recorded and any dead individuals were removed, preserved and stored. The remaining live individuals were placed back in the tubes and left for a further 12 hour tidal cycle.

In order to corroborate and compare the 72 hr laboratory acute salinity toxicity experiments (at 7 °C) with the 24 hr field tidal cycle survival studies, the toxicity of salinity at 12 and 24 hr (LC₉₉, LC₉₅, LC₅₀, LC₅ and LC₁) were calculated from the laboratory salinity toxicity data using standard logistic regression (method as in Section 6.5; Table 6.6).
6.7.4 Results

1. *Gammarus pulex*

Under all four field experiments (E1 – E4), survivorship in the control tests (0.01) (C) was high, at more than 98%. At experimental site E1, survivorship of *Gammarus pulex* was low, falling to 24% survival after 12 hrs (over tidal cycle salinities of 0.2 – 29.6 – 0.3) and to 16% after 24 hours (0.2 – 27.7 – 0.2) (Figure 6.16a, Table 6.5). The water temperature over the 24 hour experimental period ranged from 6.8 – 14.8 °C (mean of 9.7 °C) (Table 6.5). The maximum
salinities (29.6 and 27.7) and salinity ranges (29.4 and 27.5) recorded over the 24 hr tidal period were greater than the 12 and 24 hr concentration of salt lethal to 99% of *G. pulex* individuals (LC$_{99}$) recorded in the laboratory salinity toxicity experiments (Table 6.5, Table 6.6). The recorded LC$_{99}$ was 26.6 (CI range 26.3 – 27.3) after 12 hours, and 24.6 (24.1 – 25.4) after 24 hours, which corresponds to the recorded mortality of *G. pulex* in E1 (Table 6.6, Figure 6.16a). The recorded tidal cycle salinity was greater than the 12 and 24 hr LC$_{50}$ salinity tolerance range of *G. pulex* (25.6 – 26.6 and 23.4 – 24.7) (at 7 ºC) for a total of 2 hrs 40 min over the 24 hr test period (Table 6.5, Table 6.6).

At experimental sites E2, E3 and E4, survivorship of *G. pulex* was 100%. Survivorship at these sites again corresponded to the 12 and 24 hr laboratory toxicity studies (Figure 6.16a, b, c, Table 6.6). At experimental site E2, the 12 and 24 hr tidal maximum salinity was recorded at 15 and 12.3 respectively, with an associated salinity range of 14.8 and 12.1 (over a temperature range of 7 – 10.5 ºC) (Table 6.5). These maximum and range of salinities were lower than the 12 and 24 hr LC$_1$ recorded for *G. pulex* in the laboratory toxicity studies, at 22 (CI 21.7 – 22.7) and 20 (19.5 – 20.8) respectively (at 7 ºC; Table 6.6). Experimental site E3 recorded 12 and 24 hour maximum salinities of 9 and 6.5 and associated salinity ranges of 8.8 and 6.3 (over a temperature range of 7.1 – 13.6 ºC; Table 6.5). Site E4 recorded tidal limnetic minimum (0.2) and maximum (0.24 and 0.21) salinities, over a temperature range of 7.5 – 13.6 ºC (Table 6.5). Both of these sites (E3 and E4) recorded 100% survivorship, with the maximum salinities and salinity ranges recorded at these sites well below the laboratory salinity tolerance range of *G. pulex* over 12 and 24 hrs (LC$_{50}$ at 7 ºC; Table 6.6).
2. *Ephemera danica*

Survivorship of *E. danica* was high in all field experiments (>98%) (E1 – E4) and associated control tests (100%) (C) (Figure 6.17, Table 6.5). At experimental site E1, survivorship of *E. danica* was 100%, despite experiencing a 24 hr tidal maximum salinity (27.7) and salinity range (27.5), greater than the 24 hr laboratory derived concentration of salt lethal to 99% of individuals (LC$_{99}$), at 26.6 (26.3 – 27.3) (at 7 ºC) (Figure 6.17a, Table 6.5, Table 6.6). The recorded field salinity was greater than the 24 hr LC$_{50}$ salinity tolerance range of *E. danica* (25.6 – 26.6) (at 7 ºC) for a total of 24 minutes over the final 12 hr test period (Table 6.5, Table 6.6). The 12 hr maximum salinity (29.6) and salinity range (29.4) was within the 12 hr LC$_{50}$ of *Ephemera danica* recorded under laboratory conditions (29.6 – 30.7). The water temperature over the 24 hour experimental period ranged from 6.8 – 14.8 ºC (mean of 9.7 ºC) (Table 6.5).

At experimental sites E2, E3 and E4, survivorship of *E. danica* was 100% (Figure 6.17b, c, d Table 6.5). Survivorship at these sites corresponds to the 12 and 24 hr laboratory toxicity studies, with the maximum 12 and 24 hr tidal salinities (and salinity ranges) recorded at E2 (15
and 12.3), E3 (9 and 6.5) and E4 (0.24 and 0.21), below the laboratory salinity toxicity range (LC₃) of *E. danica* at 12 (25.7 – 26.8) and 24 hrs (21.7 – 22.7) (at 7 °C) (Table 6.5, Table 6.6). Over the 24 hour experimental period, the water temperatures at these sites (E2 – E4) ranged from 7 – 13.6 °C (Table 6.5).

![Figure 6.17](image)

**Figure 6.17.** The 12 and 24 hr survivorship of *Ephemera danica* under field tidal cycle conditions. a. experimental site E1, b. experimental site E2, c. experimental site E3 and d. experimental site E4. The salinity profile recorded at each site is shown.

3. *Neureclipsis bimaculata*

Survivorship of *N. bimaculata* in the field experiments E1, E2 and E3, and associated control tests (0.01) (C) was high, at more than 80% (Figure 6.18a, b, c, Table 6.5). The laboratory derived salinity toxicity results (at 7 °C) of *N. bimaculata* reveal a short-term (12 – 24 hr) tolerance to high salinities (up to 35), with more than 20% survivorship in all salinity treatments over 24 hrs (Figure 6.20), resulting in high LC₅ₐₖ tolerance values (Table 6.6). At site E1, survivorship dropped to 92% after 12 hrs (over a tidal cycle salinity range of 0.2 – 29.6 – 0.2) and to 86% after 24 hours (0.2 – 27.7 – 0.2) (Figure 6.18a, Table 6.5). Although survivorship above 80% is deemed reliable in acute toxicity studies (Greve et al 1998), the fall in survivorship of *N. bimaculata* over 12 and 24 hrs could be indicative of a slow mortality rate in relation to tidal conditions. The 12 and 24 hr laboratory salinity toxicity studies would however
support the tolerance of *N. bimaculata* to the tidal salinities recorded at site E1, with a recorded LC$_1$ of 33.4 (32.9 – 34.3) and 31.8 (31.2 – 32.7) (at 7 °C) (Table 6.5, Table 6.6).

At sites E2 and E3, survivorship of *N. bimaculata* was 100%, exhibiting tolerance to maximum salinities of up to 15 and tidal salinity ranges of up to 14.8 for 24 hours (Figure 6.18b, c, Table 6.5). This tolerance again corresponds to the 12 and 24 hr salinity toxicity studies at 7 °C (Table 6.6). Over the 24 hour experimental period, the water temperatures at these sites (E2 – E3) ranged from 7 – 13.6 °C (Table 6.5).

Survivorship at site E4 was low, despite only experiencing tidal limnetic (0.2 – 0.24) salinities over the 24 hr test period (Figure 6.18d, Table 6.5). Survivorship dropped to 80% after 12 hours and to 58% after 24 hrs (Figure 6.18d, Table 6.5). During the 24 hr experimental period at site E4, the field test cell tubes were flushed with fine sediments carried in the water column. This fine sediment covered the *N. bimaculata* specimens (pers. obs.) and may have caused the mortality observed in this experiment (E4). Survivorship in the limnetic (0.1) control was 100% (Figure 6.18d, Table 6.5).

![Figure 6.18. The 12 and 24 hr survivorship of Neureclipsis bimaculata under field tidal cycle conditions. a. experimental site E1, b. experimental site E2, c. experimental site E3 and d. experimental site E4. The salinity profile recorded at each site is shown.](image-url)
4. *Hydropsyche angustipennis*

Survivorship of *H. angustipennis* in the field experiments (E1 – E4), and associated control tests (0.5) (C) was high, at more than 98% (Figure 6.19, Table 6.5). The laboratory derived salinity toxicity results for *H. angustipennis* (at 7 °C) reveal a short-term (12 – 24 hr) tolerance to high salinities (up to 35), with more than 50% survivorship in all salinity treatments over 24 hrs (Figure 6.20), resulting in high LC₅ₐ tolerance values (as recorded for *N. bimaculata*) (Table 6.6). The high survivorship (>98%) of *H. angustipennis* at all experimental sites corresponds to this high laboratory recorded salinity tolerance (Figure 6.19, Table 6.5, Table 6.6).

At experimental site E1, survivorship of *H. angustipennis* was 98%, exhibiting a 12 and 24 hr tolerance to tidal maximum salinities of 29.6 and 27.7 and salinity ranges of 29.4 and 27.5 (Figure 6.19a, Table 6.5). This tolerance corresponds with the laboratory derived 12 and 24 hr salinity concentration lethal to 1% of the population (LC₅), recorded at 35.6 (35 – 36.6) and 35.5 (34.7 – 36.4) respectively (Table 6.6). Survivorship at experimental sites E2, E3 and E4 was 100%, with *H. angustipennis* tolerating maximum salinities of up to 15 and salinity ranges of up to 14.8 for 24 hours (Figure 6.19b, c, d, Table 6.5).

![Figure 6.19](image_url)

Figure 6.19. The 12 and 24 hr survivorship of *Hydropsyche angustipennis* under field tidal cycle conditions. a. experimental site E1, b. experimental site E2, c. experimental site E3 and d. experimental site E4. The salinity profile recorded at each site is shown.
Table 6.5. Summary table of the field tidal cycle experiments and associated controls. Results shown as proportion alive after 12 and 24 hrs. The maximum and minimum salinities (psu), salinity range and temperature (°C) range recorded at each site over the experimental period are shown.

<table>
<thead>
<tr>
<th></th>
<th>Experimental site 1 (6.35 km)</th>
<th>Experimental site 2 (8.7 km)</th>
<th>Experimental site 3 (9.18 km)</th>
<th>Experimental site 4 (11.31 km)</th>
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<tbody>
<tr>
<td>Time (hr)</td>
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<td>12</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td><strong>Min and max tidal cycle salinities (psu)</strong></td>
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<td>0.2 - 27.7 - 0.2</td>
<td>0.2 - 15 - 0.2</td>
<td>0.2 - 12.3 - 0.2</td>
</tr>
<tr>
<td>Salinity range (psu)</td>
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<td>27.5</td>
<td>14.8</td>
<td>12.1</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>6.8 - 14.8 (mean 9.7)</td>
<td>7 - 10.5 (mean 9.1)</td>
<td>7.1 - 13.6 (mean 9.5)</td>
<td>7.5 - 13.6 (mean 10.1)</td>
</tr>
<tr>
<td><em>Gammarus pulex</em></td>
<td>1 0.24 0.16 1 1 1 1 1 1 1 1 1</td>
<td>1 0.98 0.98 1 1 1 1 0.98</td>
<td>0.98 1 1 1 1 1 1 0.8 0.58</td>
<td></td>
</tr>
<tr>
<td>Control (0.1)</td>
<td>1 1 1 1 1 1 1 1 0.98 0.98 1 1 1 1 1 1</td>
<td>1 1 1 0.98 0.98 0.98 0.98 0.98 0.98 0.98</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0.98 0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0.1)</td>
<td>1 1 1 1 1 1 1 1 0.98 0.98 1 1 1 1 1 1</td>
<td>1 1 1 0.98 0.98 0.98 0.98 0.98 0.98</td>
<td></td>
<td></td>
</tr>
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<td><em>Neureclipsis bimaculata</em></td>
<td>1 0.92 0.86 1 1 1 1 1 1 1 0.8 0.58</td>
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<td>Control (0.1)</td>
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<td></td>
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<tr>
<td><em>Hydropsyche angustipennis</em></td>
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<tr>
<td>Control (0.1)</td>
<td>1 0.98 0.98 1 1 0.98 0.98 1 0.98 0.98 0.98 0.98 0.98 0.98</td>
<td>1 1 1 0.98 0.98 0.98 0.98 0.98 0.98 0.98</td>
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<td></td>
</tr>
<tr>
<td>Species</td>
<td>Time</td>
<td>Temp (°C)</td>
<td>Control mortality (%)</td>
<td>Regression model sig.</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>-----------------------</td>
</tr>
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<tr>
<td></td>
<td>24</td>
<td>7</td>
<td>0</td>
<td>0.000*</td>
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<td></td>
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<tr>
<td></td>
<td>24</td>
<td>7</td>
<td>0</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

Table 6.6. Summary table of the 12 and 24 hr laboratory salinity toxicity test results (at 7°C) for the selected field test species, including control mortality and regression model significance (*model significant at p<0.01). Salinity tolerances classified as the lethal concentration of salt to a given percentage of the test population (LC_{x}), with associated 95% confidence interval ranges.
Figure 6.20. The survival of selected macroinvertebrate species in the laboratory acute salinity toxicity studies, after 12, 24, 48 and 72 hours, a. *Gammarus pulex*, b. *Ephemera danica*, c. *Neureclipsis bimaculata* and d. *Hydropsyche angustipennis*.

6.7.5 Summary

- The tolerance of *Gammarus pulex* to field tidal cycle conditions corresponds with the salinity tolerance values (LC$_x$) determined in the laboratory salinity toxicity study, with high mortality recorded where the maximum tidal salinity exceeded the LC$_{50}$ over 12 and 24 hours. Where the maximum tidal salinity was below that of the LC$_{50}$, 100% survivorship was recorded, demonstrating both a tolerance of these maximum salinities in the field, but also the range of salinities (below the maximum) experienced under tidal cycle conditions.

- The tolerance of *Ephemera danica* to field tidal cycle conditions exceeded the salinity tolerance values (LC$_{99}$) determined in the laboratory, with 100% survival in tidal cycle salinities determined to be lethal to it in the laboratory (LC$_{99}$). This may indicate a
greater tolerance to fluctuating tidal profiles where maximum salinities are higher than those determined fatal under laboratory conditions.

- The tolerance of *Neureclipsis bimaculata* to field tidal cycle conditions corresponds with the 12 and 24 hr salinity tolerance values (LC₅₀) determined under laboratory conditions (12 and 24 hr LC₅₀ greater than 35). The low mortality at all experimental sites demonstrates a tolerance to both high maximum salinities and the large range of salinities experienced over a tidal cycle. High mortality was recorded in tidal limnetic conditions (<0.5), where individuals were covered with fine muddy sediments, highlighting the impact of additional environmental parameters on mortality in the estuarine zone.

- The tolerance of *Hydropsyche angustipennis* to field tidal cycle conditions corresponds with the very high 12 and 24 hr salinity tolerance values (LC₅₀) determined under laboratory conditions (with LC₁ greater than 35). Low mortality at all experimental sites demonstrates a tolerance to both high maximum salinities and the large range of salinities experienced over a tidal cycle.

### 6.8 Discussion

The responses of macroinvertebrate species to salinity concentrations above and/or below those that they would normally experience, is invariably determined by physiological processes (Potts, 1954; Silva & Davies, 1999; Potts, 1954; Kapoor, 1979; Colburn, 1983; Stern *et al.*, 1984; Hart *et al.*, 1991; Silva & Davies, 1999; Kefford *et al.*, 2007; Sornom *et al.*, 2010). Examining the somatic and cellular mechanisms that underpin the observed physiological responses to salinity (under both laboratory and field conditions), was however beyond the scope of this research project. For example, whilst a number of studies have indicated the importance of osmoregulatory mechanisms in the survival of macroinvertebrates in hypertonic saline conditions (Kinne, 1970; Shyamasundari, 1973; Dorgelo, 1977; Allan *et al.*, 2006; Sornom *et al.*, 2010), it is the relationships between salinity and mortality (salinity tolerance) and its relevance to actual/predicted distribution patterns that are of importance to the delivery of this project, rather than the physiological mechanisms that underpin them.

In this chapter it was hypothesised that the laboratory acute salinity tolerances (LC₅₀) of benthic macroinvertebrate species will be reduced at high temperatures (18 °C) compared to lower temperatures (7 °C). In this study, the 72 hr laboratory acute salinity toxicity studies have shown that temperature regime does have a significant impact on the degree of salinity tolerance, with
all species (tested under both temperature regimes) exhibiting significantly higher tolerances to salinity at 7 °C compared to 18 °C. These results are corroborated by studies which have shown that beyond the natural salinity range of a species, increasing temperatures significantly decreases survivorship and when combined, salinity and temperature can have seasonal effects on the salinity tolerances of macroinvertebrate species (Kinne, 1971; Lincoln, 1979; Hayward & Ryland, 1995; Barnes et al., 2008). As the temperature regimes used in the salinity toxicity studies reflect typical summer and winter water temperatures in the River Adur and River Ouse, the resultant salinity tolerance values relate directly to survival potentials over seasonal and annual cycles.

The distributions and ecology of estuarine macroinvertebrate species in the UK have been extensively studied for many decades, resulting in a large body of research upon which to base predictions of the potential ecological impacts of future increases in saline penetration (Kinne, 1971; Lincoln, 1979; Barnes, 1994; Hayward & Ryland, 1995 and the references therin). The laboratory acute toxicity tests of euryhaline marine and brackish water macroinvertebrate species commonly recorded in the estuarine zone (Gammarus salinus, Melita palmata and Gammarus zaddachi) to reductions in salinity, closely corresponds to the field distributions and tolerances of these species in published literature, but do not match the distributions of these species (Gammarus salinus and Melita palmata) in the River Adur and River Ouse estuaries. In the laboratory acute toxicity study, Gammarus salinus and Melita palmata tolerated reductions in salinity concentrations of 4.1 (2.9 – 7.6) and 4 (2.8 – 5.6) respectively (72 hr at 18 °C), but were recorded at sites in the River Adur and River Ouse estuaries that experienced tidal limnetic (<0.5) conditions at low tide. It was hypothesised (in Chapter 5) that these species may seek refuge from tidal limnetic conditions in the intertidal coarse bank sediment at low tide. Testing this hypothesis by undertaking field tidal cycle survival tests (as for selected limnic species in this chapter) could be of value in determining the field tolerances of these species to low tide tidal limnetic conditions.

The acute salinity toxicity tests of selected limnic derived species demonstrated high tolerances to the laboratory maximum salinity levels and those naturally occurring during tidal cycles in the field. The salinity tolerance values (LC₅₀) determined from the laboratory salinity toxicity studies generally corresponded well with survival under field tidal cycle conditions, irrespective of the range of fluctuations in salinity (below the maximum) that occurred during the 12 and 24 hr tidal cycles. Where the maximum tidal cycle salinity did not exceed the concentration of salinity lethal to 50% of individuals (LC₅₀), survival in the field was high. The one exception was the
ephemeropteran *Ephemera danica*, which survived tidal cycle salinities greater than the salinities determined to be lethal to it over 24 hrs in the laboratory (LC<sub>99</sub>). This therefore suggests that *E. danica* may have greater tolerance to fluctuating tidal profiles where maximum salinities are higher than those determined fatal under laboratory conditions. Further investigation of the salinity responses of this species would be required to resolve this apparent conflict.

It is commonly assumed that limnic aquatic insects (Odonata, Plecoptera, Ephemeroptera and Trichoptera), in particular the Ephemeroptera, are the most intolerant of the macroinvertebrates to increases in salinity (Short et al., 1991; Williams & Williams, 1998b; Williams & Hamm, 2002). The limnic insects selected and tested in this study (e.g. *Ephemera danica*, *Pyrrhosoma nymphula*, *Hydropsyche angustipennis* and *Neureclipsis bimaculata*) do not support this assumption, recording 72 hr salinity tolerance (LC<sub>50</sub>) ranges of 22.8 – 31.5 at 7 °C and 8 – 24 at 18 °C (including CI) and surviving tidal cycle salinity ranges of up to 31.2 over 24 hrs. Of all limnic species tested, the damselfly (Odonata) *Pyrrhosoma nymphula* recorded the highest salinity tolerance, exhibiting a haline-tolerant (0 – 35) tolerance to salinity, with high survivorship in all salinity treatments. The assumption that aquatic insects are especially sensitive to saline conditions must therefore be questioned. This study is not alone in recording high salinity tolerances in limnic aquatic insects, with several species belonging to the orders Ephemeroptera (Sutcliffe, 1960; Williams & Williams, 1998a; Piscart et al., 2005; Blinn & Ruiter, 2006; Williams, 2009), Trichoptera (Muller & Mendl, 1979), Plectoptera (Butler & Popham, 1958; Kefford et al., 2003) and Odonata (Hart et al., 1991; Bailey et al., 2002; Clunie et al., 2002; Nielsen et al., 2003) reported as tolerating high salinity concentrations.

In this chapter it was hypothesised that the laboratory acute salinity tolerances (LC<sub>50</sub>) of a species will correspond to its recorded survivorship under field tidal cycle conditions, and that these results will reflect the recorded field distributions and salinity tolerances reported in published literature. However, the high experimentally-determined salinity tolerances of the limnic derived macroinvertebrate species in this study do not correspond to the salinity preference/tolerance groupings allocated to these species in Chapter 5 (based on published literature), or the field distributions of these species in the River Adur and River Ouse. In the UK, there has been very little research focused on the tolerance of limnic macroinvertebrate species to increases in salinity under standardised laboratory or field conditions. In contrast, in Australia, secondary salinisation of limnetic water bodies has resulted in greater assessment of the impact of increasing salinity upon limnic macroinvertebrates, resulting in a large body of published research (for reviews see: Odum, 1988; Schuchardt et al., 1993; Rundle et al., 1998;
Williams & Williams, 1998b; Sousa et al., 2007). In the UK, very few studies have investigated the distributions of limnic derived species in estuaries, with both estuarine and freshwater ecologists using the estuarine tidal limnetic zone to delineate their study reaches of interest (estuarine ecologists below, freshwater ecologists above this zone; 1998a). This historical neglect of the tidal limnetic zone has resulted in limited knowledge of the tolerances of limnic species to estuarine conditions and their associated distribution patterns. The combined effect of this lack of knowledge (ecophysiological and biogeographical), has resulted in assumptions being made regarding the salinity tolerances of limnic macroinvertebrate species, based primarily on the maximum salinities at which these species are recorded in the field, a method unlikely to provide an accurate determination of salinity tolerance (Underwood et al., 2000). As the majority of limnic derived species are recorded in non-tidal limnetic water bodies (<0.5) with no salinity input, most limnic macroinvertebrate species are assumed to have no capacity for tolerance to increases in salinity. Anticipated future increases of saline penetration into river systems make consideration of salinity effects upon limnic species a valid matter for focused research. Where published studies have investigated the distributions and salinity tolerances of limnic macroinvertebrate species in estuaries, authors have also recorded high tolerances to salinity, both in the laboratory and field. In Wales, Williams and Williams (Williams & Williams, 1998a), recorded high densities (2915 and 74900 cm$^{-2}$) of freshwater insect larvae (Plecoptera, Ephemeroptera, Trichopteran, Coleoptera and Diptera), in areas of the Aber estuary, which were inundated by 33.7% and 100% of all incoming tides. Salinity trials demonstrated that in the laboratory, many of these limnic species could survive at least some level of salinity, with some trichopterans withstanding simulated 24 hr tidal cycles in seawater-strength salinity levels (32) (Williams & Williams, 1998a; Williams, 2009). Gut content analysis of two limnic trichopterans, Hydropsyche siltalai and H. instabilis recorded in the Aber estuary, determined that these species were functioning normally in suboptimal conditions, under varying degrees of saline penetration (see Chapter 1, Section 1.2.8; Williams, 2009).

The laboratory acute salinity toxicity experiments and associated tidal cycle survival tests (along with a small number of published research studies) have shown that selected limnic macroinvertebrate species have considerable capacities to tolerate saline conditions, both in the laboratory and under tidal cycle conditions (Williams & Hamm, 2002). Despite this, discrepancies occur between the experimentally assessed ranges of salt tolerance and the natural field distributions of these species. Of the limnic macroinvertebrate species tested in this study, only the amphipod Gammarus pulex and isopod (Asellus aquaticus) were recorded in the River Adur and River Ouse estuaries, and only A. aquaticus at salinities above that of freshwater. It is
likely that this is due to habitat preference type (e.g. substrate composition), with only A. aquaticus and G. pulex (out of the limnic species selected in this study) able to inhabit the tidal, deep, fine grained mud and clay sediment, lower oxygenated sites of the mid to upper River Ouse and River Adur estuaries. Despite the capacity of G. pulex to tolerate these habitat characteristics and saline estuarine conditions, this species is not recorded in estuarine sites with salinities greater than 0.5. This may indicate the impact of additional biotic and abiotic factors limiting the distribution of G. pulex in estuaries, for example competition with its sibling estuarine species Gammarus zaddachi (see Chapter 5). The majority of limnic macroinvertebrate species tested in this study could not inhabit the tidal limnetic estuarine zone of the River Adur or River Ouse (e.g. Ephemera danica, Hydropsyche angustipennis, Neureclipsis bimaculata and Pyrrhosoma nymphula), but may still be at risk from progressive increases in saline penetration above the current limit of the tide, particularly in estuaries and rivers where abiotic environmental factors (such as substrate composition) provide preferential habitat for these species. For example, in coarse grained substratum estuaries in Canada, the immature stages of limnic derived Ephemeroptera, Plectoptera, Coleoptera and Trichoptera species have been recorded inhabiting estuarine sites inundated by 25% of all incoming tides, experiencing high tide salinities of approximately 24 (Williams & Hamm, 2002).

This study indicates that the large body of research pertaining to the distribution patterns and saline tolerances of euryhaline-marine and brackish water macroinvertebrate species commonly recorded in estuaries corresponds well to results derived from laboratory salinity toxicity studies (Table 6.3). In contrast, the limited knowledge of both salinity tolerances and estuarine field distributions for limnic macroinvertebrate species, often results in assumptive salinity tolerances with no correlation to actual laboratory and field determined salinity tolerance values. The majority of macroinvertebrate species tested in this study (with the exception of G. zaddachi) have shown discrepancies between experimentally assessed tolerances to salinities (or reduced salinities) and the field distributions of these species in the River Adur and River Ouse. This could be due to both issues regarding the variances in salinity tolerances within species populations (e.g. adult life stages, may differ for other development life stages, age, reproductive state, moult cycle stage, nutritional state and parasitic infection), additional physiological impacts of salinity not recorded in this study, and/or the effects additional biotic and abiotic factors in the field (e.g. inter/intra specific competition, predation, sedimentary regime, flow rates, physicochemical changes, dissolved oxygen concentration and water depth). While this project has indicated that quantified species salinity tolerance levels would be critical elements in any attempt to predict the ecological consequences of future increases in salinity, the possible
roles of other biotic and abiotic factors should not be ignored. The identification of any such factors which may co-occur with increasing salinity and could contribute to the distributions of macroinvertebrate species in the field are beyond the scope of this current research project.

6.9 Summary

- Temperature regime has a significant impact on the degree of macroinvertebrate salinity tolerance, with selected limnic species exhibiting significantly higher tolerances to salinity at 7 ºC compared to 18 ºC. This could relate directly to survival potentials and distributions of limnic macroinvertebrate species in estuarine zones over seasonal and annual cycles.

- The acute salinity toxicity tests of selected limnic derived species, demonstrated high tolerances to both laboratory maximum salinity levels and ranges of salinity naturally occurring during tidal cycles in the field. The resulting salinity tolerances question the assumption that of all aquatic macroinvertebrates, aquatic insects are especially sensitive to increases in salinity.

- The large body of research pertaining to the distribution patterns and saline tolerances of marine and brackish water macroinvertebrate species commonly recorded in estuaries corresponds well to results derived from laboratory salinity toxicity studies.

- The limited knowledge of both salinity tolerances and estuarine field distributions for limnic macroinvertebrate species, often results in assumptive salinity tolerances with no correlation to actual laboratory and field determined salinity tolerance values.

- Discrepancies were observed between experimentally assessed tolerances to salinities (or reduced salinities) and the field distributions of selected macroinvertebrate species in the River Adur and River Ouse, which may be the result of issues regarding variances in salinity tolerance within species populations and/or the effects biotic and abiotic factors in the field.
Chapter 7. Synthesis and discussion

7.1 Introduction

To investigate the extent and ecological impact of future increases in saline penetration on the River Adur and River Ouse in Sussex, this research project investigated a progressive, cumulative series of research elements (see Chapter 1 for full breakdown). These included determination of current and future (predicted) tide and salinity profiles (Chapter 5), examination of historic (Chapter 4) and contemporary (Chapter 6) macroinvertebrate community structures, relationships with environmental variables and macroinvertebrate acute salinity tolerances in the laboratory and field (Chapter 7). In this chapter these individual elements are integrated in order to predict macroinvertebrate distribution patterns for 2080 under the UKCIP high emissions scenario (SRES:A1FI) for low freshwater discharge conditions and evaluated with respect to predictive accuracy (Section 7.2). In section 7.3, the results are discussed with reference to the impact of additional climatic and anthropogenic changes to freshwater discharge and channel morphology on the upstream extent of saline penetration. A conceptual model of the potential ecological effects of both increases in saline penetration and changes to the estuarine system (anthropogenic and climatic induced) is introduced and discussed in relation to current climate change biology literature, estuarine and riverine habitat and biodiversity legislature, future management strategies and consequences for regional and national estuarine systems. Some consideration is also given to the direction of future and follow-up research activities.

7.1.1 Aims, Objectives and Hypotheses

Specifically this chapter aims to answer the primary aim of this research project: to determine how the temporal and spatial extent of saline penetration into the River Adur and River Ouse estuaries will change under future climate scenarios and to predict the impact these changes will have upon benthic macroinvertebrate community structure and species distributions over the freshwater to marine transition. These aims will be achieved through the following objectives:

1. Predict future benthic macroinvertebrate community structure and species distributions in the River Adur and River Ouse for projected increases in saline penetration (determined in Chapter 5) under ‘worst-case’ conditions (low freshwater discharge, high emissions scenario, 2080; 95th percentile).
2. To critically assess the use of salinity tolerances alone for the prediction of future benthic macroinvertebrate community structure and species distributions in relation to projected increases in saline penetration (under ‘worst-case’ conditions; Chapter 5).

3. Discuss the influence of additional environmental variables (e.g. sedimentary characteristics, human impact) on the distribution and faunal structure of benthic macroinvertebrates in the River Adur and River Ouse estuaries, and their implications for accurate prediction of future ecological and environmental change to projected increases in saline penetration.

4. Discuss the implications of projected increases in saline penetration upon the wider ecology and ecosystem functioning of the estuarine and lower reaches of the River Adur and River Ouse.

5. Discuss the impact of additional climatic and anthropogenic changes to freshwater discharge and channel morphology on the predicted upstream extent of saline penetration in the River Adur and River Ouse estuaries and introduce and discuss a conceptual model of the potential ecological effects of both increases in saline penetration and changes to the estuarine system (anthropogenic and climatic induced).

6. Discuss the wider applications of the results presented in this study (including suggestions for future research) and consider the relevance and application to UK estuarine and river systems.

In this project it was hypothesised that current and future climate change will increase relative sea levels and reduce freshwater river discharge, resulting in the progressive upstream penetration of saline water into the River Adur and River Ouse estuaries. It was also hypothesised that the ecological consequences of increased saline penetration will include changes to the distribution and composition of resident benthic macroinvertebrate taxa as functions of a species salinity tolerance. It was further hypothesised that these changes could be predicted using the quantified salinity tolerances of representative macroinvertebrate species.

### 7.2 The future extent of saline penetration

The tide and salinity profiles of the River Adur and River Ouse estuaries have been shown to be highly spatially and temporally dynamic, resulting from large tidal ranges (MHWS of 6.1 and 5.5 m respectively), low channel bed gradients, significant seasonal changes in river discharge regimes and management strategies (e.g. channel constriction through bank stabilisation and land reclamation; see Chapter 5). Future tide and salinity profiles (MHWS and MLWS) for the River Adur and River Ouse estuaries were predicted for relative sea level rise projected by the high
emissions scenario (SRES: A1FI), over three 30-year time periods (2020s, 2050s and 2080s; 5th to 95th percentile range) under both low (summer, August 2008) and high (winter, February 2009) freshwater discharge conditions (see Chapter 5). In both the River Ouse and River Adur estuaries, these projected increases in relative sea level (above MHWS) resulted in an increase in both the upstream extent of saline penetration and the location of maximum salinity zones (compared to current tide and salinity profiles; Chapter 5, Section 5.4). However, the predicted increases in saline penetration in both the River Ouse and River Adur estuaries were modest, even under the ‘worst-case’ relative sea level rise scenario (i.e. associated with SRES: A1FI) for the year 2080 (95th percentile), under low freshwater discharge conditions (see Chapter 5). In the River Ouse estuary, this ‘worst-case’ profile predicted an increase in the upstream extent of saline penetration of 0.15 km (150 m) compared to 0.32 km (320 m) in the River Adur estuary.

The very small increase in saline penetration predicted for the River Ouse estuary is likely due to the artificial increase in bed gradient at the Hamsey cut-through channel acting as a partial in-stream barrier to the upstream ingress of saline water (as shown in the current tide and salinity profiles; Chapter 5, Section 5.4). The more natural course of the River Adur estuary (and the more representative distribution of salinity stations) suggests that the patterns of current and predicted tidal saline penetration under low and high freshwater discharge conditions is a more realistic representation of low-lying coastal river valleys with essentially consistent and gradual gradients.

7.2.1 Consequences for ecology

Salinity parameters (i.e. maximum, minimum and average) have been shown to be the dominant environmental variables driving macroinvertebrate community structure and species distributions in the both the River Adur and River Ouse estuaries, with variations in the degree of saline penetration (between low and high freshwater discharge conditions), resulting in some shifts in the distribution and structure of the macroinvertebrate community (Chapter 6). However, within the River Adur and River Ouse estuaries, additional environmental variables might be of greater local significance than salinity in determining the distributions of macroinvertebrate species (e.g. sediment characteristics; Chapter 6). Using the benthic macroinvertebrate species salinity tolerance data, field distribution patterns (under low freshwater discharge conditions; August 2008) and associations with additional environmental variables, future macroinvertebrate distributions were predicted for both rivers/estuaries for the ‘worst-case’ saline penetration profiles (i.e. an increase of 0.32 km in the River Adur, 0.15 km in the River Ouse, see above) and the accuracy of these predictions was assessed. Predicted faunal changes are discussed.
sequentially, with the estuaries divided into three arbitrary zones: lower to mid, mid-upper and upper to tidal limnetic.

7.2.1.1 Predicted changes within the lower to mid estuarine zones

There is a large volume of published literature and research on the ecology, distributions and salinity tolerances of marine and estuarine benthic macroinvertebrate species commonly recorded in the UK (Chapter 6). The acute tolerance of the amphipods *Gammarus salinus* and *Melita palmata* to reductions in salinity (LC$_{50}$ and 95% CI) recorded under laboratory conditions (in this study), corresponded with both salinity tolerances and field distributions of these two species in published literature (Lincoln, 1979; Hayward & Ryland, 1995; Barnes *et al.*, 2008) (Chapter 7). The close association between tolerance values derived from laboratory experiments and published literature might indicate that the salinity tolerances of marine and estuarine macroinvertebrates (in the lower to mid estuarine zone) are already well established and therefore could potentially be used to predict the distribution responses of these species to increases in saline penetration. This assumption, however, needs to be more thoroughly tested on additional marine and estuarine species before more confident predictions can be made. In this study, marine and brackish water macroinvertebrates were allocated to salinity tolerance groupings based on published literature (Wolf *et al.*, 2009) (euryhaline-marine 0.5 – 35 and brackish water 0.5 – 30; see Chapter 6). Despite providing some insight into the upstream extent of saline penetration into the River Adur and River Ouse estuaries (in the absence of salinity measurements), using these groupings to predict individual species distribution responses to salinity increases is inherently flawed (Chapter 6). This is due to the wide salinity ranges of these groupings (0.5 – 35 and 0.5 – 30) effectively masking individual species tolerance ranges, especially at the lower salinities (<5). This will potentially lead to predictions exaggerating the upstream distributions of these species, if based on salinity tolerance groupings alone.

In the River Adur and River Ouse estuaries, the field distributions of euryhaline-marine and brackish water species did not correspond with their salinity tolerance ranges according to published literature (both individual and grouped ranges) or tolerance to salinity reductions determined under laboratory conditions (i.e. *Gammarus salinus* and *Melita palmata*; Chapter 7). In both estuaries, the majority of euryhaline-marine and brackish water species were recorded up to 10.64 km and 12.8 km from the mouths of the River Adur and River Ouse respectively, in sites experiencing high maximum salinities (10 – 35) and tidal limnetic (<0.5) minimum salinities. Based on the tolerances of *Gammarus salinus* and *Melita palmata* to reductions in salinity (as recorded in the salinity toxicity studies and published literature; Lincoln, 1979;
Barnes, 1994; Hayward & Ryland, 1995), these species should have been restricted to the lower 5.6 km of the River Ouse estuary, due to their (assumed) inability to tolerate low minimum salinities. Conversely, based on tolerance to maximum salinities, their ranges should have extended a further 1.6 km upstream (from actual recorded location under low freshwater discharge conditions). This suggests that the actual field distributions of these two species are also influenced by other environmental parameters to some degree (e.g. sediment characteristics described below).

In the lower to mid zones of both estuaries, the distributions of the majority of recorded euryhaline-marine and brackish water species appeared to be closely associated with coarse grained sediment (<60 mm diameter), with the transition from coarse to fine grained sediment appearing to act as a barrier to the upstream distributions of these species, particularly in the River Adur estuary (e.g. *Gammarus salinus*, *Melita palmata*, *Carcinus maenas* and *Leptocheirus pilosus*, Chapter 6). This sediment boundary ‘effect’ was less apparent in the River Ouse estuary, where anthropogenic modification of the estuary banks (pole-wharfing) has resulted in the extension of coarse grained sediment upstream (Chapter 6). The observed relationship between sediment grain size and the distribution of euryhaline-marine and brackish water species might be related to the refuge potential of coarse grained sediment, providing protection against aerial exposure, desiccation, predation and access to higher interstitial salinities in the estuary channel banks at low tide (Chapter 6; Lincoln, 1979; Hayward & Ryland, 1995; Williams & Hamm, 2002). Therefore, the limits of coarse grained sediment in the River Adur and River Ouse estuaries might restrict the upstream distribution of euryhaline-marine and brackish water species, in relation to their tolerances to reductions in maximum salinities (high tide), but act as an extension to their distributions based on tolerances to minimum salinities, by providing refuge from tidal limnetic (<0.5) salinities (outside their tolerance ranges), at low tide. It is therefore likely that even under the ‘worst-case’ predicted saline penetration profiles (low freshwater discharge, 2080 high emissions scenario; 95\(^{th}\) percentile), the maximum upstream extent of the majority of euryhaline-marine and brackish water species recorded in the River Adur and River Ouse estuaries, will remain at the upstream limit of coarse grained sediment (i.e. at 10.64 km and 12.8 km from the river mouths for the River Adur and River Ouse respectively). It is also possible that the high calcium carbonate content of the sediment in the lower to mid catchments of both rivers (from both the underlying geology and chalk rip-rap) might also increase the tolerance of some species to reduced salinities (see Chapter 6, Section 6.5, Kinne, 1971).
7.2.1.2 Predicted changes within the mid to upper estuarine zones

In the majority of estuaries, the mid to upper estuarine zones are dominated by a limited number of species able to inhabit the physiologically challenging salinity boundary (5 – 8), the point at which the majority of limnic species become intolerant to increases in salinity and only a few marine and brackish species can survive (Kinne, 1971; Remane & Schlieper, 1971; Wolff, 1973; Deaton & Greenberg, 1986; Telesh, 2004; Telesh & Khlebovich, 2010). In the River Adur and River Ouse estuaries, the ‘naturally’ low species diversity of these zones might be exaggerated by the coarse to fine grained sediment transition (see above), restricting the upstream penetration of the majority of euryhaline-marine and brackish water species, irrespective of salinity tolerance. This might have been of particular importance in the River Adur estuary, where the zone of lowest diversity (and highest species dominance) was recorded immediately upstream of the transition from coarse to fine grain sediment (14.06 – 17.88 km). In the River Adur and River Ouse estuaries, the mid to upper estuarine zones were inhabited by species that had the ability to cross these sediment habitat boundaries (i.e. inhabit both coarse and fine grained sediments) and could tolerate the physiologically challenging mid to low maximum salinities (0.41 - 8) experienced at these sites (e.g. Gammarus zaddachi, Cyathura carinata, Corophium multiseta and Potamopyrgus antipodarum; Lincoln, 1979; Barnes, 1994; Gérard et al., 2003; Alonso & Castro-Díez, 2008). Under future predicted ‘worst case’ saline penetration profiles (low freshwater discharge, high emissions scenario (95th percentile), 2080), the mesohaline (5 – 18) and oligohaline (0.5 – 5) maximum salinity zones that typify the mid to upper estuarine zones of the River Adur and River Ouse estuaries are predicted to shift upstream by 0.32 and 0.15 km respectively (see Section 7.2).

The amphipod Gammarus zaddachi recorded a holeury-haline tolerance to salinity (0 – 35) under laboratory conditions (at 7 °C), which corresponded with the salinity tolerances and field distributions of this species recorded in published literature (Lincoln, 1979; Gledhill et al., 1993; Barnes, 1994; Hayward & Ryland, 1995; Wolf et al., 2009). The holeury-haline tolerance of G. zaddachi would suggest a ubiquitous field distribution (i.e. from the river mouth to above the tidal limits). However, the actual field distributions of G. zaddachi in the River Adur and River Ouse estuaries did not reflect this, as it was only recorded in the mid to upper estuarine zones in both estuaries. It is likely that this field distribution restriction is related to habitat partitioning of Gammarus zaddachi and its sibling species Gammarus salinus and Gammarus pulex along the salinity gradient (e.g. Gaston & Spicer, 2001; Korpinen & Westerbom, 2009, see Chapter 7, Section 7.2). Based on the holeury-haline salinity tolerance alone, it would not be possible to predict changes in the upstream distribution of Gammarus zaddachi for future increases in saline
penetration. However, the application of ecological restrictions (the distribution of *G. salinus* and *G. pulex*) upon the distribution of *G. zaddachi* (apparent in both estuaries) enables a general prediction of how the distribution of this species might change under future increases in saline penetration.

Within the mid to upper estuarine zones of the River Adur and River Ouse estuaries, the estuarine amphipod *Corophium multisetosum* and the limnic-derived gastropod *Potamopyrgus antipodarum* were recorded in maximum salinities beyond those of their allocated salinity tolerance groupings (brackish (0.5 – 30) and euryhaline-limnic (0 – 10), with *C. multisetosum* recorded in tidal limnetic maximum salinities (<0.5) and *P. antipodarum* in salinities of up to 27.8 (Queiroga, 1990; Barnes, 1994; Hayward & Ryland, 1995; Gérard et al., 2003; Alonso & Castro-Díez, 2008). These discrepancies highlight the issues associated with allocating ‘transitional’ species, with wide salinity tolerance to predetermined, precisely defined salinity tolerance groupings (see Chapter 6, Section 6.5). The large volume of published literature detailing the salinity tolerances and estuarine distributions of *C. multisetosum* and *P. antipodarum* corresponds to the field distributions of these species in the both the River Adur and River Ouse estuaries. Therefore, for species with wide salinity tolerances, predetermined salinity tolerance groupings should not be used, but rather predictions (under increasing saline penetration) should be based on published field and salinity tolerance data.

In this study, it is predicted that under future ‘worst-case’ increases in saline penetration, the low diversity, mid to upper estuarine zones (and the species recorded in them) could extend 0.32 km (River Adur) and 0.15 km (River Ouse) upstream in relation to the predicted increase in saline penetration. The downstream extents of these zones are, however, unlikely to change due to the sediment grain size boundaries limiting the upstream movement of a number of euryhaline-marine and brackish water species.

### 7.2.1.3 Predicted changes within the upper to tidal limnetic estuarine zones

The upper to tidal limnetic estuarine zones are the most sensitive to future increases in saline penetration as they mark the physiochemical and faunal transition between saline and limnetic waters. Under the ‘worst-case’ predicted saline penetration profiles (low freshwater discharge, high emissions scenario (95th percentile), 2080), the lower boundary of the tidal limnetic zone will be pushed by 0.32 km and 0.15 km upstream in the River Adur and River Ouse estuaries respectively (Section 7.2). The responses of existing limnic derived species (that inhabit the tidal limnetic zone) to increases in saline penetration are of critical importance for predicting the likely impacts upon the macroinvertebrate communities of tidal and non-tidal limnetic waters.
There is a lack of published literature on the salinity tolerances and estuarine field distributions of limnic derived macroinvertebrate species that inhabit the tidal limnetic zones of estuaries, therefore meaning that the likely responses of these species to predicted increases in salinity is uncertain. Where salinity tolerances have been published, many limnic derived species have recorded high tolerances to salinity, particularly for aquatic insect species that have historically been believed to be ‘most at risk’ (Hart et al., 1991; Gallardo-Mayenco, 1994; Williams & Williams, 1998b; Williams & Hamm, 2002; Piscart et al., 2010, see Chapter 6, Section 6.8).

The historic lack of research on the estuarine field distributions, coupled with limited salinity tolerance values for limnic species, both above and below the tidal limits, made the allocation of these species to salinity tolerance groupings difficult (limnic salt tolerating (0 - <5) and limnic (<0.5)). It is therefore unlikely that the allocated salinity tolerance groupings accurately depict the actual salinity tolerance capabilities of these species and their responses to future increases in saline penetration in the River Adur and River Ouse estuaries.

In this project a small, but representative number of limnic derived macroinvertebrate species were recorded in the tidal limnetic zones of the River Adur and River Ouse estuaries and as such might be subject to predicted future increases in saline penetration. Based on the available published literature, these species were allocated to the limnic salt tolerating (0 - <5) and limnic (<0.5) saline tolerance groupings (Wolf et al., 2009). These groupings were adequate in explaining the distribution of some of these species in the field, with a number of limnic, salt tolerating species recorded at the freshwater seawater interface (FSI) and therefore subject to fluctuating physiochemical conditions, over a maximum salinity range of 0.4 to 5.1 (e.g. Asellus aquaticus, Erpobdella octoculata, Erpobdella testacea, Sialis lutaria and Polycentropus flavomaculatus). The remaining limnic species were recorded upstream of the FSI in stable limnetic salinities (<0.5), but were still exposed to fluctuating physiochemical conditions over tidal cycles (e.g. Limnephilus flavicorns, Phryganea bipunctata, Plectrocnemia conspersa, Limnius volckmari, Centroptilum luteolum, Ephemera vulgata, Libella fulva, Hesperocorixa sahlbergi and Gammarus pulex). Under acute salinity toxicity studies, two limnic derived species, Asellus aquaticus (recorded at the FSI) and Gammarus pulex (recorded in the tidal limnetic zone), exhibited high tolerances to salinity, in the laboratory and for G. pulex, under field tidal cycle conditions. This is however contradictory to published salinity tolerance literature and the actual field distributions of these species in the River Adur and River Ouse estuaries (see Chapter 6). This discrepancy between laboratory derived salinity tolerance (LC50) and the field distributions of these limnic species in both estuaries, might be due to additional
physiological impacts associated with salinity not tested in this study, and/or other environmental factors that limit the distribution of these species in the field (Williams, 2009). This is supported by the upstream shift in limnic derived species in relation to increases in the extent of saline penetration, recorded in both in this study and published literature (Andrews, 1977; Attrill et al., 1996; Attrill & Power, 2000; Bessa et al., 2010).

For some macroinvertebrate species it has been demonstrated that salinity tolerances can vary in relation to developmental life cycle stage, age, reproductive state, moult cycle stage, nutritional state and parasitic infection (e.g. Lockwood, 1959a; Brock et al., 2003; Tills et al., 2010; Brooks & Mills, 2011; Piscart et al., 2010), suggesting that the salinity toxicity values determined in this study may not be representative of salinity tolerances at a population scale level. Under field conditions, the large ranges in salinity and additional sedimentological and physicochemical variables experienced over a tidal cycle might be more physiologically stressful than ‘fixed’ maximum salinities maintained during acute salinity toxicity tests (undertaken in the laboratory) (Kinne, 1971; Prandle, 2009). However, when subjected to actual tidal cycle conditions (in the field), survivorship of *Gammarus pulex* corresponded to its high laboratory-derived salinity tolerance values. Investigating the potential non-saline field variables that potentially limit the downstream distribution to *G. pulex* and *A. aquaticus* (based on salinity tolerance alone) was beyond the scope of this project. However, as previously highlighted, the downstream distribution limit of *Gammarus pulex* might be restricted by the upstream extent of its sibling species *Gammarus zaddachi* due habitat partitioning through interspecific competition (see Section 1.2.1.2, Lincoln, 1979; Gaston & Spicer, 2001; Korpinnen & Westerbom, 2009).

Without further knowledge of role played by other (non-saline) factors in limiting the field distributions of these species, it is not possible to accurately predict the responses of these species to future predicted increases in salinity based on laboratory derived salinity tolerances alone. Based on current field distributions and observed upstream shifts of these species with increases in saline penetration (i.e. difference in winter and summer freshwater discharge), it is likely that future increases in saline penetration will result in an upstream shift in distribution of these limnic derived species in both the River Adur and River Ouse estuaries, irrespective of their recorded salinity tolerance values.

The majority of limnic derived macroinvertebrate species in the River Adur and River Ouse were recorded above the tidal limits (21 km and 21.8 km). Under the future saline penetration profiles predicted in this study, it is unlikely that the lower non-tidal limnetic riverine systems of the River Adur and River Ouse would be subject to increases in tidal driven saline penetration over
the next century (see Chapter 5). However, predicted changes in river flows in south east England (caused by climate change and/or anthropogenic factors) could result in much greater future saline penetration, particularly in the summer months. Therefore, in estuarine systems where tidal limits are in range of predicted increases in saline penetration, the salinity tolerances of these species might become an important factor in determining their future distributions (for more detailed discussion, see below). In this study, the acute salinity tolerances of selected limnic species collected from above the tidal limits proved to be very high (LC50 range), both in the laboratory and under field tidal cycle conditions (e.g. Ephemera danica, Hydropsyche angustipennis, Neureclipsis bimaculata and Pyrrhosoma nymphula). These laboratory derived salinity tolerance values (LC50) did not correspond with the field distributions of these species in the River Adur or the River Ouse, possibly due to the other environmental factors (e.g. absence of coarse gravel and sand substrate, lack of highly oxygenated, shallow fast flowing channels) that are important in the life cycles of these species, precluding their occurrence in tidal saline reaches of the River Adur and River Ouse estuaries. The high saline tolerances recorded for limnic species in this study reinforces similar findings amongst other species from freshwater sources (e.g. Williams & Williams, 1998a; Chadwick & Feminella, 2001; Chadwick et al., 2002; Nielsen et al., 2003; Piscart et al., 2010). These results suggest that the existence, degree and value of salinity tolerances for limnic macroinvertebrates need to be assessed and their role in macroinvertebrate species distribution dynamics at the saline/limnic interface reviewed.

This project hypothesised that current and future climate change would increase relative sea levels and reduce freshwater river discharge, resulting in the progressive upstream penetration of saline water into the River Adur and River Ouse estuaries. It was also hypothesised that the ecological consequences of increased saline penetration would include changes to the distribution and composition of resident benthic macroinvertebrate taxa as functions of a species salinity tolerance. It was further hypothesised that these changes could be predicted using the quantified salinity tolerances of representative macroinvertebrate species. In both the River Ouse and River Adur estuaries, projected increases in relative sea level (above MHWS for scenario SRES: A1FI) resulted in modest predicted increases in both the upstream extent of saline penetration and the location of maximum salinity zones compared to present day tide and salinity profiles (Chapter 5, Section 5.4). Downstream freshwater discharge rates were shown to have a significant effect upon the extent of saline penetration, with high flows displacing salinity zones downstream and altering the composition and distributions of the resident benthic macroinvertebrate communities, particularly within the limnic and limnic salt tolerating taxa. For a number of species (particularly euryhaline-marine and brackish) additional environmental
variables (i.e. sediment grain size) were of greater local importance than salinity in determining macroinvertebrate distribution limits. Although definitive salinity tolerance values were established under laboratory conditions, these did not correspond to the distributions of the selected species in the River Adur and River Ouse estuaries. These disparities are suggestive of the influence of additional biotic and abiotic environmental variables which are of more local importance than salinity in determining macroinvertebrate species distributions and community structure. While salinity has been shown to be the dominant environmental variable determining macroinvertebrate structure along the freshwater to marine transitions of the River Adur and River Ouse estuaries, the influence of additional environmental factors is such that salinity alone cannot be used as the sole parameter for the prediction of the future consequences of increased saline penetration into estuary and river systems.

7.2.2 Summary

- Under the ‘worst-case’ saline penetration profiles (high emission scenario relative sea level (95th percentile) for the year 2080, under low freshwater discharge conditions), saline water is predicted to penetrate up to 17.3 km and 16.21 km into the River Adur and River Ouse estuaries respectively, a moderate increase of 0.32 km and 0.15 km from current profiles respectively (August 2008; Chapter 5).
- In the River Adur and River Ouse estuaries, the upstream distribution of the majority of euryhaline-marine (0.5 – 35) and brackish water (0.5 – 30) species might be restricted by the ‘junction’ of coarse to fine grained sediments at 10.64 km and 12.8 km, regardless of an upstream extension of tolerable maximum salinity conditions predicted under future saline penetration profiles.
- The low diversity, mid to upper estuarine zones of the River Adur and River Ouse estuaries and the macroinvertebrate species that inhabit them, are likely to extend in range upstream by 0.32 km and 0.15 km respectively. Their downstream distribution is likely to remain unchanged.
- Saline waters are predicted to penetrate into the lowermost reaches of the tidal limnetic zones. The responses of the limnic derived macroinvertebrate species inhabiting the FSI and lower limits of these tidal limnetic zones are currently unknown.
- Based on predicted future saline penetration profiles, it is unlikely that saline water will penetrate beyond the current tidal limits of the River Adur and River Ouse estuaries at 21 km and 21.8 km. However, the high salinity tolerances of limnic macroinvertebrate species present above the tidal limits (as determined by experimentation) suggest that
views on the assumed intolerance of limnic species to saline conditions needs to be reassessed.

- The relatively small increases in saline penetration, predicted under even ‘worst-case’ conditions, might have limited impact on the benthic macroinvertebrate ecology of the River Adur and River Ouse. Gradual overall patterns of upstream shifts might be masked by the dynamic seasonal distributions of macroinvertebrate species currently recorded in these systems.

- Future changes in river flows were not included in the determination of the predictive tide and salinity profiles of the River Adur and River Ouse estuaries. However, projected decreases in future summer flows as a consequence of climate and anthropogenic changes (especially in SE England), are likely to significantly increase the upstream extent of saline penetration predicted in this study and could result in salt water approaching the normal tidal limits.

- It was not possible to accurately determine future field distributions of macroinvertebrate species in the River Adur and River Ouse in relation to predicted increases in saline penetration, using species salinity tolerances alone, due to the likely influence of additional environmental factors.

7.3 Discussion, wider implications and future research

7.3.1 Conceptual model of saline penetration cause and effects

The degree of saline penetration into an estuary is predominantly dependent upon the topography and morphology of the estuary channel and the relative opposing forces of riverine freshwater discharge downstream and tide-driven saline forcing upstream (Chapter 4; Dyer, 1997; Savenije, 2005; Savenije & Veling, 2005). This research project primarily focussed upon the ecological consequences of increases in saline penetration predicted under the high emissions climate scenario projected relative sea level rise (SRES:A1FI). However, this project has highlighted a series of interrelated climatic and anthropogenic induced factors which could force changes in future channel morphology/topography, freshwater discharge and tidal range, which acting singly or in combination could result in a significant upstream increase in the degree of saline penetration into estuarine and riverine systems (Figure 7.1).
Figure 7.1. A conceptual model of the climatic and anthropogenic drivers and effects of future increases in saline penetration.
7.3.1.1 Anthropogenic and climatic factors

In addition to increases in relative sea level, future climate impacts are predicted to influence freshwater river discharges in the UK through increased global temperatures, decreased summer and increased winter precipitation (Figure 7.1; Murphy *et al*., 2009; Whitehead *et al*., 2009a). Whilst future saline penetration extents based on relative sea level rise were predicted under current low and high freshwater discharge conditions (recorded and averaged over the salinity sampling periods; August 2008 and February 2009), it was beyond the scope of this project to include predicted climate-induced changes to future river freshwater discharge to the modelled saline penetration profiles of the River Adur and River Ouse estuaries, which were based on relative sea level rise alone (for climate scenario SRES:A1FI; Chapter 4). Where changes in UK freshwater river discharges have been modelled for a series of future climate scenarios, a 1-32% reduction in summer flows has been predicted (Romanowicz *et al*., 2006). Under the most extreme of these reductions (i.e. 32%), the upstream extent of saline penetration in the River Adur estuary could increase by up to five times the extent predicted under ‘worst-case’ conditions (i.e. a 0.677 m increase in relative sea level under a low freshwater discharge of 0.73 m$^3$s$^{-1}$). The strong relationship between freshwater discharge (low and high) and the upstream extent of saline penetration recorded in both the current and future tide/salinity profiles of the River Adur and River Ouse estuaries (see Chapter 5), indicates that future river flow changes might have greater impact upon saline penetration than projected increases in relative sea level (even those projected under high emissions, Gillanders & Kingsford, 2002).

Under future climate change, summers are projected to be hotter and drier, resulting in reductions in freshwater river discharge both directly and indirectly through reductions in reservoir levels (through increases in evaporation), increased abstraction (for domestic, agricultural and urban use) and an increase in actual evapotranspiration and soil moisture deficits, reducing the volume and distribution of water to groundwater recharge and run-off to river systems (Figure 7.1; IPCC, 2007; Herrera-Pantoja & Hiscock, 2008; Murphy *et al*., 2009; Wilby *et al*., 2010). In the 2080s (under high emissions), long term mean annual potential groundwater recharge in the south east is predicted to fall by 40% (with decreases from 2011 to 2100 in summer and winter) along with a marked drop in summer river flows (Herrera-Pantoja & Hiscock, 2008). South east England is one of the most densely populated and driest regions in the UK, with an effective rainfall (areal rainfall minus actual evaporation) of between 260 - 280 mm per year (Marsh *et al*., 2007; Rodda, 2008), currently providing only 610 m$^3$ of water per person per year, almost half the figure (1000 m$^3$) employed by the World Bank to indicate a
developing country is suffering ‘water-stress’ (Falkenmark & Widstrand, 1992; Rodda, 2008). Surface (and ground) water resources suffer from unsustainable extraction during summer months, with surface flow rates even in winter months, deemed ‘unacceptable’ for large parts of the region (EA, 2007; Rodda, 2008). In contrast, it could be assumed that warmer, wetter winters (IPCC, 2007; Murphy et al., 2009) would result in an increase in river discharge, which would act in reducing the upstream extent of saline penetration and result in extreme seasonal differences in saline penetration extents between summer and winter (see Chapter 6). However, the impact of changing climate on winter river flows might be negated by future increased anthropogenic abstraction of freshwater to support a growing population (particularly in the South East), which has been estimated to considerably exceed any future affects of climate change on river flow regimes (Vorosmarty et al., 2000; Vorosmarty et al., 2010; Lester et al., 2011). The South East England Plan requires the region to accommodate a further one million people by 2026, raising concerns that the available freshwater resource will be exceeded by increasing population demands (Figure 7.1; SEP, 2006). The combination of these future climatic and anthropogenic pressures on the water resources of the south east are likely to result in significant changes to the River Adur and River Ouse discharge regimes, with large reductions expected in the summer months (Figure 7.1; Murphy et al., 2009).

In addition to an increase in freshwater abstraction (for domestic, agricultural and industrial use) population growth and development within the drainage basin can lead to land use change (e.g. draining of the flood plain), an increase in waste disposal (i.e. sewage effluent returns), channel management strategies (e.g. vegetation removal, impoundment, water transfers, channelization and flood control), and the construction of in-channel flood and flow control structures (i.e. dams, weirs and barrages) (Figure 7.1; Weatherhead & Knox, 2000; Bunn & Arthington, 2002; Dudgeon et al., 2006; Vorosmarty et al., 2010; Wilby et al., 2010). Removal of flood storage areas through the introduction of flood defences (e.g. intertidal areas and flood plains) and channel management strategies that reduce the cross-sectional area of the channel (i.e. channelization), increase tidal flow velocity, funnelling and propagating tidal amplitude upstream, resulting in an increase in saline penetration (Figure 7.1; Chapter 4; Savenije, 2005; Savenije & Veling, 2005). In contrast, in-stream flood and flow engineering structures (i.e. dams, weirs and barrages) can act as a barrier to the upstream extent of saline penetration (e.g. River Ouse Hamsey half-weir, Chapter 4) and where these structures are situated at the heads of estuaries (near the tidal limits) may act to squeeze out the tidal limnetic zone, resulting in the loss of freshwater fauna from below the tidal limits (Figure 7.1). In addition, these structures can act to decrease water quality by reducing flushing and increasing residence times, leading to the
stratification of impounded estuarine waters (Figure 7.1; Davies & Kalish, 1994; Chan et al., 2002). When combined with predicted reductions in freshwater river discharge, sediment (from land clearance/agriculture) and nutrient inputs (through sewage effluent returns and fertiliser run-off; particularly nitrogen and phosphorus), can result in a decrease in estuarine water quality, increasing the systems vulnerability to eutrophication, anoxia and hypoxia through phytoplankton and cyanobacteria blooms, which can have detrimental effects upon estuarine ecosystems, biodiversity and food web function (Figure 7.1; Chan et al., 2002; Wildsmith et al., 2009; Wildsmith et al., 2011). For example, in the Guadiana estuary in the Iberian Peninsula, a history of high winter nitrogen and phosphorus loads (enrichment in dissolved inorganic nitrogen and dissolved reactive phosphorus through agriculture) and spring diatom blooms, resulted in a reduction in the availability of silicon in the water column (Rocha et al., 2002). The low Si:N and N:P availability in the water column, coupled with low summer freshwater discharge rates (associated with water retention by more than 40 dams in the drainage basin) resulted in summer toxic cyanobacteria blooms leading to fish kills and deleterious health effects on the local human population (Rocha et al., 2002).

7.3.1.2 Ecological effects

In this research study the ‘worst-case’ projected relative sea level rise scenario (SRES:A1F1, 95th percentile for the year 2080, under low freshwater discharge conditions) resulted in only moderate predicted increases in saline penetration extents and small-scale changes in the benthic macroinvertebrate communities of the River Adur and River Ouse estuaries (Section 7.2). However, when combined with climatic and anthropogenic forced changes to freshwater river discharge and channel morphology/topography (Figure 7.1) the upstream extent of saline penetration is likely to significantly increase, potentially driving salt water close to the current limits of the tide (NTL). Predicting ecological responses to increases in saline penetration in complex (and highly dynamic) estuarine systems is difficult, requiring the collection of a large amount of data and combining of many different (and often considered separate) elements of estuarine research (e.g. Walther et al., 2002; Struyf et al., 2004; Kappel, 2005; Vermaat et al., 2005; Hellmann et al., 2008; Cheung et al., 2009; Dijkstra et al., 2010; Traill et al., 2010; DeLaune & White, 2011). It is however possible to use this published research to produce a broad-scale linkage model of the potential ecological effects of both increases in saline penetration and related changes to the estuarine system (anthropogenic and climatic induced i.e. reductions in river discharge, population growth and land use change; Figure 7.1).
An increase in the upstream extent of saline penetration into estuarine systems is likely to impact biotic functions (community structure, biodiversity and food web function) and affect underlying nutrient cycling and primary and secondary productivity processes through changes to sediment processes (i.e. biogeochemistry, resuspension and export), erosion rates and ‘lateral squeeze’ of the intertidal zone (Figure 7.1; Michener et al., 1997; Thomas et al., 2001; Neubauer et al., 2005; Ensign et al., 2008; Antonio et al., 2010; Weston et al., 2011). The impact that saline increases may have upon nutrient cycling, productivity (both primary and secondary) and food web function in estuarine systems could have consequences across all trophic levels (Struyf et al., 2004; Neubauer et al., 2005; Ensign et al., 2008; Antonio et al., 2010; Weston et al., 2011). For example, salinity-induced stress in freshwater macrophyte communities is predicted to decrease primary production and organic accumulation rates, stimulate microbial decomposition and accelerate the loss of organic carbon in tidal freshwater marshes (Spalding & Hester, 2007; Weston et al., 2011). A decline in plant productivity coupled with an increase in decomposition rates could limit organic matter sequestration, reducing the availability of organic matter to benthic macroinvertebrate herbivores and omnivores (shredders) resulting in a change to faunal trophic dynamics. In addition, a reduction in plant production may limit the vertical accretion of freshwater marshes, making them liable to tidal flooding (from relative sea level rise) and resulting in a shift from freshwater to salt tolerant macrophytes (Weston et al., 2011). Although not systematically surveyed in this research study, distinct patterns of macrophyte zonation were identifiable in both estuaries with distance from the river mouth (Adams et al., 1992). Species associated with saline and brackish water conditions (e.g Atriplex porulacoides, Agropyron pungens, Aster tripolium) dominated the lower estuarine zones and were replaced by salt-tolerating freshwater species (e.g. Parapholis strigosa, Bolboschoenus maritimus and Phalaris arundinacea) in short mid estuarine transition zones (Adams et al., 1992). Ubiquitous freshwater macrophytes were recorded in the upper estuarine zones and above the tidal limits (e.g. Sparganium erectum, Lythrum salicaria, Impertiens gladulifera, Schenoplectus lacustris and Salix sp.). Changes in the structure and composition of marginal macrophyte communities (from freshwater to saline tolerant) and the removal of marginal vegetation from estuary and river channels for flood defence (to prevent blockages and maintain freshwater flow) may have a direct negative impact upon fauna that exploit these resources (e.g. as a food source and refuge) and force changes to channel and bank sediment structure, resulting in bank instability (Figure 7.1; Baldwin & Mendelssohn, 1998).

In contrast, a number of channel management techniques which change the sediment structure of a site (e.g. addition of rip-rap) can subsequently positively influence macroinvertebrate biomass
and density (Fischenich, 2003). For example, pole-wharfing on the River Adur and River Ouse estuaries may have enabled euryhaline marine and brackish water macroinvertebrate species to inhabit sections of estuary that would otherwise be outside their tolerance range (tidal limnetic; Chapter 5). The addition of coarse grained substrate on the intertidal zone provided refuge, access to high interstitial salinities and protection from aerial exposure, desiccation and potential predators for these species during low tide (e.g. wading birds; Fischenich, 2003).

Where channel banks are constrained and intertidal areas limited (through the introduction of flood defences), increases in relative sea level could result in the lateral squeeze of the bank side intertidal zone, particularly in the lower estuary, resulting in the local loss of salt marsh habitats (Figure 7.1; Simas et al., 2001; Craft et al., 2009). On the lower River Adur estuary, the salt marsh and saline lagoon habitats are designated Sites of Special Scientific Interest (SSSI’s), due to a number of protected species (Clavospella navis, Edwardsia ivelli and Trifolium stellatum) and a large colony of the viviparous lizard Lacerta vivipara (Buck, 1997; Environment Agency, 2005a). These protected salt marsh and saline lagoon sites will not be affected by increases in salinity, but are at substantial risk from predicted increases in relative sea level, with salt marsh requiring sedimentation rates in excess of sea level rise to maintain bank elevation (Reed, 1990). Whilst maintaining high sedimentation rates might not be a problem in the river-dominated macrotidal estuary of the River Adur, the constrained nature of the current estuary channel banks may result in lateral squeeze and loss of protected SSSI salt marsh and lagoon habitats (Figure 7.1; Simas et al., 2001; Craft et al., 2009). Ironically schemes to protect freshwater habitats at the margin of estuaries, including embanking and channelizing further constrain estuarine channels, increasing the upstream extent of saline penetration and exacerbating lateral squeeze (Figure 7.1). On the River Ouse estuary, two freshwater SSSI’s may be at risk from increasing saline penetration; Offham Marshes and Lewes Brooks (Buck, 1997 Environment Agency, 2005a). These sites represent 173 hectares of freshwater flood plain meadow and marsh, which contain rare invertebrate species (Lewes Brooks) and large populations of amphibians (Offham Marshes). Although protected from the estuarine channel by flood banks, Lewes Brooks has experienced small saline penetration events, which have been shown to locally increase biodiversity (Environment, 2005). However, increased and prolonged salinity stress (through bank breach or tidal flooding) associated with increases in the upstream extent of saline penetration and sea level rise is likely to have deleterious effects upon the protected freshwater biota (Environment Agency, 2005a).
In addition to changes to nutrient cycling and productivity processes (primary and secondary), increases in saline penetration could alter estuarine food web function through changes to species interactions (Figure 7.1). The loss, gain and movement of species in response to increases in saline penetration is likely to change the structure of estuarine faunal communities and food webs through competition for resources (inter and intra-specific) and predator and prey interactions (Figure 7.1). Saline ingress into tidal limnetic zones is likely to result in the local loss and upstream shift of freshwater species, with the fauna reduced to species tolerant of saline conditions and the fluctuating physiochemical conditions of the saline front (Figure 7.1, see Chapter 5). In addition, where in-stream flood and flow control structures (i.e. dams and weirs) prevent the upstream penetration of saline tidal waters, tidal limnetic estuarine zones may be longitudinally squeezed out, resulting in the loss of freshwater flora and fauna below the structure and a sharp division between upstream freshwater and downstream marine habitats (Figure 7.1). Increases in saline penetration, resulting from drought-induced low freshwater flow have been shown to influence the distribution of freshwater fish species in estuaries, with species retracting to above the normal tidal limits (Baptista et al., 2010; Dolbeth et al., 2010). The River Adur and River Ouse support recreational fisheries, of which the sea trout (Salmo trutta) is the highest profile species. Sea trout physiologically adjust to both freshwater and marine conditions over their lifecycles and, as such, are unlikely to be directly affected by increases in saline penetration in the River Adur and River Ouse estuaries, unless tidal saline water penetrates into their lotic spawning and nursery grounds (though this is unlikely based on present predictions; Hayward & Ryland, 1995). For obligate freshwater fish species (e.g. chub, tench, rud, roach), the predicted increases in saline penetration (particularly in the River Adur estuary) might result in the restriction of their current downstream distributions at high tide, dependant upon salinity tolerance, capacity for adaptation and additional environmental factors that have been shown to influence habitat use (Roessig et al., 2004; Purcell et al., 2010; Gillson, 2011; Love et al., 2008).

The upstream extension of favourable saline conditions is likely to result in the upstream shift of mobile brackish and marine macroinvertebrate species, dependant upon (as recorded in this study) additional physiochemical factors (i.e. availability of suitable substrate/grain size and tolerance to physiochemical changes; Figure 7.1). In addition, increases in saline penetration is likely to result in an upstream extension in the distribution of marine and estuarine fish species that enter estuaries at high tide (e.g. grey mullet; Crenimugil labrosus). The upstream increases in range of these invertebrate and vertebrate species will potentially have a significant impact upon predator-prey interactions. For example, increases in the upstream extent of Carcinus
*maenas* associated with drought events (such as in the Mondego estuary, Portugal; Bessa *et al.*, 2010) could have a significant impact upon populations of juvenile fish species on which they feed (Ansell *et al.*, 1999; Taylor, 2005). In turn, where estuarine amphipods (e.g. *Gammarus* sp.) constitute a large proportion of fish species diets, shifts in the distributions and abundances of these amphipods could result in a local decline in fish populations. For example, changes in the distribution and faunal structure of *Gammarus zaddachi* in the River Thames estuary have been attributed to local decline in juvenile flounder (*Platichthys flesus*; Thomas, 1998).

Non-native invasive species (such as the Chinese Mitten Crab, *Eriocheir sinensis*) can have a profound effect on estuarine ecology through predation and competition with native species, significantly altering estuarine food webs and trophic structure (Hanson & Sytsma, 2008; Dittel & Epifanio, 2009). Increases in saline penetration and low freshwater discharge conditions (long flushing times) could provide ideal microhabitats for the propagation of invasive species where retention of larvae and increases in salinity play a critical role in development and maintenance (Cohen *et al.*, 1995; Herborg *et al.*, 2005; Hanson & Sytsma, 2008). For example, estuaries that support large populations of the invasive Chinese Mitten Crab (*Eriocheir sinensis*) have long flushing times and saline penetration extents (i.e. San Francisco Bay; Hanson & Sytsma, 2008). In the Thames estuary, the population of *Eriocheir sinensis* increased from 1989 to 1992, coinciding with a drought induced decrease in freshwater discharge and an increase in salinity (Attrill & Thomas, 1996). Large populations of *E. sinensis* have the potential to reduce populations of native invertebrates through predation and fish species through egg consumption, altering the structure of both fresh and brackish water benthic invertebrate communities (Dittel & Epifanio, 2009). In addition, *E. sinensis* may out-compete native species for habitat and refugia, having been recorded successfully excluding native *Carcinus maenas* from boulder shelters in the laboratory, which has implications on native estuarine invertebrate populations which rely on intertidal shelters for refuge at low tide (see Chapter 5; Gilbey *et al.*, 2008).

While the concept of sea level and climate driven increases in saline penetration into coastal systems is not new (Ippen, 1966; Komar, 1976; Sorenson, 1978; Todd, 1980), the impacts that such changes might have upon individual species and whole ecosystems, however, has only recently attracted research attention (Peterson *et al.*, 2010a). The majority of this attention has focused on past, contemporary and predictive ecosystem-level modelling approaches, analysing ecosystem health, growth and decline in relation to sea level through observational and correlational studies (Uncles, 2003; Kirwan *et al.*, 2007; Craft *et al.*, 2009; Virah-Sawmy *et al.*, 2009; Traill *et al.*, 2010). Whilst some studies have identified species tolerances to be a critical
factor in determining biological and ecological response to sea-level rise, only a few experimental approaches have been attempted and these have mainly focussed on marsh, forest and fish ecology (Spalding & Hester, 2007; Howard, 2009). More information is required assessing likely responses of species and biodiversity to changes in saline penetration using a combination of observational and experimental approaches (Petersen & Petersen, 1984; Cheung et al., 2009). The present study, with its emphasis on macroinvertebrate response to increases in saline penetration, from field and experimental observations, forms an important contribution towards improving our understanding of the impacts of future climate change on benthic macroinvertebrate community structure and species distributions in estuaries.

### 7.3.2 Estuary and river management and regulation

Despite only moderate increases in saline penetration projected for the River Adur and River Ouse estuaries under the ‘worst-case’ sea level scenario, any salinity change could have a significant impact upon estuarine and riverine management and regulation (European Communities, 2003; Environment Agency, 2010). This includes the safeguarding of protected sites (EU Habitat Directive, 1992) and requirements to achieve minimum standards of the EU Water Framework Directive (McLusky & Elliott, 2007; European Commission, 2003). Under the EU Water Framework Directive (WFD; 2000/60/EC), the highly dynamic ‘transitional waters’ of the River Adur and River Ouse estuaries are required by UK law to be classified, monitored and managed, in order to reach ‘good’ standard by 2015 (McLusky & Elliott, 2007; European Commission, 2003; European Communities, 2000). As the basis for actions to achieve improvement targets, the South East Catchment Plan (which covers the River Adur and River Ouse) predicts that many of its inclusive estuaries are unlikely to achieve the minimum standard (Good Ecological Status) by 2015, due in part to “limited knowledge about the pressures that affect many of these water bodies and how their biology responds to changes in these pressures” (Environment Agency, 2010). The Environment Agency Transitional Waters Ecology Study (Environment Agency, 2005b) undertaken during 2005/2006, attempted to relate estuarine macroinvertebrate species distributions to saline penetration, but lacked the methodological precision to produce definitive conclusions (i.e. lack of salinity measurements and sampling sites; see Chapter 3). By providing a detailed assessment of the impact of salinity on the distributions of macroinvertebrate species, the current research project has made a significant contribution and improvement towards understanding, and resolving, some of the key issues which are currently preventing ‘good’ or higher standard being achieved in the River Adur and River Ouse ‘transitional waters’ by 2021 and 2027 respectively (Environment Agency, 2010).
Despite acknowledging limited understanding of the biotic communities of the River Adur and River Ouse estuaries and the pressures they face, future proposed and accepted flood and coastal defence management strategies for these systems (devised by the Environment Agency), could have significant impacts upon the ecology and tide and salinity profiles described and predicted in this study (Chapter 4 and 5). Whilst several strategies have been suggested for both rivers, probably the most extreme proposals include managed realignment of the lower River Ouse estuary course (currently rejected for a ‘hold the line’ approach) and a proposed tidal barrier on the River Adur estuary at Shoreham (Environment Agency, 2010; Environment Agency, 2011). Both these proposals have been shown to reduce tidal and saline penetration significantly and influence benthic macroinvertebrates (Garbutt et al., 2006). It is suggested here that less extreme (and capital intensive) management approaches, such as the extension of pole wharfing in the River Ouse estuary (see Chapter 6) and formation of new habitats (e.g. mudflats, saltmarsh, floodplain grassland, reed beds) in the River Adur estuary, which can locally increase abundance and diversity of the macroinvertebrate community, are also effective strategies for coastal defence and maintenance of good ecological health (Borsje et al., 2010).

7.3.3 Wider applications and future research

All UK coasts and estuaries will experience the effects of progressive increases in eustatic sea levels, and approximately 70 will suffer the combined effects of sea level rise and significant isostatic land level decline (from Yorkshire clockwise to Pembrokeshire; Davidson et al., 1991; Shennan et al., 2006; Bradley et al., 2009). This rise in relative sea level might allow the local tidal conditions to progressively drive saline waters upstream. This project studied two low gradient, macrotidal, anthropogenically constrained estuaries selected due to their perceived susceptibility to future increases in saline penetration (see Chapter 2). Despite these initial concerns, the River Adur and River Ouse estuaries were predicted to experience only moderate saline penetration increases, resulting in small-scale ecological changes even under ‘worst-case’ conditions. However, in the River Adur and River Ouse estuaries, a strong relationship was recorded between saline penetration extent and freshwater river discharge regime, indicating that future climate and anthropogenic induced reductions in freshwater river discharge might be of more significance than projected increases in relative sea levels in determining the upstream extent of saline penetration (see Section 7.2, Figure 7.1; Vorosmarty et al., 2000; Vorosmarty et al., 2010; Lester et al., 2011). It could therefore be assumed that in river dominated estuaries where freshwater inflow is a major factor contributing to salinity changes, the potential impact from increases in saline penetration might be more severe (i.e. greater upstream extent) than in estuaries where the salinity profile is not significantly modulated by freshwater inputs and future
increases in saline penetration are related to increases in relative sea levels alone (Figure 7.1; Gillanders & Kingsford, 2002).

Although the range and upstream extent of increases in saline penetration would vary with estuary topography, morphology and location, this study has indicated that whilst potentially important at local scales (e.g. protected sites/areas, rare habitats and SSSIs), predicted increases in relative sea levels alone are unlikely to be a major cause of large scale ecological concern (e.g. wholesale ecosystem changes, loss of species and biodiversity). However, in combination with anthropogenic and climate induced changes within the drainage basin, increases in saline penetration extents could be detrimental to estuarine and riverine ecosystem function through a series of interlinked cause and effects, from changes to nutrient cycling and productivity through to consequences for food web function (Section 7.3.1; Figure 7.1). Despite this, even small (localised) increases in saline penetration must be considered under future proposals/strategies for estuarine and riverine management, as it is likely that in other UK (and European) estuaries small increases in saline penetration might have equal, or even more severe impacts upon local ecology and protected ecological habitats (e.g. SSSI’s).

Where current and/or future increases in saline penetration are recorded in relation to reductions in river flows, the artificial maintenance of set base-flow conditions (e.g. using a balancing reservoir or additional abstraction quantity limits), could reduce these impacts. Projected changes to climate (warmer, drier summers) and future population growth/development are however predicted to exert pressure on the available freshwater resource through abstraction for domestic, agricultural and industrial use (Figure 7.1). Limits placed on abstraction quantities under pressured need for freshwater (i.e. during drought conditions) would however be difficult to enforce. Irrespective of the impact to estuarine ecosystem function, significant increases in the upstream extent of saline penetration driven by reductions in freshwater discharge could result in a reduction in abstracted water quality (particularly where abstraction occurs at the tidal limits).

In addition to the maintenance of set base-flow conditions, the upstream extent of saline penetration could be reduced using natural and artificial in-stream structures that restrict or partially impede flow (such as half-weirs and gradient changes; Figure 7.1). In estuaries deemed ‘at-risk’ from increases in saline penetration, engineering structures could be developed to limit the upstream extent and effects of saline penetration, although the design and construction of artificial barriers should be considered in respect to estuarine ecology (e.g. integration of fish...
ladders). In estuaries with long flushing and residence times, in-stream structures that partially/fully restrict flow could prolong these times, leading to stratification of the water column and increased vulnerability to eutrophication, hypoxia and anoxia (Figure 7.1). In addition, the presence of these structures at the heads of estuaries (NTL) could cause tidal limnic zones to be ‘squeezed out’ by advancing zones of increasingly saline waters, resulting in the local loss of freshwater estuarine fauna and flora (Figure 7.1, Borsje et al., 2010). The potential ecological impact associated with the use of these structures as preventative saline penetration methods would require further study.

This project has highlighted a number of issues associated with the impact of future increases in saline penetration upon estuarine fauna that require further research. The application of similar experimental and observational methodological aspects used in this project to additional trophic levels and the investigation of the impacts upon nutrient cycling and productivity (both primary and secondary) would provide a broader view of the impacts of increasing saline penetration and tide levels upon estuarine ecology at an ecosystem level (Thomas et al., 2001; Struyf et al., 2004; Neubauer et al., 2005; Ensign et al., 2008; Weston et al., 2011). Further research is also required into the ecological consequences of short-term saline penetration spikes caused by unpredictable, extreme events (such as storm surges). In particular, the effect on macroinvertebrate species distributions in estuaries (Weinstein et al., 2002; Nielsen et al., 2007; Goodman et al., 2010). In addition, investigations into the habitat and biodiversity potential of bank engineering materials, with particular reference to their use as refuge for the extension of species distribution ranges (counter to their saline tolerance limitations) may also help with riverine management, ecological conservation and maintenance of good ecological status in the face of future salinity change (Borsje et al., 2010). Future research must also consider the consequences for limnic derived macroinvertebrate species in localities where tidal limnic zones are being progressively ‘squeezed’ between advancing saline waters and flood control barriers. More extensive controlled laboratory and field salinity tolerance determinations of limnic macroinvertebrate species at population levels (all life stages) are extremely important for reducing ‘uncertainties’ in the prediction of the likely effects of future relative sea level increases and resultant tidal limnetic zone squeeze. This study suggests that research emphasis should be placed upon these limnic derived species that inhabit the tidal limnetic and upstream non-tidal limnetic zones (see above, Section 7.2.1.3). It is in these zones that the benthic macroinvertebrate communities and associated dynamics are least understood and the most vulnerable to predicted increases in the upstream extent of saline penetration.
7.4 Conclusions

- Estuaries and associated rivers on the south coast of England might be amongst the most susceptible to future increases in saline penetration. This is due to the combination of eustatic sea level rise, glacial isostatic land level fall (i.e. together causing relative sea level rise) and predicted summer reductions in freshwater river discharge, the latter being caused by local climatic changes (i.e. increased temperatures and reduced precipitation) and increased anthropogenic pressures upon freshwater resources (i.e. increased freshwater abstraction for population growth).

- Salinity parameters (particularly maximum, minimum and average salinity) were the dominant environmental variables driving benthic macroinvertebrate community structure and species distributions in the River Adur and River Ouse estuaries (over the study period). The reduction in saline penetration between low (August 2008) and high (February 2009) freshwater discharge conditions, was recorded in a downstream distribution shift of limnic derived macroinvertebrate species (euryhaline-limnic and limnic salt tolerating) in both estuaries, and a downstream shift of euryhaline-marine species, brackish water species and the zone of lowest faunal diversity in the River Ouse estuary.

- Whilst salinity parameters (i.e. maximum, minimum and range) were shown to be the most important variables driving macroinvertebrate community structure and distributions in the River Adur and River Ouse estuaries, the impact of additional environmental variables (e.g. sediment characteristics) might have greater local importance than salinity in determining the distribution of benthic macroinvertebrate species. For example, the change from coarse to fine grained sediment in the River Adur estuary might determine both the upstream extent of euryhaline-marine and brackish water macroinvertebrate species and the zone of lowest faunal diversity. Anthropogenic extensions of coarse grained sediment in these systems (e.g. pole wharfing in the River Ouse estuary) might enable the upstream extension of euryhaline-marine and brackish water species, by acting as areas of refuge from adverse conditions at low tide (e.g. tidal limnetic minimum salinities (and higher interstitial salinities), aerial exposure, predation and desiccation).

- The allocation of benthic macroinvertebrate species to salinity tolerance groupings based on published literature, enabled the approximate determination of the upstream extent of saline penetration in the River Adur and River Ouse estuaries in the absence of salinity
measurements, but lacked the necessary precision for predictive applications. This approach could enable researchers to make approximate determinations of past saline penetration extents based on historic benthic macroinvertebrate data in the absence of salinity measurements (as in this study, see Chapter 4).

- Limnic derived benthic macroinvertebrate species exhibited significant levels of salinity tolerance under laboratory and field tidal cycle conditions, which did not correspond to published literature or the distributions of these species in the River Adur and River Ouse. These conflicting tolerance data could be indicative of the lack of research on estuarine distributions and salinity tolerances of limnic species (particularly within the neglected tidal limnetic zone) and/or the impact of additional biotic (e.g. competition, predation) or abiotic variables (e.g. habitat preference) and/or physiological effects of salinity not tested in this study (e.g. impact on all life stages and cycles). In contrast to limnic derived species, the laboratory salinity tolerances of the estuarine macroinvertebrate species (euryhaline-marine and brackish water), corresponded to the extensive published literature (salinity tolerances and field distributions), however, did not correspond to the field distributions of these species in the River Adur and River Ouse estuaries, likely due to the local importance of non-saline environmental variables (e.g. sediment characteristics) and species interactions (e.g. habitat partitioning of *Gammarus salinus*, *Gammarus zaddachi* and *Gammarus pulex* along the salinity gradient). Macroinvertebrate species salinity tolerances therefore do not account for actual field distributions (in the River Adur and River Ouse) with sufficient accuracy to allow for precise prediction of future distribution patterns under projected saline penetration profiles.

- In both the River Adur and River Ouse estuaries, projected increases in relative sea levels under the high emissions scenario (IPCC SRES:A1FI), resulted in an increase in both the upstream extent of saline penetration and gradient of maximum salinity zones, which differed according to predicted year, freshwater flow regime (low and high) and estuary. However, even under ‘worst-case’ conditions (low freshwater discharge, high emissions, 2080; 95th percentile) these increases were projected to be moderate at 0.32 km and 0.15 km for the River Adur and River Ouse estuaries respectively.

- Partial in-stream barriers to the upstream ingress of saline water, such as the artificial increase in bed gradient in the River Ouse estuary (Hamsey cut-through channel), significantly influences the upstream extent of saline penetration, suggesting that natural
and anthropogenic in-stream barriers can be effective in reducing the degree and extent of saline penetration, whilst still allowing upstream water flow at high tide. The more natural course of the River Adur estuary (and the more representative distribution of salinity stations) suggests that the patterns of current and predicted tidal saline penetration under low and high freshwater discharge conditions is a more realistic representation of the hydrodynamics of low-lying coastal river valleys with essentially consistent and gradual gradients.

- Freshwater river discharge rates have been shown to significantly influence the upstream extent of saline penetration in the River Adur and River Ouse estuaries, with an increase in upstream extent recorded under low summer discharge conditions compared to high winter discharge conditions. Although not factored into the predictive tide and salinity profiles for the River Adur and River Ouse estuaries, predicted climate (increased temperatures, reduced summer and increased winter precipitation) and anthropogenic (e.g. increased abstraction, population growth/development, land-use change) induced changes in future freshwater river discharge are likely to effect predicted saline penetration extents significantly under summer and winter flow regimes. For example, in the River Adur estuary, modelled decreases in summer river flows of 32%, could result in a five-fold increase in the upstream extent of saline penetration from current ‘worst-case’ saline profile predictions (high emissions, 2080; 95th percentile, low freshwater discharge). This could indicate that in the River Adur and River Ouse estuaries, changes in freshwater river discharge could be of more significance than increases in relative sea levels in determining the upstream extent of saline penetration. As a catchment variable which can to some degree be influenced (e.g. abstraction regulation and balancing reservoir discharges), these freshwater discharge effects might represent a river management feature that could be used to ameliorate a degree of future saline penetration.

- The moderate predicted increases in saline penetration determined for the River Adur and River Ouse estuaries even under ‘worst-case’ saline penetration profiles are unlikely to result in large-scale changes in benthic macroinvertebrate community structure and species distributions. However, the addition of potential climatic and anthropogenic forced changes to freshwater river discharge and channel morphology/topography could result in critical consequences for not only benthic macroinvertebrate species, but across all trophic levels. Furthermore, the impact of these potential increases upon nutrient cycling and primary/secondary production in these systems remains unknown.
• The predicted increases in saline penetration determined for the River Adur and River Ouse estuaries might have severe impacts at local level estuarine and riverine management and regulation, including the safeguarding of protected sites (especially the freshwater SSSI’s on the River Ouse estuary; Lewes Brooks and Offham Marshes) and requirements to achieve the minimum standards of the EU Water Framework Directive.

• It was not possible to determine accurately the future field distributions of benthic macroinvertebrate species in the River Adur and River Ouse in relation to predicted increases in saline penetration using species salinity tolerances alone, due to the complexity of estuarine ecosystems and the influence of additional biotic and abiotic factors limiting species distributions. However, combining benthic macroinvertebrate salinity tolerances with published literature, field distributions, and the impact of additional environmental parameters, enabled general predictions of future species distributions to be made in relation to projected increases in saline penetration. For example, regardless of an upstream extension of tolerable maximum salinity conditions, the majority of euryhaline-marine and brackish water species recorded in the River Adur and River Ouse estuaries, might be restricted in upstream distribution by the junction from coarse to fine grained sediments (at 10.64 km and 12.8 km respectively). Because of this, the low diversity mid to upper River Adur and River Ouse estuarine zones (and associated ‘hardy’ macroinvertebrate species) are likely to maintain downstream extents, while extending in range upstream to the new limit of saline penetration (0.5). Due to contradictions between measured salinity tolerance and field distributions, it was not possible to determine the responses of limnic derived species inhabiting the freshwater-seawater interface (FSI) and tidal limnetic zones to predicted increases in salinity without further knowledge of the nature of the abiotic and/or biotic factors limiting the field distributions of these species. Based on field distributions and published literature, it seems likely that regardless of recorded salinity tolerance values, increases in saline penetration will result in a related upstream shift in distribution of these limnic derived species in the River Adur and River Ouse estuaries.

• This research project has shown that predicted increases in saline penetration extents driven by relative sea level rise alone are unlikely to be a major cause of future large scale ecological concern (e.g. wholesale ecosystem changes, loss of species and biodiversity). However, this project has highlighted a series of interrelated climatic and anthropogenic induced factors which could force changes to future channel morphology/topography, freshwater discharge and tidal range, which when combined...
could result in a significant upstream increase in the degree of saline penetration into estuarine and riverine systems. These increases could be detrimental to estuarine and riverine ecosystem function through a series of interlinked cause and effects, from changes to nutrient cycling and productivity through to consequences for food web function and as such, requires urgent further investigation.
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