DEVELOPMENT OF A NOVEL INVERTEBRATE INDEXING TOOL FOR THE DETERMINATION OF SALINITY IN AQUATIC INLAND DRAINAGE CHANNELS

Alexander G. G. Pickwell

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Certificate of Originality

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own, except as specified in the acknowledgements and in references, and that neither the thesis nor the original work contained therein has been previously submitted to any institution for the award of a degree.

Signature:

Name: Alexander G. G. Pickwell

Date: 31st August 2012

Abstract

Salinisation of freshwater habitats is an issue with global implications that can have serious detrimental effects on the environment resulting in an overall loss in biodiversity. Whilst increases in salinity can occur naturally, such anthropogenic actions as the disposal of industrial and urban effluents and the disturbance of natural hydrological cycles can also result in the salinisation of freshwater habitats.

The Water Framework Directive (WFD) requires Member States to restore all freshwater habitats to "good ecological status" and to prevent any further deterioration. Macro-invertebrates are widely used as indicators of river condition for a wide range of reasons and have been designated a key biological element in the assessment of aquatic habitats by the WFD. A review of the available literature, however, found no macro-invertebrate-based biotic indices have been developed for the detection and determination of salinity increases in freshwater habitats that are suitable for application in the United Kingdom for the purposes of the WFD. To this end, a biotic index based on the aquatic macro-invertebrate community response to changes in salinity, termed the Salinity Association Group (SAG) index, was developed.

The potential of the SAG index for assessing water quality in terms of salinity in freshwater systems was investigated using data collected from survey sites in Lincolnshire and Norfolk, England, and the results compared to several published salinity indices. Whilst the SAG index was found to show both geographic and seasonal dependence, as is common among many biotic indices, the proposed metric exhibited a stronger relationship to salinity than macro-invertebrate indices employed in Europe for the purposes of the WFD show to their specific pressure. Furthermore, the SAG index was found to be highly selective to only salinity concentration, was significantly related to salinity when used with less detailed information and significantly discriminated between the salinity classes defined by the WFD. It is also highlighted that application of the SAG index with such predictive models as the River InVertebrate Prediction And Classification System (RIVPACS) can resolve the exhibited geographical and seasonal dependence.

In a comparison of the SAG index with the published indices, it was found that the SAG index was the superior metric in terms of recognising abundance as required by the WFD, reliably indicating changes in salinity, compatibility with sampling protocols employed by England's regulatory authority and producing a linear output. Consequently, it was concluded that the SAG index surpasses other published metrics for the detection and determination of salinity increases in freshwater habitats and is a viable biomonitoring tool suitable for use in England for informing aquatic habitat management decisions, research application and the purposes of the WFD. It is proposed, however, that more rigorous sampling protocols for both macro-invertebrate and environmental data may result in more accurate metric scores and reveal further issues or benefits associated with the SAG index and could also be used to further refine the metric. It is also suggested that adaptation and examination of the SAG index at a larger geographical scale would further demonstrate the validity of the proposed metric and illustrate the potential of the SAG index for worldwide application. Furthermore, intercalibration of the SAG index to harmonise WFD reference conditions and class boundaries across Europe would allow the application of the SAG index throughout Europe for the purpose of the WFD.

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List of Abbreviations and Symbols

1-D: Simpson's dominance index

ANCOVA: ANalysis of COVAriance

ANNA: Assessment by Nearest Neighbor Analysis

ANOSIM: ANalysis Of SIMilarity

ANOVA: ANalysis Of VAriance

$ASPT_{BMWP}$: Average Score Per Taxon derivation of the BMWP scoring system

AUSRIVAS: AUStralian RIVer Assessment Scheme

B: Berger-Parker dominance index

BEAST: BEnthic Assessment SedimenT

BMWP: Biological Monitoring Working Party

CC: Cophenetic correlation coefficients

CCA: Canonical Correspondence Analysis

CEN: European Committee for Standardisation

df: degrees of freedom

D_{Mg}: Margalef's richness index

E: Buzas & Gibson's Evenness

EC: Electrical Conductivity

EPT: Ephemeroptera, Plecoptera and Trichoptera index

***H*:** Kruskal-Wallis statistic

H_i: Shannon diversity index

IBI: Index of Biotic Integrity

ISO: International Organization for Standardization

LIFE: Lotic-invertebrate Index for Flow Evaluation

LOWESS: LOcally WEighted Scatterplot Smoothing

MEDPACS: MEDiterranean Prediction And Classification System

NMDS: Non-metric MultiDimensional Scaling

NO_3: nitrate

NPMANOVA: Non-parametric Permutational Multivariate ANalysis Of VAriance

$NTAXA_{BMWP}$: Number of scoring taxa derivation of the BMWP scoring system

OSGR: Ordnance Survey Grid Reference

p: significance level

PAST: PAleontological STatistics software package

PCA: Principal Component Analysis

Pers. comm.: Personal communication

PO_4: phosphate

PSU: Practical Salinity Units

RICT: River Invertebrate Classification Tool

RIVPACS: River InVertebrate Prediction And Classification System

r: Pearson product-moment correlation coefficient

r_s: Spearman's rank order correlation coefficient

SAG: Salinity Association Group

SAG.FA: SAG scores calculated using family level identification and abundance data

SAG.FnA: SAG scores calculated using family level identification and presence/absence data

SAG.MA: SAG scores calculated using mixed level identification and abundance data

SAG.MnA: SAG scores calculated using mixed level identification and presence/absence data

SAS: Salinity Association Score

SF: South Forty Foot Drain

SH: South Holland Main Drain

SIGNAL: Stream Invertebrate Grade Number – Average Level index

$SPEAR_{salinity}$: SPEcies At Risk salinity index

SSS: Salinity Sensitivity Score

SWEPAC: SWEdish invertebrate Prediction and Classification predictive model

***U*:** Mann-Whitney statistic

UKTAG: United Kingdom Technical Advisory Group

WFD: Water Framework Directive

Σ: the sum of

σ: standard deviation of a population

σ^2: variance of a population

μ: mean of a population

z: standardised score of value x

1 INTRODUCTION

Water is widely regarded as the world's most essential natural resource (Vörösmarty *et al*., 2010), providing a vital resource for humans and a unique habitat for a richly diverse, sensitive and endemic collection of flora and fauna (Strayer & Dudgeon, 2010). Humans have made use of fresh waters for a variety of reasons, such as fishing, irrigation, transportation, farming of aquatic plants and animals, industrial purposes and power production (Strayer & Dudgeon, 2010). In addition to the direct economic value of freshwater uses for humans, the ecosystem services provided by the freshwater environment beneficial to human populations have been conservatively estimated to have a global value greater than US$1.7 trillion per year (Costanza *et al*., 1997). Fresh water, however, accounts for only 0.01% of the water in the world covering approximately 0.8% of the world's surface (Dudgeon *et al*., 2006) and is facing a multitude of pressures (Carpenter *et al*., 2011).

The salinisation of freshwater habitats has reportedly affected an area of 950 million hectares (Hart *et al*., 1990) and is considered to be is an issue with global implications (Williams, 2001). Whilst the arid and semi-arid regions of the world are the areas most commonly affected by increases in salinity (Williams, 1987, 1999, 2001; Brock *et al*., 2005), temperate regions are also experiencing salinisation (Williams, 1987, 1999, 2001; Ghassemi *et al.*, 1995). Increases in salinity beyond a threshold level will result in the loss of a freshwater supply for domestic, agricultural and industrial purposes (Williams, 1999) incurring substantial economic costs (Williams, 1987, 1999, 2001) and, in the most severe circumstances, affect human health (Williams, 1999, 2001). Furthermore salinisation of freshwater habitats can have serious detrimental effects on the environment as salt sensitive taxa are replaced by salt tolerant taxa resulting in an overall loss in biodiversity (Williams, 1999, 2001; Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003), which itself can result in the loss of aquatic organisms for food and such recreational activities as fishing and eco-tourism (Costanza *et al*., 1997).

In some cases natural processes have increased salinity concentrations in inland waters, such as in terminal lakes where salts in the water basin collect and concentrate (Hart *et al*., 1990; Williams, 1999). Furthermore, some rivers and streams have naturally high salinities (Metzeling, 1993; Gallardo-Mayenco, 1994; Velasco *et al*., 2006; Dunlop *et al*., 2008).

The salinity of freshwater habitats, however, can also be increased as a result of the actions of man such as the disposal of oilfield wastewater (Short *et al*., 1991), industrial and urban effluents (Williams, 1987, 2001; Piscart *et al*., 2005a), mine waters (Kowalik & Obarska-Pempkowiak, 1997; Echols *et al*., 2009; Wolf *et al.*, 2009) and the application and subsequent washing of road salts into nearby freshwater habitats (Williams *et al*., 1999; Blasius & Merritt, 2002; Kaushal *et al*., 2005). The largest contribution to the salinisation of inland waters, however, results from such disturbance of natural hydrological cycles as the abstraction (Goetsch & Palmer, 1997) and diversion of water (Williams & Aladin, 1991; Williams, 1999, 2001), and the agricultural practices of replacing deep rooted native vegetation with shallow rooted crops and irrigation (Pillsbury, 1981; Hart *et al.*, 1990; Williams, 1999; Kay *et al.*, 2001; Marshall & Bailey, 2004).

The use of bio-indication is central to the implementation of the Water Framework Directive (WFD; European Commission, 2000), which was developed to safeguard and improve the condition of the water bodies in Europe (Kallis & Butler, 2001; Blanchet *et al*., 2008). Member States of the European Union are required by the WFD to classify the ecological status of freshwater habitats on a scale from high to bad based on the biological communities present (European Commission, 2000; UKTAG, 2007). Member States are then required to restore all habitats to "good ecological status", defined as where the biological communities only slightly deviate from that which would be present in undisturbed conditions (European Commission, 2000; UKTAG, 2007; Moss, 2008), and to prevent the deterioration of those waters already classified as in good status (European Commission, 2000; Kallis & Butler, 2001; Griffiths, 2002; UKTAG, 2007). Furthermore, the Water Framework Directive requires salinity, as well as a number of other environmental parameters such as acidification and selected pollutants, to be monitored in freshwater habitats as a supporting element of the biological data (European Commission, 2000).

Macro-invertebrates are favoured for use in bio-indication and many indices have been developed based on macro-invertebrate community responses to a wide range of environmental stressors (e.g. Chesters, 1980; Lenat, 1988; Extence *et al*., 1999; Williams *et al*., 1999; Alvarez *et al*., 2001; Chadd & Extence, 2004; Davy-Bowker, 2005; Horrigan *et al*., 2005; Palmer *et al*., 2010; Extence *et al*., 2011; Schäfer *et al*., 2011).

Despite this, the potential environmental and economic impacts resulting from salinisation and the legislative requirement, to date only the biotic index by Palmer *et al*. (2010) has been developed for the assessment of salinity in freshwater habitats in the United Kingdom. The index proposed by Palmer *et al*. (2010), however, was designed specifically for use in coastal grazing marsh drainage channels and does not make use of abundance data, which is a requirement of the Water Framework Directive in the biological assessment of European water bodies (European Commission, 2000).

1.1 Aims and Objectives

The aim of this work is to develop and test an index to assess water quality in terms of salinity pollution in freshwater systems. The following hypotheses are proposed for examination to meet this aim:

i. A biotic index can be developed to detect and quantify the impact of salinity in freshwater habitats.
ii. The proposed index will have a significant relationship with, and high selectivity to, salinity concentration.

The objectives of this study are to:

i. Assess the need and requirements for an index assessing water quality in terms of salinity in freshwater systems.
ii. Develop a new index to be compliant with the requirements of the Water Framework Directive and compatible with the sampling protocol of the regulatory authority.
iii. Investigate the influence exerted by salinity and other environmental features on the aquatic biota.
iv. Investigate the relationship between the new salinity index and salinity concentration, as well as the other measured environmental features.
v. Compare the new salinity index with any other relevant published salinity indices.

2 LITERATURE REVIEW

Salinity has been defined as the concentration of salts dissolved in a solvent (Williams, 1987; Metzeling, 1993; Goetsch & Palmer, 1997; Hart *et al*., 2003). Dissolved salts are a natural component of freshwater (Pillsbury, 1981), and some inland water systems have naturally high salinities (Dunlop *et al*., 2008). Salts can enter freshwater systems through a number of routes; incorporation by way of dissolution when water flows through soils and weathers terrestrial material such as rocks (McCaull & Crossland, 1974; Pillsbury, 1981), seawater intrusion into rivers through tidal influence (Williams *et al*., 1991; Greenwood & Wood, 2003; Wolf *et al*., 2009), saline groundwater seepage into surface waters (Pillsbury, 1981; Williams, 1987; Kefford, 1998a), and through precipitation. Precipitation contains a small amount of salts originating from the ocean (Nielsen *et al*., 2003), whilst additional salts may also become associated with the precipitation as it travels through the atmosphere (McCaull & Crossland, 1974).

Salts separate into their component ions upon dissolution (McCaull & Crossland, 1974), of which the most commonly encountered in surface waters are sodium and chloride (McCaull & Crossland, 1974; Williams, 1987; Bunn & Davies, 1992). Other major ions contributing to salinity include calcium and sulphate (Pillsbury, 1981; Williams, 1987; Bunn & Davies, 1992), magnesium, potassium, carbonate and bicarbonate (Goetsch & Palmer, 1997; Nielsen *et al.*, 2003; Kefford *et al.*, 2004a), whilst McCaull & Crossland (1974) also identified bromide, iodide, phosphate and nitrate as component ions of salts in surface waters.

2.1 Salinisation

The process whereby salinity increases is termed salinisation (Hart *et al.*, 1990; Williams *et al*., 1991; Horrigan *et al*., 2005; Velasco *et al*., 2006). Furthermore, Piscart *et al*. (2005a) and Williams (1987) both also define salinisation as the resulting condition of surface waters and land caused by increasing salinity levels. Two types of salinisation have been distinguished: primary salinisation, also termed natural salinisation (Williams, 2001), and secondary salinisation, also designated anthropogenic salinisation (Williams, 1987, 1999).

Primary, or natural, salinisation is the increase in salinity in inland waters due to natural factors and occurring at rates unaffected by human activities (Williams, 2001). Primary salinisation usually occurs in closed basins where rivers, or streams, flow into a terminal lake (Hart *et al*., 1990; Williams, 1999), for example the Caspian Sea in central Asia (Williams, 1987), Pyramid Lake in Nevada, USA (Williams, 1999) and the Aral Sea in Asia between Kazakhstan and Uzbekistan (Williams & Aladin, 1991). Some rivers and streams, frequent in the Mediterranean basin (Gallardo-Mayenco, 1994), also have naturally high salinities (Dunlop *et al*., 2008). Examples of such rivers include Deep Creek, also known as Saltwater Creek, in Australia (Metzeling, 1993) and the Rambla Salada stream in south-east Spain studied by Velasco *et al*. (2006).

The increase in salinity in inland waters resulting from human activities is termed secondary, or anthropogenic, salinisation (Williams, 1999; Marshall & Bailey, 2004). Secondary salinisation may result from human actions directly, such as the disposal of saline wastewater (Short *et al.*, 1991; Piscart *et al.*, 2005a). Secondary salinisation may also occur indirectly as a consequence of obvious human activities affecting natural hydrological cycles (Williams *et al*., 1991; Kay *et al.*, 2001; Hart *et al*., 2003), such as abstraction (Goetsch & Palmer, 1997) and diversion of water (Williams & Aladin, 1991; Williams, 1999, 2001) for irrigation purposes (Pillsbury, 1981; Williams, 1987) and the cultivation of non-native plants using intensive agricultural practices (Williams, 1987, Hart *et al.*, 1990; Williams, 1999; Kay *et al.*, 2001; Marshall & Bailey, 2004).

Whilst human activities causing secondary salinisation may be as innocuous as the application of salt to road, which can enter rivers and streams and increase the salinity of these waters (Williams *et al*., 1999; Blasius & Merritt, 2002; Kaushal *et al*., 2005), secondary salinisation may also occur from the input of mine waste waters (Kowalik & Obarska-Pempkowiak, 1997; Echols *et al*., 2009; Wolf *et al.*, 2009) or industrial (Short *et al*., 1991; Piscart *et al*., 2005a) and urban effluents (Williams, 1987, 2001). Addition of salt to inland waters from industry and urban activities, however, generally tends to be of local significance (Williams, 1987).

Disturbance of natural hydrological cycles as a result of human actions can cause the mobilisation of naturally accumulated salts held in groundwater (Hart *et al*., 1990), soil (Kay *et al*., 2001) and rocks (McCaull & Crossland, 1974; Pillsbury, 1981), and it is these disturbances which make the largest contribution to the salinisation of inland waters (Williams, 1987). Irrigation and the removal of native deep-rooted plants are the two activities with the greatest effect on hydrological cycles resulting in the mobilisation of naturally held salts (Williams, 1987). Clearing deep-rooted vegetation and replacing it with shallow rooted crops results in decreased interception of precipitation and increased groundwater recharge (Hart *et al*., 1990; Williams, 1999; Kay *et al*., 2001), resulting in a rise in groundwater tables, which may be naturally saline (Hart *et al*., 1990; Marshall & Bailey, 2004), and mobilising salts held in soil (Hart *et al*., 1990; Williams, 1999; Kay *et al*., 2001). This process has been implicated as the cause of increased salinity in Blackwood River and Gleneg River in Australia (Williams *et al*., 1991).

Evapo-transpiration by crops and natural evaporation causes irrigation water to become more saline (Pillsbury, 1981; Williams, 1987). Irrigation water may further increase in salinity by leaching salts (McCaull & Crossland, 1974; Kay *et al.*, 2001) as it filters through the soil, eventually reaching groundwater or a nearby surface water body (Pillsbury, 1981; Williams, 1987).

Abstraction of water also leads to salinity increases in inland waters as a result of concentrating water-held salts (Goetsch & Palmer, 1997). Increases in the salinity of lakes can result from the diversion of inflowing water. Lake volumes decrease as water is lost through evaporation and not replaced as inflowing water has been diverted, whilst the salt mass contained within the lake remains the same (Williams, 1999, 2001). As a consequence of the decrease in water volume, the salinity of the lake increases as the salts concentrate (Pillsbury, 1981; Goetsch & Palmer, 1997; Williams, 1999, 2001).

Despite the distinction between the two, salinisation cannot always be specifically classified as either primary or secondary. For example, salinity in near coastal inland waters elevated by tidal intrusion (e.g. Williams *et al*., 1991; Greenwood & Wood, 2003; Wolf *et al.*, 2009) may be classed as primary salinisation.

Global warming causing sea levels to rise (Short & Neckles, 1999; Edwards & Winn, 2006) will, however, result in increased seawater intrusion into rivers and saline penetration further upstream in the future (Short & Neckles, 1999). Decreased freshwater flow, which results from droughts or increased abstraction of freshwater, also results in increased seawater penetration (Attrill *et al*., 1996). Hence, whilst seawater intrusion may be classed as primary salinisation, it is also influenced, at least in part, by human activities.

2.2 Measurement of Salinity

The accurate determination of salinity requires comprehensive ionic analysis (Williams, 1987; Rice *et al*., 2012). This method, however, is time-consuming and consequently salinity is frequently determined by measuring a physical property (Rice *et al*., 2012) such as electrical conductivity (Hart *et al.*, 1990; Wood & Dykes, 2002; Kefford *et al*., 2004a; Horrigan *et al*., 2005; Kefford *et al*., 2007). Electrical conductivity measures the ability of a water sample to conduct an electrical current, and as such can be defined as a measure of the concentration of ionic material present in the sample (Goetsch & Palmer, 1997). Electrical conductivity is easily, rapidly and accurately measured (Kefford *et al*., 2003, 2004b) and is utilised by the Environment Agency (Environment Agency, 2012a) in assessing water quality. Whilst Panter *et al*. (2011) stated that using conductivity has a poor relationship with salinity for conductivities less than 1000µScm^{-1}, Williams (1987) reported that use of electrical conductivity to measure salinity does not result in any significant error. Furthermore, Rice *et al*. (2012) state that the use of electrical conductivity to determine salinity is recommended for precise field and laboratory work due to its high precision and sensitivity.

Quantification of total dissolved solids can also be used to determine salinity (Metzeling, 1993; Kefford *et al.*, 2004b; Marshall & Bailey, 2004). Total dissolved solids is the concentration of all dissolved material in a water sample (Goetsch & Palmer, 1997). Williams (1987) stated the concentration of total dissolved solids is not significantly different from salinity.

2.3 Classification of Surface Waters Based on Salinity

Mixtures of fresh water and marine water resulting in a salinity concentration between 0.3 and 35PSU (Practical Salinity Units) are termed brackish (Williams, 1987). Transitional waters, which are brackish, occur where rivers and other surface water systems are influenced in terms of salinity by both coastal waters and freshwater flows (European Commission, 2000). Several systems have been proposed to describe brackish waters, such as the classification systems of Redeke (Den Hartog, 1974), Välikangas (Den Hartog, 1974), Bulger *et al*. (1993) and Christensen *et al*. (1997). These systems were all developed based on observations of changes in biotic communities in relation to salinity. Table 2.1 displays the salinity ranges that characterise the various zones of brackish water according to the systems of Redeke (Den Hartog, 1974), Välikangas (Den Hartog, 1974) and the Venice System (Battaglia, 1959).

Table 2.1: Salinity ranges of the different zones proposed in the systems of Redeke, Välikangas and the Venice System

	Salinity range of zones (PSU)				
System	Freshwater	Oligohaline	Mesohaline	Polyhaline	Euhaline (or marine)
Venice System[1]	< 0.5	0.5 - < 5.0	5.0 - < 18.0	18.0 - < 30.0	30.0 - < 40.0
Redeke[2]	< 0.1	0.1 - 1.0	1.0 - 10.0	10.0 - 17.0	> 17.0
Välikangas[2]	< 0.3	0.3 - 1.6	1.6 - 10.0[A]	10.0 - 16.5	> 16.5

References: [1] = Battaglia, 1959; [2] = Den Hartog, 1974.

PSU = Practical Salinity Units.

[A] Välikangas separated the mesohaline zone into two distinct zones, with the salinity range (1.6-8.0PSU) termed α-mesohaline and the salinity range (8.0-10.0PSU) termed β-mesohaline (Den Hartog, 1974).

Redeke first proposed his system in 1922 and sub-divided brackish water on the basis of the biotic communities present, but also stressed the figures were approximate to be refined when new data became available (Den Hartog, 1974). In contrast, in 1933 Välikangas proposed a classification system of brackish water based only on the planktonic communities present (Den Hartog, 1974). The Venice System was proposed at the International Symposium for the Classification of Brackish Waters held in Venice in 1958 (Battaglia, 1959). This system was designed for universal application and to clearly define terms such as oligohaline, mesohaline, polyhaline and euhaline (Battaglia, 1959; Den Hartog, 1974).

The Venice System is essentially a modified amalgamation of the Redeke and Välikangas systems (Battaglia, 1959), and as such has been criticised by Den Hartog (1974) for being a compromise and having no biological basis. The Venice System has since been used in the Water Framework Directive as a tool to describe the zones of transitional waters in terms of salinity (European Commission, 2000).

Alternative classification systems have been developed which are based on the statistical analysis of the salinity tolerances and preferences of organisms present in transitional waters (Bulger *et al*., 1993; Christensen *et al*., 1997). Table 2.2 displays the salinity ranges that characterise the various zones of brackish water according to the systems of Bulger *et al*. (1993) and Christensen *et al*. (1997).

Table 2.2: Salinity ranges of the different zones proposed in the systems based on salinity tolerances and preferences of aquatic organisms

	Salinity range of zones (PSU)				
System	Component /Biozone 1	Component /Biozone 2	Component /Biozone 3	Component /Biozone 4	Component /Biozone 5
Bulger *et al*. (1993)	Freshwater - 4	2 - 14	11 - 18	16 - 27	24 - marine
Christensen *et al*. (1997)	< 0.5	> 0.5 – 8	8 – 15	15 – 25	25 – 35

PSU = Practical Salinity Units.

The different salinity zones were termed components by Bulger *et al*. (1993) and biozones by Christensen *et al*. (1997).

Bulger *et al*. (1993) used a multivariate analysis to generate their classification system. Principal component analysis was applied to known salinity ranges of fish and invertebrates found in the Chesapeake and Delaware Bays, USA, to derive the salinity zones of the classification system. Bulger *et al*. (1993) termed the salinity zones of their classification system "components", whereas Christensen *et al*. (1997) used the term "biozones". Christensen *et al*. (1997) used the same technique as Bulger *et al*. (1993) to develop the boundaries, but used the salinity tolerances and preferences of species commonly found in the estuaries of the northern Gulf of Mexico to derive the salinity "biozones".

The differences in salinity tolerances and preferences between the species present in the estuaries of the northern Gulf of Mexico and those present in Chesapeake Bay and Delaware Bay (Bulger *et al*., 1993; Christensen *et al*., 1997) may be the reason for the differences in the salinity zone boundaries of the two systems.

2.4 The Scale of Salinisation in Freshwater Habitats

Salinisation is an important global issue (Williams, 2001) that has been reported to affect an area of 950 million hectares (Hart *et al*., 1990). Although the threat and impact of salinisation is well recognised at national levels (Hassell *et al*., 2006), its global extent and importance is less well recognised (Williams, 1999). Semi-arid and arid regions of the world are the areas most commonly affected (Williams, 1987, 1999, 2001; Brock *et al*., 2005) and represent almost one third of total land area, a proportion likely to increase with global climatic change (Williams, 1999, 2001).

Water supplies in countries from Pakistan in the east to Libya in the west are afflicted by salinisation problems (Williams, 1987), as are Ethiopian and Egyptian lakes, central Asian rivers and some American reservoirs (Williams, 2001). Salinisation issues have been reported in the Prairie Provinces of Canada, large areas of North America (Williams, 1987; Kaushal *et al*., 2005) and in North American rivers (Pillsbury, 1981). The southern part of the African continent experiences issues with salinisation (Williams, 1987), whilst salinisation has also been acknowledged as a major concern in South Africa (Goetsch & Palmer, 1997), northeastern USA (Kaushal *et al*., 2005) and Australia (Williams, 1987; Bunn & Davies, 1992; James *et al.*, 2003). An estimated 5.7 million hectares of rural land in Australia is afflicted by raised salinities (Marshall & Bailey, 2004; Hassell *et al*., 2006; Dunlop *et al.*, 2008) and increased salinities have been widely documented in Australian rivers (Williams, 1987; Williams *et al.*, 1991; Williams, 2001).

The issue of salinisation, however, is not limited to the arid and semi-arid regions (Williams, 1987). Salinisation has been reported in countries with a Mediterranean climate (Piscart *et al*., 2005a), as well as in countries in temperate regions (Williams, 1999, 2001). Salinity problems are occurring in Thailand (Williams, 1987, 1999), China and Argentina (Ghassemi *et al.*, 1995).

Saline groundwaters are polluting waters in former opencast coal mines in Germany (Williams, 2001) and extraction of salt, which has occurred since before early Roman times to later than Victorian times (Cooper, 2002), has also caused salinisation of several Cheshire lakes (Williams, 1999, 2001).

2.5 The Impact of Salinisation on Freshwater Habitats

Significant economic, social and environmental costs can result from the impact of salinisation on inland aquatic ecosystems (Williams, 2001). It has been suggested that the demise of some ancient civilisations, such as the Sumerian civilisation of the Middle East (Williams, 1987, 2001), was a direct consequence of land and water degradation by salinisation (Pillsbury, 1981; Williams, 1987). The most important economic and environmental effects upon water bodies, however, concern salinity increases over only a small part of the total salinity range of inland waters (Williams, 1987) and can result in severe costs being incurred (e.g. Williams 1987; 2001). For example, Williams (1999) suggested an increase in salinity beyond approximately 1PSU renders water unsuitable for domestic, agricultural and industrial purposes.

Salts are not regarded as traditional contaminants of freshwaters such as heavy metals, oil, organic effluent and pesticides (Hynes, 1960). When present in excess, however, salts can have adverse effects on aquatic biota (Hart *et al*., 1990). Salinisation impacts on aquatic systems through direct toxic effects and through habitat loss in the water, riparian zones and adjacent floodplains (James *et al*., 2003; Horrigan *et al*., 2005). Small increases in salinity can be significant in fresh waters since the salinity tolerance of the freshwater biota is much lower than that of saltwater biota (Williams, 2001). Biota unable to tolerate an increase in salinity either perish, or disperse to re-colonise if salinity levels drop to a favourable concentration (Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003). Even small increases in salinity will result in the loss of sensitive species (James *et al*., 2003) and can lead to the gain of salt tolerant biota (Nielsen *et al*., 2003). Examples of halo-sensitive species loss and halo-tolerant species gain resulting from increasing salinity are provided by Williams (1987), who reported that the brackish mussel *Fluviolanatus subtortus* (Dunker, 1857) replaced the freshwater mussel *Westralunio carteri* (Iredale, 1944) in the Avon river, Western Australia.

Williams (1987) also recorded halo-sensitive diatom taxa being replaced by a halo-tolerant but less diverse group of diatom taxa in both the Fish River and the Sundays River in South Africa.

The replacement of the halo-sensitive biota with halo-tolerant biota, along with a decrease in biodiversity, is the general biological response to increased salinity (Williams, 1999, 2001; Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003). The addition and loss of taxa can further affect biota as the taxa removed or gained due to a salinity increase may modify refuge and food availability as well as predation pressure (Nielsen *et al*., 2003). Furthermore, the addition or loss of taxa is likely to affect the flow of energy and material through trophic webs as well as ecosystem processes such as primary productivity (Kaushal *et al*., 2005), decomposition and nutrient recycling (James *et al*., 2003). Although many taxa may be able to survive at elevated salt concentrations, chronic exposure to increased salinity may significantly reduce the recruitment and growth of juveniles, as well as the reproductive capability of the taxa, with severe consequences for subsequent generations (Hart *et al*., 2003; Nielsen *et al*., 2003).

In summary, an increase in salinity is likely to have a detrimental effect on the ecosystem processes of primary productivity (Kaushal *et al*., 2005), decomposition and nutrient recycling (James *et al*., 2003), alterations in predator/prey relationships and ecosystem resilience through the addition or loss of taxa (Williams, 1999, 2001; Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003), which itself may result in the loss of aquatic organisms for food and such recreational activities as fishing and eco-tourism (Costanza *et al*., 1997). Furthermore, increases in salinity beyond a threshold level will result in the loss of a freshwater supply for domestic, agricultural and industrial purposes (Williams, 1999). As such, it can be seen that salinity increases in the freshwater environment can result in a substantial decrease in the ecosystem services beneficial to human populations, which have been conservatively estimated to have a global value greater than US$1.7 trillion per year (Costanza *et al*., 1997).

2.6 Legislation and Water Quality

In addition to environmental and economic reasons (see Section 2.5), there is also a legislative requirement to monitor and manage salinity. Council Directive 79/409/EEC on the Conservation of Wild Birds, known as the Birds Directive, and Council Directive 79/409/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora, known as the Habitats Directive, both legislate for the protection of habitats (European Community, 1979; European Community, 1992).

The Birds Directive provides measures for the protection of wild birds and their habitats within Europe (European Community, 1979). Article 3 of the Birds Directive requires Member States of the European Union to take measures to preserve, maintain, and, if required, restore habitats for the naturally occurring wild birds of Europe (European Community, 1979).

The Habitats Directive is designed to ensure the biodiversity of the European Union (European Community, 1992; Lund, 2002) through conservation of natural habitats and the populations of species of wild fauna and flora (European Community, 1992; Domínguez Lozano *et al*., 1996). Furthermore, the Habitats Directive requires, where necessary, Member States of the European Union to maintain or restore habitats to a favourable status in areas designated as Special Areas of Conservation (European Community, 1992). Given the effect salinity has on habitats (see Section 2.5), it can be seen that salinity is a factor which must be managed in order to maintain habitats as required by both the Birds Directive and the Habitats Directive.

In addition to the Birds Directive and the Habitats Directive, Council Directive 78/569/EEC on the quality of fresh waters needing protection or improvement in order to support fish life, updated in 2006 (European Community, 2006; Petrescu-Mag, 2008) and commonly known as the Fresh Water Fish Directive (Davies *et al.*, 2004; Petrescu-Mag, 2008) requires Member States of the European Union to designate, protect and improve the quality of surface fresh water bodies that are capable of supporting certain species of fish (Davies *et al.*, 2004; European Community, 2006; Petrescu-Mag, 2008). Consequently, very large quantities of standing and flowing freshwater habitats in Britain are designated under the Fresh Water Fish Directive (Ormerod, 2003).

Member States are then required to ensure that the designated stretches of standing or flowing surface waters meet physical and chemical quality standards listed in Annex I of the Fresh Water Fish Directive (European Community, 2006; Petrescu-Mag, 2008). Despite the aim of this Directive to protect and improve fresh surface waters for fish, salinity is not among the standards listed in this Directive (European Community, 2006). Furthermore, the Fresh Water Fish Directive is among six European directives due to be fully integrated into Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy, known as the Water Framework Directive (Carter & Howe, 2006), and either phased out or repealed by 2013 (Mayes & Codling, 2009).

2.6.1 The Water Framework Directive

The Water Framework Directive (WFD) came into force in December 2000 (Kaika, 2003). The WFD complements the many water directives that already exist (Mostert, 2003; Carter & Howe, 2006) and brings together many legislative instruments in the context of water, as well as some from other environmental aspects (Chave, 2001).

Member States of the European Union are required by the WFD to classify the ecological status of aquatic habitats on a scale from high to bad by comparing the biological communities present to that which would be expected to be present in undisturbed conditions (European Commission, 2000; UKTAG, 2007). The chemical status of aquatic habitats is also required to be classified and is undertaken by examining if the water contains substances listed in Annex IX and Annex X of the WFD exceeding the concentration established as the environmental quality standard for that specific substance (European Commission, 2000; UKTAG, 2007). Chemical status is presented simply as either “good” where no substance is present at concentrations its established environmental quality standard or “failing to achieve good” where the concentration of one or more substance exceeds its established quality standard (European Commission, 2000; UKTAG, 2007). The overall “surface water status” of a water body is determined by the lower of the ecological status and chemical status of that water body (European Commission, 2000; UKTAG, 2007).

Member States are then required to restore all habitats to "good surface water status" where good status is defined as slightly different from high status (European Commission, 2000; UKTAG, 2007; Moss, 2008) and to prevent the deterioration of those waters already classified as in good status (European Commission, 2000; Kallis & Butler, 2001; Griffiths, 2002; UKTAG, 2007). Good ecological potential instead of good ecological status, however, is the minimum target for water bodies that have been designated as either "artificial" or "heavily modified" by Member States (European Commission, 2000; Griffiths, 2002). An artificial water body is defined by the WFD as a body of water created by human activity (European Commission, 2000), whilst a heavily modified water body is defined as a body of water which has been substantially altered by human activity (European Commission, 2000). Good surface water chemical status remains the target for such water bodies (European Commission, 2000).

According to the WFD, the ecological assessment of a water body must be based on the evaluation of biological elements and reinforced by the measurement of hydro-morphological and physico-chemical elements (Chave, 2001; Kallis & Butler, 2001). The reason for assessing biological elements is based on the concept of bio-indication, whereby the fauna and flora indicate the status of environmental parameters such as organic pollution (Wolf *et al*., 2009) and changes in the environment are evaluated by using the response of the biota affected (Matthews *et al*., 1982; Lemke *et al*., 1997). The biological elements to be monitored include benthic invertebrate fauna, fish fauna, macrophytes and other biological entities such as diatoms (European Commission, 2000). Hydrological conditions, river continuity and morphological regime are the hydro-morphological elements required to be monitored by the WFD (European Commission, 2000). The physico-chemical elements to be monitored include, among others, salinity, acidification and nitrification status (European Commission, 2000; Kallis & Butler, 2001). Each element is given a rating of high, good, moderate, poor or bad status according to how they correspond to the expected undisturbed conditions (European Commission, 2000; Kallis & Butler, 2001).

Transitional and coastal waters are further classified in terms of annual mean salinity (European Commission, 2000) and the concentrations of the salinity classes in the WFD correspond with those in the Venice System (Wolf *et al.*, 2009).

The Venice System, however, has been strongly criticised by Den Hartog (1974) for being a compromise and having no biological basis (see Section 2.3). The WFD is further criticised by Wolf *et al*. (2009) for not specifying the depth, season or tide in which salinity measurements in transitional waters should be taken. The WFD, however, does state that where relevant CEN/ISO standards have been developed for monitoring and/or sampling procedures, these should be used (European Commission, 2000).

2.7 The Assessment of Water Quality

Given the environmental and economic reasons and legislative requirements (see Sections 2.5 and 2.6) for monitoring and managing salinity, the efficiency of water quality assessment methods need to be considered. The assessment of water quality can be performed through physical and chemical analyses or by assessing the biota present in the water (Hynes, 1960; Lancaster & Scudder, 1987; Williams *et al*., 1999; Iliopoulou-Georgudaki *et al*., 2003), though both of these techniques have inherent advantages and disadvantages (Table 2.3). Thus, in an ideal situation both techniques would be used in the assessment of water quality (Wright, 1994; Iliopoulou-Georgudaki *et al*., 2003).

Physico-chemical assessment is the conventional technique employed in the determination of water quality (Wright, 1994). The main advantage of physico-chemical assessments is that they provide the exact concentrations of pollutants, such as salinity, in a water body (Lafferty, 1997). In comparison, biological assessments cannot reveal the precise concentration of pollutant in the water (Hynes, 1960). There are, however, several major disadvantages to the use of physico-chemical assessments of aquatic habitats. Firstly, physico-chemical assessments do not indicate the impact of pollutants, such as salinity (Wright, 1994; Clarke *et al*., 2003), on the resident ecology (Knoben *et al*., 1995; Lafferty, 1997) as required by the WFD (European Commission, 2000; UKTAG, 2007). Secondly, chemical analysis of water quality provides only a snapshot of pollutant concentration, such as salinity, at the time of sample collection (Hynes, 1960; Hellawell, 1986; Wright, 1994; Knoben *et al*., 1995; Vrana *et al*., 2002). Consequently, short-term pollution events (Hynes, 1960; Wright, 1994; Clarke & Wharton, 2001; Vrana *et al*., 2002) and variations in pollutant concentrations (Hynes, 1960; Wright, 1994; Clarke & Wharton, 2001; Vrana *et al*., 2002) may be missed.

Table 2.3: Advantages and disadvantages of physico-chemical and biological assessments of water quality

Assessment method	Advantages	Disadvantages
Physico-chemical	**1.** Cost effective **2.** Quick process **3.** Precise determination of pollutant concentrations such as salinity	**1.** Does not indicate the impact of pollutant on the resident ecology **2.** Only a snapshot of conditions at time of sampling; short term or intermittent pollution can be missed **3.** Low levels and variation in concentration of salinity may not be recorded **4.** Can introduce automatic sampling, but this is an expensive and impractical solution
Biological	**1.** Public interest in biological communities such as fish **2.** Cost effective; less equipment needed, quick sampling process and large areas can be sampled quickly **3.** Pollutants such as salinity can be detected at low levels **4.** Facilitates the tracking of temporal changes **5.** Knowledge can be taught to amateurs easily **6.** Not as dependent on when samples are collected **7.** Lower frequency of sampling required	**1.** Dependent upon the organism-type being assessed **2.** May result in a delay in preventing further damage due to length of sample analysis time **3.** Cannot give exact concentrations of a specific pollutant

See Section 2.7 for relevant references and greater detail.

Whilst high sampling frequency (Knoben *et al*., 1995; Vrana *et al*., 2002) or installation of automatic sampling systems (Vrana *et al*., 2002) can resolve this issue, both solutions increase costs and can be impractical as secure sites are required for sampling system installations (Vrana *et al*., 2002). Thus, whilst the evaluation of biotic communities can be time consuming and expensive (Lafferty, 1997; Koponen *et al*., 2002), physico-chemical assessments can be less cost and time effective (Lafferty, 1997; Lemke *et al*., 1997; Iliopoulou-Georgudaki *et al*., 2003) as it may require many series of samples to obtain the same information that can be revealed by a single series of biological samples (Hynes, 1960). Furthermore, when undertaken by trained personnel biological assessments can be rapid and relatively cheap (Freidrich *et al*., 1996; Barbour *et al*., 1999).

A further issue with the use of physico-chemical assessments arises from the fact that saline water sinks below fresh water due to the difference in density, a process termed halo-stratification (Dyer, 1973; Davidson *et al*., 1991).

For example, Muñoz & Prat (1994) noted that the salinity at the water's surface of an estuarine site was 0.7-1.1PSU, but 1.5m below the water's surface salinity was 24.9-37.7PSU. Thus to accurately determine the salinity of a section of a water body, chemical tests may need to be made at a series of depths. The depth from which salinity data are recorded, however, is not specified in the Water Framework Directive (European Commission, 2000; Wolf *et al*., 2009), nor is a standard depth stated in the relevant ISO standard (International Organization for Standardization, 1985). The only advice given regarding depth during the collection of water samples for analysis, regardless of the constituent tested, is to collect a sample from the water body surface (International Organization for Standardization, 1985; Ritter, 2010; Rice *et al*., 2012). As such, a range of depths have been used to collect salinity data. For example, depths of 10-20cm (Horrigan *et al.*, 2005; Carlsson, 2006), 0.5m (Wolf *et al*., 2009), and 1m below the surface (Lancaster & Scudder, 1987), as well as the middle (Short *et al*., 1991; Joyce *et al*., 2005), base (Greenwood & Wood, 2003; Kazanci *et al*., 2003) and top (Kazanci *et al*., 2003) of the water column have all been used in the collection of salinity data from surface waters.

In contrast to physico-chemical analyses, the results from biological analyses are less dependent upon the time of sampling (Clarke & Wharton, 2001). Aquatic biota integrate the effect of continuous, episodic and fluctuating pollution events into a single response for a given period of time (Hynes, 1960; Hellawell, 1986; Cuffney *et al*., 1993; Knoben *et al*., 1995; Wright, 1995; Barbour *et al*., 1999; Williams *et al*., 1999; Vrana *et al*., 2002), which is dependent on the organism-group used (Hellawell, 1986; Cuffney *et al*., 1993; Knoben *et al*., 1995; Friedrich *et al*., 1996; Barbour *et al*., 1999; Melzer, 1999; Nurminen, 2003). Consequently biological analyses require lower frequency of sampling in comparison to chemical analyses (Knoben *et al*., 1995). Further advantages of biological assessments over physico-chemical assessments include the requirement of less equipment, a large area can be intensively surveyed in a short time (Freidrich *et al*., 1996), as well as the fact that there is a high degree of public interest in the status of biological communities (Barbour *et al*., 1999) and the protection of threatened and endangered species (Cuffney *et al*., 1993). Furthermore, it has been shown that salinity at very low concentrations may not be revealed by chemical assessments but can be detected by biological assessments (Wolf *et al*., 2009).

As well as the fact that biological assessments do not reveal the concentrations of pollutants (Hynes, 1960), using biological assessment in favour of chemical assessment may result in delays in the prevention of further damage and the remediation of a salinised water body as biological assessments are only responsive once a detectable effect has been elicited from the organism, or organisms, under evaluation (Clarke & Wharton, 2001; Azrina *et al*., 2006). There are also further disadvantages to using biological assessments that are dependent upon the organism group used in to assess water quality and these are discussed in greater detail in Sections 2.7.1.1, 2.7.1.2, 2.7.1.3 and 2.7.1.4.

2.7.1 Biological Organisms Used for the Assessment of Water Quality

Biological assessments of water quality can be performed using different types of organisms such as micro-organisms, vegetation, invertebrates and fish (Hynes, 1960; Clarke & Wharton, 2001; Iliopoulou-Georgudaki *et al*., 2003). There is some debate about which type of organism is best suited for use in the biological assessment of water bodies (Hynes, 1960). The selection of organism type can be based on such factors as distribution, mobility, ease of sampling and identification (Hynes, 1960; Hellawell, 1986; Johnson *et al.*, 1993), sensitivity to pollution (MacNeil *et al*., 2002; Azrina *et al*., 2006), abundance (Hynes, 1960; Johnson *et al.*, 1993) and length of life cycle (Johnson *et al.*, 1993; Wright, 1994; Clarke *et al*., 2003).

The selected organism type should have a relatively large number of species with known responses to disturbance (Niemi & McDonald, 2004) and should be both abundant (Hynes, 1960; Johnson *et al.*, 1993; Linton & Warner, 2003) and well distributed (Hellawell, 1986; Johnson *et al.*, 1993; Linton & Warner, 2003). Furthermore, organism types exploited by humans should be avoided as this would confound any trends shown through monitoring of their presence and abundance (Phillips, 1980; Wenner, 1988).

Given that taxonomic uncertainties can confuse data interpretation (Hellawell, 1986), the selected organism type should be easy to identify (Johnson *et al.*, 1993; Niemi & McDonald, 2004) and have a stable taxonomy that can easily be taught to amateurs (Linton & Warner, 2003). The selected organism type should be easy and inexpensive to sample (Dale & Beyeler, 2001; Niemi & McDonald, 2004) in a quantitative and objective manner (Linton & Warner, 2003) without the need for expensive equipment or several operators (Hellawell, 1986).

Sedentary organisms reflect local water conditions more faithfully and accurately than mobile organisms (Moore, 1977). Furthermore, sedentary organisms are also more useful in detecting the precise location of pollutant sources than mobile organisms (Hellawell, 1986). As such, a sedentary nature in the selected organism type is considered advantageous (Hynes, 1960; Moore, 1977; Hellawell, 1986; Johnson *et al.*, 1993). A long life cycle in the selected organism type is also considered an advantage (Johnson *et al.*, 1993). Long life cycles allow temporal changes to be followed and integrate the effects of prolonged exposure to variable concentrations or intermittent discharges of pollutant (Hellawell, 1986). Furthermore, a long life cycle justifies the adoption of periodic sampling as such a procedure would not be valid for organisms with short life cycles (Hellawell, 1986).

The selected organism type should have a high sensitivity to the particular stress in question and respond to the stress in a predictable manner with a low variability in the response exhibited (Dale & Beyeler, 2001). Furthermore, the selected organism type should exhibit a graded response relative to the quantity of the particular stress (Linton & Warner, 2003). Ideally, the selected organism type should present measurable changes before a substantial change in ecological system integrity occurs (Dale & Beyeler, 2001). The advantages and disadvantages associated with each the organism group used in the assessment of water quality are presented in Table 2.4 and discussed in greater detail in Sections 2.7.1.1, 2.7.1.2, 2.7.1.3 and 2.7.1.4.

2.7.1.1 Fish

Fish are considered as a key biotic element in the assessment of aquatic habitats by the WFD (European Commission, 2000) and they have long been used to undertake such assessments (Karr, 1981; Fausch et al., 1990; Angermeier & Davideanu, 2004). The first fish-based multi-metric index, the index of biotic integrity (IBI), was developed in 1981 (Karr, 1981) and has since been modified for application in many countries (Hughes & Oberdorff, 1999). Furthermore, a variety of fish-based biotic indices have been developed to assess water quality with many based on the original IBI (Aparicio *et al*., 2011).

Table 2.4: Summarised advantages and disadvantages of common biological organism types used in the assessment of water quality

	Advantages	Disadvantages
Fish	**1.** Taxonomy of European species is simple **2.** Extensive knowledge regarding life-histories and ecological requirements **3.** Bio-accumulating ability **4.** Reflect conditions over large spatial areas	**1.** Less abundant than other organisms **2.** Highly mobile; exhibit seasonal migrations and capable of moving away from pollution **3.** Generally tolerant of increases in salinity **4.** Comparatively expensive and difficult to survey in fast-flowing or deep habitats **5.** Low number of species; approximately 60 in the UK
Aquatic macrophytes	**1.** Respond to a large number of environmental variables including salinity **2.** Easy to survey - little lab based ID and low manpower demands **3.** Immobile & visible to the naked eye **4.** Long lived - long term indicator potential **5.** Large number of species; over 350 in the UK	**1.** Hybridisation/lack of flowering bodies make identification difficult **2.** Seasonal variations in biomass and not visible for long periods of time **3.** Incapable of exhibiting a graded response to environmental stress tolerant of intermittent pollutions **4.** Slow response time to environmental stress **5.** Few species in any one region
Phytoplankton and periphyton	**1.** Large number of species; approximately 2400 freshwater algae and 2500 diatom species described so far in the UK **2.** Algae sensitive to some pollutants that only start to affect other organisms at higher concentrations **3.** Diatom samples can be preserved indefinitely	**1.** Only reflect water quality for 1-2 weeks prior to sample collection and recovery from pollution is rapid **2.** Specialist equipment required for preparation and analysis of algal, bacterial and protozoan samples **3.** Protozoa, algae and bacteria are taxonomically difficult **4.** Diatoms less sensitive than macroinvertebrates to salinity **5.** Well defined seasonal variation in abundance
Macro-invertebrates	**1.** Greater than 3000 aquatic species in Britain **2.** Wide variation in salinity tolerance of different macro-invertebrate taxa **3.** Reliable indicators of local conditions at the site of sampling and respond rapidly to environmental changes **4.** Sensitive life stages respond quickly to changes in environmental conditions **5.** Long-lived species exhibit a graded response to changes in pollution load and type **6.** Convenient size for field examination, storage, transport and predicatably responds to changes in water quality **7.** Taxonomy of groups is well known **8.** Comparatively easy and inexpensive to sample and identify **9.** Sedentary in nature with limited migration patterns	**1.** Issues with comparing samples collected in different seasons **2.** Flow rate - some species can drift being carried into areas where they would not usually occur **3.** Not all water quality stresses illicit a detectable response from macro-invertebrate communities **4.** Macro-invertebrate community structure influenced by substrate type **5.** Some groups are difficult to identify to species level, e.g. chironomid fly larvae, oligochaete worms

See Sections 2.7.1.1, 2.7.1.2, 2.7.1.3 and 2.7.1.4 for relevant references and greater detail.

There are many reasons to use fish in the assessment of water quality. Due to their position towards the apex of food pyramids (Maitland, 1974; Hellawell, 1986; Knoben *et al*., 1995; Barbour *et al*., 1999), fish reflect the effects of pollutants on their prey organisms as well as the direct effect on the fish themselves (Maitland, 1974; Hellawell, 1986; Friedrich *et al*., 1996; Barbour *et al*., 1999; Resh, 2008). The taxonomy of European fish species is relatively easy (Hellawell, 1986; Friedrich *et al*., 1996; Resh, 2008), allowing adult fish to be identified in the field and returned alive to the water (Barbour *et al*., 1999; Maitland & Linsell, 2009). Furthermore, fish are ideal organisms for tracking changes over time as they have a long generation time and are long-term integrators that bio-accumulate toxins within their tissues (Resh, 2008). Furthermore, fish reflect conditions over large spatial areas resulting from their mobility and longevity (Plafkin *et al*., 1989; Cuffney *et al*., 1993; Knoben *et al*., 1995). The recreational (Resh, 2008) and commercial value of fish (Hellawell, 1986; Oberdorff *et al*., 2001) and the availability of pre-existing information (Barbour *et al*., 1999; Oberdorff *et al*., 2001) are also considered advantages of using fish in water quality assessments.

There are, however, disadvantages associated with using fish for assessing water quality. Fish are very mobile organisms (Hynes, 1960; Hellawell, 1986; Knoben *et al*., 1995; Friedrich *et al*., 1996; Iliopoulou-Georgudaki *et al*., 2003; Kefford *et al*., 2004b; Resh, 2008) and many fish species exhibit seasonal migrations for spawning (Knoben *et al*., 1995). As such, fish frequently occur far from their normal habitats (Hynes, 1960) and are capable of avoiding polluting water to return if favourable conditions are restored (Hellawell, 1986; Knoben *et al*., 1995; Friedrich *et al*., 1996). Furthermore, natural fish movements can be disrupted by the presence of physical barriers such as dams (Fore & Graffe, 2002), thus influencing the affected fish community composition. There are a relatively small number of freshwater fish species present in the UK in comparison to other organism types; Davies *et al*. (2004) included only 60 species in a checklist of freshwater fish species in Britain. Furthermore, fish are also less abundant than other organisms (Hynes, 1960; Iliopoulou-Georgudaki *et al*., 2003; Kefford *et al*., 2004b; Resh, 2008). Certain fish species are exploited through angling and managed through such actions of fish farming as movement of fish stocks between locations (Maitland, 1974; Wheeler, 1983; Maitland & Linsell, 2009).

All of these actions are likely to confound any trends shown through the monitoring of the presence and abundance these species (Phillips, 1980; Wenner, 1988). The requirement of extensive manpower (Hellawell, 1986; Knoben *et al*., 1995) and the difficulty with surveying the fish community in fast-flowing or deep aquatic habitats are also considered to be disadvantages (Hellawell, 1986). It has also been noted that freshwater fish are generally tolerant of increased salinities (James *et al*., 2003; Nielsen *et al*., 2003), up to a salinity of 10PSU according to Hart *et al*. (2003). Given that a high sensitivity to the particular stress is considered advantageous (Dale & Beyeler, 2001) the general tolerance of freshwater fish to increased salinities should also be viewed as a disadvantage.

2.7.1.2 Macrophytes

Macrophytes are widely used in the assessment of aquatic habitats as many species have well-defined ecological ranges (Schneider, 2007). Furthermore, macrophytes are categorised as a key biological element for the assessment of aquatic habitats by the WFD (European Commission, 2000). Many macrophyte-based indices have been developed for assessing the trophic status of aquatic habitats (e.g. Dawson *et al*., 1999; Holmes *et al*., 1999; Melzer, 1999; Schneider & Melzer, 2003; Haury *et al*., 2006). Aquatic macrophytes, however, are known to respond to a large number of environmental factors other than nutrient concentration, such as rate and variability of water flow (Soley *et al*., 2002; Lacoul & Freedman, 2006), alkalinity and substrate (Pentecost *et al*., 2009), oxygen-concentration (Barendregt & Bio, 2003), pollutants (Lacoul & Freedman, 2006), salinity (Barendregt & Bio, 2003; Lacoul & Freedman, 2006) and shading (Barendregt & Bio, 2003; Lacoul & Freedman, 2006; Pentecost *et al*., 2009). As such, macrophyte-based indices have been developed to assess several of these features (e.g. Soley *et al*., 2002; Willby *et al*., 2009; Palmer *et al*., 2010).

There are numerous advantages associated with using macrophytes in water quality assessments. Macrophytes are not mobile (Hynes, 1960; Hellawell, 1986; Knoben *et al*., 1995; Friedrich *et al*., 1996; Clarke & Wharton, 2001; Nurminen, 2003) and are visible to the naked eye (Friedrich *et al*., 1996; Hellawell, 1986).

Whilst many macrophyte species are easy to identify (Knoben *et al*., 1995; Friedrich *et al*., 1996), hybridisation or the lack of flowering or fruiting bodies required to make accurate determinations means certain species are difficult to identify (e.g. Lansdown, 2007, 2009). For these reasons, macrophyte surveys are considered easy (Hynes, 1960), rapid with little or no laboratory-based identification (Clarke & Wharton, 2001), and have low manpower demands (Hellawell, 1986). Macrophytes are relatively long-living (Carbiener *et al*., 1990) and as such can be used as long-term indicators (Melzer, 1999; Nurminen, 2003). In relation to salinity, many aquatic macrophytes have been reported to be halo-sensitive (Hart *et al*., 2003; Nielsen *et al*., 2003) and are reported to respond to increases in salinity at the community level (Hart *et al*., 1990; Wollheim & Lovvorn, 1995), exhibiting a reduction in species diversity as salinity increases (Hart *et al*., 2003). Furthermore, aquatic plants may exhibit adverse effects such as loss of vigour (Hart *et al*., 2003), reduced growth rates and reduced root and leaf development at salinities as low as 1PSU (Nielsen *et al*., 2003). Iliopoulou-Georgudaki *et al*. (2003) reported, however, that these effects are slow to become apparent.

There are further disadvantages to using aquatic macrophytes in the assessment of water quality. Whilst there are over 350 species of macrophytes associated with one or more of standing waters, canals, rivers and streams in the UK (Hill *et al*., 2004), it has been claimed that there are relatively few species within any one region (Clarke & Wharton, 2001). Many inland aquatic habitats have sparse macrophyte growth resulting from physical factors such as high water velocity or limited light attenuation (Clarke & Wharton, 2001). Macrophytes show substantial seasonal variations in biomass (Hellawell, 1986; Knoben *et al*., 1995) and community composition resulting from the organism type dying back during winter (Clarke & Wharton, 2001). Consequently, macrophytes are not readily visible for lengthy periods of time (Hellawell, 1986; Friedrich *et al*., 1996). Macrophyte communities can be influenced by channel management actions (Knoben *et al*., 1995) such as cutting (Hellawell, 1986; Clarke & Wharton, 2001) or herbicide applications (Hellawell, 1986). Furthermore, macrophyte communities show a slow response to changes in environmental conditions (Melzer, 1999; Iliopoulou-Georgudaki *et al*., 2003; Nurminen, 2003) and consequently are frequently tolerant of intermittent pollution events (Friedrich *et al*., 1996).

It has also been reported that macrophytes are incapable of exhibiting a graded response to varying degrees of environmental stress (Iliopoulou-Georgudaki *et al*., 2003).

2.7.1.3 Phytoplankton and periphyton

Phytoplankton has been designated as a key element in assessing the biological quality of aquatic habitats by the Water Framework Directive (WFD; European Commission, 2000). Certain phytoplankton groups have a long history of use in the biological monitoring of aquatic habitats, namely bacteria (Lin *et al.*, 1974; Hellawell, 1986; Lemke *et al.*, 1997; Skraber *et al*., 2004) and diatoms (Whitton & Kelly, 1995; Stevenson & Pan, 1999; Lavoie *et al*., 2008). The use of bacteria, however, is largely limited to assessing the sanitary quality of surface waters (Hellawell, 1986; Lemke *et al*. 1997; Fong & Lipp, 2005). In contrast, diatom species are known to integrate water quality variations at a given site (Feio *et al*. 2009), are sensitive to changes in many factors (Reid *et al*., 1995; Stevenson & Pan, 1999) and have well defined ecological tolerances (Reid *et al*., 1995). As a result, a large range of indices the make use of diatom data have been developed to assess water quality features such as organic pollution (Descy & Coste, 1991; Kelly & Whitton, 1995), acidification (Eloranta, 1990), pH (Renberg & Hellberg, 1982; Hakansson, 1993) and general pollution levels (Descy, 1979; Watanbe et al., 1986). Furthermore, changes in the wider algae community assemblages have also been linked with changes in water chemistry such as nitrogen, phosphorus and pH (Fore & Graffe, 2002).

There are a number of further reasons for using microorganisms in water quality assessments. There are a large number of species within this organism group. For example, John *et al*. (2011) describe over 2400 species of freshwater algae (not including diatoms) and state that many more are likely yet to be discovered, whilst Kelly *et al*. (2005) stated that over 2500 species of diatoms have been recorded from Britain and Ireland. Microorganisms are more abundant and much less mobile in comparison to fish (Hynes, 1960). Diatoms in particular can be found in almost all aquatic habitats (Stevenson & Pan, 1999; Feio *et al*. 2009) and are abundant in most (Reid *et al*., 1995). Diatoms and algae, however, have well-defined seasonal variations in abundance and high flows may scour and move algae from a location (Barbour *et al*., 1999).

Whilst quantitative sampling of algae and bacteria is difficult, this can be overcome by the use of artificial substrates (Hellawell, 1986; Knoben *et al*., 1995). Artificial substrates have several advantages, namely that they allow sampling of typically difficult sampling sites and habitat differences are negated through provision of a standard micro-habitat (Barbour *et al*., 1999). Consequently sampling variability is decreased through reduced micro-habitat irregularities and subjective sampling techniques are eliminated (Barbour *et al*., 1999). Using artificial substrates, however, are not without drawbacks such as requiring deploy and collection trips, and that they are prone to vandalism, natural damage or loss (Barbour *et al*., 1999). Artificial substrates also introduces an unnatural habitat and the collected samples are not truly representative of the natural community present (Friedrich *et al*., 1996) as the material of the substrate influences the composition of the community (Barbour *et al*., 1999). Furthermore, the orientation and exposure length of the artificial substrate also influences the periphyton community structure and composition (Barbour *et al*., 1999). Substrate type also is known to influence diatom community composition (Reid *et al*., 1995). Different diatoms communities are prevalent on different substrates such as, for example, macrophytes, rocks, silt and sand (Reid *et al*., 1995). Regardless of this particular issue, microorganisms in general are considered easy to sample (Hynes, 1960; Hellawell, 1986; Friedrich *et al*., 1996; Barbour *et al*., 1999; Resh, 2008) and have low manpower demands associated with sampling procedures (Hellawell, 1986; Barbour *et al*., 1999; Resh, 2008). In addition, sampling of microorganisms is inexpensive and causes minimal impact to resident biota (Barbour *et al*., 1999). Diatom samples in particular have relatively simple sampling and preparation methods (Feio *et al*. 2009) and the cost of sampling and analysing diatoms are relatively low when compared to other organisms (Stevenson & Pan, 1999). Furthermore, prepared diatom samples can be preserved indefinitely (Feio *et al*. 2009). There are further advantages associated with certain microorganism groups. As primary producers, algae are most directly affected by physical and chemical factors due to being primary producers (Barbour *et al*., 1999). Furthermore, algae communities are sensitive to some pollutants which only affect other organism groups at higher concentrations, such as herbicides (Rosenberg & Resh, 1993; Barbour *et al*., 1999).

Protozoa exhibits high sensitivity to pollutants resulting from their small stature and large relative surface area (Zhou *et al*., 2008). Furthermore, most Protozoan species have a global distribution and are not influenced by seasonal variations (Zhou *et al*., 2008).

Rapid responses to changes in environmental conditions are advantages of protozoa (Friedrich *et al*., 1996), bacteria (Hellawell, 1986; Friedrich *et al*., 1996; Lemke *et al.*, 1997), algae and diatoms (Knoben *et al*., 1995; Barbour *et al*., 1999; Stevenson & Pan, 1999; Lavoie *et al*., 2008) that result from the short generation time of these organism types (Hellawell, 1986; Reid *et al*., 1995; Barbour *et al*., 1999; Stevenson & Pan, 1999; Lavoie *et al*., 2008). This short generation time, however, means that microorganisms only reflect water quality for approximately 1-2 weeks prior to sample collection (Friedrich *et al*., 1996; Barbour *et al*., 1999) and that recovery from pollution events is rapid (Hellawell, 1986), both of which are considered to be disadvantages. There are also further disadvantages associated with using microorganisms in water quality assessments. Algae and diatoms may be sparse in heavily shaded streams due to their light-dependence (Knoben *et al*., 1995; Barbour *et al*., 1999). Whilst diatoms are relatively easy to identify based on morphological features (Stevenson & Pan, 1999) and have a number of taxonomic guides to assist identification (Reid *et al*., 1995; Feio *et al*. 2009), protozoa, bacteria and algae are all considered taxonomically difficult (Hellawell, 1986; Friedrich *et al*., 1996; Resh, 2008) and accurate identification to species, genus or family level requires specialist training (Hynes, 1960; Knoben *et al*., 1995). Analysis of algal and bacterial samples can be further complicated by the difficulty in distinguishing between living and dead cells (Hellawell, 1986; Knoben *et al*., 1995). Specialist equipment is needed for the preparation and analysis of samples of algae, bacteria and protozoa (Friedrich *et al*., 1996). With respect to bacteria, some species are known to be difficult or even impossible to culture (Lemke *et al.*, 1997). Furthermore, the culturing of bacterial samples results in a delay, up to several days, in obtaining results (Hellawell, 1986). The results of bacterial cultures are further complicated by not knowing if the cells which grow on the culture media were active when the sample was collected (Hellawell, 1986). A further disadvantage arises from the fact that the origin of bacterial and protozoan cells may not be from the sampling location and is unknown (Hellawell, 1986; Friedrich *et al*., 1996). With respect to salinity, the majority of freshwater micro-organism taxa do not appear to be tolerant of increasing salinity (Nielsen *et al*., 2003).

Diatoms, however, are known to be strongly influenced by salinity and concentrations of major ions (Poyapova & Charles, 2003). Whilst diatoms exhibit a graded response to pollution increases, they are less sensitive to these changes than macro-invertebrates (Iliopoulou-Georgudaki *et al*., 2003).

2.7.1.4 Macro-invertebrates

Macro-invertebrates are considered to be sensitive indicators of water quality (Larimore, 1974; Goetsch & Palmer, 1997; Clarke *et al.*, 2003) and their use as an indicator of water quality has long been recognised as effective (Williams *et al*., 1991), the validity of which has been repeatedly confirmed (Rutt *et al*., 1989). The use of macro-invertebrate assemblages is considered one of the best understood, most convenient, and most economical water quality monitoring systems (Olive *et al*., 1988). As such, macro-invertebrates have been designated a key biological element in the assessment of aquatic habitats by the WFD (European Commission, 2000). Macro-invertebrates are also widely used as indicators of river condition in North America (Azrina *et al*., 2006) and many other parts of the world (Kay *et al*., 2001; Li *et al*., 2010). Consequently there is more information on macro-invertebrates than many other biotic groups (Wright, 1994; Hering *et al*., 2003; Nielsen *et al*., 2003; Bonada *et al*., 2006). For example, the responses of many common species to different pollutant types have been established (Rosenberg & Resh, 1993; Bonada *et al*., 2006). Many indices have been developed to use macro-invertebrates (Chessman, 2003; Azrina *et al*., 2006) to assess many features of the aquatic habitat, such as acidification (Davy-Bowker, 2005), conservation value (Chadd & Extence, 2004), flow (Extence *et al*., 1999), organic pollution (Chesters, 1980; Lenat, 1988; Alvarez *et al*., 2001), pesticide pollution (Schäfer *et al*., 2011), salinity (Williams *et al*., 1999; Horrigan *et al*., 2005; Palmer *et al*., 2010; Schäfer *et al*., 2011) and sedimentation (Extence *et al*., 2011).

There are many reasons why macro-invertebrates are used in the biological assessment of water. Many macro-invertebrate species have an essentially sedentary nature (Hynes, 1960; Hellawell, 1986; Cuffney *et al*., 1993; Rosenberg & Resh, 1993; Wright, 1994; Chessman, 1995; Clarke *et al*., 2003; Bonada *et al*., 2006; De Pauw *et al*., 2006) with limited migration patterns (Barbour *et al*., 1999) and as such are reliable indicators of local conditions at the site of sampling (Plafkin *et al*., 1989; Knoben *et al*., 1995; Friedrich *et al*., 1996; Barbour *et al*., 1999; De Pauw *et al*., 2006). There are a large number of macro-invertebrate species (Rosenberg & Resh, 1993; Bonada *et al*., 2006); for example Davies & Edwards (2011) list over 3000 species of macro-invertebrates occurring in aquatic habitats in Britain. Macro-invertebrates frequently exhibit greater taxonomic variety than other organism types (Barbour *et al*., 1999; Lammert & Allan, 1999). They are also abundant (Hynes, 1960; Barbour *et al*., 1999; Hering *et al*., 2003; Iliopoulou-Georgudaki *et al*., 2003) and widespread (Wright, 1994; Clarke *et al*., 2003; Iliopoulou-Georgudaki *et al*., 2003; Bonada *et al*., 2006). Macro-invertebrates can be found in all river types (Barbour *et al*., 1999; Lammert & Allan, 1999; Hering *et al*., 2003) as well as aquatic environments ranging from thermal springs to salt lakes (Grandjean *et al*., 2003).

Macro-invertebrates are relatively long-living organisms (Wright, 1994; Knoben *et al*., 1995; Clarke *et al*., 2003; Bonada *et al*., 2006) that have life cycles that range from a few weeks to a few years in length (Cuffney *et al*., 1993; Chessman, 1995; De Pauw *et al*., 2006). Macro-invertebrates generally respond rapidly to environmental changes (Wright, 1994; Iliopoulou-Georgudaki *et al*., 2003). Whilst sensitive life stages of macro-invertebrate species will respond quickly to changes in environmental conditions, the overall community will respond more slowly (Barbour *et al*., 1999). Long-lived species can indicate the integrated effects of regular and intermittent discharges of pollutants that may vary in concentration over time (Cook, 1976; Milbrink, 1983; Hellawell, 1986; Olive *et al*., 1988; Cuffney *et al*., 1993; Rosenberg & Resh, 1993; Knoben *et al*., 1995; Friedrich *et al*., 1996; Barbour *et al*., 1999). Macro-invertebrates are also capable of exhibiting a graded response to changes in pollution load and type (Iliopoulou-Georgudaki *et al*., 2003) as communities are composed of many taxa among which there is a wide range of tolerances to different pollutants (Friedrich *et al*., 1996; Barbour *et al*., 1999; Li *et al*., 2010).

Further advantages of macro-invertebrates include the facts that they are an important food source for aquatic predators such as fish (Barbour *et al*., 1999; Grandjean *et al*., 2003), they are of convenient size for field examination, storage and transport (Chessman, 1995) and that macro-invertebrate assemblages respond to changes in water quality in a predictable fashion (Grandjean *et al*., 2003).

The taxonomy of many groups is well known (Rosenberg & Resh, 1993; Bonada *et al*., 2006) and good taxonomic keys to identification exist (Hellawell, 1986; Rosenberg & Resh, 1993; Friedrich *et al*., 1996). As a result, the identification of macro-invertebrate taxa is relatively simple (Barbour *et al*., 1999; Clarke *et al*., 2003; Hering *et al*., 2003) and many taxa can be identified to low taxonomic levels, i.e. genus or species, with ease (Barbour *et al*., 1999), even after preservation (Hynes, 1960). There are, however, certain groups which are taxonomically difficult (Hellawell, 1986; Rosenberg & Resh, 1993; Friedrich *et al*., 1996), e.g. the larvae of Chironomid flies and oligochaete worms (Hellawell, 1986; Rosenberg & Resh, 1993), that require great expertise to identify to species level (Basset *et al*., 2004).

Qualitative sampling of macro-invertebrates is easy (Hynes, 1960; Hellawell, 1986; Rosenberg & Resh, 1993; Friedrich *et al*., 1996; Barbour *et al*., 1999; Clarke *et al*., 2003; Iliopoulou-Georgudaki *et al*., 2003) and uses inexpensive equipment (Hellawell, 1986; Rosenberg & Resh, 1993; Friedrich *et al*., 1996; Barbour *et al*., 1999; Bonada *et al*., 2006). Furthermore, sampling requires few people and has only a slight detrimental effect on the biota (Barbour *et al*., 1999). Substrate type is important when sampling macro-invertebrates (Friedrich *et al*., 1996; De Pauw *et al*., 2006) and quantitative sampling can be difficult (Hellawell, 1986; Knoben *et al*., 1995; Friedrich *et al*., 1996; De Pauw *et al*., 2006) resulting from the irregular distribution within the substrate (Hellawell, 1986; Knoben *et al*., 1995; De Pauw *et al*., 2006). Consequently, quantitative sampling of macro-invertebrates may require a large number of samples to be collected to account for this irregular distribution (Basset *et al*., 2004).

There are further disadvantages to macro-invertebrates as well as issues with the identification of certain macro-invertebrate groups and quantitative sampling. Factors other than water quality, for example flow rate and substrate type, can influence macro-invertebrate abundance and distribution (Rosenberg & Resh, 1993; De Pauw *et al*., 2006).

Not all water quality stresses illicit a detectable response from macro-invertebrates (Rosenberg & Resh, 1993). Furthermore, some macro-invertebrate species exhibit drift behaviour (Rosenberg & Resh, 1993; Knoben *et al*., 1995; Friedrich *et al*., 1996) which in flowing habitats can carry specimens into areas in which they would not usually occur (Rosenberg & Resh, 1993). Macro-invertebrates and in particular the insect taxa present well-defined seasonal variations in abundance and distribution (Rosenberg & Resh, 1993; Knoben *et al*., 1995). Consequently, there may be difficulties with comparing samples collected in different seasons (Rosenberg & Resh, 1993) and knowledge of the life cycles of certain macro-invertebrates is required to interpret the absence of species from samples (Friedrich *et al*., 1996).

Many studies have shown that there is a wide variation in the salinity tolerances of different macro-invertebrate taxa (Lancaster & Scudder, 1987; Metzeling, 1993; Berezina, 2003; Greenwood & Wood, 2003; Kefford *et al*., 2004a, 2004b). The salinity tolerances of individual macro-invertebrate species have been observed to be similar regardless of geographic sampling location (Kefford *et al*., 2003; Dunlop *et al*., 2008). It has long been established that salinity can influence the presence, and thus distribution, of macro-invertebrate taxa within the aquatic habitat (Lancaster & Scudder, 1987; Bulger *et al*., 1993; Attrill *et al*., 1996; Iliopoulou-Georgudaki *et al*., 2003). For example, Macan (1977) indicated that salinity is an important environmental factor influencing the aquatic taxa of the order Gastropoda by separating species on the basis of those found only in brackish water and those found in freshwater habitats. Elliott & Mann (1979) reported that salinity was one of eleven environmental factors influencing the distribution of leeches, but also stated that it is most frequently the combination of all eleven environmental factors that ultimately determines each leech species' distribution. Savage (1989) showed that species of the Heteropteran family Corixidae with lentic habitat preferences are each distributed within defined ranges of electrical conductivity. Savage (1989) further stated that there is a succession of changes in the species present within the lake-dwelling Corixidae community as conductivity increases from 100µScm^{-1} to 30000µScm^{-1} (salinity equivalent is 0.05PSU to 18.56PSU). In coastal habitats Wolf *et al*. (2009) stated that salinity, along with tidal influence, determines the distribution of benthic macro-invertebrates.

As such, macro-invertebrates also respond to salinity increases at the wider community level (Muñoz & Prat, 1994; Greenwood & Wood, 2003; Piscart *et al*., 2005a; Velasco *et al*., 2006; Wolf *et al*., 2009). It has also been reported that macro-invertebrate community structure and salinity concentration are related even when considered over narrow ranges of salinity (Kefford *et al*., 2006a).

The ubiquitous nature, general ease and inexpensiveness of identification and sampling, as well as the temporally-integrating ability of the macro-invertebrate community and the rapid and graded response to increases in salinity, appears to make macro-invertebrates the most suitable candidates for use in the detection of increasing salt concentrations.

2.8 Approaches to Biological Monitoring

A variety of approaches have been developed use in the biological monitoring of aquatic habitats, such as the functional approach, biotic indices, multimetric indices and the multivariate approach (Bonada *et al*., 2006; Li *et al*., 2010). The nature and effectiveness of each of these approaches to biological monitoring are discussed in greater detail in Sections 2.8.1, 2.8.2, 2.8.3 and 2.8.4.

2.8.1 Functional Approach

The functional approach depends on morphological and behavioural traits rather taxonomic distinctions (Cummins *et al*., 2005). A trait is defined as an attribute that reflects an adaptation by a species to its environment (Menezes *et al*., 2010). Such traits are usually separated into two categories, namely ecological traits which are related to habitat preferences, such as pollution, pH and temperature tolerance, and biological traits which include behavioural and physiological characteristics among other traits (Menezes *et al*., 2010). The use of the functional approach in monitoring programs is a relatively recent development and may still be considered largely experimental (Dolédec *et al*., 1999; Charvet *et al*., 2000; Statzner *et al*., 2001; Gayraud *et al*., 2003; Vandewalle *et al*., 2010).

The functional approach has largely been used to examine and assess ecological integrity and ecosystem health (Castela *et al*., 2008; Young & Collier, 2009). A number of ecosystem processes have been suggested to meet this particular requirement, such as rates of nutrient uptake (Sabater *et al*., 2000; Hall & Tank, 2003), organic matter decomposition and ecosystem metabolism (Young & Collier, 2009), benthic microbial respiration (Niyogi *et al*., 2001; Hill *et al*., 2002), denitrification (Bernhardt *et al*., 2002; Udy *et al*., 2006), fine particulate organic matter export (Wallace *et al*., 1996), organic matter retention (Speaker *et al*., 1984; Quinn *et al*., 2007), and invertebrate production (Woodcock & Huryn, 2007).

One advantage of the functional approach is its capability to reduce a large amount of species-specific information into a small number of categories of attributes (Rader, 1997). Given that the functional approach is based on easily recognisable behavioural and morphological characteristics of the biotic group being utilised (Cummins *et al*., 2005), a further advantage of the functional approach is the greatly reduced taxonomic effort required for analysis compared to a taxonomic approach (Cummins *et al*., 2005). Furthermore, the functional approach can allow comparisons across different eco-regions as it does not rely on specific taxa (Statzner *et al*., 2001; Bonada *et al*., 2006; Vandewalle *et al*., 2010). It has also been suggested that the functional approach may have the ability to discriminate between low levels of impairment (Young & Collier, 2009). The disadvantage with the functional approach is describing traits on the same scale consistently due to the lack of information for many parts of the world (Bonada *et al*., 2006).

Cummins *et al*. (2005) stated that whilst the functional approach is rapid and appropriate to characterise ecosystem condition, the taxonomic approach is the most useful in the assessment of chemical contaminants. Despite this assessment, the functional approach has been integrated into indices (e.g. Schäfer *et al*., 2007, 2011; Extence *et al*., 2011) under the principle that certain environmental stressors only draw a response from specific traits and as such these traits can be used to determine that environmental stressor (Schäfer *et al*., 2007). Furthermore, given that ecological traits are one of the two major aspects of the functional approach that are directly related to habitat preference (Menezes *et al*., 2010), biotic indices may be considered products of the functional approach. The functional approach has also been combined with both multimetric indices and multivariate approaches (Li *et al*., 2010).

2.8.2 Biotic Indices

Biotic indices are utilised in the assessment of the biological integrity of ecosystems (Pinto *et al*., 2009), where biological integrity is defined as how closely the current biotic community composition mirrors one which would be present in the habitat in its natural state (Karr & Dudley, 1981). Biotic indices perform this task by reducing biotic community data into simple measurements which can be used to assess alterations in the biotic community in relation to environmental and physico-chemical parameters (Griffith *et al*., 2001).

Despite the advantages in utilising aquatic biota in the assessment of aquatic habitats, biotic indices are not without their restrictions. Geographical, seasonal and diurnal differences result in variation in the macro-invertebrate community that can influence the scores of biotic indices (e.g. Hellawell, 1978; Murphy, 1978; Chesters, 1980; Armitage *et al*., 1983; Washington, 1984; Leunda *et al*., 2009) and constitute the main arguments against the use of biotic indices (Rosenberg & Resh, 1993). Biotic indices can also be confounded by physical and chemical variables other than the factor being assessed. For example, the physical characteristics of a river system, such as width, depth, velocity, sediment load and particle size (Vannote *et al*., 1980), naturally alter from source to mouth and this results in continual changes in the community composition along the river (Vannote *et al*., 1980; Statzner & Higler, 1985; Montgomery, 1999) which can manifest in the scores resulting from the application of biotic indices. Furthermore, the introduction of such invasive species as the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and the shrimp *Dikerogammarus villosus* (Sowinsky, 1894) can also influence the native macro-invertebrate community composition (e.g. Nyström *et al*., 2001; Dick *et al*., 2002; Crawford *et al*., 2006) and thus biotic index scores (e.g. MacNeil & Briffa, 2009), as can alterations in a water body's adjacent land use (Paul & Meyer, 2001; Walsh *et al*., 2005).

Biotic indices have been developed to assess many features of aquatic habitats. For example, the Lotic-invertebrate Index for Flow Evaluation (LIFE) system was developed by Extence *et al*. (1999) to evaluate river flow rate. The Biological Monitoring Working Party (BMWP) system was devised for quick assessment of a location for organic pollution (Wright, 1994), whilst the Average Score Per taxon ($ASPT_{BMWP}$) derivation of the BMWP system is widely regarded as one of the most reliable indices for the detection of organic pollution (MacNeil *et al*., 2002).

The Ephemeroptera, Plecoptera and Trichoptera (EPT) index is widely used in the United States to assess the water quality at a location (Leland & Fend, 1998; Compin & Céréghino, 2003), whilst indices for the detection of sewage effluent and trace metals have been designed by Chessman & McEvoy (1998).

Biotic indices range in design from the simple to the complex. Some indices are calculated purely by the number of taxa present in a sample belonging to certain orders. An example of such an index is the EPT index (Wallace *et al*., 1996; Leland & Fend, 1998). Many indices, such as the $ASPT_{BMWP}$ system (MacNeil *et al*., 2002) and the Stream Invertebrate Grade Number – Average Level (SIGNAL) index (Chessman, 2003), have expanded on this basis by introducing a scoring system (Wallace *et al*., 1996). Such indices assign numerical values to individual taxa based on their ability to inhabit aquatic environments differing in water quality and then average the values for all the taxa present in a sample (Chessman & McEvoy, 1998). These types of indices are favoured as they accommodate variations in sample size (Chessman & McEvoy, 1998), sampling effort and duration (MacNeil *et al*., 2002). Furthermore, these indices can be continually reviewed and refined (Chessman & McEvoy, 1998). Some indices, such as the LIFE system (Extence *et al*., 1999), have further expanded on scoring systems to incorporate abundance data. The recognition of abundance is a requirement of the Water Framework Directive (WFD; European Commission, 2000) and it has been reported that abundance data is as informative as occurrence of species (Hynes, 1960). It has also been suggested the use of abundance data can improve the accuracy and precision of indices (Horrigan *et al*., 2005).

Whilst numerous indices have been produced for the assessment of many aquatic habitat parameters, relatively few indices have been proposed for the detection and determination of salinity increases in freshwater habitats. One such index is the chloride contamination index proposed by Williams *et al*. (1999). Due to the fact that Australia has a serious issue with salinisation of inland waters (Williams, 1987; Bunn & Davies, 1992; James *et al.*, 2003; Marshall & Bailey, 2004; Hassell *et al*., 2006; Dunlop *et al.*, 2008), much of the research into the ecological effects of salinity on freshwater communities has been undertaken in Australia. The production of the salinity index by Horrigan *et al*. (2005) and the Species At Risk salinity index (denoted by $SPEAR_{salinity}$) developed by Schäfer *et al*. (2011) are two results of this research.

The introduction of the WFD (European Commission, 2000) has led to increased interest in quantifying and monitoring the effects of salinity in Europe, two outcomes of which are the salinity index proposed by Wolf *et al*. (2009) and the ditch salinity index developed by Palmer *et al*. (2010).

2.8.3 Multimetric Indices

Multimetric indices combine several individual biological indices to assess and communicate the biological condition (Davis *et al*., 1996; Hughes *et al*., 1998; Barbour *et al*., 1999; Emery *et al*., 2003; Karr & Chu, 2006; Gabriels *et al*., 2010) of a water body (Blocksom, 2003). Individual metrics designed to be a measure of such features as behavioural traits, functional feeding guilds, pollution tolerance, taxonomic composition or richness, among others, may be integrated into a multimetric index (Blocksom, 2003). As such, multimetric indices have the capacity to consider multiple stressors (Hering *et al*., 2006).

The multimetric method was first developed to use fish for the assessment of stream quality by Karr (1981). Since this initial development, numerous additional multimetric indices have also been proposed (Plafkin *et al*., 1989; Hering *et al*., 2004). Multimetric methods are commonly and widely used in the assessment of the biological condition of water bodies in the USA (Davis *et al*., 1996; Hughes & Oberdorff, 1999) and the United States Environmental Protection Agency has since developed rapid biological assessment protocols specifically for use with multimetric indices (Plafkin *et al*., 1989). Multimetric indices have also been, or are being, developed for use in Australia (Boulton, 1999), several European countries (e.g. De Pauw & Vanhooren, 1983; Böhmer *et al*., 2004; Hering *et al.*, 2004) and are used on six continents in terrestrial, marine and freshwater environments (Karr & Chu, 2006). The use of multimetric indices has been recommended for the overall assessment of biological condition as they are considered to improve data interpretation and consequently reduce the judgement errors caused by the use of individual biological measures (Davis *et al*., 1996).

The flexibility of multimetric indices which results from adding or removing individual metrics or adjusting the integration system of the multimetric index is an important advantage (Gabriels *et al*., 2010). There are, however, disadvantages inherent with multimetric indices.

The major disadvantage is that whilst multimetric indices can distinguish between impacted sites and sites with no or minimal impact, multimetric indices do not determine the cause of the impact (Fore, 2003; Hering *et al*., 2006). The development of pressure-specific multimetric indices, however, has been suggested (Hering *et al*., 2006). Given that multimetric indices are composed from individual indices, the issues of geographical, seasonal and diurnal differences (e.g. Hellawell, 1978; Murphy, 1978; Chesters, 1980; Armitage *et al*., 1983; Washington, 1984; Leunda *et al*., 2009), the introduction of invasive species (e.g. Nyström *et al*., 2001; Dick *et al*., 2002; Crawford *et al*., 2006) and alterations in a water body's adjacent land use (Paul & Meyer, 2001; Walsh *et al*., 2005) can also manifest in the scores resulting from the application of multimetric indices. Furthermore, useful individual indices do not always integrate successfully into a multimetric index (Boulton, 1999).

2.8.4 Multivariate Approaches

Multivariate approaches use statistical analyses to predict the aquatic site-specific faunal composition which would be present at the site in the absence of any environmental stress (Wright, 2000; Bonada *et al*., 2006; Li *et al*., 2010), referred to as expected fauna (Norris & Hawkins, 2000; Li *et al*., 2010). Predictive models are constructed using reference sites in pristine condition that are assigned to groups based on similarities in faunal community composition and finally correlated with natural environmental variables (Bonada *et al*., 2006) such as altitude, slope and alkalinity (Poquet *et al*., 2009), distance from source substratum, river width and depth (Wright, 2000). Biological evaluations for the site are then undertaken by comparing the observed faunal composition with the expected faunal composition (Norris & Hawkins, 2000; Wright, 2000; Bonada *et al*., 2006). This method of using multivariate approaches has proven to be an effective tool in biological monitoring (Li *et al*., 2010). For example, the WFD (European Commission, 2000) requires Member States in Europe to assess ecological status for all water bodies as deviation from the reference condition for biological quality elements (see Section 2.6.1).

Many Member States undertake these assessments using predictive models based on the multivariate approach (Poquet *et al*., 2009), examples of which include the SWEdish invertebrate Prediction and Classification predictive models (SWEPAC; Johnson & Sandin, 2001), the PERLA predictive model (named after the Plecoptera genus) of the Czech Republic (Kokeš et al., 2006), the predictive model developed by Ferréol *et al*. (2008) for use in Luxembourg and the MEDiterranean Prediction And Classification System (MEDPACS; Poquet *et al*., 2009). Many of these multivariate approaches are based on the British River InVertebrate Prediction And Classification System (RIVPACS; Wright *et al*., 1984; Moss *et al*., 1987; Wright, 2000) and its Australian derivative AUStralian RIVer Assessment Scheme (AUSRIVAS; Simpson & Norris, 2000). Further examples of multivariate predictive models include the Canadian BEnthic Assessment SedimenT (BEAST; Reynoldson *et al*., 1995, Rosenberg *et al*., 2000) and Assessment by Nearest Neighbor Analysis (ANNA; Linke *et al*., 2005).

Generally, multivariate approaches predict faunal composition as taxon identity (Bonada *et al*., 2006), although a key difference between the RIVPACS model II and the RIVPACS model III was the addition in the latter model to predict $\log_{10}$ abundance categories (Wright, 2000). Nonetheless, the multivariate approach is typically used in conjunction with biotic indices or simple measures diversity such as taxon richness (Bonada *et al*., 2006). Furthermore, multivariate approaches are designed to act as measures of change in overall community composition resulting from environmental stresses and as tools to identify the stress causing the impairment in the community composition (Bonada *et al*., 2006). It has been stated, however, that the ability to identify the environmental stressor could be conferred to multivariate approaches if they are used in conjunction with suitable biotic indices (Wright, 2000). As such, it can be seen that the development of effective and suitable biotic indices is a pre-requisite for the multivariate approach to be used in the identification of environmental stressors.

2.9 Salinity Indices

The published salinity indices are essentially based on the fact that as salinity increases, halo-sensitive macro-invertebrate species decrease in abundance until they disappear whilst halo-tolerant species become increasingly abundant.

This reaction in the macro-invertebrate community to increases in salinity has been documented in many research papers (e.g. Lancaster & Scudder, 1987; Short *et al*., 1991; Gallardo-Mayenco, 1994; Muñoz & Prat, 1994; Wollheim & Lovvorn, 1995; Piscart *et al*., 2005a; Silberbush *et al*., 2005; Velasco *et al*., 2006).

Horrigan *et al*. (2005) utilised an artificial neural network in order to assess the sensitivity of macro-invertebrate taxa to salinity. Artificial neural networks, in this context, are non-linear mapping structures used to develop models that predict the response of a macro-invertebrate taxon to changes in water quality variables such as flow rate, pH or dissolved oxygen (Goethals *et al*., 2007). The network constructs a predictive model based on data containing the information required to establish the relationship (Goethals *et al*., 2007). In the case of Horrigan *et al*. (2005), the relationship for one predictive model was the probability of a macro-invertebrate taxon being present relative to the salinity concentration. The output of the predictive model was plotted against conductivity and the shape of the resulting graph, along with the mean conductivity of the habitats where a taxon was recorded, was used to assign taxa to one of three salinity sensitivity scores (Horrigan *et al*., 2005). A score of 10 was attributed to sensitive taxa, 5 to generally tolerant taxa whilst a score of 1 was ascribed to very tolerant taxa (Horrigan *et al*., 2005). The salinity index score for a sample was calculated using the following formula (Horrigan *et al*., 2005):

$$Salinity\ Index\ score = \frac{\sum(Xi\ \times SSSi)}{n}$$

where Σ = the sum of, Xi = 1 if taxon *i* present and 0 if absent, $SSSi$ = salinity sensitivity score of taxon i, and n = total number of taxa in the sample.

In simple terms, the index score is calculated by taking the average of the salinity sensitivity scores of all the taxa present in a sample, and as such the salinity index can theoretically vary between 1 for very high salinity sites and 10 for very low salinity sites.

Horrigan *et al.* (2005) applied the salinity index to samples taken where the water quality, except salinity, was good in order to determine whether it reflected changes in the macro-invertebrate communities. The results indicated that the salinity index values decreased as salinity increased (Horrigan *et al.*, 2005).

This was also the case when the salinity index was applied to samples taken where water quality was not good (Horrigan *et al*., 2005). These findings demonstrate the index could be used to detect sites with high salinity. It appears, however, that Horrigan *et al*. (2005) used the same data obtained from a Queensland (Australia) government agency to both test the salinity index and develop the salinity sensitivity scores employed by the index. This may have introduced a beneficial bias into the assessment of the salinity index.

The chloride contamination index was developed by Williams *et al*. (1999) in order to assess the salinity concentration of freshwater springs, acting as a proxy of assessing the groundwater itself. Freshwater springs in and around the Greater Toronto Area, Canada, were sampled twice in a single month for macro-invertebrates and monthly for a year to determine chloride concentrations (Williams *et al*., 1999). Due to the difficulty in identifying some taxonomic groups to species level and the desire to keep the index simple enough to be used by non-specialists, macro-invertebrates were identified to varying levels of taxonomic resolution (Williams *et al*., 1999). The relationship between the sampled invertebrates and chloride concentration was examined using Canonical Correspondence Analysis (CCA) (Williams *et al*., 1999), a multivariate statistical method which directly relates a set of taxa to a set of environmental factors (Ter Braak, 1986, 1987). Average Euclidean Distance and Ward Linkage, a type of cluster analysis (Unal *et al*., 2003), was then applied to the taxon scores obtained from the first CCA axis, which resulted in the identification of two groups of invertebrates which differed in their association with chloride concentrations (Williams *et al*., 1999). Each group was assigned a tolerance value according to their association with chloride concentrations (Williams *et al*., 1999). The taxa in the group associated with low chloride concentrations were attributed a score of 10, whilst the taxa in the group associated with high chloride concentrations scored 5 and no other scores were assigned (Williams *et al*., 1999). The final index score was calculated using the following formula:

$$Chloride\ Contamination\ Index\ score = \frac{\sum(Xi \times Ti)}{n}$$

where Σ = the sum of, Xi = 1 if taxon *i* present and 0 if absent, Ti = tolerance of taxon i, and n = total number of taxa in the sample.

Simply put, the chloride contamination index score for a sample is calculated by averaging the tolerance values of all the taxa present in the sample. As no other scores were assigned, the chloride contamination index can theoretically vary between 5 for high chloride locations and 10 for low chloride locations.

Williams *et al*. (1999) plotted the chloride contamination index scores calculated for macro-invertebrate samples collected during spring against the average chloride concentration of the sampled sites for the entire season in order to establish the significance of the relationship between the index scores and chloride concentration. The resulting graph revealed a strong relationship between the index scores and chloride concentration, indicating that the index could be used to discriminate between sites with high chloride concentrations and those with low chloride concentrations (Williams *et al*., 1999). This conclusion was validated by applying the chloride contamination index to three rural springs with low chloride levels and three urban springs with much higher chloride levels (Williams *et al*., 1999). The three rural springs demonstrated high index scores whilst the urban springs achieved lower index scores, indicating the index is successful in predicting chloride concentration levels (Williams *et al*., 1999).

The Water Framework Directive (WFD) requires good ecological status to be attained for all inland and coastal waters (European Commission, 2000), not just rivers and lakes. Grazing marsh ditch systems, though classified as artificial water bodies under the WFD, are important for biodiversity as they can accommodate rich communities of both fauna and flora (Buisson *et al*., 2008). In response to the requirements of the WFD, and with the general biological response of a decrease in biodiversity as salinity increases (Williams, 1999, 2001; Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003) to be accounted for, Palmer *et al*. (2010) developed a methodology for the detailed evaluation of grazing marsh ditch vegetation and invertebrate communities which included the ditch salinity index. Palmer *et al*. (2010) defined three scores, displayed in Table 2.5, and classified invertebrate taxa according to these definitions.

Table 2.5: Definitions developed by Palmer *et al*. (2010) to assign invertebrate taxa salinity index scores

Score	Definition
0	Freshwater species tolerant of only mildly brackish water. Species are not routinely found in brackish conditions or close to the coast.
1	Species tolerant of mildly brackish conditions. Found more often in brackish conditions than in completely fresh water, or near the coast more often than inland.
2	Species that are obligately dependent upon mild to moderately brackish conditions. Absent from completely fresh water except as strays from nearby brackish sites.

The final index score is calculated by simply adding all of the scores for the species present in a sample (Palmer *et al*., 2010). Whilst Palmer *et al*. (2010) stated this method produced a useful index for gauging the salinity of a site using the macro-invertebrate assemblage, no evidence was provided to indicate the extent of the accuracy of the ditch salinity index.

Schäfer *et al*. (2011) utilised the physiological trait-based species at risk (SPEAR) approach in favour of taxonomy-based indicators in order to make their index selective to salinity, terming the metric $SPEAR_{salinity}$. Data expressing macro-invertebrate taxa physiological sensitivity to salinity were utilised by Schäfer *et al*. (2011) to classify taxa in favour of macro-invertebrate records and contemporaneous salinity readings, as were used by Williams *et al*. (1999) and Horrigan *et al*. (2005). A salinity sensitivity trait database for 172 taxa at the family-level was developed by consulting with experts in aquatic ecology, a further database and 85 references in the literature about macro-invertebrate taxa traits (Schäfer *et al*., 2011). Macro-invertebrate families were classified as sensitive if their physiological salinity tolerance was lower than medium on an ordinal scale, or if the majority of the taxa within a family had a laboratory derived salinity tolerance of less than $26gL^{-1}$ (Schäfer *et al*., 2011). Taxa that were not deemed as sensitive to salinity were classified as tolerant (Schäfer *et al*., 2011). Laboratory derived salinity tolerances were determined by experimentally deriving the salinity at which 50% of a population of a taxon died after 72 hours of exposure (e.g. Kefford *et al*., 2003, 2006a; Dunlop *et al*., 2008).

Schäfer *et al*. (2011) used the following formula to calculate the final index score, termed %SPEAR:

$$\%SPEAR = \frac{\sum_i^n (x_i y_i)}{\sum_i^n x_i}$$

where Σ = the sum of, n = the total number of species in the sample, x_i = the logarithm of the abundance +1 of species i, and y = 1 for a sensitive taxon and 0 for all other taxa.

Thus the calculation of the final index score is simply the percent of the sensitive individuals of a community in a sample. Schäfer *et al*. (2011) used data collected from the Australian states of Victoria and South Australia to determine the accuracy of the index. The results indicated a linear relationship between %SPEAR and the common logarithm ($\log_{10}$) of electrical conductivity, resulting in a Pearson's product moment correlation of between 0.62 and 0.71 depending on the habitat type sampled (Schäfer *et al*., 2011). Schäfer *et al*. (2011) also determined that the index did not respond to water quality variables other than salinity and, as such, concluded that it is highly selective.

Wolf *et al.* (2009) developed an ordination technique to determine how the composition of the salinity tolerances and preferences of the benthic macro-invertebrate community changes in relation to salinity. Wolf *et al*. (2009) defined six biotic classes of macro-invertebrate salinity preferences/tolerances (Table 2.6), to which macro-invertebrate taxa were classified based on 137 references in the literature.

Table 2.6: Biotic classes of the Salinity Classification System of Wolf *et al*. (2009)

Biotic salinity preference/ tolerance class	Description
Limnic	Freshwater taxa; do not tolerate even low salinity
Limnic, tolerates salt	Freshwater taxa; tolerate salinity below $5gL^{-1}$
Euryhaline-limnic	Freshwater taxa; tolerate salinity up to $10gL^{-1}$ (even higher salinity for a short time)
Brackish water	Brackish water taxa; permanently living and reproducing in brackish waters, tolerate varying salinity between 0.5 and $30gL^{-1}$
Euryhaline marine	Marine taxa with a wide affinity for salinity, tolerates salinity between 0.5 and $35gL^{-1}$
Holeuryhaline	Taxa with a marine origin but tolerate the entire range of salinity from freshwater to seawater

Wolf *et al*. (2009) also developed a scoring system based on the combination of the five abiotic salinity classes in the Water Framework Directive (European Commission, 2000) and six classes of macro-invertebrate salinity preferences/tolerances (Table 2.7). The scores in the scoring system were apportioned according to the main focus of species distribution with respect to the salinity classes (Wolf *et al*., 2009).

Table 2.7: Scoring system employed by the Salinity Classification System of Wolf *et al*. (2009)

Salinity range (gL^{-1})	Biotic salinity preference/ tolerance class	WFD salinity classification, salinity range in brackets				
		(<0.5gL^{-1}) Freshwater	(0.5-<5gL^{-1}) Oligohaline	(5-<18gL^{-1}) Mesohaline	(18-<30gL^{-1}) Polyhaline	(30-<40gL^{-1}) Euhaline
<0.5	Limnic	10				
0-5	Limnic, tolerates salt	7	3			
0-10	Euryhaline-limnic	6	2	2		
0.5-<30	Brackish water		2	5	3	
0.5-35	Euryhaline marine		2	2	3	3
0-35	Holeuryhaline	2	2	2	2	2

The scoring system was used by Wolf *et al*. (2009) in conjunction with the following formula to calculate the percentage tolerance of the community to a particular salinity class:

$$\%\ tolerance\ of\ community\ in\ class\ j\ = \left(\frac{\sum\left(Score_{ij} \times AB_i\right)}{AB}\right) \times 10$$

where Σ = the sum of, $Score_{ij}$ = tolerance score of taxon i and salinity class j, AB_i = abundance of taxon i, and AB = total abundance of all taxa.

The application of the salinity classification system developed by Wolf *et al*. (2009) to data derived from two official surveys carried out in several brackish water systems in Germany resulted in the system detecting salinity gradients that were not detected by chemical assessment (Wolf *et al*., 2009). Salinity readings, however, were only taken at a depth of 0.5m below the surface (Wolf *et al*., 2009). Salinity can increase greatly with a small increase in depth due to halo-stratification (Dyer, 1973; Davidson *et al*., 1991).

Furthermore, periodic increases in salinity may not have been detected as salinity readings were only collected once (Wolf *et al*., 2009). As such, a more thorough examination by taking a series of chemical readings over a period of time and from a range of depths may well have revealed the salinity gradients that Wolf *et al*. (2009) concluded were detected by the salinity classification system.

2.9.1 Comparison of Salinity Indices

Each of the salinity indices presented in Section 2.9 have inherent advantages and disadvantages (Table 2.8) associated with their respective applications which are further discussed here.

Table 2.8: Advantages and disadvantages of five macro-invertebrate based salinity indices

Index	Advantages	Disadvantages
Horrigan *et al*. (2005)	**1.** Produces a single output for each sample data set **2.** Uses qualitative data	**1.** Uses presence/absence data **2.** Uses family-level identification **3.** Uses qualitative data
Williams *et al*. (1999)	**1.** Uses varying levels of taxonomic identification **2.** Uses qualitative data **3.** Produces a single output for each sample data set	**1.** Uses presence/absence data **2.** Uses qualitative data
Palmer *et al*. (2010)	**1.** Uses species-level identification **2.** Uses qualitative data **3.** Produces a single output for each sample data set	**1.** Uses presence/absence data **2.** Uses qualitative data
Wolf *et al*. (2009)	**1.** Uses abundance data **2.** Uses fully quantitative data **3.** Uses species-level identification	**1.** Produces five outputs for each sample data set **2.** Uses fully quantitative data
Schäfer *et al*. (2011)	**1.** Uses abundance data **2.** Uses fully quantitative data **3.** Produces a single output for each sample data set	**1.** Uses family-level identification **2.** Uses fully quantitative data

It is evident that Williams *et al*. (1999), Horrigan *et al*. (2005) and Palmer *et al*. (2010) all developed their respective indices to evaluate presence/absence data. As such, these indices all essentially operate by applying a scoring system to a list of taxa present at a location. Any change in the scores of the indices proposed by Williams *et al*. (1999), Horrigan *et al*. (2005) and Palmer *et al*. (2010) would have to be facilitated by the loss or gain of a macro-invertebrate taxon, whilst a rise in salinity that affects the abundance of a taxon without affecting its presence would not be detected. Furthermore, changes in the number of individuals of each taxon are considered to be more significant than changes in the lists of taxa present at a location (Hynes, 1960). For example, Extence *et al*. (1999) found that scores calculated by the LIFE system exhibited a stronger correlation with flow rate when relative abundance data were utilised in favour of presence/absence data. Thus, the use of abundance data allows the detection of the subtle changes in the macro-invertebrate community which precede the loss or gain of taxa (Extence, 2012). The recognition of abundance in water quality assessments using biological elements is also a requirement of the WFD (European Commission, 2000). Horrigan *et al*. (2005) acknowledged the benefit of abundance data by proposing its use in order to improve the accuracy and precision their index. The incorporation of abundance data may also improve the indices of Williams *et al*. (1999) and Palmer *et al*. (2010). In comparison to the indices proposed by Williams *et al*. (1999), Horrigan *et al*. (2005) and Palmer *et al*. (2010), abundance data were incorporated into the indices developed by Wolf *et al*. (2009) and Schäfer *et al*. (2011).

Due to the fact that both indices utilise abundance data, the salinity classification system of Wolf *et al*. (2009) and the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) do not depend on the loss or gain of taxa, but react to changes in the abundances of macro-invertebrate taxa. These indices, however, require fully quantitative sampling and sample analysis methods, the use of which are not without their criticisms. Fully quantitative sampling and sample analysis methods are both time consuming and labour intensive in comparison to qualitative and semi-quantitative sampling and sample analysis methods, such as those described in Murray-Bligh *et al*. (1997) and utilised by the Environment Agency (Chadd, 2011). Surber samplers, cylinder or box corers, and Ekman bottom grabs can all be deployed to obtain fully quantitative data (British Standards Institution, 2012).

These methodologies, however, may not completely incorporate the different local habitat types present at a sampling site (Chadd, 2010). For example, surber samplers, box samplers and cylinder samplers can only be utilised in flowing water conditions and therefore are not suitable for use in still or slowly-flowing water conditions (Chadd, 2010; British Standards Institution, 2012). Table 2.9 illustrates further issues related with a variety of macro-invertebrate sample collection methods that collect qualitative, semi-quantitative and fully quantitative data.

Table 2.9: Comparison of macro-invertebrate sample collection methods

Conditions	Hand net	Surber sampler	Box & cylinder samplers	Ekman-Birge grab	Ponar & van Veen grabs	Polyp grab	Air-lift sampler	Core and tube samplers	Colonisation sampler
Suitable in still water	✓	✗	✗	✓	✗	✗	✓	✓	✓
Suitable in flowing waters	✓	✓	✓	✓	✓	✓	✓	✓	✓
Suitable in shallow waters	✓	✓	✓	✓	✓	✓	✗	✓	✓
Suitable in deep waters	✓[A]	✓[B]	✓[B]	✓	✓	✓	✓	✓	✓
Suitable in soft substrate	✓	✓	✓	✓	✓	✓	✓	✓	✓
Suitable in hard substrate	✓	✓	✓	✓[C]	✓[C]	✓	✗	✓	✓
Suitable in boulders and bedrock	✓	✗	✗	✗	✗	✓	✗	✗	✓
Suitable in macrophytes	✓	✗	✓	✗	✗	✗	✗	✗	✓
Collect surface-dwelling inverts	✓	✗	✗	✗	✗	✗	✗	✗	✗
Types of data produced	QL, SQ	QL, SQ, QN	QL, SQ, QN	QL, SQ, QN	QL, SQ, QN	QL, SQ, QN	QL, SQ, QN	QL, SQ, QN	QL, SQ

[A] = limited to handle length, [B] = Limited to sampler height, [C] = where particle size is less than 16mm.

QL =Qualitative data, SQ = Semi-quantitative data, QN = Quantitative data.

Table 2.9 adapted from British Standards Institution (2012).

It can be seen that sampling by hand net is the only macro-invertebrate sample collection method that successfully collects surface-dwelling invertebrates, such as the water measurer *Hydrometra stagnorum* (Linnaeus, 1758) or Gyrinidae whirligig beetles (Table 2.9).

Sampling by hand net can also be applied in any habitat type and on any type of substrate (Table 2.9; British Standards Institution, 2012). In comparison, surface dwelling macro-invertebrates are not collected by any of the methods used to collect fully quantitative data (Table 2.9; British Standards Institution, 2012). Box and cylinder samplers are the only fully quantitative sampling methods that are suitable for use in macrophytes, whilst the polyp grab is the only one of these methods that can be used with a boulder substrate (Table 2.9; British Standards Institution, 2012). Furthermore, all of the fully quantitative sampling methods that make use of grabs may require a winch to collect samples (British Standards Institution, 2012).

The chloride contamination index proposed by Williams *et al.* (1999) does not identify to the species level, but instead uses varying levels of taxonomic resolution. This was due to the difficulty in identifying some taxonomic groups and the desire to keep the index simple enough to be used by non-specialists (Williams *et al*., 1999). In contrast, Horrigan *et al*. (2005) and Schäfer *et al*. (2011) both developed their respective indices to be used in conjunction with identification of macro-invertebrates made to family level. This was likely due to the data used by Horrigan *et al*. (2005) to generate the salinity sensitivity scores also being at family level identification, and this data could not be used to assign salinity sensitivity scores to genus or species levels of identification. This was also the case with Schäfer *et al*. (2011). Family level identification is both quicker and cheaper than identification to species (Armitage *et al*., 1990). Furthermore, Kefford (1998b) reported that similar results are obtained regardless of whether family level identification or species level identification is used, whilst Chessman *et al*. (2007) found that an index using genus level identification of invertebrates was slightly more sensitive than one which employed family level identification. Chessman *et al*. (2007) concluded that a tiered approach, where only the families with a wide variation in tolerance are identified with greater taxonomic resolution, would likely be the most cost and time effective solution.

Armitage *et al*. (1990) stated, however, that identification to species level produces the most detailed ecological data. For example, Extence *et al*. (1999) found that LIFE scores obtained from family level data were more weakly correlated with flow rate than scores obtained using species level data, whilst Chessman *et al*. (2007) stated that the use of greater resolution is justified for the detection of subtle impacts.

This indicates that the use of species level data results in the generation of the most accurate scores. Furthermore, it has also been documented that some families contain species that have different salinity tolerances (Dunlop *et al*., 2008). For example, *Notonecta obliqua* (Gallén in Thunberg, 1787) is found at salinities less than $1.6gL^{-1}$ (Savage, 1989), approximately 1.08PSU, whereas *Notonecta viridis* (Delcourt, 1909) has been recorded at salinities ranging from $4gL^{-1}$ to $23gL^{-1}$ (Greenwood & Wood, 2003), approximately 2.85-18.91PSU. Increased taxonomic resolution would resolve this potential source of error in an index. This was a proposal made by Horrigan *et al*. (2005) to improve the accuracy and precision of their index. The same proposal could also be applied to the chloride contamination index of Williams *et al*. (1999) and the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) to achieve the same improvements. Horrigan *et al*. (2005) noted that their salinity index appeared to be unreliable when calculated for samples with less than fifteen families present. Increased taxonomic resolution to species level may solve this issue simply by increasing the number of taxa used in the index calculation.

In comparison to the indices of Williams *et al*. (1999), Horrigan *et al*. (2005) and Schäfer *et al*. (2011), the salinity classification system proposed by Wolf *et al*. (2009) and the ditch salinity index developed by Palmer *et al*. (2010) both require identification to species level. The major criticism of the salinity classification system of Wolf *et al*. (2009) is that the result following the application of the system is not a single figure which can be directly related to the salinity of the sampling location. Instead the system produces a series of numbers which can be used to produce graphs that describe the salinity preference/tolerance of the macro-invertebrate community present at the site at the time of sampling (Wolf *et al*., 2009). It is this which reflects the salinity of the location. Furthermore, the salinity classification system requires several calculations to be performed for each sample. In contrast the indices of Williams *et al*. (1999), Horrigan *et al*. (2005), Palmer *et al*. (2010) and Schäfer *et al*. (2011) require only one calculation to be performed for each sample, thus making these indices easier and quicker to use than the salinity classification system of Wolf *et al*. (2009).

2.10 Rationale for a New Salinity Index

Increases in salinity can have serious detrimental effects on both the economy and the environment of the areas affected (see Section 2.5). Despite the existence of European legislation which requires the use of fauna to monitor salinity as well as other physico-chemical elements (European Commission, 2000), only the index of Palmer *et al*. (2010) was developed in the UK. Palmer *et al*. (2010), however, specify that the ditch salinity index is only for use in coastal and near-coastal flood plain grazing marsh drainage ditches. Furthermore, the sampling and analysis protocol required to use an index should be compatible for use with other existing biotic indices and surveys (Bonada *et al*., 2006). The Environment Agency employs a semi-quantitative sampling and sample analysis methodology (Murray-Bligh *et al*., 1997; Chadd, 2011) and as such meets the requirement of the Water Framework Directive for recognition of abundance in the assessment of water quality (European Commission, 2000). However, the indices developed by Williams *et al*. (1999), Horrigan *et al*. (2005) and Palmer *et al*. (2010) use qualitative data and as such fail to meet the aforementioned requirement of the WFD. Whilst the indices developed by Wolf *et al*. (2009) and Schäfer *et al*. (2011) use fully quantitative data and thus meet the WFD requirement for recognising abundance, neither of these indices are compatible with the sampling and sample analysis protocol currently employed by the Environment Agency due to the requirement for fully quantitative data. As such there is a lack of diagnostic indices for the detection and determination of salinity increases in freshwater habitats that are suitable for use, nor have been developed, in the UK.

Developing an index which incorporates a scoring system and the use of abundance data, whilst keeping it simple enough to be used by both specialists and non-specialists, would make the index much more sensitive and accessible (Hynes, 1960; Extence *et al*., 1999; MacNeil *et al*., 2002; Horrigan *et al*., 2005). The literature also indicates that use of mixed level identification where families with a wide variation in tolerance are identified with greater taxonomic resolution, as opposed to general family level identification, would also increase the sensitivity of the index (Extence *et al*., 1999; Horrigan *et al*., 2005; Chessman *et al*., 2007) whilst retaining a level of accessibility for both specialists and non-specialists (Williams *et al*., 1999).

Whilst it is recognised that the indices developed by Williams *et al*. (1999), Horrigan *et al*. (2005), Wolf *et al*. (2009) and Schäfer *et al*. (2011) may work in the UK, no known studies have yet been undertaken to confirm or refute this. Furthermore, the index of Williams *et al*. (1999) may be difficult to test due to the small number of taxa attributed scores in the study.

3 DEVELOPMENT OF THE SALINITY ASSOCIATION GROUP INDEX

Following the principles adopted in devising the Lotic-invertebrate Index for Flow Evaluation (LIFE) (Extence *et al*., 1999), Community Conservation Index (Chadd & Extence, 2004) and the Proportion of Sediment-sensitive Invertebrates metric (Extence *et al*., 2011), taxa (species, genera and families) of British benthic macro-invertebrates were classified into five groups, defined in Table 3.1, termed Salinity Association Groups (SAGs).

Table 3.1: Definitions of the Salinity Association Groups

Salinity Association Group (SAG)	Group definition*
I	Macro-invertebrate taxa which tolerate only salinities below 2.5gL^{-1}, approximately 1.73PSU. *Typically freshwater taxa; may be tolerant of slightly brackish conditions, or completely intolerant.*
II	Macro-invertebrate taxa which can tolerate salinities over 2.5gL^{-1} (1.73PSU) up to a salinity of 10gL^{-1} (7.63PSU). Taxa may be present at slightly higher salinities, but only in small numbers. *Freshwater taxa tolerant of mild brackish conditions.*
III	Macro-invertebrate taxa which are characterised by the largest abundance occurring in the salinity range 8-20gL^{-1} (5.99-16.22PSU). Taxa are tolerant of the salinity range 4-25gL^{-1} (2.85-20.73PSU), but may also be recorded at salinities greater, or less, than those specified in this range. *Characteristic brackish water taxa, tolerant of a wide range of salinity conditions from long term brackish to near freshwater.*
IV	Macro-invertebrate taxa which tolerate salinities below 20gL^{-1} (16.22PSU) down to 14gL^{-1} (14.99PSU). Taxa may be present at slightly lower salinities, but only in small numbers. *Long-term brackish taxa tolerant of lower salinities, i.e. transition zones.*
V	Macro-invertebrate taxa which tolerate only salinities greater than 20gL^{-1}, approximately 16.22PSU. *Full coastal seawater taxa rarely moving into nominally freshwater habitats.*

***Definitions using salinity concentrations are in regular font style, whilst the descriptive definitions of the groups are in italic font style.**

Whilst it is recognised that species level identification gives the most detailed ecological data (Armitage *et al*., 1990), a mixed level of identification was employed in order that the Salinity Association Group (SAG) index could be utilised by both specialists and non-specialists.

This approach is also considered to be the most cost and time effective with regards to the identification of macro-invertebrates for use in biotic indices (Chessman *et al*., 2007). Furthermore, this method has been successfully utilised by several published biotic indices (e.g. Extence *et al*., 1999, 2011) which are employed by the regulatory bodies in the UK. Classification of the taxa was accomplished by undertaking an extensive literature review to determine the association of taxa with salinity (see Appendix 1 for list of taxa assignments to Salinity Association Groups (SAGs), and Appendix 2 for justifications of assignments of macro-invertebrate taxa).

It has been stated that changes in the abundance of each taxon are more informative than changes in the lists of taxa present at a location (Hynes, 1960). Evidence of this is provided by Extence *et al*. (1999), who found that utilisation of abundance data with the LIFE system resulted in scores which exhibited a stronger correlation with flow rate than the scores obtained using presence/absence data. The use of abundance data was a proposal by Horrigan *et al*. (2005) to improve their salinity index, whilst the use abundance data was incorporated into the indices developed by Wolf *et al*. (2009) and Schäfer *et al*. (2011). Williams (1987) stated that the influence of salinity of the aquatic macro-invertebrate community may be so subtle as to only be evident by gradual changes in the abundances of the taxa present. Furthermore, recognition of abundance in the assessment of water quality is a requirement of the Water Framework Directive (European Commission, 2000). Thus, the use of abundance data was incorporated into the SAG index methodology in order to improve the accuracy and precision of the metric. The abundance categories integrated into the SAG index method (Table 3.2) are the same as those utilised as part of the operating procedure of the Environment Agency for analysing macro-invertebrate samples (Murray-Bligh *et al*., 1997).

A scoring matrix incorporating the Salinity Association Groups and the Environment Agency abundance categories was developed by assigning the arbitrary scores 1 to 20, termed Salinity Association Scores (SAS). The SAG index calculation is based upon the sum of the individual salinity association scores for each scoring taxon in a sample. A taxon score is determined by reference to the scoring matrix presented in Table 3.2.

Table 3.2: Scoring matrix for determining Salinity Association Scores

Salinity Association Group (SAG)	Abundance category (number of individuals)			
	A (1-9)	B (10-99)	C (100-999)	D/E (1000+)
I	4	3	2	1
II	5	6	7	8
III	9	10	11	12
IV	13	14	15	16
V	17	18	19	20

Thus, for a theoretical sample which contains 300 individuals of a taxon (abundance category C) that has been assigned to SAG III, based on the taxon's association with salinity as reported in the literature, a SAS of 11 would be awarded for that taxon. A taxon which has been assigned to SAG V and had nine individuals present in the sample (abundance category A) would be awarded a SAS of 17. To calculate the final SAG index score for a sample, the following formula is applied.

$$\text{SAG index} = \Sigma SAS/n$$

where ΣSAS = the sum of individual taxon salinity association scores present in the sample and n = the number of taxa used to calculate ΣSAS.

Hence, the final SAG index score for a sample is simply the arithmetic mean of all the scores obtained for the taxa present in the sample (see Appendix 3 for a worked example of the SAG index).

4 SURVEY SITES

Surveys were undertaken at locations in both Lincolnshire and Norfolk in order to collect environmental data, as well as macro-invertebrate community data over a range of salinity concentrations.

Within Lincolnshire two separate study locations were chosen; namely the South Forty Foot Drain and the South Holland Main Drain. The South Forty Foot Drain and South Holland Main Drain were selected as study locations as both water bodies are believed to be subject to increased salinity (Chadd, 2009). Furthermore, examination of macro-invertebrate data collected by the Environment Agency (data collected from February 1987 to May 2009) provided evidence of variable salinity in both drains. The data revealed that taxa indicative of brackish conditions, such as *Palaemontes varians* (Leach, 1837), *Gammarus zaddachi* Sexton, 1912, *Haliplus apicalis* C.G. Thomson, 1868 and *Enochrus bicolor* (Fabricius, 1792), have been recorded from both the South Forty Foot Drain and South Holland Main Drain.

The majority of the sites surveyed in Lincolnshire were large fen drains. In order to assess the suitability of the Salinity Association Group (SAG) index in a different habitat type, a total of eight sites located within the Norfolk Broads area were surveyed in order to obtain macro-invertebrate community data over a range of salinity concentrations. All of the survey sites were located on small, non-flowing drainage ditches. Much of the Norfolk Broads area is flat (Gilvear *et al*., 1997) and only slightly higher than sea level (Wheeler & Giller, 1982). The defining characteristic of the Broads area is an extensive river system inter-linked by a number of shallow lakes termed Broads (Wheeler & Giller, 1982; Matless, 1994). The river system is tidal up to 40-50km from the mouth of Breydon Water estuary (Baker & Howlett, 2010) as a result of very shallow gradients (Wheeler & Giller, 1982). For example, Birkett *et al*. (2002) stated the River Yare has a bed gradient of approximately 3cm per km. The Broads themselves are shallow lakes formed from the flooding of peat-diggings (Phillips, 1977; Wheeler & Giller, 1982; Matless, 1994) undertaken in the 12th and 13th centuries (Gilvear *et al*., 1997). All of the Broads are susceptible to flooding as they lie below the highest river levels (Baker & Howlett, 2010).

All of the surveyed water bodies would be classified as artificial or heavily modified water bodies by the Water Framework Directive (European Commission, 2000) and as such have the target of achieving good ecological potential. Sections 4.1 and 4.2 describe the specific study areas of both Lincolnshire and Norfolk and their survey sites in detail.

4.1 Study Areas: Lincolnshire

Figure 4.1 illustrates the survey sites of the South Forty Foot Drain and the South Holland Main Drain study locations. Selection of the survey sites for the South Forty Foot Drain and South Holland Main Drain was based on collecting macro-invertebrate community samples and environmental data over a range of salinity concentrations, spatial evenness along the length of the water bodies and safety during collection of macro-invertebrate community samples and environmental data.

Figure 4.1: Study locations and survey sites in Lincolnshire. Green dots represent the survey sites on the South Forty Foot Drain and red dots denote the survey sites of the South Holland Main Drain. (Figure 4.1 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Consideration was also given to rights of way for access and, consequently, the majority of the sites had road bridges in close proximity. Each of the sites within Lincolnshire were surveyed three times, once in spring, once in summer and again in autumn (see Appendix 4 for specific dates). Spring was defined as March April and May, summer as June, July and August, and autumn as September, October and November, following the definitions specified in Murray-Bligh *et al*. (1997) and utilised by the Environment Agency. Given that seasonal variation is a major criticism of biotic indices (see Section 2.8.2), surveys were undertaken in spring, summer and autumn in order that any seasonal differences in the macro-invertebrate fauna may be detected and any resulting effect upon the SAG index examined.

4.1.1 South Forty Foot Drain

The South Forty Foot Drain is located in the Lincolnshire Fens and is managed by Black Sluice Internal Drainage Board. The South Forty Foot Drain is 33.3km long and currently drains a total of 750km^2 of land (Faulkner, 2009). The water body starts near to Guthram Gowt (Ordnance Survey Grid Reference (OSGR): TF-17500-22500) and terminating in Boston (OSGR: TF-33700-42600). The first section of the drain was constructed in the 1630s (Taylor, 1999). This was later extended and revised in the late eighteenth century, resulting in the current course of the South Forty Foot Drain (Barnwell, 1998). Drainage water is pumped directly into the channel by 21 pumping stations, whilst Black Sluice pumping station pumps water out of the South Forty Foot Drain and into the tidal section of the River Witham, known as The Haven, at Boston (Faulkner, 2009).

The four survey sites on the South Forty Foot Drain are all currently, or have been, utilised as macro-invertebrate community sample collection points for routine monitoring of water quality by the Environment Agency. The survey sites were, in order of upstream to downstream, SF1 (Casswell's Bridge; OSGR: TF-16500-27500), SF2 (Donington Bridge; OSGR: TF-17300-35600), SF3 (Swineshead Bridge; OSGR: TF-21800-42900) and SF4 (Wyberton Chain Bridge; OSGR: TF-30400-43400) (Figure 4.2). Further detail for each individual survey site of the South Forty Foot Drain is available in Appendix 5.

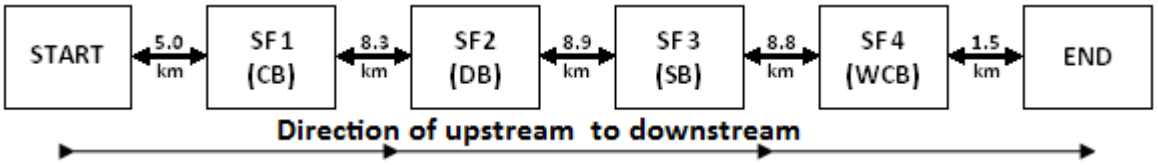


Figure 4.2: Diagrammatic sketch of the South Forty Foot Drain, Lincolnshire, illustrating the sequence of the survey sites. CB = Casswell's Bridge, DB = Donington Bridge, SB = Swineshead Bridge, and WCB = Wyberton Chain Bridge.

4.1.2 South Holland Main Drain

The South Holland Main Drain is located between the River Welland and the River Nene (Moriarty & French, 1975) and is managed by the South Holland Internal Drainage Board. The drain is 22.5 kilometres long, running from Cowbit (OSGR: TF-26500-17900) to Sutton Bridge (OSGR: TF-47500-21500) where it discharges into the tidal River Nene (South Holland Internal Drainage Board, 1984). In contrast to the South Forty Foot Drain, where water levels are maintained by a pumping station, the water level of the South Holland Main Drain is controlled by a tidal sluice gate, Nene Outfall Sluice, at the confluence with the River Nene. The South Holland Main Drain was constructed as a result of the South Holland Drainage Act 1793 and currently, with its subsidiaries, drains a total of 216km^2 of land (South Holland Internal Drainage Board, 1984).

The four survey sites on the South Holland Main Drain are all currently, or have been, utilised by the Environment Agency as survey sites for routine monitoring of water quality. In order of furthest upstream to furthest downstream, the survey sites located on the South Holland Main Drain were SH1 (Weston Fen; OSGR: TF-27600-15900), SH2 (Clifton's Bridge; OSGR: TF-38000-18900), SH3 (A1101 Road Bridge; OSGR: TF-44300-19800) and SH4 (Nene Outfall Sluice; OSGR: TF-47400-19900) (Figure 4.3).

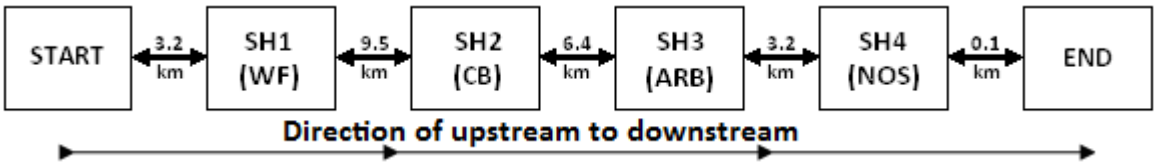


Figure 4.3: Diagrammatic sketch of the South Holland Main Drain, Lincolnshire, illustrating the sequence of the survey sites. WF = Weston Fen, CB = Clifton's Bridge, ARB = A1101 Road Bridge, and NOS = Nene Outfall Sluice.

Further detail for each individual survey site of the South Holland Main Drain is available in Appendix 5.

4.2 Study Area: Norfolk

The Norfolk Broads area is managed by the Broads Authority, a statutory body which was established following the 1988 Norfolk and Suffolk Broads Act (Norfolk Wildlife Services Ltd. & the Broads Authority, 2009). As well as rivers and Broads, the Broads Authority Executive Area is comprised of a wide range of habitats (Norfolk Wildlife Services Ltd. & the Broads Authority, 2009). Such habitats include grazing marsh and dykes originating from drainage and reclamation of estuarine land, estuarine and coastal habitat (Norfolk Wildlife Services Ltd. & the Broads Authority, 2009). Selection of the survey sites within Norfolk was based on collecting macro-invertebrate community samples and environmental data over a range of salinity concentrations and safety during collection of macro-invertebrate community samples and environmental data. Consideration was also given to rights of way for access. The sites located within Norfolk were each surveyed twice to obtain macro-invertebrate community samples and environmental data; once in spring and again in summer as defined by Murray-Bligh *et al*. (1997) (see Appendix 4 for specific dates).

The survey sites within Norfolk were separated into two broad geographic regions based on their locations within river catchments; the River Yare catchment and the Upper Thurne catchment (Figure 4.4).

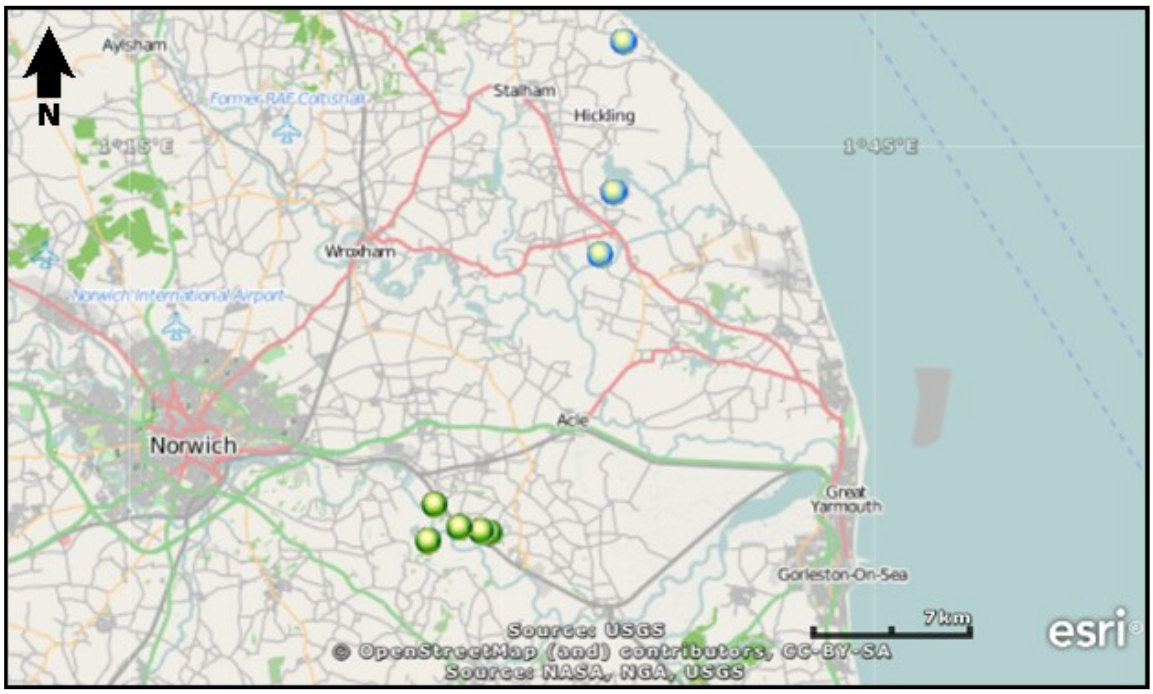


Figure 4.4: Study locations and survey sites in Norfolk. Survey sites are indicated by coloured dots, with green dots representing the survey sites of the River Yare and the blue dots indicating the survey sites of the Upper Thurne catchment. (Figure 4.4 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

4.2.1 Survey Sites of the Upper Thurne Catchment

The Upper Thurne catchment was located in the north-east of Norfolk (Drake, 2011). Examination of historical data has shown that electrical conductivity was one of the most important variables in explaining differences in the macro-invertebrate communities of this region through time (Drake, 2011). The survey sites within the Upper Thurne catchment are illustrated in Figure 4.5 and were LD (Long Dyke; OSGR: TG-41300-27700), MM (Middle Marsh; OSGR: TG-41300-20800) and LM (Ludham Marsh; TG-40900-17900). Whilst the LM (Ludham Marsh) survey site was not strictly within the boundaries of the Upper Thurne catchment, it was included here due to its proximity to the catchment. Further detail for each of the survey sites located within Norfolk is available in Appendix 6.

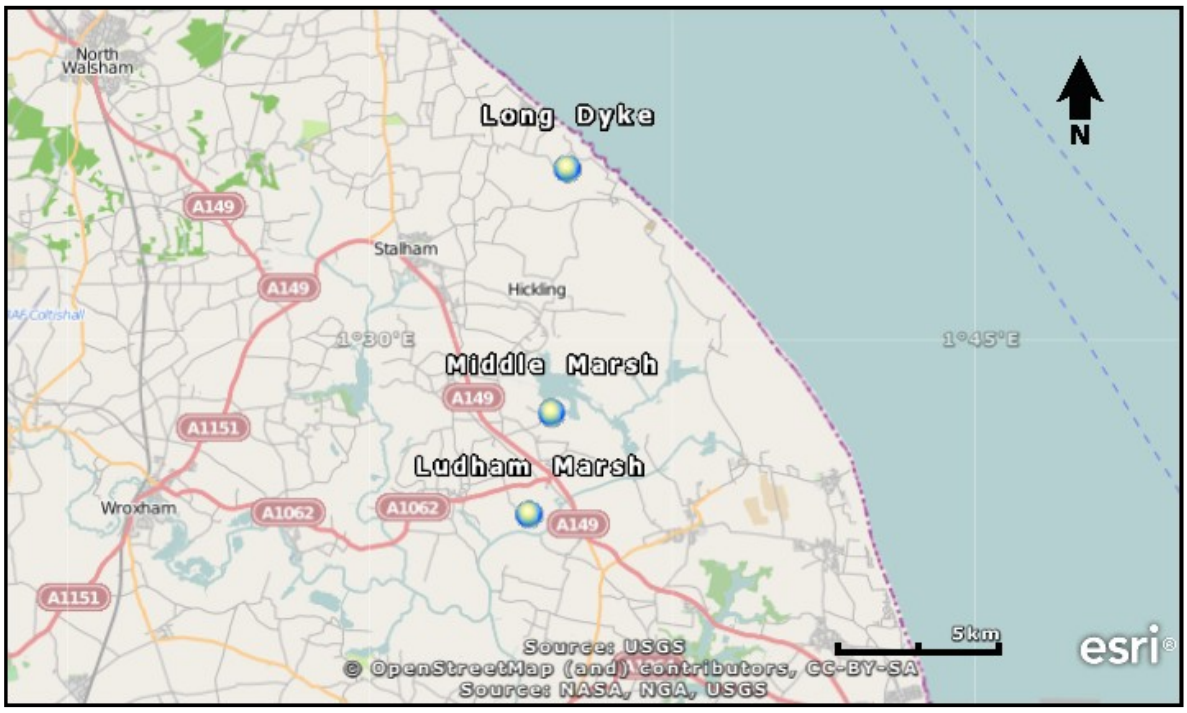


Figure 4.5: The survey sites of the Upper Thurne catchment. (Figure 4.5 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

4.2.2 Survey Sites of the River Yare Catchment

The River Yare catchment was located to the south-east of Norwich (Figure 4.4). The River Yare has been known to flood within this area, resulting in brackish waters intruding nearby ditches (Strudwick, 2011). As such, the area appeared ideal for investigating the impacts of salinity on macro-invertebrate ditch fauna. The survey sites located within the River Yare catchment were SM (Strumpshaw Meadow; OSGR: TG-33800-06700), BM (Buckenham Marsh; OSGR: TG-35300-05400), ND (Near Dry Dyke; TG-35900-05300), HM (Hatchet Marsh; TG-36500-05100) and RM (Rockland Marsh; OSGR: TG-33500-04900) and the locations of these survey sites are displayed in Figure 4.6. Further detail for each of the survey sites located within Norfolk is available in Appendix 6.

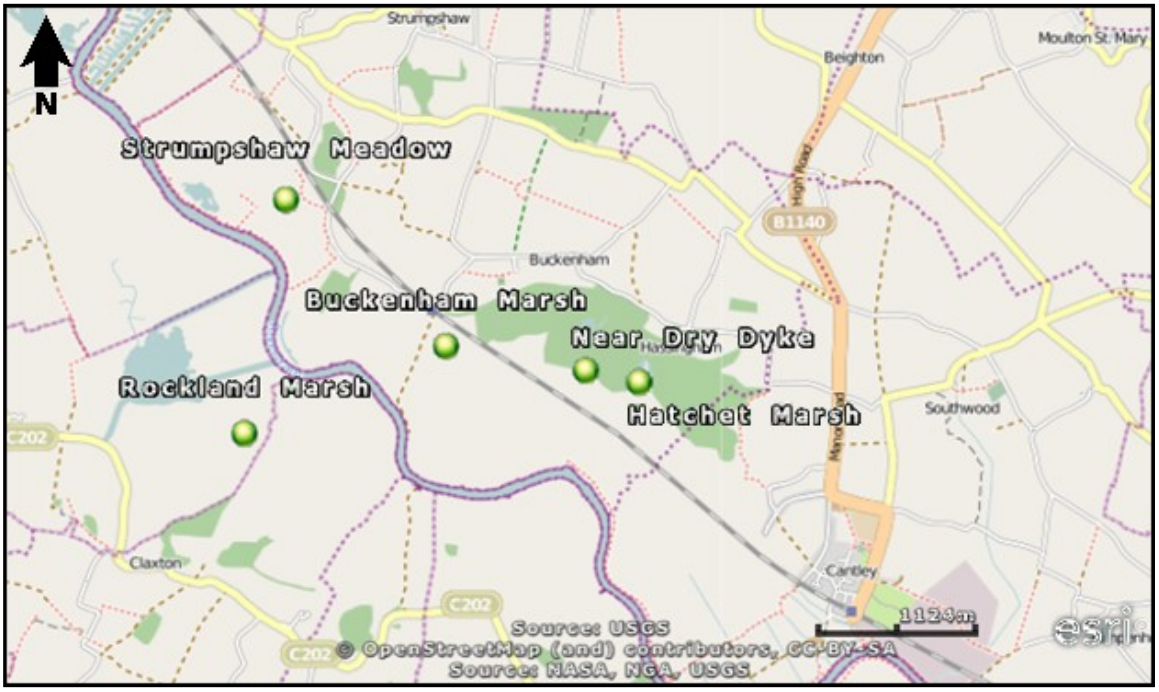


Figure 4.6: The survey sites of the River Yare Catchment. (Figure 4.6 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

5 METHODS

The methods employed in the collection and analysis of macroinvertebrate community samples, environmental data and sediment samples are detailed in the following sections. The techniques utilised to analyse the resulting data are also described. The coding system developed to label samples, and used hereinafter, is comprised of prefixes denoting the specific survey site (Table 5.1) and suffixes denoting the specific season during which the sample was collected (Table 5.2).

Table 5.1: Prefixes and meanings used in sample coding system

Prefix	Site Name	Location
SF1	Casswell's Bridge, South Forty Foot Drain	Lincolnshire
SF2	Donington Bridge, South Forty Foot Drain	
SF3	Swineshead Bridge, South Forty Foot Drain	
SF4	Wyberton Chain Bridge, South Forty Foot Drain	
SH1	Weston Fen, South Holland Main Drain	
SH2	Clifton's Bridge, South Holland Main Drain	
SH3	A1101 Road Bridge, South Holland Main Drain	
SH4	Nene Outfall Sluice, South Holland Main Drain	
HM	Hatchet Marsh	Norfolk
ND	Near Dry Dyke	
SM	Strumpshaw Meadow	
LM	Ludham Marsh	
LD	Long Dyke	
BM	Buckenham Marsh	
MM	Middle Marsh	
RM	Rockland Marsh	

Table 5.2: Suffixes and meanings used in sample coding system

Suffix	Season
SPR	Spring
SUM	Summer
AUT	Autumn

5.1 Sampling Procedure

Surveys of the selected sites consisted of collecting environmental data and macro-invertebrate community samples, as well as surveying the vegetation taxa present during each visit. Macro-invertebrate community sampling was undertaken after the collection of environmental data. This order was chosen to negate the possibility of the macro-invertebrate sampling procedure creating disturbances, which could result in the higher freshwater water layer and the lower salinity layer to mix, resulting in the introduction of error into the chemical data. It was recognised, however, that the collection of environmental data may cause macro-invertebrates to enter drift prior to sampling due to disturbances created by the collection of environmental data. To minimise this potential source of error, the collection of environmental data was performed slightly downstream of the areas to be sampled for macro-invertebrates. The survey sites of the South Forty Foot Drain and South Holland Main Drain were visited starting at the furthest downstream site and working upstream.

5.2 Environmental Data

Water body depth was measured at the centre of the channel using a length of cable marked at measured intervals and weighted at one end. Water body width was measured as the width of the water surface using a tape measure where possible, or otherwise estimated by counting the number of steps required to walk from one bank to the other and measuring the length of a step. The methods employed to measure watercourse width are the same as those detailed in Murray-Bligh *et al*. (1997), which are standard techniques employed by the Environment Agency.

There is not a standard depth from which to collect chemical data (see Section 2.7). As such, it was decided to collect chemical data at each survey site from the surface, mid-depth and base of the water column from the middle of the water bodies and the surface and base at one bank of the water bodies where feasible. These positions were determined following the measurement of the water body depth. Chemical data were only collected from the surface and base of the water column from the middle of the water bodies during the surveys of the sites located within Norfolk due to the lack of depth and width of these water bodies.

An YSI-556 multi-probe field meter, supplied by Van Walt Ltd., was used to measure electrical conductivity (accuracy: the greater of 0.001mScm^{-1} or ±0.5% of reading), redox potential (accuracy ±20 mV), dissolved oxygen (accuracy: the greater of ±0.2 mg/L or ±2% of reading) and water temperature (accuracy ±0.15°C) at the Lincolnshire survey sites in all survey seasons and the Norfolk survey sites in the spring surveys. A Hanna Instruments HI-98312 Dist6 Conductivity & TDS and temperature meter was used to determine conductivity (accuracy ±0.4 mS/cm) and water temperature (accuracy ±0.5°C) at the Norfolk survey sites in the summer surveys. Conductivity was later transformed into Practical Salinity Units (PSU) using the 6th-order polynomial equation described by Schemel (2001), which is based the equation given in Lewis (1980) and developed for a single temperature (25°C) and atmospheric pressure (760 mm):

$$Salinity\ (PSU) = 0.012 + \left(-0.2174\left(\frac{\left(\frac{\mathrm{EC}}{1000}\right)}{53.087}\right)^{0.5}\right) + \left(25.3283\left(\frac{\left(\frac{\mathrm{EC}}{1000}\right)}{53.087}\right)^{1}\right) + \left(13.7714\left(\frac{\left(\frac{\mathrm{EC}}{1000}\right)}{53.087}\right)^{1.5}\right) + \left(-6.4788\left(\frac{\left(\frac{\mathrm{EC}}{1000}\right)}{53.087}\right)^{2}\right) + \left(2.5842\left(\frac{\left(\frac{\mathrm{EC}}{1000}\right)}{53.087}\right)^{2.5}\right)$$

where EC = specific conductivity compensated to 25°C in µScm^{-1}.

Given that nitrates and phosphates can make significant contributions to conductivity (Panter *et al*., 2011), both nitrate and phosphate concentration were determined during the autumn surveys of the Lincolnshire sites and all surveys of the Norfolk sites. Phosphate and nitrate concentrations were determined using a Palintest Photometer 5000 (accuracy: ±0.5% transmittance) and water samples collected from the base at one bank of the survey sites. Photometers operate by measuring the intensity of colour at a predetermined wavelength when reagents are added to a sample solution to produce a coloured complex with a target chemical (Palintest instruction sheet PHOT.1). The intensity of colour produced is proportional to the concentration of the chemical being analysed (Palintest instruction sheet PHOT.1). Phosphate concentration was determined as PO_4, whilst nitrate was measured as N and converted to NO_3 by multiplying by 4.4 as stated in the Palintest instruction sheet PHOT.23.

5.3 Macro-invertebrate Community Samples

Macro-invertebrate community samples were collected in accordance with the procedure defined within the UK Technical Advisory Group methodology for macro-invertebrate sampling and analysis (Murray-Bligh *et al*., 1997). The same procedure is used by the Environment Agency to collect invertebrate data for use with the River Invertebrate Classification Tool (RICT) for classifying water bodies in accordance with the requirements of the WFD (WFD-UKTAG, 2008) and is compliant with the international standard BS EN ISO 10870:2012 (British Standards Institution, 2012), which has superseded the European standards EN 27828:1994 (European Committee for Standardization, 1994a) and EN 28265:1994 (European Committee for Standardization, 1994b). The procedure consisted of a three minute pond net kick/sweep sampling and a one minute manual search which was split into two parts; one undertaken prior to the kick/sweep sampling and the other performed after. The first part of the manual search was to seek and collect water surface-dwelling animals that would rapidly leave the sampling area or seek refuge if they are disturbed. The second part of the manual search was to seek and collect animals from habitats that were not sampled effectively by the kick/sweep sampling method. During the three minute pond net kick/sweep sampling, each invertebrate micro-habitat within the sampling area was sampled with effort in proportion to its cover. The aperture of the net used during the collection of macro-invertebrate community samples was 670cm² and the mesh size was 1mm. The net was cleared of vegetation, sediment and other material periodically during each three minute sampling run. This prevented blockages building up in the mesh of the net and thus maintaining the effectiveness of the sampling. Material removed from the net was retained as it constitutes part of the sample.

Once collected, samples were stored in a refrigerator at temperatures between 1 and 3°C until sorting and identification could be performed. Sorting was achieved by washing samples through a bank of sieves with mesh sizes 2mm, 1mm and 710µm to remove debris and plant matter. The material retained in each sieve was washed into a tray from which macro-invertebrates were picked using forceps. Specimens of the classes Gastropoda and Bivalvia were preserved in 70% ethanol, whilst all other macro-invertebrates were preserved in Kahle's solution.

Macro-invertebrates were identified generally to species or genus-level, with the exception of Diptera larvae and pupae which were identified to family-level. Identification of specimens was determined with reference to Janus (1982), Macan (1977), Ellis (1978), Reynoldson (1978), Elliott & Mann (1979), Lincoln (1979), Croft (1986), Fitter & Manuel (1986), Elliott *et al*. (1988), Friday (1988), Unwin (1988), Savage (1989), Wallace *et al*. (1990), Gledhill *et al*. (1993), Barnes (1994), Edington & Hildrew (1995), Miller (1995), Brooks & Lewington (1997) and Cham (2007, 2009).

5.3.1 Macro-invertebrate Diversity and Water Quality Measures

A variety of diversity indices and water quality measures were used to summarise macro-invertebrate community composition. Diversity indices are quantitative measures that provide more information about community composition than taxa richness by taking into account the relative abundances of the different taxa present (Okpiliya, 2012). Diversity indices generally quantify either taxon richness or the proportional representation of the taxa present (Derksen *et al.*, 1995), also known as evenness or equitability (Peet, 1975). One concept that is frequently considered is that diversity combines both taxon richness and evenness (Peet, 1974, 1975), and diversity indices that combine measures of both these features have also been developed (Peet, 1975; Derksen *et al*., 1995). Different features of the relative abundances between taxa are measured by different indices (Hill, 1973) as each index differs in their sensitivity to each aspect (Boyle *et al*., 1990). As a consequence, it has been recommended that different types of diversity indices are used together (Boyle *et al*., 1990). As such, the different aspects of diversity were measured using taxon richness (relative number of taxa), total number of individuals (abundance), Margalef's index (taxon richness), Simpson's index of dominance (dominance and evenness), Shannon index (taxon richness and evenness), Buzas & Gibson's evenness (also known as Sheldon's evenness) and Berger-Parker dominance index (dominance). In order to assess general water quality in biological terms, the Average Score Per Taxon ($ASPT_{BMWP}$) derivative of the Biological Monitoring Working Party (BMWP) scoring system (BMWP, 1978; Chesters, 1980; Hawkes, 1997) was also applied to the macro-invertebrate data.

Taxon richness is the simplest index of diversity (Pianka, 1966; Peet, 1974; Okpiliya, 2012). Species richness is one of the most widespread diversity indices (Gallardo *et al.*, 2011) and is still frequently used (Peet, 1974). Taxon richness, however, is not independent of sample size and larger samples can be expected to yield a greater number of taxa (Peet, 1974; Okpiliya, 2012). Furthermore, taxon richness is strongly affected by rare taxa (Hill, 1973) and does not account for differing abundances of taxa (Pianka, 1966). As a consequence, more complex diversity indices which account for relative abundance among taxa have been developed (Pianka, 1966), such as the commonly used Margalef's index, Shannon index and Simpson's index (Pullin, 2002), and are used in this study.

Margalef's index (Margalef, 1958) is a well known (Magurran, 2004; Okpiliya, 2012) index designed to simply measure taxon richness (Death & Winterbourn, 1995; Derksen *et al*., 1995; Hamer *et al*., 2003; Magurran, 2004; Okpiliya, 2012). The index is simple to interpret (Lexerød & Eid, 2006) as the Margalef index value increases as diversity in a sample increases (Smale *et al*., 2003). Margalef's richness index was calculated using the following formula:

$$\text{Margalef's richness index } (D_{Mg}) = \frac{(n-1)}{\ln A}$$

where n = taxon richness, and A = total number of individuals.

Whilst Margalef's richness index is designed to compensate for sample size (Magurran, 2004), the metric is sensitive to sampling size (Magurran, 2004). Nonetheless, Margalef's index can be used in tandem with diversity indices designed to be sensitive to changes in dominant taxa or evenness (Magurran, 2004), such as Simpson's index (Hill, 1973; Peet, 1974; Krebs, 1985; Death & Winterbourn, 1995). Furthermore, Margalef's index has remained in use in relatively recent years (e.g. Fisher & Petrini, 1990; Ogbeibu & Oribhabor 2002; Cheng, 2004; Azrina *et al*., 2006; Velasco *et al*., 2006; Teixeira *et al*., 2009; Ren *et al*., 2011).

Simpson's index (Simpson, 1949) is commonly used (Pullin, 2002) and has long been considered the principal diversity metric not from the subject field of communication (McDonald & Dimmick, 2003).

The index is sensitive to changes in abundant taxa (Peet, 1974; Hill, 1973; Magurran, 2004) resulting from the relative weighting given to rare species and abundant species by the index (Krebs, 1985; Magurran, 2004; Okpiliya, 2012). The addition of a rare species results in only a small difference in the Simpson's index value (Okpiliya, 2012). As such, Simpson's index has been described as an index of dominance (Hill, 1973; Krebs, 1985), although Death & Winterbourn (1995) described the index as a measure of evenness. Simpson's index was calculated using the following formula:

$$\text{Simpson's index } (1 - \text{D}) = 1 - \sum \left(\frac{A_i}{A}\right)^2$$

where A_i = the number of individuals in taxon i, and A = total number of individuals.

The original formulation of Simpson's index varied inversely with diversity, but subtracting the calculated index value from its maximum possible value of 1 (as in the formula above), as suggested by Greenberg (1956) and Berger & Parker (1970) avoids any difficulty in interpretation. Simpson's index is considered by Magurran (2004) to be among the most robust and meaningful diversity metrics, which may explain the prolonged use of Simpson's index (e.g. Terman, 1997; Kaiser *et al*., 1998; Cheng, 2004; Azrina *et al*., 2006; Velasco *et al*., 2006; Gómez-Anaya & Novelo-Gutiérrez, 2010; Naigaga *et al*., 2011).

The Shannon index (Shannon & Weaver, 1949) is a commonly used diversity measure (Pullin, 2002; McDonald & Dimmick, 2003; Smale *et al*. 2003; Spellerberg & Fedor, 2003; Magurran, 2004; Tataranni & Lardicci, 2010; Gallardo *et al*., 2011), despite being originally developed within the subject field of communication (McDonald & Dimmick, 2003; Smale *et al*. 2003; Spellerberg & Fedor, 2003; Okpiliya, 2012). The Shannon-Wiener index takes into account taxon richness and the evenness of the abundances of the taxa (Krebs, 1985; Magurran, 2004; Tataranni & Lardicci, 2010). The Shannon index was calculated using the following formula:

$$\text{Shannon index } (\text{H}_\text{i}) = -\sum \left(\left(\frac{A_i}{A}\right) \ln \left(\frac{A_i}{A}\right)\right)$$

where A_i = the number of individuals in taxon i, and A = total number of individuals.

Whilst sample size does not significantly influence the Shannon index (Johnson *et al*., 2007), error is introduced in the metric if not all taxa in a community are represented in a sample collected from that community (Peet, 1974). Furthermore, the size of this error increases as the proportion of the species missing from the sample increases (Magurran, 2004). Despite this issue, the Shannon index is regarded as one of the best measures of community diversity (Baker *et al*., 1987) and is still widely used (e.g. Clair & Paterson, 1976; Lancaster & Scudder, 1987; Naidu *et al*., 1990; Gallardo-Mayenco, 1994; Marmonier *et al*., 2000; Kirkman *et al*., 2004; Leponce *et al.*, 2004; Bonner *et al*., 2005; Richardson, 2006; Schouten *et al*., 2010; Tataranni & Lardicci, 2010; Gallardo *et al*., 2011).

Evenness has been defined as the ratio between the number of abundant taxa and total taxon richness (Alatalo, 1981; Smale *et al*., 2003). Evenness measures can be expected to show a response to a change in community composition even when there is no change in taxon richness (Johnston & Roberts, 2009). One such metric that is easy to both calculate and interpret is Buzas & Gibson's evenness (Webb & Leighton, 2011). Buzas & Gibson's evenness (Buzas & Gibson, 1969) quantifies the deviation from complete evenness between the abundances of all taxa in a sample (Peet, 1974; Buzas *et al*., 2007a) by using the Shannon index value and taxon richness of the sample (Webb & Leighton, 2011). Buzas & Gibson's evenness was calculated using the following formula:

$$\text{Buzas \& } Gibson'\text{s Evenness (E)} = \frac{e^{H_i}}{n}$$

where n = taxon richness, and H_i = Shannon index value.

Buzas & Gibson's evenness is designed as such that the calculated value should not change when the number of individuals of each taxon present is multiplied with a constant, which is a vital requirement for an evenness index according to Heip & Engels (1974). Buzas & Gibson's evenness is reported to not be sensitive to rare taxa in a sample (Kirkman *et al*., 2004). This particular diversity measure has been employed in studies by the likes of Naidu *et al*. (1990), Terman (1997), Leponce *et al*. (2004), Kirkman *et al*. (2004), Bonner *et al*. (2005), Richardson (2006), Buzas *et al*. (2007a, 2007b), Webb *et al*. (2009) and Amini Yekta *et al*. (2012).

The Berger-Parker index is a simple dominance metric (Berger & Parker, 1970; May, 1975; Death & Winterbourn, 1995; Lexerød & Eid, 2006) that measures the proportional abundance of the most abundant taxon (Colunga-Garcia *et al*., 1997; Smale *et al*., 2003; Magurran, 2004). The Berger-Parker index was calculated using the following formula:

$$\text{Berger} - \text{Parker dominance index (B)} = 1 - \left(\frac{A_{max}}{A}\right)$$

where A_{max} = number of individuals in the dominant taxon, and A = total number of individuals.

The Berger-Parker index is independent of taxon richness (Southwood, 1978; Lexerød & Eid, 2006) and is generally insensitive to sample size (Southwood, 1978). May (1975) concluded the Berger-Parker index is among the most acceptable diversity metrics as a result of the aforementioned advantages, the ecological significance and simplicity of the index. the Berger-Parker index has been used by the likes of Colunga-Garcia *et al*. (1997), Sures *et al*. (1999), Alyokhin & Sewell (2004), Wilsey *et al*. (2005) Wood *et al*. (2001) and Wood *et al*. (2005).

The Biological Monitoring Working Party (BMWP) score system (BMWP, 1978; Chesters, 1980; Hawkes, 1997) has formed the basis of the river invertebrate status classification system used by the relevant authorities in the UK since 1980 and, as such, has been extensively used for nationally reporting water quality in biological terms (Extence *et al*., 1986; Extence & Ferguson, 1989). The BMWP scoring system has also been published as a standard method by the International Organization of Standardization (ISO-BMWP, 1979). The number of BMWP-scoring families (shown in Table 5.3) present in a sample ($NTAXA_{BMWP}$) and the average score per taxon ($ASPT_{BMWP}$) present, which is calculated by summing all of the scores of all taxa present in a sample and dividing by $NTAXA_{BMWP}$ (Mason, 1991; Friedrich *et al*., 1996), is still currently used in the UK in the assessment of ecological status for the WFD (Clarke, 2009).

Whilst the BMWP score system and it's $ASPT_{BMWP}$ derivative were originally devised for the detection of organic pollution (Wright, 1994; MacNeil *et al*., 2002), these indices can also respond to inorganic pollutants and give an indication of water quality (Chadd, 2010).

The $ASPT_{BMWP}$ derivative, however, is considered to be less sensitive to sample size (Armitage *et al*., 1983; Mason, 1991; Demers & Reynolds, 2002), sampling effort and seasonal changes than BMWP (Hawkes, 1997), which is calculated by simply summing the scores of all the families present (Friedrich *et al*., 1996; Hawkes, 1997; Artemiadou & Lazaridou, 2005).

Table 5.3: Scores allocated to macro-invertebrate families in the Biological Monitoring Working Party score system

Score	Macro-invertebrate families
10	Siphlonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae Taeniopterygidae Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae, Aphelocheiridae, Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae
8	Astacidae, Lestidae, Agriidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae, Libellulidae, Philopotamidae, Psychomyiidae (Ecnomidae)
7	Caenidae, Nemouridae, Rhyacophilidae (Glossosomatidae), Polycentropodidae, Limnephilidae
6	Neritidae, Viviparidae, Ancylidae (Acroloxidae), Unionidae, Corophiidae, Gammaridae (Crangonyctidae), Platycnemididae, Coenagriidae, Hydroptilidae
5	Planariidae (Dugesiidae), Dendrocoelidae, Mesovelidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae, Haliplidae, Hygrobiidae, Dytiscidae (Noteridae), Gyrinidae, Hydrophilidae(Helophoridae, Hydraenidae), Clambidae, Scirtidae, Dryopidae, Elmidae, Hydropsychidae, Tipulidae (Pediciidae, Limoniidae), Simuliidae
4	Pisicolidae, Baetidae, Sialidae
3	Valvatidae, Hydrobiidae (Bithyniidae), Lymnaeidae, Physidae, Planorbidae, Sphaeriidae, Glossiphoniidae, Hiruadinidae, Erpobdellidae, Asellidae
2	Chironomidae
1	Oligochaeta

N.B. Families in brackets are new families that were previously contained in the preceding family in the list. New families originate from developments in taxonomy since the Biological Monitoring Working Party (BMWP) system was originally prepared.

Table 5.3 adapted from Friedrich *et al*. (1996).

The success of the BMWP scoring system at indicating water quality has led to it being adapted for use in Argentina (Capítulo *et al*., 2001), Brazil (Cota *et al*., 2003), Greece (Artemiadou & Lazaridou, 2005) and Thailand (Mustow, 2002), whilst a version adapted for the Iberian peninsula has become widely used in Spain (Zamora-Muñoz *et al*., 1995). Furthermore, BMWP or the $ASPT_{BMWP}$ derivative have been used in scientific studies to assess the effects of metal pollution (De Jonge *et al*., 2008) and drought (Attrill *et al*., 1996), as well as organic pollution (MacNeil *et al*., 2002) and general water quality (Beavan *et al*., 2001; Iliopoulou-Georgudaki *et al*., 2003; Azrina *et al*., 2006).

5.4 Statistical Analysis

Environmental data were tested for normality using the Shapiro-Wilk test as first described by Shapiro & Wilk (1965). This normality test has been used in many studies such as Gray *et al*. (1999), Echols *et al*. (2009), Kovalenko & Dibble (2011) and Sala *et al*. (2012), despite the fact that for small datasets the power of preliminary normality tests is low and for large datasets the tests for normality are sensitive to small deviations (Läärä, 2009). Furthermore, the central limit theory implies approximate normality for large datasets and many statistical techniques based on normality are also robust against violation of this assumption (Läärä, 2009). Where necessary, data were transformed by $Log_{10}(X+1)$, as has been used in many studies (e.g. Kefford, 1998a; Griffith *et al*., 2001; Marshall & Bailey, 2004; Azrina *et al*., 2006; Pinto *et al*., 2009; Maltchik *et al*., 2010), in order to improve the assumptions of normality and homogeneity of variance (Kefford, 1998a, Kefford *et al*., 2006b). Transformed data were back-transformed where required to display the original data (Lang & Murphy, 2012).

Scatter plots with a smoothing curve, computed using LOWESS (LOcally WEighted Scatterplot Smoothing) algorithm (Cleveland, 1979), added to show the general trend of the plotted data and the non-parametric Spearman's rank order correlation coefficient (r_s) was used to examine the environmental data for covariance. Where there were missing values, the paired value for the other environmental variable in the test was removed.

Principal Component Analysis (PCA) using a correlation matrix was used to investigate the environmental data in order to determine any differences between the samples based on the environmental variables measured at the time of sampling, a process that has also been used by Metzeling (1993), Marmonier *et al*. (2000), Piscart *et al*. (2005b), Chessman *et al*. (2007), Sousa *et al*. (2007) and Dunlop *et al*. (2008) among others. PCA is one of the most commonly used tools in the analysis of ecological data (Randerson, 1993; Peres-Neto *et al*., 2003). The purpose of PCA is to explain as much of the total variation in a dataset (Kleinbaum *et al*., 1988) by transforming the variables in the dataset to a new set of variables referred to as principal components (Chatfield & Collins, 1980; Fowler *et al*., 1998), each of which is a particular linear combination of the original variables (Chatfield & Collins, 1980; Everitt & Dunn, 1991). The first principal component is derived to explain the maximum amount of the variation in the original dataset (Chatfield & Collins, 1980; Fowler *et al*., 1998; Everitt & Dunn, 1991), the second principal component is derived to be as different as possible from the first and then to explain the maximum amount of the variation remaining in the original dataset and so on (Fowler *et al*., 1998). Environmental data, transformed where necessary, were standardised to *z*-scores prior to PCA using the following formula:

$$z = \frac{(x - \mu)}{\sigma}$$

where z = standardised score of value x, μ = mean of the population, and σ = standard deviation of the population.

Missing values were accommodated by iterative imputation, whereby an initial PCA run is performed which is used to compute regression values for the missing data and the procedure is iterated until convergence occurs (Ilin & Raiko, 2010).

Non-parametric Permutational Multivariate ANalysis Of VAriance (NPMANOVA; Anderson, 2001; McArdle & Anderson, 2001) was used to test the hypotheses generated by the PCA. NPMANOVA is a statistical technique based on a measure of distance or similarity that compares the distance or similarity within a group of observations of potentially non-independent variables, such as taxa in an assemblage, against those in different groups and tests for a significant difference (Anderson, 2001; McArdle & Anderson, 2001).

NPMANOVA allows comparisons between uneven sample sizes (Andrew *et al*., 2003) and has been used by, for example, Andrew *et al*. (2003), Commito *et al*. (2008), Peck *et al*. (2008), Carver *et al*. (2009) and Tataranni & Lardicci (2010). All tests using NPMANOVA employed the Bray-Curtis similarity measure (Bray & Curtis, 1957) and were run using 10000 permutations. Significant results were further explored by undertaking Kruskal-Wallis and Mann-Whitney *U* tests, as appropriate, in order to determine which of the environmental variables were contributing to the significant differences.

Cluster analysis using the Unweighted Pair-Group Method using arithmetic Averages (UPGMA) alogorthim and the Bray-Curtis similarity measure (Bray & Curtis, 1957), as employed by Kaiser *et al*. (1998), Kay *et al*. (2001), Carlsson (2006), Velasco *et al*. (2006), Akbulut *et al*. (2009), Gómez-Anaya & Novelo-Gutiérrez (2010) and Rawson *et al*. (2010), was initially undertaken to reveal similarity between samples (Kay *et al*., 2001; Carlsson, 2006; Akbulut *et al*., 2009) in terms of the macro-invertebrate taxa present (Lancaster & Scudder, 1986; Kay *et al*., 2001; Velasco *et al*., 2006; Rawson *et al*., 2010). Cluster analysis is a multivariate method for classifying data points into a set of groups (Chatfield & Collins, 1980; Everitt & Dunn, 1991; Ennos, 2007) based on their differences or similarities (Bridge, 1993) such that data points within a group are similar while those in different groups are dissimilar (Chatfield & Collins, 1980). Macro-invertebrate data were fourth root transformed in order to reduce the impact of highly abundant taxa (Attrill *et al*., 1996; Chessman, 2003) and this approach has been used by Kaiser *et al*. (1998), Hampel *et al*. (2009) and Rawson *et al*. (2010) among others. Cophenetic correlation coefficients (*CC*) are used to assess the quantity of distortion associated with dendrograms, with a perfect correlation (*CC* = 1.00) indicating no distortion on converting data into a dendrogram (Lancaster & Scudder, 1986). A cophenetic correlation of 0.80 is considered the lowest acceptable limit (Lancaster & Scudder, 1986).

The similarity of the macro-invertebrate communities between samples were further analysed using Non-metric MultiDimensional Scaling (NMDS) based on the Bray-Curtis similarity measure (Bray & Curtis, 1957), as employed by Kaiser *et al*. (1998), Brock *et al*. (2005), Joyce *et al*. (2005), Carlsson (2006), Sousa *et al*. (2007), Commito *et al*. (2008), Rawson *et al*. (2010) and Tataranni & Lardicci (2010).

Multidimensional scaling is a multivariate method for organising samples into a two-dimensional ordination in which the proximity of any two items is representative of their similarity (Chatfield & Collins, 1980; Everitt & Dunn, 1991; Everitt, 1993). Similar samples are organised close together in a multidimensional scaling plot whilst very dissimilar samples are placed far apart (Everitt & Dunn, 1991; Everitt, 1993). The plots produced by NMDS represent the rank order of similarities using the Bray-Curtis similarity measure and the accuracy of the plots is shown as a stress level (Joyce *et al*., 2005). Stress levels below 0.20 in NMDS provide a useful ordination (Joyce *et al*., 2005), whilst stress levels higher than 0.20 indicate an unreliable ordination (Commito *et al*., 2008). Macro-invertebrate data were fourth root transformed prior to analysis.

ANalysis Of SIMilarity (ANOSIM; Clarke & Green, 1988; Clarke, 1993) using the Bray-Curtis similarity measure (Bray & Curtis, 1957) and run using 10000 permutations was used on fourth root transformed macro-invertebrate data to test if clusters identified by the cluster analysis and NMDS were significantly different. ANOSIM is a non-parametric, multivariate statistical technique that uses a similarity measure to compare average similarities within groups of samples against the average similarities of all pairs between groups (Clarke & Warwick, 1994; Chapman & Underwood, 1999) in order to determine if there is a significant difference between groups (Brock *et al*., 2005; Akbulut *et al*., 2009). ANOSIM was also used to examine if the hypotheses generated by the PCA of the environmental data were reflected in the macroinvertebrate data. Examinations of macro-invertebrate data by ANOSIM has been undertaken by Kaiser *et al*. (1998), Moore *et al*. (2004), Brock *et al*. (2005), Sousa *et al*. (2007), Akbulut *et al*. (2009) and Rawson *et al*. (2010), among others. All tests using ANOSIM were carried out using the Bray-Curtis similarity measure (Bray & Curtis, 1957) and run using 10000 permutations on fourth root transformed macro-invertebrate data.

Canonical Correspondence Analysis (CCA; Ter Braak, 1986, 1987; Ter Braak & Verdonschot, 1995) was undertaken to examine the influences of the measured environmental variables on the macro-invertebrate assemblages at the survey sites. This method has been used for the same purpose by the likes of Parr & Mason (2003), Williams *et al*. (2003), Velasco *et al*. (2006), Akbulut *et al*. (2009), Gómez-Anaya & Novelo-Gutiérrez (2010), Maltchik *et al*. (2010) and Rawson *et al*. (2010) among others.

CCA is a multivariate direct gradient analysis (Ter Braak, 1986, 1987; Lelend & Fend, 1998; Griffith *et al*., 2001; Sato & Riddiford, 2008) wherein both biotic and environmental data are analysed (Ter Braak, 1986, 1987; Penczak *et al*., 2002) in order to detect patterns of variation in the biotic data that can be best explained by the measured environmental data (Ter Braak, 1986, 1987; Griffith *et al*., 2001; Sato & Riddiford, 2008). The algorithm used in CCA is based on reciprocal averaging (Lelend & Fend, 1998; Penczak *et al*., 2002) and results in an ordination diagram where sample scores are positioned at the weighted averages of the taxon scores present in the sample and taxon scores are positioned at the weighted averages of the sample scores in which the taxon is present (Lelend & Fend, 1998). Environmental variables are also represented in a CCA ordination diagram by vectors which point to the maximum variation of the represented variable (Ter Braak & Verdonschot, 1995; César *et al*., 2009). The length of the vectors is proportional to the importance of the represented environmental variable in the ordination diagram (Ter Braak & Verdonschot, 1995; César *et al*., 2009). $Log_{10}(X+1)$ transformed environmental data were used in the examination by CCA as the algorithms for CCA standardise the environmental data to a mean of zero and standard deviation of one to remove the effect of differences in measurement units among these variables (Ter Braak, 1986; Griffith *et al*., 2001). Macro-invertebrate data were fourth root transformed in order to reduce the impact of highly abundant taxa (Attrill *et al*., 1996; Chessman, 2003).

Linear regression models, constructed during the examination of the Salinity Association Group (SAG) index and other published salinity indices, were validated by examination of the residuals produced by the models for normality and homogeneity of variance, as recommended by Zuur *et al*. (2010). The residuals were tested for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965), whilst the Breusch-Pagan test (Breusch & Pagan, 1979) was used to examine the residuals for homogeneity of variance. The Breusch-Pagan test is a test for heterogeneity of variance (Breusch & Pagan, 1979). As such, *p*-values generated by the test that are greater than 0.05 show that the assumption of homogeneity of variance in the residuals is satisfied. The Breusch-Pagan test has been used by Pandit & Laband (2007), Pavelsky & Smith (2009), Paerl *et al*. (2010), Krumhansl & Scheibling (2011) and Murphy *et al*. (2011), among others, for the same purpose.

Furthermore, positive autocorrelation of the residuals was examined using the Durbin-Watson test (Durbin & Watson, 1950, 1951) as used by, for example, Verschuren *et al*. (2000), Hampton *et al*. (2008) and Murphy *et al*. (2011). An exact *p*-value for the Durbin-Watson test was calculated using Pan's algorithm (Farebrother, 1980, 1984).

Pearson product-moment correlation coefficient (*r*), Spearman's rank correlation coefficient (r_s) and partial correlations were used to further examine the relationship between various published salinity indices, the SAG index and salinity. Partial correlation is a technique wherein the relationship between two variables is examined whilst statistically controlling the influence of a third variable (Heino *et al*., 2005) and has been used by Thorp *et al*. (1979), Bell (1984), Rempel *et al*. (2000), Heino *et al*. (2005), McAbendroth *et al*. (2005) and Nislow & Lowe (2006) among others.

Correlation coefficients for the SAG index calculated using data of varying degrees of resolution and other published salinity indices were compared using Hotelling's t-test for correlated correlations, calculated by the following equation given in Field (2009):

$$t_{Difference} = (r_{xy} - r_{zy}) \sqrt{\frac{(n-3)(1+r_{xz})}{2(1-r_{xy}^2 - r_{xz}^2 - r_{zy}^2 + 2r_{xy}r_{xz}r_{zy})}}$$

where r_{xy} = the correlation coefficient for first index and salinity, r_{zy} = the correlation coefficient for second index and salinity, r_{xz} = the correlation coefficient for first index and second index and *n* = sample size. Degrees of freedom for the test are given by *n*-3.

ANalysis of COVAriance (ANCOVA) was used to examine the influence of season of the SAG index. ANCOVA is a statistical method that uses linear regression to evaluate the influence of a potentially confounding scale-level variable (Hawkins, 2009) and has been used by, for example, Gewurtz *et al*. (2000), Elliott (2003), Spooner & Vaughn (2006), Effenberger *et al*. (2008), Yule *et al*. (2009), Mormul *et al*. (2011). The assumption of homogeneity of variance for ANCOVA was examined using Levene's test for equality of error variances as used by, for example, Tiemann *et al*. (2004), Hargeby *et al*. (2005), Liess & Ohe (2005), Effenberger *et al*. (2008), and Beketov *et al*. (2009).

The ability of the SAG index to discriminate between the brackish water zones defined by the Water Framework Directive (European Commission, 2000) was statistically examined using an ANalysis Of VAriance (ANOVA). ANOVA is a powerful statistical technique in which groups of data are tested for a significant difference in the means of the groups (Elliott, 1977). Post-hoc pairwise comparisons using Tukey's Honestly Significant Difference (*HSD*) test (Kramer, 1956) were undertaken where a significant difference was found by ANOVA in order to determine which groups were significantly different (Hawkins, 2009). The combination of ANOVA with post-hoc pairwise comparisons undertaken by Tukey's HSD test has been employed by the likes of Arocena (2007), Muturi *et al*. (2008), Gardner & Royer (2010) and Waterkeyn *et al*. (2010), among others.

The Shaprio-Wilk test, PCA, NPMANOVA, Kruskal-Wallis test, Mann-Whitney *U* test, cluster analysis, NMDS, ANOSIM, CCA, Breusch-Pagan test, Durbin-Watson test, Pearson product-moment correlation coefficient (*r*), Spearman's rank correlation coefficient (r_s) and partial correlations were all performed using the statistical software package PAleontological STatistics (PAST) version 2.17 (Hammer *et al*., 2001). PAST has been used by, for example, Heino *et al*. (2005), Effenberger *et al*. (2008), Kasangaki *et al*. (2008) and Akbulut *et al*. (2009), among others. PCA, NMDS and CCA ordination diagrams were also produced using PAST version 2.17, as were scatterplots with LOWESS smoothing curves, cluster analysis and boxplot diagrams. Regression and trend graphs were produced in Microsoft® Excel® 2007, which was also used to calculate the correlation coefficient difference test described in Field (2009). ANCOVA, ANOVA and post-hoc Tukey's HSD analyses were undertaken using IBM® SPSS® Statistics version 21.

6 RESULTS

The environmental data were tested for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). The results, displayed in Table 6.1, showed that none of the environmental data were normally distributed.

Table 6.1: Shapiro-Wilk test results for untransformed environmental variables

Environmental variable	Number of observations	Shapiro-Wilk statistic	*p*-value
Water body width	40	0.85	<0.01
Water body depth	40	0.88	<0.01
Water temperature	40	0.93	<0.05
Salinity	40	0.75	<0.01
Dissolved oxygen	32	0.82	<0.01
Redox potential	32	0.91	<0.05
Phosphate	24	0.81	<0.01
Nitrate	24	0.76	<0.01

Transformation of environmental data by $Log_{10}(X+1)$ was performed to improve the assumptions of normality and homogeneity of variance (Kefford, 1998a, Kefford *et al*., 2006b) and resulted in salinity, dissolved oxygen and phosphate meeting the assumptions of normality according to the Shapiro-Wilk test (Table 6.2).

Table 6.2: Shapiro-Wilk test results for $Log_{10}(X+1)$ transformed environmental variables

Environmental variable	Number of observations	Shapiro-Wilk statistic	*p*-value
Water body width	40	0.88	<0.01
Water body depth	40	0.83	<0.01
Water temperature	40	0.92	<0.05
Salinity	40	0.95	0.10
Dissolved oxygen	32	0.98	0.64
Redox potential	32	0.72	<0.01
Phosphate	24	0.93	0.09
Nitrate	24	0.81	<0.01

6.1 Environmental Data

The environmental data collected from the survey sites in Lincolnshire are summarised in Table 6.3. The data presented are average figures for the measurements collected at the range of depths and positions at that site (see Section 5.2 for positions of measurements and Appendix 7 for raw data). Following investigations of the sites in Norfolk during which phosphate and nitrate were determined to be possible important environmental variables, phosphate and nitrate readings were additionally collected during the autumn 2011 survey.

Table 6.3: Environmental data for the survey sites in Lincolnshire

Season	Survey Site	Temperature (°C)	Salinity (PSU)	Dissolved oxygen (mgL^{-1})	Redox potential (mV)	Phosphate (mgL^{-1})	Nitrate (mgL^{-1})
Spring 2010	SF1 (Casswell's Bridge)	8.94	0.56	9.17	137.54	N/A	N/A
	SF2 (Donington Bridge)	9.24	0.86	12.12	139.76	N/A	N/A
	SF3 (Swineshead Bridge)	9.20	0.70	13.68	161.18	N/A	N/A
	SF4 (Wyberton Chain Bridge)	9.80	0.66	11.68	38.35	N/A	N/A
	SH1 (Weston Fen)	8.26	2.14	13.37	168.54	N/A	N/A
	SH2 (Clifton's Bridge)	10.10	3.10	10.12	111.86	N/A	N/A
	SH3 (A1101 Road Bridge)	9.96	3.81	13.95	185.04	N/A	N/A
	SH4 (Nene Outfall Sluice)	10.09	3.82	16.81	133.80	N/A	N/A
Summer 2010	SF1 (Casswell's Bridge)	21.21	0.69	9.29	52.12	N/A	N/A
	SF2 (Donington Bridge)	19.98	0.53	9.09	109.58	N/A	N/A
	SF3 (Swineshead Bridge)	20.50	4.45	9.48	94.80	N/A	N/A
	SF4 (Wyberton Chain Bridge)	21.27	4.84	10.72	121.58	N/A	N/A
	SH1 (Weston Fen)	19.33	1.13	5.84	12.64	N/A	N/A
	SH2 (Clifton's Bridge)	21.98	4.83	6.51	45.00	N/A	N/A
	SH3 (A1101 Road Bridge)	21.11	8.11	9.58	140.04	N/A	N/A
	SH4 (Nene Outfall Sluice)	24.14	6.17	10.70	158.10	N/A	N/A
Autumn 2011	SF1 (Casswell's Bridge)	11.90	0.50	8.53	58.52	0.00	0.48
	SF2 (Donington Bridge)	13.82	0.97	5.86	-6.98	1.23	0.44
	SF3 (Swineshead Bridge)	13.33	9.76	7.30	-54.92	2.92	0.00
	SF4 (Wyberton Chain Bridge)	13.65	10.90	9.59	8.94	3.35	1.23
	SH1 (Weston Fen)	10.85	0.45	9.84	37.46	0.00	0.84
	SH2 (Clifton's Bridge)	13.87	13.71	7.74	112.46	0.54	2.24
	SH3 (A1101 Road Bridge)	13.55	13.36	25.19	97.40	0.34	0.48
	SH4 (Nene Outfall Sluice)	13.00	9.27	6.28	98.75	0.86	1.41

SF = South Forty Foot Drain, SH = South Holland Main Drain, N/A = Not Available.

All figures in Table 6.3 are averages. See Appendix 7 for raw data.

The environmental data collected from the survey sites in Norfolk are summarised in Table 6.4. The data presented are the average figures for the measurements collected at the range of depths and positions at that site (see Section 5.2 for positions of measurements and Appendix 8 for raw data).

Table 6.4: Environmental data for the survey sites in Norfolk

Season	Survey Site	Temperature (°C)	Salinity (PSU)	Dissolved oxygen (mgL^{-1})	Redox potential (mV)	Phosphate (mgL^{-1})	Nitrate (mgL^{-1})
Spring 2011	BM (Buckenham Marsh)	16.95	0.33	31.70	-91.25	1.88	0.00
	LM (Ludham Marsh)	13.56	0.39	4.58	-144.90	1.00	0.00
	ND (Near Dry Dyke)	14.01	0.40	1.79	-219.90	0.34	0.00
	HM (Hatchet Marsh)	11.52	0.43	3.25	-165.05	0.00	0.00
	RM (Rockland Marsh)	16.10	0.46	5.71	-113.05	1.38	0.30
	SM (Strumpshaw Meadow)	16.43	1.63	3.77	-107.30	0.42	0.00
	MM (Middle Marsh)	13.82	3.40	4.50	-201.50	0.58	0.00
	LM (Long Dyke)	13.90	19.88	4.83	-26.20	0.00	0.00
Summer 2011	BM (Buckenham Marsh)	22.90	0.14	N/A	N/A	1.06	1.10
	LM (Ludham Marsh)	19.00	0.26	N/A	N/A	0.36	0.00
	ND (Near Dry Dyke)	18.60	0.20	N/A	N/A	0.44	0.00
	HM (Hatchet Marsh)	19.50	0.22	N/A	N/A	0.28	3.34
	RM (Rockland Marsh)	20.80	0.28	N/A	N/A	1.09	1.58
	SM (Strumpshaw Meadow)	20.70	0.98	N/A	N/A	0.44	1.63
	MM (Middle Marsh)	21.80	2.25	N/A	N/A	0.75	0.00
	LD (Long Dyke)	19.90	14.63	N/A	N/A	0.67	0.00

N/A = Not Available.

All figures in Table 6.4 are averages. See Appendix 8 for raw data.

The environmental data were initially inspected for covariance between variables by producing scatter plots. Significance of relationships between environmental variables were tested using the non-parametric Spearman's rank order correlation coefficient (r_s) as the majority of the environmental variables were not normally distributed, even after transformation (Table 6.1). Scatter plots, degrees of freedom (*df*) and r_s values are displayed in Figure 6.1, with scatter plots in the lower triangle of the matrix and both degrees of freedom (*df*) and r_s values in the upper triangle.

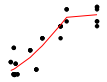
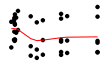
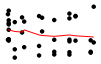
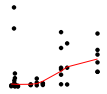
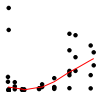
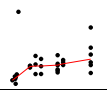
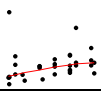
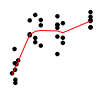
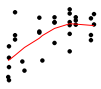
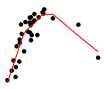
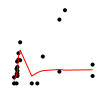
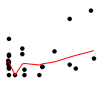
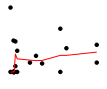
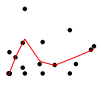
Water body width		0.84** *df*=38	-0.19 *df*=38	0.50** *df*=38	0.63** *df*=30	0.67** *df*=30	0.43* *df*=22	0.38 *df*=22
Water body depth			-0.16 *df*=38	0.52** *df*=38	0.46** *df*=30	0.55** *df*=30	0.11 *df*=22	0.58** *df*=22
Water temperature				-0.02 *df*=38	-0.30 *df*=30	-0.25 *df*=30	0.21 *df*=22	0.04 *df*=22
Salinity					0.15 *df*=30	0.34 *df*=30	0.03 *df*=22	-0.02 *df*=22
Dissolved oxygen						0.70** *df*=30	0.23 *df*=14	0.58* *df*=14
Redox potential							-0.06 *df*=14	0.85** *df*=14
Phosphate								0.04 *df*=22
Nitrate								
	Water body width	Water body depth	Water temperature	Salinity	Dissolved oxygen	Redox potential	Phosphate	Nitrate

Figure 6.1: Matrix of scatter plots and Spearman's rank order correlation coefficient (r_s) values. Significant correlations indicated by * when $p<0.05$ and ** when $p<0.01$.

The results displayed in Figure 6.1 shows that there is a high degree of covariance within the environmental data, as demonstrated by the significant correlations indicated. For example, salinity is significantly correlated with both water body depth (r_s=0.52, *df*=38, $p<0.01$) and water body width (r_s=0.50, *df*=38, $p<0.01$).

The environmental data were investigated by undertaking a Principal Component Analysis (PCA) in order to determine any differences between the samples based on the environmental variables measured at the time of sampling. It has been stated that principal components with eigenvalues of 1 or greater are considered to be significant (Ho, 2006), although it has also been suggested that only principal components with eigenvalues less than 0.7 should be excluded from subsequent analyses (Joliffe, 1972). Application of PCA to the environmental data resulted in only two components that had eigenvalues greater than 0.7.

These components explained 79% of the variance, with component 1 accounting for 49.54% and component 2 accounting for 29.95% of the total variance within the correlation matrix (Table 6.5).

Table 6.5: Principal components, eigenvalues and percent variance explained from the principal component analysis of environmental variables

Principal component	Eigenvalue	Variance explained (%)
1	3.96	49.54
2	2.40	29.95
3	0.60	7.53
4	0.41	5.07
5	0.37	4.61
6	0.12	1.54
7	0.08	1.02
8	0.06	0.75

Plotting of the sample scores from components 1 and 2 (Figure 6.2) did not separate the samples collected from the survey sites located on the South Forty Foot Drain from those collected from the survey sites located on the South Holland Main Drain (samples labelled with SF*x*- and SH*x*- prefixes respectively in Figure 6.2), but did result in the separation of the samples collected at the Lincolnshire survey sites from the samples collected at the Norfolk survey sites along component 1 (Lincolnshire samples indicated by blue asterisks and Norfolk samples indicated by purple circles in Figure 6.2). Furthermore, Figure 6.2 also indicates a separation of the samples based on season during which the data were collected along component 2 (samples collected in spring season, summer season and autumn season indicated by -SPR, -SUM and AUT suffixes respectively in Figure 6.2).

Correlations of scores for component 1 and component 2 with the environmental variables (Table 6.6) shows that water body width, redox potential, water body depth, dissolved oxygen and salinity contributed to the differences found in component 1, whilst water temperature, phosphate and nitrate contributed to the differences found in component 2.

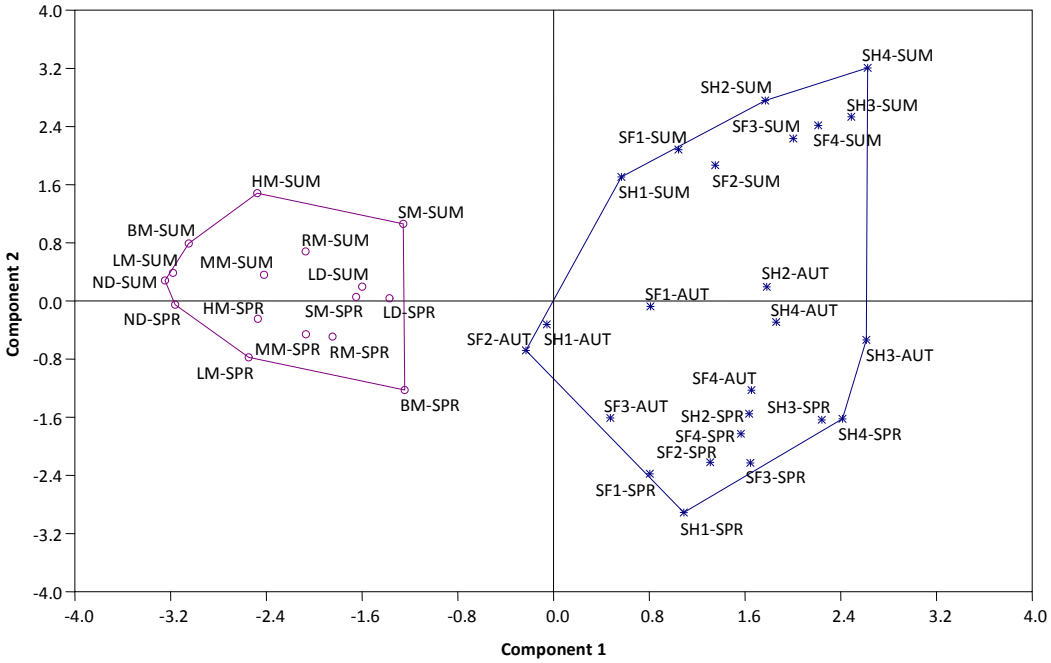


Figure 6.2: Ordination plot resulting from principal component analysis of environmental variables. Samples collected at survey sites in Lincolnshire, indicated by blue asterisks and enclosed blue polygon, and samples collected at survey sites in Norfolk, indicated by purple circles and enclosed by purple polygon. Component 1 explained 49.54% variance and component 2 explained 29.95% variance. See Table 5.1 and Table 5.2 for sample code definitions.

Table 6.6: Correlations of scores for component 1 and component 2 resulting from principal component analysis with the environmental variables

Environmental variable	Component 1	Component 2
Water body width	0.95	-0.04
Redox potential	0.95	-0.03
Water body depth	0.91	0.03
Dissolved oxygen	0.80	-0.25
Salinity	0.70	0.03
Nitrate	0.41	0.87
Water temperature	-0.20	0.89
Phosphate	-0.04	-0.88

As such, the results from the PCA suggest that the difference between the samples collected at the Lincolnshire survey sites and those collected from the Norfolk survey sites, as illustrated in Figure 6.2, results from either one, or a combination, of the physical structure of the sites (i.e. water body width and depth) or water quality (redox potential, dissolved oxygen, salinity). Furthermore, the results from the PCA suggest that the differences between the samples collected in spring, summer and autumn samples results from either one, or a combination of water temperature, nitrate or phosphate.

A two-way Non-parametric Permutational Multivariate ANalysis Of VAriance (NPMANOVA; Anderson, 2001; McArdle & Anderson, 2001) was used to test the hypotheses indicated by the PCA that there is not a significant difference in the environmental variables between the samples collected at the survey sites of the South Forty Foot Drain and those of the South Holland Main Drain and that there is a significant difference between the seasons in which the samples were collected. A non-parametric test was used as the majority of the environmental variables were not normally distributed after transformation (see Table 6.2). Environmental variables with missing data values (phosphate and nitrate) were omitted from the analysis. A one-way NPMANOVA was employed to test the hypothesis that there is a significant difference in the environmental data between the samples collected in spring season and those collected in the summer season at the Norfolk sites. Variables with missing data values (dissolved oxygen and redox potential) were omitted from the analysis. These data were tested separately from the Lincolnshire data as the PCA also indicated a difference between the environmental data for the samples collected at the Lincolnshire sites and those collected at the Norfolk sites, as well as the fact that no data were collected at the Norfolk sites for the autumn season. The hypothesis generated by the PCA, that the Norfolk samples are different from the Lincolnshire samples, was also tested by a one-way NPMANOVA. Variables with missing data values (dissolved oxygen, redox potential, phosphate and nitrate) were omitted from the analysis, as was the data collected from the survey sites in Lincolnshire during autumn due to the lack of comparable data for the survey sites in Norfolk in the same season.

The results of the two-way NPMANOVA showed that there is not a significant difference in the environmental variables between the samples collected at the survey sites of the South Forty Foot Drain and those of the South Holland Main Drain (NPMANOVA, F=2.18, p=0.15) and that there is a significant difference in the environmental variables between the seasons in which the samples were collected (NPMANOVA, F=2.50, p<0.05). Furthermore, a significant difference in the environmental variables between spring and summer seasons was also found when data collected at the Norfolk sites were examined (NPMANOVA, F=2.85, p<0.05). These results were further explored by undertaking Kruskal-Wallis and Mann-Whitney U tests, as appropriate, in order to determine which of the environmental variables were contributing to the significant differences between seasons. Non-parametric tests were used as the majority of the environmental variables were found to not be normally distributed after $\log_{10}$ transformation (Table 6.2). Variables with missing data values were omitted from the analyses.

The results of the Mann-Whitney U tests show that water temperature was the only measured environmental variable that was significantly different between spring season and summer season for samples collected at the Norfolk survey sites (Table 6.7).

Table 6.7: Results of Mann-Whitney *U* tests on environmental data for differences between spring and summer at Norfolk survey sites

Environmental variable	Mann-Whitney statistic (*U*)	*p*-value
Water body width	32	1.00
Water body depth	32	1.00
Water temperature	0	<0.01**
Salinity	18	0.16
Phosphate	29	0.78
Nitrate	18	0.08

**** = Highly significant difference.**

In comparison, the results of the Kruskal-Wallis tests for differences between seasons using environmental data collected at the Lincolnshire survey sites show that dissolved oxygen and redox potential were significantly different, as well as water temperature (Table 6.8).

Table 6.8: Results of Kruskal-Wallis tests on environmental data for differences between spring, summer and autumn at Lincolnshire survey sites

Environmental variable	Kruskal-Wallis statistic (*H*)	*p*-value
Water body width	0.46	0.79
Water body depth	4.75	0.09
Water temperature	19.57	<0.01**
Salinity	1.94	0.38
Dissolved oxygen	10.05	<0.01**
Redox potential	9.47	<0.01**

**** = Highly significant difference.**

The results of post-hoc pairwise Mann-Whitney *U* tests for these environmental variables are presented in Table 6.9 and show that water temperature was found to significantly different between every season, whilst redox potential was found to be significantly different between only the spring and autumn seasons. Dissolved oxygen was significantly different between the spring season and both summer and autumn seasons (Table 6.9).

Table 6.9: *P*-values of post-hoc pairwise Mann-Whitney *U* tests on water temperature, dissolved oxygen and redox potential data for differences between spring, summer and autumn at Lincolnshire survey sites

	Water temperature		Dissolved oxygen		Redox potential	
	Spring	Summer	Spring	Summer	Spring	Summer
Summer	<0.01**		<0.01**		0.10	
Autumn	<0.01**	<0.01**	<0.05*	0.13	<0.05*	0.13

**** = Significant difference, ** = Highly significant difference.**

The results shown in Table 6.7 and Table 6.9 are in agreement with the PCA exploration of the environmental data in determining water temperature as the main variable driving the differences between samples collected in different seasons when the environmental data are considered.

The results of the one-way NPMANOVA showed a significant difference in the environmental variables between the Norfolk samples and the Lincolnshire samples (NPMANOVA, F=22.47, p<0.01).

These results were further explored by undertaking Mann-Whitney *U* tests in order to determine which of the environmental variables were contributing to the significant differences between the samples collected at the Norfolk survey sites and those collected from the Lincolnshire survey sites when the environmental data are considered. Variables with missing data values were omitted from the analyses. The results of the tests determined that salinity and both water body width and depth was significantly different between the samples collected from the Lincolnshire survey sites and those collected from the Norfolk survey sites, whilst water temperature was not significantly different (Table 6.10).

Table 6.10: Results of Mann-Whitney *U* tests on environmental data for differences between Lincolnshire survey sites and Norfolk survey sites

Environmental variable	Mann-Whitney statistic (*U*)	*p*-value
Water body width	0	<0.01**
Water body depth	0	<0.01**
Water temperature	117	0.69
Salinity	49	<0.01**

**** = Highly significant difference.**

These results largely agree with with the PCA exploration of the environmental data in determining salinity, water body width and depth as variables that explain the difference between samples collected in Norfolk and those collected in Lincolnshire when the environmental data are considered.

6.1.1 Spatial and Temporal Trends in Environmental Data

The environmental data for both the South Forty Foot Drain and South Holland Main Drain were examined for spatial and temporal trends by plotting average measurements against survey sites organised in order of furthest upstream to furthest downstream to illustrate the environmental profiles of both water bodies. Temperature, salinity, dissolved oxygen and redox potential profiles are displayed in Figure 6.3, whilst phosphate and nitrate profiles are illustrated in Figure 6.4. The survey sites of the South Forty Drain are on the left of each graph and those of the South Holland Main Drain are on the right of each graph.

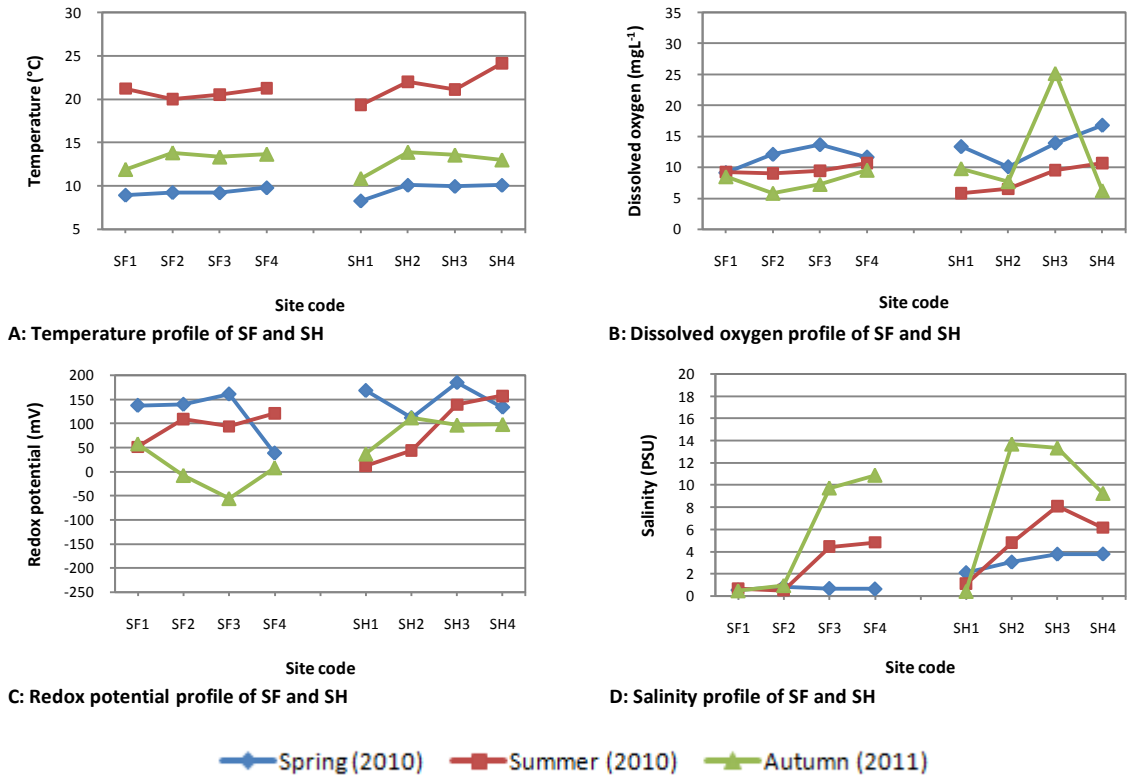


Figure 6.3: Temperature (A), dissolved oxygen (B), redox potential (C) and salinity (D) profiles of the South Forty Foot Drain (SF) and South Holland Main Drain (SH). Graphs are arranged with South Forty Foot Drain survey sites (SF*x*) on the left and South Holland Main Drain survey sites (SH*x*) on the right, with survey sites arranged in order of furthest upstream to furthest downstream. See Table 5.1 for site code definitions.

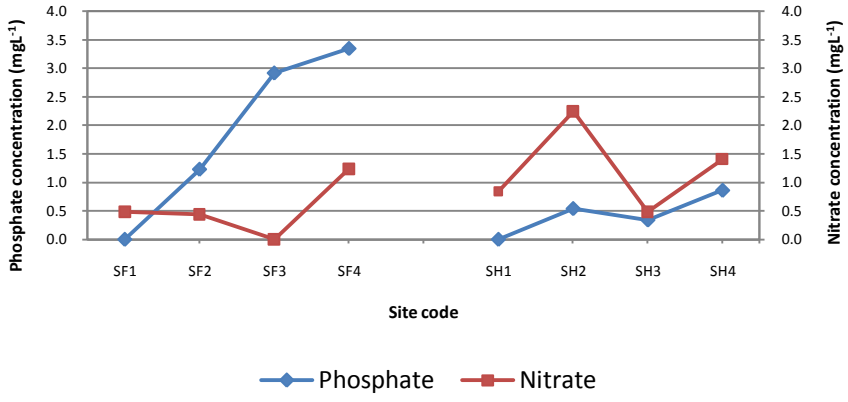


Figure 6.4: Phosphate (PO_4) and nitrate (NO_3) profiles of the South Forty Foot Drain and South Holland Main Drain. Graph is arranged with South Forty Foot Drain survey sites (SF*x*) on the left and South Holland Main Drain survey sites (SH*x*) on the right, with survey sites arranged in order of furthest upstream to furthest downstream. See Table 5.1 for site code definitions.

Temperature remained relatively consistent along both the South Forty Foot Drain and the South Holland Main Drain in all seasons (Figure 6.3A). The one exception was the South Holland Main Drain in summer, where temperature increased between the SH1 (Weston Fen) and the SH4 (Nene Outfall Sluice) survey sites. Furthermore, there were distinct differences shown in the temperature profiles of both drains between the different seasons, with summer temperatures highest and spring temperatures lowest. The dissolved oxygen profiles of the South Forty Foot Drain and South Holland Main Drain vary both between the water bodies and seasonally (Figure 6.3B). The South Holland Main Drain appeared to experience greater variation in dissolved oxygen concentration between the seasons than the South Forty Foot Drain. The large peak in dissolved oxygen concentration at SH3 (A1101 Road Bridge) in autumn may have resulted from the release of fresh water into the South Holland Main Drain by the Environment Agency and South Holland Internal Drainage Board in June 2011 in response to reports of dead fish (Environment Agency, 2012b). The redox potential profiles of the South Forty Foot Drain and South Holland Main Drain show considerable differences both seasonally and between the two water bodies (Figure 6.3C). The only increasing gradient in redox potential along either drain was recorded in summer for the South Holland Main Drain.

A narrow range of salinity concentrations were recorded at the survey sites of the South Forty Foot Drain in spring (Figure 6.3D), from 0.56PSU at the SF1 (Casswell's Bridge) survey site to 0.86PSU at the SF2 (Donington Bridge) site. The decrease in salinity between the SF2 (Donington Bridge) survey site and the SF3 (Swineshead Bridge) site, from 0.86PSU to 0.70PSU, may be due to a diluting freshwater inflow from the North Beck watercourse entering the South Forty Foot Drain between these two sites. Similarly, the decrease in salinity concentration from 0.70PSU at the SF3 (Swineshead Bridge) site to 0.66PSU at the SF4 (Wyberton Chain Bridge) survey site may also result from a diluting freshwater inflow, originating from either or both of Skerth Drain or Clay Dike, entering the main watercourse between these two sites. The salinity concentration of the South Forty Foot Drain in summer ranged from 0.53PSU at the SF2 (Donington Bridge) site to 4.84PSU at the SF4 (Wyberton Chain Bridge) site. A small decrease in salinity from 0.69PSU to 0.53PSU between the SF1 (Casswell's Bridge) and the SF2 (Donington Bridge) survey sites was recorded in summer.

This decrease may have resulted from an input of fresh water, originating from one or more of the eight inflowing drains between the two survey sites, diluting the concentration of salts held in the water of the South Forty foot Drain. In contrast to spring and summer, the South Forty Foot Drain in autumn showed a consistent increase in salinity between each survey site (Figure 6.3D), increasing from 0.50PSU at the SF1 (Casswell's Bridge) site to 10.90PSU at the SF4 (Wyberton Chain Bridge) site.

The South Holland Main Drain showed a consistent increase in salinity between each survey site in spring (Figure 6.3D), increasing from 2.14PSU at the SH1 (Weston Fen) site to 3.82PSU at the SH4 (Nene Outfall Sluice) site. Figure 6.3D, however, indicates that the SH4 (Nene Outfall Sluice) survey site was less saline than the SH3 (A1101 Road Bridge) site in summer (8.11PSU at SH3, 6.17PSU at SH4) and autumn (13.36PSU at SH3, 9.27PSU at SH4). This is most likely a result of the fact that it was not possible to collect environmental measurements from the centre of the channel at the SH4 (Nene Outfall Sluice) survey site due to safety concerns. Salinity can be expected to be greatest at the base of the centre of the channel due to halo-stratification (Dyer, 1973; Davidson *et al*., 1991).Thus it is believed that the SH4 (Nene Outfall Sluice) survey site would have recorded a higher salinity concentration than the SH3 (A1101 Road Bridge) site if environmental data could have been collected from the centre of the channel. Alternatively, the decrease in salinity concentration between the SH3 (A1101 Road Bridge and the SH4 (Nene Outfall Sluice) survey sites may have resulted from an inflow of fresh water, decreasing the salinity at the latter site. The Environment Agency implemented such an action to decrease salinity in the South Holland Main Drain in June 2011 in response to reports of dead fish (Environment Agency, 2012b).

It can be seen from Figure 6.3D that salinity was affected by season and that, generally, salinity concentration was greatest in autumn. This is to be expected as increased air temperature and decreased rainfall during spring and summer results in a decrease in the volume of water (Pillsbury, 1981; Goetsch & Palmer, 1997; Wolf *et al*., 2009) thereby concentrating the salts present in the water. Figure 6.3D also shows that salinity remained at a relatively similar concentration at the SF1 (Casswell's Bridge) and SF2 (Donington Bridge) survey sites between the three surveyed seasons. Salinity varied between 0.50PSU and 0.69PSU at the SF1 (Casswell's Bridge) site across the three seasons, whilst the SF2 (Donington Bridge) site recorded salinity concentrations between 0.53PSU and 0.97PSU.

In contrast, the SF3 (Swineshead Bridge) and SF4 (Wyberton Chain Bridge) survey sites both recorded substantial increases in salinity concentration between spring and summer, as well as between summer and autumn. The SF3 (Swineshead Bridge) site showed an increase in salinity of 3.74PSU between the spring and summer surveys, and an increase of 5.31PSU between the summer and autumn surveys. The SF4 (Wyberton Chain Bridge) site increased in water salinity by 4.18PSU between spring and summer, whilst an increase in salinity of 6.07PSU was recorded between summer and autumn. In contrast, three of the four survey sites of the South Holland Main Drain showed wide variations in salinity concentration between the three seasons. Only the SH1 (Weston Fen) survey site showed a narrow range of salinity concentrations, varying between 0.45PSU and 2.14PSU. The SH2 (Clifton's Bridge) site showed a small increase in salinity of 1.73PSU between spring and summer, followed by a large increase of 8.88PSU between summer and autumn. Large increases in salinity were also recorded at the SH3 (A1101 Road Bridge) survey site, which showed an increase of 4.30PSU between spring and summer, as well as an increase of 5.25PSU between summer and autumn. The SH4 (Nene Outfall Sluice) survey site experienced an increase in salinity of 2.34PSU between spring and summer, as well as an increase of 3.10PSU between summer and autumn. Thus, a range of salinities was experienced by the macro-invertebrate communities present at each of the surveyed sites.

Phosphate (PO_4) and nitrate (NO_3) measurements were only collected during the autumn 2011 season. Both phosphate and nitrate showed variation along the South Forty Foot Drain and the South Holland Main Drain (Figure 6.4). Despite both water bodies being drainage channels, the South Forty Foot Drain and South Holland Main Drain appear to be dissimilar in terms of nitrate and phosphate profiles. Figure 6.4 illustrates that phosphate increased at a greater rate along the profile of the South Forty Foot Drain, whilst nitrate varied much more along the South Holland Main Drain.

Overall, these data show that the Salinity Association Group index is being tested with data collected from linearly-connected sites which provide a range of conditions within a salinity concentration range from 0.50PSU to 13.71PSU with unrestricted aquatic movement possible between the sites along the individual drains.

As such, macro-invertebrate taxa were able to colonise in response to water conditions within each season through such vectors as upstream and downstream migration (drift), vertical movements from within the substrate and aerial sources such as oviposition by flying adult life stages (Williams & Hynes, 1976). Further vectors of colonisation include accidental or deliberate releases (Grigorovich *et al.*, 2002) or via internal or external transport by birds (Green & Figuerola, 2005).

The environmental data for the survey sites located in Norfolk were examined by plotting average measurements for temperature, salinity, phosphate and nitrate in Figure 6.5, whilst the dissolved oxygen and redox potential measurements for the Norfolk survey sites in summer are presented in Figure 6.6.

Figure 6.5 and Figure 6.6 show that the environmental condition of the water at the survey sites varied both seasonally and between the sites, showing that the macro-invertebrate community samples were collected from a wide range of environmental conditions. For example, salinity concentration in spring varied between 0.33PSU at the Buckenham Marsh survey site and 19.88PSU at the Long Dyke site. The same survey sites recorded the lowest and highest salinity concentrations in summer (Figure 6.5D).

Nitrate was consistently low during the spring surveys at the Norfolk survey sites, with the Rockland Marsh survey site recording a nitrate concentration of 0.30mgL^{-1} and no other survey site recording a nitrate concentration. Whilst nitrate concentrations generally increased during the summer surveys, four of the survey sites that did not record a nitrate concentration during the spring surveys also did not record a nitrate concentration in summer (the Long Dyke, Middle Marsh, Near Dry Dyke and Ludham Marsh survey sites). The highest nitrate concentration recorded during the summer surveys was 3.34mgL^{-1} at the Hatchet Marsh survey site.

A range of phosphate concentrations were recorded at the Norfolk survey sites. No phosphates were detected at the Long Dyke and Hatchet Marsh survey sites in spring, whilst the highest phosphate concentration recorded in spring was 1.88mgL^{-1} at the Buckenham Marsh survey site. The highest phosphate concentration recorded in summer was 1.09mgL^{-1} at the Rockland Marsh site, whereas the lowest phosphate concentration was 0.28mgL^{-1} at the Hatchet Marsh survey site.

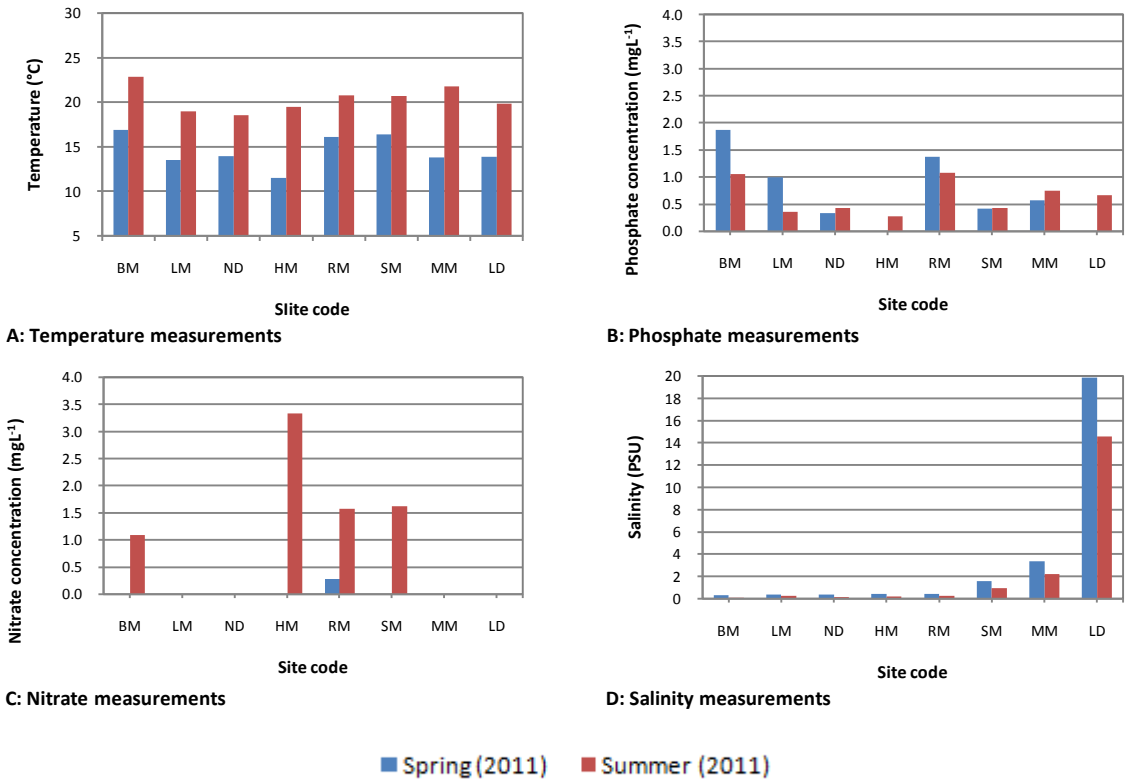


Figure 6.5: Temperature (A), phosphate (B), nitrate (C) and salinity (D) measurements collected from the Norfolk survey sites. See Table 5.1 for site code definitions.

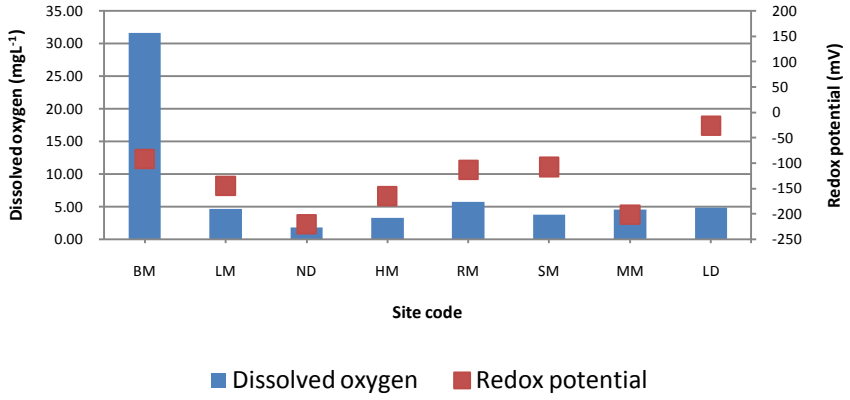


Figure 6.6: Dissolved oxygen and redox potential measurements collected from the Norfolk survey sites. See Table 5.1 for site code definitions.

Thus, the potential influence of phosphate on the Salinity Association Group (SAG) index can be fully explored using the range of phosphate concentrations recorded at the Norfolk survey sites. Furthermore, the data collected from the Norfolk survey sites can be used to test the SAG index in a significantly different habitat type and geographic region from the Lincolnshire survey sites (see Section 6.1).

6.2 Macro-invertebrate Data

A total of 25901 specimens were identified to 162 taxa (136 species, eight genera and 18 families) during the analysis of the macro-invertebrate samples. Analysis of the macro-invertebrate community samples collected at the survey sites in Lincolnshire yielded 18453 specimens identified to 112 taxa (94 species, six genera and 12 families). Analysis of the samples collected from the survey sites in Norfolk resulted in the identification of 7448 specimens to 101 taxa (84 species, six genera and 11 families). The macro-invertebrate community data for the survey sites in Lincolnshire are summarised by the application of various diversity and water quality measures in Table 6.11 (see Appendix 9 for complete table of macro-invertebrate data), whilst the macro-invertebrate community data for the survey sites in Norfolk are summarised in Table 6.12 (see Appendix 10 for complete tables of macro-invertebrate data).

Cluster analysis of fourth root transformed macro-invertebrate data, undertaken to reveal similarity between samples (Kay *et al*., 2001; Carlsson, 2006; Akbulut *et al*., 2009) in terms of the macro-invertebrate taxa present (Lancaster & Scudder, 1987; Kay *et al*., 2001; Velasco *et al*., 2006; Rawson *et al*., 2010), revealed that few samples showed a similarity of greater than 70% (Figure 6.7) and consequently indicating a relatively high degree of variation in the macro-invertebrate samples. The samples collected at the LD (Long Dyke) survey site in spring and summer showed a similarity greater than 85%, whilst the samples collected during the summer surveys at the SH3 and SH4 survey sites showed a similarity greater than 70%. A cut-off at 40% similarity resulted in six clusters of two or more samples and two outlying samples not connected to a cluster (Figure 6.7).

Table 6.11: Summarised macro-invertebrate data for Lincolnshire survey sites

Season	Biological measure	Survey site code							
		SF1	SF2	SF3	SF4	SH1	SH2	SH3	SH4
Spring (2010)	Taxon richness	24	35	27	22	22	7	10	10
	Number of individuals	320	240	741	68	269	199	67	54
	Margalef richness index	3.99	6.20	3.94	4.98	3.75	1.13	2.14	2.26
	Shannon diversity index	2.02	2.66	2.30	2.45	2.35	0.61	1.70	1.65
	Berger-Parker dominance index	0.45	0.22	0.24	0.32	0.25	0.84	0.37	0.46
	Simpson dominance index	0.76	0.89	0.86	0.85	0.86	0.28	0.76	0.72
	Evenness	0.31	0.41	0.37	0.53	0.48	0.26	0.55	0.52
	$ASPT_{BMWP}$	4.35	4.42	4.07	4.31	4.29	3.50	3.50	4.00
Summer (2010)	Taxon richness	32	35	38	25	29	10	8	10
	Number of individuals	901	730	829	877	1306	206	218	389
	Margalef richness index	4.56	5.16	5.51	3.54	3.90	1.69	1.30	1.51
	Shannon diversity index	2.07	2.36	2.28	1.93	1.77	1.62	1.07	0.76
	Berger-Parker dominance index	0.49	0.25	0.28	0.40	0.41	0.38	0.57	0.82
	Simpson dominance index	0.74	0.86	0.84	0.75	0.74	0.75	0.56	0.32
	Evenness	0.25	0.30	0.26	0.27	0.20	0.51	0.36	0.21
	$ASPT_{BMWP}$	4.53	4.73	4.63	4.46	4.50	3.33	3.67	3.67
Autumn (2011)	Taxon richness	24	29	9	8	29	6	7	8
	Number of individuals	272	446	192	3367	606	559	3538	2059
	Margalef richness index	4.10	4.59	1.52	0.86	4.37	0.79	0.73	0.92
	Shannon diversity index	2.30	2.20	0.81	0.32	2.43	1.08	0.45	0.44
	Berger-Parker dominance index	0.22	0.24	0.79	0.91	0.22	0.60	0.90	0.91
	Simpson dominance index	0.86	0.84	0.36	0.16	0.88	0.57	0.19	0.18
	Evenness	0.42	0.31	0.25	0.17	0.39	0.49	0.23	0.19
	$ASPT_{BMWP}$	4.59	4.82	5.00	3.80	4.39	3.67	3.67	4.00

See Table 5.1 for site code definitions.

Table 6.12: Summarised macro-invertebrate data for Norfolk survey sites

Season	Biological measure	Survey site code							
		BM	**LM**	**ND**	**HM**	**RM**	**SM**	**MM**	**LD**
Spring (2011)	Taxon richness	25	26	22	23	34	30	20	4
	Number of individuals	278	194	181	166	395	529	563	369
	Margalef richness index	4.27	4.75	4.04	4.30	5.52	4.62	3.00	0.51
	Shannon diversity index	2.69	2.71	2.26	2.42	2.54	2.18	1.67	0.98
	Berger-Parker dominance index	0.14	0.17	0.31	0.24	0.24	0.38	0.38	0.60
	Simpson dominance index	0.91	0.91	0.84	0.87	0.88	0.81	0.74	0.57
	Evenness	0.59	0.58	0.44	0.49	0.37	0.30	0.27	0.67
	$ASPT_{BMWP}$	4.18	4.19	4.47	4.47	4.50	4.39	4.42	4.39
Summer (2011)	Taxon richness	26	42	33	39	24	27	19	3
	Number of individuals	693	919	543	388	530	311	820	569
	Margalef richness index	3.82	6.01	5.08	6.38	3.67	4.53	2.68	0.32
	Shannon diversity index	1.59	2.01	2.45	2.78	2.45	1.97	1.65	1.02
	Berger-Parker dominance index	0.61	0.52	0.32	0.24	0.23	0.49	0.40	0.47
	Simpson dominance index	0.61	0.71	0.85	0.89	0.88	0.73	0.74	0.62
	Evenness	0.19	0.18	0.35	0.42	0.48	0.27	0.27	0.93
	$ASPT_{BMWP}$	4.06	4.90	4.53	5.10	3.67	4.87	4.45	4.08

See Table 5.1 for site code definitions.

The similarity of the macro-invertebrate communities between samples were further analysed using Non-metric MultiDimensional Scaling (NMDS) based on the Bray-Curtis similarity measure (Bray & Curtis, 1957). The clusters of the macro-invertebrate samples defined by the cluster analysis at 40% similarity are highlighted in the ordination plot resulting from the NMDS analysis of macro-invertebrate data (Figure 6.8) by differing symbols and polygons of different colours, as summarised in Table 6.13.

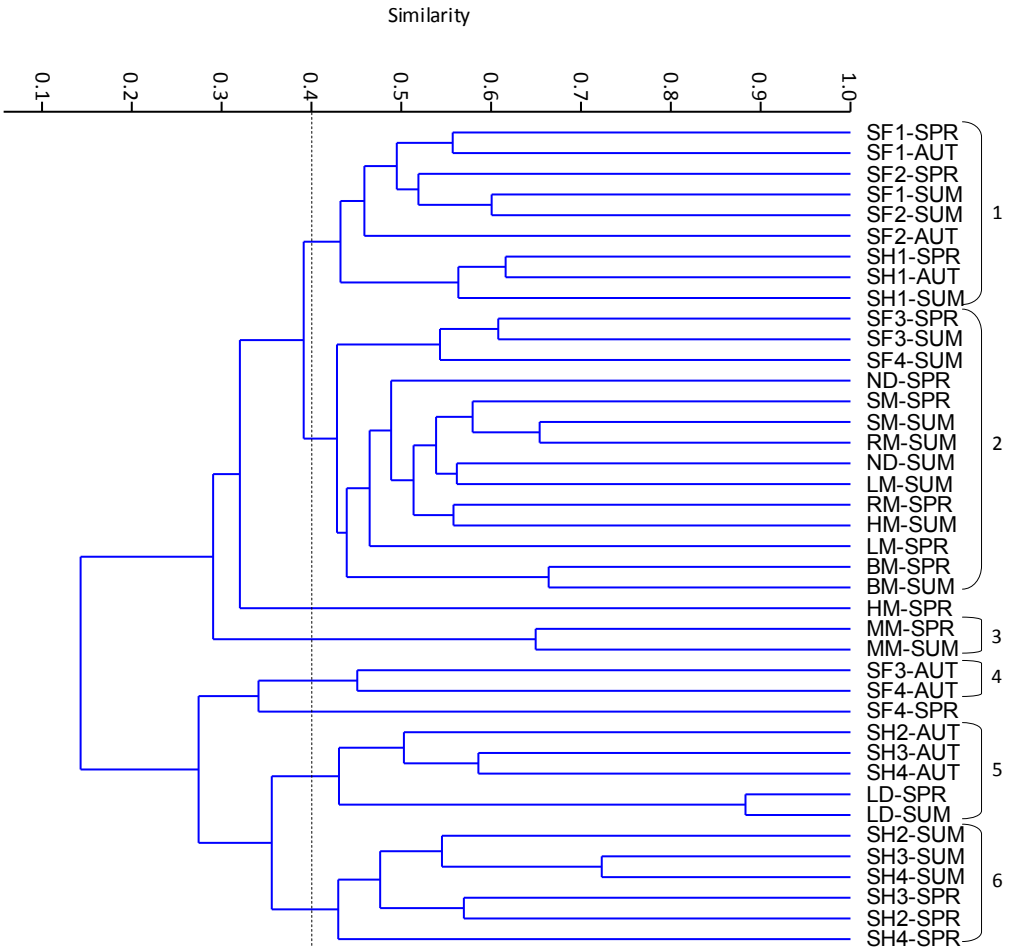


Figure 6.7: Cluster analysis dendrogram of samples based on fourth-root transformed macro-invertebrate data (Unweighted Pair-Group Method using arithmetic Averages (UPGMA) algorithm, Bray-Curtis similarity measure). Cophentic correlation coefficient (*CC*) of dendrogram = 0.89. See Table 5.1 and Table 5.2 in Section 5 for sample code definitions.

Table 6.13: Symbols and colours representing clusters in non-metric multidimensional scaling ordination plot

Cluster	Symbol	Colour
1	Hollow Diamond (◇)	Green
2	Asterisk (∗)	Dark blue
3	Hollow circle (O)	Violet
4	Diagonal cross (**X**)	Orange
5	Hollow square (□)	Pale blue
6	Upright cross (+)	Red
Outlying samples	Filled circle (●)	Black

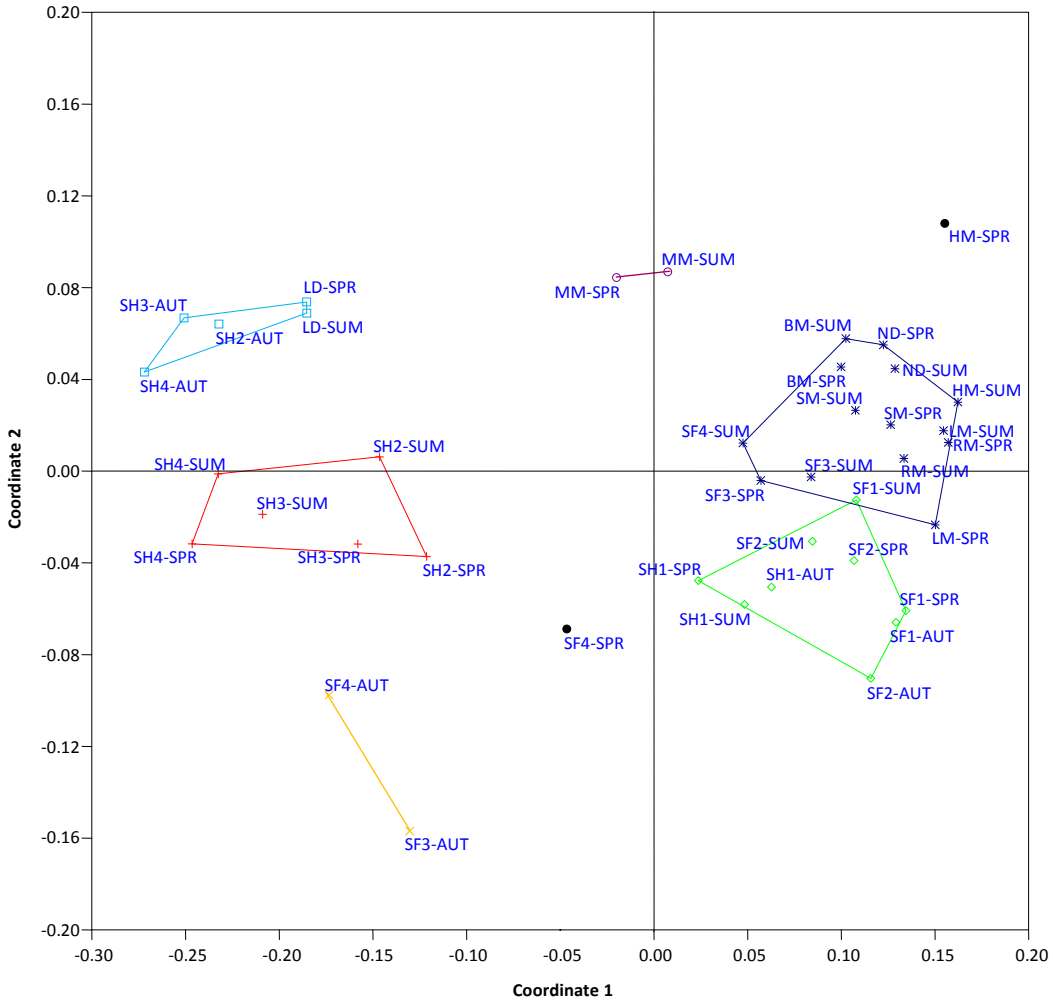


Figure 6.8: Non-metric multidimensional scaling ordination plot of samples based on fourth-root transformed macro-invertebrate data (Bray-Curtis similarity measure). Clusters of samples defined by the cluster analysis at 40% similarity are highlighted by differing symbols and polygons of different colours. Stress level of ordination plot = 0.14. See Table 5.1 and Table 5.2 in Section 5 for sample code definitions.

A one-way ANalysis Of SIMilarity (ANOSIM; Clarke & Green, 1988; Clarke, 1993) using the Bray-Curtis similarity measure (Bray & Curtis, 1957) and run using 10000 permutations was used on fourth root transformed macro-invertebrate data to test if the six clusters identified by the cluster analysis and NMDS were significantly different. The results of the analysis showed that there is a highly significant difference between the six clusters defined by the cluster analysis and NMDS (ANOSIM, R=0.87, p<0.01). The results of post-hoc pairwise ANOSIM tests between all pairs of clusters (Table 6.14) show that all clusters were significantly different from each other, except when cluster 3 and cluster 4 were examined.

Table 6.14: *P*-values of post-hoc pairwise ANOSIM tests between all pairs of clusters defined by cluster analyses of macro-invertebrate data

		Cluster number				
		1	**2**	**3**	**4**	**5**
Cluster number	**2**	<0.01**				
	3	<0.01**	<0.05*			
	4	<0.01**	<0.05*	0.34		
	5	<0.01**	<0.01**	<0.05*	<0.05*	
	6	<0.01**	<0.01**	<0.05*	<0.05*	<0.01**

*** = Significant difference, ** = Highly significant difference.**

Summary statistics (mean average, standard deviation and range) for the environmental variables associated with the samples of each cluster are shown in Table 6.15, whilst the samples associated with each cluster are listed in Table 6.16.

Table 6.15: Summary statistics of environmental variables for each cluster defined by clustering analyses

Environmental variable		Cluster (number of samples in cluster)					
		1 (n =14)	**2 (n = 9)**	**3 (n = 2)**	**4 (n = 2)**	**5 (n = 5)**	**6 (n = 6)**
Water body width	Average (± 1 SD)	6.27 (±6.75)	10.00 (±1.73)	2.80 (±0.00)	19.00 (±1.41)	16.60 (±13.77)	26.00 (±6.20)
(m)	Range (min - max)	1.60 - 20.00	8.00 - 12.00	2.80 - 2.80	18.00 - 20.00	2.50 - 30.00	18.00 - 30.00
Water body depth	Average (± 1 SD)	1.00 (±0.83)	1.20 (±0.57)	0.28 (±0.00)	2.65 (±0.49)	1.70 (±1.31)	2.50 (±0.49)
(m)	Range (min - max)	0.30 - 2.50	0.50 - 1.80	0.28 - 0.28	2.30 - 3.00	0.30 - 3.10	1.80 - 3.10
Water temperature	Average (± 1 SD)	17.82 (±3.72)	13.73 (±5.14)	17.81 (±5.64)	13.49 (±0.23)	14.84 (±2.85)	16.23 (±6.84)
(°C)	Range (min - max)	9.20 - 22.90	8.26 - 21.21	13.82 - 21.80	13.33 - 13.65	13.00 - 19.90	9.96 - 24.14
Salinity	Average (± 1 SD)	1.09 (±1.56)	0.87 (±0.53)	2.82 (±0.81)	10.33 (±0.81)	14.17 (±3.80)	4.97 (±1.87)
(PSU)	Range (min - max)	0.14 - 4.84	0.45 - 2.14	2.25 - 3.40	9.76 - 10.90	9.27 - 19.88	3.10 - 8.11
Dissolved oxygen	Average (± 1 SD)	10.18 (±9.55)	9.24 (±2.48)	N/A	8.44 (±1.62)	11.01 (±9.53)	11.28 (±3.60)
(mgL^{-1})	Range (min - max)	1.79 - 31.70	5.84 - 13.37	N/A	7.30 - 9.59	4.83 - 25.19	6.51 - 16.81
Redox potential	Average (± 1 SD)	-37.36 (±141.70)	78.80 (±61.98)	N/A	-22.99 (±45.16)	70.60 (±64.89)	128.97 (±47.92)
(mV)	Range (min - max)	-219.90 - 161.18	-6.98 - 168.54	N/A	-54.92 - 8.94	-26.20 - 112.46	45.00 - 185.04
Phosphate	Average (± 1 SD)	0.79 (±0.53)	0.41 (±0.71)	0.67 (±0.12)	3.14 (±0.30)	0.48 (±0.33)	N/A
(mgL^{-1})	Range (min - max)	0.28 - 1.88	0.00 - 1.23	0.58 - 0.75	2.92 - 3.35	0.00 - 0.86	N/A
Nitrate	Average (± 1 SD)	0.72 (±1.09)	0.59 (±0.22)	0.00 (N/A)	0.62 (±0.87)	0.83 (±0.98)	N/A
(mgL^{-1})	Range (min - max)	0.00 - 3.34	0.44 - 0.84	0.00 - 0.00	0.00 - 1.23	0.00 - 2.24	N/A

Individual samples not associated to a cluster were omitted.

Large overlaps were evident in the ranges of water body width, water temperature, dissolved oxygen, redox potential and nitrate between all clusters. Furthermore, large overlaps were present in the ranges of phosphate concentration for all clusters except cluster 4, and in the ranges of water body depth for all clusters except cluster 3. These results suggest that the clusters defined by the cluster analyses are not the result of the influence of one environmental variable on the macro-invertebrate assemblages, but may result from the influence of a combination of environmental variables on the macro-invertebrate assemblages. Of particular interest is that clusters 1, 2 and 3 appear to be largely characterised by low salinities, whilst clusters 4, 5 and 6 are characterised by higher salinities.

Table 6.16: Samples associated with each cluster defined by clustering analyses

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
SF3-SPR	SF1-SPR	MM-SPR	SF3-AUT	SH2-AUT	SH2-SPR
SF3-SUM	SF1-AUT	MM-SUM	SF4-AUT	SH3-AUT	SH3-SPR
SF4-SUM	SF2-SPR			SH4-AUT	SH4-SPR
ND-SPR	SF1-SUM			LD-SPR	SH2-SUM
SM-SPR	SF2-SUM			LD-SUM	SH3-SUM
SM-SUM	SF2-AUT				SH4-SUM
RM-SUM	SH1-SPR				
ND-SUM	SH1-AUT				
LM-SUM	SH1-SUM				
RM-SPR					
HM-SUM					
LM-SPR					
BM-SPR					
BM-SUM					

Individual samples not associated to a cluster were omitted.

See Table 5.1 and Table 5.2 in Section 5 for sample code definitions.

Table 6.16 shows that cluster analyses did not separate the macro-invertebrate samples collected from the survey sites located on the South Forty Foot Drain (samples labelled with SF*x*- prefixes in Table 6.16) from those collected at survey sites located on the South Holland Main Drain (samples labelled with SH*x*- prefixes in Table 6.16).

Furthermore, the cluster analysis also did not separate the macro-invertebrate samples collected in different seasons (samples labelled with suffixes -SPR for spring, -SUM for summer and -AUT for autumn in Table 6.16). A two-way ANOSIM, however, found a highly significant difference between the macro-invertebrate samples collected from the survey sites located on the South Forty Foot Drain and those collected at survey sites located on the South Holland Main Drain (ANOSIM, R=0.44, p<0.01), but no significant difference resulting from differences in seasonality when only macro-invertebrate data for the survey sites in Lincolnshire were considered (ANOSIM, R=0.11, p=0.12). Macro-invertebrate samples collected from the survey sites in Norfolk were omitted from the analysis as no data were collected at these sites for the autumn season and the cluster analyses suggested a difference between the macro-invertebrate data for the collected at the sites within Lincolnshire and those collected at the sites located within Norfolk. A one-way ANOSIM found no significant difference between the spring and summer seasons when only macro-invertebrate data for the survey sites in Norfolk were considered (ANOSIM, R=-0.04, p=0.68).

The clusters defined by the cluster analyses are largely composed of samples collected at either survey sites located within Lincolnshire or those located within Norfolk. Only cluster 1 and cluster 5 are composed of samples collected from survey sites within both Lincolnshire and Norfolk (Table 6.16). This suggests that the difference between the survey sites in Lincolnshire and those in Norfolk, and thus the associated differences in habitat structure and water quality found by PCA and subsequent tests by NPMANOVA of the environmental variables (see Section 6.1), may be a major factor influencing the macro-invertebrate assemblages of the survey sites. A one-way ANOSIM found a highly significant difference between the macro-invertebrate assemblages for the survey sites in Lincolnshire and those in Norfolk (ANOSIM, R=0.25, p<0.01). Data collected for the autumn season at survey sites within Lincolnshire were omitted as no comparable data were collected for the same season at the survey sites located within Norfolk.

The significant difference between the macro-invertebrate assemblages of the survey sites located on the South Forty Foot Drain and those of the survey sites of the South Holland Main Drain is unexpected given that no signficant difference was found when the associated environmental data for these samples were similarly examined (see Section 6.1).

As such, these results suggest that the difference between the macro-invertebrate assemblages of the survey sites for these two water bodies may occur due to a difference in one or more unmeasured environmental variables between the two water bodies. In contrast, the significant difference between the macro-invertebrate assemblages of the survey sites located within Lincolnshire and those of the survey sites located in Norfolk most likely results from the significant differences found when the environmental variables when the associated environmental data for these samples were similarly examined (see Section 6.1). As such, it appears that the data collected from the survey sites in Lincolnshire and those in Norfolk form two distinct datasets differing in terms of habitat structure and water quality with which to examine the proposed salinity index at sites.

6.2.1 Spatial and Temporal Trends in Macro-invertebrate Data

Spatial and temporal trends in the macro-invertebrate communities of the South Forty Foot Drain and South Holland Main Drain were investigated by plotting untransformed diversity and water quality measures (taxon richness, number of individuals, Margalef richness index, Shannon diversity index, Berger-Parker and Simpson dominance indices, evenness and $ASPT_{BMWP}$ index) against the survey sites for both the South Forty Foot Drain and South Holland Main Drain. The survey sites were arranged in order of furthest upstream to furthest downstream, with the survey sites of the South Forty Foot Drain on the left of each graph and those of the South Holland Main Drain on the right of each graph (Figure 6.9).

Many of the macro-invertebrate diversity and water quality indices showed similar profiles and were found to be significantly correlated with each other when examined using Spearman's rank order correlation coefficient (r_s), as indicated in Table 6.17. For example, taxon richness, the Margalef richness index and the $ASPT_{BMWP}$ index were all significantly correlated with each other in each season (Table 6.17). The Margalef richness index was also significantly correlated with both the Shannon diversity index and Simpson dominance index when data for the spring and summer season were considered (Table 6.17). Furthermore, the Shannon diversity index, Simpson dominance index and Berger-Parker dominance index were also all significantly correlated with each other in each season (Table 6.17).

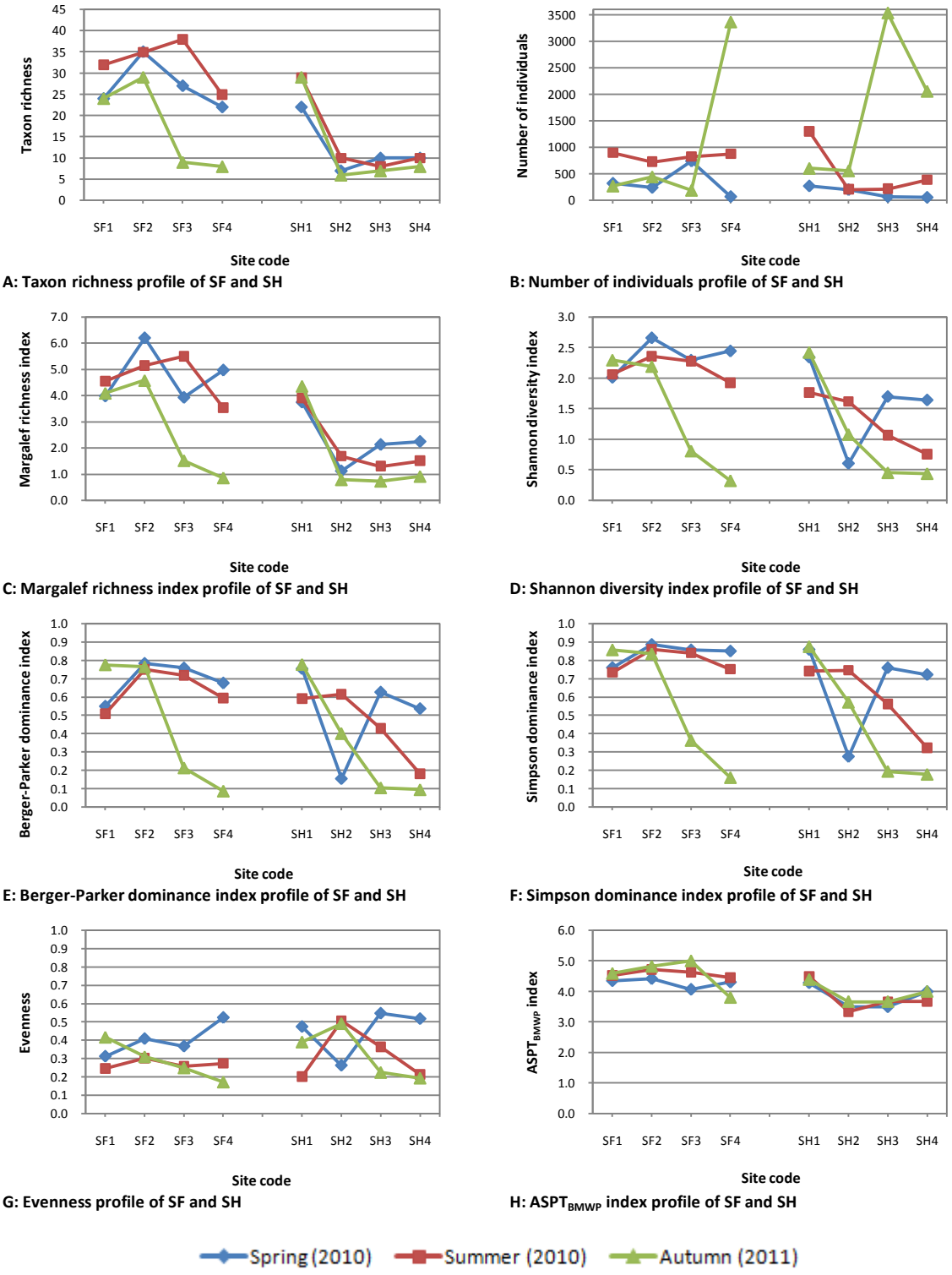


Figure 6.9: Taxon richness (A), number of individuals (B), Margalef richness index (C), Shannon diversity index (D), Berger-Parker dominance index (E), Simpson dominance index (F), evenness (G) and $ASPT_{BMWP}$ index (H) profiles of the South Forty Foot Drain (SF) and South Holland Main Drain (SH). Graphs are arranged with South Forty Foot Drain survey sites (SF*x*) on the left and South Holland Main Drain survey sites (SH*x*) on the right, with survey sites arranged in order of furthest upstream to furthest downstream. See Table 5.1 in Section 5 for site code definitions.

Table 6.17: Spearman's rank order correlation coefficient (r_s) and *p*-values resulting from tests between macro-invertebrate diversity and water quality indices for data collected at survey sites in Lincolnshire

	Taxon richness	Number of individuals	Shannon diversity index	Simpson dominance index	Evenness	Margalef richness index	Berger-Parker dominance index	$ASPT_{BMWP}$
Taxon richness		0.08 0.14 0.25	<0.05* <0.01** 0.10	<0.05* 0.06 0.10	0.69 0.45 0.69	<0.01** <0.01** <0.01**	<0.05* 0.08 0.10	<0.05* <0.01** <0.05*
Number of individuals	0.68 0.58 -0.47		0.39 0.24 0.17	0.17 0.70 0.17	0.08 <0.05* 0.13	0.36 0.17 0.13	0.22 0.98 0.17	0.26 0.13 <0.05*
Shannon diversity index	0.80 0.91 0.64	0.36 0.45 -0.55		<0.01** <0.05* <0.01**	0.58 0.98 <0.05*	<0.01** <0.01** 0.07	<0.01** <0.05* <0.01**	<0.05* <0.01** 0.32
Simpson dominance index	0.86 0.70 0.64	0.55 0.14 -0.55	0.93 0.83 1.00		0.84 0.46 <0.05*	<0.05* <0.05* 0.07	<0.01** <0.01** <0.01**	<0.05* 0.12 0.32
Evenness	-0.17 -0.31 0.17	-0.64 -0.74 -0.57	0.21 0.02 0.83	0.07 0.31 0.83		0.98 0.54 0.46	0.67 0.36 <0.05*	0.76 0.47 0.81
Margalef richness index	0.87 0.99 0.96	0.38 0.55 -0.60	0.88 0.93 0.67	0.76 0.74 0.67	0.02 -0.24 0.29		0.06 0.06 0.07	<0.01** <0.01** <0.05*
Berger-Parker dominance index	0.83 0.66 0.64	0.48 0.02 -0.55	0.88 0.79 1.00	0.95 0.98 1.00	0.17 0.38 0.83	0.69 0.71 0.67		0.14 0.18 0.32
$ASPT_{BMWP}$	0.83 0.92 0.80	0.46 0.59 -0.79	0.81 0.90 0.41	0.74 0.61 0.41	-0.13 -0.30 0.11	0.95 0.90 0.83	0.59 0.54 0.41	

Spearman's rank correlation coefficient values shown in lower left triangle, *p*-values shown in upper right triangle. Significant correlations indicated by * when $p<0.05$ and ** when $p<0.01$. In all cases $df=6$. Blue text relates to data collected in spring season, red text to data collected in summer season and green text to data collected in autumn season.

The shape of the taxon richness profile of the South Forty Foot Drain is similar in spring and autumn, although there were large differences in magnitude between the two seasons at three of the four survey sites. Furthermore, taxon richness at the sites along the South Forty Foot Drain in summer showed a different profile to that for spring and autumn (Figure 6.9A). In contrast, the number of macro-invertebrate taxa profile along the South Holland Main Drain appeared to be unaffected by changes in season (Figure 6.9A). The South Holland Main Drain consistently showed a large decrease in the number of macro-invertebrate taxa between the SH1 (Weston Fen) and SH2 (Clifton's Bridge) survey sites followed by a slight increase along the remaining survey sites.

The number of individuals profile along the South Forty Foot Drain (Figure 6.9B) in spring showed a large increase at SF3 (Swineshead Bridge) followed by a decrease at SF4 (Wyberton Chain Bridge). This profile remained relatively constant along the South Forty Foot Drain in summer but showed a large increase between SFFD-3 (Swineshead Bridge) and SF4 (Wyberton Chain Bridge) in autumn (Figure 6.9B). In contrast the number of individuals profile along the South Holland Main Drain (Figure 6.9B) showed a slight decrease along the water body in spring. In summer, the number of individuals decreased between SH1 (Weston Fen) and SH2 (Clifton's Bridge) followed by a slight increase along the remaining survey sites. The number of individuals profile along the South Holland Main Drain remained somewhat constant between SH1 (Weston Fen) and SH2 (Clifton's Bridge) in autumn, but showed a large increase at SH3 (A1101 Road Bridge) followed by a decrease at SH4 (Nene Outfall Sluice).

The profiles of the Margalef richness index, Shannon diversity index, Berger-Parker and Simpson dominance indices for the South Forty Foot Drain showed differences between the seasons (Figure 6.9C, Figure 6.9D, Figure 6.9E, and Figure 6.9F respectively), despite no significant differences being found between different seasons in the macro-invertebrate data for the South Forty Foot Drain and South Holland Main Drain. The Margalef richness and Shannon diversity profiles both fluctuated along the South Forty Foot Drain in the spring season, increasing between the SF1 (Caswell's Bridge) and SF2 (Donington Bridge) survey sites before decreasing to the SF3 (Swineshead Bridge) site and finally increasing at the SF4 (Wyberton Chain Bridge) site.

In contrast, the Berger-Parker and Simpson dominance indices both showed higher values at the SF2 (Donington Bridge) and SF3 (Swineshead Bridge) survey sites while the two terminal survey sites attained lower scores in spring, with the SF1 (Caswell's Bridge) site showing lower values that the SF4 (Wyberton Chain Bridge) site for both indices. The profiles of the Margalef richness index, Shannon diversity index, Berger-Parker and Simpson dominance indices for the South Forty Foot Drain in summer all showed a peak at the SF2 (Donington Bridge) and SF3 (Swineshead Bridge) survey sites, while the two terminal survey sites attained lower scores. In autumn, the profile of the Shannon diversity index, Berger-Parker and Simpson dominance indices of the South Forty Foot Drain consistently decreased between each site, with the largest decrease occurring between the SF2 (Donington Bridge) and SF3 (Swineshead Bridge) survey sites in all cases. The Margalef richness index showed a slight increase between the SF1 (Caswell's Bridge) and SF2 (Donington Bridge) survey sites, but decreased between all other sites with the largest decrease occurring between the SF2 (Donington Bridge) and SF3 (Swineshead Bridge) survey sites as with Shannon diversity index, Berger-Parker and Simpson dominance indices. The profiles of Margalef richness index, Shannon diversity index, Berger-Parker and Simpson dominance indices along the South Holland Main Drain (Figure 6.9C, Figure 6.9D, Figure 6.9E, and Figure 6.9F respectively) in spring all showed a large decrease between the SH1 (Weston Fen) and SH2 (Clifton's Bridge) survey sites followed by an increase at the SH3 (A1101 Road Bridge) site. In contrast, the profiles of these four indices all decreased consistently along the South Holland Main Drain in both summer and autumn, with the exception of the Berger-Parker and Simpson dominance indices which both exhibited a slight increase between the SH1 (Weston Fen) and SH2 (Clifton's Bridge) survey sites in summer.

Evenness showed distinctly different profiles for each season along both drains (Figure 6.9G). Evenness generally increased along the South forty foot Drain in spring, remained relatively constant along the drain in summer and consistently decreased along the drain in autumn. Evenness showed a large decrease between the SH1 (Weston Fen) and the SH2 (Clifton's Bridge) survey sites, exhibited a larger increase between the SH2 (Clifton's Bridge) and SH3 (A1101 Road Bridge) sites and a slight decrease to the SH4 (Nene Outfall Sluice) site of the South Holland Main Drain in spring.

The evenness profiles of the South Holland Main Drain in summer and autumn were somewhat similar, with both exhibiting increases between the SH1 (Weston Fen) and the SH2 (Clifton's Bridge) sites and decreases between all the remaining sites. There were, however, differences in the magnitude of the increases and decreases for the evenness profiles of the South Holland Main Drain in summer and autumn. The autumn profile showed a larger decrease between the SH2 (Clifton's Bridge) and SH3 (A1101 Road Bridge) sites and smaller decreases between the SH1 (Weston Fen) and the SH2 (Clifton's Bridge) sites and between the SH3 (A1101 Road Bridge) and the SH4 (Nene Outfall Sluice) sites.

The $ASPT_{BMWP}$ index remained relatively constant along the South Forty Foot Drain in both spring and summer (Figure 6.9H), attaining scores between 4.0 and 4.5 in spring and between 4.4 and 4.8 in summer. The SF2 (Donington) Bridge survey site attained the highest scores in both spring and summer (4.42 and 4.73 respectively). the $ASPT_{BMWP}$ index exhibited a different profile along the South Forty Foot Drain in the autumn season, indicating a slight increase between each of the first three upstream survey sites before showing a large decrease at the SF4 (Wyberton Chain Bridge) survey site. In contrast, the $ASPT_{BMWP}$ index showed distinctly similar profiles along the South Holland Main Drain in each season (Figure 6.9H). A large decrease between the SH1 (Weston Fen) and SH2 (Clifton's Bridge) survey sites followed by small increases or the same scores occurring between each of the remaining sites of the South Holland Main drain was exhibited by the $ASPT_{BMWP}$ index in each season.

The macro-invertebrate diversity and water quality measures were examined using Kruskal-Wallis and Mann-Whitney *U* tests, as appropriate, to determine if there is a significant difference between summary macro-invertebrate data of the South Forty Foot Drain and the South Holland Main Drain, as well as between different seasons. Non-parametric methods were used in order to avoid making assumptions of normality and homogeneity of variance, as stated by Kay *et al*. (2001). The results of these analyses are presented in Table 6.18.

Table 6.18: Results of Kruskal-Wallis and Mann-Whitney *U* tests on macro-invertebrate diversity and water quality measures from Lincolnshire survey sites for differences between seasons and the Lincolnshire water bodies

Test for difference:	Between SF and SH		Between seasons	
Diversity/water quality measure	Mann-Whitney statistic (*U*)	*p*-value	Kruskal-Wallis statistic (*H*)	*p*-value
Taxon richness	27	<0.01**	3.58	0.16
Number of individuals	60	0.51	7.31	<0.05*
Margalef richness index	22	<0.01**	2.75	0.25
Shannon index	35	<0.05*	2.77	0.25
Berger-Parker index	44	0.11	1.51	0.47
Simpson index	41	0.08	2.68	0.26
Evenness	63	0.62	6.64	<0.05*
$ASPT_{BMWP}$	14	<0.01**	1.43	0.49

SF = South Forty Foot Drain, SH = South Holland Main Drain.

*** = Significant difference, ** = Highly significant difference.**

It is apparent from Table 6.18 that there are significant differences between the macro-invertebrate communities of the South Forty Foot Drain and South Holland Main Drain for four of the eight diversity and water quality measures considered. This difference was also found when the macro-invertebrate data for the two drains were examined directly using ANOSIM (see Section 6.2). Whilst no significant difference was found between different seasons when the macro-invertebrate data were directly examined using ANOSIM (see Section 6.2), Table 6.18 shows a significant difference between the number of individuals recorded and the evenness of the macro-invertebrate communities between different seasons.

The taxon richness, number of individuals, Margalef richness index, Shannon diversity index, Berger-Parker dominance index, Simpson dominance index, evenness and $ASPT_{BMWP}$ index results in both spring and summer for the survey sites located in Norfolk are presented in Figure 6.10.

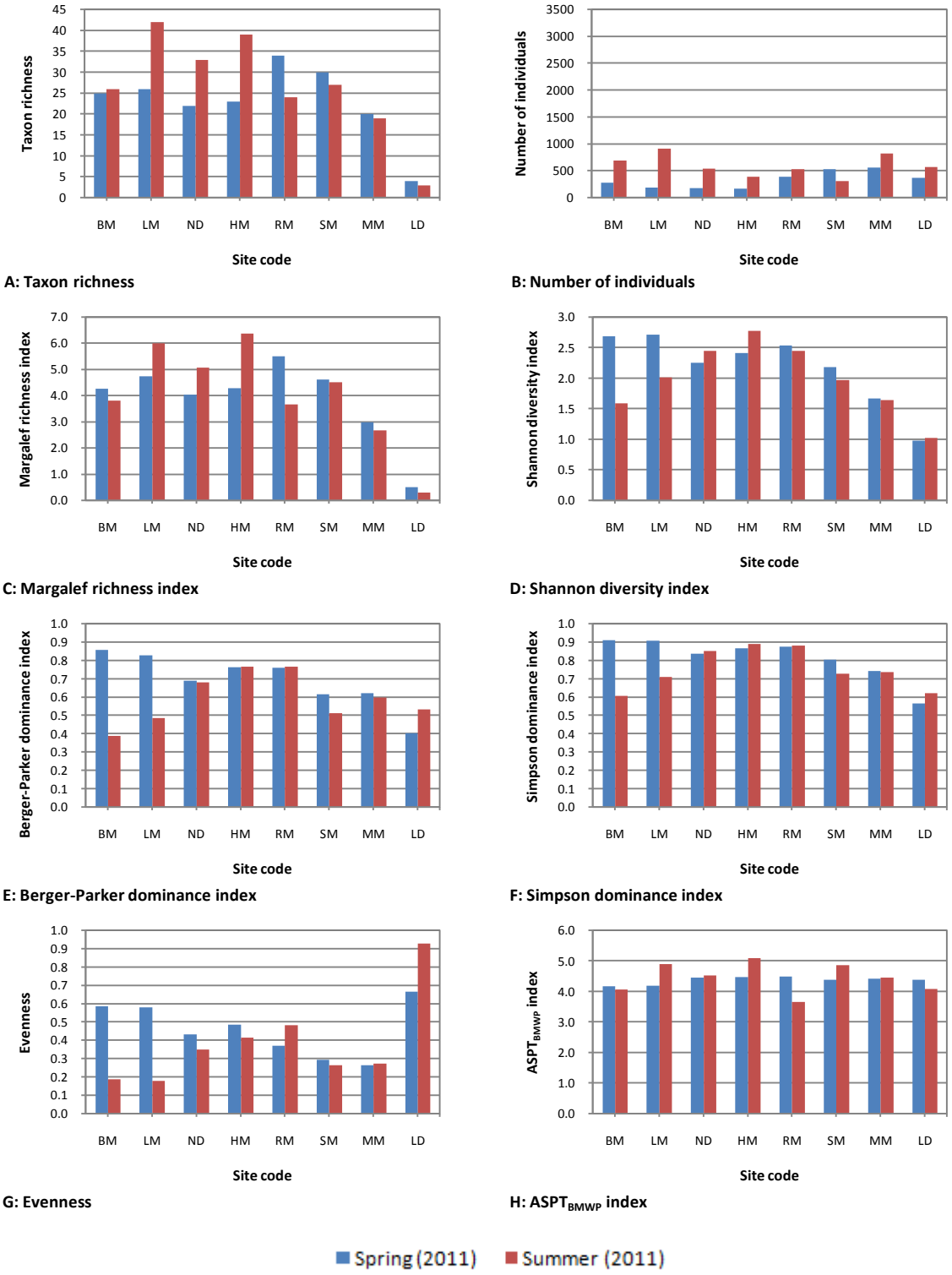


Figure 6.10: Taxon richness (A), number of individuals (B), Margalef richness index (C), Shannon diversity index (D), Berger-Parker dominance index (E), Simpson dominance index (F), evenness (G) and $ASPT_{BMWP}$ index (H) results for the Norfolk survey sites. See Table 5.1 in Section 5 for site code definitions.

Several of the macro-invertebrate diversity and water quality indices were found to be significantly correlated with each other when examined using Spearman's rank order correlation coefficient (r_s), as shown in Table 6.19 and as was found when the Lincolnshire data were examined (Table 6.17). For example, the Margalef richness index was significantly correlated with taxon richness in both spring and summer, as well as the Shannon diversity index in summer (Table 6.19). The Shannon diversity index was significantly correlated with the Simpson dominance index in both seasons and the Berger-Parker dominance index in spring (Table 6.19). Furthermore, the Berger-Parker dominance index was also significantly correlated with the Simpson dominance index in both seasons and evenness when summer data were considered (Table 6.19).

Table 6.19: Spearman's rank order correlation coefficient (r_s) and *p*-values resulting from tests between macro-invertebrate diversity and water quality indices for data collected at survey sites in Norfolk

	Taxon richness	Number of individuals	Shannon diversity index	Simpson dominance index	Evenness	Margalef richness index	Berger-Parker dominance index	$ASPT_{BMWP}$
Taxon richness		0.84 0.84	0.08 0.06	0.11 0.54	0.54 0.20	<0.01** <0.01**	0.33 0.84	0.83 0.13
Number of individuals	0.10 -0.07		0.24 0.27	0.27 0.20	0.17 0.27	0.84 0.58	0.20 0.22	0.16 0.33
Shannon diversity index	0.64 0.69	-0.45 -0.43		<0.01** <0.05*	0.54 0.84	0.06 <0.05*	<0.01** 0.08	0.42 0.70
Simpson dominance index	0.62 0.26	-0.43 -0.52	0.98 0.86		0.54 0.27	0.08 0.36	<0.01** <0.01**	0.32 0.54
Evenness	-0.26 -0.52	-0.55 -0.43	0.24 0.10	0.26 0.43		0.58 0.33	0.46 <0.05*	0.69 <0.05*
Margalef richness index	0.95 0.98	-0.07 -0.21	0.71 0.76	0.64 0.38	-0.21 -0.41		0.22 0.98	0.72 0.24
Berger-Parker dominance index	0.41 -0.10	-0.52 -0.48	0.93 0.64	0.95 0.91	0.29 0.74	0.48 0.02		0.20 0.22
$ASPT_{BMWP}$	-0.10 0.60	0.55 0.41	-0.34 0.14	-0.41 -0.26	0.17 -0.83	-0.16 0.45	-0.51 -0.50	

Spearman's rank correlation coefficient (r_s) values shown in lower left triangle, p-values shown in upper right triangle. Significant correlations indicated by * when *p*<0.05 and ** when *p*<0.01. In all cases *df*=6. Blue text relates to data collected in spring season and red text to data collected in summer season.

The macro-invertebrate communities showed substantial variation between the survey sites located in Norfolk in both spring and summer (Figure 6.10). Furthermore, seasonal differences in the macro-invertebrate diversity and water quality measures of the sites are suggested in Figure 6.10, despite no significant difference being found in the macro-invertebrate communities when examined directly by ANOSIM (see Section 6.2). Taxon richness, the Margalef richness index and the Shannon diversity index all showed increases at the RM (Rockland Marsh), SM (Strumpshaw Meadow), MM (Middle Marsh) and LD (Long Dyke) survey sites between spring and summer. The Margalef richness index and Shannon diversity index both also increased at the BM (Buckenham Marsh), with the Shannon diversity index also increasing at the LM (Ludham Marsh) survey sites between spring and summer. The number of individuals increased at all survey sites except for the SM (Strumpshaw Meadow) site between spring and summer. The Berger-Parker and Simpson dominance indices both showed decreases at the BM (Buckenham Marsh), LM (Ludham Marsh), SM (Strumpshaw Marsh) and MM (Middle Marsh) survey sites between spring and summer, with the Berger-Parker dominance index also decreasing at the ND (Near Dry Dyke) survey site. Evenness decreased at the BM (Buckenham Marsh), LM (Ludham Marsh) ND (Near Dry Dyke), HM (Hatchet Marsh) and SM (Strumpshaw Marsh) survey sites between spring and summer, whilst the $ASPT_{BMWP}$ water quality index only showed relatively small decreases at the BM (Buckenham Marsh), RM (Rockland Marsh) and the LD (Long Dyke) survey sites between spring and summer.

The macro-invertebrate diversity and water quality measures were examined using Mann-Whitney *U* tests to determine if there is a significant difference between the spring season and the summer season. Non-parametric methods were used in order to avoid making assumptions of normality and homogeneity of variance, as stated by Kay *et al*. (2001). The results of these analyses are presented in Table 6.20 and largely agree with the direct analysis of the macro-invertebrate communities using ANOSIM (see Section 6.2) in not showing a significant difference between spring and summer. The sole exception is the number of individuals recorded, which was found to be significantly different between the spring and summer seasons (Table 6.20). The same macro-invertebrate community measure was also found to be significantly different between seasons when the Lincolnshire data were examined (Table 6.18).

Table 6.20: Results of Mann-Whitney *U* tests on macro-invertebrate diversity and water quality measures from Norfolk survey sites for differences between seasons

Diversity/water quality measure	Mann-Whitney statistic (*U*)	*p*-value
Taxon richness	25	0.46
Number of individuals	9	<0.05*
Margalef richness index	31	0.96
Shannon index	25	0.49
Berger-Parker index	19	0.19
Simpson index	21	0.27
Evenness	19	0.19
$ASPT_{BMWP}$	27	0.64

*** = Significant difference.**

Overall, the differences in the number of individuals shown in Table 6.18 and Table 6.20 may result from the significant differences in the environmental variables between the different seasons (see Section 6.1) as well as the change in season.

6.2.2 Effect of Environmental Variables on Macro-invertebrate Fauna

Significant differences were found between the macro-invertebrate communities of the survey sites located within Lincolnshire and those of the survey sites located in Norfolk (see Section 6.2) that may result from the significant differences between the environmental variables (see Section 6.1) or from the general difference in the geographical location and habitat structure of the two sets of survey sites. Furthermore, subtle differences were also found the macro-invertebrate diversity and water quality measures between different seasons (see Section 6.2.1) that may also result from the significant differences in the environmental variables between the different seasons (see Section 6.1) as well as the change in season. Thus Canonical Correspondence Analysis (CCA; Ter Braak, 1986, 1987; Ter Braak & Verdonschot, 1995) was undertaken to examine the influences of the measured environmental variables on the macro-invertebrate assemblages at the survey sites.

Application of CCA results in an ordination diagram in which sample scores are positioned at the weighted averages of the taxon scores present in the sample and taxon scores are positioned at the weighted averages of the sample scores in which the taxon is present (Lelend & Fend, 1998). Environmental variables are represented in a CCA ordination diagram by vectors which point to the maximum variation of the represented variable (Ter Braak & Verdonschot, 1995; César *et al*., 2009) and the length of which is proportional to the importance of the represented environmental variable in the ordination diagram (Ter Braak & Verdonschot, 1995; César *et al*., 2009). Environmental variables with missing values were omitted from the analysis. The data for each season was examined separately by CCA and the resulting ordination diagrams are presented in Figure 6.11 for the spring season, Figure 6.12 for the summer season and Figure 6.13 for the autumn season. In addition, the eigenvalues and percentage of the variation of the taxon-environmental structure explained for the derived axes of each CCA ordination plot are presented in Table 6.21.

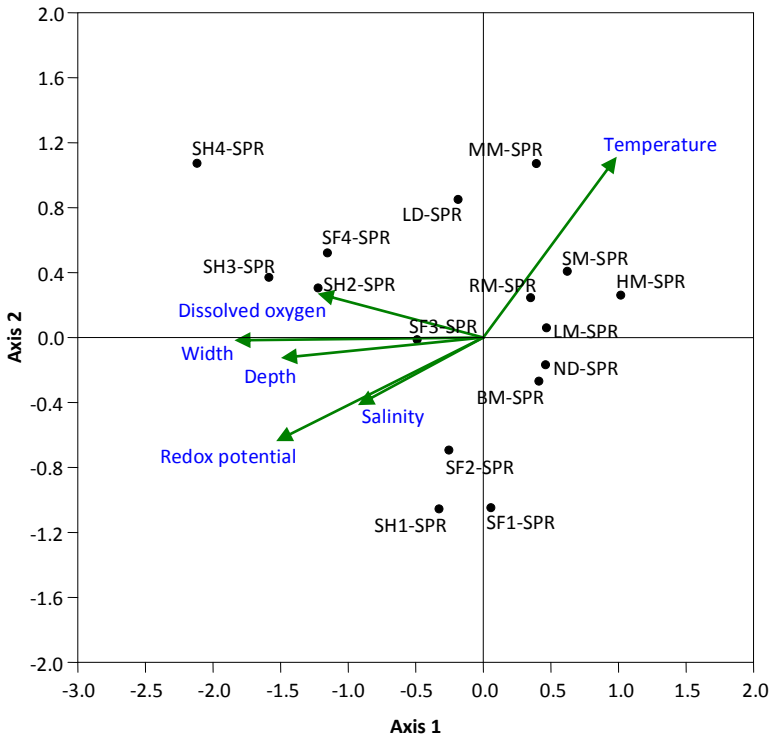


Figure 6.11: Canonical Correspondence Analysis ordination diagram from macro-invertebrate and environmental data collected during spring season. Environmental vectors are amplified by a factor of 2. Axis 1 explained 31.75% of the variation in the taxon-environmental structure, axis 2 explained 24.48% of the variation. See Table 5.1 and Table 5.2 in Section 5 for sample code definitions.

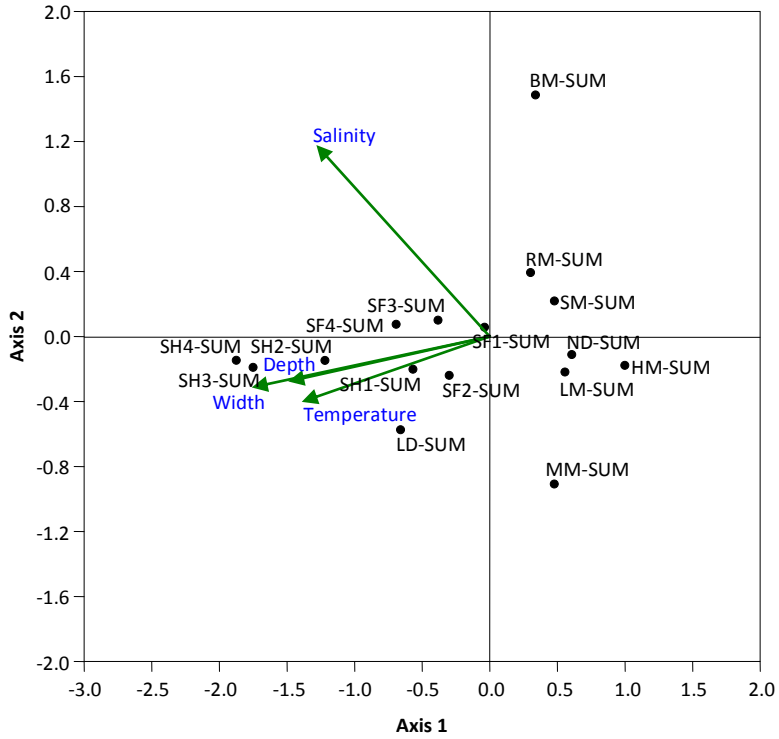


Figure 6.12: Canonical Correspondence Analysis ordination diagram from macro-invertebrate and environmental data collected during summer season. Environmental vectors are amplified by a factor of 2. Axis 1 explained 51.57% of the variation in the taxon-environmental structure, axis 2 explained 26.04% of the variation. See Table 5.1 and Table 5.2 in Section 5 for sample code definitions.

Table 6.21: Eigenvalues and variation of the taxon-environmental structure explained for the derived axes of Canonical Correspondence Analysis ordination plots

Axis	Spring		Summer		Autumn	
	Eigenvalue	Variation explained (%)	Eigenvalue	Variation explained (%)	Eigenvalue	Variation explained (%)
1	0.45	31.75	0.39	51.57	0.80	31.74
2	0.34	24.48	0.20	26.04	0.48	18.99
3	0.27	19.49	0.17	22.39	0.35	13.80
4	0.22	15.40	2.91×10^{-6}	3.86×10^{-4}	0.29	11.72
5	0.13	8.88	N/A	N/A	0.29	11.42
6	8.81×10^{-6}	6.26×10^{-4}	N/A	N/A	0.21	8.56
7	N/A	N/A	N/A	N/A	0.09	3.77

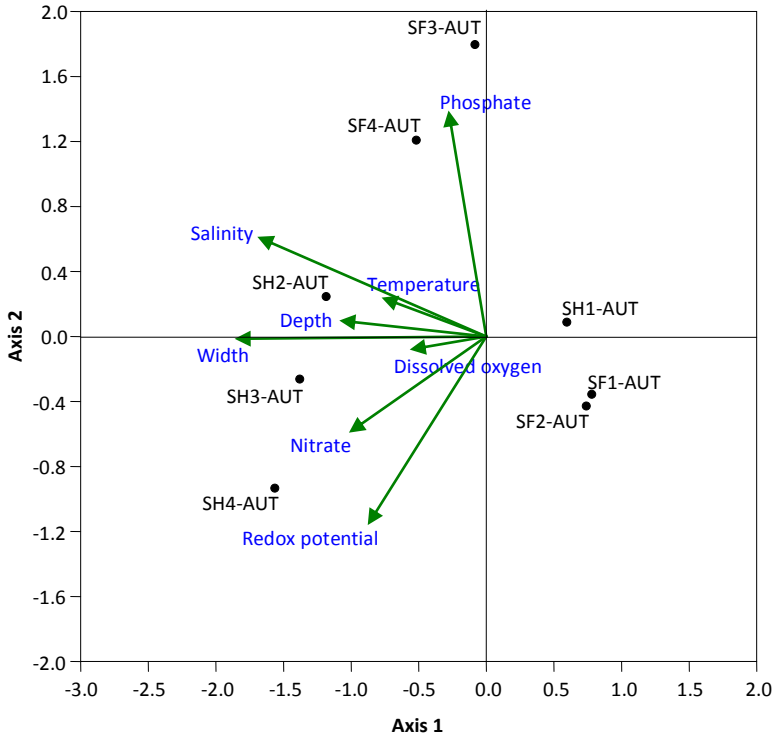


Figure 6.13: Canonical Correspondence Analysis ordination diagram from macro-invertebrate and environmental data collected during autumn season. Environmental vectors are amplified by a factor of 2. Axis 1 explained 31.74% of the variation in the taxon-environmental structure, axis 2 explained 18.99% of the variation. See Table 5.1 and Table 5.2 in Section 5 for sample code definitions.

The results displayed in Table 6.21 show that greater than 50% of the variation in taxon-environmental structure was explained by the first two axes in each of the CCA ordination diagrams. As such, the CCA diagrams provide a useful ordination of the biotic and environmental data and the patterns of variation contained therein for each season.

The CCA ordination diagrams of the biotic and environmental data collected during the spring season (Figure 6.11) and the summer season (Figure 6.12) both showed a broad continuum between the macro-invertebrate assemblages collected at the survey sites located within Lincolnshire and Norfolk along a water body width and depth gradient. Given that salinity was found to be highly significantly correlated with both variables (Figure 6.1), the macro-invertebrate assemblages may also be responding to a gradient in salinity. Furthermore, the ordination diagram for the data collected during the spring season (Figure 6.11) also indicates that redox potential and water temperature are both important variables in explaining the variation in the macro-invertebrate assemblages.

In contrast, the ordination diagram for the data collected during the summer season (Figure 6.12) indicates that salinity, as well as water body width and depth, is an important environmental variable in explaining the variation in the biotic data.

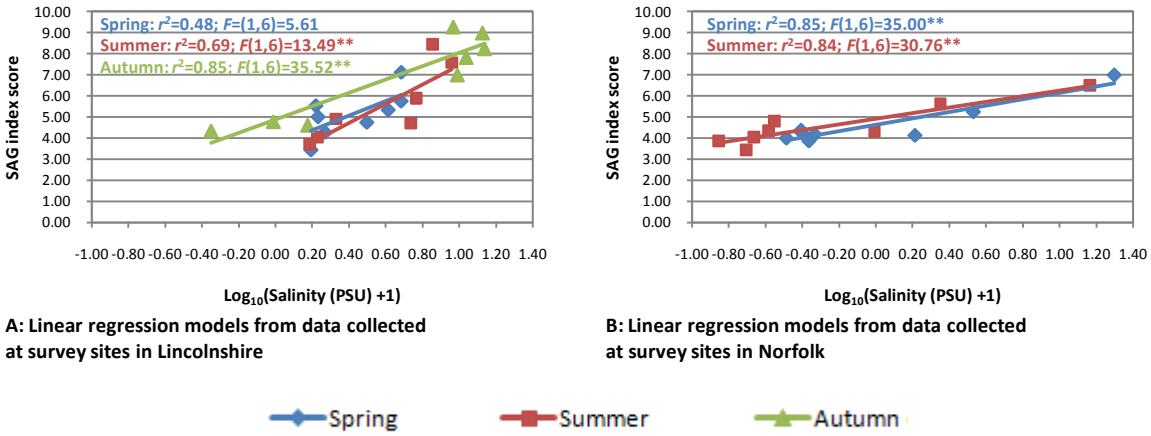
The CCA ordination diagram for the biotic and environmental data collected during the spring season (Figure 6.11) showed that macro-invertebrate samples collected at survey sites located within Lincolnshire were distinct from those collected at the survey sites located within Norfolk, as was also found by investigation of the environmental data by PCA and subsequent tests by NPMANOVA (see Section 6.1) and examination of the macro-invertebrate data by cluster analyses and subsequent tests by ANOSIM (see Section 6.1.1). This was also the case when data collected during the summer season was examined by CCA (Figure 6.12). The relatively close grouping of the samples collected at the survey sites in Norfolk during the spring season evident in the CCA diagram (Figure 6.11) indicate that macro-invertebrate assemblages and environmental data were relatively similar across these samples. In contrast, samples collected from the survey sites in Lincolnshire during the same season showed a relatively high degree of dispersion and as such suggests a greater degree of variation in the macro-invertebrate assemblages and environmental data for these samples. This pattern was reversed when biotic and environmental data collected during the summer season were examined by CCA (Figure 6.12), with samples collected from survey sites located within Lincolnshire showing a close grouping and the samples collected from survey sites located within Norfolk showing a relatively high degree of dispersion. The CCA diagrams for both the spring season (Figure 6.11) and summer season (Figure 6.12) indicate that water body width was the environmental variable that had the largest correlation with Axis 1 of the CCA, along which the samples collected from the survey sites located within Lincolnshire and those collected from the survey sites at Norfolk largely separate. It is noticeable, however, that water body depth also had a substantial correlation with Axis 1 in both CCA diagrams. As such, these results suggests that the differences in these two groups of samples largely results from differences in the habitat structure of the survey sites located within Lincolnshire, which were all large fenland drains, and those located in Norfolk which were small drainage ditches.

The CCA ordination diagram for the biotic and environmental data collected during the autumn season (Figure 6.13) showed a broad continuum between the macro-invertebrate assemblages collected at the survey sites located within Lincolnshire along a redox potential and phosphate gradient. Salinity and water body width, however, are also shown to be important variables in explaining the variation in the macro-invertebrate assemblages during the autumn season, as was also found when data for both the spring season and summer season were examined by CCA.

6.3 Examination of the Salinity Association Group Index

The Salinity Association Group (SAG) index (see Section 3) was applied to the macro-invertebrate data obtained from the survey sites and the resulting index scores were plotted against the transformed average salinity concentration recorded at the time of sampling in order to determine the accuracy of the index. Statistical differences in the environmental data were found between the survey sites in Lincolnshire and those in Norfolk (see Section 6.1). As such, the SAG index scores and associated environmental data for these two groups of survey sites were considered separately. Furthermore, due to the statistical differences that were also found between the environmental data collected in different seasons (see Section 6.1) and to determine if the SAG index is influenced by seasonality, the SAG index scores and environmental data for different seasons were also initially considered separately. Graphs of the linear regression models resulting from plotting the SAG index scores against the transformed average salinity concentration recorded at the time of sampling in order to determine the accuracy of the index are presented in Figure 6.14. The model assumptions of all linear regression models were validated by the results of the Shapiro-Wilk test for normality, Durbin-Watson test for autocorrelation and Breusch-Pagan test for homogeneity of variance on the residuals of the models (Table 6.22).

Figure 6.14 shows that the SAG index scores calculated for the macro-invertebrate samples collected at the survey sites in Lincolnshire (Figure 6.14A) and those in Norfolk (Figure 6.14B) increased linearly as the transformed salinity concentration increased in each season.



A: Linear regression models from data collected at survey sites in Lincolnshire

B: Linear regression models from data collected at survey sites in Norfolk

Figure 6.14: Linear regression models of Salinity Association Group (SAG) index scores calculated from spring, summer and autumn macro-invertebrate samples collected at the survey sites in Lincolnshire (A) and Norfolk (B) correlated against salinity concentration for each season. Correlation of determination (r^2) and *F* values for each model are shown in the top left of each graph. Significant results indicated by * when $p<0.05$ and ** when $p<0.01$. See Table 6.22 for results of model assumption validation tests on residuals.

Table 6.22: Results of model assumption validation tests on the residuals of models

Model	Number of observations	Shapiro-Wilk statistic (*p*-value)	Durbin-Watson statistic (*p*-value)	Breusch-Pagan statistic (*p*-value)
Lincolnshire, spring season	8	0.87 (0.15)	1.49(0.22)	0.22 (0.64)
Lincolnshire, summer season	8	0.97 (0.87)	1.30(1.20)	1.51 (0.22)
Lincolnshire, autumn season	8	0.96 (0.76)	1.05(0.06)	0.38 (0.54)
Norfolk, spring season	8	0.89 (0.23)	2.65(0.96)	0.57 (0.45)
Norfolk, summer season	8	0.91 (0.37)	1.00(0.13)	0.28 (0.60)

The relationship between the SAG index scores and transformed salinity when data collected from the survey sites in Lincolnshire were considered was found to be significant in summer (Pearson's product-moment correlation (r)=0.83, df=6, $p<0.05$) and highly significant in autumn (r=0.92, df=6, $p<0.01$). Furthermore, the relationship between the SAG index scores and transformed salinity when data collected from the survey sites in Lincolnshire during the spring season were considered was found to be approaching significance (r=0.70, df=6, p=0.06). Consideration of the SAG index scores and transformed salinity concentration for the survey sites in Norfolk shows a highly significant correlation in both the spring season (r=0.92, df=6, $p<0.01$) the summer season (r=0.92, df=6, $p<0.01$).

The intercept, slope and the results of significance tests on these coefficients for each linear regression model are displayed in Table 6.23.

Table 6.23: Estimated coefficients and significance test results for linear regression models of Salinity Association Group index scores and salinity concentration

Model	Intercept (*t*-statistic, *p*-value)	Slope (*t*-statistic, *p*-value)
Lincolnshire, spring season	3.65 (5.25, $p<0.01$)**	3.51 (2.37, $p=0.06$)
Lincolnshire, summer season	2.90 (3.51, $p<0.05$)*	4.55 (3.67, $p<0.05$)*
Lincolnshire, autumn season	4.88 (10.73, $p<0.01$)**	3.16 (5.88, $p<0.01$)**
Norfolk, spring season	4.62 (30.30, $p<0.01$)**	1.52 (5.92, $p<0.01$)**
Norfolk, summer season	4.92 (29.81, $p<0.01$)**	1.33 (5.55, $p<0.01$)**

Significant results indicated by * when $p<0.05$ and ** when $p<0.01$.

Salinity was found to significantly co-vary with both water body depth (r_s=0.52, df=38, $p<0.01$; see Section 6.1) and water body width (r_s=0.50, df=38, $p<0.01$; see Section 6.1). As such, partial correlations were undertaken in order to determine if salinity, and not water body depth or width, is significantly correlated with the SAG index. Given that water body depth and water body width were found to be significantly correlated (r_s=0.84, df=38, $p<0.01$; see Section 6.1) and salinity had a stronger relationship (1.3% higher) with water body depth than water body width, the partial correlations were undertaken whilst statistically controlling water body depth. The results of the partial correlation analyses on the data collected at the survey sites in Lincolnshire showed a significant correlation between the SAG index scores and transformed salinity when controlling for water body depth in both spring ($r_{(SAGI)(Salinity).Depth}$=0.83, df=5, $p<0.05$) and autumn ($r_{(SAGI)(Salinity).Depth}$=0.87, df=5, $p<0.05$), whilst the partial correlation was approaching significance when data collected at the survey sites in Lincolnshire during the summer season were considered ($r_{(SAGI)(Salinity).Depth}$=0.73, df=5, p=0.06). Examination of the data collected at the survey sites in Norfolk with partial correlation controlling for water body depth showed a highly significant correlation between the SAG index scores and transformed salinity in both spring ($r_{(SAGI)(Salinity).Depth}$=0.97, df=5, $p<0.01$) and summer ($r_{(SAGI)(Salinity).Depth}$=0.92, df=5, $p<0.01$).

6.3.1 The Influence of Season and Habitat on the Salinity Association Group Index

Figure 6.14 suggests that the Salinity Association Group (SAG) index scores showed a similar response to salinity regardless of season when data collected from the survey sites in Lincolnshire (Figure 6.14A) and those in Norfolk (Figure 6.14B) were considered separately.
The homogeneity of the regression slopes and the effect of seasonality on the relationship between the SAG index and salinity were statistically examined using a one-way ANalysis of COVAriance (ANCOVA) with transformed salinity as the covariate and season as the fixed factor. Data collected from the survey sites in Lincolnshire were considered separately from the data collected at the survey sites in Norfolk due to the statistical differences in the environmental data between these two groups of survey sites (see Section 6.1).

Examination of the data collected at the survey sites in Lincolnshire showed that assumption of homogeneity of variance for ANCOVA was not violated (Levene's test, $F(2,21)=0.03$, $p=0.97$) and that the slopes of the three regression lines in Figure 6.14A do not differ significantly (ANCOVA, $F(2,18)=0.61$, $p=0.56$). The results of the ANCOVA showed that whilst season has a significant effect on the SAG index (ANCOVA, $F(2,20)=3.92$, $p<0.05$), transformed salinity showed a highly significant correlation to the SAG index (ANCOVA, $F(1,20)=52.94$, $p<0.01$). Similar results were found when the data collected at the survey sites in Norfolk were examined; the assumption of homogeneity of variance for ANCOVA was not violated (Levene's test, $F(1,14)=0.02$, $p=0.89$), the slopes of the two regression lines in Figure 6.14B do not differ significantly (ANCOVA, $F(2,13)=0.30$, $p=0.60$) and transformed salinity showed a highly significant correlation to the SAG index (ANCOVA, $F(1,13)=69.12$, $p<0.01$). Furthermore, the results of the ANCOVA on the data collected at the survey sites in Norfolk also showed that season does not have a significant effect on the SAG index (ANCOVA, $F(1,13)=2.11$, $p=0.17$), the sole case which contrasts with the results of the examination of the data collected at the survey sites in Lincolnshire.

The results of the ANCOVA analyses and the fact that the SAG index scores are significantly correlated with salinity in each season when the data for the survey sites in Lincolnshire and the data for the survey sites in Norfolk are considered separately (see Section 6.3) justified combining the data collected during different seasons from the survey sites in Norfolk, but not the data collected in Lincolnshire.

The linear regression model of the pooled data for the survey sites in Norfolk, the assumptions of which were validated by tests on residuals for normality (Shapiro-Wilk=0.96, n=32, p=0.31), autocorrelation (Durbin-Watson=1.51, n=16, p=0.24) and homogeneity of variance (Breusch-Pagan=2.21, n=32, p=0.14), is presented in Figure 6.15.

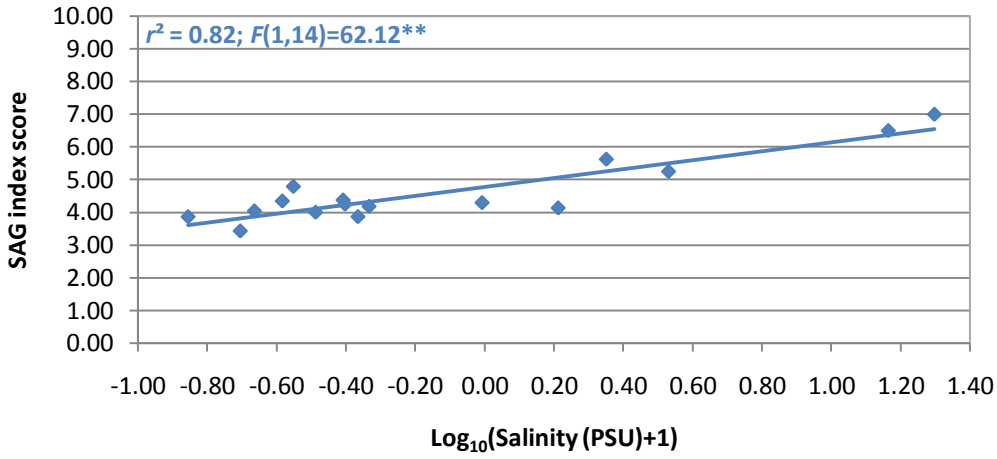


Figure 6.15: Linear regression model of Salinity Association Group (SAG) index scores calculated from macro-invertebrate samples collected at the survey sites located in Norfolk correlated against salinity concentration. Correlation of determination (r^2) and F value the model are shown in the top left of the graph. Significant results indicated by * when p<0.05 and ** when p<0.01. Assumptions of model validated by tests on residuals for normality (Shapiro-Wilk=0.96, n=16, p=0.31), autocorrelation (Durbin-Watson=1.51, n=16, p=0.24) and homogeneity of variance (Breusch-Pagan=2.21, n=16, p=0.14).

The coefficients of the regression model using data collected at the survey sites in Norfolk were found to be significant for both the intercept (4.78; t=42.74, p<0.01) and the slope (1.37; t=7.88, p<0.01). Furthermore, the model using data collected at the survey sites in Norfolk showed a highly significant relationship between the SAG index scores and transformed salinity (r=0.90, df=14, p<0.01) that remained significant when analysed using partial correlation controlling for water body depth ($r_{(SAGI)(Salinity).Depth}$=0.92, df=13, p<0.01).

Significant differences were found in the environmental data between the survey sites in Lincolnshire and those in Norfolk (see Section 6.1). As such, the effect of these differences, summed up as habitat type, on the relationship between the SAG index and salinity were statistically examined using a one-way ANCOVA with transformed salinity as the covariate and habitat type as the fixed factor.

Data collected in autumn season at the survey sites in Lincolnshire were excluded from the analysis due to the lack of comparable data for the survey sites in Norfolk in the same season and to retain a balanced design in the analysis. Furthermore, data collected in spring were analysed separately from yhose collected in the summer season to avoid any potential influence due to seasonality.The results of the analysis showed that the assumption of homogeneity of variance for ANCOVA was not violated when using the data collected in the spring season (Levene's test, $F(1,14)=2.63$, $p=0.13$) or when using the data collected in summer (Levene's test, $F(1,14)=2.46$, $p=0.14$). The results of the analyses, however, showed that the slopes of the regression lines were significantly different when data collected in the spring season were considered (ANCOVA, $F(2,13)=10.23$, $p<0.01$) and when the summer data were considered (ANCOVA, $F(2,13)=10.03$, $p<0.01$). As such, these results show that the SAG index shows a significantly different response when applied to data collected in a different habitat and in a different geographic location.

6.3.2 The Discriminative Ability of the Salinity Association Group Index

The discriminative ability of the Salinity Association Group (SAG) index was examined using all the data collected from the survey sites in both Lincolnshire and Norfolk. The SAG index scores calculated from macro-invertebrate samples were assigned to bins based on the untransformed average salinity concentration recorded at the time of the sample collection. Bin sizes were based on the brackish water classes defined by the Venice System (Battaglia, 1959) and used by the Water Framework Directive (WFD) to describe the zones of transitional waters in terms of salinity (European Commission, 2000). No data were assigned to the euhaline class (18.0- <30.0PSU), whilst only one datum was assigned to the polyhaline class (30.0- <40.0PSU). As such, both of these classes, and the one associated datum, were omitted from the analysis. The distributions of the data for the bins are presented as box plots in Figure 6.16 and show that the analysis did not detect any outliers in any of the groups of data.

The ability of the SAG index to discriminate between the brackish water zones shown in Figure 6.16 was statistically examined using a one-way ANalysis Of VAriance (ANOVA) after $\log_{10}$ transformation of the SAG index scores to meet the assumption of homogeneity of variance (Levene's test, $F(2,36)=2.64$, $p=0.09$). Furthermore, examination of the residuals for normality (Shapiro-Wilk=0.98, $n=39$, $p=0.82$) validated the use of ANOVA.

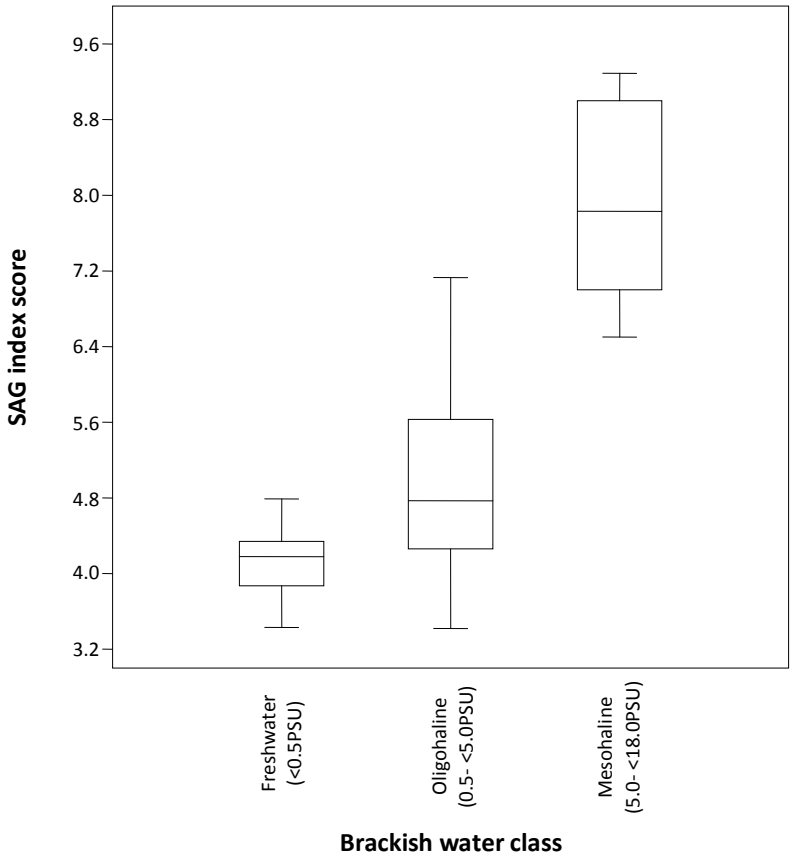


Figure 6.16: Box plots showing relationship between brackish water classes, defined by the Venice System (Battaglia, 1959) and used by the Water Framework Directive (WFD), and grouped Salinity Association Group index scores calculated all from macro-invertebrate samples. No outliers were identified.

The results of the analysis showed a highly significant difference between the SAG index scores, grouped by the brackish water zones of the Venice System (ANOVA, $F(2,36)=48.9$, $p<0.01$). Post-hoc pairwise comparisons using Tukey's Honestly Significant Difference (*HSD*) test (Kramer, 1956) showed a significant difference between the freshwater and oligohaline groups ($p<0.05$) and a highly significant difference between the oligohaline and mesohaline groups ($p<0.01$) based on the mean transformed SAG index scores of the groups.

A highly significant difference was also found between the freshwater and mesohaline groups (p<0.01).

6.3.3 The Selectivity of the Salinity Association Group Index

The selectivity of the Salinity Association Group (SAG) index was examined by performing correlations between SAG index scores and environmental variables other than salinity. Spearman's rank order correlation coefficient (r_s) was used as many of the environmental variables were not normally distributed after $\log_{10}$ (x+1) transformation (Table 6.1). Data from different survey sites and seasons were considered separately to avoid the confounding factors of season, geography and habitat type (see Section 6.1 and Section 6.3.1). The results of the analysis are displayed in Table 6.24.

Table 6.24: Spearman's rank order correlation coefficients (r_s) for correlations between Salinity Association Group index scores and environmental variables

Log_{10}(x+1) transformed environmental variable	Lincolnshire data			Norfolk data	
	Spring	Summer	Autumn	Spring	Summer
Water body width	0.96**	0.82*	0.93**	-0.05	-0.22
Water body Depth	0.80*	0.76*	0.83*	-0.67	-0.25
Water temperature	0.74*	0.64	0.40	-0.24	0.26
Dissolved oxygen	0.64	0.43	-0.10	0.10	N/A
Redox potential	-0.17	0.48	0.55	0.00	N/A
Phosphate	N/A	N/A	0.32	-0.16	0.30
Nitrate	N/A	N/A	0.44	-0.08	-0.29

Significant results indicated by * when p<0.05 and ** when p<0.01.

The results shown in Table 6.24 indicate that the SAG index was significantly correlated with both water body width and depth in all seasons when data collected at the survey sites in Lincolnshire were considered. This is unsurprising given that salinity was found to significantly co-vary with both water body depth (r_s=0.52, df=38, p<0.01; see Section 6.1) and water body width (r_s=0.50, df=38, p<0.01; see Section 6.1). In contrast, the SAG index was not significantly correlated with either width or depth when data collected at the survey sites in Norfolk were considered.

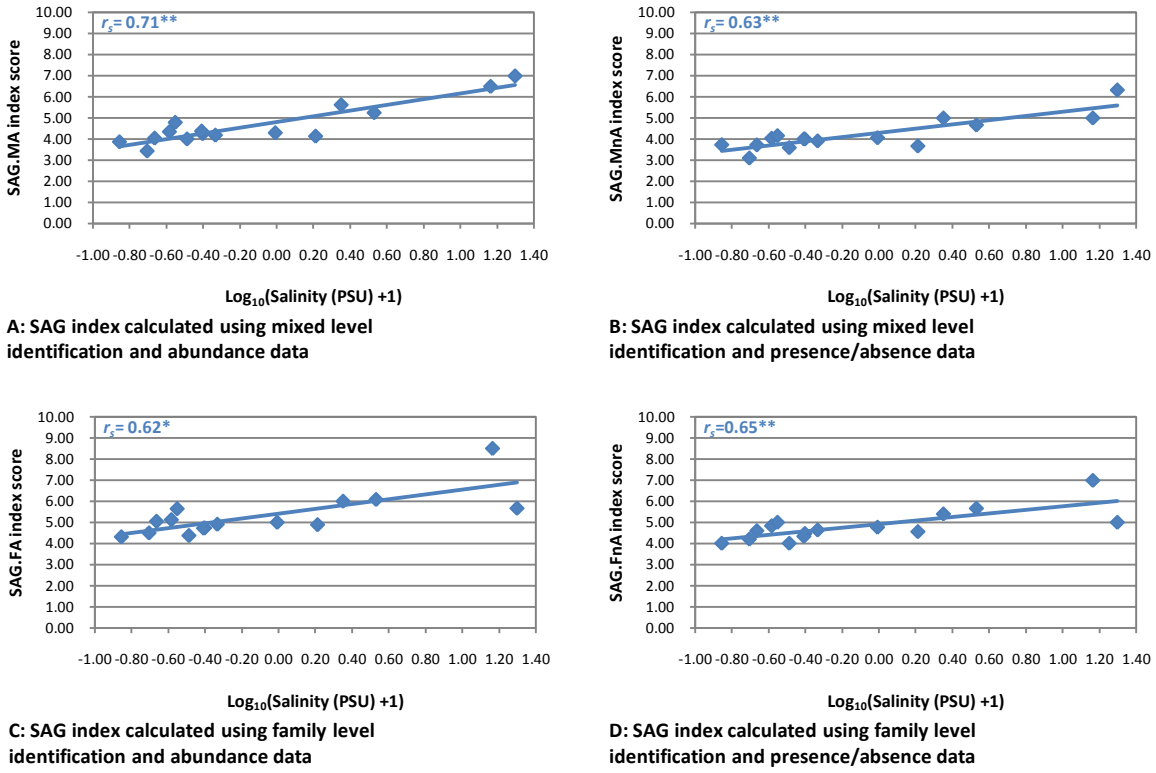
This may be explained by the fact that width and depth both varied considerably less between the survey sites in Norfolk (width, σ^2=0.39; depth, σ^2=0.16) in comparison to the survey sites in Lincolnshire (width, σ^2=70.21; depth, σ^2=0.71). The SAG index was also found to be significantly correlated with water temperature only when data collected in the spring season at the Lincolnshire survey sites were examined. Given that no other significant correlations were found between water temperature and the SAG index scores (Table 6.24), this would suggest that the significant result for water temperature and SAG index scores appears to be incidental. No other significant correlations were found by the analysis. Overall, these results indicate that the SAG index has a high selectivity towards salinity.

6.3.4 The Effect of Data Resolution on the Salinity Association Group Index

In order to assess the potential of the Salinity Association Group (SAG) index for use with data of varying degrees of resolution, SAG index scores were calculated using all the data collected at all of the survey sites located in Norfolk at mixed level taxonomic resolution both with abundance data (indicated by SAG.MA) and without abundance data (indicated by SAG.MnA). SAG index scores were also calculated at family level identification, again both with abundance data (indicated by SAG.FA) and without abundance data (indicated by SAG.FnA). Where abundance scores were not used, taxon scores were determined using the first column of the scoring matrix (Table 3.2). Families were assigned to Salinity Association Groups by calculating the average Salinity Association Group value for all the taxa assigned within a family and rounding to the nearest integer. The resulting SAG index scores were plotted against transformed salinity concentration (Figure 6.17). Spearman's rank order correlation coefficient (r_s) was calculated in each case as the assumption of homogeneity of variances of the residuals was violated for correlations using SAG index scores calculated with family level data both with abundance data (SAG.FA; Breusch-Pagan=7.82, n=16, p<0.01) and without abundance data (SAG.FnA; Breusch-Pagan=9.22, n=16, p<0.01).

The r_s values displayed in Figure 6.17 show that the SAG index scores are significantly correlated to the transformed salinity concentration (p<0.05) regardless of the resolution of the data utilised in the index calculation.

Significance tests of the correlation coefficients of the SAG index calculated using data of varying degrees of resolution showed that there was not a significant difference between the correlation coefficients in any of the pairwise comparisons (Table 6.25). Furthermore, the same results were found when the data collected from survey sites in Lincolnshire were examined in the same manner (Table 6.26).



A: SAG index calculated using mixed level identification and abundance data

B: SAG index calculated using mixed level identification and presence/absence data

C: SAG index calculated using family level identification and abundance data

D: SAG index calculated using family level identification and presence/absence data

Figure 6.17: Salinity Association Group (SAG) index calculated using data of varying resolution from Norfolk survey sites correlated against transformed salinity concentration. Index scores calculated using mixed level identification and abundance data indicated by SAG.MA (A), mixed level identification and presence/absence data indicated by SAG.MnA (B), family level identification and abundance data indicated by SAG.FA (C), and family level identification and presence/absence data indicated by SAG.FnA (D). Spearman's rank order correlation coefficient (r_s) for each correlation are shown in the top left of each graph, in all cases *df*=14. Significant results indicated by * when *p*<0.05 and ** when *p*<0.01.

Table 6.25: Results of pairwise comparisons of correlation coefficients for correlations of transformed salinity concentration and Salinity Association Group index scores calculated using varying degrees of data resolution from survey sites in Norfolk

	SAG.MA	SAG.MnA	SAG.FA
SAG.MnA	1.17		
SAG.FA	0.87	0.12	
SAG.FnA	0.64	0.10	0.79

SAG.MA indicates SAG index calculated using mixed level identification with abundance data.

SAG.MnA indicates SAG index calculated using mixed level identification without abundance data.

SAG.FA indicates SAG index calculated using family level identification with abundance data.

SAG.FnA indicates SAG index calculated using family level identification without abundance data.

Significant differences indicated by * when $p<0.05$ and ** when $p<0.01$, $df=13$ in all cases.

Table 6.26: Results of pairwise comparisons of correlation coefficients for correlations of transformed salinity concentration and Salinity Association Group index scores calculated using varying degrees of data resolution from survey sites in Lincolnshire

	Data collected in spring season			Data collected in summer season			Data collected in autumn season		
	SAG.MA	SAG.MnA	SAG.FA	SAG.MA	SAG.MnA	SAG.FA	SAG.MA	SAG.MnA	SAG.FA
SAG.MnA	2.23			1.18			0.59		
SAG.FA	1.25	1.86		0.00	1.18		0.17	0.29	
SAG.FnA	1.63	2.33	0.25	0.80	0.42	0.80	0.35	0.19	1.17

SAG.MA indicates SAG index calculated using mixed level identification with abundance data.

SAG.MnA indicates SAG index calculated using mixed level identification without abundance data.

SAG.FA indicates SAG index calculated using family level identification with abundance data.

SAG.FnA indicates SAG index calculated using family level identification without abundance data.

Significant differences indicated by * when $p<0.05$ and ** when $p<0.01$, $df=5$ in all cases.

Given that the results displayed in Figure 6.17 show that show that the SAG index scores are significantly correlated to the transformed salinity concentration (p<0.05) regardless of the resolution of the data and Table 6.25 and Table 6.26 indicate that there are no significant differences in the correlation coefficients when data of different resolution are used to calculate the SAG index, it is apparent that the SAG index can be used with less detailed information without resulting in a significant loss of accuracy.

6.4 Comparison of the Salinity Association Group Index to Published Salinity Indices

Several diagnostic indices have been proposed for the detection and determination of salinity increases in freshwater habitats. The salinity index of Horrigan *et al*. (2005), the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) and the ditch salinity index of Palmer *et al*. (2010) were applied to the macro-invertebrate data obtained from the survey sites located in Norfolk and the resulting index scores were plotted against the transformed average salinity concentration recorded at the time of sampling in order to determine the accuracy of the indices. The salinity classification system of Wolf *et al*. (2009) was not examined as this metric was developed for application in transitional waters, defined as surface waters which are characterised by salinity tidal influences (European Commission, 2000), rather than for detection and determination of saline intrusion in freshwater habitats. The chloride contamination index proposed by Williams *et al*. (1999) was not applied to the data as to the low number of taxa attributed scores made examination of this index unfeasible. The accuracy of the examined indices was compared against that of the Salinity Association Group (SAG) index, calculated using mixed level identification and abundance data, proposed in this work.

Scores resulting from the application of the salinity index of Horrigan *et al*. (2005) were plotted against the transformed salinity concentration recorded at the survey sites at the time of sampling to determine the accuracy of the index (Figure 6.18; see Appendix 12 for table of index results). Two data points were omitted from the analysis as application of the salinity index of Horrigan *et al*. (2005) to the macro-invertebrate samples for these data points did not results in an index score due to the lack of any scoring taxa.

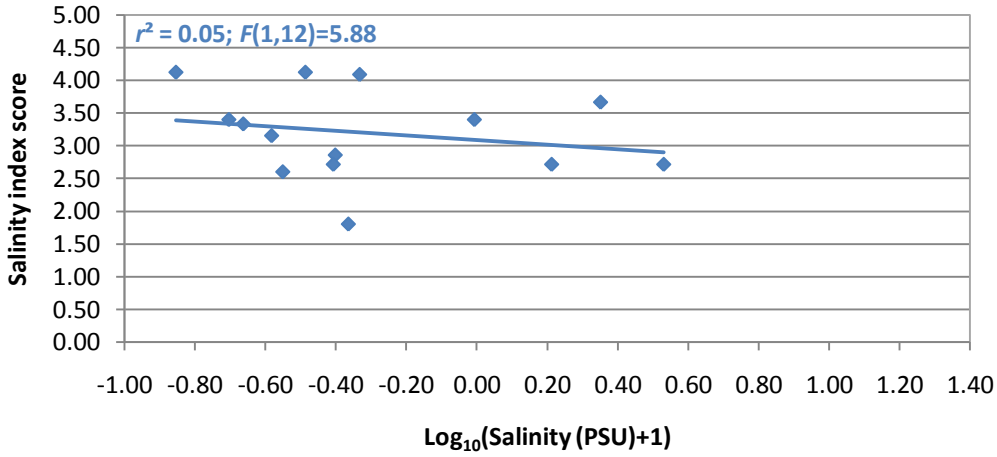


Figure 6.18: Scores for the salinity index of Horrigan *et al*. (2005) calculated using data from Norfolk survey sites correlated against transformed salinity concentration. Correlation of determination (r^2) and *F* values for the model are shown in the top left of the graph. Significant results indicated by * when $p<0.05$ and ** when $p<0.01$. Assumptions of model validated by tests on residuals for normality (Shapiro-Wilk=0.95, $n=16$, $p=0.51$), autocorrelation (Durbin-Watson=1.59, $n=16$, $p=0.28$) and homogeneity of variance (Breusch-Pagan=0.16, $n=16$, $p=0.69$).

The coefficients of the regression model for the salinity index of Horrigan *et al*. (2005) using data collected at the survey sites in Norfolk were found to be significant for the intercept (3.09; t=-13.34, $p<0.01$) but not for the slope (-0.351; t=-4.64, p=0.46). Furthermore, the model for the salinity index of Horrigan *et al*. (2005) showed a weak, non-significant relationship between index scores and transformed salinity concentration (r=-0.22, df=12, p=0.46). In comparison, application of the SAG index to the same data resulted in a highly significant relationship between SAG index scores and transformed salinity concentration (r=0.71, df=12, $p<0.01$). A comparison of the correlation coefficients for the salinity index of Horrigan *et al*. (2005), which was made positive for the analysis, and the correlation coefficient for the SAG index applied to the same data did not reveal a significant difference ($t_{Difference}$=1.77, df=11, $p>0.05$).

Scores resulting from the application of the $SPEAR_{salinity}$ metric of Schäfer *et al*. (2011) were plotted against the transformed salinity concentration recorded at the survey sites at the time of sampling to determine the accuracy of the index (Figure 6.19; see Appendix 12 for table of index results).

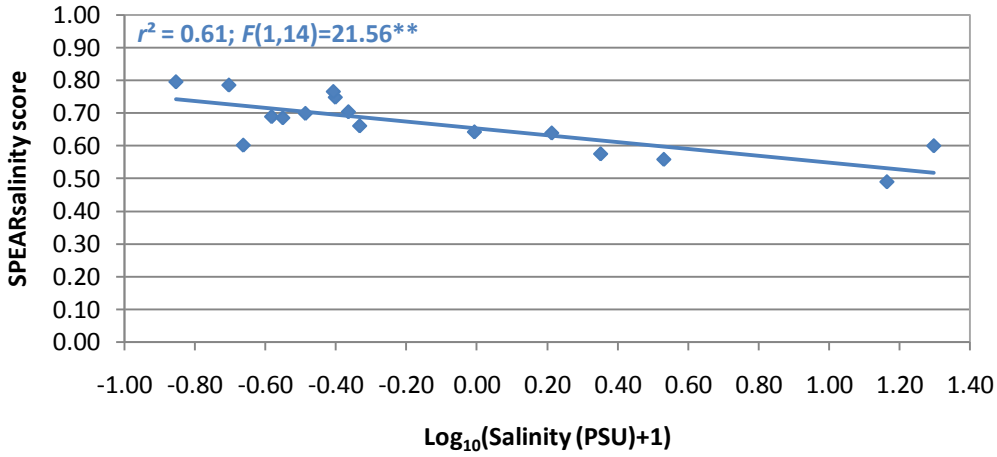


Figure 6.19: Scores for the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) calculated using data from Norfolk survey sites correlated against transformed salinity concentration. Correlation of determination (r^2) and *F* values for the model are shown in the top left of the graph. Significant results indicated by * when $p<0.05$ and ** when $p<0.01$. Assumptions of model validated by tests on residuals for normality (Shapiro-Wilk=0.93, $n=16$, $p=0.29$), autocorrelation (Durbin-Watson=1.93, $n=16$, $p=0.57$) and homogeneity of variance (Breusch-Pagan=0.08, $n=16$, $p=0.77$).

The coefficients of the regression model for the $SPEAR_{salinity}$ metric using data collected at the survey sites in Norfolk were found to be significant for both the intercept (0.65; t=45.08, p<0.01) and the slope (-0.11; t=-4.64, p<0.01). Furthermore, the model showed a highly significant relationship between the $SPEAR_{salinity}$ index scores and transformed salinity concentration (r=-0.78, df=14, p<0.01). The same result was also found when the SAG index was applied to the same data (r=0.90, df=14, p<0.01). Whilst the calculated correlation coefficient for the $SPEAR_{salinity}$ index is lower than that for the SAG index, this difference was not found to be significant ($t_{Difference}$=1.56, df=13, p>0.05).

Scores resulting from the application of the ditch salinity index of Palmer *et al*. (2010) were plotted against the transformed salinity concentration recorded at the survey sites at the time of sampling to determine the accuracy of the index (Figure 6.20; see Appendix 12 for table of index results).

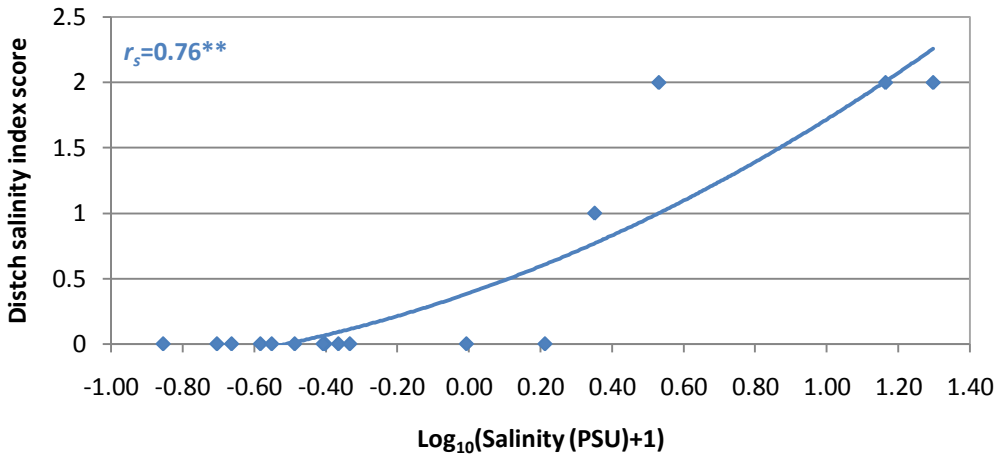


Figure 6.20: Ditch salinity index of Palmer *et al*. (2010) calculated using data from Norfolk survey sites correlated against transformed salinity concentration. Spearman's rank order correlation coefficient (r_s) is shown in the top left of the graph, *df*=14. Significant result indicated by * when *p*<0.05 and ** when *p*<0.01.

Spearman's rank order correlation coefficient (r_s) was calculated as the correlation between transformed salinity concentration and the ditch salinity index of Palmer *et al*. (2010) showed a non-linear relationship. Figure 6.20 shows a highly significant relationship between the ditch salinity index of Palmer *et al*. (2010) and transformed salinity concentration (r_s=0.76, *df*=14, *p*<0.01). In order to compare the SAG index with the ditch salinity index of Palmer *et al*. (2010), r_s values were calculated following application of the SAG index to the same data and also showed a highly significant relationship (r_s=0.71, *df*=14, *p*<0.01). Whilst the calculated correlation coefficient for the SAG index with salinity is less than that for the ditch salinity index of Palmer *et al*. (2010) with salinity, this difference was not found to be significant ($t_{Difference}$=0.40, *df*=13, *p*>0.05).

The salinity index of Horrigan *et al*. (2005), the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) and the ditch salinity index of Palmer *et al*. (2010) were examined using the data collected from the survey sites in Lincolnshire. Data collected in different seasons were considered separately to avoid the confounding influence of seasonality (see Section 6.3.1). The results of the analysis showed that the salinity index of Horrigan *et al*. (2005), $SPEAR_{salinity}$ index and the ditch salinity index of Palmer *et al*. (2010) all had weak, non-significant relationships with transformed salinity concentration in each season (Table 6.27).

Table 6.27: Correlation results of published salinity indices and the Salinity Association Group index with transformed salinity concentration using data collected at survey sites in Lincolnshire

Season	Salinity index of Horrigan *et al*. (2005)	$SPEAR_{salinity}$ index of Schäfer *et al*. (2011)	Ditch salinity index of Palmer *et al*. (2010)	Salinity Association Group index
Spring	r=-0.37, df=6, p=0.37	r=0.35, df=6, p=0.39	r_s=0.23, df=6, p=0.59	r=0.70, df=6, p=0.06
Summer	r=-0.32, df=5, p=0.48	r=-0.37, df=6, p=0.37	r_s=0.62, df=6, p=0.11	r=0.83, df=6, p<0.05*
Autumn	r=-0.25, df=3, p=0.69	r=-0.29, df=6, p=0.49	r_s=0.60, df=6, p=0.14	r=0.92, df=6, p<0.01**

Significant result indicated by * when p<0.05 and ** when p<0.01.

See Appendix 11 for table of index results.

In comparison, the SAG index was significantly correlated with transformed salinity concentration in both summer and autumn (Table 6.27). Furthermore, the correlation between the SAG index and salinity concentration was approaching significance in spring (Table 6.27).

7 DISCUSSION

The results of the current study have shown a number of significant differences between the groupings of the survey sites. Statistical differences were found between the survey sites located in Lincolnshire and those in Norfolk in both the macro-invertebrate (see Section 6.2) and environmental data (see Section 6.1). Furthermore, a significant difference between the survey sites of the South Forty Foot Drain and the South Holland Main Drain, both Lincolnshire, was found in the macro-invertebrate data (see Section 6.2) despite the lack of a corresponding difference in the environmental data between the two water bodies (see Section 6.1). Whilst a statistical difference was found between the environmental data collected in different seasons (see Section 6.1), a corresponding difference was not discovered in the macro-invertebrate data (see Section 6.2). Finally, analysis of both the environmental and macro-invertebrate data indicated that water body width and depth, redox potential, temperature, phosphate and salinity were the most important environmental variables influencing the macro-invertebrate community composition (see Section 6.2.2).

7.1 Differences Between Survey Sites in Lincolnshire and Survey Sites in Norfolk

The observed significant difference in the macro-invertebrate data between the survey sites in Lincolnshire and those in Norfolk is unsurprising given that differences in macro-invertebrate community structure resulting from geographic differences have been reported (e.g. Sponseller *et al*., 2001; Townsend *et al*., 2003) even over small spatial scales (Bunn *et al*., 1986; Downes *et al*., 1993). As such, the difference observed in the macro-invertebrate data between the two regions in the current study may result from geographic range restrictions of macro-invertebrate taxa (Hellawell, 1978; Chesters, 1980; Washington, 1984; Ode *et al*., 2008) such as those reported in, for example, Hammond (1985), Friday (1988), Savage (1989), Brooks & Lewington (1997) and Cham (2007, 2009).

Habitat structure is also known to influence the spatial distribution of freshwater invertebrate populations (Downing, 1991) and both Briers & Biggs (2005) and Zimmer *et al*. (2000) found that environmental conditions contributed to the spatial difference in macro-invertebrate community structures. Given that a significant difference between the survey sites in Lincolnshire and those in Norfolk was observed in the environmental variables, specifically water body depth, water body width and salinity, the same argument may also be applied to explain the results of the current study. The importance of salinity concentration to macro-invertebrate community structure has been highlighted in many other studies (e.g. Lancaster & Scudder, 1987; Williams, 1987; Hart *et al*., 1990, 2003; Short *et al*., 1991; Gallardo-Mayenco, 1994; Muñoz & Prat, 1994; Wollheim & Lovvorn, 1995; Williams, 2001; James *et al*., 2003; Nielsen *et al*., 2003; Piscart *et al*., 2005a; Silberbush *et al*., 2005; Velasco *et al*., 2006). Furthermore, both water body depth (e.g. Zimmer *et al*., 2000; Williams *et al*., 2003) and width (e.g. Tate & Heiny, 1995; Carter *et al*., 1996) are also known to influence macro-invertebrate community composition (Furse *et al*., 1984; Wright *et al*., 1984, 1989; Moss *et al*., 1987).

7.2 Differences Between Lincolnshire Water Bodies

Whilst no significant difference was found in the environmental data between the survey sites of the South Forty Foot Drain and the South Holland Main Drain in the current study, a significant difference was observed in the macro-invertebrate data of the two water bodies. This result corroborates the reports of differences in macro-invertebrate community composition over small spatial scales (Bunn *et al*., 1986; Downes *et al*., 1993) and there are several possible reasons for the observed difference.

Environmental conditions have been reported to influence macro-invertebrate community composition (e.g. Zimmer *et al*., 2000; Briers & Biggs, 2005). This is unlikely to explain the lack of similarity between the macro-invertebrate communities of the South Forty Foot Drain and South Holland Main Drain as no difference was observed in the environmental data collected from the two water bodies. It is recognised, however, that a difference may exist in an environmental variable that was not measured in the current study, such as the aquatic macrophyte community composition.

Macrophyte community structure is known to influence macro-invertebrate community composition (Gregg & Rose, 1985; Strayer *et al*., 2003; Warfe & Barmuta, 2006). Furthermore, the influence of such catchment scale variables as land use (Helms *et al*., 2009), surface geology (Richards *et al*., 1997; Death & Joy, 2004) anthropogenic developments adjacent to water bodies (Sponseller *et al*., 2001; Townsend *et al*., 2003) are well reported and may also explain the lack of similarity between the macro-invertebrate communities of the two water bodies. Alternatively, the observed difference between the macro-invertebrate communities of the South Forty Foot Drain and South Holland Main Drain may result from the observed geographic range restrictions of macro-invertebrate taxa (e.g. Hammond, 1985; Friday, 1988; Savage, 1989; Brooks & Lewington, 1997; Cham, 2007, 2009).

7.3 Differences Between Seasons

The absence of an observed significant difference in the macro-invertebrate between different seasons in the current study is surprising given that many insect taxa have seasonal life cycles which affect aquatic macro-invertebrate community composition (Gaufin & Tarzwell, 1952; Hynes, 1970; Furse *et al*., 1984; Wright *et al*., 1984; Soulsby *et al*., 2001). For example, Odonata species leave freshwater habitats in spring and summer to emerge as adults (Brooks & Lewington, 1997). Trichoptera larvae generally emerge as adults at varying times of spring, summer and autumn depending on the species (Wallace *et al*., 1990; Edington & Hildrew, 1995), whereas the majority of Hemiptera species overwinter as adults (Savage, 1989). Non-insect macro-invertebrate taxa have also been reported to present seasonal variations in abundance and distribution that influence community composition (Elliott & Mann, 1979; Gledhill *et al*., 1993; Rosenberg & Resh, 1993; Knoben *et al*., 1995; De Jonge *et al*., 2008). Furthermore, seasonal differences in macro-invertebrate community composition have been well reported (e.g. Bunn & Davies, 1992; Metzeling, 1993; Greenwood & Wood, 2003; Velasco *et al*., 2006; Gómez-Anaya & Novelo-Gutiérrez, 2010). The lack of a significant seasonal difference in the macro-invertebrate data collected from the survey sites in Norfolk may result from the proximity of the spring season sampling date (16^{th}-18^{th} May 2011) to the summer season sampling date (15^{th}-21^{st} June 2011).

The same rationalisation, however, cannot be used to explain the lack of a seasonal difference in the macro-invertebrate data collected from the survey sites located in Lincolnshire, where survey dates were substantially separated for the spring (29th-30th March 2010), summer (26th-28th June 2010) and autumn (8th-9th October 2011) seasons.

In contrast to the macro-invertebrate data, a significant seasonal difference was observed in the environmental variables as has been reported in many studies (e.g. Lancaster & Scudder, 1987; Bunn & Davies, 1992; Metzeling, 1993; Leland & Fend, 1998; Greenwood & Wood, 2003; Azrina *et al*., 2006; Velasco *et al*., 2006; Akbulut *et al*., 2009; Gómez-Anaya & Novelo-Gutiérrez, 2010). Specifically in the current study, seasonal differences were observed in water temperature, dissolved oxygen concentration and redox potential (see Section 6.1). The seasonal difference in water temperature is to be expected and is well reported (e.g. Lancaster & Scudder, 1987; Metzeling, 1993; Akbulut *et al*., 2009; Gómez-Anaya & Novelo-Gutiérrez, 2010). It is well known that water temperature and dissolved oxygen concentration are related (Chapman & Kimstach, 1996; Horrigan *et al*., 2005; Kefford, 1998b; Williams, 1998), despite the results of the current study which did not find such a relationship (see Section 6.1). Dissolved oxygen, along with organic compounds such as nitrates and nitrites, are among the most influential chemical species in determining the redox potential (Chapman & Kimstach, 1996; Delaune & Reddy, 2005) and as such it is little surprise that redox potential was found to co-vary with dissolved oxygen (see Section 6.1). Furthermore, these findings explain the observed significant seasonal differences in the environmental variables.

7.4 Influence of Environmental Variables on Macro-invertebrate Fauna

The results of the current study suggested that water body width and depth, redox potential, temperature, phosphate and salinity were the most important environmental variables influencing the macro-invertebrate community composition (see Section 6.2.2). Temperature is reported to influence macro-invertebrate communities (Jacobsen *et al.*, 1997; Stone & Wallace, 1998; Vinson & Hawkins, 1998; Daufresne *et al*., 2004) and as such, corroborates this result from the current study.

In comparison, no studies appear to have reported redox potential as a prominent environmental influencer of macro-invertebrate community composition. Water temperature and dissolved oxygen concentration are known to be related (Chapman & Kimstach, 1996; Horrigan *et al*., 2005; Kefford, 1998b; Williams, 1998), whilst dissolved oxygen is one of the major influencers of redox potential (Chapman & Kimstach, 1996; Delaune & Reddy, 2005) and these two environmental variables were found to significantly co-vary in the current study (see Section 6.1). Furthermore, redox potential was also found to have a highly significant relationship with both water body depth and width (see Section 6.1), both of which were also found to be important in determining macro-invertebrate community composition. As such, the finding of redox potential as a prominent environmental influencer of macro-invertebrate community composition may result from covariance between redox potential and other environmental variables found to be important in determining macro-invertebrate community composition.

Increases in phosphate concentration and other nutrients have been well reported to modify macro-invertebrate assemblages (e.g. Richardson & Qian, 1999; Smith *et al*., 1999; Parr & Mason, 2003; Steinman *et al*., 2003), as well as the wider biological community including fish and macrophytes (Carpenter *et al*., 1998), substantiating the results of the current study. Given the macro-invertebrate community composition may also be influenced by the aquatic macrophyte community structure (Gregg & Rose, 1985; Strayer *et al*., 2003; Warfe & Barmuta, 2006) and the influence of nutrient enrichment over macrophytes communities (e.g. Dawson *et al*., 1999; Holmes *et al*., 1999; Melzer, 1999; Schneider & Melzer, 2003; Haury *et al*., 2006), it is evident that macro-invertebrate communities can be influenced by phosphate concentration both directly and indirectly.

The influence of salinity on macro-invertebrate community composition was been extensively reported (e.g. Lancaster & Scudder, 1987; Williams, 1987; Hart *et al*., 1990, 2003; Short *et al*., 1991; Gallardo-Mayenco, 1994; Muñoz & Prat, 1994; Wollheim & Lovvorn, 1995; Williams, 2001; James *et al*., 2003; Nielsen *et al*., 2003; Piscart *et al*., 2005a; Silberbush *et al*., 2005; Velasco *et al*., 2006) and, in brief, increases in salinity result in halo-sensitive macro-invertebrate species decreasing in abundance until they disappear whilst halo-tolerant species become increasingly abundant (for a full account see Section 2.5).

Furthermore, both water body depth (e.g. Zimmer *et al*., 2000; Williams *et al*., 2003) and width (e.g. Tate & Heiny, 1995; Carter *et al*., 1996) are known to be important influencers of macro-invertebrate community composition to the extent the both variables are required data for the River InVertebrate Prediction And Classification System (RIVPACS) to make site-specific predictions of the expected macro-invertebrate community in the absence of stressors (Furse *et al*., 1984; Wright *et al*., 1984, 1989; Moss *et al*., 1987). As such, the findings that salinity, water body depth and width corroborates the results presented in other published studies.

7.5 The Salinity Association Group Index

The results of the current study show that the Salinity Association Group (SAG) index is significantly correlated with salinity in all cases examined except one (see Section 6.3). Only the correlation coefficient between the SAG index and salinity using data collected from the survey sites in Lincolnshire in spring was not significant, but was approaching significance. The correlation coefficients between SAG index scores and salinity using data collected at survey sites located in Norfolk were generally greater in comparison with the correlation coefficients resulting from application of the SAG index to the data collected at the survey sites in Lincolnshire. This may result from the fact that the environmental variables, other than salinity, varied considerably less at the survey sites in Norfolk. In comparison the survey sites in Lincolnshire showed considerably greater variation in the environmental variables, such as water body width and depth, and consequently the macro-invertebrate communities of these sites could also be responding to these changes (Ormerod *et al*., 2010) which are confounding the relationship between the macro-invertebrate community and salinity. The correlation coefficients between the SAG index and salinity in this study ranged from 0.70 to 0.92, with a median value also of 0.92. In comparison, Birk *et al*. (2012) examined 33 macro-invertebrate assessment techniques employed in Europe for the delivery of the Water Framework Directive in response to their respective pressure and found that 0.64 was the median correlation coefficient. As such, these results show that the SAG index is more robust than indices that are used to deliver the Water Framework Directive (WFD).

It has been stated that a good biomonitoring tool should reliably indicate changes in its respective pressure and respond only to its specific pressure(s) (Dolédec *et al*., 1999; Birk *et al*., 2012), be compatible with a sampling protocol employed by other biomonitoring tools and surveys and that a linear output is desirable to aid interpretation of results (Bonada *et al*., 2006). It is apparent that the SAG index is compatible with the sampling protocol used by the regulatory authority (see Section 3), the Environment Agency, and is compliant with the international standard BS EN ISO 10870:2012 (British Standards Institution, 2012). The same protocol is used by the Environment Agency to report water quality in biological terms by calculating number of BMWP-scoring families present in a sample ($NTAXA_{BMWP}$) and the average score per taxon ($ASPT_{BMWP}$). These indices are further employed to assess water bodies in accordance with the requirements of the WFD (WFD-UKTAG, 2008). Furthermore, the results of this study show that the SAG index has a linear relationship with $\log_{10}(X+1)$ transformed salinity, as such meeting this recommendation of Bonada *et al*. (2006). The results of this study have also shown that the SAG index is highly selective to salinity (see Section 6.3.3). Although significant correlations between SAG index scores and both water body width and depth were also found when data collected from survey sites in Lincolnshire were examined, these correlations could be explained by significant covariance between these two variables and salinity. Furthermore, examination of the data collected in Norfolk, where both water body depth and width showed substantially less variation, found that SAG index scores were significantly correlated only with salinity.

A broad geographic application has been defined as a requirement of a good biomonitoring tool (Dolédec *et al*., 1999; Bonada *et al*., 2006). The results of the current study show that the SAG index is highly correlated to salinity when applied to data collected in Lincolnshire and when applied to data collected in Norfolk, demonstrating the applicability of the SAG index across this geographic region. It was, however, found that the SAG index showed a different response to salinity when applied to data collected in Lincolnshire in comparison to when applied to data collected in Norfolk (see Section 6.3.1). Schäfer *et al*. (2011) found similar results during the examination of the $SPEAR_{salinity}$ index, producing different regression models for application in Victoria, Australia and South Australia. Furthermore, geographic dependence is a common criticism of biotic indices (Rosenberg & Resh, 1993).

Whilst it is possible that the difference shown by the SAG index when applied in different geographic regions results from the resident macro-invertebrate fauna showing local adaptations to salinity, this is unlikely as it has been shown that salinity tolerances of macro-invertebrate taxa are similar regardless of geographic sampling location (Kefford *et al*., 2003; Dunlop *et al*., 2008). The observed difference in the response of the SAG index to salinity when applied to data collected in Lincolnshire in comparison to when applied to data collected in Norfolk may result from the greater variability in other environmental variables observed at the survey sites in Lincolnshire confounding the response of the SAG index, or may result from the difference in the macro-invertebrate communities between the survey sites in Lincolnshire and those in Norfolk. Variation in the macro-invertebrate community resulting from geographical differences has been shown to influence the scores of biotic indices (e.g. Hellawell, 1978; Chesters, 1980; Washington, 1984; Ode *et al*., 2008). Alternatively, the observed difference may result from the observed significant difference in habitat structure between the survey sites in Lincolnshire and those in Norfolk (see Section 7.1). This is supported by the fact that comparable results were also obtained by Horrigan *et al*. (2005) and Schäfer *et al*. (2011) in examinations of their respective indices. Horrigan *et al*. (2005) produced separate regression models for samples collected from edge habitats and riffle habitats. Similarly, Schäfer *et al*. (2011) developed models to examine the $SPEAR_{salinity}$ metric with samples collected from riffle habitats and pool habitats separately.

The issue of geographical dependence shown by the SAG index could potentially be resolved through the use of predictive multivariate approaches such as River InVertebrate Prediction And Classification System (RIVPACS; Wright *et al*., 1984; Moss *et al*., 1987; Wright, 2000). Such approaches are able to derive site and season-specific predictions of the macro-invertebrate fauna that would be present in the absence of environmental and physical stressors which can be used to calculate observed/expected index score ratios that are standardised across both site and season (Wright *et al*., 1998; Wright, 2000; Clarke *et al*., 2003), thus negating both geographical and seasonal influences.

7.5.1 Influence of Season on the Salinity Association Group Index

Seasonal dependence is one of the main issues related to the use of biotic indices (Rosenberg & Resh, 1993; Zamora-Muñoz *et al*., 1995; Šporka *et al*., 2006) and it has been shown that many biological metrics are susceptible to seasonal differences (e.g. Zamora-Muñoz *et al*., 1995; Leunda *et al*., 2009; Álvarez-Cabria *et al*., 2010; Johnson *et al*., 2012). To quantify this, Šporka *et al*. (2006) examined 76 biotic indices and found that 31 of the metrics exhibited statistically significant seasonal variations. It has been shown in the current study that the Salinity Association Group (SAG) index also exhibited significant seasonal variation when examined using the data collected in Lincolnshire. When examined using the data collected in Norfolk, however, the SAG index showed no significant difference between the spring and summer seasons. It is recognised that the lack of significant difference in this case may result from the proximity of the spring season sampling date (16^{th}-18^{th} May 2011) to the summer season sampling date (15^{th}-21^{st} June 2011) for the survey sites in Norfolk. Nonetheless, use of such predictive multivariate approaches as River InVertebrate Prediction And Classification System (RIVPACS; Wright *et al*., 1984; Moss *et al*., 1987; Wright, 2000) to produce site- and season-standardised observed/expected index score ratios could potentially resolve the issue of the seasonal dependence shown by the SAG index.

The life histories of the macro-invertebrate taxa have been related to the seasonal variations in biotic indices (Soulsby *et al*., 2001; Šporka *et al*., 2006; Johnson *et al*., 2012). It has long been recognised that many insect taxa have seasonal life cycles which influence aquatic macro-invertebrate community composition throughout the year (Hynes, 1970; Furse *et al*., 1984; Wright *et al*., 1984; Soulsby *et al*., 2001). It has also been recognised that non-insect macro-invertebrate taxa also present well-defined seasonal variations in abundance and distribution (Rosenberg & Resh, 1993; Knoben *et al*., 1995). Whilst seasonal differences in the macro-invertebrate communities were evident in the current study these differences were not found to be significant (see Section 6.2.1). The seasonal variation in macro-invertebrate community composition may also result from seasonal changes in physical and environmental variables (Marchant, 1982) such as hydrological regime, light levels, temperature and water chemistry (Šporka *et al*., 2006). Significant seasonal differences were found in the environmental variables measured in this study (see Section 6.1).

7.5.2 Discriminative Ability of the Salinity Association group Index

Statzner *et al*. (2005) stated that a viable biotic monitoring tool should be able to assign samples to groups correctly in around 70% of instances. The results of the ANOVA and post-hoc Tukey Honestly Significant Difference tests in this study (see Section 6.3.2) showed that the Salinity Association Group (SAG) index successfully and significantly discriminates between all of the examined brackish water classes defined by the Venice System (Battaglia, 1959) and used by the Water Framework Directive (WFD; European Commission, 2000). It is apparent, however, that there are overlaps in the SAG index scores between the brackish water classes. The overlaps between the brackish water classes may result from the examined macro-invertebrate communities responding to multiple pressures (Ormerod *et al*., 2010). Further potential sources of error in the SAG index include the lack of accounting of the integrating ability of long-lived macro-invertebrate species, as well as the inability of the sampling protocol to fully collect a sample representative of the maximum diversity of a location.

Whilst sensitive macro-invertebrate species respond rapidly to environmental changes (Wright, 1994; Barbour *et al*., 1999; Iliopoulou-Georgudaki *et al*., 2003), the overall community responds more slowly and long-lived species indicate the integrated effects of environmental changes over a period of time (Cook, 1976; Milbrink, 1983; Hellawell, 1986; Olive *et al*., 1988; Cuffney *et al*., 1993; Rosenberg & Resh, 1993; Knoben *et al*., 1995; Friedrich *et al*., 1996; Barbour *et al*., 1999). The sampling protocol employed to attain environmental data, however, only provided a snapshot of the environmental conditions at the time of sample collection (Hynes, 1960; Hellawell, 1986; Wright, 1994; Knoben *et al*., 1995; Vrana *et al*., 2002) and, as such, does not represent conditions of the same period of time as the integrated picture provided by the macro-invertebrate data.

The macro-invertebrate sampling protocol employed in the current study was the procedure defined within the UK Technical Advisory Group methodology for macro-invertebrate sampling and analysis (Murray-Bligh *et al*., 1997). It has been stated, however, that this procedure collects around 70% of the macro-invertebrate families present at a site (Chadd, 2010), whilst a separate study found that the same procedure collected approximately 62% of families and 50% of species in comparison to the collection of six replicate samples at the same site (Furse *et al*., 1981).

As such, it is likely the employment of a more rigorous sampling protocol which collects data indicating close to the maximum diversity of a site would, in turn, result in more accurate metric scores. The sampling procedure, however, is used by the regulatory authority, the Environment Agency, to collect invertebrate data to assess water bodies in accordance with the requirements of the WFD (WFD-UKTAG, 2008) and is compliant with the international standard BS EN ISO 10870:2012 (British Standards Institution, 2012).

7.5.3 Effect of Data Resolution on the Salinity Association Group Index

The advantages and disadvantages of taxonomic resolution have been examined in numerous studies. Family level identification has been recognised to be both quicker and less expensive than identification to species level (Armitage *et al*., 1990), is likely to result in less identification errors and does not require extensive taxonomic expertise (Furse *et al*., 1984; Bailey *et al*., 2001). Furthermore, several studies have reported similar results when comparing family-level data with species- or genus-level data (e.g. Kefford, 1998b; Clements *et al*., 2000; Chessman *et al*., 2007). Salinity Association Group (SAG) index scores calculated using family level and mixed (species, genus and family) level identification in this study were all significantly correlated to the transformed salinity concentration (p<0.05) there were no significant differences in the correlation coefficients between the SAG index scores calculated at family level and mixed level identification (see Section 6.3.4). As such, these results indicate that the SAG index can be used with less detailed information without resulting in a significant loss of accuracy. Armitage *et al*. (1990) stated, however, that identification to species level produces the most detailed ecological data. Both Melo (2005) and Chessman *et al*. (2007) stated that the use of greater resolution is justified for the detection of subtle impacts, whilst Jones (2008) concluded in a review that species level identification should be the default for bioassessment purposes. Furthermore, increased taxonomic resolution was a proposal made by Horrigan *et al*. (2005) to improve the accuracy and precision of their index.

For example, Pond *et al*. (2008) found that correlations between a genus-level multimetric index and water-quality variables were stronger than correlations between the family-level multimetric index and those variables in an investigation of mining disturbance on West Virginia streams, whilst Hawkins *et al*. (2000) found that predictive models based on species-level data gave better predictions of watershed alterations by logging than models based on family-level data. Furthermore, Extence *et al*. (1999) found that Lotic-invertebrate Index for Flow Evaluation (LIFE) scores obtained from family level data were more weakly correlated with flow rate than scores obtained using species level data. The decreases in the r_s values in the relationship between SAG index scores and salinity as data resolution decreases, albeit non-significant, suggest that where saline effects are subtle, the use of the best taxonomic resolution with the SAG index is justified and support the findings of Extence *et al*. (1999), Melo (2005) and Chessman *et al*. (2007).

It has also been stated that changes in the number of individuals of each taxon are considered to be more significant than changes in the lists of taxa present at a location (Hynes, 1960). Furthermore, recognition of abundance in the assessment of water quality is also a requirement of the Water Framework Directive (WFD; European Commission, 2000). Extence *et al*. (1999) examined the effect of using presence/absence data instead of relative abundance data in the calculation of LIFE scores and found that the resulting LIFE scores exhibited weaker correlations with flow rate. In a study comparing the use of numeric and presence/absence resolutions, Melo (2005) also concluded that using simplified data in local datasets results in a significant loss of information. SAG index scores calculated with and without abundance data in this study showed no significant differences in correlation coefficients were all significantly correlated to the transformed salinity concentration (p<0.05; see Section 6.3.4). It was apparent, however, that there is a substantial, although non-significant, difference between the correlation coefficients of SAG index scores calculated at mixed-level identification with and without abundance data. These results indicate that the use of abundance data with the SAG index is recommended to detect subtle saline effects, and as such tend to support the conclusions of Extence *et al*. (1999) and Melo (2005).

7.5.4 Comparison of the Salinity Association Group Index to Published Salinity Indices

The current study has shown that the salinity index of Horrigan *et al*. (2005) had a weak, non-significant relationship between index scores and transformed salinity concentration when examined with both data collected at survey sites in Norfolk and data collected in Lincolnshire (see Section 6.4). Both the ditch salinity index of Palmer *et al*. (2010) and the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) were found to be significantly correlated with transformed salinity concentration when examined using the data collected in Norfolk (see Section 6.4). Neither of these indices, however, were found to be significantly correlated to transformed salinity concentration when examined using the data collected in separate seasons at the survey sites located in Lincolnshire (see Section 6.4). In comparison, the Salinity Association Group (SAG) index proposed in the current study was found to be significantly correlated with salinity in all cases examined except one (see Section 7.5). Despite this, no significant difference was found in the correlation coefficients of index scores and salinity for any of the published metrics and the SAG index.

Palmer *et al*. (2010) specifically developed the ditch salinity index for use with the macro-invertebrate assemblages present in the ditches of coastal and flood plain grazing marshes. Given that the survey sites in Norfolk were all drainage ditches located on grazing marshes whilst the survey sites in Lincolnshire were all large fenland drains, thus not suitable habitats for application of this index, the results shown in this study related to the examination of the index of Palmer *et al*. (2010) are not unexpected. There are also several possible explanations for the lack of a significant correlation between salinity concentration and both the index of Horrigan *et al*. (2005) and the $SPEAR_{salinity}$ metric. The index scores attributed to the taxa in the development of both indices were derived from macro-invertebrate data collected in Australia where the metrics were both originally developed and assessed. Whilst many macro-invertebrate families found in Australia are also present in the United Kingdom, few species are common to both countries. For example, Abell *et al*. (2008) attributed areas of the United Kingdom and Australia to distinctly different regions whilst delineating global freshwater ecoregions based on the distinctness of the freshwater communities and species present in the freshwater systems within each region.

Consequently, the scoring taxa of the both the index of Horrigan *et al*. (2005) and the $SPEAR_{salinity}$ metric will most likely have different index values when considered in the United Kingdom. Biotic index scores are known to be influenced by variation in macro-invertebrate communities resulting from geographical differences (e.g. Hellawell, 1978; Chesters, 1980; Washington, 1984; Ode *et al*., 2008). Reassignment of index values to macro-invertebrate taxa based on locally collected data may improve the performance of both the $SPEAR_{salinity}$ index and the salinity index of Horrigan *et al*. (2005) in the United Kingdom. Horrigan *et al*. (2005) also proposed increased taxonomic resolution to improve accuracy and precision of the salinity index, the use of which has been shown to improve the performance of metrics in several studies (Extence *et al*., 1999; Hawkins *et al*., 2000; Pond *et al*., 2008). The same proposal may also improve the accuracy of the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011). Integration of abundance data, which has also been shown to improve the accuracy of index scores (e.g. Extence *et al*., 1999; Melo, 2005), may also improve the index of Horrigan *et al*. (2005).

Further issues are also related to the salinity index of Horrigan *et al*. (2005) It appears that during the initial examination of the index, Horrigan *et al*. (2005) used the same data to both derive the salinity sensitivity scores for taxa and to examine the validity of the index (see Section 2.9) thus introducing bias into the assessment of the metric. As such, it is possible that the initial examination of the salinity index of Horrigan *et al*. (2005) produced a false positive result and the lack of functionality of the index has been shown in the current study. Alternatively, the taxa could have been assigned the erroneous Salinity Sensitivity Scores and the examination of the index undertaken by Horrigan *et al*. (2005) may not have detected this fault. Horrigan *et al*. (2005) also reported that the index produces odd values when fewer than 15 macro-invertebrate families are present in a sample. No sample collected during this study possessed more than 13 families. The use of increased taxonomic resolution would likely increase the number of scoring taxa used in the index calculation and thus also resolve this issue. Any one, or a combination, of these reasons may explain the failure of the salinity index of Horrigan *et al*. (2005) to function as designed when applied to the data collected in this study.

The Water Framework Directive (WFD) requires the recognition of abundance in the assessment of water quality (European Commission, 2000), whilst compatibility with the sampling protocol used by other biomonitoring tools and surveys (Bonada *et al*., 2006), reliable indication of change in the targeted pressure (Dolédec *et al*., 1999; Birk *et al*., 2012) and a linear output (Bonada *et al*., 2006) have all been also defined as requirements or desirable benefits of good biomonitoring tools. The results of the current study have shown that whilst the salinity index of Horrigan *et al*. (2005) has a linear output and can potentially be used with the sampling protocol employed by the regulatory authority in England, the metric does not reliably indicate changes in salinity concentration (see Section 6.4) and also does not recognise abundance data. The ditch salinity index of Palmer *et al*. (2010) does not produce a linear output and whilst the metric was significantly correlated with transformed salinity concentration when examined using the data collected in Norfolk, it was not found to reliably indicate changes in salinity when examined using the data collected in separate seasons at the survey sites located in Lincolnshire (see Section 6.4). The ditch salinity index of Palmer *et al*. (2010) shows potential for use with the sampling protocol employed by the regulatory authority but, as with the salinity index of Horrigan *et al*. (2005), does not recognise abundance data. The $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) has been shown in the current study to have a linear output and, as with the ditch salinity index of Palmer *et al*. (2010), was also found to reliably indicate changes in salinity concentration when examined using data in Norfolk but not when examined using data collected in Lincolnshire (see Section 6.4). Furthermore, the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) requires the use of fully quantitative abundance data which, whilst meeting this particular requirement of the WFD, is currently incompatible with the sampling and sorting protocols used by England's regulatory authority. Whilst it is recognised that further development of the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011), the ditch salinity index of Palmer *et al*. (2010) and the salinity index of Horrigan *et al*. (2005) may remedy their respective deficiencies, none of the published salinity indices would be considered to be suitable for use in England for the purposes of the WFD in their current state. In comparison, the current study shows that the Salinity Association group (SAG) index reliably indicates changes in salinity concentration and has a linear output, recognises abundance data and is compatible with the sampling and sorting protocols of the regulatory authority in England (see Section 7.5).

8 CONCLUSIONS

Water is widely regarded as the world's most essential natural resource (Vörösmarty *et al*., 2010) and, in addition to the direct economic value of such services as fishing, irrigation, transportation, farming of aquatic plants and animals, industrial purposes and power production (Strayer & Dudgeon, 2010) provided by freshwater bodies, the ecosystem services provided by the freshwater environment beneficial to human populations have been conservatively estimated to have a global value greater than US$1.7 trillion per year (Costanza *et al*., 1997). Salinisation of freshwater habitats has reportedly affected an area of 950 million hectares (Hart *et al*., 1990) and is considered to be is an issue with global implications (Williams, 2001), with increases in salinity occurring in temperate, arid and semi-arid regions around the globe (Williams, 1987, 1999, 2001; Ghassemi *et al.*, 1995; Brock *et al*., 2005). Increased salinities can be a natural feature of inland waters (Hart *et al*., 1990; Metzeling, 1993; Gallardo-Mayenco, 1994; Williams, 1999; Velasco *et al*., 2006; Dunlop *et al*., 2008), but may also result from anthropogenic actions such as the disposal of industrial (Short *et al*., 1991; Kowalik & Obarska-Pempkowiak, 1997; Piscart *et al*., 2005a; Echols *et al*., 2009; Wolf *et al.*, 2009) and urban effluents (Williams, 1987, 2001), application and subsequent washing of road salts into nearby freshwater habitats (Williams *et al*., 1999; Blasius & Merritt, 2002; Kaushal *et al*., 2005) and the disturbance of natural hydrological cycles (Pillsbury, 1981; Hart *et al.*, 1990; Williams & Aladin, 1991; Goetsch & Palmer, 1997; Williams, 1999, 2001; Kay *et al.*, 2001; Marshall & Bailey, 2004).

Salinisation of freshwater habitats can have serious detrimental effects on the environment as salt sensitive taxa are replaced by salt tolerant taxa, resulting in an overall loss in biodiversity (Williams, 1999, 2001; Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003). The Water Framework Directive (WFD) requires Member States to restore all freshwater habitats to "good ecological status" where the biological communities are only slightly different from that which would be present in undisturbed conditions (European Commission, 2000; UKTAG, 2007; Moss, 2008) and to prevent the deterioration of those waters already classified as in good status (European Commission, 2000; Kallis & Butler, 2001; Griffiths, 2002; UKTAG, 2007).

Macro-invertebrates are widely used as indicators of river condition (Kay *et al*., 2001; Azrina *et al*., 2006; Li *et al*., 2010) for a variety of reasons (see Section 2.7.1.4) and have been designated a key biological element in the assessment of aquatic habitats by the WFD (European Commission, 2000). A review of the available literature, however, found that few macro-invertebrate-based biotic indices have been developed for the detection and determination of salinity increases in freshwater habitats (see Section 2.9). Furthermore, none of these indices appeared to be suitable for application in the United Kingdom for the purposes of the WFD. To this end, a biotic index based on the aquatic macro-invertebrate community response to changes in salinity, termed the Salinity Association Group (SAG) index, was developed.

The potential of the SAG index for assessing water quality in terms of salinity in freshwater systems was investigated using data collected from survey sites in Lincolnshire and Norfolk, England. The influence exerted by salinity and other environmental features on the aquatic macro-invertebrate communities at these survey sites were investigated. Salinity indices proposed by Horrigan *et al*. (2005), Palmer *et al*. (2010) and Schäfer *et al*. (2011) were also examined using the same data and the results of these published indices were compared against that of the SAG index.

8.1 The Salinity Association Group Index

The Salinity Association Group (SAG) scores resulting from application of the index to the macro-invertebrate data were significantly related to salinity in all cases examined except one (see Section 6.3), exhibiting a stronger relationship than macro-invertebrate indices employed for the purposes of the Water Framework Directive (WFD) in Europe (see Section 7.5). Furthermore, the SAG index was found to significantly discriminate between the salinity classes defined by the WFD (see Section 6.3.2). Despite the results showing that water temperature, phosphate water body depth and width, as well as salinity, were the major influencers of macro-invertebrate community structure in the current study (see Section 6.2.2), the SAG index was found to be highly selective to only salinity concentration (see Section 7.5).

The result finding the SAG index showed geographic (see Section 7.5) and seasonal dependence (see Section 7.5.1) corroborates the current knowledge regarding macro-invertebrate index dependence on geographical location (e.g. Hellawell, 1978; Chesters, 1980; Washington, 1984; Ode *et al*., 2008) and season (e.g. Zamora-Muñoz *et al*., 1995; Leunda *et al*., 2009; Álvarez-Cabria *et al*., 2010; Johnson *et al*., 2012). Whilst undesirable, both of these potential issues could be resolved by application of the SAG index with such predictive models as the River InVertebrate Prediction And Classification System (RIVPACS; Wright *et al*., 1984; Moss *et al*., 1987; Wright, 2000). Given the aforementioned findings, it was concluded that the SAG index is a viable biomonitoring tool suitable for use in England for the purposes of the WFD, application to research and for informing aquatic habitat management decisions.

It was determined that the SAG index scores remain significantly related to salinity even when the index is applied without abundance data and using family level identification instead of greater taxonomic resolution (see Section 6.3.4). As such, it was concluded that the SAG index can be used with less detailed information without resulting in a severe loss of accuracy, illustrating the potential for the SAG index to be used by non-experts in macro-invertebrate identification. Furthermore, this finding adds further weight to the conclusion that the SAG index is suitable for application for the WFD. Although the decreases in the correlation coefficients as less detailed information was used to calculate SAG scores were non-significant, it was concluded that the use of abundance data and the best taxonomic resolution is justified where saline effects are subtle.

8.2 Comparison of the Salinity Association Group Index to Published Salinity Indices

The current study found that the salinity index of Horrigan *et al*. (2005), the ditch salinity index of Palmer *et al*. (2010) and the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) were not significantly related to salinity concentration when examined using the data collected in separate seasons at the survey sites located in Lincolnshire (see Section 6.4), although both the ditch salinity index of Palmer *et al*. (2010) and the $SPEAR_{salinity}$ index of Schäfer *et al*. (2011) showed a significant relationship with salinity when examined using the data collected in Norfolk (see Section 6.4).

It was concluded that the findings relating to the index proposed by Palmer *et al*. (2010) results from the fact that the metric was designed for use in a specific habitat, namely coastal and flood plain grazing marsh ditches. It was also concluded that the respective findings relating to the metrics proposed by Horrigan *et al*. (2005) and Schäfer *et al*. (2011) is largely due to the difference in macro-invertebrate species present in Australia and the United Kingdom.

A comparison of the SAG index with the published indices found that the SAG index surpasses the other metrics in terms of recognising abundance as required by the Water Framework Directive (WFD), reliably indicating changes in salinity, compatibility with sampling protocols employed by England's regulatory authority and producing a linear output. As such, it was concluded that the SAG index is the superior metric for the detection and determination of salinity increases in freshwater habitats.

8.3 Limitations

The macro-invertebrate sampling protocol employed in the current study was the same as that defined within the UK Technical Advisory Group methodology for macro-invertebrate sampling and analysis (Murray-Bligh *et al*., 1997) and employed by the regulatory authority, the Environment Agency, in the assessment of water bodies for the Water Framework Directive (WFD; WFD-UKTAG, 2008). It has been reported that the protocol collects approximately 60-70% of the macro-invertebrate families (Furse *et al*., 1981; Chadd, 2010) and 50% of species (Furse *et al*., 1981) present at a site. The employment of a more rigorous sampling protocol which collects data indicating close to the maximum diversity of a site may result in more accurate metric scores. Nonetheless, the procedure recommended by the UK Technical Advisory Group and employed by the Environment Agency for WFD purposes is compliant with the international standard BS EN ISO 10870:2012 (British Standards Institution, 2012).

It is well reported that the macro-invertebrate community reflects the integrated environmental condition over a period of time (Cook, 1976; Milbrink, 1983; Hellawell, 1986; Olive *et al*., 1988; Cuffney *et al*., 1993; Rosenberg & Resh, 1993; Knoben *et al*., 1995; Friedrich *et al*., 1996; Barbour *et al*., 1999). The sampling protocol employed in the current study, however, comprised of only one visit to collect environmental data.

As such, these data only provided a snapshot of the environmental conditions at the time of sample collection (Hynes, 1960; Hellawell, 1986; Wright, 1994; Knoben *et al*., 1995; Vrana *et al*., 2002) and does not reflect the environmental conditions of the same period of time as provided by the macro-invertebrate data. A more temporally encompassing method of environmental data collection may reveal further issues or benefits associated with the SAG index and could also be used to further refine the metric.

9 FURTHER WORK

Macroinvertebrate taxa were assigned to the Salinity Association Groups (SAGs) following an extensive literature review (see Section 3). Assignment of taxa using primary data, such as testing the salinity tolerances of macro-invertebrate taxa or using field records of taxa and associated salinity records taken at the same time as macro-invertebrate community samples, may enhance the legitimacy and accuracy of the Salinity Association Group (SAG) index. As such, a comparison between the index scores obtained following application of the SAG index based on the literature taxa assignments and the SAG index based on the field data taxa assignments is also recommended.

The collection of a greater quantity of macro-invertebrate data with associated salinity measurements recorded simultaneously, followed by a detailed examination of the data, would allow the identification of the macro-invertebrate taxa with wide salinity tolerances. These taxa appear to detract accuracy from the SAG index by only generating noise when used within the index. Thus identification of these taxa and their removal from the SAG index may improve the accuracy of index.

The SAG index has so far only been tested in the east of England. Thus testing of the SAG index on a much larger geographical scale, such as in Wales and Scotland, would provide a much more rigorous examination of the index's validity. Adaptation of the SAG index for application in another country, such as Spain or Australia, followed by an examination of the validity of the adapted index would also illustrate the potential of the SAG index for worldwide adaptation and application. Furthermore, intercalibration of the SAG index to harmonise WFD reference conditions and class boundaries across Europe would allow the application of the SAG index throughout Europe for the purpose of the WFD.

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11 GLOSSARY OF TERMS

Anthropogenic (secondary) salinisation: The increase in salinity in water resulting from human activities (Williams, 1999; Marshall & Bailey, 2004).

Benthic macro-invertebrate: Macro-invertebrate that dwells on the bottom of an aquatic habitat (Velasco *et al*., 2006).

Bio-indication: A concept whereby the fauna and flora indicate the status of environmental parameters such as organic pollution (Wolf *et al*., 2009).

Biota: ogranisms (Velasco *et al*., 2006).

Euhaline: Water with mean annual salinity in the range 30PSU to <40PSU (European Commission, 2000).

Euryhaline: The ability to tolerate a wide range of salinities (Wolf *et al*., 2009).

Freshwater: Water with mean annual salinity less than 0.5PSU (European Commission, 2000).

Groundwater: Water that is below the surface of the ground and in direct contact with the ground or subsoil (European Commission, 2000).

Halo-sensitive: Sensitive to increases in salt concentration (Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003).

Halo-stratification: The process whereby saline water sinks below fresh water due to the difference in density (Dyer, 1973; Davidson *et al*., 1991).

Halo-tolerant: Tolerant of high salt concentrations (Hart *et al.*, 2003; James *et al*., 2003; Nielsen *et al*., 2003).

Hypersaline: Term to describe salinities greater than 100PSU (Velasco *et al*., 2006).

Kahle's solution: A solution for the preservation of larvae and adult macro-invertebrates, composed of 1 part glacial acetic acid, 4 parts formaldehyde, 7.5 parts 95% ethyl alcohol and 15 parts water (Gennard, 2007).

Mesohaline: Water with mean annual salinity in the range 5PSU to 18PSU (European Commission, 2000).

Oligohaline: Water with mean annual salinity in the range 0.5PSU to 5PSU (European Commission, 2000).

Polyhaline: Water with mean annual salinity less than 18PSU to 30PSU (European Commission, 2000).

Primary (natural) salinisation: The increase in salinity in water due to natural factors and occurring at rates unaffected by human activities (Williams, 2001)

Salinisation: The process whereby the salinity of water increases (Hart *et al.*, 1990; Williams *et al*., 1991; Horrigan *et al*., 2005; Velasco *et al*., 2006).

Total dissolved solids: The concentration of all dissolved material in a water sample (Goetsch & Palmer, 1997).

Transitional waters: Bodies of surface water which are partly saline as a result of their proximity to coastal waters, but which are substantially influenced by freshwater flows (European Commission, 2000).

12 APPENDIX

Appendix 1: List of Taxa Assignments to Salinity Association Groups

The following is a list of macro-invertebrate taxa and the Salinity Association Group (SAG) to which they were assigned.

Tricladida					
Planariidae	**II**				
Crenobia alpina	II	*Planaria torva*	III	*Polycelis nigra*	I
Polycelis tenuis	I				
Dugesiidae	**II**				
Dugesia lugubris	II	*Dugesia polychroa*	II	*Dugesia tigrina*	I
Dendrocoelidae	**II**				
Dendrocoelum lacteum	II				
Nemertea					
Tetrastemmatidae	**III**	*Tetrastemma melanocephalum*	IV		
Prostoma sp.	II				
Lineidae	**V**				
Lineus longissimus	V	*Lineus ruber*	III	*Lineus viridis*	V
Ramphogordius sanguineus	V				
Amphiporidae	**V**				
Amphiporus lactifloreus	V				
Gastropoda					
Trochidae	**V**	**Patellidae**	**V**	**Cerithiidae**	**IV**
Gibbula cineraria	V	*Patella vulgata*	V	*Bittium reticulatum*	IV
Littorinidae	**IV**				
Lacuna pallidula	IV	*Lacuna vincta*	IV	*Littorina littorea*	V
Littorina saxatilis	III				
Nassariidae	**IV**	**Neritidae**	**II**		
Nassarius reticulatus	IV	*Theodoxus fluviatilis*	II		
Viviparidae	**I**				
Viviparus contectus	I	*Viviparus viviparus*	I		
Valvatidae	**I**				
Valvata cristata	I	*Valvata piscinalis*	I		

Hydrobiidae	**III**				
Hydrobia acuta	IV	*Peringia ulvae*	IV	*Potamopyrgus antipodarum*	II
Ventrosia ventrosa	III				
Bithyniidae	**II**				
Bithynia leachii	I	*Bithynia tentaculata*	II		
Assimineidae	**III**	**Muricidae**	**V**	**Buccinidae**	**IV**
Assiminea grayana	III	*Nucella lapillus*	V	*Buccinum undatum*	IV
Ellobiidae	**IV**				
Auriculinella bidentata	IV	*Ovatella myosotis*	IV		
Physidae	**II**				
Physa fontinalis	II				
Lymnaeidae	**II**				
Galba truncatula	II	*Lymnaea stagnalis*	II	*Radix auricularia*	II
Radix balthica	II	*Stagnicola palustris*	II		
Planorbidae	**II**				
Anisus spirorbis	V	*Anisus vortex*	II	*Bathyomphalus contortus*	I
Gyraulus albus	II	*Gyraulus crista*	II	*Gyraulus laevis*	I
Hippeutis complanatus	I	*Planorbarius corneus*	I	*Planorbis carinatus*	I
Planorbis planorbis	II				
Ancylidae	**I**	**Acroloxidae**	**I**		
Ancylus fluviatilis	I	*Acroloxus lacustris*	I		
Bivalvia					
Mytilidae	**IV**	**Ostreidae**	**V**		
Mytilus edulis	IV	*Ostrea edulis*	V		
Cardiidae	**IV**				
Cerastoderma edule	IV	*Cerastoderma glaucum*	III	*Parvicardium exiguum*	IV
Parvicardium ovale	V				
Veneridae	**V**	**Mactridae**	**V**	**Scrobiculariidae**	**III**
Venerupis senegalensis	V	*Spisula subtruncata*	V	*Scrobicularia plana*	III
Semelidae	**IV**				
Abra alba	IV	*Abra tenuis*	III		
Corbulidae	**IV**	**Tellinidae**	**III**		
Corbula gibba	IV	*Macoma balthica*	III		
Myidae	**IV**				
Mya arenaria	III	*Mya truncata*	IV		

Pholadidae	**V**				
Barnea candida	V				
Unionidae	**I**				
Anodonta anatina	I	*Anodonta cygnea*	II	*Pseudanodonta complanata*	I
Unio pictorum	I				
Sphaeriidae	**II**				
Pisidium amnicum	II	*Pisidium casertanum*	II	*Pisidium subtruncatum*	I
All other *Pisidium* sp.	I				
Dreissenidae	**III**				
Dreissena polymorpha	II	*Mytilopsis leucophaeata*	III		
Corbiculidae	**II**				
Corbicula fluminea	II				
Sessilia					
Balanidae	**IV**				
Balanus improvisus	II	*Semibalanus balanoides*	V		
Elminiidae	**IV**				
Elminius modestus	IV				
Polychaeta					
Aphroditidae	**IV**				
Aphrodita aculeata	IV				
Polynoidae	**V**				
Gattyana cirrhosa	V	*Harmothoe extenuata*	V	*Harmothoe imbricata*	IV
Harmothoe impar	V	*Lepidonotus squamatus*	IV		
Sigalionidae	**V**				
Sthenelais boa	V				
Phyllodocidae	**IV**				
Eteone longa	IV	*Eulalia viridis*	IV	*Mysta picta*	V
Glyceridae	**V**				
Glycera tridactyla	V				
Hesionidae	**V**				
Kefersteinia cirrata	V	*Magelona mirabilis*	V		
Syllidae	**V**				
Eusyllis blomstrandi	V				
Nereidae	**IV**				
Neanthes (Attila) virens	IV	*Nereis (Hediste) diversicolor*	III	*Nereis pelagica*	V
Perinereis cultrifera	V	*Platynereis dumerilii*	V		

Nephtyidae	**V**				
Nephtys caeca	IV	*Nephtys cirrosa*	V	*Nephtys hombergii*	IV
Nephtys longosetosa	V				
Cirratulidae	**V**				
Cirratulus cirratus	V	*Cirriformia tentaculata*	V	*Tharyx marioni*	IV
Orbiniidae	**IV**				
Scoloplos (Scoloplos) armiger	IV				
Spionidae	**IV**				
Malacoceros fuliginosus	V	*Polydora ciliata*	IV	*Pygospio elegans*	III
Scolelepis foliosa	V	*Scolelepis squamata*	IV	*Spiophanes bombyx*	V
Streblospio shrubsoli	III				
Capitellidae	**IV**				
Capitella capitata	IV	*Heteromastus filiformis*	IV		
Arenicolidae	**IV**	**Opheliidae**	**IV**	**Oweniidae**	**V**
Arenicola marina	IV	*Ophelia rathkei*	IV	*Owenia fusiformis*	V
Pectinariidae	**V**				
Lagis koreni	V				
Sabellariidae	**V**				
Sabellaria alveolata	V	*Sabellaria spinulosa*	V		
Ampharetidae	**V**				
Alkmaria romijni	IV	*Melinna palmata*	V		
Terebellidae	**V**				
Eupolymnia nebulosa	V	*Lanice conchilega*	V	*Neoamphitrite figulus*	V
Sabellidae	**IV**				
Manayunkia aestuarina	III	*Myxicola infundibulum*	IV	*Sabella pavonina*	V
Serpulidae	**V**				
Hydroides norvegicus	V	*Pomatoceros triqueter*	V	*Protula tubularia*	V
Hirudinea					
Piscicolidae	**II**				
Piscicola geometra	II				
Glossiphoniidae	**II**				
Glossiphonia sp.	I	*Helobdella stagnalis*	II	*Hemiclepsis marginata*	II
Theromyzon tessulatum	II				
Erpobdellidae	**I**				
Erpobdella octoculata	II	*Erpobdella testacea*	I	*Trocheta bykowskii*	I
Trocheta subviridis	I				

Araneae					
Cybaeidae	**II**				
Argyroneta aquatica	II				
Decapoda					
Palaemonidae	**III**				
Palaemon elegans	IV	*Palaemon longirostris*	III	*Palaemonetes varians*	III
Crangonidae	**III**	**Portunidae**	**IV**	**Astacidae**	**I**
Crangon crangon	III	*Carcinus maenas*	IV	*Austropotamobius pallipes*	I
Cambaridae	**I**				
Orconectes limosus	I	*Procambarus clarkii*	I		
Mysidacea					
Mysidae	**IV**				
Gastrosaccus spinifer	V	*Mesopodopsis slabberi*	III	*Neomysis integer*	III
Praunus flexuosus	V	*Praunus inermis*	V	*Schistomysis ornata*	V
Schistomysis spiritus	V				
Isopoda					
Gnathiidae	**IV**				
Paragnathia formica	IV				
Asellidae	**II**				
Asellus aquaticus	II	*Proasellus meridanus*	I		
Anthuridae	**III**	**Cirolanidae**	**IV**	**Janiridae**	**III**
Cyathura carinata	III	*Eurydice pulchra*	IV	*Jaera nordmanni*	III
Sphaeromatidae	**IV**				
Lekanesphaera monodi	IV	*Lekanosphaera hookeri*	III	*Lekanosphaera rugicauda*	III
Sphaeroma serratum	IV				
Idoteidae	**IV**				
Idotea balthica	IV	*Idotea chelipes*	III	*Idotea granulosa*	IV
Amphipoda					
Corophiidae	**III**				
Corophium arenarium	III	*Corophium curvispinum*	III	*Corophium insidiosum*	IV
Corophium multisetosum	III	*Corophium volutator*	III		
Talitridae	**IV**				
Orchestia cavimana	IV	*Orchestia gammarellus*	IV		
Crangonyctidae	**II**	**Haustoriidae**	**III**		
Crangonyx pseudogracilis	II	*Haustorius arenarius*	III		

Gammaridae	**II**				
Gammarus chevreuxi	II	*Gammarus duebeni*	III	*Gammarus lacustris*	I
Gammarus locusta	IV	*Gammarus oceanicus*	IV	*Gammarus pulex*	I
Gammarus salinus	III	*Gammarus tigrinus*	II	*Gammarus zaddachi*	II
Melitidae	**III**	**Hyalidae**	**V**		
Melita palmata	III	*Hyale prevostii*	V		
Pontoporeiidae	**III**				
Bathyporeia pelagica	III	*Bathyporeia pilosa*	III	*Bathyporeia sarsi*	III
Chilopoda					
Geophilidae	**II**				
Strigamia maritima	II				
Ephemeroptera					
Baetidae	**I**				
Alainites muticus	I	*Baetis fuscatus*	I	*Baetis rhodani*	I
Baetis scambus	I	All other *Baetis sp.*	I	*Centroptilum luteolum*	I
Cloeon dipterum	II				
Cloeon simile	I				
Heptageniidae	**I**				
Heptagenia sulphurea	I	*Rhithrogena semicolorata*	I		
Leptophlebiidae	**I**	**Ephemeridae**	**I**	**Ephemerellidae**	**I**
Paraleptophlebia submarginata	I	*Ephemera danica*	I	*Serratella ignita*	I
Caenidae	**I**				
Caenis horaria	II	*Caenis luctosa*	I	*Caenis pseudorivulorum*	I
Caenis pusilla	I	All other *Caenis* sp.	I		
Plecoptera					
Nemouridae	**I**				
Nemoura cinerea	I	*Nemoura erratica*	II	*Protonemura* sp.	I
Leuctridae	**I**				
Leuctra fusca	I	*Leuctra geniculata*	I		
Perlodidae	**I**				
Isoperla grammatica	II				
Perlidae	**II**				
Dinocras cephalotes	II	*Perla bipunctata*	II		
Odonata					
Platycnemididae	**II**				
Platycnemis pennipes	II				

Coenagrionidae	**II**				
Coenagrion puella	II	*Enallagma cyathigerum*	II	*Ischnura elegans*	II
Ischnura pumilio	II				
Lestidae	**II**				
Lestes dryas	II	*Lestes sponsa*	II		
Calopterygidae	**I**				
Calopteryx splendens	I	*Calopteryx virgo*	I		
Gomphidae	**I**	**Cordulegastridae**	**I**		
Gomphus sp.	I	*Cordulegaster boltonii*	I		
Aeshnidae	**II**				
Aeshna grandis	II	*Aeshna juncea*	I	*Aeshna mixta*	III
Hemianax ephippiger	II				
Libellulidae	**III**				
Libellula depressa	II	*Libellula quadrimaculata*	II	*Orthetrum cancellatum*	II
Pantala flavescens	IV	*Sympetrum danae*	II	*Sympetrum nigrescens*	II
Sympetrum sanguineum	II	*Sympetrum striolatum*	II		
Hemiptera					
Hebridae	**I**	**Hydrometridae**	**I**		
Hebrus ruficeps	I	*Hydrometra stagnorum*	I		
Veliidae	**I**				
Microvelia pygmaea	I	*Microvelia reticulata*	II	*Velia (Plesiovelia) caprai*	I
Velia (Plesiovelia) saulii	I				
Gerridae	**I**				
Aquarius najas	I	*Gerris argentatus*	I	*Gerris costae*	I
Gerris gibbifer	I	*Gerris lacustris*	I	*Gerris odontogaster*	I
Gerris thoracicus	II				
Nepidae	**II**				
Nepa cinerea	II				
Naucoridae	**II**				
Ilyocoris cimicoides	II	*Naucoris maculatus*	I		
Aphelocheiridae	**I**				
Aphelocheirus aestivalis	I				
Notonectidae	**II**				
Notonecta glauca	II	*Notonecta maculata*	I	*Notonecta obliqua*	I
Notonecta viridis	III				

Corixidae	**I**				
Arctocorisa carinata	I	*Arctocorisa germari*	I	*Callicorixa praeusta*	I
Callicorixa wollastoni	I	*Corixa affinis*	II	*Corixa dentipes*	I
Corixa panzeri	II	*Corixa punctata*	II	*Cymatia bonsdorffii*	I
Cymatia coleoptrata	I	*Glaenocorisa propinqua*	I	*Hesperocorixa castanea*	I
Hesperocorixa linnaei	II	*Hesperocorixa moesta*	I	*Hesperocorixa sahlbergi*	II
Micronecta poweri	I	*Paracorixa concinna*	II	*Sigara distincta*	I
Sigara dorsalis	II	*Sigara falleni*	II	*Sigara fossarum*	I
Sigara iactans	I	*Sigara lateralis*	II	*Sigara limitata*	I
Sigara nigrolineata	I	*Sigara scotti*	I	*Sigara selecta*	III
Sigara semistriata	II	*Sigara stagnalis*	III	*Sigara venusta*	I

Coleoptera

Haliplidae	**II**				
Haliplus apicalis	II	*Haliplus confinis*	II	*Haliplus lineatocollis*	II
All other *Haliplus* sp.	II				
Noteridae	**III**				
Noterus clavicornis	III				
Dytiscidae	**II**				
Agabus conspersus	III	*Agabus didymus*	I	*Colymbetes fuscus*	III
Dytiscus circumflexus	II	*Graptodytes pictus*	I	*Hydroporus palustris*	II
Hydroporus pubescens	II	*Hydroporus tessellatus*	II	*Hygrotus impressopunctatus*	I
Hygrotus parallellogrammus	III	*Ilybius subaeneus*	II	*Laccophilus hyalinus*	II
Rhantus frontalis	III	*Rhantus suturalis*	III		
Gyrinidae	**II**				
Gyrinus caspius	II	*Gyrinus marinus*	II	*Gyrinus substriatus*	II
Orectochilus villosus	I				
Helophoridae	**II**				
Helophorus alternans	II	*Helophorus brevipalpis*	I	*Helophorus fulgidicollis*	III
Helophorus granularis	II	*Helophorus minutus*	III		
Hydrophilidae	**II**				
Anacaena limbata	II	*Berosus affinis*	III	*Cercyon depressus*	III
Cercyon littoralis	III	*Enochrus bicolor*	III	*Enochrus melanocephalus*	II
Hydrobius fuscipes	II	*Laccobius atratus*	II	*Laccobius bipunctatus*	II
Hydraenidae	**III**				
Hydraena testacea	I	*Ochthebius auriculatus*	III	*Ochthebius dilatatus*	III
Ochthebius marinus	III	*Ochthebius punctatus*	III	*Ochthebius viridis*	III
Scirtidae	**I**	**Dryopidae**	**I**		
Hydrocyphon deflexicollis	I	*Dryops* sp.	I		

Elmidae	**I**				
Elmis aenea	I	*Esolus parallelepipedus*	I	*Limnius* sp.	I
Oulimnius sp.	I	*Riolus subviolaceus*	I		
Heteroceridae	**III**				
Augyles maritimus	III				
Megaloptera					
Sialidae	**I**				
Sialis fuliginosa	I	*Sialis lutaria*	I	*Sialis nigripes*	I
Trichoptera					
Rhyacophilidae	**I**				
Rhyacophila dorsalis	I	*Rhyacophila munda*	I		
Glossosomatidae	**II**				
Agapetus delicatulus	I	*Agapetus fuscipes*	I	*Glossosoma boltoni*	II
Glossosoma conformis	II				
Hydroptilidae	**I**				
Hydroptila sp.	I	*Ithyrichia lamellaris*	I		
Philopotamidae	**II**				
Chimarra sp.	I	*Philopotamus montanus*	II		
Psychomyiidae	**I**				
Lype reducta	I	*Psychomyia pusilla*	I	*Tinodes waeneri*	I
Ecnomidae	**II**				
Ecnomus tenellus	II				
Polycentropodidae	**I**				
Cyrnus trimaculatus	I	*Neurecilpsis bimaculata*	I	*Plectrocnemia geniculata*	II
Polycentropus flavomaculatus	II	All other *Polycentropus* sp.	I		
Hydropsychidae	**II**				
Cheumatopsyche lepida	II	*Hydropsyche contubernalis*	I	*Hydropsyche instabilis*	II
Hydropsyche pellucidula	I	*Hydropsyche siltalai*	II		
Phryganeidae	**I**	**Brachycentridae**	**II**	**Lepidostomatidae**	**I**
Oligotricha striata	I	*Brachycentrus subnilus*	II	*Lepidostoma* sp.	I
Limnephilidae	**I**				
Halesus radiatus	I	*Limnephilus affinis*	III	*Limnephilus decipiens*	I
Limnephilus flavicornis	I	*Limnephilus rhombicus*	I	*Potamophylax cingulatus*	I
Goeridae	**II**				
Goera pilosa	I	*Silo pallipes*	II		

Sericostomatidae	**II**	**Odontoceridae**	**II**	**Molannidae**	**I**
Sericostoma personatum	II	*Odontocerum albicorne*	II	*Molanna* sp.	I
Leptoceridae	**I**				
Adicella reducta	I	*Athripsodes aterrimus*	I	*Athripsodes cinerus*	I
Ceraclea annulicornis	I	*Ceraclea* sp.	I	*Leptocerus tineiformis*	I
Mystacides azurea	I	*Mystacides longicornis*	II	*Oecetis ochracea*	II

Lepidoptera

Pyralidae	**II**				
Elophila nymphaeata	II				

Diptera

Athericidae	**I**	**Ceratopogonidae**	**II**	**Culicidae**	**II**
Dixidae	**I**	**Dolichopodidae**	**III**	**Limoniidae**	**II**
Psychodidae	**II**	**Simuliidae**	**II**	**Stratiomyidae**	**II**
Tabanidae	**II**				

In order to show the connections between macro-invertebrate taxa relating to the Salinity Association Group (SAG) assignments, the taxa are also presented below according to their SAG assignment.

Salinity Association Group I (Species/Genera)

Acroloxus lacustris
Adicella reducta
Aeshna juncea
Agabus didymus
Agapetus delicatulus
Agapetus fuscipes
Alainites muticus
Ancylus fluviatilis
Anodonta anatina
Aphelocheirus aestivalis
Aquarius najas
Arctocorisa carinata
Arctocorisa germari
Athripsodes aterrimus
Athripsodes cinerus
Austropotamobius pallipes
Baetis fuscatus
Baetis rhodani
Baetis scambus
Bathyomphalus contortus
Bithynia leachii
Caenis luctosa
Caenis pseudorivulorum
Caenis pusilla
Callicorixa praeusta
Callicorixa wollastoni
Calopteryx splendens
Calopteryx virgo
Centroptilum luteolum
Ceraclea annulicornis
Ceraclea sp.
Chimarra sp.
Cloeon simile
Cordulegaster boltonii
Corixa dentipes
Cymatia bonsdorffii
Cymatia coleoptrata
Cyrnus trimaculatus
Dryops sp.
Dugesia tigrina
Elmis aenea
Ephemera danica
Erpobdella testacea
Esolus parallelepipedus
Gammarus lacustris
Gammarus pulex
Gerris argentatus
Gerris costae
Gerris gibbifer
Gerris lacustris
Gerris odontogaster
Glaenocorisa propinqua
Glossiphonia sp.
Goera pilosa
Gomphus sp.
Graptodytes pictus
Gyraulus laevis
Halesus radiatus
Hebrus ruficeps
Helophorus brevipalpis
Heptagenia sulphurea
Hesperocorixa castanea
Hesperocorixa moesta
Hippeutis complanatus
Hydraena testacea
Hydrocyphon deflexicollis
Hydrometra stagnorum
Hydropsyche contubernalis
Hydropsyche pellucidula
Hydroptila sp.
Hygrotus impressopunctatus
Ithyrichia lamellaris
Lepidostoma sp.
Leptocerus tineiformis
Leuctra fusca
Leuctra geniculata
Limnephilus decipiens
Limnephilus flavicornis
Limnephilus rhombicus
Limnius sp.
Lype reducta
Micronecta poweri
Microvelia pygmaea
Molanna sp.
Mystacides azurea
Naucoris maculatus
Nemoura cinerea
Neurecilpsis bimaculata
Notonecta maculata
Notonecta obliqua
Oligotricha striata
Orconectes limosus
Orectochilus villosus
Oulimnius sp.
Paraleptophlebia submarginata
Pisidium subtruncatum
Planorbarius corneus
Planorbis carinatus
Polycelis nigra
Polycelis tenuis
Potamophylax cingulatus
Proasellus meridanus
Procambarus clarkii
Protonemura sp.
Pseudanodonta complanata
Psychomyia pusilla
Rhithrogena semicolorata
Rhyacophila dorsalis
Rhyacophila munda
Riolus subviolaceus
Serratella ignita
Sialis fuliginosa
Sialis lutaria
Sialis nigripes
Sigara distincta
Sigara fossarum
Sigara iactans
Sigara limitata
Sigara nigrolineata
Sigara scotti
Sigara venusta
Tinodes waeneri
Trocheta bykowskii
Trocheta subviridis
Unio pictorum
Valvata cristata
Valvata piscinalis
Velia (Plesiovelia) caprai
Velia (Plesiovelia) saulii
Viviparus contectus
Viviparus viviparus

Salinity Association Group I (Families)

Acroloxidae
Ancylidae
Aphelocheiridae
Astacidae
Athericidae
Baetidae
Caenidae
Calopterygidae
Cambaridae
Cordulegastridae
Corixidae
Dixidae
Dryopidae
Elmidae
Ephemerellidae
Ephemeridae
Erpobdellidae
Gerridae
Gomphidae
Hebridae
Heptageniidae
Hydrometridae
Hydroptilidae
Lepidostomatidae
Leptoceridae
Leptophlebiidae
Leuctridae
Limnephilidae
Molannidae
Nemouridae
Perlodidae
Phryganeidae
Polycentropodidae
Psychomyiidae
Rhyacophilidae
Scirtidae
Sialidae
Unionidae
Valvatidae
Veliidae
Viviparidae

Salinity Association Group II (Species/Genera)

Aeshna grandis
Anacaena limbata
Anisus vortex
Anodonta cygnea
Argyroneta aquatica
Asellus aquaticus
Balanus improvisus
Bithynia tentaculata
Brachycentrus subnilus
Caenis horaria
Cheumatopsyche lepida
Cloeon dipterum
Coenagrion puella
Corbicula fluminea
Corixa affinis
Corixa panzeri
Corixa punctata
Crangonyx pseudogracilis
Crenobia alpina
Dendrocoelum lacteum
Dinocras cephalotes
Dreissena polymorpha
Dugesia lugubris
Dugesia polychroa
Dytiscus circumflexus
Ecnomus tenellus
Elophila nymphaeata
Enallagma cyathigerum
Enochrus melanocephalus
Erpobdella octoculata
Galba truncatula
Gammarus chevreuxi
Gammarus tigrinus
Gammarus zaddachi
Gerris thoracicus
Glossosoma boltoni
Glossosoma conformis
Gyraulus albus
Gyraulus crista
Gyrinus caspius
Gyrinus marinus
Gyrinus substriatus
Haliplus apicalis
Haliplus confinis
Haliplus lineatocollis
Helobdella stagnalis
Helophorus alternans
Helophorus granularis
Hemianax ephippiger
Hemiclepsis marginata
Hesperocorixa linnaei
Hesperocorixa sahlbergi
Hydrobius fuscipes
Hydroporus palustris
Hydroporus pubescens
Hydroporus tessellatus
Hydropsyche instabilis
Hydropsyche siltalai
Ilybius subaeneus
Ilyocoris cimicoides
Ischnura elegans
Ischnura pumilio
Isoperla grammatica
Laccobius atratus
Laccobius bipunctatus
Laccophilus hyalinus
Lestes dryas
Lestes sponsa
Libellula depressa
Libellula quadrimaculata
Lymnaea stagnalis
Microvelia reticulata
Mystacides longicornis
Nemoura erratica
Nepa cinerea
Notonecta glauca
Odontocerum albicorne
Oecetis ochracea
Orthetrum cancellatum
Paracorixa concinna
Perla bipunctata
Philopotamus montanus
Physa fontinalis
Piscicola geometra
Pisidium amnicum
Pisidium casertanum
Planorbis planorbis
Platycnemis pennipes
Plectrocnemia geniculata
Polycentropus flavomaculatus
Potamopyrgus antipodarum
Prostoma sp.
Radix auricularia
Radix balthica
Sericostoma personatum
Sigara dorsalis
Sigara falleni
Sigara lateralis
Sigara semistriata
Silo pallipes
Stagnicola palustris
Strigamia maritima
Sympetrum danae
Sympetrum nigrescens
Sympetrum sanguineum
Sympetrum striolatum
Theodoxus fluviatilis
Theromyzon tessulatum

Salinity Association Group II (Families)

Aeshnidae
Asellidae
Bithyniidae
Brachycentridae
Ceratopogonidae
Coenagrionidae
Corbiculidae
Crangonyctidae
Culicidae
Cybaeidae
Dendrocoelidae
Dugesiidae
Dytiscidae
Ecnomidae
Gammaridae
Geophilidae
Glossiphoniidae
Glossosomatidae
Goeridae
Gyrinidae
Haliplidae
Helophoridae
Hydrophilidae
Hydropsychidae
Lestidae
Limoniidae
Lymnaeidae
Naucoridae
Nepidae
Neritidae
Notonectidae
Odontoceridae
Perlidae
Philopotamidae
Physidae
Piscicolidae
Planariidae
Planorbidae
Platycnemididae
Psychodidae
Pyralidae
Sericostomatidae
Simuliidae
Sphaeriidae
Stratiomyidae
Tabanidae

Salinity Association Group III (Species/Genera)

Abra tenuis
Aeshna mixta
Agabus conspersus
Assiminea grayana
Augyles maritimus
Bathyporeia pelagica
Bathyporeia pilosa
Bathyporeia sarsi
Berosus affinis
Cerastoderma glaucum
Cercyon depressus
Cercyon littoralis
Colymbetes fuscus
Corophium arenarium
Corophium curvispinum
Corophium multisetosum
Corophium volutator
Crangon crangon
Cyathura carinata
Enochrus bicolor
Gammarus duebeni
Gammarus salinus
Haustorius arenarius
Helophorus fulgidicollis
Helophorus minutus
Hygrotus parallellogrammus
Idotea chelipes
Jaera nordmanni
Lekanosphaera hookeri
Lekanosphaera rugicauda
Limnephilus affinis
Lineus ruber
Littorina saxatilis
Macoma balthica
Manayunkia aestuarina
Melita palmata
Mesopodopsis slabberi
Mya arenaria
Mytilopsis leucophaeata
Neomysis integer
Nereis (Hediste) diversicolor
Noterus clavicornis
Notonecta viridis
Ochthebius auriculatus
Ochthebius dilatatus
Ochthebius marinus
Ochthebius punctatus
Ochthebius viridis
Palaemon longirostris
Palaemonetes varians
Planaria torva
Pygospio elegans
Rhantus frontalis
Rhantus suturalis
Scrobicularia plana
Sigara selecta
Sigara stagnalis
Streblospio shrubsoli
Ventrosia ventrosa

Salinity Association Group III (Families)

Anthuridae
Assimineidae
Corophiidae
Crangonidae
Dolichopodidae
Dreissenidae
Haustoriidae
Heteroceridae
Hydraenidae
Hydrobiidae
Janiridae
Libellulidae
Melitidae
Noteridae
Palaemonidae
Pontoporeiidae
Scrobiculariidae
Tellinidae
Tetrastemmatidae

Salinity Association Group IV (Species/Genera)

Abra alba
Alkmaria romijni
Aphrodita aculeata
Arenicola marina
Auriculinella bidentata
Bittium reticulatum
Buccinum undatum
Capitella capitata
Carcinus maenas
Cerastoderma edule
Corbula gibba
Corophium insidiosum
Elminius modestus
Eteone longa
Eulalia viridis
Eurydice pulchra
Gammarus locusta
Gammarus oceanicus
Harmothoe imbricata
Heteromastus filiformis
Hydrobia acuta
Idotea balthica
Idotea granulosa
Lacuna pallidula
Lacuna vincta
Lekanesphaera monodi
Lepidonotus squamatus
Mya truncata
Mytilus edulis
Myxicola infundibulum
Nassarius reticulatus
Neanthes (Attila) virens
Nephtys caeca
Nephtys hombergii
Ophelia rathkei
Orchestia cavimana
Orchestia gammarellus
Ovatella myosotis
Palaemon elegans
Pantala flavescens
Paragnathia formica
Parvicardium exiguum
Peringia ulvae
Polydora ciliata
Scolelepis squamata
Scoloplos (Scoloplos) armiger
Sphaeroma serratum
Tetrastemma melanocephalum
Tharyx marioni

Salinity Association Group IV (Families)

Aphroditidae
Arenicolidae
Balanidae
Buccinidae
Capitellidae
Cardiidae
Cerithiidae
Cirolanidae
Corbulidae
Ellobiidae
Elminiidae
Gnathiidae
Idoteidae
Littorinidae
Myidae
Mysidae
Mytilidae
Nassariidae
Nereidae
Opheliidae
Orbiniidae
Phyllodocidae
Portunidae
Sabellidae
Semelidae
Sphaeromatidae
Spionidae
Talitridae

Salinity Association Group V (Species/Genera)

Amphiporus lactifloreus
Hyale prevostii
Nephtys cirrosa
Protula tubularia

Anisus spirorbis
Barnea candida
Cirratulus cirratus
Cirriformia tentaculata
Eupolymnia nebulosa
Eusyllis blomstrandi
Gastrosaccus spinifer
Gattyana cirrhosa
Gibbula cineraria
Glycera tridactyla
Harmothoe extenuata
Harmothoe impar
Hydroides norvegicus
Kefersteinia cirrata
Lagis koreni
Lanice conchilega
Lineus longissimus
Lineus viridis
Littorina littorea
Magelona mirabilis
Malacoceros fuliginosus
Melinna palmata
Mysta picta
Neoamphitrite figulus
Nephtys longosetosa
Nereis pelagica
Nucella lapillus
Ostrea edulis
Owenia fusiformis
Parvicardium ovale
Patella vulgata
Perinereis cultrifera
Platynereis dumerilii
Pomatoceros triqueter
Praunus flexuosus
Praunus inermis
Ramphogordius sanguineus
Sabella pavonina
Sabellaria alveolata
Sabellaria spinulosa
Schistomysis ornata
Schistomysis spiritus
Scolelepis foliosa
Semibalanus balanoides
Spiophanes bombyx
Spisula subtruncata
Sthenelais boa
Venerupis senegalensis

Salinity Association Group V (Families)

Ampharetidae
Amphiporidae
Cirratulidae
Glyceridae
Hesionidae
Hyalidae
Lineidae
Mactridae
Muricidae
Nephtyidae
Ostreidae
Oweniidae
Patellidae
Pectinariidae
Pholadidae
Polynoidae
Sabellariidae
Serpulidae
Sigalionidae
Syllidae
Terebellidae
Trochidae
Veneridae

Appendix 2: Justifications for Salinity Association Group (SAG) Assignment of Macro-invertebrate Taxa

The macro-invertebrate taxa were assigned to specific Salinity Association Groups (SAGs) for the following reasons.

Tricladida

Tricladida have been reported to be among the most salt-sensitive invertebrates (Kefford *et al*., 2003).

Planariidae

- *Planaria torva* (Müller, 1774) is common in coastal areas such as ports and connecting canals (Reynoldson, 1978). As such, *P. torva* was assigned to Salinity Association Group (SAG) III.
- *Polycelis nigra* (Müller, 1774) is found in lowland streams and lakes (Reynoldson, 1978) and has been recorded in a lake with a salinity of $1gL^{-1}$ (Johnson *et al*., 2007), approximately 0.66PSU. Consequently, *P. nigra* was assigned to SAG I.
- *Polycelis tenuis* Ijima, 1884 competes with *P. nigra* for food (Reynoldson, 1978), and thus occupies similar habitats. Therefore it can be assumed that *P. tenuis* has a similar salinity tolerance to *P. nigra*, thus it was also assigned to SAG I.
- *Crenobia alpina* (Dana, 1766) is found in underground water, streams and at sea-level where a suitable habitat for *C. alpina* exists (Reynoldson, 1978), indicating that the species has some tolerance to salinity. As such, *C. alpina* was assigned to SAG II.

Dugesiidae

- Species of the genus *Dugesia* are distributed in salinities less than $1.6gL^{-1}$ (approximately 1.08PSU) according to Gallardo-Mayenco (1994). *Dugesia tigrina* (Girard, 1850) has a salinity tolerance range of $0.1\text{-}2.2gL^{-1}$ (Berezina, 2003), approximately 0.07-1.51PSU, and so it was assigned to SAG I.

- Barnes (1994) reported that whilst *Dugesia lugubris* (Schmidt, 1861) and *Dugesia polychroa* (Schmidt, 1861) are essentially freshwater invertebrates, both species may penetrate dilute brackish waters with salinities below $8gL^{-1}$ (Barnes, 1994), approximately 5.99PSU. Furthermore, Piscart *et al*. (2005a) found that species of the genus *Dugesia* to be relatively insensitive to increases in salinity. Thus *D. lugubris* and *D. polychroa* are assigned to SAG II.

Dendrocoelidae

- *Dendrocoelum lacteum* (O.F. Müller, 1774) is found in lakes containing greater than $10mgL^{-1}$ of calcium (Reynoldson, 1978) and has been found to be tolerant of increases in salinity (Piscart *et al*., 2005a). As such, *D. lacteum* was assigned to SAG II.

Nemertea

Species of Nemertea are most often marine in nature (Fitter & Manuel, 1986). A few genera of Nemertea, however, are freshwater inhabitants and the genus *Prostoma* is the only freshwater genus of Nemertea encountered in north-west Europe (Fitter & Manuel, 1986).

Tetrastemmatidae

- Leland & Fend (1998) reported that the genus *Prostoma* tolerates salinities within the range $2\text{-}5gL^{-1}$, approximately 1.37-3.62PSU. Hence the genus *Prostoma* was assigned to Salinity Association Group (SAG) II.
- *Tetrastemma melanocephalum* (Johnston, 1837) was added to the list as the species is widely distributed around Britain, inhabiting sand flats, mud flats and salt marshes (Fish & Fish, 1996). Barnes (1994) stated that the species also occurs in the mouths of estuaries. It has been reported that *T. melanocephalum* feeds on *Corophium* species (Barnes, 1994; Fish & Fish, 1996) and as such it can be assumed that *Tetrastemma melanocephalum* tolerates similar salinities. Thus, *T. melanocephalum* was assigned to SAG IV.

Lineidae

- *Lineus ruber* (Müller, 1774) was added to the list as the species is common in estuarine sands and mud, as well as on salt marshes (Fish & Fish, 1996). *Lineus ruber* tolerates brackish waters with salinities as low as $8gL^{-1}$ (Barnes, 1994; Fish & Fish, 1996), approximately 5.99PSU, and as such the species was assigned to SAG III.

- *Lineus longissimus* (Gunnerus, 1770) was added to the list as the species is common around Britain (Fish & Fish, 1996) and may be recorded in brackish waters (Barnes, 1994). Barnes (1994) stated that whilst *L. longissimus* is recorded in the brackish Baltic Sea, it is not otherwise often recorded in brackish waters. Hence, *L. longissimus* was assigned to SAG V.
- *Lineus viridis* (Müller, 1774) was added to the list as the species is commonly found around Britain (Fish & Fish, 1996), distributed in muddy estuarine sediments and brackish waters (Barnes, 1994). Commito *et al*. (2008) found that *L. viridis* was among the most abundant invertebrates at a location with a salinity of $30gL^{-1}$, approximately 25.36PSU. Thus, *L. viridis* was assigned to SAG V.
- *Ramphogordius sanguineus* (Rathke, 1799) was added to the list as the species is common around Britain (Fish & Fish, 1996) and may be recorded in brackish waters and estuarine mud (Barnes, 1994). Barnes (1994) stated that *R. sanguineus* is the least euryhaline species when compared to *L. ruber* and *L. viridis*. As such, *R. sanguineus* was assigned to SAG V.

Amphiporidae

- *Amphiporus lactifloreus* (Johnston, 1828) was added to the list as the species is widely distributed around Britain (Fish & Fish, 1996). According to Barnes (1994), *Amphiporus lactifloreus* is distributed where salinities are greater than $20gL^{-1}$, approximately 16.22PSU. Thus, *A. lactifloreus* was assigned to SAG V.

Gastropoda

It has been suggested that Gastropoda are among the more halo-sensitive taxa (Hart *et al*., 1990; Kefford, 1998a; Kefford *et al*., 2003; Hassell *et al*., 2006). This proposal has been verified by the research of Wollheim & Lovvorn (1995) and Piscart *et al*. (2005a). Wollheim & Lovvorn (1995) found that Gastropoda were rare in mesohaline lakes but were major components in oligohaline lakes, whilst Piscart *et al*. (2005a) concluded that pulmonate Gastropoda are not tolerant of increases in salinity. Furthermore, Dunlop *et al*. (2008) compared the salinity tolerances of taxa at the order and sub-order levels and concluded that Gastropoda are more sensitive to salinity increases than Hemiptera and Odonata but are less sensitive than Ephemeroptera.

Trochidae

- *Gibbula cineraria* (Linnaeus, 1758) was added to the list as the species is widely distributed around Britain (Fish & Fish, 1996) and has long been recorded (Crisp, 1964). The species tolerates only salinities above 20gL^{-1} (Barnes, 1994), approximately 16.22PSU. As such, *G. cineraria* was assigned to Salinity Association Group (SAG) V.

Patellidae

- *Patella vulgata* Linnaeus, 1758 was added to the list as the species is widely distributed in north-western Europe (Barnes, 1994; Fish & Fish, 1996), as well as in Britain (Fish & Fish, 1996). *Patella vulgata* is found in habitats where salinity does not go below 20gL^{-1} (Barnes, 1994; Fish & Fish, 1996), approximately 16.22PSU. Hence *P. vulgata* was assigned to SAG V.

Cerithiidae

- *Bittium reticulatum* (da Costa, 1778) was added to the list as the species has been recorded in the south and west of Britain (Fish & Fish, 1996) and is expected to spread quickly (Jones & Carpenter, 2009). Kazanci *et al*. (2003) recorded that the largest abundance of *B. reticulatum* occurred at a salinity of 24gL^{-1} (approximately 19.81PSU) and that the species also occurred at higher salinities. Akbulut *et al*. (2009), however, also recorded *B. reticulatum* in the salinity range 10-18gL^{-1} (7.63-14.45PSU). Consequently the species was assigned to SAG IV.

Littorinidae

- *Lacuna pallidula* (da Costa, 1778) was added to the list as the species is frequently encountered around Britain (Fish & Fish, 1996). Barnes (1994) stated that *L. pallidula* is found only in habitats with salinities greater than 15gL^{-1} (approximately 11.84PSU). As such *L. pallidula* was assigned to SAG IV.
- *Lacuna vincta* (Monatgu, 1803) was added to the list as the species is common around Britain (Fish & Fish, 1996). Barnes (1994) reported that *L. vincta* tolerates only salinities greater than 15gL^{-1} (approximately 11.84PSU). Hence *L. vincta* was assigned to SAG IV.

- *Littorina littorea* (Linnaeus, 1758) was added to the list as the species is common in Britain (Fish & Fish, 1996) and frequently occurs in estuaries and on mud flats (Fish & Fish, 1996; Joyce *et al*., 2005). *Littorina littorea* has been recorded from a salt marsh where salinity ranged from 17gL^{-1} to 37gL^{-1} (Frid & James, 1989), approximately 13.57-32.04PSU. Barnes (1994) reported that *L. littorea* is associated with high salinities as the eggs of the species require a salinity of 20gL^{-1} (16.22PSU) to develop. As such, *L. littorea* was assigned to SAG V.
- *Littorina saxatilis* (Olivi, 1792) was added to the list as the species is common in Britain (Fish & Fish, 1996), frequently occurring in estuaries and mud flats (Fish & Fish, 1996; McCorry & Otte, 2001) as well as salt marshes (Mason *et al.*, 1991). *Littorina saxatilis* tolerates salinities as low as 8gL^{-1} (Barnes, 1994; Fish & Fish, 1996), approximately 5.99PSU, and as such the species was assigned to SAG III.

Nassariidae

- *Nassarius reticulatus* (Linnaeus, 1758) was added to the list as the species is frequently encountered around Britain and tolerates salinities down to 16gL^{-1} (Barnes, 1994; Fish & Fish, 1996), approximately 12.71PSU. Thus *N. reticulatus* was assigned to SAG IV.

Neritidae

- *Theodoxus fluviatilis* (Linnaeus, 1758) is a common, hard water species (Macan, 1977) that has been found to be absent from estuarine locations by Muñoz & Prat (1994). However, Carlsson (2006) recorded *T. fluviatilis* in salinities in the range 0.7-7.4gL^{-1} (approximately 0.46-5.51PSU). Furthermore, Kazanci *et al*. (2003) documented the largest abundance of the species occurred at a salinity of 8gL^{-1} (5.99PSU) and was present in salinities up to 15gL^{-1} (11.84PSU), but only in very small numbers. Hence *T. fluviatilis* was assigned to SAG II.

Viviparidae

- *Viviparus contectus* (Millet, 1813) was assigned to SAG I as Crothers (1997) described the species as freshwater.
- *Viviparus viviparus* (Linnaeus, 1758) is a common hard water species according to Macan (1977). Ezhova *et al*. (2005) described *V. viviparus* as a freshwater species and presented records that indicated the species has a low tolerance to salinities above 1.5-2.9gL^{-1} (approximately 1.01-2.03PSU). Thus *V. viviparus* was assigned to SAG I.

Valvatidae

- *Valvata cristata* O.F. Müller, 1774 has been recorded at a salinity of 0.7gL^{-1} (approximately 0.46PSU) by Carlsson (2006), whilst Akbulut *et al*. (2009) recorded the species in the salinity range 0.1-0.2gL^{-1} (0.07-0.13PSU). As such, *V. cristata* was assigned to SAG I.
- *Valvata piscinalis* (O.F. Müller, 1774) is common in all kinds of running water (Macan, 1977) and has been recorded at salinity less than 1gL^{-1} (Sousa *et al*., 2007), approximately 0.66PSU. Akbulut *et al*. (2009) recorded the largest abundance of *V. piscinalis* in the salinity range 0.1-0.2gL^{-1} (0.07-0.13PSU), whilst Carlsson (2006) recorded the species in a lake with a salinity of 0.7gL^{-1} (0.46PSU). Thus *V. piscinalis* was assigned to SAG I.

Hydrobiidae

- It has been reported that *Hydrobia acuta* (Draparnaud, 1805) tolerates salinities in the range 10-50gL^{-1} (Bamber *et al*., 2001), approximately 7.63-45.03PSU. Thus, *H. acuta* was assigned to SAG IV.
- *Potamopyrgus antipodarum* (J.E. Gray, 1843) is found in running waters of all types and is recorded in brackish waters (Macan, 1977; Velasco *et al*., 2006). The species is relatively insensitive to salinity according to Piscart *et al*. (2005a), whilst Wolf *et al*. (2009) indicated that *P. antipodarum* can tolerate salinities up to 10gL^{-1} (approximately 7.63PSU) and higher for a short time. Carlsson (2006) recorded the presence of *P. antipodarum* in the salinity range 0.7-7.4gL^{-1} (0.46-5.51PSU). Furthermore, Barnes (1994) stated that whilst the species mostly occupies freshwater habitats, the species can tolerate salinities up to 20gL^{-1} (16.22PSU). As such *P. antipodarum* was assigned to SAG II.
- *Ventrosia ventrosa* (Montagu, 1803) is found in brackish water and is relatively common in estuaries, ditches, and brackish lagoons (Macan, 1977; Joyce *et al*., 2005). Bamber *et al*. (2001) reported *V. ventrosa* tolerates salinities in the range 4-40gL^{-1} (approximately 2.85-34.97PSU), whilst Fish & Fish (1996) stated that the species is most commonly found in the salinity range 6-20gL^{-1} (4.40-16.22PSU). *Ventrosia ventrosa* has also been recorded at salinities as low as 5gL^{-1} (Carlsson, 2006), approximately 3.62PSU. Thus *V. ventrosa* was assigned to SAG III.

- *Peringia ulvae* (Pennant, 1777) is found in brackish water (Joyce *et al*., 2005), and is commonly found on mud flats (McCorry & Otte, 2001), salt marshes (Mason *et al*., 1991) and in estuaries (Macan, 1977). Frid & James (1989) recorded *P. ulvae* on a salt marsh where salinity ranged from $17gL^{-1}$ to $37gL^{-1}$ (approximately 13.57-32.04PSU) The species tolerates salinities as low as $1.5gL^{-1}$ (1.01PSU) but has a preference for salinities in the range $10\text{-}33gL^{-1}$ (7.63-28.20PSU) according to Fish & Fish (1996). Kazanci *et al*. (2003) recorded that the largest abundance of *P. ulvae* occurred at a salinity of $25gL^{-1}$ (20.73PSU), whilst Ysebaert *et al*. (2003) noted large numbers of the species at a salinity of $20gL^{-1}$ (16.22PSU). Kazanci *et al*. (2003) also reported that the species was present at salinities as low as $8gL^{-1}$ (5.99PSU), whereas Barnes (1994) stated *P. ulvae* tolerates salinities as low as $4gL^{-1}$ (2.85PSU). Hence the species was assigned to SAG IV.

Bithyniidae

- *Bithynia tentaculata* (Linnaeus, 1758) is a common hard water species (Macan, 1977) that has been found to be relatively insensitive to salinity increases (Piscart *et al*., 2005a), tolerating salinities up to $10gL^{-1}$ (approximately 7.63PSU) and possibly higher for a short time (Wolf *et al*., 2009). Berezina (2003) reported that the salinity tolerance range of the species is within the range $45\text{-}6300mgL^{-1}$ (0.03-4.64PSU), whilst Carlsson (2006) recorded the species in lakes varying in salinity between $0.7gL^{-1}$ and $7.4gL^{-1}$ (0.46-5.51PSU). As such, *B. tentaculata* was assigned to SAG II.
- Ezhova *et al*. (2005) described *Bithynia leachii* (Sheppard, 1823) as a freshwater species and presented records indicating the species tolerates only salinities below $2.9gL^{-1}$, approximately 2.03PSU. Hence *B. leachii* was assigned to SAG I.

Assimineidae

- *Assiminea grayana* Fleming, 1828 is found in brackish water and is common in the Thames estuary and north of the Wash (Macan, 1977). *Assiminea grayana* has been recorded on salt marshes (Mason *et al*., 1991; Barnes, 1994) and is common in brackish water habitats but can survive fresh water for several days (Barnes, 1994). Consequently, *A. grayana* was assigned to SAG III.

Muricidae

- *Nucella lapillus* (Linnaeus, 1758) was added to the list as the species is commonly encountered in Britain (Fish & Fish, 1996). *Nucella lapillus* penetrates the more saline parts of estuaries (Barnes, 1994) and as such the species was assigned to SAG V.

Buccinidae

- *Buccinum undatum* Linnaeus, 1758 was included as the species is widely distributed in Britain (Fish & Fish, 1996). *Buccinum undatum* penetrates estuaries down to salinities of $15gL^{-1}$ (Barnes, 1994), approximately 11.84PSU. Thus *B. undatum* was assigned to SAG IV.

Ellobiidae

- *Auriculinella bidentata* (Montagu, 1808) was added to the list as the species is relatively common on British salt marshes (Mason *et al*., 1991; Crothers, 1997) and in estuaries (Barnes, 1994; Fish & Fish, 1996). Hence *A. bidentata* was assigned to SAG IV.
- *Ovatella myosotis* (Draparnaud, 1801) was added to the list as the species is frequently recorded from estuaries (Fish & Fish, 1996) and salt marshes (Mason *et al*., 1991; Crothers, 1997). Barnes (1994) reported that *O. myosotis* survives the entire brackish water salinity range, whilst Kazanci *et al*. (2003) recorded the species at a salinity concentration of $15gL^{-1}$ (approximately 11.84PSU). As such *O. myosotis* was assigned to SAG IV.

Physidae

- *Physa fontinalis* (Linnaeus, 1758) is commonly found in clean running water and occasionally lakes (Macan, 1977). Wolf *et al*. (2009) indicated that *P. fontinalis* only tolerates salinity below $5gL^{-1}$ (approximately 3.62PSU), whilst Carlsson (2006) recorded the species in lakes varying in salinity between $0.7gL^{-1}$ and $5.3gL^{-1}$ (0.46-3.85PSU). Consequently, *P. fontinalis* was assigned to SAG II.

Lymnaeidae

- *Lymnaea stagnalis* (Linnaeus, 1758) is a common hard water species (Macan, 1977) which Wollheim & Lovvorn (1995) reported was restricted to lakes with salinity less than $5gL^{-1}$ (approximately 3.62PSU). Carlsson (2006), however, recorded *L. stagnalis* in lakes varying in salinity from $0.7gL^{-1}$ to $7.4gL^{-1}$ (0.46-5.51PSU). Thus *L. stagnalis* was assigned to SAG II.

- *Galba truncatula* (O.F. Müller, 1774) is commonly found at the edges of ditches, streams and rivers (Macan, 1977). Piscart *et al*. (2005a) found *G. truncatula* to be greatly affected by increases in salinity, whilst Gallardo-Mayenco (1994) reported the largest abundance of the species occurred where salinity was 3.7gL^{-1} (approximately 2.63PSU). Akbulut *et al*. (2009) recorded that the largest abundance of *G. truncatula* occurred in the salinity range 0.2-3.5gL^{-1} (0.13-2.47PSU) and the presence of the species at higher salinities. Consequently *G. truncatula* was assigned to SAG II.
- *Stagnicola palustris* (O.F. Müller, 1774) has been recorded in lakes with salinities ranging from 0.7gL^{-1} to 7.4gL^{-1} (approximately 0.46-5.51PSU) by Carlsson (2006), whilst Akbulut *et al*. (2009) recorded the species in the salinity range 0.2-3.5gL^{-1} (0.13-2.47PSU). Consequently *S. palustris* was assigned to SAG II.
- *Radix auricularia* (Linnaeus, 1758) is a common hard water species according to Macan (1977). Akbulut *et al*. (2009) recorded the largest abundance of *R. auricularia* occurred in the salinity range 0.2-3.5gL^{-1} (approximately 0.13-2.47PSU) and also noted the presence of the species at higher salinities. As such, *R. auricularia* was assigned to SAG II.
- *Radix balthica* (Linnaeus, 1758) is a freshwater to brackish species (Joyce *et al*., 2005) which tolerates salinities below 5gL^{-1} (Wolf *et al*., 2009), approximately 3.62PSU. Akbulut *et al*. (2009) recorded the largest abundance of *R. balthica* in the salinity range 0.1-3.5gL^{-1} (0.07-2.47PSU) and also noted the presence of the species at higher salinities. Kazanci *et al*. (2003) recorded *R. balthica* at salinities up to 8.4gL^{-1} (6.32PSU) and noted the largest abundance of the species occurred at 8gL^{-1} (5.99PSU). Hence *R. balthica* was assigned to SAG II.

Planorbidae

- *Planorbis carinatus* (O. F. Müller, 1774) has been recorded at salinities within the range 0.1-0.2gL^{-1} (approximately 0.07-0.13PSU) by Akbulut *et al*. (2009). Hence *P. carinatus* was assigned to SAG I.
- *Anisus spirorbis* (Linnaeus, 1758) was described as a marine species by Joyce *et al*. (2005) and as such *A. spirorbis* was assigned to SAG V.

- *Planorbis planorbis* (Linnaeus, 1758) is a common hard water species (Macan, 1977), which Berezina (2003) documented had a salinity tolerance range of 20-4200mgL^{-1}, approximately 0.02-3.01PSU. Akbulut *et al*. (2009) recorded *P. planorbis* in the salinity range 0.1-0.3gL^{-1} (0.07-0.19PSU), whereas Carlsson (2006) recorded the species in lakes with salinities from 0.7gL^{-1} to 3.8gL^{-1} (0.46-2.70PSU). As such, *P. planorbis* was assigned to SAG II.
- It has been reported that *Anisus vortex* (Linnaeus, 1758) is a common hard water species (Macan, 1977). Carlsson (2006) recorded the presence of *A. vortex* in lakes with salinities in the range 2.1-7.4gL^{-1}, approximately 1.44-5.51PSU. Consequently, *A. vortex* was assigned to SAG II.
- Macan (1977) stated that *Bathyomphalus contortus* (Linnaeus, 1758) is widespread and occupies a wide range of habitats. Carlsson (2006) recorded *B. contortus* in a lake with a salinity of 0.7gL^{-1} (approximately 0.46PSU). Hence *B. contortus* was assigned to SAG I.
- Macan (1977) reported *Gyraulus albus* (O.F. Müller, 1774) inhabits all types of freshwater, regardless of flow regime and Wolf *et al*. (2009) described the species as a purely freshwater inhabitant. *Gyraulus albus*, however, has been recorded in lakes with salinities in the range 2.1-5.3gL^{-1} (Carlsson, 2006), approximately 1.44-3.85PSU. As such, *G. albus* was assigned to SAG II.
- Macan (1977) stated the common habitats of *Gyraulus laevis* (Alder, 1838) are lakes and ponds. *Gyraulus laevis* has been recorded at a salinity of less than 1gL^{-1} (approximately 0.66PSU) by Sousa *et al*. (2007). Thus *G. laevis* was assigned to SAG I.
- *Gyraulus crista* (Linnaeus, 1758) occurs in a variety of habitats (Macan, 1977). Carlsson (2006) recorded the species in lakes ranging in salinity from 0.7-4.7gL^{-1}, approximately 0.46-3.39PSU. Hence *G. crista* was assigned to SAG II.
- *Hippeutis complanatus* (Linnaeus, 1758) has been recorded in freshwater lakes by Carlsson (2006), who also noted the absence of the species from lakes with salinities greater than 0.7gL^{-1} (approximately 0.46PSU). As such *H. complanatus* was assigned to SAG I.

- *Planorbarius corneus* (Linnaeus, 1758) has been found to be highly tolerant of salinity by Piscart *et al*. (2005a), whereas Gallardo-Mayenco (1994) stated that the largest abundance of the species is found at a salinity of 1.1gL^{-1} (approximately 0.73PSU). Consequently *P. corneus* was assigned to SAG I.
- *Ancylus fluviatilis* (O.F. Müller, 1774) has been reported to be tolerant of salinities up to 2.45gL^{-1} (Muñoz & Prat, 1994), approximately 1.70PSU, but is greatly affected by further increases in salinity (Piscart *et al*., 2005a). Furthermore, Gallardo-Mayenco (1994) found that the largest abundance of *A. fluviatilis* occurred where salinity was 1.4gL^{-1} (0.94PSU). Hence *A. fluviatilis* was assigned to SAG I.

Acroloxidae

- It has been reported that *Acroloxus lacustris* (Linnaeus, 1758) is a common hard water species (Macan, 1977). Carlsson (2006) recorded *A. lacustris* in a lake with a salinity of 0.7gL^{-1} (approximately 0.46PSU). As such, *A. lacustris* was assigned to SAG I.

Bivalvia

Bivalvia species are frequently encountered in a wide range of surface habitats and are often abundant in canals and slow-flowing rivers (Fitter & Manuel, 1986).

Mytilidae

- *Mytilus edulis* Linnaeus, 1758 was added to the list as the species is common in coastal and estuarine waters tolerating salinities down to 4-5gL^{-1} (Fish & Fish, 1996), approximately 2.85-3.62PSU. Barnes (1994) also reported *M. edulis* survives salinities as low as 4gL^{-1} (2.85PSU) but Barnes (1994) noted that growth rates of *M. edulis* are reduced in salinities below 15-20gL^{-1} (11.84-16.22PSU). Verween *et al*. (2007) reported that the species is commonly found in the salinity range 15-40gL^{-1} (11.84-34.97PSU). Thus *M. edulis* was assigned to Salinity Association Group (SAG) IV.

Ostreidae

- *Ostrea edulis* (Linnaeus, 1758) was added to the list as the species is common in Britain (Crothers, 1997). *Ostrea edulis* tolerates salinities down to 23gL^{-1} (approximately 18.91PSU) and extends into estuaries (Fish & Fish, 1996). Verween *et al*. (2007) stated that the species inhabits the salinity range 28-32gL^{-1} (23.49-27.25PSU), whereas Barnes (1994) reported that *O. edulis* survives in salinities down to 20gL^{-1} (16.22PSU). Consequently *O. edulis* was assigned to SAG V.

Cardiidae

- *Cerastoderma edule* (Linnaeus, 1758) was added to the list as the species is often abundant in UK estuaries living in salinities between 15-35gL^{-1} (Fish & Fish, 1996), approximately 11.84-30.11PSU. Ysebaert *et al*. (2003) described *C. edule* as a polyhaline species, whilst Verween *et al*. (2007) stated that the species survives in the salinity range 18-40gL^{-1} (14.45-34.97PSU). Furthermore, Brady (1943) recorded *C. edule* in salinities in the range 32-38gL^{-1} (27.25-33.01PSU), whereas Barnes (1994) reported that *C. edule* occurs in salinities as low as 15gL^{-1} (11.84PSU). Hence *C. edule* was assigned to SAG IV.
- *Cerastoderma glaucum* (Poiret, 1789) was added to the list as the species is common in Britain in the salinity range of 5-38gL^{-1} (Fish & Fish, 1996), approximately 3.62-33.01PSU. Joyce *et al*. (2005) stated that *C. glaucum* is a brackish water species, whilst Barnes (1994) reported that the species occurs in salinities down to 4gL^{-1} (2.85PSU). Bamber *et al*. (2001) stated that whilst *C. glaucum* tolerates salinities in the range 5-40gL^{-1} (3.62-34.97PSU), the species prefers salinities of 10-35gL^{-1} (7.63-30.11PSU). Thus *C. glaucum* was assigned to SAG III.
- *Parvicardium exiguum* (Gmelin, 1791) was added to the list as the species is commonly extends into estuaries where it tolerates salinities as low as 17gL^{-1} (Fish & Fish, 1996), approximately 13.57PSU. Barnes (1994) stated that *P. exiguum* tolerates salinities down to 20gL^{-1} (16.22PSU), whereas Kazanci *et al*. (2003) recorded the presence of *P. exiguum* in the salinity range 8-24gL^{-1} (5.99-19.81PSU). The species, however, was only recorded in very small numbers at the lower salinity. Thus *P. exiguum* was assigned to SAG IV.

- *Parvicardium ovale* (G.B. Sowerby, 1840) was added to the list as the species has been recorded in British waters (Tooley & Smith, 2005; Nickell *et al*., 2009). Kazanci *et al*. (2003) noted that the largest abundance of *P. ovale* occurred at a salinity of $25gL^{-1}$ (approximately 20.73PSU) and that the species was present at a salinity of $8gL^{-1}$ (5.99PSU), though it was only recorded in very small numbers at this low salinity. Hence *P. ovale* was assigned to SAG V.

Veneridae

- *Venerupis senegalensis* (Gmelin, 1791) was added to the list as the species is widely distributed around Britain (Fish & Fish, 1996). *Venerupis senegalensis* tolerates salinities down to $20gL^{-1}$ (Barnes, 1994), approximately 16.22PSU. Thus *V. senegalensis* was assigned to SAG V.

Mactridae

- *Spisula subtruncata* (da Costa, 1778) was added to the list as the species is frequently found around Britain (Fish & Fish, 1996). *Spisula subtruncata* is tolerant of salinities as low as $15gL^{-1}$ (approximately 11.84PSU) according to Barnes (1994), whereas Meijera & Cleveringab (2009) considered the species to be fully marine. Camusso *et al*. (1998) found that the species is the dominant filter-feeder of the marine section of the River Po delta in Italy. Consequently *S. subtruncata* was assigned to SAG V.

Scrobiculariidae

- *Scrobicularia plana* (da Costa, 1778) was added to the list as the species is common in estuarine and intertidal habitats where it survives salinities down to $10gL^{-1}$ (Fish & Fish, 1996), approximately 7.63PSU. Hence *S. plana* was assigned to SAG III.

Semelidae

- *Abra tenuis* (Montagu, 1803) was added to the list as the species is frequently encountered in northern Britain (Barnes, 1994; Dekker & Beukema, 1999). Barnes (1994) stated that *A. tenuis* tolerates salinities as low as $10gL^{-1}$, approximately 7.63PSU. Consequently *A. tenuis* was assigned to SAG III.

- *Abra alba* (Wood W., 1802) was added to the list as the species is widely distributed around Britain (Rees *et al.*, 1999; Van Hoey *et al*., 2005). Barnes (1994) documented that *A. alba* tolerates only salinities greater than 20gL^{-1} (approximately 16.22PSU), whilst Akbulut *et al*. (2009) recorded the species in the salinity range 10-18gL^{-1} (7.63-14.45PSU). As such *A. alba* was assigned to SAG IV.

Corbulidae

- *Corbula gibba* (Olivi, 1792) was added to the list as the species is frequently encountered around Britain (Crothers, 1997). Barnes (1994) reported that *C. gibba* tolerates salinities as low as 15gL^{-1} (approximately 11.84PSU). Hence *C. gibba* was assigned to Salinity Association Group (SAG) IV.

Tellinidae

- *Macoma balthica* (Linnaeus, 1758) was added to the list as the species is often abundant in estuaries tolerating salinities as low as 5gL^{-1} (Fish & Fish, 1996), approximately 3.62PSU. *Macoma balthica* has been recorded in Essex salt marshes (Mason *et al*., 1991), the southern Baltic Sea (Zettler *et al.*, 2007), an euhaline marsh (Hampel *et al*., 2009) and mud flats (McCorry & Otte, 2001). Brady (1943) recorded *M. balthica* in the salinity range 27-39gL^{-1} (22.57-33.99PSU) from the River Tyne. Furthermore, Frid & James (1989) also recorded *M. balthica* from a salt marsh with a salinity range of 17-37gL^{-1} (13.57-32.05PSU), whilst Ysebaert *et al*. (2003) reported large numbers of the species in the salinity range 14-20gL^{-1} (10.99-16.22PSU). Thus *M. balthica* was assigned to SAG III.

Myidae

- *Mya arenaria* Linnaeus, 1758 was added to the list as the species is common in estuaries and mud flats (Fish & Fish, 1996). Fish & Fish (1996) stated *M. arenaria* is known to feed in salinities of 15gL^{-1} (approximately 11.84PSU) and survive in salinities of 4gL^{-1} (2.85PSU) for some time. Barnes (1994) also reported that *M. arenaria* survives salinities of 4gL^{-1} (2.85PSU) but requires salinities of 10-15gL^{-1} (7.63-11.84PSU) for active life functions such as movement and feeding. *Mya arenaria* has been described as a mesohaline species by Ysebaert *et al*. (2003), who also recorded large numbers of the species at a salinity of 20gL^{-1}, (16.22PSU). As such *M. arenaria* was assigned to SAG III.

- *Mya truncata* Linnaeus, 1758 was added to the list as the species is widely distributed throughout Britain (Fish & Fish, 1996). Barnes (1994) reported that *M. truncata* only tolerates salinities down to $10gL^{-1}$ (7.63PSU). Hence *M. truncata* was assigned to SAG IV.

Pholadidae

- *Barnea candida* (Linnaeus, 1758) was added to the list as the species is common around Britain and extends into estuaries down to salinities $20gL^{-1}$ (Fish & Fish, 1996), 16.22PSU. Barnes (1994) also reported that the species tolerates salinities down to $20gL^{-1}$ (16.22PSU). Thus *B. candida* was assigned to SAG V.

Unionidae

- *Unio pictorum* (Linnaeus, 1758) has been recorded at salinities less than $1gL^{-1}$, approximately 0.66PSU, by Sousa *et al*. (2007). As such *U. pictorum* was assigned to SAG I.
- *Anodonta anatina* (Linnaeus, 1758) has been recorded at salinities less than $1gL^{-1}$, approximately 0.66PSU, by Sousa *et al*. (2007). Hence *A. anatina* was assigned to SAG I.
- *Anodonta cygnea* (Linnaeus, 1758) has been found at salinities less than $1gL^{-1}$, approximately 0.66PSU, by Sousa *et al*. (2007) and in the salinity range 0.1-$3.5gL^{-1}$ (0.07-2.47PSU) by Akbulut *et al*. (2009). Attrill *et al*. (1996) suggested that this species of Unionidae is more tolerant of increases in salinity than other members of Unionidae. Thus *A. cygnea* was assigned to SAG II.
- Attrill *et al*. (1996) reported that *Pseudanodonta complanata* (Rossmässler, 1835) were not recorded from the Thames when salinity increased above $1.5gL^{-1}$, approximately 1.01PSU. As such the species was assigned to SAG I.
- Species of the family Unionidae appear intolerant of salinities above $2gL^{-1}$, approximately 1.37PSU, according to Attrill *et al*. (1996). Furthermore, Sousa *et al*. (2007) stated that species of the family Unionidae are usually present in limnetic estuarine zones. Thus the remaining members of the family not yet assigned to a Salinity Aassociation Group were assigned to SAG I.

Sphaeriidae

- *Pisidium amnicum* (Müller, 1774) has been recorded at a salinity of less than $1gL^{-1}$ (Sousa *et al*., 2007), approximately 0.66PSU, whilst Berezina (2003) reported that the salinity tolerance of *P. amnicum* is 45-$4200mgL^{-1}$ (0.03-3.01PSU). Hence *P. amnicum* was assigned to SAG II.

- *Pisidium casertanum* (Poli, 1791) has been recorded at a salinity of less than $1gL^{-1}$, approximately 0.66PSU, by Sousa *et al*. (2007), whilst Akbulut *et al*. (2009) recorded that the largest abundance of *Pisidium casertanum* occurred in the salinity range 0.2-$3.5gL^{-1}$ (0.13-2.47PSU). As such, *P. casertanum* was assigned to SAG II.
- *Pisidium subtruncatum* Malm, 1855 has been found at a salinity of less than $1gL^{-1}$ (Sousa *et al*., 2007), approximately 0.66PSU. Thus *P. subtruncatum* was assigned to SAG I.
- Whilst Sphaeriidae appear to be tolerant of changes in salinity over the range 0-$6gL^{-1}$ (Attrill *et al*., 1996), approximately 0-4.40PSU, species of the genus *Pisidium* are greatly affected by increases in salinity (Piscart *et al.*, 2005a). Gallardo-Mayenco (1994) stated that the genus *Pisidium* is distributed where salinity is less than $1.6gL^{-1}$ (1.08PSU) whilst species of the genus have been recorded in the salinity range 24-$170mgL^{-1}$ (Short *et al*., 1991) and 0-$600mgL^{-1}$ (Williams *et al*., 1999), approximately 0.02-0.11PSU and 0-0.39PSU. Thus species of the genus *Pisidium* not yet assigned to a Salinity Association Group were assigned to SAG I.

Dreissenidae

- Akbulut *et al*. (2009) recorded *Dreissena polymorpha* (Pallas, 1771) in the salinity range 0.1-$0.3gL^{-1}$, approximately 0.07-0.19PSU. The presence of *D. polymorpha*, however, was first found in the Meurthe River, France, at a salinity of $2.6gL^{-1}$ (Piscart *et al*., 2005a), approximately 1.81PSU, and a large population of the species inhabits the Aral Sea (Williams & Aladin, 1991). Wolf *et al*. (2009) indicated that *D. polymorpha* tolerates salinities up to $5gL^{-1}$ (3.62PSU), whilst Berezina (2003) reported that the species has a salinity tolerance range of $45mgL^{-1}$ to $8100mgL^{-1}$ (0.03-6.08PSU). Hence *D. polymorpha* was assigned to SAG II.
- *Mytilopsis leucophaeata* (Conrad, 1831) has been reported to have a salinity tolerance range of 0.1-$31gL^{-1}$, approximately 0.07-26.30PSU, by Verween *et al*. (2007). Consequently *M. leucophaeata* was assigned to SAG III.

Corbiculidae

- *Corbicula fluminea* (Müller, 1774) is commonly found in estuaries (Williams & Williams, 1998b) and is reported to be insensitive to salinity increases (Piscart *et al*., 2005a). Sousa *et al*. (2007) recorded *C. fluminea* at a salinity concentration less than $1gL^{-1}$, approximately 0.03PSU, whilst Wolf *et al*. (2009) indicated that the species tolerates salinities up to $5gL^{-1}$ (3.62PSU). Furthermore, Morton & Tong (1985) found that the species can tolerate salinities up to $13gL^{-1}$ (10.13PSU) for several days. Thus *C. fluminea* was assigned to SAG II.

Sessilia

Balanidae

- *Balanus improvisus* Darwin, 1854 was added to the list as the species is common in large British estuaries where it tolerates salinities down to $15gL^{-1}$ (Fish & Fish, 1996), approximately 11.84PSU. Barnes (1994) reported that *B. improvisus* is characteristic of brackish waters and that the species is capable of surviving salinities close to that of freshwater. As such, *Balanus improvisus* was assigned to Salinity Association Group (SAG) II.
- *Semibalanus balanoides* (Linnaeus, 1758) was added to the list as the species is widespread in Britain (Fish & Fish, 1996). The species tolerates salinities as low as $20gL^{-1}$ (Barnes, 1994; Fish & Fish, 1996), approximately 16.22PSU. Hence *S. balanoides* was assigned to SAG V.

Elminiidae

- *Elminius modestus* Darwin 1854 was added to the list as this Australasian species has become well established around Britain (Fish & Fish, 1996). *Elminius modestus* displaces *Balanus improvisus* where the two species compete (Barnes, 1994). The lower limit of the salinity range that *E. modestus* can tolerate and survive appears to be $12gL^{-1}$ (Lance, 1964), approximately 9.29PSU. Thus *E. modestus* was assigned to SAG IV.

Polychaeta

According to Crothers (1997), all the British species of Polychaeta are only found in marine habitats. Fitter & Manuel (1986), however, stated that whilst Polychaeta are mostly marine in nature, some species may also be found in brackish water. Furthermore, several authors such as Fish & Fish (1996), Ysebaert *et al*. (2003), Johnson *et al*. (2007) and Zettler *et al*. (2007) have reported that some species of Polychaeta are also tolerant of salinities lower than that of seawater.

Aphroditidae

- *Aphrodita aculeata* Linnaeus, 1758 was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). The species may occur in salinities as low as 18gL^{-1} (Barnes, 1994), approximately 14.45PSU. Thus *A. aculeata* was assigned to Salinity Association Group (SAG) IV.

Polynoidae

- *Gattyana cirrhosa* (Pallas, 1766) was added to the list as the species has been recorded in Britain (Kaiser *et al*., 1998) and is common in north-western Europe (Barnes, 1994). Barnes (1994) reported that *G. cirrhosa* inhabits the mouths of estuaries, tolerating salinities as low as 22gL^{-1} (approximately 18.00PSU). As such *G. cirrhosa* was assigned to SAG V.
- *Harmothoe extenuata* (Grube, 1840) was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996). Thus *H. extenuata* was assigned to SAG V.
- *Harmothoe imbricata* (Linnaeus, 1767) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) reported that *H. imbricata* frequently inhabits locations with salinities as low as 16gL^{-1} (approximately 12.71PSU) and very occasionally occupies habitats with salinities down to 5gL^{-1} (3.62PSU), although Barnes (1994) noted that this is a rare occurrence. Hence *H. imbricata* was assigned to SAG IV.
- *Harmothoe impar* (Johnston, 1839) was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996) and as such *H. impar* was assigned to SAG V.

- *Lepidonotus squamatus* (Linnaeus, 1758) was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996). Barnes (1994) noted that *L. squamatus* can occur in habitats with a salinity as low as 18gL^{-1}, approximately 14.45PSU. Thus *L. squamatus* was assigned to SAG IV.

Sigalionidae

- *Sthenelais boa* (Johnston, 1833) was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996). Thus *S. boa* was assigned to SAG V.

Phyllodocidae

- *Eteone longa* (Fabricius, 1780) was added to the list as the species has been recorded in the Humber estuary, North Yorkshire (Stillman *et al*., 2005) and is common in north-western Europe (Barnes, 1994). Barnes (1994) reported *E. longa* occupies habitats with salinities as low as 18gL^{-1}, approximately 14.45PSU. Consequently, *E. longa* was assigned to SAG IV.
- *Eulalia viridis* (Johnston, 1829) was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996), inhabiting waters with a salinity as low as 18gL^{-1} (Barnes, 1994), approximately 14.45PSU. As such, *E. viridis* was assigned to SAG IV.
- *Mysta picta* (Quatrefagues, 1865) was added to the list as the species may occasionally be found in brackish waters (Barnes, 1994) and has long been recorded in Britain (Brady, 1943). *Mysta picta* has been recorded in the salinity range 31-35gL^{-1} by Brady (1943), approximately 26.30-30.11PSU. Hence *M. picta* was assigned to SAG V.

Glyceridae

- *Glycera tridactyla* Schmarda, 1861 was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Thus *G. tridactyla* was assigned to SAG V.

Hesionidae

- *Kefersteinia cirrata* (Keferstein, 1862) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Hence *K. cirrata* was assigned to SAG V.
- *Magelona mirabilis* (Johnston, 1865) was added to the list as the species is common in north-western Europe (Barnes, 1994) and has been recorded in large numbers in County Durham. According to Barnes (1994), *M. mirabilis* tolerates only salinities above 22gL^{-1}, approximately 18.00PSU. Thus *M. mirabilis* was assigned to SAG V.

Syllidae

- *Eusyllis blomstrandi* Malmgren, 1867 was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996). Thus *E. blomstrandi* was assigned to SAG V.

Nereidae

- *Nereis (Hediste) diversicolor* (O.F. Müller, 1776) was added to the list as the species is common coastal and estuarine waters (Fish & Fish, 1996; Crothers, 1997). The species has been recorded in the Essex salt marshes by Mason *et al*. (1991) and in a lake with a salinity of $2gL^{-1}$, approximately 1.37PSU, by Johnson *et al*. (2007). Kazanci *et al*. (2003) recorded the largest abundance of the species occurred at a salinity of $8gL^{-1}$ (5.99PSU), whilst Ysebaert *et al*. (2003) noted large abundances of *N. diversicolor* in the salinity range 10-$14gL^{-1}$ (7.63-10.99PSU). Frid & James (1989) recorded the species on a salt marsh with a salinity range of 17-$37gL^{-1}$ (13.57-32.04PSU), whilst Brady (1943) recorded *N. diversicolor* from the River Tyne at a salinity of $31gL^{-1}$ (26.30PSU). Wolf *et al*. (2009) attributed a salinity tolerance range of 0.5-$35gL^{-1}$ (0.32-30.11PSU) to *N. diversicolor*, whilst Fish & Fish (1996) stated that the species tolerates salinities as low as $1gL^{-1}$ (0.66PSU). Barnes (1994) reported that *N. diversicolor* can tolerate salinities lower than $5gL^{-1}$ (3.62PSU). Consequently, *N. diversicolor* was assigned to Salinity Association Group (SAG) III.
- *Nereis pelagica* Linnaeus, 1758 was added to the list as the species is common in coastal waters (Fish & Fish, 1996). Brady (1943) recorded the presence of the species in the salinity range 32-$34gL^{-1}$, approximately 27.25-29.15PSU. Thus *N. pelagica* was assigned to SAG V.
- *Neanthes (Attila) virens* (M. Sars, 1835) was added to the list as the species is distributed around Britain and can be abundant locally (Fish & Fish, 1996). Brady (1943) recorded the species from the River Tyne in the salinity range 31-$34gL^{-1}$, approximately 26.30-29.15PSU. Barnes (1994) documented that *N. virens* occupies habitats with salinities greater than $17gL^{-1}$ (13.57PSU). Hence *N. virens* was assigned to SAG IV.
- *Platynereis dumerilii* (Audouin & Milne Edwards, 1833) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Thus *P. dumerilii* was assigned to SAG V.

- *Perinereis cultrifera* (Grube, 1840) was added to the list as the species is widely distributed around Britain (Fish & Fish, 1996). *Perinereis cultrifera* tends to replace *Nereis diversicolor* in habitats where salinities are over $20gL^{-1}$ (Barnes, 1994), approximately 16.22PSU. Thus, *P. cultrifera* was assigned to SAG V.

Nephtyidae

- *Nephtys caeca* (Fabricius, 1780) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). The species can tolerate salinities as low as $20gL^{-1}$, approximately 16.22PSU, and occasionally penetrates habitats with salinities down to $18gL^{-1}$ (Barnes, 1994), equivalent to 14.45PSU. Hence *N. caeca* was assigned to SAG IV.
- *Nephtys cirrosa* (Ehlers, 1868) was added to the list as the species is frequently encountered in north-western Europe (Barnes, 1994) and has long been recorded in the UK (Clark *et al*., 1962; Bamber, 1993). Barnes (1994) reported that *N. cirrosa* tolerates salinities down to $20gL^{-1}$, approximately 16.22PSU. Thus *N. cirrosa* was assigned to SAG V.
- *Nephtys hombergii* Savigny in Lamarck, 1818 was added to the list as the species is common in coastal waters (Fish & Fish, 1996). *Nephtys hombergii* has been recorded on a salt marsh with a salinity range of $17\text{-}37gL^{-1}$ (approximately 13.57-32.04PSU) by Frid & James (1989), whilst Brady (1943) recorded the species from the River Tyne in a salinity range of $30\text{-}34gL^{-1}$ (25.36-29.15PSU). Barnes (1994) reported that *N. hombergii* tolerates salinities down to $20gL^{-1}$ (16.22PSU) and occasionally occupies habitats with salinities down to $18gL^{-1}$ (14.45PSU). As such, *N. hombergii* was assigned to SAG IV.
- *Nephtys longosetosa* Örsted, 1843 was added to the list as the species is widely distributed in north-western Europe (Barnes, 1994) and has long been recorded in Britain (Clark *et al*., 1962). Barnes (1994) documented that the species tolerates salinities down to $20gL^{-1}$, approximately 16.22PSU. Hence *N. longosetosa* was assigned to SAG V.

Cirratulidae

- *Cirratulus cirratus* (O. F. Müller, 1776) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) stated that *C. cirratus* tolerates only salinities above $20gL^{-1}$, approximately 16.22PSU. As such, the species was assigned to SAG V.

- *Cirriformia tentaculata* (Montagu, 1808) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) reported that the species is only recorded from the mouths of estuaries. Consequently *C. tentaculata* was assigned to SAG V.
- *Tharyx marioni* (Saint-Joseph, 1894) was added to the list as the species has long been recorded in British estuarine waters (Gibbs *et al*., 1983). Barnes (1994) noted that the species is recorded in salinities as low as to $12gL^{-1}$ (approximately 9.29PSU), whilst Ysebaert *et al*. (2003) found that *T. marioni* was dominant, in terms of numbers, in the invertebrate community at a salinity of $28gL^{-1}$ (23.49PSU). Thus *T. marioni* was assigned to SAG IV.

Orbiniidae

- *Scoloplos (Scoloplos) armiger* (Müller, 1776) was added to the list as the species is common in coastal waters (Fish & Fish, 1996). The species has been recorded on mudflats (McCorry & Otte, 2001), and is also found in the southern Baltic Sea (Zettler *et al*., 2007), indicating it is tolerant of reduced salinities. Brady (1943) recorded *S. armiger* from habitats with salinities in the range 29-$35gL^{-1}$, approximately 24.42-30.11PSU. Ysebaert *et al*. (2003) described *S. armiger* as characteristic of polyhaline waters, whilst Barnes (1994) reported that the species frequently inhabits locations with salinities as low as $15gL^{-1}$ (11.84PSU). As such, *S. armiger* was assigned to SAG IV.

Spionidae

- *Polydora ciliata* (Johnston, 1838) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) stated that *P. ciliata* inhabits waters with salinities as low as $18gL^{-1}$, approximately 14.45PSU. Thus *P. ciliata* was assigned to SAG IV.
- *Malacoceros fuliginosus* (Claparède, 1869) was added to the list as the species is common in coastal waters (Fish & Fish, 1996). Barnes (1994) documented that *M. fuliginosus* is most frequently recorded where salinity is above $20gL^{-1}$, approximately 16.22PSU. As such, the species was assigned to SAG V.

- *Pygospio elegans* Claparède, 1863 was added to the list as the species is common in coastal and estuarine waters (Fish & Fish, 1996; Crothers, 1997). The species has also been recorded in the southern Baltic Sea (Zettler *et al*., 2007), whilst Barnes (1994) reported that *P. elegans* is found in lagoonal and estuarine habitats with salinities as low as $4gL^{-1}$ (approximately 2.85PSU). Barnes (1994) further stated that the species may tolerate a salinity of $2gL^{-1}$ (1.37PSU) for a short period of time. Johnson *et al*. (2007) recorded *P. elegans* in a lake with salinity of $2gL^{-1}$ (1.37PSU) and also stated that this species is characteristic of salinities above $4gL^{-1}$ (2.85PSU). Furthermore, *P. elegans* has been recorded in large abundances in the salinity range 14-$28gL^{-1}$ (Ysebaert *et al*., 2003), approximately 10.99-23.49PSU. Hence *P. elegans* was assigned to SAG III.
- *Scolelepis foliosa* (Audouin & Milne Edwards, 1833) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) noted that the species is generally found in the mouths of estuaries with salinities as low as $23gL^{-1}$, approximately 18.91PSU. As such, *S. foliosa* was assigned to SAG V.
- *Scolelepis squamata* (O.F. Muller, 1806) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). *Scolelepis squamata* has been recorded from the Northumberland coast in the salinity range of 28-$35gL^{-1}$ (Brady, 1943), approximately 23.49-30.11PSU. Barnes (1994) reported that *S. squamata* is recorded in estuaries at salinities as low as $18gL^{-1}$ (14.45PSU). Consequently *S. squamata* was assigned to SAG IV.
- *Streblospio shrubsoli* (Buchanan, 1890) was added to the list as the species has been recorded in England (Soulsby *et al*., 1982; Dauer, 2003). Barnes (1994) reported that *Streblospio shrubsoli* inhabits locations in both estuaries and lagoons with salinities down to $4gL^{-1}$, approximately 2.85PSU. Hence *S. shrubsoli* was assigned to SAG III.
- *Spiophanes bombyx* (Claparède, 1870) was added to the list as the species is common in brackish north-western European waters (Barnes, 1994) and has been recorded in large numbers in English north-eastern estuaries (Shillabeer & Tapp, 1990). Barnes (1994) reported that *S. bombyx* is only tolerant of salinities down to $22gL^{-1}$, approximately 18.00PSU. As a result *S. bombyx* was assigned to SAG V.

Capitellidae

- *Capitella capitata* (Fabricius, 1780) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). The species has been recorded from a salt marsh with salinities ranging from 17gL^{-1} to 37gL^{-1} (Frid & James, 1989), approximately 13.57-32.04PSU. Barnes (1994) stated the species occurs in salinities as low as 18gL^{-1} (14.45PSU). Hence *C. capitata* was assigned to SAG IV.
- *Heteromastus filiformis* (Claparède, 1864) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996; Crothers, 1997). The species has also been recorded in freshwater, mesohaline, polyhaline and euhaline marshes by Hampel *et al*. (2009). Ysebaert *et al*. (2003) noted large abundances of the species in the salinity range 10-28gL^{-1}, approximately 7.63-23.49PSU. Barnes (1994) noted that *H. filiformis* survives salinities down to 5gL^{-1} (3.62PSU) but also reported that the species is rarely recorded below 18gL^{-1} (14.45PSU). Consequently *H. filiformis* was assigned to SAG IV.

Arenicolidae

- *Arenicola marina* Lamarck, 1801 was added to the list as species is frequently found on mud flats (McCorry & Otte, 2001; Joyce *et al*., 2005) as well as in coastal and estuarine waters, tolerating salinities as low as 12gL^{-1} (Fish & Fish, 1996), approximately 9.29PSU. In contrast, Barnes (1994) stated that *A. marina* is only abundant in salinities above 18gL^{-1} (14.45PSU). Brady (1943) recorded the species from the River Tyne in salinities which ranged from 28gL^{-1} to 38 gL^{-1} (23.49-33.01PSU). Frid & James (1989) recorded *A. marina* from a salt marsh where salinity varied from 17 gL^{-1} to 37gL^{-1} (13.57-32.04PSU), whilst Ysebaert *et al*. (2003) noted that large numbers of the species at a salinity concentration of 20gL^{-1} (16.22PSU). As such, *A. marina* was assigned to SAG IV.

Opheliidae

- *Ophelia rathkei* McIntosh, 1908 was added to the list as Barnes (1994) reported that the species occurs around Britain tolerating salinities down to 18gL^{-1}, approximately 14.45PSU. Hence *O. rathkei* was assigned to SAG IV.

Oweniidae

- *Owenia fusiformis* Delle Chiaje, 1844 was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996). Thus *O. fusiformis* was assigned to SAG V.

Pectinariidae

- *Lagis koreni* Malmgren, 1866 was added to the list as the species is common in coastal and marine waters around Britain (Fish & Fish, 1996). Hence *L. koreni* was assigned to SAG V.

Sabellariidae

- *Sabellaria alveolata* (Linnaeus, 1767) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). *Sabellaria alveolata* only occurs in salinities over $22gL^{-1}$, approximately 18.00PSU, according to Barnes (1994). Thus *S. alveolata* was assigned to Salinity Association Group (SAG) V.
- *Sabellaria spinulosa* Leuckart, 1849 was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). The lowest salinity *S. spinulosa* is distributed at appears to be $22gL^{-1}$ (Barnes, 1994), approximately 18.00PSU. Consequently *S. spinulosa* was assigned to SAG V.

Ampharetidae

- *Alkmaria romijni* Horst, 1919 was added to the list as the species is distributed along the eastern coast of Britain (Barnes, 1994). It has been reported *A. romijni* is rarely recorded out of the salinity range 4-$25gL^{-1}$ (Barnes, 1994), approximately 2.85-20.83PSU. As such, *A. romijni* was assigned to SAG III.
- *Ampharete grubei* Malmgren, 1865 was added to the list as the species is frequently encountered in north-western Europe (Barnes, 1994) and has been recorded in the Humber estuary, North Yorkshire (Stillman *et al.*, 2005), and Blackwater estuary, Essex (Garbutt *et al*., 2006). Barnes (1994) reported that *A. grubei* is most frequently recorded where salinity is greater than $18gL^{-1}$, approximately 14.45PSU. Hence, *A. grubei* was assigned to SAG IV.
- *Melinna palmata* Grube, 1870 was added to the list as the species is well distributed and frequently encountered around Britain (Dauvin *et al*., 2007). Barnes (1994) noted that the species is present only in the marine sections of estuaries. Thus, *M. palmata* was assigned to SAG V.

Terebellidae

- *Eupolymnia nebulosa* (Monatgu, 1818) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Hence *E. nebulosa* was assigned to SAG V.

- *Lanice conchilega* Pallas, 1766 was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) reported that *L. conchilega* inhabits only waters with salinities greater than $22gL^{-1}$, approximately 18.00PSU. As such, the species was assigned to SAG V.
- *Neoamphitrite figulus* (Dalyell, 1853) was added to the list as Fish & Fish (1996) described the species as common in estuarine waters and tolerant of low salinities. Barnes (1994) documented that *N. figulus* does not occur in salinities below $22gL^{-1}$, approximately 18.00PSU. Thus *N. figulus* was assigned to SAG V.

Sabellidae

- *Manayunkia aestuarina* (Bourne, 1883) was added to the list as the species is common in coastal and estuarine waters (Fish & Fish, 1996; Crothers, 1997). The species has been recorded in a lake with salinity of $2gL^{-1}$ (Johnson *et al*., 2007), whilst Wolf *et al*. (2009) indicated that it is a brackish water species tolerating salinities of $0.5\text{-}30gL^{-1}$, approximately 0.32-25.36PSU. Furthermore, Barnes (1994) noted that *M. aestuarina* occurs throughout the brackish water range down to salinities of less than $1gL^{-1}$ (0.66PSU). Hence *M. aestuarina* was assigned to SAG III.
- *Myxicola infundibulum* (Montagu, 1808) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) noted that the species can be found in salinities as low as $18gL^{-1}$ (approximately 14.45PSU). As such *M. infundibulum* was assigned to SAG IV.
- *Sabella pavonina* Savigny in Sars, 1835 was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Barnes (1994) reported that *S. pavonina* is found where salinities are greater than $20gL^{-1}$, approximately 16.22PSU. Thus *S. pavonina* was assigned to SAG V.

Serpulidae

- *Hydroides norvegicus* Gunnerus, 1768 was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Hence *H. norvegicus* was assigned to SAG V.
- *Pomatoceros triqueter* (Linnaeus, 1758) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). As such, *P. triqueter* was assigned to SAG V.

- *Protula tubularia* (Montagu, 1803) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Thus *P. tubularia* was assigned to SAG V.
- *Serpula vermicularis* (Linnaeus, 1767) was added to the list as the species is common in coastal and marine waters (Fish & Fish, 1996). Hence *S. vermicularis* was assigned to SAG V.

Hirudinea

In a survey of Backwood River and Gleneg River, Australia, Williams *et al*. (1991) found that Hirudinea were rare or absent in these salinised rivers, indicating a lack of tolerance to increases in salinity in this particular taxon. Kefford *et al*. (2003) reported that Hirudinea are among the least halo-tolerant taxa, whilst Fitter & Manuel (1986) stated that Hirudinea are frequently encountered in freshwater habitats.

Piscicolidae

- *Piscicola geometra* (Linnaeus, 1761) has been found to be relatively insensitive to salinity increases by Piscart *et al*. (2005a) and Wolf *et al*. (2009) indicated that the species is tolerant of salinities up to $5gL^{-1}$, approximately 3.62PSU. Elliott & Mann (1979), however, stated that *P. geometra* has been recorded at salinities up to $8gL^{-1}$ (5.99PSU). Consequently *P. geometra* was assigned to Salinity Association Group (SAG) II.

Glossiphoniidae

- *Theromyzon tessulatum* (O.F. Müller, 1774) has been recorded in numbers in freshwater habitats by De Jonge *et al*. (2008) and in small numbers in fresh to slightly brackish water by Zettler & Daunys (2007). Hohenstein & Shain (2006) maintained populations of the species at a salinity of $3gL^{-1}$ (approximately 2.10PSU), indicating that *T. tessulatum* tolerates some salinity in water. Hence *T. tessulatum* was assigned to SAG II.
- Zettler & Daunys (2007) recorded *Hemiclepsis marginata* (O.F. Müller, 1774) from sites where salinity varied between 0-$8gL^{-1}$, approximately 0-5.99PSU. As such, *H. marginata* was assigned to SAG II.
- Piscart *et al.* (2005a) reported that the genus *Glossiphonia* is greatly affected by increases in salinity. Coupled with the knowledge that British species of *Glossiphonia* occupy freshwater habitats (Elliott & Mann, 1979), *Glossiphonia complanata* (Linnaeus, 1758) and *Alboglossiphonia heteroclita* (Linnaeus, 1758) were both assigned to SAG I.

- *Helobdella stagnalis* (Linnaeus, 1758) is found in all types of freshwater, regardless of the type of flow (Elliott & Mann, 1979) and has been found to be greatly affected by salinity increases (Piscart *et al*., 2005a). Wolf *et al*. (2009) indicated that *H. stagnalis* is tolerant of salinities up to $5gL^{-1}$ (approximately 3.62PSU), whereas Berezina (2003) found that the species has a salinity tolerance range of 0.1-$6.3gL^{-1}$ (0.07-4.64PSU). Furthermore, Barnes (1994) noted that the species may be recorded in dilute brackish water with a salinity less than $8gL^{-1}$ (5.99PSU). Hence *H. stagnalis* was assigned to SAG II.

Erpobdellidae

- *Erpobdella octoculata* (Linnaeus, 1758) is found in freshwater according to Elliott & Mann (1979). Wolf *et al*. (2009) implied that the species is tolerant of salinities up to $5gL^{-1}$ (approximately 3.62PSU), whilst Berezina (2003) reported that *E. octoculata* has a salinity tolerance within the range 45-$6300mgL^{-1}$ (0.03-4.64PSU). As such *E. octoculata* was assigned to SAG II.
- *Erpobdella testacea* (Savigny, 1812) is found in lakes, rivers, ditches and eutrophic ponds (Elliott & Mann, 1979). *Erpobdella testacea* has been recorded in a lake with a salinity of $1gL^{-1}$, approximately 0.66PSU, by Johnson *et al*. (2007). Thus *E. testacea* was assigned to SAG I.
- Attrill *et al*. (1996) recorded that Erpobdellidae disappeared when salinity increased above $1.5gL^{-1}$, approximately 1.01PSU. As such all other species of the family, namrly *Dina lineata* (O.F. Müller, 1774), *Trocheta bykowskii* (Gedroyc, 1913) and *Trocheta subviridis* (Dutrochet, 1817), were assigned to SAG I.

Araneae

Cybaeidae

- *Argyroneta aquatica* (Clerck, 1758) has been recorded at a salinity of $4.4gL^{-1}$ (approximately 3.16PSU) by Batty *et al*. (2005), hence the species was assigned to SAG II.

Decapoda

Kefford (1998a) stated that Decapoda are suspected to be affected by changes in salinity. In contrast, Dunlop *et al*. (2008) found that Decapoda are among the most salt tolerant freshwater taxa with only Isopoda more tolerant of salinity increases.

Palaemonidae

- *Palaemonetes varians* (Leach, 1837) is found in brackish waters and estuaries with salinity as low as 1gL^{-1} (Barnes, 1994; Fish & Fish, 1996), approximately 0.66PSU. *Palaemonetes varians* has also been recorded in the salinity range 4-8gL^{-1} (2.85-5.99PSU) by Johnson *et al*. (2007). Hence *P. varians* was assigned to Salinity Association Group (SAG) III.
- *Palaemon elegans* Rathke, 1837 was added to the list as the species is common around Britain and reported to tolerate salinities down to 6gL^{-1} (Fish & Fish, 1996), approximately 4.40PSU. *Palaemon elegans* was successfully introduced into the Aral Sea (Williams & Aladin, 1991) and as such is capable of surviving at higher salinities. Barnes (1994) reported that *P. elegans* has a mostly marine distribution, but added the species can occur in salinities as low as 4gL^{-1}, approximately 2.85PSU. *Palaemon elegans* has been recorded from a salt marsh with a salinity range of 17-37gL^{-1} (Frid & James, 1989), approximately 13.57-32.04PSU. Thus *P. elegans* was assigned to SAG IV.
- *Palaemon longirostris* H. Milne Edwards, 1837 is a brackish water species according to Wolf *et al*. (2009), who also indicated that it inhabits the salinity range 0.5-30gL^{-1}, approximately 0.32-25.36PSU. Barnes (1994) noted that *P. longirostris* adults can penetrate freshwater habitats. As such, the species was assigned to SAG III.

Crangonidae

- *Crangon crangon* (Linnaeus, 1758) is found in coastal and estuarine waters (Fish & Fish, 1996). *Crangon crangon* has been recorded in the salinity range 4-35gL^{-1} (approximately 2.85-30.11PSU) by Attrill & Thomas (1996), whilst Wolf *et al*. (2009) implied that the species is tolerant of salinities in the range 0.5-35gL^{-1} (0.32-30.11PSU). Barnes (1994) reported that *C. crangon* tolerates salinities down to 6gL^{-1} (4.40PSU). Hence *C. crangon* was assigned to SAG III.

Portunidae

- *Carcinus maenas* (Linnaeus, 1758) was added to the list as the species has been recorded in the Essex salt marshes by Mason *et al*. (1991). *Carcinus maenas* has also been recorded from a salt marsh with a salinity range of 17-37gL^{-1} (approximately 13.57-32.04PSU) by Frid & James (1989). Both Barnes (1994) and Fish & Fish (1996) stated that the adults of *C. maenas* can survive salinities down to 4gL^{-1} (2.85PSU), whilst Barnes (1994) further reported that the eggs of *C. maenas* are killed by salinities lower than 20gL^{-1} (16.22PSU). Thus *C. maenas* was assigned to SAG IV.

Astacidae

- *Austropotamobius pallipes* (Lereboullet, 1858) is commonly found in clean standing and running water according to Gledhill *et al*. (1993), who added that the species is sensitive to both organic and inorganic pollution. As such, *A. pallipes* was assigned to SAG I.

Cambaridae

- Gallardo-Mayenco (1994) found the largest abundance of *Procambarus clarkii* (Girard, 1852) at a salinity of 2.1gL^{-1}, approximately 1.44PSU. Hence *P. clarkia* was assigned to SAG I.
- Piscart *et al*. (2005a) stated that *Orconectes limosus* (Rafinesque, 1817) is greatly affected by increases in salinity. Thus *O. limosus* was assigned to SAG I.

Mysidacea

Mysidae

- *Gastrosaccus spinifer* (Goës, 1864) was added to the list as the species is commonly found in brackish waters (Crothers, 1997) at salinities as low as 20gL^{-1} (Fish & Fish, 1996), approximately 16.22PSU. *Gastrosaccus spinifer* has been described by Ysebaert *et al*. (2003) as a polyhaline species. As such, *G. spinifer* was assigned to Salinity Association Group (SAG) V.
- *Mesopodopsis slabberi* (van Beneden, 1861) was added to the list as the species is common in estuarine waters with salinities as low as to 0.5gL^{-1} (Fish & Fish, 1996), approximately 0.32PSU. Barnes (1994) reported that *M. slabberi* tolerates salinities down to 0.5gL^{-1} (0.32PSU) and further stated that the species can tolerate fresh water for short periods of time. Hence *M. slabberi* was assigned to SAG III.

- *Neomysis integer* (Leach, 1814) is commonly found in estuarine waters and salt marsh pools (Crothers, 1997), tolerating salinities as low as $1gL^{-1}$ (Fish & Fish, 1996), approximately 0.66PSU. Wolf *et al*. (2009) implied that *N. integer* is tolerant of salinities ranging from 0.5-$30gL^{-1}$ (0.32-25.36PSU), a tolerance range that Barnes (1994) agreed the species possessed. Thus *N. integer* was assigned to SAG III.
- *Praunus flexuosus* (Müller, 1776) was added to the list as the species is common in coastal waters where it tolerates salinities down to $5gL^{-1}$ (Fish & Fish, 1996), approximately 3.62PSU. Barnes (1994) reported that whilst *P. flexuosus* can tolerate salinities as low as $6gL^{-1}$ (4.40PSU), it is most frequently encountered in the salinity range 20-$35gL^{-1}$ (16.22-30.11PSU) where it replaces *Neomysis integer*. As such, *P. flexuosus* was assigned to SAG V.
- *Praunus inermis* (Rathke, 1843) was added to the list as the species is common in Britain and extends into estuaries (Fish & Fish, 1996). However, it has been reported the species is most frequently recorded from the mouths of estuaries (Barnes, 1994). Consequently *P. inermis* was assigned to SAG V.
- *Schistomysis ornata* (G.O. Sars, 1864) was added to the list as Barnes (1994) reported that even though the species has a mainly marine distribution, it has been recorded in some estuaries in salinities down to $20gL^{-1}$ (approximately 16.22PSU). Thus *S. ornata* was assigned to SAG V.
- *Schistomysis spiritus* (Norman, 1860) was added to the list as the species is common in marine and coastal waters and extends into estuaries (Fish & Fish, 1996). Barnes (1994) reported that *S. spiritus* just extends into the mouths of estuaries. As such the species was assigned to SAG V.

Isopoda

It has been reported by Dunlop *et al*. (2008) that Isopoda are the most halo-tolerant freshwater taxa.

Gnathiidae

- *Paragnathia formica* (Hesse, 1864) was added to the list as the species is distributed in Britain and tolerates salinities down to $18gL^{-1}$ (Fish & Fish, 1996), approximately 14.45PSU. Barnes (1994) described *P. formica* as characteristic of estuarine habitats and also stated that the species tolerates salinities as low as to $18gL^{-1}$ (14.45PSU). Consequently *P. formica* was assigned to Salinity Association Group (SAG) IV.

Asellidae

- It has been stated that *Asellus aquaticus* (Linnaeus, 1758) is tolerant of high salinities (Gledhill *et al*., 1993). Piscart *et al*. (2005a) found that *A. aquaticus* is insensitive to increases in salinity, whilst Attrill *et al*. (1996) found that the species is not tolerant of increased salinities. However, Johnson *et al*. (2007) reported that *A. aquaticus* is most frequently found in the salinity range of $1\text{-}2gL^{-1}$ (approximately 0.66-1.37PSU), whilst Wolf *et al*. (2009) indicated that the species tolerates salinities up to $5gL^{-1}$ (3.62PSU). Furthermore, Berezina (2003) reported that the salinity tolerance range of *A. aquaticus* is $20mgL^{-1}$ to $8100mgL^{-1}$ (0.02-6.08PSU). Thus the species was assigned to SAG II.
- Gledhill *et al*. (1993) stated that *Proasellus meridanus* (Racovitza, 1919) is less tolerant of high salinities than *A. aquaticus.* As such *P. meridanus* was assigned to SAG I.

Anthuridae

- *Cyathura carinata* (Krøyer, 1847) inhabits sites where freshwater and salt water mix (Burbank, 1959) and has been recorded from sites which range in salinity from $15gL^{-1}$ to $30gL^{-1}$ (approximately 11.84-25.36PSU) by Ferreira *et al*. (2004). Hampel *et al*. (2009) reported that *C. carinata* occurs in large numbers in oligohaline environments, whilst Barnes (1994) stated that *C. carinata* can occur sporadically in salinities as low as $1gL^{-1}$ 9.066PSU0. As such, the species was assigned to SAG III.

Cirolanidae

- *Eurydice pulchra* Leach, 1815 was added to the list as the species is common in British marine and estuarine waters (Fish & Fish, 1996). Ysebaert *et al*. (2003) reported that the species is most often found in mesohaline waters and that *E. pulchra* can also be found in polyhaline and oligohaline waters. Brady (1943) recorded *E. pulchra* in salinities of $35gL^{-1}$ (approximately 30.11PSU), whilst Barnes (1994) stated that the species tolerates salinities as low as $18gL^{-1}$ (14.45PSU). Consequently *E. pulchra* was assigned to SAG IV.

Janiridae

- *Jaera nordmanni* Rathke, 1837 is usually found in salinities less than $5gL^{-1}$ (approximately 3.62PSU) according to Barnes (1994). However, Jones (1974) observed *J. nordmanni* tolerating salinities fluctuating from $10gL^{-1}$ to $30gL^{-1}$ (7.63-25.36PSU), whilst Naylor & Slinn (1958) recorded the species in salinities ranging from $2gL^{-1}$ to $34gL^{-1}$ (1.37-29.15PSU). Jones (1974) further stated that the species can also be found in freshwater sites, brackish waters and estuaries. Thus *J. nordmanni* was assigned to SAG III.

Sphaeromatidae

- *Lekanesphaera monodi* (Arcangeli, 1934) was added to the list as the species is common on salt marshes and in estuaries where it tolerates salinities down to $14gL^{-1}$ (Barnes, 1994; Fish & Fish, 1996), approximately 10.99PSU. Naylor (1972) described *L. monodi* as a brackish water species. Hence *L. monodi* was assigned to SAG IV.
- *Lekanesphaera hookeri* Leach, 1814 is most commonly found in habitats where salinity is within the range of $1\text{-}10gL^{-1}$ (approximately 0.66-7.63PSU) and higher (Fish & Fish, 1996). Naylor (1972) stated that the species can be found in brackish ditches as well as at the head of sheltered estuaries. Bamber *et al*. (2001) stated that the species is tolerant of salinities in the range $2\text{-}40gL^{-1}$ (1.37-34.97PSU), whereas Barnes (1994) reported that *L. hookeri* may be found in salinities ranging from $1gL^{-1}$ to $10gL^{-1}$ (0.66-7.63PSU) and occasionally up to $35gL^{-1}$ (30.11PSU). As such, *L. hookeri* was assigned to SAG III.
- *Lekanesphaera rugicauda* Leach, 1814 is found in estuaries and on salt marshes (Fish & Fish, 1996). Barnes (1994) reported that *L. rugicauda* is frequently found where salinity is over $8gL^{-1}$ (approximately 5.99PSU) and that rarely in salinities as low as $4gL^{-1}$ (2.85PSU). Consequently *L. rugicauda* was assigned to SAG III.

- *Sphaeroma serratum* (Fabricius, 1787) was added to the list as the species is common in marine and estuarine waters (Fish & Fish, 1996). Naylor (1972) reported that *Sphaeroma serratum* generally inhabits the mouths of estuaries. Kazanci *et al*. (2003) recorded that the largest abundance of *S. serratum* occurred at a salinity of $15gL^{-1}$ (approximately 11.84PSU) and also noted that the species occurred at salinities as low as $8gL^{-1}$ (5.99PSU). The species, however, was only present in very small numbers at this salinity. Consequently *S. serratum* was assigned to SAG IV.

Idoteidae

- *Idotea chelipes* (Pallas, 1766) was added to the list as the species has been recorded in large numbers in Britain (Jolly *et al*., 2003) and has been found in estuaries (Fish & Fish, 1996). Naylor & Slinn (1958) recorded *I. chelipes* in salinities ranging from $2gL^{-1}$ to $34gL^{-1}$, approximately 1.37-29.15PSU. Barnes (1994) stated that *I. chelipes* has been recorded in salinities as low as $4gL^{-1}$ (2.85PSU), whilst Bamber *et al*. (2001) reported that the species tolerates salinities in the range 5-$40gL^{-1}$ (3.62-34.97PSU) but prefers salinities in the range 15-$40gL^{-1}$ (11.84-34.97PSU). Hence *I. chelipes* was assigned to SAG III.
- *Idotea balthica* (Pallas, 1772) was added to the list as the species is commonly found in Britain (Fish & Fish, 1996). The species can tolerate salinities as low as $18gL^{-1}$ (approximately 14.45PSU) according to Barnes (1994). Thus *I. balthica* was assigned to SAG IV.
- *Idotea granulosa* Rathke, 1843 was added to the list as it has been suggested that tha animal is the most common species of the genus *Idotea* in Britain (Fish & Fish, 1996). Barnes (1994) stated that *I. granulosa* inhabits waters with salinities greater than $18gL^{-1}$, approximately 14.45PSU. As such, *I. granulosa* was assigned to SAG IV.

Amphipoda

Kefford (1998a) stated that Amphipoda are suspected to be affected by changes in salinity, a view which is supported by Wollheim & Lovvorn (1995). Wollheim & Lovvorn (1995) found Amphipoda were present in much smaller numbers in mesohaline lakes than in oligohaline lakes, indicating a low tolerance to high salinity.

Corophiidae

- *Corophium volutator* (Pallas, 1766) survives salinities down to $2gL^{-1}$ (approximately 1.37PSU) in estuaries according to Fish & Fish (1996), whereas Wolf *et al*. (2009) indicated that the species tolerates salinities in the range 0.5-$35gL^{-1}$ (0.32-30.11PSU). Barnes (1994) reported that *C. volutator* may occur in habitats with salinities as low as $1gL^{-1}$ (0.66PSU). Hampel *et al*. (2009) stated that the species is also found in oligohaline, mesohaline and polyhaline marshes. The species has also been recorded from salt marshes (Mason *et al*., 1991). Brady (1943) recorded the presence of *C. volutator* in the salinity range 32-$34gL^{-1}$ (27.25-29.15PSU), whilst Ysebaert *et al*. (2003) reported large abundances of the species in the salinity range 10-$14gL^{-1}$ (7.63-10.99PSU). Consequently *C. volutator* was assigned to Salinity Association Group (SAG) III.
- *Corophium arenarium* Crawford, 1937 was added to the list as the species is common in British estuaries and has a slightly narrower salinity range than *C. volutator* (Fish & Fish, 1996). Barnes (1994) reported that the preferred salinity range of *C. arenarium* is between $6gL^{-1}$ and $35gL^{-1}$ (approximately 4.40-30.11PSU). Thus *C. arenarium* was assigned to SAG III.
- *Chelicorophium curvispinum* Sars, 1895 occurs in both fresh and brackish water (Barnes, 1994). The species was first found in a river with a permanent salinity gradient at a salinity of $2.6gL^{-1}$ (Piscart *et al*., 2005a), approximately 1.81PSU, whilst *C. curvispinum* has also been recorded at a salinity of $4.5gL^{-1}$ (Herkül & Kotta, 2007), equivalent to 3.23PSU. As such, *C. curvispinum* was assigned to SAG III.
- *Monocorophium insidiosum* Crawford, 1937 has been described by Ysebaert *et al*. (2003) as a mesohaline to oligohaline species. Bamber *et al*. (2001) stated that *M. insidiosum* tolerates salinities in the range 15-$40gL^{-1}$, approximately 11.84-34.97PSU. Barnes (1994) reported that the species occurs in the salinity range 12-$35gL^{-1}$ (9.29-30.11PSU). Hence *M. insidiosum* was assigned to SAG IV.

- *Corophium multisetosum* Stock, 1952 has been described as a brackish water species by Wolf *et al*. (2009), who further indicated that the species can inhabit the salinity range 0.5-30gL^{-1}, approximately 0.32-25.36PSU. Cunha *et al*. (2000) stated that *C. multisetosum* tolerates salinities up to 20gL^{-1} (16.22PSU), whereas Barnes (1994) reported that the species occurs in fresh and brackish water up to a salinity of 16gL^{-1} (12.71PSU). Consequently *C. multisetosum* was assigned to salinity SAG III.

Talitridae

- *Orchestia cavimana* Heller, 1865 is commonly found in estuarine and brackish waters (Gledhill *et al*., 1993) and as such *O. cavimana* was assigned to SAG IV.
- *Orchestia gammarellus* (Pallas, 1766) was added to the list as the species is common in coastal and estuarine waters (Fish & Fish, 1996). The species has been recorded from salt marshes in Essex (Mason *et al*., 1991). Hence *O. gammarellus* was assigned to SAG IV.

Crangonyctidae

- *Crangonyx pseudogracilis* Bousfield, 1958 inhabits most types of water courses and is tolerant of saline water (Gledhill *et al*., 1993). Thus *C. pseudogracilis* was assigned to SAG II.

Haustoriidae

- *Haustorius arenarius* (Slabber, 1767) is the only British species of the family and was added to the list as it is frequently recorded in coastal waters and estuary mouths (Fish & Fish, 1996). Brady (1943) recorded *H. arenarius* at a salinity of 35gL^{-1}, approximately 30.11PSU, whereas Barnes (1994) stated that the species can tolerate salinities as low as 10gL^{-1} (7.63PSU). Ysebaert *et al*. (2003) reported that *H. arenarius* is mainly found in mesohaline water, but can also be found in polyhaline and oligohaline waters. Consequently *H. arenarius* was assigned to SAG III.

Gammaridae

- According to Bamber *et al*. (2001), *Gammarus chevreuxi* Sexton, 1913 tolerates salinities in the range 1-15gL^{-1}, approximately 0.66-11.84PSU, whilst Barnes (1994) reported that the species has been recorded in the salinity range 1-10gL^{-1} (0.66-7.63PSU). Subsequently *G. chevreuxi* was assigned to SAG II.

- *Gammarus duebeni* Liljeborg, 1852 primarily occurs in brackish water such as estuaries and salt marsh pools according to Gledhill *et al*. (1993). Jażdżewski *et al*. (2005) described *G. duebeni* as a brackish water species and stated that it has been recorded in salinities of 0.5-1.5gL^{-1}, approximately 0.32-1.01PSU, whilst Naylor & Slinn (1958) recorded the species in salinities ranging from 2gL^{-1} to 34gL^{-1} (1.37-29.15PSU). Thus *G. duebeni* was assigned to SAG III.
- *Gammarus lacustris* Sars, 1863 is tolerant only of slightly brackish water according to Gledhill *et al*. (1993). Wollheim & Lovvorn (1995) noted that *G. lacustris* was frequently found in oligohaline lakes, but rare in mesohaline lakes. Thus *G. lacustris* was assigned to SAG I.
- *Gammarus locusta* Linnaeus, 1758 is common in coastal and estuarine waters where it tolerates salinities down to 4gL^{-1} (Fish & Fish, 1996), approximately 2.85PSU. Brady (1943) recorded *G. locusta* from the River Tyne at a salinity of 31gL^{-1} (26.30PSU), whilst Correia & Costa (2000) described the species as marine and held specimens at a salinity of 33gL^{-1} (28.20PSU) prior to performing experiments investigating metal toxicity. Barnes (1994) stated that *G. locusta* is most frequently found in salinities over 12gL^{-1} (9.29PSU) and added that the species can tolerate salinities down to 8gL^{-1} (5.99PSU). Consequently *G. locusta* was assigned to SAG IV.
- *Gammarus oceanicus* Segerstrale, 1947 is found in the open sea, brackish waters and some fresh waters, surviving in salinities as low as 5gL^{-1} (Normant & Lamprecht, 2006), approximately 3.62PSU. Barnes (1994), however, reported that *G. oceanicus* is only found where salinity is over 18gL^{-1} (14.45PSU). Thus *G. oceanicus* was assigned to SAG IV.
- *Gammarus pulex* (Linnaeus, 1758) is frequently found in flowing and standing freshwater (Gledhill *et al*., 1993) in salinities of 1-2gL^{-1} (Johnson *et al*., 2007), approximately 0.66-1.37PSU. Barnes (1994) noted that *G. pulex* may occasionally be washed into dilute brackish waters. Piscart *et al*. (2005a) reported that *G. pulex* is greatly affected by salinity increases, a conclusion which is supported by Wood & Dykes (2002). Wood & Dykes (2002) recorded that *G. pulex* entered drift when a salt solution was added to a river as part of the dilution gauging technique to measure stream velocity and flow discharge. As such, *G. pulex* was assigned to SAG I.

- *Gammarus salinus* Spooner, 1942 is reported to be confined to brackish and marine habitats (Gledhill *et al*., 1993). Johnson *et al*. (2007) found *G. salinus* in a lake with salinity ranging from $2gL^{-1}$ to $8gL^{-1}$ (approximately 1.37-5.99PSU) and also stated that *G. salinus* is replaced by *Gammarus locusta* at higher salinities. Barnes (1994) stated that *G. salinus* tolerates salinities in the range 3-$25gL^{-1}$ (2.10-20.73PSU). Hence *G. salinus* was assigned to SAG III.
- *Gammarus tigrinus* Sexton, 1939 inhabits brackish water from slightly saline waters to coastal estuarine habitats (Gledhill *et al*., 1993) and was first recorded in the Meurthe River, France, when salinity rose to $2.6gL^{-1}$ (Piscart *et al*., 2005a), approximately 1.81PSU. *Gammarus tigrinus* has also been recorded in large numbers at salinities ranging from 0.5-$10gL^{-1}$ (Normant *et al*., 2007), 0.32-7.63PSU. Thus *G. tigrinus* was assigned to SAG II.
- *Gammarus zaddachi* Sexton, 1912 is a brackish water species which is tolerant of freshwater (Gledhill *et al*., 1993). *Gammarus zaddachi* is most active in the middle reaches of estuaries (Attrill *et al*., 1996) and is found in the salinity range 1-$8gL^{-1}$ (Williams & Williams, 1998b), approximately 0.66-5.99PSU. Barnes (1994) stated that *G. zaddachi* is only found where salinity is less than $10gL^{-1}$ (7.63PSU), whilst Johnson *et al*. (2007) also stated that the species is replaced by *G. salinus* at higher salinities. As such, *G. zaddachi* was assigned to SAG II.

Melitidae

- *Melita palmata* (Montagu, 1804) was added to the list as the species is widely distributed in Britain (Fish & Fish, 1996). Barnes (1994) reported that *M. palmata* tolerates salinities down to $5gL^{-1}$, approximately 3.62PSU. Hence *M. palmata* was assigned to SAG III.

Hyalidae

- *Hyale prevostii* (Milne-Edwards, 1830) was added to the list as the species is common in coastal and estuarine waters (Fish & Fish, 1996). Thus *H. prevostii* was assigned to SAG V.

Pontoporeiidae

- *Bathyporeia pelagica* (Bate, 1856) was added to the list as the species is common in coastal waters and estuary mouths (Fish & Fish, 1996). Ysebaert *et al*. (2003) described the species as an inhabitant of mesohaline conditions which may also be found in polyhaline and oligohaline waters. As such, *B. pelagica* was assigned to SAG III.

- *Bathyporeia pilosa* Lindström, 1855 was added to the list as the species is commonly found in coastal and estuarine waters tolerating salinities down to $5gL^{-1}$ (Fish & Fish, 1996), approximately 3.62PSU. Barnes (1994) stated that *B. pilosa* can tolerate salinities as low as $4gL^{-1}$ (2.85PSU), whilst Ysebaert *et al*. (2003) described the species as mesohaline which can also be found in oligohaline and polyhaline waters. Hence *B. pilosa* was assigned to SAG III.
- *Bathyporeia sarsi* Watkin, 1938 was added to the list as the species is frequently found in coastal waters and estuary mouths (Fish & Fish, 1996). As with *B. pelagica* and *B. pilosa*, Ysebaert *et al*. (2003) described *B. sarsi* as a mesohaline species which may also be found in polyhaline and oligohaline waters. Thus *B. sarsi* was assigned to SAG III.

Chilopoda

Geophilidae

- *Strigamia maritima* (Leach, 1817) is frequently found around the high-water mark on beaches and estuaries where it hides in crevices and under seaweed (Crothers, 1997). As such, *S. maritima* was assigned to SAG II.

Ephemeroptera

James *et al*. (2003), Kefford *et al*. (2004a) and Hassell *et al*. (2006) all stated that mayflies are salt sensitive species, whilst Dunlop *et al*. (2008) reported Ephemeroptera are the most salt sensitive species. These views are supported by the findings of Short *et al*. (1991), Bunn & Davies (1992) and Piscart *et al*. (2005a). Short *et al*. (1991) studied the effect that the disposal of saline waste water had on the macro-invertebrate community of a river system in Kentucky, USA, and found that Ephemeroptera were the most severely affected taxon. During an investigation of a salinised river system in Australia, Bunn & Davies (1992) found no mayflies were present. In a study along a permanent salinity gradient in the Meurthe River, France, Piscart *et al*. (2005a) reported that abundances of Ephemeroptera decreased as salinity increased.

Baetidae

- *Baetis fuscatus* (Linnaeus, 1761) is found in running water and may have a preference for calcareous waters (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Gallardo-Mayenco (1994) noted larger abundances of *B. fuscatus* occurring at low salinities, with the largest abundance occurring at 1.7gL^{-1}, approximately 1.15PSU. Hence *B. fuscatus* was assigned to Salinity Association Group (SAG) I.
- *Baetis rhodani* (Pictet, 1843-1845) is found in running water, mostly in riffles in streams and rivers (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Gallardo-Mayenco (1994) reported that larger abundances of *B. rhodani* occur at lower salinities and the largest abundance occurred at a salinity of 1.3gL^{-1}, approximately 0.87PSU. Thus *B. rhodani* was assigned to SAG I.
- *Baetis scambus* Eaton, 1870 is found in running water (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Gallardo-Mayenco (1994) documented larger abundances of *B. scambus* occurring at lower salinities and also noted that the largest abundance occurred at 0.97gL^{-1}, approximately 0.64PSU. As such, *B. scambus* was assigned to SAG I.
- Short *et al*. (1991) recorded the genus *Baetis* in the salinity range 48-88mgL^{-1}, approximately 0.04-0.06PSU, whilst Piscart *et al*. (2005a) found that the genus *Baetis* is greatly affected by increases in salinity. Hence the remaining species of the genus not yet assigned to a Salinity Association Group were assigned to SAG I.
- *Centroptilum luteolum* (Müller, 1776) is frequently found in slow-flowing water and on the wave-washed shores of lakes (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Wolf *et al*. (2009) described the species as purely freshwater, whilst Gallardo-Mayenco (1994) observed that *C. luteolum* is distributed where salinity is below 1.63gL^{-1}, approximately 1.10PSU, and noted that the largest abundance of the species occurred at a salinity of 1.22gL^{-1} (0.81PSU). Thus *C. luteolum* was assigned to SAG I.
- *Cloeon dipterum* (Linnaeus, 1761) inhabits ponds, shallow lakes and slow flowing water (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Silberbush *et al*. (2005) found that the species tolerates salinities from freshwater to a concentration of 30gL^{-1}, approximately 25.36PSU, with no preference, whereas Wolf *et al*. (2009) indicated that *C. dipterum* tolerates salinities up to 5gL^{-1} (3.62PSU). Barnes (1994) reported that *C. dipterum* is occasionally recorded from low salinity sites. As such, the species was assigned to SAG II.

- *Cloeon simile* (Eaton, 1870) inhabits slow flowing and standing waters (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Wolf *et al*. (2009) described *C. simile* as a freshwater species. Furthermore, Gallardo-Mayenco (1994) noted that larger abundances of the species occur at lower salinities and that the largest abundance occurred at a salinity of $1.5gL^{-1}$, approximately 1.01PSU. Hence *C. simile* was assigned to SAG I.
- *Alainites muticus* (Linnaeus, 1758) is found in small stony streams and rivers (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Gallardo-Mayenco (1994) recorded that the largest abundance of *A. muticus* occurred at a salinity of $1.22gL^{-1}$, approximately 0.81PSU. Thus *A. muticus* was assigned to SAG I.

Heptageniidae

- *Rhithrogena semicolorata* (Curtis, 1834) is chiefly found in riffles in running water and in stony streams and rivers (Macan, 1979; Elliott & Humpesch, 1983; Elliott *et al*., 1988). Williams & Williams (1998a) reported that *R. semicolorata* was confined to the upper sections of the Aber Estuary, Wales. Williams & Williams (1998a) also found that the species only survived one hour immersion in undiluted seawater. Hence *R. semicolorata* was assigned to SAG I.
- *Heptagenia sulphurea* (Müller, 1776) inhabits riffles in large rivers and may also be found on the wave-washed shores of calcareous rivers (Macan, 1979; Elliott & Humpesch, 1983; Elliott *et al*., 1988). Wolf *et al*. (2009) described the species as purely freshwater. As such *H. sulphurea* was assigned to SAG I.

Leptophlebiidae

- *Paraleptophlebia submarginata* (Stephens, 1835) is found in stony streams and rivers (Elliott & Humpesch, 1983; Elliott *et al*., 1988). Gallardo-Mayenco (1994) recorded that the species is distributed at salinities below $1.63gL^{-1}$, approximately 1.10PSU, and that the largest abundance occurred where salinity was $1.5gL^{-1}$ (1.01PSU). Wolf *et al*. (2009) classified *P. submarginata* as a purely freshwater species. Thus the species was assigned to SAG I.

Ephemeridae

- *Ephemera danica* Müller, 1764 is distributed in lakes, as well as in the sand and gravel of fast-flowing rivers and streams (Macan, 1979; Elliott & Humpesch, 1983; Elliott *et al*., 1988). Gallardo-Mayenco (1994) noted that the largest abundance of *E. danica* occurred where salinity was $1.47gL^{-1}$ (approximately 0.99PSU) and that the species is distributed where salinity is below $1.63gL^{-1}$ (1.10PSU). As such, *E. danica* was assigned to SAG I.

Ephemerellidae

- *Serratella ignita* (Poda, 1761) inhabits fast-flowing rivers and streams (Macan, 1979; Elliott & Humpesch, 1983; Elliott *et al*., 1988). It has been documented that *S. ignita* is distributed at salinities below $1.63gL^{-1}$ (approximately 1.10PSU) with the largest abundance occurring at a salinity of $1.47gL^{-1}$ (Gallardo-Mayenco, 1994), approximately 0.99PSU. Hence *S. ignita* was assigned to SAG I.

Caenidae

- *Caenis horaria* (Linnaeus, 1758) is found in the mud and silt of lakes, canals and large rivers (Macan, 1979; Elliott & Humpesch, 1983; Elliott *et al*., 1988). Wolf *et al*. (2009) stated that *C. horaria* is tolerant of salinities up to $5gL^{-1}$, approximately 3.62PSU, whilst Lingdell & Müller (1981) reported that the species occurs in the salinity range $2.5\text{-}5.5gL^{-1}$ (1.73-4.01PSU). Thus *C. horaria* was assigned to SAG II.
- *Caenis luctosa* (Burmeister, 1839) is distributed in the silt of standing and flowing water (Macan, 1979; Elliott & Humpesch, 1983; Elliott *et al*., 1988). Muñoz & Prat (1994) found that the mean density of *C. luctosa* decreases severely when salinity reaches $2.45gL^{-1}$, approximately 1.70PSU, whilst Gallardo-Mayenco (1994) noted that the largest abundance of the species occurred at a salinity of $1.1gL^{-1}$ (0.73PSU). As such, *C. luctosa* was assigned to SAG I.
- *Caenis pseudorivulorum* Keffermüller, 1960 has been described by Wolf *et al*. (2009) as a purely freshwater species. Hence *C. pseudorivulorum* was assigned to SAG I.
- *Caenis pusilla* Navás, 1913 inhabits the pools and margins of stony streams and rivers (Elliott *et al*., 1988). Muñoz & Prat (1994) recorded that the mean density of *C. pusilla* decreased severely at estuarine sites. Thus *C. pusilla* was assigned to SAG I.

- Piscart *et al*. (2005a) reported that species of the genus *Caenis* are relatively insensitive to increases in salinity. However, Leland & Fend (1998) recorded the presence of the genus in the salinity range 0-2gL^{-1}, approximately 0-1.37PSU, whilst Short *et al*. (1991) found species of the genus *Caenis* in the salinity range 860-1350mgL^{-1} (0.56-0.90PSU). Hence species of the genus *Caenis* not yet assigned to a Salinity Association Group were assigned to SAG I.

Plecoptera

It has been proposed that stoneflies are among the most salt sensitive invertebrates (Hart *et al*., 1990; Kefford, 1998a; James *et al*., 2003) and the findings of both Williams *et al*. (1991) and Bunn & Davies (1992) support this suggestion. In a survey of Backwood River and Gleneg River, Australia, Williams *et al*. (1991) found that Plecoptera were rare or absent in these salinised rivers. Whilst surveying Thirty-four Mile Brook and Hotham River in Australia, where salinity ranged from 0.2gL^{-1} to 2.4gL^{-1} (0.13-1.66PSU), Bunn & Davies (1992) only recorded one specimen of stonefly from conditions.

Nemouridae

- It has been reported that the genus *Protonemura* is distributed at salinities less than 1.6gL^{-1} (Gallardo-Mayenco, 1994), approximately 1.08PSU. As such, the genus *Protonemura* was assigned to Salinity Association Group (SAG) I.
- *Nemoura cinerea* (Retzius, 1783) inhabits still or slow-flowing water with emergent vegetation (Hynes, 1977). Wood & Dykes (2002) studied the effects on macro-invertebrates of using a salt solution in implementing the gulp injection technique and found that *N. cinerea* was one of the species which entered drift as a result. This indicates that *N. cinerea* has a low tolerance to even small increases in salinity. Hence *N. cinerea* was assigned to SAG I.
- *Nemoura erratica* Claassen, 1936 is typically found in small stony streams (Hynes, 1977). Gallardo-Mayenco (1994) recorded the species in a stream with a salinity ranging from 5.5 gL^{-1} to 8.8gL^{-1} (approximately 4.01-6.64PSU). Thus *N. erratica* was assigned to SAG II.

Leuctridae

- *Leuctra fusca* (Linnaeus, 1758) can be found in all types of water courses with a stony substratum (Hynes, 1977). *Leuctra fusca* is distributed at salinities less than $1.6gL^{-1}$, approximately 1.08PSU, according to Gallardo-Mayenco (1994), who also found that the largest abundance of the species occurred at a salinity of $1.3gL^{-1}$ (0.87PSU) in the Guadalete and Guadaira rivers, Spain. As such, *L. fusca* was assigned to SAG I.
- *Leuctra geniculata* (Stephens, 1836) inhabits the stony beds of rivers and large streams (Hynes, 1977). Gallardo-Mayenco (1994) reported that the species is distributed in salinities less than $1.6gL^{-1}$, approximately 1.08PSU. Hence *L. geniculata* was assigned to SAG I.

Perlodidae

- *Isoperla grammatica* (Poda, 1761) is found in rivers and streams with a stony substratum (Hynes, 1977). The species has been recorded from locations in Guadalete and Guadaira rivers, Spain, with salinity ranging from 5.5 gL^{-1} to $8.8gL^{-1}$ by Gallardo-Mayenco (1994), approximately 4.01-6.64PSU. Thus *I. grammatica* was assigned to SAG II.

Perlidae

- *Dinocras cephalotes* (Curtis, 1827) can be found in rivers, and occasionally in streams, with a stony substratum (Hynes, 1977; Chinery, 1986). Williams & Williams (1998a) noted that *D. cephalotes* was confined to only freshwater sites in the Aber Estuary, Wales. Williams & Williams (1998a) also reported that the species survived a four hour immersion in undiluted seawater. As such, *D. cephalotes* was assigned to SAG II.
- *Perla bipunctata* Pictet, 1833 inhabits rivers and streams with a stony substratum (Hynes, 1977). Following a study of the Aber Estuary, Wales, Williams & Williams (1998a) reported that *P. bipunctata* was confined to the upper parts of the estuary. Williams & Williams (1998a) also found that the species survived a four hour immersion in undiluted seawater. Consequently *P. bipunctata* was assigned to SAG II.

Odonata

Hart *et al*. (1990) stated that larvae of Odonata are salt sensitive, whereas Kefford *et al*. (2003) reported that Odonata are, in general, more salt tolerant than other insect orders such as Ephemeroptera, Trichoptera and Hemiptera. Whilst Berezina (2003) found that the larvae of Odonata were among some of the insect larvae least tolerant to salinity increases, thus agreeing with the view of Hart *et al*. (1990), Piscart *et al*. (2005a) noted that Odonata contains both salt tolerant and salt sensitive species and, as such, is in contrast with both suggestions.

Platycnemididae

- *Platycnemis pennipes* (Pallas, 1771) occupies slow-flowing water habitats (Chinery, 1986) in weedy streams and rivers (Hammond, 1985). Piscart *et al*. (2005a) found *P. pennipes* to be relatively insensitive to increases in salinity. Thus *P. pennipes* was assigned to Salinity Association Group (SAG) II.

Coenagrionidae

- *Ischnura elegans* (Vander Linden, 1820) can be found in slow-flowing and still water habitats, including brackish and polluted water (Hammond, 1985; Chinery, 1986; Miller, 1995). Greenwood & Wood (2003) reported that *I. elegans* has been recorded in the salinity range 4-23gL^{-1}, approximately 2.85-18.91PSU, whilst Barnes (1994) documented that the nymphs of *I. elegans* can develop in habitats with salinities up to, and very occasionally over, 18gL^{-1} (14.45PSU). Beschovski & Marinov (2007) stated that *I. elegans* prefers salinities below 5gL^{-1} (3.62PSU), but can also tolerate salinities up to 13gL^{-1} (10.13PSU). As such *I. elegans* was assigned to SAG II.
- *Ischnura pumilio* (Charpentier, 1825) tolerates brackish conditions according to Miller (1995). Thus, *I. pumilio* was assigned to SAG II.
- *Enallagma cyathigerum* (Charpentier, 1840) inhabits slow-flowing water and tolerates brackish conditions (Hammond, 1985; Chinery, 1986). Wollheim & Lovvorn (1995) noted that higher salinities corresponded with an increased biomass of *Enallagma cyathigerum*, whilst Corbet (1999) reported that the species is tolerant of all salinities below 7.4gL^{-1}, approximately 5.51PSU. Hence *E. cyathigerum* was assigned to SAG II.

- *Coenagrion puella* (Linnaeus, 1758) is found in weedy ponds, lakes, dykes and canals (Hammond, 1985; Chinery, 1986). Johnson *et al*. (2007) reported that *C. puella* tolerates salinities in the range 1-8gL^{-1}, approximately 0.66-5.99PSU. Thus *C. puella* was assigned to SAG II.

Lestidae

- *Lestes dryas* Kirby, 1890 is tolerant of brackish water conditions (Miller, 1995). Hence the species was assigned to SAG II.
- *Lestes sponsa* (Hansemann, 1823) tolerates brackish conditions (Miller, 1995). Consequently *L. sponsa* was assigned to SAG II.

Calopterygidae

- *Calopteryx splendens* (Harris, 1782) inhabits sluggish streams and occasionally ponds with a muddy substratum (Hammond, 1985). The species is greatly affected by salinity increases (Piscart *et al*., 2005a). As such, *C. splendens* was assigned to SAG I.
- The genus *Calopteryx* is distributed at salinities less than 1.6gL^{-1}, approximately 1.08PSU, according to Gallardo-Mayenco (1994). Thus *Calopteryx virgo* (Linnaeus, 1758) was assigned to SAG I.

Gomphidae

- Short *et al*. (1991) recorded the genus *Gomphus* in the salinity range 860-1350mgL^{-1} (approximately 0.56-0.90PSU), whilst Piscart *et al*. (2005a) reported that the genus is greatly affected by increases in salinity. Hence the genus *Gomphus* was assigned to SAG I.

Cordulegastridae

- *Cordulegaster boltonii* (Donovan, 1807) inhabits moorland streams and occasionally boggy pools (Hammond, 1985). Gallardo-Mayenco (1994) noted that the species is distributed at salinities less than 1.6gL^{-1}, approximately 1.08PSU. Thus *C. boltonii* was assigned to SAG I.

Aeshnidae

- *Aeshna grandis* (Linnaeus, 1758) has been recorded at a site with a salinity of 3.8gL^{-1} (approximately 2.70PSU) by Vuori *et al*. (1999). Thus *A. grandis* was assigned to SAG II.
- *Aeshna juncea* (Linnaeus, 1758) can be found in weedy ponds, lakes and peat pools (Hammond, 1985). Corbet (1999) reported that *A. juncea* only tolerates salinities up to 1.8gL^{-1}, approximately 1.22PSU. As such, *A. juncea* was assigned to SAG I.

- *Aeshna mixta* Lattreille, 1805 is found mainly in still waters such as ponds and lakes (Chinery, 1986), but can breed in brackish water conditions (Miller, 1995). The species has been recorded in the salinity range 4-23gL^{-1} (Greenwood & Wood, 2003), approximately 2.85-18.91PSU. Consequently *A. mixta* was assigned to SAG III.
- *Hemianax ephippiger* (Burmeister, 1839) is reportedly tolerant of salinity (Corbet, 1999). Thus *H. ephippiger* was assigned to SAG II.

Libellulidae

- *Orthetrum cancellatum* (Linnaeus, 1758) occupies a wide range of habitats (Hammond, 1985; Chinery, 1986). Barnes (1994) reported that *O. cancellatum* has been recorded from salinities up to 13gL^{-1}, approximately 10.13PSU. As such, *O. cancellatum* was assigned to SAG II.
- *Libellula depressa* Linnaeus, 1758 inhabits ponds, lakes and canals (Hammond, 1985; Chinery, 1986). Berezina (2003) reported that the salinity tolerance range of the species is 20-6300mgL^{-1}, approximately 0.02-4.64PSU. Hence *L. depressa* was assigned to SAG II.
- *Libellula quadrimaculata* Linnaeus, 1758 is frequently found in brackish water in ponds, lakes and canals and often occupies waters close to the sea (Hammond, 1985; Chinery, 1986). Corbet (1999) stated that the species tolerates salinities up to 9gL^{-1}, approximately 6.81PSU. Consequently *L. quadrimaculata* was assigned to SAG II.
- *Sympetrum nigrescens* Lucas, 1912 favours waters close to the coast (Hammond, 1985) and thus must have some tolerance to increased salinity. As such *S. nigresens* was assigned to SAG II.
- *Sympetrum sanguineum* Müller, 1764 can be found in a wide range of habitats (Hammond, 1985). The species has been recorded from habitats with salinities of up to 8gL^{-1} (Barnes, 1994), approximately 5.99PSU. Thus *S. sanguineum* was assigned to SAG II.
- *Sympetrum danae* (Sulzer, 1776) inhabits moorland bog-holes and rush-filled pools (Hammond, 1985). It has been reported that *S. danae* tolerates salinities up to 5gL^{-1} (Corbet, 1999), approximately 3.62PSU. Hence *S. danae* was assigned to SAG II.
- *Sympetrum striolatum* (Charpentier, 1840) occurs in brackish water conditions according to Miller (1995). Thus *S. Striolatum* was assigned to SAG II.
- It has been stated that *Pantala flavescens* (Fabricius, 1798) occasionally inhabits brackish intertidal pools (Corbet, 1999). As such, *P. flavescens* was assigned to SAG IV.

Hemiptera

Hart *et al*. (1990) proposed that certain genera of Hemiptera are halo-sensitive. James *et al*. (2003) agreed with this proposal, stating that certain Hemiptera appear to be tolerant of salinity increases whereas other Hemiptera are sensitive to even slight increases in salinity. Dunlop *et al*. (2008) found that Hemiptera are more tolerant of salinity increases than Gastropoda and Ephemeroptera, but less halo-tolerant than Odonata. Kefford *et al*. (2003) also found that Hemiptera are more halo-tolerant than Ephemeroptera and also reported that Hemiptera are less halo-tolerant than Coleoptera and Odonata.

Hebridae

- It has been documented that *Hebrus ruficeps* Thomson, 1871 is only found where salinities are less than $0.9gL^{-1}$ (Savage, 1989), approximately 0.59PSU. Hence *H. ruficeps* was assigned to Salinity Association Group (SAG) I.

Hydrometridae

- *Hydrometra stagnorum* (Linnaeus, 1758) occupies the vegetation at the edge of still or slow moving water (Macan, 1965). Savage (1989) stated that *H. stagnorum* is found at salinities less than $1.6gL^{-1}$ (approximately 1.08PSU), whilst Gallardo-Mayenco (1994) recorded the largest abundance of the species at a salinity of $1.1gL^{-1}$ (0.73PSU). Consequently *H. stagnorum* was assigned to SAG I.

Veliidae

- *Velia (Plesiovelia) caprai* (Tamanini, 1947) is found in running water and ponds (Macan, 1965). It has been noted that *V. caprai* is found where salinity is less than $1.6gL^{-1}$ (Savage, 1989), approximately 1.08PSU. As such, *V. caprai* was assigned to SAG I.
- *Velia (Plesiovelia) saulii* (Tamanini, 1947) inhabits large, open water bodies according to Macan (1965). Savage (1989) reported that *V. saulii* only occurs where salinity is not greater than $1.6gL^{-1}$, approximately 1.08PSU. Hence *V. saulii* was assigned to SAG I.
- *Microvelia pygmaea* (Dufour, 1833) is only found where salinity is below $1.6gL^{-1}$ (Savage, 1989), approximately 1.08PSU. Thus *M. pygmaea* was assigned to SAG I.
- It has been documented by Savage (1989) that *Microvelia reticulata* (Burmeister, 1835) tolerates salinities up to $9gL^{-1}$, approximately 6.81PSU. As such, *M. reticulata* was assigned to SAG II.

Gerridae

- Savage (1989) stated that *Gerris argentatus* Schummel, 1832 only inhabits waters where salinity is not greater than $1.6gL^{-1}$, approximately 1.08PSU. Hence *G. argentatus* was assigned to SAG I.
- *Gerris costae* (Herrich-Schäffer, 1850) is found where salinity is below $0.9gL^{-1}$ (Savage, 1989), approximately 0.59PSU. Thus *G. costae* was assigned to SAG I.
- *Gerris gibbifer* Schummel, 1832 inhabits waters where salinity is below $0.9gL^{-1}$, approximately 0.59PSU, and may also occur in salinities up to $1.6gL^{-1}$ (Savage, 1989), approximately 1.08PSU. As such, *G. gibbifer* was assigned to SAG I.
- *Gerris lacustris* (Linnaeus, 1758) is commonly found where salinity is below $1.6gL^{-1}$, approximately 1.08PSU, according to Savage (1989). Gallardo-Mayenco (1994) recorded that the largest abundance of the species occurred at a salinity of $1.1gL^{-1}$ (0.73PSU). Hence *G. lacustris* was assigned to SAG I.
- *Gerris odontogaster* (Zetterstedt, 1828) inhabits the salinity range 0.9-$1.6gL^{-1}$ according to Savage (1989), approximately 0.59-1.08PSU, who further stated that the species may also occur at slightly higher salinities. Thus *G. odontogaster* was assigned to SAG I.
- Macan (1965) described *Gerris thoracicus* Schummel, 1832 as an inhabitant of brackish water. Savage (1989) stated that the species may be found in the salinity range 0.9-$9gL^{-1}$, approximately 0.59-6.81PSU, whereas Gallardo-Mayenco (1994) recorded that the largest abundance of *G. thoracicus* occurred at a salinity of $1.3gL^{-1}$ (0.87PSU). Consequently the species was assigned to SAG II.
- *Aquarius najas* (DeGeer, 1773) is found in large bodies of still water as well as in running water (Macan, 1965). It has been noted that *A. najas* occurs most frequently where salinity is not more than $0.9gL^{-1}$ (Savage 1989), approximately 0.59PSU. Savage (1989) also stated that the species may occasionally be present in salinities up to $1.6gL^{-1}$ (1.08PSU). Hence *A. najas* was assigned to SAG I.

Nepidae

- Macan (1965) stated that *Nepa cinerea* (Linnaeus, 1758) inhabits the mud or thick undergrowth of shallow water. The species is frequently found in where salinity ranges from $0.9gL^{-1}$ to $1.6gL^{-1}$ (approximately 0.59-1.08PSU) and also tolerates up to $9gL^{-1}$ (Savage, 1989), approximately 6.81PSU. Thus *N. cinerea* was assigned to SAG II.

Naucoridae

- *Ilyocoris cimicoides* (Linnaeus, 1758) inhabits still water according to Macan (1965). It is most commonly found in the salinity range 0.9-1.6gL^{-1} (approximately 0.59-1.08PSU) and may also be found at salinities up to 9gL^{-1} (Savage, 1989), approximately 6.81PSU. As such, *I. cimicoides* was assigned to SAG II.
- Gallardo-Mayenco (1994) recorded that the largest abundance of *Naucoris maculatus* (Fabricius, 1758) occurred at a salinity of 1.3gL^{-1}, approximately 0.87PSU. Consequently *N. maculatus* was assigned to SAG I.

Aphelocheiridae

- *Aphelocheirus aestivalis* (Fabricius, 1794) inhabits waters with a salinity of 0.9-1.6gL^{-1} (Savage, 1989), approximately 0.59-1.08PSU, and is greatly affected by increases in salinity (Piscart *et al*., 2005a). Consequently *A. aestivalis* was assigned to SAG I.

Notonectidae

- It has been reported that *Notonecta glauca* (Linnaeus, 1758) is found in the salinity range 0.9-9gL^{-1} (Savage, 1989), approximately 0.59-6.81PSU. Barnes (1994) stated that the species can occupy habitats with relatively low salinities. As such, *N. glauca* was assigned to SAG II.
- According to Savage (1989), *Notonecta maculata* Fabricius, 1794 is found in the salinity range 0.9-1.6gL^{-1}, approximately 0.59-1.08PSU. Gallardo-Mayenco (1994) recorded that the largest abundance of the species occurred at a salinity of 1.1gL^{-1} (0.73PSU). Hence *N. maculata* was assigned to SAG I.
- *Notonecta obliqua* Gallén in Thunberg, 1787 is frequently found where salinity is less than 0.9gL^{-1} (approximately 0.59PSU) and can be found in salinities up to 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Thus *N. obliqua* was assigned to SAG I.
- Macan (1965) stated that *Notonecta viridis* Delcourt, 1909 is found mostly in brackish water but has also been recorded in freshwater. Greenwood & Wood (2003) reported that *N. viridis* has been recorded in the salinity range 4-23gL^{-1}, approximately 2.85-18.91PSU, whilst Barnes (1994) stated that the species can occupy habitats with relatively low salinities. As such *Notonecta viridis* was assigned to SAG III.

Corixidae

- *Micronecta poweri* (Douglas & Scott, 1869) inhabits lakes and rivers (Macan, 1965) with salinities less than 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Hence *M. poweri* was assigned to SAG I.
- *Cymatia bonsdorffii* (C.R. Sahlberg, 1819) is found in heath and moorland ponds, lakes and rivers (Macan, 1965) where salinity is below 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Thus the species was assigned to SAG I.
- *Cymatia coleoptrata* (Fabricius, 1777) is an inhabitant of heath and moorland ponds, lakes and rivers (Macan, 1965) where salinity is in the range of 0.9-1.6gL^{-1} (Savage, 1989), approximately 0.59-1.08PSU. As such, *C. coleoptrata* was assigned to SAG I.
- *Glaenocorisa propinqua* (Fieber, 1860) is frequently found where salinity is less than 0.9gL^{-1}, approximately 0.59PSU, but may also be present in habitats with salinities up to 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Consequently *G. propinqua* was assigned to SAG I.
- *Callicorixa praeusta* (Fieber, 1848) inhabits rivers and small lakes (Macan, 1965), generally in the salinity range 0.9-1.6gL^{-1} (Savage, 1989), approximately 0.59-1.08PSU. Thus *C. praeusta* was assigned to SAG I.
- *Callicorixa wollastoni* (Douglas & Scott, 1865) is found primarily in peat pools (Macan, 1965) and at salinities less than 0.9gL^{-1} (Savage, 1989), approximately 0.59PSU. As such, *C. wollastoni* was assigned to SAG I.
- Macan (1965) stated that *Corixa affinis* Leach, 1817 inhabits ponds which are often close to the sea. Savage (1989) noted that *C. affinis* usually occupies habitats with water salinity in the range 0.9-1.6gL^{-1} (approximately 0.59-1.08PSU). Savage (1989), however, also stated that the species may occur in salinities up to 9gL^{-1} (6.81PSU). Hence *C. affinis* was assigned to SAG II.
- It has been documented that *Corixa dentipes* (Thomson, 1869) only occurs at salinities less than 1.6gL^{-1} (Savage, 1989), approsimately 1.08PSU. Thus *C. dentipes* was assigned to SAG I.
- *Corixa panzeri* (Fieber, 1848) typically occurs in lakes, rivers and ponds (Macan, 1965) at salinities up to 9gL^{-1} (Savage, 1989), approximately 6.81PSU. As such, *C. panzeri* was assigned to SAG II.

- Macan (1965) noted that *Corixa punctata* (Illiger, 1807) usually occurs in ponds. Savage (1989) stated that the species typically inhabits waters in the salinity range 0.9-1.6gL^{-1}, approximately 0.59-1.08PSU, and is also tolerant of salinities up to 9gL^{-1} (6.81PSU). Hence *C. punctata* was assigned to SAG II.
- *Hesperocorixa castanea* (Thomson, 1869) is typically found in waters with salinity less than 0.9gL^{-1}, approximately 0.59PSU, but may also be present in salinities up to 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Consequently *H. castanea* was assigned to SAG I.
- *Hespercorixa linnaei* (Fieber, 1848) usually inhabits lakes and ponds (Macan, 1965). It has been reported that *H. linnaei* tolerates salinities up to 9gL^{-1} (approximately 6.81PSU) but usually occurs in the salinity range 0.9-1.6gL^{-1} (Savage, 1989), approximately 0.59-1.08PSU. As such, *H. linnaei* was assigned to SAG II.
- *Hesperocorixa moesta* (Fieber, 1848) usually inhabits ponds (Macan, 1965). Savage (1989) reported that *H. moesta* occupies habitats with a salinity range of 0.9-1.6gL^{-1}, approximately 0.59-1.08PSU. Consequently *H. moesta* was assigned to SAG I.
- *Hesperocorixa sahlbergi* (Fieber, 1848) is an inhabitant of small bodies of water (Macan, 1965). Savage (1989) stated that *H. sahlbergi* tolerates salinities up to 9gL^{-1}, approximately 6.81PSU, but is more frequently found in the salinity range 0.9-1.6gL^{-1} (0.59-1.08PSU). Thus *H. sahlbergi* was assigned to SAG II.
- *Arctocorisa carinata* (C.R. Sahlberg, 1819) is usually found in peat pools (Macan, 1965) and at a salinity less than 0.9gL^{-1} (Savage, 1989), approximately 0.59PSU. Consequently, *A. carinata* was assigned to SAG I.
- *Arctocorisa germari* (Fieber, 1848) is frequently found in calcareous lakes and peat pools (Macan, 1965) where salinity is less than 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Hence *A. germari* was assigned to SAG I.
- Lakes and rivers are the usual habitats of *Sigara dorsalis* (Leach, 1817) according to Macan (1965). Savage (1989) noted that *S. dorsalis* inhabits waters with a salinity less than 1.6gL^{-1}, approximately 1.08PSU, but also stated that the species may occasionally be found in salinities up to 9gL^{-1} (6.81PSU). Thus *S. dorsalis* was assigned to SAG II.
- *Sigara distincta* (Fieber, 1848) is an inhabitant of lakes and ponds (Macan, 1965) where salinities are not greater than 1.6gL^{-1} (Savage, 1989), approximately 1.08PSU. Consequently *S. distincta* was assigned to SAG I.

- *Sigara falleni* (Fieber, 1848) is found in lakes and ponds (Macan, 1965). Wolf *et al*. (2009) indicated that *S. falleni* tolerates salinities up to $5gL^{-1}$, approximately 3.62PSU. Savage (1989) reported that the species occurs in the salinity range 0.9-$1.6gL^{-1}$ (0.59-1.08PSU) and may occur in habitats with salinities as high as $9gL^{-1}$ (6.81PSU). Thus *S. falleni* was assigned to SAG II.
- *Sigara fossarum* (Leach, 1817) inhabits lakes, ponds and rivers (Macan, 1965) with salinities less than $1.6gL^{-1}$ (Savage, 1989), approximately 1.08PSU. As such, *S. fossarum* was assigned to SAG I.
- Macan (1965) noted that ponds and pools are the usual habitats of *Sigara scotti* (Douglas & Scott, 1868). Savage (1989) stated that the species only occurs where salinity is below $1.6gL^{-1}$, approximately 1.08PSU. Hence *S. scotti* was assigned to SAG I.
- *Sigara iactans* Jansson, 1983 has been described by Wolf *et al*. (2009) as a purely freshwater species. Hence *S. iactans* was assigned to SAG I.
- Savage (1989) stated that *Sigara lateralis* (Leach, 1817) is usually found in the salinity range 0.9-$9gL^{-1}$, approximately 0.59-6.81PSU, and occasionally at higher salinities. Wolf *et al*. (2009) indicated that the species tolerates salinities up to $10gL^{-1}$ (7.63PSU) and can also tolerate higher salinities for a short time. Thus *S. lateralis* was assigned to SAG II.
- *Sigara nigrolineata* (Fieber, 1848) usually inhabits ponds and pools of all types (Macan, 1965). Savage (1989) stated that *S. nigrolineata* only tolerates salinities less than $1.6gL^{-1}$, approximately 1.08PSU. As such, *S. nigrolineata* was assigned to SAG I.
- *Sigara limitata* (Fieber, 1848) is usually found in ponds (Macan, 1965) and where salinity is in the range 0.9-$1.6gL^{-1}$ (Savage, 1989), approximately 0.59-1.08PSU. Hence *S. limitata* was assigned to SAG I.
- *Sigara semistriata* (Fieber, 1848) is found where salinity is below $1.6gL^{-1}$ (approximately 1.08PSU) according to Savage (1989). Wolf *et al*. (2009), however, stated that the species tolerates salinities up to $10gL^{-1}$ (7.63PSU) and can survive higher salinities for a short time. Thus *S. semistriata* was assigned to SAG II.
- Macan (1965) stated that streams, peat pools and lake shores are usual the habitats of *Sigara venusta* (Douglas & Scott, 1869). Savage (1989) reported that the species is only present at these habitats if salinity is less than $0.9gL^{-1}$, approximately 0.59PSU. Consequently, *S. venusta* was assigned to SAG I.

- *Sigara selecta* (Fieber, 1848) is described as a brackish water species by Macan (1965), whilst Velasco *et al*. (2006) recorded that the species was present in a hypersaline stream at salinity as high as 75gL^{-1}, approximately 72.17PSU. Savage (1989) stated that *S. selecta* is distributed in salinities greater than 9gL^{-1} (6.81PSU), which is supported by the findings of Gallardo-Mayenco (1994). Gallardo-Mayenco (1994) recorded that the largest abundance of *S. selecta* occurred at a salinity of 9.1gL^{-1} (6.89PSU), whilst Greenwood & Wood (2003) noted that the species has been recorded in the salinity range 18-65gL^{-1} (14.45-60.96PSU). Hence *S. selecta* was assigned to SAG III.
- Macan (1965) described *Sigara stagnalis* (Leach, 1817) as a brackish water species. Greenwood & Wood (2003) stated that *S. stagnalis* has been recorded in the salinity range 4-23gL^{-1} (approximately 2.85-18.91PSU), whilst Savage (1989) reported that the species is distributed at salinities greater than 9gL^{-1} (6.81PSU). Thus *S. stagnalis* was assigned to SAG III.
- *Paracorixa concinna* (Fieber, 1848) inhabits lakes and rivers with slightly brackish waters (Macan, 1965). The species tolerates salinities in the range 1.6-9gL^{-1} (approximately 1.08-6.81PSU) and occasionally occurs in salinities as low as 0.9gL^{-1} (Savage, 1989), approximately 0.59PSU. As such, *P. concinna* was assigned to SAG II.

Coleoptera

Coleoptera appear to be relatively tolerant to salinity increases (James *et al*., 2003; Kefford *et al*., 2003). In contrast to this view, both Kefford (1998a) and Piscart *et al*. (2005a) found that Coleoptera appear to be sensitive to increases in salinity. Piscart *et al*. (2005a), however, also noted that Coleoptera contains both halo-sensitive and halo-tolerant species. For example, Velasco *et al*. (2006) noted that the Hydraenidae, Dytiscidae and Hydrophilidae families of Coleoptera contain species which survive in a broad range of salinity. After comparing the salinity tolerances of taxa at the order and sub-order level, Dunlop *et al*. (2008) concluded that Coleoptera are not sensitive to salinity increases and that Coleoptera are more halo-tolerant than Hemiptera, Odonata, Gastropoda and Ephemeroptera.

Haliplidae

- Friday (1988) described *Haliplus apicalis* C.G. Thomson, 1868 as a brackish water species. Greenwood & Wood (2003) stated that *H. apicalis* has been recorded in the salinity range 1-16gL^{-1}, approximately 0.66-12.71PSU. Hence *H. apicalis* was assigned to Salinity Association Group (SAG) II.
- *Haliplus confinis* Stephens, 1828 is commonly found in fen ditches and dykes (Friday, 1988). *Haliplus confinis* has been recorded in the salinity range 2-6gL^{-1} (1.37-4.40PSU) by Johnson *et al*. (2007). Thus *H. confinis* was assigned to SAG II.
- *Haliplus lineatocollis* (Marsham, 1802) inhabits mainly slow running waters according to Friday (1988). Gallardo-Mayenco (1994) reported that larger abundances of the species occurred at higher salinities and also noted that the largest abundance occurred at a salinity of 9.1gL^{-1}, approximately 6.89PSU. As such, *H. lineatocollis* was assigned to SAG II.
- Species of the genus *Haliplus* can occur in habitats with salinities up to 10gL^{-1} (Barnes, 1994), approximately 7.63PSU. Hence the remaining members of the genus are all assigned to SAG II.

Noteridae

- *Noterus clavicornis* (DeGeer, 1774) is found in still water (Friday, 1988), and has been recorded at a salinity of 18.4gL^{-1} (approximately 14.80PSU) by Greenwood & Wood (2003). Barnes (1994) noted that *N. clavicornis* has been recorded from low salinity habitats. Thus *N. clavicornis* was assigned to SAG III.

Dytiscidae

- *Laccophilus hyalinus* (DeGeer, 1774) inhabits mainly slow running waters according to Friday (1988). Gallardo-Mayenco (1994) noted larger abundances of the species occurring at higher salinities and that the largest abundance of *L. hyalinus* occurred at a salinity of 7.7gL^{-1}, approximately 5.75PSU. As such *L. hyalinus* was assigned to SAG II.
- *Hygrotus impressopunctatus* (Schaller, 1783) is found in the vegetation of ponds and drains (Friday, 1988). Lancaster & Scudder (1987) recorded the species in a Canadian lake with an average salinity of 2gL^{-1}, approximately 1.37PSU. Hence *H. impressopunctatus* was assigned to SAG I.

- It has been stated that *Hygrotus parallellogrammus* (Ahrens, 1812) is found in brackish water (Friday, 1988). Greenwood & Wood (2003) reported that the species has been recorded at salinities in the range 9-22gL^{-1}, approximately 6.81-18.00PSU. Consequently *H. parallellogrammus* was assigned to SAG III.
- Barnes *et al*. (1971) reported that *Hydroporus palustris* (Linnaeus, 1761) is known from sites with salinities up to 8gL^{-1} (approximately 5.99PSU) and as such the species was assigned to SAG II.
- Friday (1988) documented that *Hydroporus pubescens* (Gyllenhal, 1808) can be found in peaty, fresh or brackish water (Friday, 1988). As such, *H. pubescens* was assigned to SAG II.
- *Hydroporus tessellatus* (Drapiez, 1819) is frequently found in running water, pools and on salt marshes (Friday, 1988). Hence *H. tessellatus* was assigned to SAG II.
- *Graptodytes pictus* (Fabricius, 1787) has been recorded from sites ranging in salinity from 0.2gL^{-1} to 1.55gL^{-1} (Kowalik & Bucyński, 2003), approximately 0.13-1.05PSU. Consequently *G. pictus* was assigned to SAG I.
- *Agabus conspersus* (Marsham, 1802) inhabits brackish pools and drains (Friday, 1988). The species has been recorded in the salinity range 8.4-21gL^{-1} (approximately 6.32-17.11PSU) according to Greenwood & Wood (2003). Thus *A. conspersus* was assigned to SAG III.
- *Agabus didymus* (Olivier, 1795) has been recorded from a site with a salinity in the range 0.2-0.26gL^{-1} (approximately 0.13-0.17PSU) by Kowalik & Bucyński (2003), but was not recorded from sites with higher salinities. Hence *A. didymus* was assigned to SAG I.
- Friday (1988) noted that *Ilybius subaeneus* Erichson, 1837 is an inhabitant of detritus ponds. Lancaster & Scudder (1987) have recorded the species in lakes ranging in salinity from 0.86gL^{-1} to 4.5gL^{-1} (approximately 0.56-3.23PSU). As such, *I. subaeneus* was assigned to SAG II.
- *Rhantus frontalis* (Marsham, 1820) is found in sandy pools and subsidence ponds on peat according to Friday (1988). Lancaster & Scudder (1987) recorded *R. frontalis* in lakes ranging in salinity from 1.6gL^{-1} to 4.5gL^{-1} (approximately 1.08-3.23PSU), whereas Greenwood & Wood (2003) reported that the species has been recorded in the salinity range 3-27gL^{-1} (2.10-22.57PSU). Hence *R. frontalis* was assigned to SAG III.

- *Rhantus suturalis* (Macleay, 1825) can be found in silt and detritus pools (Friday, 1988) and has been recorded at a salinity of 18.4gL^{-1} (Greenwood & Wood, 2003), approximately 14.80PSU. Consequently *R. suturalis* was assigned to SAG III.
- *Colymbetes fuscus* (Linnaeus, 1758) inhabits ponds and ditches (Friday, 1988). Barnes (1994) stated that *C. fuscus* can occupy habitats with salinities up to 20gL^{-1}, approximately 16.22PSU. Consequently *C. fuscus* was assigned to SAG III.
- Friday (1988) stated that *Dytiscus circumflexus* Fabricius, 1801 inhabits brackish inland pools. Hence *D. circumflexus* was assigned to SAG II.

Gyrinidae

- *Gyrinus caspius* Ménétriés, 1832 is found in fen drains and pools usually in coastal locations (Friday, 1988). Thus *G. caspius* was assigned to SAG II.
- Friday (1988) stated that *Gyrinus marinus* Gyllenhal, 1808 survives in fresh, peaty and brackish water. As such, *G. marinus* was assigned to SAG II.
- It has been reported that *Gyrinus substriatus* Stephens, 1828 inhabits fresh, peaty and occasionally brackish waters (Friday, 1988). Hence *G. substriatus* was assigned to SAG II.
- *Orectochilus villosus* (O.F. Müller, 1776) is an inhabitant of running waters and the wave-washed shores of lakes (Friday, 1988). Gallardo-Mayenco (1994) reported that *O. villosus* is distributed where salinity is below 1.6gL^{-1} (approximately 1.08PSU) and also recorded that the largest abundance of *O. villosus* occurred at a salinity of 1.4gL^{-1} (0.94PSU), Piscart *et al*. (2005a) found that the species is greatly affected by increases in salinity. Consequently *O. villosus* was assigned to SAG I.

Helophoridae

- Friday (1988) documented that *Helophorus alternans* Gené, 1836 occupies weedy ponds usually in coastal areas. As such, *H. alternans* was assigned to SAG II.
- *Helophorus brevipalpis* Bedel, 1881 was recorded from freshwater habitats by Martinoy *et al*. (2006), who also noted that the species was absent from brackish water sites. Copp *et al*. (2010) also recorded *H. brevipalpis* from freshwater sites. Thus *H. brevipalpis* was assigned to SAG I.
- *Helophorus fulgidicollis* Motschulsky, 1860 inhabits salt marshes according to Friday (1988). Hence *H. fulgidicollis* was assigned to SAG III.

- *Helophorus granularis* (Linnaeus, 1760) can be found in grassy ponds according Friday (1988). Wolf *et al*. (2009) stated that the species tolerates salinities up to 10gL^{-1}, approximately 7.63PSU, and can survive higher salinities for a short period of time. Thus *H. granularis* was assigned to SAG II.
- *Helophorus minutus* Fabricius, 1775 inhabits grassy ponds (Friday, 1988). Wolf *et al*. (2009) indicated that the species tolerates salinities up to 5gL^{-1}, approximately 3.62PSU. Greenwood & Wood (2003) reported that *H. minutus* has been recorded in the salinity range 4-23gL^{-1} (2.85-18.91PSU). As such, *H. minutus* was assigned SAG III.

Hydrophilidae

- Friday (1988) noted that *Hydrobius fuscipes* (Linnaeus, 1758) is usually found in detritus pools. Johnson *et al*. (2007) recorded the species at a salinity of 6gL^{-1}, approximately 4.40PSU. Hence *H. fuscipes* was assigned to SAG II.
- *Anacaena limbata* (Fabricius, 1792) has been recorded from a freshwater location by Gerend & Callot (2001) and from several freshwater sites by Copp *et al*. (2010). Ranta (1982), however, reported *A. limbata* tolerates salinities up to 6.1gL^{-1} (approximately 4.48PSU) and as such the species was assigned to SAG II.
- Gallardo-Mayenco (1994) recorded that the largest abundance of *Laccobius atratus* (Rottenburg, 1874) occurred at a salinity of 7.7gL^{-1}, approximately 5.75PSU. Consequently *L. atratus* was assigned to SAG II.
- İncekara (2009) recorded *Laccobius bipunctatus* (Fabricius, 1775) from a lake only when salinity dropped to 10.2gL^{-1}, approximately 7.79PSU, indicating that the species is not tolerant of salinities greater than this concentration. The species has also been recorded from sites with salinities of 2.5gL^{-1} (1.73PSU) and 2.0gL^{-1} (1.37PSU) by Kowalik & Bucyński (2003). Hence *L. bipunctatus* was assigned to SAG II.
- Friday (1988) described *Enochrus bicolor* (Fabricius, 1792) as an inhabitant of brackish water. Greenwood & Wood (2003) reported that *E. bicolor* tolerates salinities in the range 4.7-62.6gL^{-1}, approximately 3.39-58.34PSU, whilst Velasco *et al*. (2006) stated that the species has been recorded from salinities as high as 76.4gL^{-1} (73.77PSU). Consequently *E. bicolor* was assigned to SAG III.
- *Enochrus melanocephalus* (Olivier, 1792) occupies brackish water according to Friday (1988). Hence *E. melanocephalus* was assigned to SAG II.

- *Berosus affinis* Brullé, 1835 is found in silt ponds and drains which may be brackish (Friday, 1988). Greenwood & Wood (2003) stated that *B. affinis* has been recorded in the salinity range 12-23gL^{-1}, approximately 9.29-18.91PSU. Thus *B. affinis* was assigned to SAG III.
- *Cercyon depressus* Stephens, 1830 can be found under decaying seaweed (Friday, 1988) and thus must survive in a wide range of salinities. As such, *C. depressus* was assigned to SAG III.
- *Cercyon littoralis* (Glyllenhal, 1808) can be found under decaying seaweed (Friday, 1988) and as such must be able to tolerate a wide range of salinities. Hence *C. littoralis* was assigned to SAG III.

Hydraenidae

- *Ochthebius auriculatus* Rey, 1886 inhabits brackish water according to Friday (1988). Thus *O. auriculatus* was assigned to SAG III.
- *Ochthebius dilatatus* Stephens, 1829 is found in muddy water (Friday, 1988). Gallardo-Mayenco (1994) recorded that the largest abundance of *O. dilatus* occurred at a salinity of 12.7gL^{-1}, approximately 9.88PSU. As such, *O. dilatatus* was assigned to SAG III.
- *Ochthebius marinus* (Paykull, 1798) occupies brackish pools (Friday, 1988) and has been recorded in the salinity range 7-34gL^{-1} (Greenwood & Wood, 2003), approximately 5.19-29.15PSU. Hence *O. marinus* was assigned to SAG III.
- Friday (1988) stated that *Ochthebius punctatus* Stephens, 1829 tolerates brackish water, whilst Greenwood & Wood (2003) reported that *O. punctatus* has recorded at a salinity of 18gL^{-1} (approximately 14.45PSU). Thus *O. punctatus* was assigned to SAG III.
- *Ochthebius viridis* Peyron, 1858 inhabits brackish and heath pools (Friday, 1988) and has been recorded in salinities ranging from 8.4gL^{-1} to 34.1gL^{-1} (Greenwood & Wood, 2003), approximately 6.32-29.25PSU. As such, *O. viridis* was assigned to SAG III.
- *Hydraena testacea* Curtis, 1831 is an inhabitant of stagnant water and muddy streams according to Friday (1988). Wolf *et al*. (2009) described *H. testacea* as a purely freshwater species. Hence *H. testacea* was assigned to SAG I.

Scirtidae

- The genus *Hydrocyphon* is distributed at salinities less than 1.6gL^{-1} (approximately 1.08PSU) according to Gallardo-Mayenco (1994). Thus *Hydrocyphon deflexicollis* (Müller, 1821), the only British member of the genus, was assigned to SAG I.

Dryopidae

- Despite the fact that Piscart *et al*. (2005a) found the genus *Dryops* to be greatly tolerant of increases in salinity, Muñoz & Prat (1994) only recorded the genus in the salinity range 1.5-2.0gL^{-1} (approximately 1.01-1.37PSU). Thus the genus *Dryops* was assigned to SAG I.

Elmidae

- *Elmis aenea* (Müller, 1806) is found in riffles in running water (Friday, 1988). In a study of the Aber Estuary, Wales, Williams & Williams (1998a) only recorded low numbers of the species at sites located at the upper estuary, indicating a low tolerance to increased salinity. This conclusion is supported by Wood & Dykes (2002), who found that *E. aenea* entered drift due to the addition of saline solution to a stream. Hence *E. aenea* was assigned to SAG I.
- *Esolus parallelepipedus* (Müller, 1806) occupies running water (Friday, 1988), and was only found in low numbers at upper estuary sites by Williams & Williams (1998a). Consequently *E. parallelepipedus* was assigned to SAG I.
- Gallardo-Mayenco (1994) reported that species of the genus *Limnius* are distributed at salinities less than 1.6gL^{-1} (approximately 1.08PSU), whilst Piscart *et al*. (2005a) determined that the genus is greatly affected by increases in salinity. As such, the genus *Limnius* was assigned to SAG I.
- Muñoz & Prat (1994) recorded the genus *Oulimnius* from salinities within the range of 1.5-2.0gL^{-1} (approximately 1.01-1.37PSU), whilst Short *et al*. (1991) reported the presence the genus in the salinity range 48-88mgL^{-1} (0.04-0.06PSU). Furthermore, Piscart *et al*. (2005a) found that the genus *Oulimnius* is greatly affected by increases in salinity. Hence the genus *Oulimnius* was assigned to SAG I.

- Friday (1988) noted that *Riolus subviolaceus* (Müller, 1817) is generally found in running water. Gallardo-Mayenco (1994) reported that *R. subviolaceus* is distributed where salinity is below 1.6gL^{-1} (approximately 1.08PSU) and that the largest abundance of the species occurred at a salinity of 1.1gL^{-1} (0.73PSU). Thus *R. subviolaceus* was assigned to SAG I.

Heteroceridae

- *Augyles maritimus* Guerin-Meneville, 1844 has been recorded on the Essex salt marshes by Mason *et al*. (1991). As such, *A. maritimus* was assigned to SAG III.

Megaloptera

Megaloptera were rare or absent from the survey of salinised rivers Blackwood River and Gleneg River, Australia (Williams *et al*., 1991), indicating that this order of insects generally have a low salinity tolerance.

Sialidae

- *Sialis fuliginosa* F. Pictet, 1836 has been recorded in a stream with an average salinity of 0.8gL^{-1} (Schmid-Araya *et al*., 2002), approximately 0.52PSU. As such, *S. fuliginosa* was assigned to Salinity Association Group (SAG) I.
- Johnson *et al*. (2007) recorded *Sialis lutaria* (Linnaeus, 1758) at a salinity of 1gL^{-1} (approximately 0.66PSU), whilst Piscart *et al*. (2005a) determined that the species is greatly affected by increases in salinity. Thus *S. lutaria* was assigned to SAG I.
- Gallardo-Mayenco (1994) reported that *Sialis nigripes* (Pictet, 1865) is distributed in waters where salinity is not greater than 1.6gL^{-1}, approximately 1.08PSU. Consequently, *S. nigripes* was assigned to SAG I.

Trichoptera

Hart *et al*. (1990) stated that Trichoptera appear to be among the more salt sensitive invertebrates. This appears to be supported by the findings of Bunn & Davies (1992), who noted during a study of a salinised river system that most species of Trichoptera found were represented by single specimens. This indicates a lack of tolerance to high concentrations of salinity in this order. Furthermore, James *et al*. (2003) reported that some species of Trichoptera are sensitive to even slight increases in salinity. Kefford *et al*. (2003), however, reported that Trichoptera are more halo-tolerant than Ephemeroptera, but less halo-tolerant than Coleoptera and Odonata. In contrast, Piscart *et al*. (2005a) documented that Trichoptera are more tolerant of salinity than other major invertebrate groups such as Ephemeroptera, Coleoptera and Heteroptera, but also stated that Trichoptera contains both halo-tolerant and halo-sensitive species.

Rhyacophilidae

- Williams & Williams (1998a) found *Rhyacophila dorsalis* (Curtis, 1834) only in the upper parts of the Aber Estuary, Wales, and also discovered that the species survived only two hours immersion in undiluted seawater. Piscart *et al*. (2005a) reported that *R. dorsalis* is greatly affected by increases in salinity. Hence *R. dorsalis* was assigned to Salinity Association Group (SAG) I.
- *Rhyacophila munda* McLachlan, 1862 is found in small streams and rivers (Edington & Hildrew, 1995). Gallardo-Mayenco (1994) noted that the largest abundance of *R. munda* occurred at a salinity of 0.97gL^{-1}, approximately 0.64PSU. Thus *R. munda* was assigned to SAG I.

Glossosomatidae

- *Glossosoma boltoni* Curtis, 1834 inhabits the stony substrata in rivers and large streams (Wallace *et al*., 1990). Williams & Williams (1998a) recorded *G. boltoni* at all the sampling stations on the Aber Estuary, Wales, and also discovered that the species survived a four hour immersion in undiluted seawater. As such, *G. boltoni* was assigned to SAG II.

- *Glossosoma conformis* Neboiss, 1963 is an inhabitant of the stony substrata of streams and rivers (Wallace *et al*., 1990). Williams & Williams (1998a) recorded *G. conformis* at all parts of the Aber Estuary, Wales, and also found that the species survived a four hour immersion in undiluted seawater. Hence *G. conformis* was assigned to SAG II.
- *Agapetus delicatulus* McLachlan, 1884 is found in rivers and large streams with a stony substratum according to Wallace *et al*. (1990). Williams & Williams (1998a) documented the presence of *A. delicatulus* from only the upper sampling stations of the Aber Estuary, Wales, and also noted that the species only survived one and a half hours of immersion in undiluted seawater. Thus *A. delicatulus* was assigned to SAG I.
- *Agapetus fuscipes* Curtis, 1834 can be found on lake shores and in the stony substratum of permanent streams and rivers (Wallace *et al*., 1990). Wood & Dykes (2002) noted that *A. fuscipes* entered drift due to the addition of a salt solution to a stream, indicating a low tolerance to small increases in salinity. Consequently *A. fuscipes* was assigned to SAG I.

Hydroptilidae

- The genus *Hydroptila* has been recorded in the salinity range 1.5-2.0gL^{-1} (approximately 1.01-1.37PSU) by Muñoz & Prat (1994). Piscart *et al*. (2005a) reported that the genus is relatively insensitive to increases in salinity, whereas Wolf *et al*. (2009) classified the genus *Hydroptila* as tolerant of only freshwater. Thus the genus *Hydroptila* was assigned to SAG I.
- According to Piscart *et al*. (2005a), *Ithyrichia lamellaris* (Eaton, 1873) is greatly affected by increases in salinity. Hence *I. lamellaris* was assigned to SAG I.

Philopotamidae

- *Philopotamus montanus* (Donovan, 1813) is found in the rapids of headwaters and tributaries and other swiftly flowing waters (Chinery, 1986; Edington & Hildrew, 1995). The species has been recorded by Williams & Williams (1998a) at the upper sampling stations of the Aber Estuary, Wales, and extending into the middle reaches of the estuary. Williams & Williams (1998a) also documented that *P. montanus* survived four hours of immersion in undiluted seawater. As such, *P. montanus* was assigned to SAG II.

- The genus *Chimarra* have been recorded in the salinity range 24-3850mgL^{-1} (approximately 0.02-2.74PSU) by Short *et al.* (1991), who also noted that the largest abundance of the genus occurred in the salinity range 48-88mgL^{-1} (0.04-0.06PSU). Hence the genus *Chimarra* was assigned to SAG I.

Psychomyiidae

- *Lype reducta* (Hagen, 1868) inhabits streams, rivers, ponds and lakes according to Edington & Hildrew (1995). Piscart *et al*. (2005a) found that *L. reducta* is greatly affected by increases in salinity. Thus *L. reducta* was assigned to SAG I.
- *Psychomyia pusilla* (Fabricius, 1781) can be found in rivers and large streams (Edington & Hildrew, 1995). Piscart *et al*. (2005a) concluded that *P. pusilla* is insensitive to increases in salinity. Gallardo-Mayenco (1994), however, reported that the species is distributed at salinities below 1.6gL^{-1} (approximately 1.08PSU) and also noted that the largest abundance of *P. pusilla* occurred at a salinity of 1.2gL^{-1} (0.80PSU). Furthermore, Muñoz & Prat (1994) noted that *P. pusilla* was absent from estuarine locations, indicating a lack of tolerance to salinity. As such, *P. pusilla* was assigned to SAG I.
- It has been reported that *Tinodes waeneri* (Linnaeus, 1758) is found in streams and rivers as well as on the wave-washed stony shores of lakes and (Edington & Hildrew, 1995). *Tinodes waeneri* is greatly affected by increases in salinity (Piscart *et al*., 2005a). Consequently *T. waeneri* was assigned to SAG I.

Ecnomidae

- Wolf *et al*. (2009) stated that *Ecnomus tenellus* (Rambur, 1842) is a purely freshwater species, whilst Piscart *et al*. (2005a) concluded that *E. tenellus* is highly tolerant of salinity increases. In contrast, Muñoz & Prat (1994) found that the mean density of the species decreased only slightly as salinity increased. Thus *E. tenellus* was assigned to SAG II.

Polycentropodidae

- *Cyrnus trimaculatus* (Curtis, 1834) inhabits ponds, lakes and the slow flowing sections of rivers according to Edington & Hildrew (1995). Piscart *et al*. (2005a) reported that *Cyrnus trimaculatus* is greatly affected by increases in salinity. As such, *C. trimaculatus* was assigned to SAG I.

- *Neurecilpsis bimaculata* (Linnaeus, 1758) inhabits streams draining from lakes (Edington & Hildrew, 1995). Piscart *et al*. (2005a) found *N. bimaculata* to be greatly affected by increases in salinity. Hence *N. bimaculata* was assigned to SAG I.
- *Plectrocnemia geniculata* McLachlan, 1871 is generally found in small headwater streams (Edington & Hildrew, 1995). Williams & Williams (1998a) recorded *P. geniculata* at sampling stations located on the upper parts of the Aber Estuary, Wales. Williams & Williams (1998a) also reported that the species survived four hours of immersion in undiluted seawater. Thus *P. geniculata* was assigned to SAG II.
- Edington & Hildrew (1995) stated *Polycentropus flavomaculatus* (Pictet, 1934) can be found on stony lake shores and in the lower reaches of rivers and streams. The species has been recorded at all the sampling stations of the Aber Estuary, Wales, except the most seaward station by Williams & Williams (1998a). Williams & Williams (1998a) also reported that *P. flavomaculatus* survived a four hour immersion in undiluted seawater. Furthermore, Wolf *et al*. (2009) described the species as tolerant of salinities up to $5gL^{-1}$, approximately 3.62PSU. Thus *P. flavomaculatus* was assigned to SAG II.
- The genus *Polycentropus* is distributed at salinities less than $1.6gL^{-1}$ (approximately 1.08PSU) according to Gallardo-Mayenco (1994). As such, all other species of the genus *Polycentropus* were assigned to SAG I.

Hydropsychidae

- Edington & Hildrew (1995) stated that *Cheumatopsyche lepida* (Pictet, 1834) inhabits the lower reaches of rivers and the outflows from lakes. Gallardo-Mayenco (1994) recorded the largest abundance of *C. lepida* at a salinity of $3.4gL^{-1}$, approximately 2.40PSU. Hence *C. lepida* was assigned to SAG II.
- *Hydropsyche contubernalis* McLachlan, 1865 can be found in the lower reaches of large rivers according to Edington & Hildrew (1995). Piscart *et al*. (2005a) determined that the species is affected substantially by increases in salinity, whilst Wolf *et al*. (2009) described *H. contubernalis* as a purely freshwater species. Thus *H. contubernalis* was assigned to SAG I.

- Gallardo-Mayenco (1994) documented that *Hydropsyche instabilis* (Curtis, 1834) is distributed at salinities less than 1.6gL^{-1} (approximately 1.08PSU) and also recorded that the largest abundance of the species occurred at 1.5gL^{-1} (1.01PSU). Williams & Williams (1998a), however, recorded the species in all parts of the Aber Estuary, Wales, and also found that *H. instabilis* survived six hours of immersion in undiluted sea water. As such, *H. instabilis* was assigned to SAG II.
- Still and slow flowing water is the preferred habitat of *Hydropsyche pellucidula* (Curtis, 1834) according to Chinery (1986). Piscart *et al*. (2005a) found that the species is greatly affected by increases in salinity. Hence *H. pellucidula* was assigned to SAG I.
- *Hydropsyche siltalai* Döhler, 1963 is found in fast flowing rivers and streams (Edington & Hildrew, 1995). Piscart *et al*. (2005a) concluded that the species is greatly affected by increases in salinity. Williams & Williams (1998a), however, recorded *H. siltalai* in the upper and middle reaches of the Aber Estuary, Wales. Furthermore, Williams & Williams (1998a) also found that the species survived four hours of immersion in undiluted seawater. Thus *H. siltalai* was assigned to SAG II.

Phryganeidae

- *Oligotricha striata* (Linnaeus, 1758) inhabits deep pools and ditches (Wallace *et al*., 1990). Berezina (2003) reported that the salinity tolerance range of *O. striata* is 20-2200mgL^{-1}, approximately 0.02-1.51PSU. As such, *O. striata* was assigned to SAG I.

Brachycentridae

- *Brachycentrus subnubilus* Curtis, 1834 can be found in slow flowing sections of streams and rivers (Chinery, 1986; Wallace *et al*., 1990). Piscart *et al*. (2005a) reported that the species is relatively insensitive to increases in salinity. Hence *B. subnubilus* was assigned to SAG II.

Lepidostomatidae

- The genus *Lepidostoma* has been recorded in salinities below 250mgL^{-1} (approximately 0.16PSU) by Williams *et al*. (1999). Thus the genus *Lepidostoma* was assigned to SAG I.

Limnephilidae

- *Halesus radiatus* (Curtis, 1834) is found in streams, rivers and lake shores according to Wallace *et al*. (1990). Piscart *et al*. (2005a) found *H. radiatus* to be greatly affected by increases in salinity. As such, *H. radiatus* was assigned to SAG I.

- *Potamophylax cingulatus* (Stephens, 1837) inhabits streams and rivers with a stony substratum (Wallace *et al*., 1990). Williams & Williams (1998a) reported that the species is confined to the extreme upper reaches of the Aber Estuary in Wales, indicating a low tolerance to increased salinity. Hence *P. cingulatus* was assigned to SAG I.
- *Limnephilus affinis* Curtis, 1834 is an inhabitant of lakes and slow flowing ditches (Wallace *et al*., 1990). Crothers (1997) stated that *L. affinis* can tolerate salinities up to $24gL^{-1}$ (approximately 19.81PSU), whilst Williams & Williams (1998a) documented that the species can survive several months at a salinity of $26gL^{-1}$ (21.64PSU). Furthermore, Barnes (1994) reported that *L. affinis* can develop in habitats with salinities up to almost $20gL^{-1}$ (16.22PSU) and that the species can survive salinities over $25gL^{-1}$ (20.73PSU). Consequently *L. affinis* was assigned to SAG III.
- Wallace *et al*. (1990) reported that *Limnephilus decipiens* (Kolenati, 1848) can be found in lakes, canals and dykes. Wolf *et al*. (2009) described *L. decipiens* as a purely freshwater species. As such, *L. decipiens* was assigned to SAG I.
- Wood & Dykes (2002) noted that *Limnephilus flavicornis* (Fabricius, 1787) entered drift when a salt solution was added to a stream as part of the dilution gauging technique to measure stream velocity and flow discharge. This indicates *L. flavicornis* has a very low tolerance to salinity increases. Hence *L .flavicornis* was assigned to SAG I.
- According to Piscart *et al*. (2005a), *Limnephilus rhombicus* (Linnaeus, 1758) is greatly affected by increases in salinity. Thus *L. rhombicus* was assigned to SAG I.

Goeridae

- *Goera pilosa* (Fabricius, 1775) usually inhabits gravelly lake shores and the fast flowing sections of streams and rivers (Chinery, 1986; Wallace *et al*., 1990). Piscart *et al*. (2005a) reported that *G. pilosa* is greatly affected by increases in salinity. As such, *G. pilosa* was assigned to SAG I.
- *Silo pallipes* (Fabricius, 1781) inhabits streams and rivers (Wallace *et al*., 1990). Williams & Williams (1998a) recorded *S. pallipes* in the upper sections of the Aber Estuary, Wales, and also discovered that the species survived six hours immersion in undiluted seawater. Hence *S. pallipes* was assigned to SAG II.

Sericostomatidae

- *Sericostoma personatum* (Spence in Kirby & Spence, 1826) is found in streams, rivers and lakes with a stony substratum (Chinery, 1986; Wallace *et al*., 1990). Williams & Williams (1998a) recorded *S. personatum* at all the sampling stations on the Aber Estuary, Wales, and also noted that the species survived eight hours immersion in undiluted seawater. Thus *S. personatum* was assigned to SAG II.

Odontoceridae

- *Odontocerum albicorne* (Scopoli, 1763) is an inhabitant of streams and rivers with a stony substratum (Chinery, 1986; Wallace *et al*., 1990). Williams & Williams (1998a) recorded *O. albicorne* from the upper reaches of the Aber Estuary, Wales, but also discovered that the species survived sixteen hours immersion in undiluted seawater. As such, *O. albicorne* was assigned to SAG II.

Molannidae

- Williams *et al*. (1999) only recorded the genus *Molanna* in salinities below 25mgL^{-1}, approximately 0.02PSU. Hence the genus *Molanna* was assigned to SAG I.

Leptoceridae

- *Athripsodes aterrimus* (Stephens, 1836) can be found in ponds, lakes and slow flowing sections of rivers and streams according to Wallace *et al*. (1990). Berezina (2003) discovered that the salinity tolerance range of *A. aterrimus* is 45-2200mgL^{-1}, approximately 0.03-1.51PSU. Consequently *A. aterrimus* was assigned to SAG I.
- *Athripsodes cinerus* (Curtis, 1834) inhabits rivers, streams, lakes and canals with a stony or sandy substratum (Wallace *et al*., 1990). Piscart *et al*. (2005a) discovered that *Athripsodes cinerus* is greatly affected by increases in salinity. Thus, *A. cinerus* was assigned to SAG I.
- *Ceraclea annulicornis* (Stephens, 1836) is found in rivers and occasionally on lake shores (Wallace *et al*., 1990). The species is greatly affected by increases in salinity according to Piscart *et al*. (2005a). Hence *C. annulicornis* was assigned to SAG I.
- The greatest density of the genus *Ceraclea* was recorded in the salinity range 1.5-2.5gL^{-1} (approximately 1.01-1.37PSU) by Muñoz & Prat (1994), who also noted only a very low density of the genus at salinities over 2.5gL^{-1} (1.73PSU). Thus all other species of the genus *Ceraclea* were assigned to SAG I.

- Piscart *et al*. (2005a) found that *Leptocerus tineiformis* Curtis, 1834, an inhabitant of lakes and large weedy ponds (Wallace *et al*., 1990), is greatly affected by increases in salinity. As such, *L. tineiformis* was assigned to SAG I.
- *Mystacides azurea* (Linnaeus, 1761) can be found in lakes, canals and the still and slow flowing sections of rivers and streams (Wallace *et al*, 1990). Wolf *et al*. (2009) described *M. azurea* as a purely freshwater species, whilst Piscart *et al*. (2005a) concluded that the species is greatly affected by increases in salinity. Hence *M. azurea* was assigned to SAG I.
- Piscart *et al*. (2005a) found *Mystacides longicornis* (Linnaeus, 1758), an inhabitant of large ponds, lakes, canals and very slowly flowing rivers (Wallace *et al*., 1990), to be greatly affected by increases in salinity. Barnes (1994), however, reported that *Mystacides longicornis* has occasionally been recorded from habitats with salinities as high as $10gL^{-1}$ (approximately 7.63PSU). Thus *M. longicornis* was assigned to SAG II.
- *Adicella reducta* (McLachlan, 1865) inhabits rivers, canals, streams and flowing marshes (Wallace *et al*., 1990). Williams & Williams (1998a) found that *A. reducta* was restricted to the freshwater sampling station above the Aber Estuary, Wales, indicating a very low tolerance to increasing salinities. As such, *A. reducta* was assigned to SAG I.
- *Oecetis ochracea* (Curtis, 1825) can be found in lakes, large ponds and canals (Wallace *et al*., 1990). Piscart *et al*. (2005a) found this species to be intolerant of increases in salinity, whilst Barnes (1994) reported that *O. ochracea* has occasionally been recorded from habitats with salinities up to $10gL^{-1}$ (approximately 7.63PSU). Hence *O. ochracea* was assigned to SAG II.

Lepidoptera

Pyralidae

- *Elophila nymphaeata* (Linnaeus, 1758) has been recorded from a low salinity habitat (Barnes, 1994). Thus *E. nymphaeata* was assigned to SAG II.

Diptera

Aquatic Diptera larvae are difficult to identify to species and it has been reported that salinity tolerances within families tend to vary less than the salinity tolerances between families as a whole (Dunlop *et al*., 2008). Consequently it was decided to only score the families of Diptera instead of species, thus negating the requirement to identify to species level.

The scores obtained from these families during the application of the index should be used with caution due to the lack of literature relating to the salinity tolerances of aquatic Dipteran larvae and the fact that species within the same families may vary widely in salinity tolerances.

Limoniidae

- The larvae of Limoniidae species can be found in a range of salinities. For example, Short *et al*. (1991) recorded *Hexatoma* and *Antocha* species in the salinity range 24-3850mgL^{-1} (approximately 0.02-2.74PSU), and further noted the largest abundance of these species occurred in the salinity range 48-1350mgL^{-1} (0.04-0.90PSU). In contrast, *Dicranomyia sera* (Walker, 1848), *Dicranomyia ventralis* (Schummel, 1829) and *Geranomyia unicolor* (Haliday 1833) are all tolerant of brackish water and can be found in habitats where a concentration of salinity greater than 2.5gL^{-1} (1.37PSU) can be expected (Stubbs, 1978). Thus the family Limoniidae was assigned to Salinity Association Group (SAG) II.

Psychodidae

- It has been reported that two genera of the family Psychodidae have wide distributions across the brackish water range (Short *et al*., 1991; Gallardo-Mayenco, 1994). Gallardo-Mayenco (1994) found that whilst the genus *Pericoma* is distributed across a wide range of salinities, the genus was present in larger abundances at lower salinities. Short *et al*. (1991) recorded the genus *Psychoda* across the salinity range 2.7-22.7gL^{-1} (approximately 1.88-18.64PSU) and also noted that the largest abundance of the genus occurred in the range 12.1-22.7gL^{-1} (9.38-18.64PSU). As such, the family Psychodidae was assigned to SAG II.

Dixidae

- *Dixa* and *Dixella* are the only genera of Dixidae found in Britain. Gallardo-Mayenco (1994) found that the genus *Dixa* is distributed at salinities less than 1.6gL^{-1} (approximately 1.08PSU), whilst the genus *Dixella* has been recorded from locations in the salinity range 0-175mgL^{-1} (0-0.11PSU) by Williams *et al*. (1999). Hence the family Dixidae was assigned to SAG I.

Culicidae

- It has been stated that species of the family Culicidae are generally found in freshwater to low salinity brackish water habitats (Cogan, 1978; Cranston *et al*., 1987; Silberbush *et al*., 2005). For example, Silberbush *et al*. (2005) found that during a mesocosm experiment in the Dead Sea, *Ochlerotatus caspius* (Pallas, 1771) was most abundant in pools with a salinity of 10gL^{-1} (approximately 7.63PSU) and least abundant in pools with a salinity of 30gL^{-1} (25.36PSU). Cranston *et al*. (1987) stated that *Anopheles claviger* (Meigen, 1804) tolerates freshwater and brackish water up to a salinity of 13gL^{-1} (10.13PSU), and that *Culiseta annulata* (Schrank, 1776), *Culiseta litorea* (Shute, 1928), *Culiseta morsitans* (Theobald, 1901), *Culex pipiens* (Linnaeus, 1758) and *Culex territans* (Walker, 1856) can all inhabit freshwater and brackish water habitats with salinities up to up to 5gL^{-1} (3.62PSU). Thus, the family Culicidae was assigned to SAG II.

Ceratopogonidae

- Some of the species of Ceratopogonidae are reported to tolerate salinities from freshwater up to 6.3gL^{-1} (Berezina, 2003), approximately 4.64PSU. Furthermore, the genus *Culicoides* has been recorded in the salinity range 0.9-22.7gL^{-1} (0.59-18.64PSU) with the largest abundance occurring in the range 2.7-8.9gL^{-1} (Short *et al*., 1991), approximately 1.88-6.72PSU. As such, the family Ceratopogonidae was assigned to SAG II.

Simuliidae

- It has been reported that some of the species of Simuliidae are tolerant of salinities up to 4.7gL^{-1} (Gallardo-Mayenco, 1994), approximately 3.39PSU, and can occur in the upper, and occasionally middle, reaches of estuaries (Williams & Williams, 1998a). Hence the family Simuliidae was assigned to SAG II.

Stratiomyidae

- Gallardo-Mayenco (1994) reported that the species of the genus *Odontomyia* are distributed where salinity is less than 1.6gL^{-1} (approximately 1.08PSU) and also stated that the genus *Stratiomys* is distributed at salinities greater than 3.6gL^{-1} (2.55PSU), whilst Williams *et al*. (1999) recorded species of the genus *Stratiomys* at sites with salinities in the range 350-500mgL^{-1} (0.22-0.32PSU). Furthermore, it has been reported that several species of *Nemotelus* can be found on salt marshes, indicating a tolerance to increased salinities (Stubbs, 1978; Mason *et al*., 1991). Thus the family Stratiomyidae was assigned to SAG II.

Tabanidae

- It has been reported that some species of Tabanidae, such as *Chrysops relictus* Meigen, 1820 and *Haematopota bigoti* Gobert, 1880, can be found on salt marshes (Stubbs, 1978). Other species of the family, for example *Chrysops caecutiens* (Linnaeus, 1758), *Tabanus bromius* Linnaeus, 1758 and *Tabanus cordiger* Meigen, 1820, can be found in large abundances in the salinity range 7-10gL^{-1} (Gallardo-Mayenco, 1994), approximately 5.19-7.63PSU. As such, the family Tabanidae was assigned to SAG II.

Athericidae

- Two of the three species of Athricidae which occur in Britain, namely *Atrichops crassipes* (Meigen, 1820) and *Ibisia marginata* (Fabricius, 1791), are reported to only be distributed in habitats where salinity is less than 1.6gL^{-1} (Gallardo-Mayenco, 1994), approximately 1.08PSU. Hence the family Athericidae was assigned to SAG I.

Dolichopodidae

- Many species of the family Dolichopodidae, such as *Hydrophorus oceanus* (Macquart, 1838), *Dolichopus plumipes* (Scopoli, 1763), and *Rhaphium consobrinum* Zetterstedt, 1843, have been recorded in salt marshes (Mason *et al*., 1991) as this is their natural habitat (Stubbs, 1978). Thus the family Dolichopodidae was assigned to SAG III.

Appendix 3: Salinity Association Group (SAG) Index Calculation Example

A worked example of the calculation performed to obtain the SAG index score for a sample is shown in Table 12.1. The spring macro-invertebrate sample collected from the SF1 (Casswell's Bridge) survey site on the South Forty Foot Drain is used in the example.

Table 12.1: Worked example of a Salinity Association Group index calculation using the macro-invertebrate community sample collected in spring from the SF1 (Casswell's Bridge) survey site, South Forty Foot Drain

Taxon	Salinity Association Group (SAG)	Recorded abundance	Abundance category	Salinity Association Score (SAS)
Bithynia tentaculata	II	11	B	6
Bithynia leachii	I	16	B	3
Physa fontanilis	II	13	B	6
Anisus vortex	II	8	A	5
Hippeutis complanatus	I	1	A	4
Sphaeriidae	II	1	A	5
Piscicola geometra	II	1	A	5
Glossiphonia complanata	I	1	A	4
Abloglossiphonia heteroclita	I	1	A	4
Erpobdella octoculata	II	1	A	5
Erpobdella testacea	I	1	A	4
Asellus aquaticus	II	34	B	6
Crangonyx pseudogracilis	II	144	C	7
Gammarus pulex	I	1	A	4
Centroptilum luteolum	I	1	A	4
Coenagrion puella	II	2	A	5
Haliplus laminatus	II	1	A	5
Nebrioporus elegans	N/A	1	A	N/A
Sialis lutaria	I	2	A	4
Limnephilus lunatus	N/A	33	B	N/A
Athripsodes aterrimus	I	4	A	4
Triaenodes bicolor	N/A	5	A	N/A
Chrysomelidae	N/A	1	A	N/A
Chironomidae	N/A	26	B	N/A
	SAG index score for sample (Arithmetic mean of SAS) =			4.74

Appendix 4: Survey Dates for Lincolnshire and Norfolk Sites

The dates when surveys were undertaken at the Lincolnshire sites are displayed in Table 12.2. The dates when surveys were undertaken at the Norfolk sites are displayed in Table 12.3.

Table 12.2: Survey dates of sites located within Lincolnshire

Survey site	Water body	Survey date and season		
		Spring	Summer	Autumn
SF1 (Casswell's Bridge)	South Forty Foot Drain	30-Mar-10	26-Jun-10	08-Oct-11
SF2 (Donington Bridge)		30-Mar-10	26-Jun-10	08-Oct-11
SF3 (Swineshead Bridge)		29-Mar-10	26-Jun-10	09-Oct-11
SF4 (Wyberton Chain Bridge)		29-Mar-10	26-Jun-10	09-Oct-11
SH1 (Weston Fen)	South Holland Main Drain	29-Mar-10	28-Jun-10	08-Oct-11
SH2 (Clifton's Bridge)		29-Mar-10	28-Jun-10	08-Oct-11
SH3 (A1101 Road Bridge)		29-Mar-10	28-Jun-10	08-Oct-11
SH4 (Nene Outfall Sluice)		29-Mar-10	28-Jun-10	08-Oct-11

Table 12.3: Survey dates of sites located within Norfolk

Survey site	Survey date and season	
	Spring	Summer
HM (Hatchet Marsh)	17-May-11	21-Jun-11
ND (Near Dry Dyke)	17-May-11	21-Jun-11
SM (Strumpshaw Meadow)	17-May-11	21-Jun-11
LM (Ludham Marsh)	16-May-11	15-Jun-11
LD (Long Dyke)	18-May-11	15-Jun-11
BM (Buckenham Marsh)	17-May-11	21-Jun-11
MM (Middle Marsh)	16-May-11	15-Jun-11
RM (Rockland Marsh)	18-May-11	21-Jun-11

Appendix 5: Further detail for survey sites located in Lincolnshire

South Forty Foot Drain survey sites

SF1 (Casswell's Bridge) survey site

The SF1 (Casswell's Bridge) survey site (OSGR: TF-16500-27500; Figure 12.1) was located east of Dunsby Fen Farm on the boundaries of Pinchbeck North Fen and Dunsby Fen, approximately five kilometres below the start of the South Forty Foot Drain. The water body was determined to be 1.3m deep at its centre and eight metres wide at the SF1 (Casswell's Bridge) survey site.

Figure 12.1: The location of the SF1 (Casswell's Bridge) survey site (OSGR: TF-16500-27500), indicated by the green dot. (Figure 12.1 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

The dominant species of vegetation at the SF1 (Casswell's Bridge) survey site was *Phragmites australis* (Cav.) Trin. ex Steud. (Plate 12.1), commonly known as Common Reed (Lambert *et al.*, 2007).

Plate 12.1: The SF1 (Casswell's Bridge) survey site (OSGR: TF-16500-27500) was sampled directly beneath the bridge, where a large abundance of *Phragmites australis* (Cav.) Trin. ex Steud. can be seen. (Photograph taken 30th March, 2010)

Sagittaria sagittifolia Linnaeus (Arrowhead) was also recorded during all the visits to the SF1 (Casswell's Bridge) survey site. *Lemna trisulca* Linnaeus (Ivy-leaved Duckweed) and other *Lemna* species were present during the autumn survey of the site.

SF2 (Donington Bridge) survey site

The SF2 (Donington Bridge) survey site (OSGR: TF-17300-35600; Figure 12.2) was located where the A52 road crosses the South Forty Foot Drain, approximately 8.3km downstream from the SF1 (Casswell's Bridge) survey site. The South Forty Foot Drain was 12m wide and 1.8m deep at its centre at the SF2 (Donington Bridge) survey site (Plate 12.2)

Glyceria maxima (Hartm.) Holmberg, commonly known as Reed Sweet-grass (Stace, 1991), was the dominant species of vegetation present at the SF2 (Donington Bridge) survey site, whilst *Nymphoides peltata* Kuntze (Fringed Water-lily), *Sparganium* (Bur-reed) and *Myriophyllum* (Water Milfoil) were also recorded during the spring and summer surveys. Gutweed (*Enteromorpha*) was also present at the SF2 (Donington Bridge) site when sample and data collection was undertaken during the summer survey. *Sparganium*, *Myriophyllum* and *N. peltata* were not recorded during the autumn survey, although several new taxa of vegetation were recorded; namely *Lemna*, *Azolla filiculoides* Lam. (Water Fern), *Ceratophyllum demersum* L. (Rigid Hornwort) and *Elodea nuttallii* (Planch.) H. St. John (Nuttall's Waterweed).

Figure 12.2: The location of the SF2 (Donington Bridge) survey site (OSGR: TF-17300-35600), represented by the green dot. (Figure 12.2 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Plate 12.2: The SF2 (Donington Bridge) survey site (OSGR: TF-17300-35600). *Glyceria maxima* (Hartm.) Holmberg was the dominant vegetation at the SF2 (Donington Bridge) survey site. (Photograph taken 30th March, 2010)

SF3 (Swineshead Bridge) survey site

The SF3 (Swineshead Bridge) survey site (OSGR: TF-21800-42900; Figure 12.3) was located where the A17 road crosses the South Forty Foot Drain, 8.9km downstream from the SF2 (Donington Bridge) survey site. The South Forty Foot Drain was determined to be 18m wide and 2.3m deep at its centre at the SF3 (Swineshead Bridge) survey site (Plate 12.3).

Figure 12.3: The Location of the SF3 (Swineshead Bridge) survey site (OSGR: TF-21800-42900), denoted by the green dot. (Figure 12.3 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Glyceria maxima was the dominant vegetation at the survey site, whilst *N. peltata* was also present during both the spring and summer surveys. *Enteromorpha* was present in the margins of the channel during the summer survey but absent during autumn. The presence of *A. filiculoides* and *Myriophyllum spicatum* L., commonly known as Spiked Water Milfoil (Stace, 1991), was noted during the autumn survey.

Plate 12.3: The SF3 (Swineshead Bridge) survey site (OSGR: TF-21800-42900), looking downstream from the bridge. The site was sampled just before the bridge, in the bottom right corner of the photograph. (Photograph taken 29th March, 2010)

SF4 (Wyberton Chain Bridge) survey site

The SF4 (Wyberton Chain Bridge) survey site (OSGR: TF-30400-43400; Figure 12.4) was located where the A52 crosses over the South Forty Foot Drain, on the outskirts of Boston. The site was 8.8km downstream from the SF3 (Swineshead Bridge) survey site. At the SF4 (Wyberton Chain Bridge) survey site, the South Forty Foot Drain was measured as 20m wide and had a depth of greater than three metres at its centre (Plate 12.4).

Plate 12.4: The SF4 (Wyberton Chain Bridge) survey site (OSGR: TF-30400-43400). The site was sampled before the bridge, in the foreground of the photograph. The presence of *Phragmites australis* (Cav.) Trin. ex Steud. can also be observed in the photograph in the bottom left corner. (Photograph taken 29th March, 2010)

Figure 12.4: The location of the SF4 (Wyberton Chain Bridge) survey site (OSGR: TF-30400-43400), indicated by the green dot. (Figure 12.4 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Large rocks and an abundance of *P. australis* were noted at the side of the channel, though no submerged vegetation was apparent during the spring and summer surveys. *Enteromorpha* was recorded at the margins of the SF4 (Wyberton Chain Bridge) survey site when sample and data collection was undertaken during the summer survey, whilst *Myriophyllum spicatum* was additionally recorded in autumn.

South Holland Main Drain survey sites

SH1 (Weston Fen) survey site

The SH1 (Weston Fen) survey site (OSGR: TF-27600-15900; Figure 12.5) was located in Weston Fen, approximately 3.2km downstream from the source of the South Holland Main Drain. At the SH1 (Weston Fen) survey site (Plate 12.5), the South Holland Main Drain was determined to be 10m wide and 0.5m deep at its centre.

Figure 12.5: The Location of the SH1 (Weston Fen) survey site (OSGR: TF-27600-15900), represented by the red dot. (Figure 12.5 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Plate 12.5: The SH1 (Weston Fen) survey site (OSGR: TF-27600-15900). The sparseness of margins of the channel during the spring survey is evident in the photograph. (Photograph taken 29th March, 2010)

During the spring survey at the SH1 (Weston Fen) survey site, it was noted that the channel had recently been dredged. Whilst *G. maxima* was present at the site, the marginal vegetation was sparse as a result of this action.

It was noted during the summer survey at the SH1 (Weston Fen) survey site that the vegetation had increased in both diversity and abundance. Whilst *G. maxima* was apparently absent, *Phragmites australis*, *E. canadensis* (Canadian Pondweed), *M. spicatum*, *Rumex hydrolapathum* Hudson (Water Dock) and *Enteromorpha* were all recorded at the survey site. During the autumn survey of the SH1 (Weston Fen) survey site both *E. nuttallii* and *Lemna* were recorded, although *M. spicatum*, *E. canadensis* and *Rumex hydrolapathum* were all found to be absent.

SH2 (Clifton's Bridge) survey site

The SH2 (Clifton's Bridge) survey site (OSGR: TF-38000-18900; Figure 12.6) was located where the B1165 road crosses the South Holland Main Drain on the outskirts of Sutton St James. The site was 9.5km downstream from the SH1 (Weston Fen) survey site. The South Holland Main Drain was measured as being 18m wide and 2.3m deep at its centre at the site.

Figure 12.6: The Location of the SH2 (Clifton's Bridge) survey site (OSGR: TF-38000-18900), denoted by the red dot. (Figure 12.6 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Whilst no submerged vegetation was evident during the spring and summer surveys of the SH2 (Clifton's Bridge) survey site, *P. australis* was present at the margins at this point along the drain. The presence of *M. spicatum* was additionally recorded during the autumn survey. There was a lack of vegetation under the bridge, where it was moderately dark (Plate 12.6).

Plate 12.6: The SH2 (Clifton's Bridge) survey site (OSGR: TF-38000-18900). The site was surveyed before the bridge, in the bottom centre of the photograph. The presence of *Phragmites australis* (Cav.) Trin. ex Steud. can be seen in the photograph. (Photograph taken 29th March, 2010)

SH3 (A1101 Road Bridge) survey site

The SH3 (A1101 Road Bridge) survey site (OSGR: TF-44300-19800; Figure 12.7) was located where the A1101 road crosses the South Holland Main Drain, on the outskirts of Tydd St Mary. The site was 6.4km downstream from the SH2 (Clifton's Bridge) survey site. The South Holland Main Drain was determined to be 30m wide and the depth was greater than three metres at its centre at the SH3 (A1101 Road Bridge) survey site. *Phragmites australis* was the dominant species of vegetation (Plate 12.7), whilst *Myriophyllum spicatum* and *Equisetum fluviatile* L. (Water Horsetail) were also recorded during both the spring and summer surveys. *Equisetum fluviatile*, however, was absent during the autumn survey.

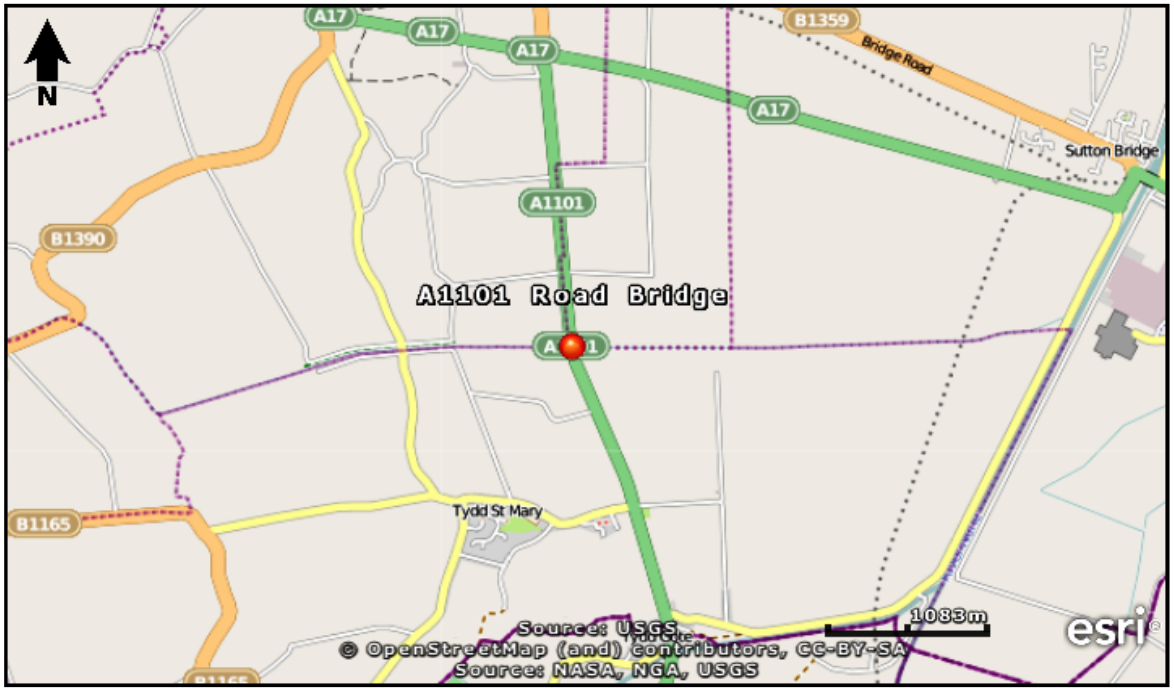

Figure 12.7: The location of the SH3 (A1101 Road Bridge) survey site (OSGR: TF-44300-19800), indicated by the red dot. (Figure 12.7 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

Plate 12.7: The SH3 (A1101 Road Bridge) survey site (OSGR: TF-44300-19800). The presence of *Phragmites australis* (Cav.) Trin. ex Steud. can be seen in the photograph. (Photograph taken 29th March, 2010)

SH4 (Nene Outfall Sluice) survey site

The SH4 (Nene Outfall Sluice) survey site (OSGR: TF-47400-19900; Figure 12.8) was located where the South Holland Main Drain meets the River Nene, on the outskirts of Sutton Bridge. The site was 3.2km downstream from the SH3 (A1101 Road Bridge) survey site.

Figure 12.8: The location of the SH4 (Nene Outfall Sluice) survey site (OSGR: TF-47400-19900), represented by the red dot. (Figure 12.8 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

The South Holland Main Drain was measured at 30m wide at the SH4 (Nene Outfall Sluice) survey site. *Phragmites australis* formed the majority of the vegetation along the length of the drain at the survey site (Plate 12.8) during all the surveys. Both *Enteromorpha* and *M. spicatum* were additionally recorded during the autumn survey of the SH4 (Nene Outfall Sluice) site.

Plate 12.8: The SH4 (Nene Outfall Sluice) survey site (OSGR: TF-47400-19900). The sluice can be seen in the background, as can a power station. *Phragmites australis* (Cav.) Trin. ex Steud. can be seen extending along the banks of the South Holland Main Drain. (Photograph taken 29th March, 2010)

Appendix 6: Further detail for survey sites located in Norfolk

Survey Sites of the Upper Thurne Catchment

The locations of the survey sites of the Upper Thurne catchment in Norfolk are displayed in Figure 12.9.

Figure 12.9: The survey sites of the Upper Thurne catchment. (Figure 12.9 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

The LD (Long Dyke) survey site (OSGR: TG-41300-27700; Plate 12.9) was located on the Brograve Level, close to the north-east coast of Norfolk and between the villages of Hempstead and Sea Palling. It has been stated that the water bodies of the Brograve Level are influenced by coastal saline groundwater inflow (Simpson, 2007), which has resulted in elevated salinity readings being recorded in drain surface waters (e.g. Holman, 1994; Simpson, 2007). The LD (Long Dyke) survey site was determined to be 300mm deep and 2.5m wide. The only vegetation recorded at the site in both spring and summer was *Phragmites australis*.

Plate 12.9: The LD (Long Dyke) survey site (OSGR: TG-41300-27700). *Phragmites australis* (Cav.) Trin. ex Steud. was the only plant species recorded at the site. (Photograph taken 18th May, 2011)

The MM (Middle Marsh) survey site (OSGR: TG-41300-20800; Plate 12.10) was located near Hickling Broad. It was considered that the site may be influenced in terms of salinity given the proximity to Hickling Broad, which itself is brackish (Irvine *et al*., 1993). The water depth at the MM (Middle Marsh) site was measured at 275mm and the width at 2.8m. The dominant vegetation in both spring and summer was *P. australis*. Two species of *Carex*, namely *Carex acutiformis* Ehrh. (Lesser Pond-sedge) and *C. riparia* Curtis (Greater Pond-sedge), were also both recorded at a substantially lower percentage cover of the water body at this site.

Plate 12.10: The MM (Middle Marsh) survey site (OSGR: TG-41300-20800). *Phragmites australis* (Cav.) Trin. ex Steud. was the dominant plant species recorded at the site. (Photograph taken 16th May, 2011)

LM (Ludham Marsh) was declared a National Nature Reserve in 1987 (Blunden & Curry, 1996) and was located on the north bank of the River Thurne (Figure 12.9), between Potter Heigham (OSGR: TG-41500-19300) and Ludham (OSGR: TG-38900-18300).

Plate 12.11: The LM (Ludham Marsh) survey site (OSGR: TG-40900-17900). A wide range of aquatic plant taxa were recorded at the site. (Photograph taken 16th May, 2011)

The LM (Ludham Marsh) site (TG-40900-17900; Plate 12.11) was surveyed in both spring and summer, during which water depth was determined to be 300mm and width was found to be 2.8m. It was noted that a wide range of aquatic plant taxa were present at the survey site (Table 12.4), of which *Stratiotes aloides* L. was the dominant member.

Table 12.4: Plant taxa recorded at the LM (Ludham Marsh) survey site

Plant taxon	Common name
Epilobium hirsutum (L.)	Great Willowherb
Equisetum sp.	Horsetail
Glyceria maxima (Hartm.) Holmb.	Reed Sweet-grass
Hydrocharis morsus-ranae L.	Frogbit
Juncus sp.	Rushes
Lemna trisulca L.	Ivy-leaved Duckweed
Mentha aquatica L.	Water Mint
Rorippa sp.	Water-cresses
Stratiotes aloides L.	Water Soldier
Typha latifolia L.	Bulrush
Veronica anagallis-aquatica/catenata	Water-Speedwells

Survey Sites of the River Yare Catchment

The locations of the survey sites of the River Yare catchment in Norfolk are displayed in Figure 12.10.

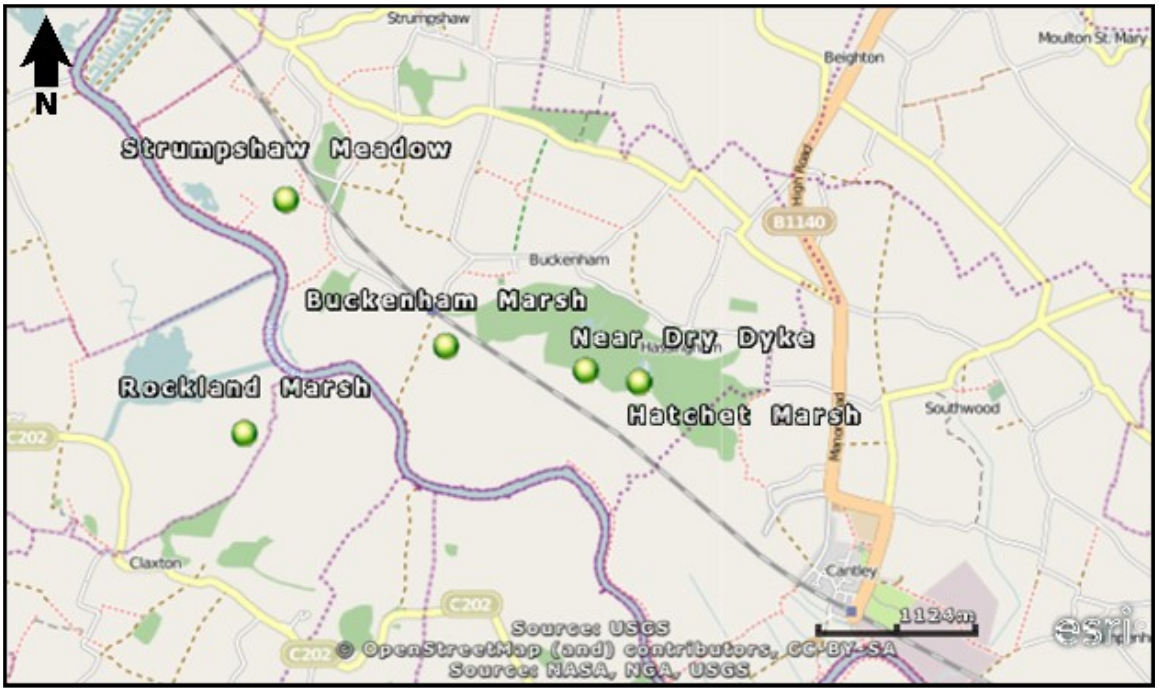

Figure 12.10: The survey sites of the River Yare Catchment. (Figure 12.10 contains Ordnance Survey data © Crown copyright and database right 2010, and was produced using ArcGIS Explorer software and data provided by OpenStreetMap [Map data © OpenStreetMap contributors, CC-BY-SA]).

The SM (Strumpshaw Meadow) survey site (OSGR: TG-33800-06700; Plate 12.12) and the BM (Buckenham Marsh) survey site (OSGR: TG-35300-05400; Plate 12.13) are both managed by the Royal Society for the Protection of Birds (RSPB) and form part of the Mid-Yare Reserve (Smart & Coutts, 2004; Bingham, 2006) located on the north side of the River Yare (Figure 12.10). Isolation of the reserve from the River Yare and the major mechanical management undertaken since 1978 has resulted in a large improvement in the water quality of the formerly eutrophic fen and Broad (Pickess, 1995). SM (Strumpshaw Meadow), however, has suffered two saline intrusion events in recent years, once in December 2006 and again in December 2007 (Strudwick, 2011). The BM (Buckenham Marsh) and SM (Strumpshaw Meadow) survey sites were both surveyed in spring and summer.

Plate 12.12: The SM (Strumpshaw Meadow) survey site (OSGR: TG-34000-06200). A diverse aquatic plant community was recorded at the site. (Photograph taken 17th May, 2011)

Plate 12.13: The BM (Buckenham Marsh) survey site (OSGR: TG-35200-05400). *Ceratophyllum demersum* L. was the dominant aquatic plant at the site in both spring and summer. (Photograph taken 17th May, 2011)

The BM (Buckenham Marsh) survey site had a water depth of 300mm and a width of 3.8m. Whilst *Eleocharis palustris* (L.) Roemer & Schultes (Common Spike-rush), *G*. *maxima*, *Juncus effusus* L. (Soft-rush), *Lemna*, *L. trisulca*, *Mentha aquatica* L. (Water Mint) and *Rorippa* were all recorded at the site, *C. demersum* was noted to be the dominant aquatic plant. The SM (Strumpshaw Meadow) survey site also supported a wide range of aquatic plants (Table 12.5). Water depth at the SM (Strumpshaw Meadow) site was 1400mm, whilst the width was 2.6m.

Table 12.5: Plant taxa recorded at the SM (Strumpshaw Meadow) survey site

Plant taxon	Common name
Carex acutiformis Ehrh.	Lesser Pond-sedge
Carex riparia Curtis	Greater Pond-sedge
Ceratophyllum demersum L.	Rigid Hornwort
Hydrocharis morsus-ranae L.	Frogbit
Iris pseudacorus L.	Yellow Iris
Lemna trisulca L.	Ivy-leaved Duckweed
Phragmites australis (Cav.) Trin. ex Steud.	Common Reed
Rorippa sp.	Water-cresses
Stratiotes aloides L.	Water Soldier
Typha latifolia L.	Bulrush
Vaucheria sp.	Mole-Pelt Algae

The RM (Rockland Marsh) survey site (OSGR: TG-33500-04900; Plate 12.14) was located to the south of Rockland Broad (Figure 12.10) and to the east of the village Rockland St Mary. Rockland Broad itself is connected to the River Yare by a dyke and may be subjected to increased levels of salinity due to the influence of tidal waters (Lambert, 1946; Croston *et al*., 1996).

Plate 12.14: The RM (Rockland Marsh) survey site (OSGR: TG-33500-04900). A diverse aquatic plant community was present at the site. (Photograph taken 18th May 2011)

The RM (Rockland Marsh) site was surveyed in both spring and summer. *Agrostis stolonifera* L. (Creeping Bent), *Carex rostrata* Stokes (Bottle Sedge), *Myriophyllum spicatum* L., *L. trisulca*, *Hydrocharis morsus-ranae* and *S. aloides* were all recorded at the RM (Rockland Marsh) site. *Stratiotes aloides* was the dominant plant species. Water depth was determined to be 740mm at the RM (Rockland Marsh) survey site, whilst width was 3.2m.

The ND (Near Dry Dyke) survey site (TG-35900-05300; Plate 12.15) and the HM (Hatchet Marsh) survey site (TG-36500-05100; Plate 12.16) were both located within Cantley Marshes on the north bank of the River Yare (Figure 12.10), located between the villages of Cantley, Hassingham and Buckenham.

Plate 12.15: The ND (Near Dry Dyke) survey site (TG-35900-05300). *Ceratophyllum demersum* L. was the dominant member of the aquatic plant community. (Photograph taken 17th May 2011)

Cantley Marshes occurs on the floodplain of the River Yare (Smart & Coutts, 2004) and thus the surface water bodies of the area may be subject to brackish floodwater originating the river. The HM (Hatchet Marsh) and ND (Near Dry Dyke) survey sites were both surveyed in spring and summer. The HM (Hatchet Marsh) survey site was found to have a water depth of 810mm and a water width of 1.6m. The aquatic vegetation community of the HM (Hatchet Marsh) site was composed of *Carex rostrata*, *H. morsus-ranae*, *M*. *spicatum*, *P*. *australis*, *Lemna* and dominated by *S. aloides* (Plate 12.16). The water depth at the ND (Near Dry Dyke) survey site was determined to be 300mm and width was 2.8m. The aquatic plant community of the ND (Near Dry Dyke) survey site was composed of *Carex riparia*, *Lemna*, *L. trisulca*, *Juncus*, *P. australis*, *S. aloides*, *T. latifolia* and the dominant member was *C. demersum*.

Plate 12.16: The HM (Hatchet Marsh) survey site (TG-36500-05100). *Stratiotes aloides* L. was the dominant member of the aquatic plant community. (Photograph taken 17th May 2011)

Appendix 7: Environmental Data Collected at Survey Sites in Lincolnshire

Table 12.6: Lincolnshire raw environmental data collected during spring season

		South Forty Foot Drain				South Holland Main Drain			
Position	Parameter	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
N/A	Width (m)	8.00	12.00	18.00	20.00	10.00	17.50	30.00	30.00
N/A	Depth, at centre (m)	1.30	1.80	2.30	3.00	0.50	2.30	2.70	3.00
Side, surface	Temperature (°C)	8.78	9.25	9.24	9.90	8.23	9.79	9.89	10.10
	Conductivity (mS/cm³)	0.92	1.71	1.41	1.31	4.04	4.84	6.10	6.74
	Dissolved oxygen (mgL^{-1})	9.44	12.08	13.79	12.17	13.46	10.81	14.80	17.33
	Redox potential (mV)	162.00	164.10	232.30	25.50	166.10	106.70	169.70	139.30
Side, base	Temperature (°C)	8.89	9.25	9.22	9.84	8.36	10.27	9.90	10.08
	Conductivity (mS/cm³)	1.00	1.71	1.41	1.31	4.05	6.21	6.14	7.22
	Dissolved oxygen (mgL^{-1})	8.79	11.68	12.65	11.43	11.81	9.45	14.25	16.28
	Redox potential (mV)	144.10	164.80	193.60	31.60	160.40	108.10	164.90	128.30
Middle, surface	Temperature (°C)	8.76	9.24	9.21	9.85	8.24	9.79	9.93	N/A
	Conductivity (mS/cm³)	0.88	1.71	1.40	1.31	4.04	4.81	6.13	N/A
	Dissolved oxygen (mgL^{-1})	9.60	12.60	14.41	12.09	14.48	11.10	15.47	N/A
	Redox potential (mV)	135.60	118.60	104.90	36.90	177.70	118.30	205.90	N/A
Middle, middle	Temperature (°C)	8.83	9.24	9.16	9.61	8.24	10.28	9.97	N/A
	Conductivity (mS/cm³)	0.95	1.71	1.40	1.33	4.05	6.20	6.20	N/A
	Dissolved oxygen (mgL^{-1})	9.06	12.03	13.56	11.03	13.79	9.94	14.29	N/A
	Redox potential (mV)	114.90	133.10	159.80	59.40	170.50	116.80	186.60	N/A
Middle, base	Temperature (°C)	9.42	9.24	9.18	N/A	8.25	10.35	10.12	N/A
	Conductivity (mS/cm³)	1.89	1.72	1.41	N/A	4.05	6.59	10.25	N/A
	Dissolved oxygen (mgL^{-1})	8.97	12.22	13.99	N/A	13.30	9.31	10.92	N/A
	Redox potential (mV)	131.10	118.20	115.30	N/A	168.00	109.40	198.10	N/A
Average	Temperature (°C)	8.94	9.24	9.20	9.80	8.26	10.10	9.96	10.09
	Conductivity (mS/cm³)	1.13	1.71	1.41	1.32	4.04	5.73	6.96	6.98
	Salinity (PSU)	0.56	0.86	0.70	0.66	2.14	3.10	3.81	3.82
	Dissolved oxygen (mgL^{-1})	9.17	12.12	13.68	11.68	13.37	10.12	13.95	16.81
	Redox potential (mV)	137.54	139.76	161.18	38.35	168.54	111.86	185.04	133.80

Table 12.7: Lincolnshire raw environmental data collected during summer season

		South Forty Foot Drain				South Holland Main Drain			
Channel position	**Parameter**	**SF1 (Casswell's Bridge)**	**SF2 (Donington Bridge)**	**SF3 (Swineshead Bridge)**	**SF4 (Wyberton Chain Bridge)**	**SH1 (Weston Fen)**	**SH2 (Clifton's Bridge)**	**SH3(A1101 Road Bridge)**	**SH4 (Nene Outfall Sluice)**
N/A	Width (m)	8.00	12.00	18.00	20.00	10.00	17.50	30.00	30.00
N/A	Depth, at centre (m)	1.30	1.80	2.30	3.00	0.50	2.30	2.70	3.00
Side, surface	Temperature (°C)	21.66	20.45	21.82	22.08	19.08	23.66	22.82	24.17
	Conductivity (mS/cm³)	1.39	1.08	2.44	3.74	2.23	7.08	9.97	10.86
	Dissolved oxygen (mgL^{-1})	8.80	10.58	10.81	12.41	7.33	10.17	10.11	10.58
	Redox potential (mV)	68.50	136.60	159.80	133.80	27.60	19.20	159.20	160.90
Side, base	Temperature (°C)	21.01	20.31	19.86	21.90	18.59	21.19	22.78	24.10
	Conductivity (mS/cm³)	1.35	1.07	14.11	3.73	2.23	9.37	9.99	10.92
	Dissolved oxygen (mgL^{-1})	7.10	10.45	13.44	11.49	3.16	5.08	10.08	10.82
	Redox potential (mV)	16.30	134.10	143.50	118.60	10.90	15.20	153.50	155.30
Middle, surface	Temperature (°C)	21.61	20.03	21.82	22.32	20.61	23.93	23.00	N/A
	Conductivity (mS/cm³)	1.41	1.07	2.46	3.81	2.17	7.12	10.06	N/A
	Dissolved oxygen (mgL^{-1})	9.70	10.71	11.10	12.54	8.74	9.46	10.57	N/A
	Redox potential (mV)	53.70	127.80	121.30	119.50	14.00	23.30	141.40	N/A
Middle, middle	Temperature (°C)	21.24	20.28	20.73	22.05	19.86	21.22	21.47	N/A
	Conductivity (mS/cm³)	1.39	1.07	5.13	3.87	2.20	9.44	13.23	N/A
	Dissolved oxygen (mgL^{-1})	10.32	9.85	9.05	10.69	7.22	4.14	12.45	N/A
	Redox potential (mV)	63.60	14.90	44.40	91.00	5.30	52.80	65.00	N/A
Middle, base	Temperature (°C)	20.55	18.83	18.27	18.00	18.52	19.91	15.48	N/A
	Conductivity (mS/cm³)	1.34	1.13	16.04	28.30	2.24	10.38	26.93	N/A
	Dissolved oxygen (mgL^{-1})	10.54	3.84	3.00	6.48	2.73	3.71	4.67	N/A
	Redox potential (mV)	58.50	134.50	5.00	145.00	5.40	114.50	181.10	N/A
Average	Temperature (°C)	21.21	19.98	20.50	21.27	19.33	21.98	21.11	24.14
	Conductivity (mS/cm³)	1.38	1.08	8.04	8.69	2.21	8.68	14.04	10.89
	Salinity (PSU)	0.69	0.53	4.45	4.84	1.13	4.83	8.11	6.17
	Dissolved oxygen (mgL^{-1})	9.29	9.09	9.48	10.72	5.84	6.51	9.58	10.70
	Redox potential (mV)	52.12	109.58	94.80	121.58	12.64	45.00	140.04	158.10

Table 12.8: Lincolnshire raw environmental data collected during autumn season

		South Forty Foot Drain				South Holland Main Drain			
Channel position	Parameter	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
N/A	Width (m)	8.00	12.00	18.00	20.00	10.00	17.50	30.00	30.00
N/A	Depth, at centre (m)	1.30	1.80	2.30	3.00	0.50	2.30	2.70	3.00
Side, surface	Temperature (°C)	11.92	13.79	12.93	13.23	10.83	11.42	12.93	12.99
	Conductivity (mS/cm³)	1.01	1.30	15.04	14.74	0.91	13.46	18.47	15.67
	Dissolved oxygen (mgL^{-1})	8.73	7.03	9.58	11.61	9.93	9.57	8.22	5.86
	Redox potential (mV)	85.40	24.20	14.80	22.90	40.80	104.50	93.00	101.90
Side, base	Temperature (°C)	11.90	13.80	13.24	13.24	10.86	15.06	12.94	13.00
	Conductivity (mS/cm³)	1.01	1.37	16.39	15.73	0.91	28.76	18.46	16.09
	Dissolved oxygen (mgL^{-1})	7.40	6.50	6.19	10.22	9.75	6.84	8.07	6.70
	Redox potential (mV)	46.00	-140.00	-227.20	27.10	46.20	110.10	95.60	95.60
	Phosphate (PO_4, mg/L)	0.00	1.23	2.92	3.35	0.00	0.54	0.34	0.86
	Nitrate (N, mg/L)	0.11	0.10	0.00	0.28	0.19	0.51	0.11	0.32
	Nitrate NO_3 (mgL^{-1})	0.48	0.44	0.00	1.23	0.84	2.24	0.48	1.41
Middle, surface	Temperature (°C)	11.92	13.80	12.91	13.26	10.83	11.38	12.96	N/A
	Conductivity (mS/cm³)	1.01	1.31	14.50	13.98	0.92	12.67	17.87	N/A
	Dissolved oxygen (mgL^{-1})	9.84	8.17	10.92	12.37	10.45	11.54	99.39	N/A
	Redox potential (mV)	70.90	97.50	106.00	166.80	69.70	116.10	110.90	N/A
Middle, middle	Temperature (°C)	11.89	13.81	12.91	13.19	10.84	15.95	12.99	N/A
	Conductivity (mS/cm³)	1.01	1.33	15.22	15.77	0.91	28.89	18.58	N/A
	Dissolved oxygen (mgL^{-1})	8.56	6.41	8.78	12.26	9.74	4.38	7.80	N/A
	Redox potential (mV)	82.60	9.20	3.20	13.20	32.50	112.80	90.30	N/A
Middle, base	Temperature (°C)	11.88	13.88	14.66	15.33	10.91	15.56	15.95	N/A
	Conductivity (mS/cm³)	1.01	4.31	22.10	31.99	0.91	29.96	37.70	N/A
	Dissolved oxygen (mgL^{-1})	8.14	1.21	1.01	1.47	9.34	6.37	2.46	N/A
	Redox potential (mV)	7.71	-25.80	-171.40	-185.30	-1.90	118.80	97.20	N/A
Average	Temperature (°C)	11.90	13.82	13.33	13.65	10.85	13.87	13.55	13.00
	Conductivity (mS/cm³)	1.01	1.92	16.65	18.44	0.91	22.75	22.22	15.88
	Salinity (PSU)	0.50	0.97	9.76	10.90	0.45	13.71	13.36	9.27
	Dissolved oxygen (mgL^{-1})	8.53	5.86	7.30	9.59	9.84	7.74	25.19	6.28
	Redox potential (mV)	58.52	-6.98	-54.92	8.94	37.46	112.46	97.40	98.75
	Phosphate (PO_4, mg/L)	0.00	1.23	2.92	3.35	0.00	0.54	0.34	0.86
	Nitrate (N, mg/L)	0.11	0.10	0.00	0.28	0.19	0.51	0.11	0.32
	Nitrate NO_3 (mgL^{-1})	0.48	0.44	0.00	1.23	0.84	2.24	0.48	1.41

Appendix 8: Environmental Data Collected at Survey Sites in Norfolk

Table 12.9: Environmental data from spring survey at Norfolk sites

Survey Site	Width (m)	Depth (mm)	Water column position	Conductivity ($mScm^{-1}$)	Salinity (PSU)	Temperature (°C)	Dissolved oxygen (mgL^{-1})	pH	Redox potential (mV)	Phosphate PO_4 (mgL^{-1})	Nitrate NO_3 (mgL^{-1})
LD (Long Dyke)	2.5	300	Top	31.92	19.88	13.90	4.83	6.88	-26.20	0.00	0.00
			Bottom	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MM (Middle Marsh)	2.8	275	Top	6.25	3.40	13.82	4.50	6.84	-201.50	0.58	0.00
			Bottom	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SM (Strumpshaw Meadow)	2.6	1400	Top	3.14	1.63	16.69	5.42	7.45	-80.40	0.42	0.00
			Bottom	3.13	1.63	16.16	2.12	7.34	-134.20	N/A	N/A
HM (Hatchet Marsh)	1.6	810	Top	0.84	0.41	11.73	2.80	6.91	-155.70	0.00	0.00
			Bottom	0.92	0.45	11.30	3.70	6.85	-174.40	N/A	N/A
LM (Ludham Marsh)	2.7	300	Top	0.80	0.39	13.56	4.58	7.03	-144.90	1.00	0.00
			Bottom	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RM (Rockland Marsh)	3.2	740	Top	0.90	0.44	17.75	9.06	7.60	-77.10	1.38	0.30
			Bottom	0.99	0.49	14.45	2.35	6.97	-149.00	N/A	N/A
BM (Buckenham Marsh)	3.8	300	Top	0.66	0.32	17.60	13.34	8.47	-48.90	1.88	0.00
			Bottom	0.68	0.33	16.30	50.06	7.34	-133.60	N/A	N/A
ND (Near Dry Dyke)	2.8	300	Top	0.81	0.40	14.01	1.79	7.09	-219.90	0.34	0.00
			Bottom	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 12.10: Environmental data from summer survey at Norfolk sites

Survey Site	Depth (mm)	Width (m)	Conductivity ($mScm^{-1}$)	Salinity (PSU)	Temperature (°C)	pH	Phosphate PO_4 (mgL^{-1})	Nitrate NO_3 (mgL^{-1})
LD (Long Dyke)	300	2.5	24.14	14.63	19.90	7.06	0.67	0.00
MM (Middle Marsh)	275	2.8	4.24	2.25	21.80	7.20	0.75	0.00
SM (Strumpshaw Meadow)	1400	2.6	1.94	0.98	20.70	7.77	0.44	1.63
HM (Hatchet Marsh)	810	1.6	0.45	0.22	19.50	7.64	0.28	3.34
LM (Ludham Marsh)	300	2.7	0.54	0.26	19.00	7.31	0.36	0.00
RM (Rockland Marsh)	740	3.2	0.58	0.28	20.80	7.79	1.09	1.58
BM (Buckenham Marsh)	300	3.8	0.29	0.14	22.90	9.75	1.06	1.10
ND (Near Dry Dyke)	300	2.8	0.41	0.20	18.60	7.85	0.44	0.00

Appendix 9: Macro-invertebrate Data Collected at Survey Sites in Lincolnshire

Table 12.11: Macro-invertebrate data from spring surveys at survey sites in Lincolnshire

Taxon	Site	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
Dugesia lugubris/polychroa		-	-	1	-	-	-	-	-
Valvata piscinalis		-	5	-	-	12	-	-	-
Potamopyrgus antipodarum		-	2	13	1	66	1	3	-
Peringia ulvae		-	-	-	-	-	-	-	1
Bithynia tentaculata		11	6	105	3	9	1	-	-
Bithynia leachii		16	6	21	-	-	-	-	-
Assiminea grayana		-	-	-	-	-	-	-	-
Physa fontanilis		13	1	19	-	13	-	-	-
Physella sp.		-	-	3	-	-	-	-	-
Lymnaea stagnalis		-	-	4	1	-	-	-	-
Galba truncatula		-	2	-	-	1	-	-	-
Stagnicola palustris		-	-	4	-	-	-	-	-
Radix balthica		-	1	32	5	9	3	2	-
Planorbis carinatus		-	-	1	-	-	-	-	-
Planorbis planorbis		-	3	47	-	6	-	1	-
Anisus vortex		8	52	50	1	-	-	-	-
Bathyomphalus contortus		-	2	1	-	-	-	-	-
Gyraulus albus		-	2	-	-	-	-	-	-
Hippeutis complanatus		1	3	-	-	-	-	-	-
Sphaerium sp.		-	4	-	-	-	-	-	-
Pisidium sp.		1	13	-	-	29	-	-	-
Dreissena polymorpha		-	-	7	2	-	168	5	10
Piscicola geometra		1	-	-	-	-	-	1	-
Hemiclepsis marginata		-	2	-	-	-	-	-	-
Glossiphonia complanata		1	-	-	-	-	-	-	-
Helobdella stagnalis		-	-	1	-	-	-	-	-
Alboglossiphonia heteroclita		1	-	-	-	-	-	-	-
Erpobdella octoculata		1	-	-	-	-	-	-	-
Erpobdella testacea		1	1	-	-	-	-	-	-
Argyroneta aquatica		-	-	-	1	-	-	-	-
Neomysis integer		-	-	-	-	17	-	-	-
Asellus aquaticus		34	29	69	1	17	-	-	-
Cyathura carinata		-	-	-	-	-	-	-	1
Lekanesphaera rugicauda		-	-	-	-	-	-	-	1
Corophium multisetosum		-	-	-	2	-	-	11	7
Crangonyx pseudogracilis		144	42	153	-	10	1	-	-
Gammarus pulex		1	1	10	-	-	-	-	5
Gammarus tigrinus		-	-	2	1	-	5	17	-
Gammarus zaddachi		-	-	1	-	-	-	-	2
Isotomurus palustris		-	2	-	-	-	-	1	1

Table 12.11: continued

Taxon	Site	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
Centroptilum luteolum		1	-	-	-	-	-	-	-
Caenis horaria		-	1	-	-	-	-	-	-
Caenis robusta		-	2	-	-	-	-	-	-
Coenagrion puella		2	-	7	1	-	-	-	-
Notonecta glauca		-	1	4	-	-	-	-	-
Notonecta viridis		-	-	-	1	-	-	-	-
Sigara dorsalis		-	-	1	1	-	-	-	1
Haliplus larvae		-	-	-	1	-	-	-	-
Haliplus confinis		-	-	1	-	-	-	-	-
Haliplus lineatocollis		-	2	-	6	5	-	-	-
Haliplus laminatus		1	1	-	-	-	-	-	-
Haliplus "ruficollis group"		-	4	2	2	-	-	-	-
Noterus clavicornis		-	2	3	-	-	-	-	-
Laccophilus hyalinus		-	-	-	-	1	-	-	-
Hydroporus palustris		-	-	-	-	3	-	-	-
Graptodytes pictus		-	-	-	-	2	-	-	-
Nebrioporus elegans		1	-	-	10	1	-	-	-
Stictotarsus duodecimpustulatus		-	-	-	3	-	-	-	-
Agabus didymus		-	-	-	-	1	-	-	-
Gyrinus caspius		-	-	-	1	-	-	-	-
Anacaena limbata		-	1	-	-	-	-	-	-
Laccobius colon		-	-	-	1	-	-	-	-
Laccobius bipunctatus		-	1	-	-	-	-	-	-
Sialis lutaria		2	-	-	-	1	-	-	-
Cyrnus flavidus		-	1	-	-	-	-	-	-
Limnephilus lunatus		33	-	-	-	3	-	-	-
Athripsodes aterrimus		4	-	-	-	-	-	-	-
Mystacides longicornis		-	-	-	-	1	-	-	-
Triaenodes bicolor		5	1	-	-	-	-	-	-
Tipulidae		-	3	-	-	-	-	-	-
Chrysomelidae		1	17	-	1	-	-	-	-
Chironomidae		26	23	179	22	61	20	25	25
Stratiomyidae		-	1	-	-	-	-	-	-
Tabanidae		-	-	-	-	1	-	1	-
Number of taxa		24	35	27	22	22	7	10	10
Number of individuals		320	240	741	68	269	199	67	54

Table 12.12: Macro-invertebrate data from summer surveys at survey sites in Lincolnshire

Taxon	Site	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
Polycelis nigra/tenuis		-	1	-	-	-	-	-	-
Dendrocoelum lacteum		-	-	-	2	4	-	-	-
Viviparus contectus		-	1	-	-	-	-	-	-
Valvata piscinalis		3	6	-	-	-	-	-	-
Potamopyrgus antipodarum		4	117	1	14	531	55	2	-
Peringia ulvae		-	-	-	-	-	-	5	6
Bithynia tentaculata		29	6	147	41	14	-	-	-
Bithynia leachii		36	3	16	5	46	-	-	-
Assiminea grayana		-	-	-	-	-	-	-	-
Physa fontanilis		21	-	15	-	18	1	-	-
Lymnaea stagnalis		2	5	1	-	-	-	-	-
Galba truncatula		-	-	36	-	-	2	-	-
Stagnicola palustris		7	-	5	25	-	-	-	-
Radix balthica		9	14	45	238	344	15	-	-
Planorbis carinatus		7	-	-	-	-	-	-	-
Planorbis planorbis		23	9	19	26	107	-	-	-
Anisus vortex		77	114	152	29	-	-	-	-
Bathyomphalus contortus		-	1	1	-	-	-	-	-
Gyraulus albus		9	-	-	-	-	-	-	-
Gyraulus crista		-	-	1	-	-	2	-	-
Hippeutis complanatus		4	5	-	-	-	-	-	-
Planorbarius corneus		-	-	6	-	-	-	-	-
Sphaerium sp.		19	11	-	-	12	-	-	-
Pisidium sp.		3	46	1	-	10	-	-	-
Dreissena polymorpha		-	-	-	-	-	12	4	1
Piscicola geometra		-	1	-	-	-	-	-	-
Theromyzon tessulatum		-	1	-	-	-	-	-	-
Hemiclepsis marginata		1	-	-	-	-	-	-	-
Glossiphonia complanata		1	-	1	-	-	-	-	-
Helobdella stagnalis		-	1	-	-	-	-	-	-
Alboglossiphonia heteroclita		1	3	-	-	-	-	-	-
Argyroneta aquatica		3	-	9	-	-	-	-	-
Palaemonetes varians		-	-	-	-	-	-	-	1
Crangon crangon		-	-	-	-	-	-	1	2
Carcinus maenas		-	-	-	-	-	-	-	-
Neomysis integer		-	-	-	-	-	1	-	1
Asellus aquaticus		441	181	71	45	156	-	-	-
Lekanesphaera rugicauda		-	-	-	-	-	-	-	1
Corophium multisetosum		-	-	-	-	-	-	2	28
Crangonyx pseudogracilis		56	69	28	13	11	-	-	-
Gammarus pulex		81	4	3	-	-	-	-	-
Gammarus tigrinus		-	-	-	-	6	32	124	20
Gammarus zaddachi		-	-	1	-	1	7	7	11
Caenis horaria		-	3	-	-	-	-	-	-
Coenagrion puella		1	-	1	-	-	-	-	-
Lestes sponsa		-	-	1	-	-	-	-	-

Table 12.12: continued

Taxon	Site	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
Aeshna grandis		1	-	-	-	-	-	-	-
Aeshna mixta		-	-	3	1	-	-	-	-
Microvelia reticulata		-	-	2	-	-	-	-	-
Gerridae nymphs		-	3	2	-	-	-	-	-
Ilyocoris cimicoides		-	-	1	-	-	-	-	-
Notonecta nymphs		13	1	2	8	3	-	-	-
Haliplus larvae		-	4	4	25	1	-	-	-
Haliplus lineatocollis		-	-	-	1	3	-	-	-
Haliplus mucronatus		-	-	-	2	-	-	-	-
Haliplus "*ruficollis* group"		1	1	3	-	-	-	-	-
Noterus larvae		-	-	2	2	-	-	-	-
Noterus clavicornis		-	-	1	2	-	-	-	-
Laccophilus hyalinus		-	-	-	-	1	-	-	-
Hygrotus versicolor		-	-	-	-	2	-	-	-
Hydroporinae larvae		-	-	-	26	-	-	-	-
Hydroporus marginatus		-	-	-	-	1	-	-	-
Hydroporus pubescens		-	-	-	-	1	-	-	-
Graptodytes pictus		-	1	-	-	7	-	-	-
Nebrioporus elegans		-	-	2	6	2	-	-	-
Stictotarsus duodecimpustulatus		-	-	-	2	-	-	-	-
Agabus conspersus		-	-	-	-	1	-	-	-
Agabus didymus		-	-	-	-	1	-	-	-
Gyrinus caspius		-	5	-	-	-	-	-	-
Hydrophilidae larvae		-	-	3	3	-	-	-	-
Helophorus brevipalpis		-	-	-	-	1	-	-	-
Laccobius colon		-	-	-	2	-	-	-	-
Dryopidae larvae		-	-	7	-	-	-	-	-
Sialis lutaria		2	23	-	-	16	-	-	-
Cyrnus flavidus		-	2	-	-	-	-	-	-
Phryganea bipunctata		-	1	1	-	2	-	-	-
Limnephilus lunatus		21	1	-	-	1	-	-	-
Athripsodes aterrimus		3	-	-	-	-	-	-	-
Mystacides longicornis		2	-	-	-	-	-	-	-
Tipulidae		-	-	-	4	-	-	-	-
Ceratopogonidae		-	-	1	1	-	-	-	-
Chironomidae		19	79	232	354	3	79	73	318
Stratiomyidae		-	-	2	-	-	-	-	-
Tabanidae		1	1	-	-	-	-	-	-
Ephydridae		-	6	-	-	-	-	-	-
Number of taxa		30	31	37	23	27	9	6	9
Number of individuals		894	605	828	861	771	151	211	383

Table 12.13: Macro-invertebrate data from autumn surveys at survey sites in Lincolnshire

Taxon	Site	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
Polycelis nigra/tenuis		-	1	-	-	-	-	-	-
Dugesia lugubris/polychroa		-	1	-	-	-	-	-	-
Valvata piscinalis		-	-	-	-	2	-	-	-
Potamopyrgus antipodarum		-	-	-	3074	135	1	40	-
Peringia ulvae		-	-	-	-	-	-	-	-
Bithynia tentaculata		52	12	-	-	21	-	-	-
Bithynia leachii		-	1	-	-	-	-	-	-
Physa fontanilis		3	-	-	-	26	-	-	-
Physella sp.		-	2	1	-	36	-	-	-
Radix balthica		-	-	-	-	103	-	-	-
Planorbis planorbis		5	-	-	-	8	-	-	-
Anisus vortex		3	2	-	-	-	-	-	-
Gyraulus albus		3	-	-	-	-	-	-	-
Sphaerium sp.		2	61	-	-	4	-	-	-
Pisidium sp.		61	4	-	-	89	-	-	-
Dreissena polymorpha		-	-	-	-	-	-	-	2
Mytilopsis leucophaeta		-	-	3	2	-	335	52	-
Hediste diversicolor		-	-	-	-	-	-	-	-
Piscicola geometra		-	1	-	-	-	-	-	-
Glossiphonia complanata		-	-	-	-	1	-	-	-
Helobdella stagnalis		1	1	-	-	-	-	-	-
Erpobdella octoculata		1	1	-	-	-	-	-	-
Erpobdella testacea		3	-	-	-	-	-	-	-
Palaemonetes varians		-	-	-	-	-	-	-	1
Palaemon longirostris		-	-	-	-	-	-	-	-
Crangon crangon		-	-	-	-	-	-	-	-
Carcinus maenas		-	-	-	-	-	-	-	2
Neomysis integer		-	-	-	1	-	-	241	1864
Asellus aquaticus		46	105	-	2	51	-	-	-
Lekanesphaera rugicauda		-	-	-	-	-	-	2	10
Corophium multisetosum		-	-	-	3	-	133	20	49
Crangonyx pseudogracilis		36	78	-	-	29	-	-	-
Gammarus pulex		12	-	-	-	-	-	-	-
Gammarus tigrinus		-	-	151	277	-	-	-	-
Gammarus zaddachi		-	-	-	-	-	43	3167	86
Cloeon dipterum		-	19	-	-	-	-	-	-
Caenis horaria		1	6	-	-	-	-	-	-
caenis luctosa		-	-	-	-	1	-	-	-
Coenagrion puella		1	6	1	-	11	-	-	-
Brachytron pratense		-	-	1	-	-	-	-	-
Notonecta glauca		-	1	-	-	5	-	-	-
Plea minutissima		1	-	-	-	-	-	-	-
Sigara dorsalis		-	5	-	-	2	-	-	-
Sigara falleni		-	6	-	-	-	-	-	-
Haliplus larvae		3	-	-	-	1	-	-	-
Haliplus lineatocollis		-	1	-	-	1	-	-	-

Table 12.13: continued

Taxon	Site	SF1 (Casswell's Bridge)	SF2 (Donington Bridge)	SF3 (Swineshead Bridge)	SF4 (Wyberton Chain Bridge)	SH1 (Weston Fen)	SH2 (Clifton's Bridge)	SH3(A1101 Road Bridge)	SH4 (Nene Outfall Sluice)
Haliplus "*ruficollis* group"		8	3	3	2	1	-	-	-
Hyphydrus ovatus		1	-	-	-	-	-	-	-
Hygrotus versicolor		-	-	-	-	1	-	-	-
Graptodytes pictus		-	-	-	-	4	-	-	-
Nebrioporus elegans		1	-	2	-	1	-	-	-
Dytiscus marginalis		-	-	-	-	1	-	-	-
Gyrinus substriatus		-	12	-	-	-	-	-	-
Laccobius colon		-	-	5	-	-	-	-	-
Sialis lutaria		17	3	-	-	7	-	-	-
Cyrnus flavidus		1	1	-	-	-	-	-	-
Phryganea bipunctata		-	2	-	-	-	-	-	-
Athripsodes aterrimus		-	-	-	-	7	-	-	-
Mystacides longicornis		-	1	-	-	2	-	-	-
Triaenodes bicolor		3	2	-	-	-	-	-	-
Ceratopogonidae		-	4	-	-	1	-	-	-
Chironomidae		7	104	25	6	50	45	16	45
Tabanidae		-	-	-	-	5	-	-	-
Empididae		-	-	-	-	-	2	-	-
Number of taxa		24	29	9	8	29	6	7	8
Number of individuals		272	446	192	3367	606	559	3538	2059

Appendix 10: Macro-invertebrate Data Collected at Survey Sites in Norfolk

Table 12.14: Macro-invertebrate data from spring surveys at survey sites in Norfolk

Taxon	Site	LD (Long Dyke)	MM (Middle Marsh)	SM (Strumpshaw Meadow)	HM (Hatchet Marsh)	LM (Ludham Marsh)	RM (Rockland Marsh)	BM (Buckenham Marsh)	ND (Near Dry Dyke)
Polycelis nigra/tenuis		-	-	-	-	-	1	-	-
Dugesia polychroa		-	-	-	-	-	1	-	-
Viviparus viviparus		-	-	5	-	-	-	-	-
Valvata cristata		-	-	-	7	-	1	-	4
Valvata piscinalis		-	-	-	-	-	-	1	-
Potamopyrgus antipodarum		76	27	-	-	-	-	-	-
Bithynia tentaculata		-	-	53	10	-	94	34	11
Bithynia leachii		-	-	25	-	33	32	-	7
Physa fontinalis		-	-	1	-	11	-	4	1
Lymnaea stagnalis		-	1	2	-	3	5	-	2
Stagnicola palustris		-	-	-	1	-	-	-	-
Radix balthica		-	2	21	1	3	27	16	-
Planorbis planorbis		-	12	-	2	-	2	35	1
Anisus vortex		-	-	-	-	7	54	39	1
Bathyomphalus contortus		-	-	-	-	-	3	-	-
Hippeutis complanatus		-	-	1	5	-	7	5	14
Planorbarius corneus		-	-	1	2	1	1	-	-
Acroloxus lacustris		-	-	2	15	-	-	-	5
Sphaerium sp.		-	-	12	-	-	3	18	1
Pisidium sp.		-	-	9	-	17	-	-	-
Theromyzon tessulatum		-	-	-	-	-	-	1	-
Hemiclepsis marginata		-	-	-	-	-	-	-	1
Glossiphonia complanata		-	-	-	-	2	-	-	-
Glossiphonia heteroclita		-	-	-	2	-	1	1	1
Helobdella stagnalis		-	-	-	-	2	-	-	-
Erpobdella octoculata		-	-	-	1	2	1	2	-
Argyroneta aquatica		-	-	-	-	-	3	-	-
Asellus aquaticus		-	212	63	-	17	24	8	-
Proasellus meridianus		-	124	-	-	1	-	15	-
Crangonyx pseudogracilis		-	-	64	39	13	66	1	36
Gammarus zaddachi		71	12	-	-	-	-	-	-
Caenis robusta		-	-	-	-	-	-	15	-
Coenagrion puella		-	-	12	3	10	5	4	18
Coenagrion pulchellum		-	-	1	-	4	3	-	-
Aeshna cyanea		-	-	-	-	-	3	-	-
Aeshna isosceles		-	-	-	-	-	2	-	-
Sympetrum sanguineum		-	2	-	-	-	-	-	-
Nepa cinerea		-	1	-	-	-	-	-	-
Ilyocoris cimicoides		-	-	-	-	-	-	-	1
Notonecta nymphs		-	5	14	-	13	1	3	-

Table 12.14: continued

Taxon	Site	LD (Long Dyke)	MM (Middle Marsh)	SM (Strumpshaw Meadow)	HM (Hatchet Marsh)	LM (Ludham Marsh)	RM (Rockland Marsh)	BM (Buckenham Marsh)	ND (Near Dry Dyke)
Notonecta viridis		-	1	-	-	-	-	-	1
Corixidae nymphs		-	3	-	-	1	-	2	-
Callicorixa praeusta		-	1	-	-	-	-	-	-
Peltodytes caesus		-	-	-	-	-	-	1	-
Haliplus fluviatilis		-	-	-	1	-	-	-	-
Haliplus "*ruficollis* group"		-	1	1	-	1	-	13	3
Hygrobia hermanni		-	-	-	-	-	-	4	-
Noterus clavicornis		-	-	-	-	1	1	-	-
Noterus crassicornis		-	1	5	-	-	10	-	-
Dytiscidae larvae		-	4	1	-	-	-	4	1
Hyphydrus ovatus		-	-	1	-	1	1	-	-
Hygrotus inaequalis		-	4	2	8	-	-	-	-
Hydroporus pubescens		-	7	-	-	-	-	-	-
Porhydrus lineatus		-	-	1	-	-	-	-	-
Copelatus haemorrhoidalis		-	-	-	1	-	-	-	-
Rhantus grapii		-	1	1	-	-	-	-	-
Hydaticus seminger		-	-	-	1	-	-	-	-
Hydaticus transversalis		-	-	1	-	-	-	-	-
Dytiscus marginalis		-	-	-	1	-	-	-	-
Suphrodytes dorsalis		-	-	-	1	-	-	-	-
Enochrus testaceus		-	-	-	-	-	1	-	-
Holocentropus picicornis		-	-	3	11	7	7	-	-
Limnephilus affinis		2	-	-	-	-	-	-	-
Limnephilus flavicornis		-	-	-	-	3	-	-	-
Limnephilus lunatus		-	-	-	-	-	1	-	9
Limnephilus marmoratus		-	-	2	-	3	-	-	-
Leptocerus tineiformis		-	-	-	-	-	-	9	-
Triaenodes bicolor		-	-	-	-	3	-	-	-
Tipulidae		-	-	-	-	-	3	5	1
Chaoboridae		-	-	20	37	-	-	-	6
Culicidae		-	-	-	4	-	4	-	-
Ceratopogonidae		-	-	1	-	-	-	-	-
Chironomidae		220	142	203	12	32	18	38	56
Stratiomyidae		-	-	1	1	-	8	-	-
Sciomyzidae		-	-	-	-	3	1	-	-
Number of taxa		4	20	30	23	26	34	25	22
Number of individuals		369	563	529	166	194	395	278	181

Table 12.15: Macro-invertebrate data from summer surveys at survey sites in Norfolk

Taxon	Site	LD (Long Dyke)	MM (Middle Marsh)	SM (Strumpshaw Meadow)	HM (Hatchet Marsh)	LM (Ludham Marsh)	RM (Rockland Marsh)	BM (Buckenham Marsh)	ND (Near Dry Dyke)
Planaria torva		-	1	-	-	-	-	-	-
Polycelis nigra/tenuis		-	-	-	-	-	1	-	-
Dugesia lugubris		-	-	-	1	-	-	1	-
Viviparus viviparus		-	-	-	-	-	-	-	-
Valvata cristata		-	-	2	3	-	-	-	8
Valvata piscinalis		-	-	-	-	-	-	2	3
Potamopyrgus antipodarum		265	147	-	-	-	-	-	-
Bithynia tentaculata		-	-	26	12	-	77	49	19
Bithynia leachii		-	-	5	7	9	26	-	9
Physa fontinalis		-	-	1	1	77	51	-	12
Physella sp.		-	1	-	-	-	-	-	-
Lymnaea stagnalis		-	-	-	1	9	-	-	2
Stagnicola palustris		-	-	1	6	1	3	1	-
Radix balthica		-	29	8	-	26	48	34	37
Planorbis carinatus		-	-	-	4	1	-	1	-
Planorbis planorbis		-	39	1	4	17	35	49	31
Anisus vortex		-	-	-	34	473	58	29	19
Bathyomphalus contortus		-	-	-	6	-	-	-	-
Gyraulus albus		-	-	-	-	-	-	1	-
Hippeutis complanatus		-	-	2	25	2	15	1	37
Segmentina nitida		-	-	-	1	-	-	-	-
Planorbarius corneus		-	-	3	-	49	14	-	4
Acroloxus lacustris		-	-	-	13	-	-	-	2
Sphaerium sp.		-	-	8	2	-	4	9	2
Pisidium sp.		-	-	2	2	-	13	-	-
Theromyzon tessulatum		-	-	-	-	-	-	423	-
Glossiphonia complanata		-	-	-	-	1	-	-	-
Glossiphonia heteroclita		-	-	-	-	1	-	3	2
Helobdella stagnalis		-	-	-	-	1	-	-	2
Erpobdella octoculata		-	-	-	6	5	-	-	-
Erpobdella testacea		-	-	-	1	3	-	-	-
Argyroneta aquatica		-	-	-	2	3	1	-	-
Palaemonetes varians		-	-	-	-	-	-	-	-
Asellus aquaticus		-	212	27	14	33	123	1	-
Proasellus meridianus		-	25	-	19	2	-	5	2
Crangonyx pseudogracilis		-	-	41	68	83	28	31	59
Gammarus zaddachi		96	-	-	-	-	-	-	-
Caenis robusta		-	-	-	-	1	-	7	2
Pyrrhosoma nymphula		-	-	-	10	-	-	-	-
Ischnura elegans		-	-	2	-	3	-	-	-
Coenagrion puella		-	-	2	1	7	2	1	9
Coenagrion pulchellum		-	-	-	-	2	-	-	-
Lestes sponsa		-	-	2	-	3	1	-	-
Brachytron pratense		-	-	-	-	1	-	-	-
Aeshna sp.		-	-	2	-	-	-	-	-
Aeshna mixta		-	-	-	-	2	-	-	-

Table 12.15: continued

Taxon	Site	LD (Long Dyke)	MM (Middle Marsh)	SM (Strumpshaw Meadow)	HM (Hatchet Marsh)	LM (Ludham Marsh)	RM (Rockland Marsh)	BM (Buckenham Marsh)	ND (Near Dry Dyke)
Sympetrum sanguineum		-	1	-	-	2	-	-	2
Nepa cinerea		-	-	-	-	2	-	-	-
Ilyocoris cimicoides		-	-	1	-	2	1	1	-
Notonecta nymphs		-	8	7	6	6	-	-	11
Plea minutissima		-	-	1	-	-	-	-	-
Corixidae nymphs		-	5	1	-	-	-	1	1
Corixa punctata		-	-	-	-	-	-	1	-
Haliplidae larvae		-	-	-	-	-	-	-	1
Haliplus "*ruficollis* group"		-	3	1	-	2	-	3	-
Hygrobia hermanni larvae		-	-	-	-	-	-	-	1
Noterus clavicornis		-	-	-	-	5	-	-	-
Noterus crassicornis		-	-	-	2	3	-	-	-
Dytiscidae larvae		-	1	2	-	1	-	-	4
Hyphydrus ovatus		-	-	-	1	3	-	-	2
Hydroglyphus geminus		-	2	-	-	-	-	-	-
Hygrotus inaequalis		-	10	-	1	-	-	-	3
Hydroporus angustatus		-	-	-	10	-	-	-	-
Ilybius ater		-	1	-	-	-	-	-	-
Hydrophilidae larvae		-	-	2	2	1	3	-	1
Hydrobius fuscipes		-	2	-	4	-		-	-
Anacaena limbata		-	-	-	2	-	1	-	-
Helochares punctatus		-	-	-	1	-	-	-	-
Enochrus coarctatus		-	-	-	9	-	-	-	-
Enochrus halophilus		-	1	-	-	-	-	-	-
Holocentropus picicornis		-	-	-	4	-	-	-	-
Agrypnia pagetana		-	-	-	-	-	6	1	1
Limnephilus lunatus		-	-	-	-	2	-	-	-
Limnephilus marmoratus		-	-	-	-	5	-	-	-
Leptocerus tineiformis		-	-	-	-	-	-	5	4
Triaenodes bicolor		-	-	-	-	1	-	-	5
Chaoboridae		-	4	7	1	6	7	-	73
Ceratopogonidae		-	-	-	-	-	-	1	-
Chironomidae		208	328	151	91	53	11	32	173
Stratiomyidae		-	-	3	10	10	1	-	-
Tabanidae		-	-	-	1	-	-	-	-
Number of taxa		3	19	27	39	42	24	26	33
Number of individuals		569	820	311	388	919	530	693	543

Appendix 11: Results of Application of Salinity Indices to Data Collected at Survey Sites in Lincolnshire

Table 12.16: Results following application of the Salinity Association Group Index, the salinity index of Horrigan *et al*. (2005), the ditch salinity index of Palmer *et al*. (2010) and $SPEAR_{salinity}$ (Schäfer *et al*., 2011) to Lincolnshire data

		Salinity Association Group Index						
Season	**Survey site**	Mixed-level identification with abundance data	Mixed level identification without abundance data	Family-level identification with abundance data	Family-level identification without abundance data	**Salinity index (Horrigan *et al.*, 2005)**	**$SPEAR_{salinity}$ (Schäfer *et al.*, 2011)**	**Ditch Salinity Index (Palmer *et al.*, 2010)**
Spring (2010)	SF1 (Casswell's Bridge)	3.42	3.11	4.19	3.75	3.00	0.96	0
	SF2 (Donington Bridge)	4.26	4.11	4.65	4.40	4.63	0.77	0
	SF3 (Swineshead Bridge)	5.00	4.50	5.88	5.50	3.83	0.93	2
	SF4 (Wyberton Chain Bridge)	5.53	5.53	5.56	5.50	3.00	0.57	1
	SH1 (Weston Fen)	4.74	4.37	5.25	4.75	1.80	0.54	2
	SH2 (Clifton's Bridge)	5.33	5.00	6.67	6.33	1.00	0.96	0
	SH3 (A1101 Road Bridge)	5.75	5.50	6.75	6.50	1.00	0.91	0
	SH4 (Nene Outfall Sluice)	7.13	7.00	8.00	7.86	5.00	0.94	6
Summer (2010)	SF1 (Casswell's Bridge)	4.04	3.67	5.00	4.40	2.71	0.97	0
	SF2 (Donington Bridge)	3.69	3.31	4.08	3.83	3.00	0.68	1
	SF3 (Swineshead Bridge)	4.70	4.20	5.16	4.84	3.00	0.96	2
	SF4 (Wyberton Chain Bridge)	5.87	5.27	6.21	5.57	4.13	0.87	0
	SH1 (Weston Fen)	4.90	4.20	5.35	4.53	2.00	0.47	3
	SH2 (Clifton's Bridge)	5.89	5.44	7.86	7.29	1.00	0.64	4
	SH3 (A1101 Road Bridge)	7.57	7.29	8.60	8.20	N/A	0.91	6
	SH4 (Nene Outfall Sluice)	8.44	8.11	9.75	9.50	5.00	0.98	12
Autumn (2011)	SF1 (Casswell's Bridge)	4.60	4.20	4.25	3.75	3.00	0.78	0
	SF2 (Donington Bridge)	4.77	4.45	3.96	3.61	4.70	0.92	0
	SF3 (Swineshead Bridge)	7.00	6.33	5.75	5.50	3.00	0.71	0
	SF4 (Wyberton Chain Bridge)	7.83	7.00	8.57	7.86	N/A	0.00	2
	SH1 (Weston Fen)	4.33	3.86	4.70	4.20	3.00	0.60	0
	SH2 (Clifton's Bridge)	8.25	7.00	9.25	8.00	N/A	0.99	2
	SH3 (A1101 Road Bridge)	9.00	7.67	11.00	9.67	N/A	0.46	6
	SH4 (Nene Outfall Sluice)	9.29	8.43	10.86	10.14	5.00	0.90	10

Appendix 12: Results of Application of Salinity Indices to Data Collected at Survey Sites in Norfolk

Table 12.17: Results following application of the Salinity Association Group Index, the salinity index of Horrigan *et al*. (2005), the ditch salinity index of Palmer *et al*. (2010) and $SPEAR_{salinity}$ (Schäfer *et al*., 2011) to Norfolk data

Season	Survey site	Salinity Association Group Index: Mixed-level identification with abundance data	Salinity Association Group Index: Mixed level identification without abundance data	Salinity Association Group Index: Family-level identification with abundance data	Salinity Association Group Index: Family-level identification without abundance data	Salinity index (Horrigan *et al*., 2005)	$SPEAR_{salinity}$ (Schäfer *et al*., 2011)	Ditch Salinity Index (Palmer *et al*., 2010)
Spring (2011)	BM (Buckenham Marsh)	4.82	4.65	5.19	4.94	4.13	0.70	0
	LM (Ludham Marsh)	5.19	4.94	5.56	5.17	2.71	0.77	0
	ND (Near Dry Dyke)	5.06	4.94	5.33	5.07	2.86	0.75	0
	HM (Hatchet Marsh)	4.73	4.67	5.15	5.00	1.80	0.70	0
	RM (Rockland Marsh)	5.05	4.86	5.45	5.18	4.09	0.66	0
	SM (Strumpshaw Meadow)	5.00	4.67	5.56	5.22	2.71	0.64	0
	MM (Middle Marsh)	5.50	5.17	6.33	5.92	2.71	0.56	2
	LD (Long Dyke)	7.00	6.33	6.67	6.00	N/A	0.60	2
Summer (2011)	BM (Buckenham Marsh)	5.00	4.68	5.25	4.38	4.13	0.80	0
	ND (Near Dry Dyke)	4.76	4.52	5.30	5.10	3.40	0.79	0
	HM (Hatchet Marsh)	4.76	4.68	5.55	5.20	3.33	0.60	0
	LM (Ludham Marsh)	5.21	4.97	5.63	5.33	3.15	0.69	0
	RM (Rockland Marsh)	5.11	4.79	5.86	5.21	2.60	0.68	0
	SM (Strumpshaw Meadow)	5.00	4.76	5.35	5.12	3.40	0.64	0
	MM (Middle Marsh)	5.75	5.38	6.00	5.40	3.67	0.57	1
	LD (Long Dyke)	6.50	5.00	8.50	7.00	N/A	0.49	2