QUANTIFYING INHABITATION, FEEDING AND CONNECTIVITY BETWEEN ADJACENT ESTUARINE AND COASTAL REGIONS FOR THREE COMMERCIALLY IMPORTANT MARINE FISHES

By

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Abstract

1. Estuaries are regarded as valuable nursery habitats for many commercially important marine fishes. Recruitment of fish from estuarine nursery habitats to adult marine populations is considered important for maintenance of fishable stocks, but most evidence for this is qualitative. Effective and timely implementation of estuarine conservation and fisheries management plans may be aided by quantitative assessment of this habitat connectivity.

2. This thesis reports upon the quantification of inhabitation, feeding and connectivity between adjacent estuarine and coastal regions for Common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and Whiting (*Merlangius merlangus*). Sample collection was focused in the Thames Estuary and adjacent coastal regions.

3. Two techniques were employed: (i) stable isotope analysis of soft tissues for tracing feeding signals; and (ii) elemental analysis of fish otoliths for tracing patterns of fish movement and residency.

4. Analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data identified significant differences in isotopic signatures between estuarine and coastal invertebrates, and allowed re-classification to sample sites with 98.8% accuracy.

5. Using invertebrate data as source indicators, stable isotope data classified juvenile fishes to the region in which they fed using stable isotope data. Feeding signals primarily reflected physiological (freshwater tolerance) and functional (mobility) differences between species.

6. Mixing models calculated estuarine contributions to adult muscle tissue isotopic composition. Juvenile bass have an affinity for estuarine feeding, followed by greater plasticity (individual level) in habitat choice as older fish, facilitated by their mobility and tolerance of low salinities. Sole show this plasticity (population level) in estuarine-coastal feeding as juveniles, and then lower plasticity with more consistently marine diets as adults. Whiting exhibited plasticity (individual level) as both juveniles and adults.

7. Chemical composition of juvenile fish otoliths reflected their region of collection (95-100% accuracy). Misclassifications were indicative of between-habitat movement by whiting. Only juvenile sole showed significant energetic benefits of an estuarine existence.

8. Adult otolith chemistry data supported the stable isotope results. Variable plasticity in the use of estuarine and coastal resources was revealed, depending upon the species.

9. This research provides quantitative insight into resource use and estuarine-coastal habitat connectivity for these three species, as well as valuable guidance for similar future applications of soft-tissue stable isotope analysis and elemental analysis of fish otoliths.

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1. Introduction

Estuaries are commonly believed to be important nursery grounds for juvenile marine fishes, which then migrate offshore for adulthood, transporting estuarine material that they have assimilated. This connectivity between estuaries and offshore adult populations has been established and quantified in some places. However, for most regions of the world the importance of estuaries is assumed from anecdotal evidence of large numbers of juveniles in estuaries, with their respective adult populations found offshore. North Western Europe is one such area. Quantification of the utility of estuaries by marine fishes and the seaward transport of estuarine energy resources would be beneficial in fisheries management and estuarine conservation, particularly in consideration of the vulnerability of estuaries to degradation and loss as a result of human impacts.

1.1 Transport pathways from estuarine to coastal habitat.

There are three principal pathways through which biomass, nutrients and energy can be transferred from estuarine to coastal habitats: (1) as particulate organic matter (POM), (2) dissolved in the water (e.g. nitrate), and (3) through consumption and assimilation of estuarine food sources, followed by movement of organisms out of the estuary to offshore habitats. Particulate detritus is of low nutritive value as much of it settles rapidly or is respired by bacteria without entering higher levels of the marine food-web (Deegan, 1993). The latter two pathways are likely to be of considerable importance. Phytoplankton blooms caused by offshore transport of dissolved nutrients (i.e. nitrogen and phosphorus) can enhance secondary production and lead to increased fish biomass (e.g. Nixon & Buckley, 2002; Nixon, 2003), while species that consume and assimilate estuarine material and organisms can transport substantial quantities of highly nutritive energy in the event of migratory activity (Deegan, 1993). This third pathway can be split into two sub-categories: those individuals that feed periodically or over-winter in estuaries at intervals throughout their lives, and those that only use estuaries as nursery habitat. The latter is thought to be of particular importance as it is commonly assumed that growth and survival of juveniles is enhanced within estuaries, supporting recruitment to offshore adult populations (e.g. Gillanders & Kingsford, 2000; Power *et al.*, 2000a,b). For instance,

the biological production of estuarine fish in the Forth Estuary in SE Scotland has been calculated at 4.3 $gWWm^{-2}yr^{-1}$ (WW = wet-weight), compared to 2.5 $gWWm^{-2}yr^{-1}$ in the North Sea (Costa *et al.*, 2002).

With the growth of human populations, estuaries have become intensively exposed to anthropogenic impacts. Agricultural runoff, urban runoff and industrial discharges pollute the water column and sediments; boat and fishing activities disturb substrata and damage habitats; and human developments result in canalisation and habitat loss. For instance, land-claim on the Forth Estuary in Scotland resulted in the loss of almost 50% of the intertidal area, removing 24% of natural fish habitat and 40% of fish food supplies (McLusky *et al.*, 1992). It is important to understand the impact of these anthropogenic disturbances on individual organisms, populations, communities and whole ecosystems. However, in order to support and direct effective management and conservation initiatives, it is also necessary to determine the functional importance of estuaries, preferably in a quantitative manner. The ecological function of estuaries as nursery habitats for juveniles of marine species is an established phenomenon with a rapidly developing suite of scientific techniques and applications.

Issues concerning the connectivity between the estuarine and marine environment have recently become emphasised, particularly with respect to the movements and dependencies of fishes (e.g. Dame and Allen, 1996; Able, 2005; Gillanders, 2005; Herzka, 2005; Ray, 2005; Secor and Rooker, 2005). In studying the connectivity between estuaries and marine fisheries we can attempt to answer some important questions facing those responsible for managing coastal habitats and fisheries. For example, how important are estuaries for maintaining commercially important fish stocks? Or, more specifically, what proportion of an adult stock used an estuary as a nursery habitat? The production sources of the energy (i.e. terrestrial, estuarine or marine) and their representation through the food chain is another important issue facing fisheries managers and conservationists. Similarly, if anthropogenic nutrients from a terrestrial source have significant impacts on fish stocks, it is important to identify and locate the sources of these nutrient discharges. Hjerne and Hansson (2002) noted that fisheries remove large quantities of nutrients from the oceans. This raises an interesting perspective on anthropogenic nutrient discharges: are fisheries helping to dampen the deleterious effects that these discharges could have?

1.2 The nursery role concept

Numerous studies report high numbers of juvenile fish and invertebrates in shallow-water biotopes (e.g. Riley *et al.*, 1986; Connolly, 1994; Potter *et al.*, 1997; Dann *et al.*, 2002). Juveniles of species benefiting from these biotopes can be split into three categories: (1) permanent residents, (2) transient visitors and (3) nursery species spending only juvenile life-stages there (Hemminga and Duarte, 2000; Jennings *et al.*, 2001). Hypotheses explaining the high abundance of juveniles in these biotopes compared to adult habitats are based on predator avoidance, reduced predator efficiency, food abundance and larval interception (Elliott *et al.*, 1990; Nagelkerken et al., 2000a,b). These factors, and the assumption that the juvenile habitat holds advantages offsetting the predation risk and energy expenditure of ontogenetic migrations between juvenile and adult habitat (Adams and Ebersole, 2002), form the basis of the nursery-role concept. Firstly, because large predator access is often limited to times of high tide and predator habitats are far away, predatory activity is reduced (Nagelkerken et al., 2000a). Also, the physical complexity of coastal habitats such as mangroves, sea grasses and kelps reduce predator efficiency (Gotceitas et al., 1997; Laegdsgaard and Johnson, 2001). This is also true of turbid estuarine waters (Baran and Hambrey, 1998; Nagelkerken et al., 2000b). As the early life stages of most marine organisms experience extremely high mortality, reduction of this by inhabiting areas of low predator density could have an important bearing on population size. Coastal habitats, and estuaries in particular, are highly productive, providing juvenile fishes with abundant food and resulting in elevated growth rates (Baran & Hambrey, 1998). Large sheltered estuaries such as the Thames Estuary and Dutch Waddensea are thought to support particularly large numbers of juvenile fishes, consuming highly productive invertebrate prey such as polychaetes and copepods (Rogers *et al.*, 1998b). Furthermore, shallow-water biotopes are more likely to intercept and facilitate settlement of planktonic larvae than deeper offshore habitat (Nagelkerken et al., 2000a,b), making them more likely to have large juvenile populations. The use of estuaries as thermal resources is also an important consideration, since higher temperatures are expected to lead to faster juvenile growth rates. Attrill and Power (2004) present evidence raising the importance of estuaries as a thermal resource, in some cases over and above benefits relating to food resources. Factors controlling all these benefits are, however,

inextricably linked to hydrographic, climatic and biological drivers. As these drivers experience considerable spatial and temporal variation at multiple scales, the ability to generalise nursery habitat benefits is limited.

Although the presence of large numbers of juveniles in an area may be indicative of a nursery ground, such evidence is not unequivocal as it does not demonstrate the importance of that habitat to the adult population. Beck *et al.* (2001) defined the nursery habitat with this connectivity to adult populations in mind: "A habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur".

This definition may, however, misinterpret the importance of some habitats for production of juveniles. Dahlgren *et al.* (2006) discuss hypothetical scenarios where large habitats with low production per unit area would not be considered nursery habitat compared to smaller but highly productive habitats (per unit area), despite a greater overall contribution to productivity from the larger habitat. Dahlgren *et al.* (2006) introduced the concept of Effective Juvenile Habitat (EJH), which is classified on the basis of greater than average overall contribution to adult populations.

The evaluation of the importance of particular habitats for the production of juveniles of marine fishes, and their respective contributions to adult populations, is also influenced by temporal variation in estuaries. Different year-classes recruit to and from habitats with different strengths, reflecting variation in density-dependent and density–independent processes. Consequently, the year-classes included in analyses influence the evaluation of nursery habitat and EJH (Dahlgren *et al.*, 2006). The scale at which habitats are assessed is also an important consideration. In many cases, use of one of the above definitions to evaluate sub-habitats within estuaries (e.g. subtidal and intertidal soft substrata and hard substrata, seagrass, saltmarsh), may be appropriate in order to prioritise conservation efforts. A species will often have an affinity for a particular habitat type, saturating it until the benefits are outweighed by density-dependent processes. Other species, however, may exhibit greater plasticity in their life-history and utilise a wide range of habitats which should all be included in conservation and management initiatives. Plasticity may also be seen on a larger scale, such that juveniles of marine fishes may or may not utilise estuarine habitat. In these circumstances it is beneficial to consider the relative

importance of habitats, rather than to focus efforts solely on the habitat that makes a greater than average contribution to adult populations. Furthermore, plasticity may occur at both the individual and population levels. Individual level plasticity in the utility of resources and habitats may occur in highly mobile and opportunistic fishes, while population level plasticity describes distinct behaviours between groups of fish, but not between individuals within the respective groups.

1.3 Marine fishes in estuaries in NW Europe

Quantitative evidence of connectivity between estuaries and offshore adult populations and commercial landings are not common place. However, there is extensive evidence of use of estuarine habitat by the juveniles of commercially important fishes in North-Western Europe. Elliott *et al.* (1990), for example, identified species using the Forth estuary as a nursery and over-wintering habitat, taking particular note of the gadoids whiting (*Merlangius merlangus)* and cod (*Gadus morhua*), the flatfish plaice (*Pleuronectes platessa*) and common dab (*Limanda limanda*), and the clupeids herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). In the middle Thames estuary, seasonal abundance peaks have been observed for juveniles of whiting, bass (*Dicentrarchus labrax*), plaice, dab, herring, sprat, poor cod (*Trisopterus minutus*) and flounder (*Platichtys flesus*) (Araújo *et al.*, 2000). Many of the studies that have reported large abundances of juveniles of commercially important fishes in inshore habitats were designed to reveal how such species have and will respond to various environmental variables (e.g. Rogers and Millner, 1996; Power *et al.*, 2000a,b).

Both the Severn and the Thames estuaries in England have been closely linked with urban and industrial growth during the 1950s and '60s. Consequently, these estuaries were subject to heavy pollution, causing fish populations to slump. These populations have shown some recovery in the last few decades, probably due to the introduction of water treatment facilities (Araújo *et al.*, 2000; Power *et al.*, 2000b; Potter *et al.*, 2001; Attrill and Power, 2004). However, in the last 5 to 10 years there has been a declining trend for some species (e.g. flounder, plaice, cod) (Rogers *et al.*, 1998b; Dann *et al.*, 2002), potentially due to elevated fishing pressure on spawning adults.

Pihl *et al.* (2002) accumulated data from numerous sources in order to summarise the presence of fish in various habitats within estuaries across Europe. While soft-substratum is often revealed as a habitat frequented by fishes (particularly in the Baltic and Boreal/NW Atlantic regions), Pihl *et al.* (2002) describe the use of other habitats, including hard substratum, reed-beds, salt-marsh, seagrass beds and biogenic reefs.

In tropical waters, the rapid and visible destruction of estuarine and mangrove habitat during coastal development has received extensive coverage. The loss of these habitats is assumed to have a deleterious effect on offshore fisheries due to the extensive use of these habitats by the young of commercial species. As such, strong correlations have been found between total mangrove area and commercial shrimp catch off parts of Australia, Malaysia and Indonesia (Chong *et al.*, 1990; Mann, 2000). The adult shrimp distribution often mirrors the geographic extent of mangroves (Hogarth, 1999), and in the Indus Delta of Pakistan the loss of mangroves due to the construction of a major dam in 1995 was linked to a slump in offshore shrimp fisheries (Hogarth, 1999). Mangroves have also been found to support offshore stocks of spiny lobsters (*Panulirus argus*) in the Caribbean and fishes such as mullet (*Mugil* spp.), barramundi (*Lates calcarifer*) and mackerel (*Rastrelliger kanagurata*) (Gopinath and Gabriel, 1997; Nyabakken, 2001).

Temperate seagrass systems are also considered important nursery grounds. Newly recruited Atlantic cod (*Gadus morhua*) on the Newfoundland coast of Canada can be found almost exclusively in eelgrass beds (*Zostera marina*) (Gotceitas *et al.*, 1997). This is consistent with reports from other parts of the West Atlantic and Scandinavia. Correlative studies showed declines in juvenile cod with reduction of eelgrass habitat, and higher growth rates in eelgrass than out of it (Gotceitas *et al.*, 1997). In Australia, seagrass beds are believed to be important nursery habitats for commercially important penaeid shrimp, King George whiting (*Sillaginodes punctatus*) and the multimillion dollar fishery of the lobster *Panulinus cygnus* (Connolly, 1994; Heminga and Duarte, 2000; Mann, 2000). In the USA, the state of the blue crab (*Callinectes sapidus*) fishery, which was worth US$101million in 1989 alone, relies on annual recruitment of juveniles to beds of *Z. marina* (Perkins-Visser *et al*, 1996).

Positive correlations have also been found between commercial fish catches and the use of estuaries for estuarine biotopes such as mudflats and salt marshes

(Barnes and Hughes, 1998; Hogarth, 1999; Nagelkerken *et al.*, 2001). In 1961, 97% of the biomass of US fisheries in the Gulf of Mexico relied upon species using estuarine biotopes as nurseries. Despite development of deepwater and pelagic fisheries, estuarine biotopes still played an important role in 1990, supporting 50% of commercial landings (Baran and Hambrey, 1998). Similar statistics exist for Australia and South Africa. Shallow coral reefs (Nagelkerken *et al.*, 2000a), boulder reefs (Nagelkerken *et al.*, 2000b), kelp beds (Mann, 2000) and sheltered rocky coasts (Henriques and Almada, 1998) are also thought to be nursery habitats for some fisheries species.

Evidently, estuaries and their sub-habitats provide habitat for large numbers of juvenile fish. However, these studies are mostly correlative rather than mechanistic, describing relationships but not their function. In NW Europe, there has been very little research investigating the connectivity between these nursery grounds and the offshore adult populations of fishes that are available to fisheries. Quantification of the importance of estuaries and the material transported from them to the adult stocks would support and direct effective fisheries and estuarine management activities.

1.4 Studying fish movement and habitat connectivity

For many years the only convincing evidence for movement of fishes between estuaries and offshore habitats was through the use of artificial tags and mark-recapture methods (e.g. Wallace and Watson, 1980; Pawson and Eaton, 1999) (see Gillanders *et al.*, 2003 for review). Jennings *et al.* (2001) and Gillanders *et al.* (2003) provide summaries of the history of techniques used, and evidence accumulated, in tracing fish movements and migratory behaviours. Pickett and Pawson (1994) provide a review of artificial tagging research for European sea bass. However, the attachment of artificial tags on small juvenile fish can be problematic and the rate of tag return makes it difficult and expensive to generate sufficient data to show convincing patterns (Gillanders & Kingsford, 2000; Gillanders, 2002b). Data storage tags that record measurements on depth, temperature and geographic location have revealed interesting migratory behaviours (e.g. Stensholt, 2001; Hunter *et al.*, 2005). However, these are particularly expensive and studies using them have inherently small sample sizes. Recently, however, there have been considerable advances in the use of naturally occurring biomarkers. In particular, the elemental and isotopic chemistry of

hard parts of fishes (e.g. otoliths, scales, vertebrae) is becoming an established technique for tracing migratory patterns, as is the use of stable isotope analysis for revealing food web structure and the feeding habits of organisms. These natural 'tags' are a key tool for comprehensive studies of habitat use and the transport of energy resources.

1.4.1 Stable isotope analysis of soft tissues

Stable isotopes are forms of an element that have different atomic masses (due to the number of neutrons) and do not undergo radio-active decay. Isotopic composition is expressed in terms of δ (delta) values, which are parts per thousand (‰) differences from a standard reference material (Peterson and Fry, 1987) and expressed as the ratio of heavy-to-light isotopes (i.e. $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$):

$$\delta\ (‰) = [(R_{SAMPLE} - R_{STANDARD}) / R_{STANDARD}] \times 1000$$

where R_{SAMPLE} is the isotope ratio of the sample, and $R_{STANDARD}$ is the isotope ratio of the standard. Standard reference materials include carbon in the Pee Dee limestone, nitrogen gas in the atmosphere and sulphur from Canyon Diablo meteorite (Peterson and Fry, 1987). Larger δ values denote an increase in the abundance of the heavy isotope relative to the light isotope. Such samples are referred to as 'enriched' or 'heavier', as oppose to samples with less of the heavy isotope, which are referred to as 'depleted' or 'lighter' (Lajtha and Marshall, 1994).

The ratio of stable isotopes in the natural environment varies spatially as a result of chemical and biological processes (see Owens, 1987; Peterson and Fry, 1987). Different isotopes of the same element take part in the same reactions, but because of their mass differences they react at different rates, resulting in isotopic ratios that are enriched or depleted, relative to their precursor. Whilst the potentially informative content of molecules becomes scrambled by chemical and metabolic processes, isotopic variations are often preserved (Fry and Sherr, 1984). Diet has been shown to be a key determinant of the isotopic composition of animal soft tissues (Peterson and Fry, 1987). The length of time for which information is retained in the tissues of living organisms depends upon the elemental turnover rates of the tissue (Hobson, 1999), thus allowing the researcher to put a time scale on feeding activities.

Ecologists now widely use stable isotopes to provide clues regarding the origins and transformations of organic matter (Fry and Sherr, 1984). Stable isotopes enable the estimation of trophic position and food sources while overcoming the limitations of stomach content analysis. For example, partly digested prey from stomach samples only provide a snapshot of the diet and are often difficult to identify. Also, there is no way to determine how much of the consumed material would actually have been assimilated (Doucett *et al.*, 1999; Polunin *et al.*, 2001). Tracing origins and migrations of fishes is possible for animals that move between isotopically distinct food webs, which usually exist in distinct geographic regions (Hobson, 1999).

The most useful and commonly analysed stable isotopes are those of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$. Although the extent of trophic fractionation is a matter of debate (see McCutchan *et al.*, 2003), ^{13}C is relatively weakly enriched with increasing trophic level and has consequently been used frequently as an indicator of food sources (Stephenson and Lyon, 1982; Riera and Richard, 1996; Jennings *et al.*, 1997). Differences in the $\delta^{13}C$ of primary producing organisms occur during the fixation of carbon by photosynthesis (Stephenson and Lyon, 1982). The $\delta^{13}C$ values of particular types of primary producing organisms can vary between geographic regions due to variation in synthesis processes (i.e. C_3 vs C_4; planktonic Vs benthic; terrestrial Vs marine), thus allowing the relative importance of different primary production sources to higher trophic levels to be established. For example, Fry (1981) surveyed the $\delta^{13}C$ of populations of Texas brown shrimp (*Panaeus aztecus*) off the Texan coast, revealing that they have four isotopically distinct prey sources. These were then matched to four geographically distinct shrimp feeding grounds, for which the relative contribution to offshore shrimp fisheries could then be estimated.

$\delta^{15}N$ usually becomes enriched by approximately 3‰ relative to a prey item (i.e. per trophic level) (Post, 2002). Consequently, $\delta^{15}N$ values have been used regularly to define the trophic positions of aquatic organisms, helping to decipher food webs in systems where feeding relationships are unknown (Peterson and Fry, 1987; Jennings *et al.*, 1997). For example, Griffin and Valiella (2001) used $\delta^{15}N$ to study the variation in trophic position of common killifish (*Fundulus heteroclitus*) and Atlantic silverside (*Menidia menidia*) between estuarine and offshore habitats. Although the range of values is fairly narrow there can be an apparent increase in mean $\delta^{15}N$ values along the gradient of atmosphere-terrestrial-freshwater-estuarine-marine (Owens, 1987). A more recent study identified the spatial variation in the $\delta^{15}N$

of some phytoplankton-feeding bivalve molluscs (queen scallops *Aequipecten opercularis*), and therefore of the phytoplankton itself (Jennings and Warr, 2003). This was found to be correlated with environmental variables such as salinity, depth and temperature. In turn, this led to the development of a linear model, which could predict and map large-scale spatial patterns in $\delta^{15}N$ for scallops, particularly in coastal and estuarine areas where these environmental gradients are more pronounced. France (1994) compiled data from the literature to show that marine invertebrates are enriched in $\delta^{15}N$ relative to their freshwater counterparts, while the estuarine varieties had intermediate $\delta^{15}N$ values. The same pattern was found for fishes across freshwater, estuarine and marine habitats (France, 1995a). Consequently, $\delta^{15}N$ values can support conclusions on continental-marine coupling made by traditional $\delta^{13}C$ analysis. Despite some potentially useful applications for stable isotopes of nitrogen in isolation, it is generally considered that the analysis of several stable isotopes together provides a more thorough analysis and helps avoid the ambiguities which are common in single isotope studies.

Like ^{13}C, ^{34}S exists in relatively similar composition in animals to their prey item (Peterson and Fry, 1987). Stable isotopes of sulphur are useful in conjunction with carbon and nitrogen isotopes, providing an additional tool in studies of energy flow, further eliminating ambiguities associated with studies analysing fewer isotopes (Peterson *et al.*, 1985). Fry (1983) studied the migrations of fish and shrimp in the Gulf of Mexico using stable isotopes of carbon, nitrogen and sulphur. Hesslein *et al.* (1991) found that $\delta^{34}S$, in combination with $\delta^{13}C$ and $\delta^{15}N$, was a valuable tool in the interpretation of food-webs leading to the production of fish in the Mackenzie River (Canada). Similarly, Newell *et al.* (1995) used stable isotopes of C, N and S together, in order to improve the precision with which the relative importance of various primary production sources for panaeid prawn nutrition could be determined.

1.4.2 Otolith chemistry

Otoliths, commonly referred to as the 'ear stones', are present in pairs in all teleost fish, being used for balance and hearing. There are three pairs of otoliths in all teleosts: sagittal, lapilli and asteriscii. Sagittal otoliths, being the largest, are the most commonly used in scientific research. Otoliths are composed largely of calcium carbonate in a non-collagenous organic matrix (Campana, 1999; Wright *et al.*, 2002).

Concentric layers of material are deposited on a regular basis throughout life, with these growths corresponding to daily, seasonal and annual increments (Elsdon and Gillanders, 2002). For fisheries biologists, the interest in otoliths stemmed from these chronological properties, allowing fish to be aged for population studies. In many species the annual growth increments are more accurate and precise than those of scales and vertebrae. This is a consequence of the metabolically inert and acellular properties of otoliths (Campana, 1999). Otoliths are metabolically inert because they are non-skeletal, being isolated by the semi-permeable inner ear membrane and suspended in endolymphatic fluid. Therefore, unlike vertebrae, their growth is not inhibited during periods of non-feeding or poor body-growth, allowing reliable growth increments (Campana, 1999; Campana and Thorrold, 2001; Thorrold *et al.*, 2001). Fish scales exhibit poor growth rates during such conditions, resulting in a non-continuous growth pattern. In addition, the elemental signature of some scales degrades over time, limiting their suitability for studies of natal origin (Wells *et al.*, 2003). The acellular nature of the bio-mineralization process of otoliths layers means that, once deposited, they are unlikely to be resorbed or reworked. This allows a complete growth record to exist, unlike any other calcified structures, all of which can be resorbed during periods of limited feeding (Campana, 1999; Campana and Thorrold, 2001; Thorrold *et al.*, 2001). The width of the increments can differ due to variation in the accretion rate of otolith material. Research continues as to the cause of this variation, though links with temperature and metabolism are common (for further details refer to Secor *et al.*, 1995; Fossum *et al.*, 2000; Wright *et al.*, 2002).

The endolymphatic fluid contains elements and compounds that have been extracted from the surrounding water mass (Campana and Thorrold, 2001). From this endolymphatic fluid, the aragonite form of calcium carbonate crystallises on to the otolith's surface, and ions of various elements substitute for calcium or bind to proteins in the organic matrix (Dove and Kingsford, 1998; de Pontual and Geffen, 2002). This, in combination with the inert and acellular properties of otoliths, facilitates the use of otoliths to study migration patterns of fishes (Campana, 1999). Being able to accurately identify growth rings from particular stages in a fishes life history means that the chemistry of the otoliths at these particular loci can be analysed. If particular water bodies, or regions along an aquatic gradient, are sufficiently distinct from one another in their chemistry, then the movement of a fish over time can be established.

The ratio of strontium to calcium (Sr:Ca) in the otoliths of fishes has often been correlated with salinity gradients in water masses, proving very useful for tracing the environmental history of fishes (Secor and Rooker, 2000; Kraus and Secor, 2004). For example, Tzeng *et al.* (2002) studied the migratory behaviour of Japanese eels (*Anguilla japonica*) between freshwater, brackish and marine environments using Sr:Ca of otoliths. Similarly, Limburg (1995; 1996) traced the movement of American shad (*Alosa sapidissima*) from the freshwater reaches of the Hudson River to its outer estuary. Sr:Ca ratios of otoliths have also proved useful in investigations into the anadromous migrations of striped bass (*Morone saxatilis*) in rivers and estuaries of the north-eastern USA and Canada (e.g. Secor, 1992; Secor and Piccoli, 1996).

Although otolith strontium has been used as an indicator of water salinity, elucidating patterns of habitat use and migration for many species, many other elements are incorporated into otoliths from surrounding water, and may record more subtle environmental gradients (Secor *et al.*, 2001). Some elements (e.g. Al, Ba, Mn, Pb, Co and Zn) occur naturally in freshwater runoff and so will be in higher concentrations in otoliths of fish inhabiting estuaries than offshore sites (Gillanders and Kingsford, 1996). Various contaminants also tend to be found in higher concentrations in rivers and estuaries, due to the favoured locations of industrial facilities and urban developments. Forrester and Swearer (2002) identified that the water in bays of Southern California holds a greater concentration of Cu and Pb than that of the open coast. This pattern is reflected in the otoliths of California halibut (*Paralichthys californicus*) and allowed a comparison of the use of nursery habitats in bays versus those on the open-coast. Furthermore, rivers or groups of rivers draining land of particular soil and geology types will likely contain different elements than those from different geological regions. Some studies have therefore attempted to simultaneously assay many elements in otoliths, hoping to improve resolution in descriptions of migratory movements and habitat use. Multi-elemental approaches have been praised regularly in the literature (e.g. Gillanders & Kingsford, 2000; Elsdon & Gillanders, 2002), improving interpretation and reducing error rates in analyses, allowing the complex life-histories, including plasticity of migratory behaviours, to be revealed.

Gillanders and Kingsford (2000) analysed the elemental fingerprints of juvenile trumpeter (*Pelates sexlineatus*) collected from several sites within each of

seven estuaries in New South Wales, Australia, allowing adults to be reclassified to their nursery habitats at both within- and between- estuary scales. Juvenile sampling took place over two successive years, so as to test for temporal variation. However, the variation in elemental fingerprints between years, and therefore water chemistry, means that discriminant functions must be generated from the appropriate year class of recruits. Water chemistry is likely to vary between years as a consequence of rainfall patterns, point-source and non-point source pollution. Recent work has revealed the benefits in considering both within-year (Elsdon and Gillanders, 2006) and between-year (Gillanders, 2002a) variation in otolith chemistry. Gillanders (2002a) carried out an extensive study of the implications of temporal and spatial variability in elemental composition of otoliths, determining that if ignored temporal differences could confound spatial differences. Variation was found for some elements over time periods of less than a year, but these variations were very small compared to between-year differences. Hamer *et al.* (2003) also revealed temporal variation in natural chemical tags, finding that between-year variation was sufficient to confound spatial discrimination between sites but that shorter-term variation (between-months) had a negligible effect on the accuracy of spatial discrimination.

Isotopes of strontium in otoliths have also been used as fish markers. Kennedy *et al.* (1997; 2000; 2002) used the $^{87}Sr/^{86}Sr$ ratio of calcified tissues of Atlantic salmon (*Salmo salar*) from the Connecticut River, USA, to differentiate between populations and life-cycle stages. Different populations can be identified by their association with a particular branch of the river. The strontium isotope ratios in the river branches were defined by the age and composition of soil and rocks in the watershed, demonstrating sufficient variation to leave distinguishable signatures in otoliths (Kennedy *et al.*, 2000). Clearly, isotopes of strontium hold potential as indicators of natal origin, although there is some uncertainty as to how effective use of these isotopes can be, due to issues concerning the range of salinities across which differences in the otoliths can be easily detected (Chesney *et al.*, 1998; Kennedy *et al.*, 2000). However, because of the heavy nature of strontium isotopes, they do not fractionate significantly, meaning that the ratios differ little between source and otolith signal. Even more beneficial, however, is the fact that strontium isotope ratios do not suffer from any major physiological or environmental effect, the primary cause of scepticism in the use of elemental signatures (Kennedy *et al.*, 2000).

Stable isotope ratios of carbon and oxygen in otoliths have also been used in conjunction with elemental data to reveal information on environmental conditions at the time of deposition. Isotopes of oxygen can be used to trace temperature conditions (Radtke *et al.*, 1996; Edmonds *et al.*, 1999; Høie *et al.*, 2003), while carbon isotopes, although not necessarily representative of environmental conditions, can be indicative of fish growth and condition at particular life-stages (Thorrold *et al.*, 1998; Høie *et al.*, 2003). These indicators can often be related to geographic position and may help to refine and/or support information on the migratory habits of fishes. Although most commonly known for reconstruction of temperature histories, Thorrold *et al.* (1998) also found that oxygen isotopes were negatively correlated to salinity, as well as being influenced by a latitudinal gradient. This helped to reduce the overall error in classification of weakfish (*Cynoscion regalis*) to natal estuaries by between 19% and 27%. However, compared to elemental research, isotope studies of otoliths have been less successful. Further technological developments and advances in our understanding of the behaviour of isotopes will aid in the success of these techniques.

1.5 Aims and Hypotheses

The aim of this research is to quantify inhabitation and feeding in the estuaries of the North Sea by marine fishes. With the Thames estuary acting as a proxy for North Sea estuaries, the use of otolith chemistry techniques and stable isotope analysis of soft tissues may be used independently to quantify use of estuaries by marine juvenile fishes. However, coupling of these techniques (Herzka, 2005) may improve data interpretation. Not only can the inhabitancy of the estuary be quantified, but the extent to which fish use the estuary as a feeding ground during this period, and the representation of these estuarine energy resources in coastal fish stocks, can be investigated. Study species are common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and whiting (*Merlangius merlangus*), thus considering several commercially important taxa.

The specific aims and hypotheses of each results chapter are outlined below. Chapter 2 describes general methodologies used in sample collection that are relevant to all later results chapters. Chapter 3 describes and discusses the development of some specific methodologies used in this research, providing important background

information for the understanding of later analytical processes. Chapter 7 provides a synthesis of the results chapters, considering the implications of coupling the stable isotope and otolith microchemistry techniques and drawing conclusions with reference to the management implications of this research.

Chapter 4: Variation in stable isotope signatures of the invertebrate prey of fishes.

Hypothesis: *Invertebrates will show stable isotope signatures indicative of their position along the estuarine-marine gradient.*

Chapter 5: Quantification of estuarine feeding activity by marine fishes.

Hypothesis 1: *Juvenile fishes show stable isotope signatures indicative of feeding along the estuarine-coastal gradient.*

Hypothesis 2: *Many adult fishes reveal stable isotope signals indicative of estuarine feeding.*

Chapter 6: Otolith chemistry of juvenile and adult fishes.

Hypothesis 1: *Juvenile fishes show otolith chemistry signatures indicative of their position along the estuarine-marine gradient.*

Hypothesis 2: *Many adult fishes reveal otolith chemistries indicative of estuarine residency during the juvenile phase.*

2. General Materials and Methods

2.1 Study Species

Reports by CEFAS (The Centre for Environment, Fisheries and Aquaculture Science) and MAFF (Ministry of Agriculture, Fisheries and Food – now DEFRA) give an indication of the extent of fish surveys in estuaries and other coastal habitats in the UK. Since the 1960's, CEFAS (Centre for Environment, Fisheries and Aquaculture Science) have surveyed young fish populations around the coast of England, dividing the coast into sectors based around major geographical features likely to be important nursery habitats, such as bays and estuaries (Rogers *et al.*, 1998a; Dann *et al.*, 2002). Juveniles of many marine species have been caught in bays and estuaries, including common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and whiting (*Merlangius merlangus*). Pawson (1995) describes the biogeographical distribution and general biology of fish and shellfish stocks in the English Channel, including use of estuarine nursery habitats by juveniles of marine fishes. Most of these species arrive as post-larvae or fry and are fairly uncommon as second-year or older fish (Colclough, 2001).

Based on these reports I have identified three species that (i) widely use estuaries, (ii) use the Thames Estuary, and (iii) are important in commercial fisheries. These were chosen as subjects for further investigation.

2.1.1 European sea bass (*Dicentrarchus labrax*)

Popular with both commercial and recreational fisheries, adult bass (*Dicentrarchus labrax*) may be found from shallow coastal waters to approximately 100m depth, moving offshore during the winter. Offshore spring spawning is followed by post-larvae entering estuaries between June and August (Costa *et al.*, 2002), and are often abundant as 0+ fish as far upstream as the freshwater extent of rivers. Bass larvae are thought to respond to environmental cues when they reach about 15mm in length, actively swimming into estuarine nursery habitats from June onwards (Jennings and Pawson, 1992; Pickett and Pawson, 1994). Although in progressively lesser abundance beyond the 0+ group, many bass remain in estuaries during their first and second years, and in the summer months may be found in

estuaries as 4+ and occasionally 5+ fish (Claridge and Potter, 1983; Kelley, 1988). Bass are a truly euryhaline species, tolerant of freshwater conditions at any age (Pickett and Pawson, 1994). Adult *D. labrax* feed mainly on small fishes, while juveniles more than 3cm total length (TL) consume worms, crustaceans and fish larva. Previous studies have revealed shrimp, prawns and various polychaete worm species to feature heavily in the diet of juvenile and sub-adult bass (Pickett and Pawson, 1994).

The relative abundance of bass in the Thames Estuary is high, being the fifth most abundant fish species between 1985 and 1991 (Thomas, 1998). Also, over this period, the abundance of bass appearing on sampling screens at West Thurrock power station increased more than three-fold (Thomas, 1998).

2.1.2 Common Sole (*Solea solea*)

An important commercial species, sole (*Solea solea*) are also targeted by some recreational fishers. Although they do not travel as far upriver as bass, most sole recruits are first found in estuaries and coastal nursery grounds between May and August (Coggan and Dando, 1988; Thomas, 1998; Costa *et al.*, 2002). Unlike most other marine species in temperate estuaries, sole tend to leave estuaries during the winter months, returning in early spring (Thomas, 1998; Power *et al.*, 2000b). Most, but not all, of these individuals move to inhabit coastal areas before their first birthday. Sole nurseries have been identified in estuaries, tidal inlets and shallow sandy bays on both the English and continental coasts of the English Channel. In the Tamar estuary sole were found to stay in deeper estuarine water during their first year, moving to shallower tidal areas in their second year (Pawson, 1995). As demersal flatfish, sole feed primarily on worms, small crustaceans and molluscs (Molinero and Flos, 1991; Cabral, 2000; Amara *et al.*, 2001; Darnaude, 2005). Despite the commercial importance of sole in the North Sea, biological production is relatively low at less than 0.06 $gWWm^{-2}yr^{-1}$ (WW = wet-weight), in both estuarine and open-sea habitats (Costa *et al.*, 2002).

Sole were uncommon in the Thames Estuary during the 1970s, due to the poor water quality. Their numbers have since increased, however, and were found to be the seventh most abundant fish species in the Thames between 1985 and 1991 (Thomas, 1998).

2.1.3 Whiting (*Merlangius merlangus*)

A bentho-pelagic predator with an important commercial fishery and occasional recreational fishery, whiting (*Merlangius merlangus*) has a varied carnivorous diet, including polychaetes and a large quantity of crangonid shrimp (Greenstreet, 1995; Singh-Renton and Bromley, 1999). In the North Sea, whiting spawn from January through to May. 0-group whiting are documented as having an affinity for estuaries, adopting a demersal existence at shallow inshore sites throughout the English Channel by September (Pawson, 1995). In the Forth estuary, biological production of whiting has been calculated as varying between 0.3 and 0.9 $gWWm^{-2}yr^{-1}$ (Costa *et al.*, 2002). They are often abundant in inshore sites during the autumn and winter, then leaving during the first quarter, though they tend not to travel as far up the estuary as bass (Power *et al.*, 2002).

Whiting are the most common gadoid in the Thames Estuary and the sixth most abundant fish species between 1985 and 1991 (Thomas, 1998). Whiting abundances can be linked to seasonal factors and temperature, but are also limited by the range of salinities they can tolerate (Power *et al.*, 2002).

2.2 Study Area

2.2.1 The North Sea

The North Sea is a relatively shallow (mean depth 90m) semi-enclosed sea on the continental shelf of NW Europe. With a catchment area of 8,415,000 km^2, stretching across many urbanised and industrialised nations, the 296-354km^3yr^{-1} of freshwater runoff (Ducrotoy *et al.*, 2000; OSPAR, 2000) is expected to have a considerable impact on ecosystem dynamics.

The location of the North Sea has resulted in a history of heavy fishing activity. It has experienced increasingly intensive exploitation by the European fishing community ever since the advent of the commercial harvesting of fishes. The North Sea now holds the world's most active fisheries, accounting for 30-40% of NE Atlantic commercial landings (Ducrotoy *et al.*, 2000). Presently, more detailed surveys and a better understanding of stock dynamics and fish biology are bringing to

light both the direct and indirect influences mankind has on the fish populations of the North Sea.

2.2.2 The Thames Estuary

The Thames estuary (Figure 2.1) is a large turbid estuary of approximately 99.7 km^2, including 60.1 km^2 of intertidal area (Pihl *et al.*, 2002), the majority of which is soft substratum, with only 0.2% and 4.73% intertidal hard substratum and saltmarsh, respectively (Pihl *et al.*, 2002). The Thames estuary extends from the suburbs of west London, through densely populated regions of central London and heavily industrialised regions of North Kent and South Essex, and on to relatively natural seascape on its outer margins. Although the undesirable effects of human impact are still evident in many places, the Thames estuary has shown impressive recovery from very heavy pollution in the 1950s and '60s. Tinsley (1998) provides a detailed history of the impact of humans on the Thames estuary. Since the 1970s there has been a particular effort to monitor its fish populations and various environmental factors on a routine basis. Colclough *et al.*, (2000) and Colclough (2001) summarise population and environmental monitoring programs over the last few decades. Currently, in the tidal reaches of the Thames estuary, the Environment Agency regularly collects size and frequency distribution data on the fishes present (e.g. Colclough *et al.*, 2000; Colclough, 2001). Species commonly found in the tidal reaches of the estuary include whiting, sprat, herring, sole, flounder, bass, plaice, smelt, cod and dab (Colclough, 2001). Overall, 110 species of fish have been found in the Thames estuary, including 16 of commercial value (Pihl *et al.*, 2002). It is estimated that 19.1% of the fish species found in the Thames utilise it as nursery habitat (Pihl *et al.*, 2002). Consequently, the Thames estuary is considered a very important nursery ground for such species.

Other large estuaries around the North Sea such as the Oostershelde, Westershelde, Elbe and Forth estuaries are also important nursery grounds. However, practical, logistic and economic limitations make the sampling of a large number of estuaries impractical. Thus, while stable isotopes and otolith chemistry techniques have been used elsewhere to discriminate between estuaries (e.g. Gillanders and Kingsford, 2000; Gillanders, 2002a,b), the methodology used here aims to use the Thames Estuary as a proxy. It is hoped that the generality of the isotopes and

elements used in the analysis allows data from the Thames to act as a suitably robust representative of estuaries in the Southern North Sea, and possibly the Greater North Sea region.

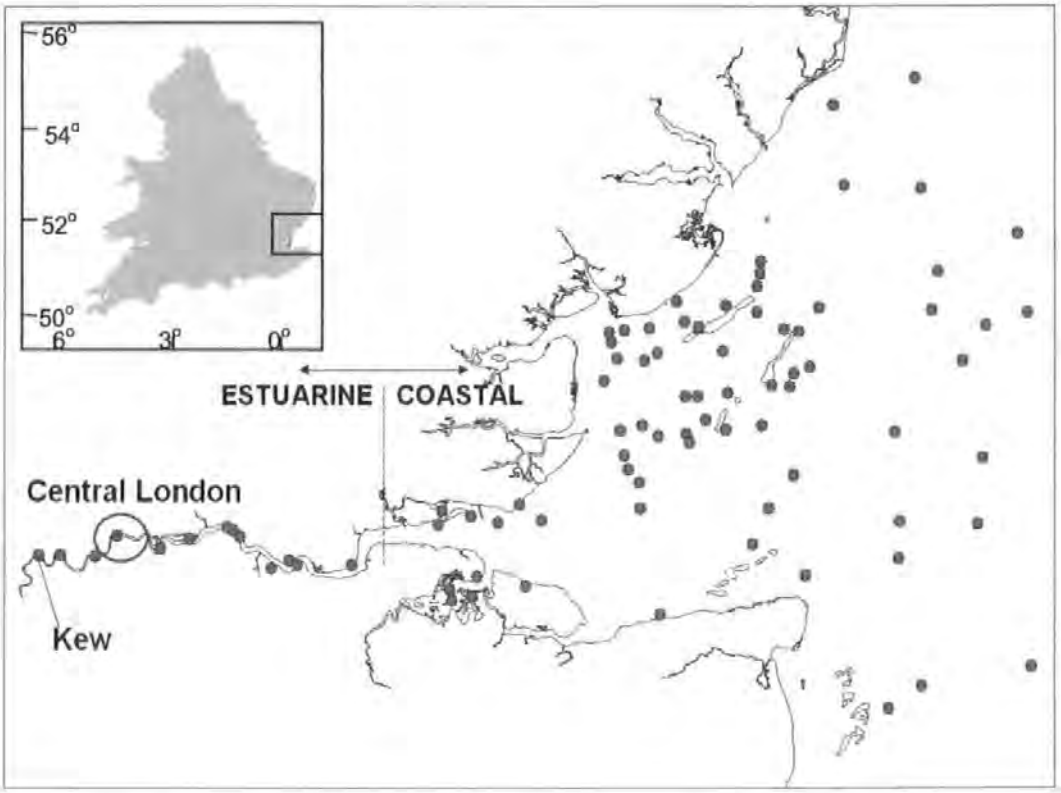


Figure 2.1 The Thames Estuary and adjacent coastal area, showing estuarine-coastal division as classified for this study (see Section 2.3.2). Dots show sampling locations.

2.3 Sampling design and methodology

Sampling efforts targeted sites and specimens needed to support the objectives of the study, with the intention of creating five data sets: (1) variation in the stable isotope signals ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of low trophic level invertebrates (i.e. likely prey items of juvenile fishes) along the estuarine-coastal gradient; (2) for young-of the-year bass, sole and whiting, the stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in the muscle tissue of fish caught along the estuarine-marine gradient; (3) for adult and sub-adult bass, sole and whiting caught in coastal and offshore regions, the stable

isotope signatures ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in the muscle tissue of individuals; (4) for bass, sole and whiting, the spatial variation in elemental signatures of otoliths from young-of-the-year fishes caught along the estuarine-marine gradient; (5) for bass, sole and whiting adults and sub-adults caught in coastal and offshore regions (i.e. available to commercial fisheries), the elemental signatures of the juvenile core of their otoliths and recently deposited edge material.

2.3.1 Sampling events, equipment and sample processing.

Sites for collection of samples were selected to give good representation of sites along the estuarine-coastal continuum. These sites ranged from near the salt-water extent of the Thames Estuary at Kew Bridge (salinity <5) to offshore marine sites in the Southern North Sea (Figure 2.1), falling under the divisions of estuarine and coastal regions (see Section 2.3.2). Sample sites are shown in Figure 2.1.

Sites in the estuary were sampled using a variety of techniques. It was not necessary for sampling to be quantitative, so samples were taken wherever sufficient material was available. Specimens of sole, bass, whiting and any available invertebrates were collected. Depending on substratum type, infaunal samples were collected by sieving mud through a 1 mm mesh, or through use of a kick-net. *Nereis diversicolor* was also collected at some sites from under rocks in the intertidal zone. Hand operated push nets were used to sample epifaunal and benthic organisms below the waterline, though some sedentary organisms were hand-collected from adjacent exposed rocks. Where possible, three sediment samples were also collected, scraping the top 1 cm of material to avoid anoxic layers and removing any large fragments of inorganic material. These sampling procedures were repeated across three seasons, in early June and September-October 2004, and March-April 2005. Fish and invertebrates were also collected from the Tilbury, Littlebrook and Barking power stations of the Thames region. The intake screens from the water cooling systems of these sites were inspected and useful material collected. In October 2004, a small beam-trawl and seine-netting was also used at selected sites to collect organisms, operating in conjunction with the Environment Agency's annual surveys. In May 2005, the frames (whole bodied minus fillets) of 28 bass between 355 and 470 mm total length (TL), collected off a beach near Bridlington (East Yorkshire), were donated to the study by a local fisherman.

Within the coastal region immediately adjacent to the Thames estuary (see 2.3.2), 42 sites were sampled from a small commercial fishing trawler (vessel: Fisher Lassie), using a 2 metre beam-trawl (10 minute trawls), chartered by CEFAS for their annual Young Fish Surveys (August 2004). Nine sites in the same area were also sampled as part of the CEFAS/EA Thames bass survey (November 2004) using 20 minute otter trawls (vessel: Ina K). These sampling efforts were mostly successful in the collection of species of shrimp, prawns and juvenile fish.

Other coastal and relatively offshore sites were sampled from the CEFAS vessels Corystes and Endeavour. A grid of 27 sites in the coastal regions adjacent to the outer regions of the Thames estuary was sampled in April 2004. At each of these sites 5 Hamon Grabs were deployed, and organisms sieved in and picked from a mesh of 1 mm aperture. Beam-trawls (2 and 4 metre) were also deployed at each site (10 and 20 minute trawls, respectively) for collection of epifaunal and benthic organisms, particularly sole, whiting and decapod crustaceans. Additional specimens were collected on the CEFAS Beamtrawl and Groundfish (semi-pelagic trawl) Surveys (August 2004).

All samples were bagged according to species, sample site, date of collection, and chilled upon collection. All samples were frozen as soon as possible to avoid any fractionation of the stable isotopes of tissues or change in the elemental composition of otoliths. In the laboratory, specimens were thawed sufficiently for processing. More than 900 fishes, ranging in size from 45 to 470 mm TL, were measured, weighed and, where possible, sexed. All sole older than 0+ were successfully sexed, but whiting and bass sexes could not all be determined so were not distinguished in later analyses. Otoliths were removed, cleaned of any adhering tissue, placed in multi-well plates to dry, and stored for later use (see Chapter 6). A small sample of muscle tissue was taken dorso-ventrally and above the lateral line from each fish, and refrozen in a small clean glass vial, ready for further preparations prior to stable isotope analysis (Chapter 5). Where tissue from one fish was deemed insufficient, tissues from several individuals from the same site were pooled.

Where a pair of otoliths had been successfully removed from a fish, one of these was used for ageing purposes. These otoliths were prepared for ageing at the CEFAS Lowestoft laboratory. They were set in polyester resin and cut using a diamond blade saw to acquire a transverse section through the core of the otolith. For ageing, sole otoliths were stained using a pink dye. The otoliths were aged using a

dissection microscope and double-checked by an experienced otolith reader. The standard method for ageing bass, however, involves the reading of growth rings in the scales. Unfortunately, as the bass used in this study were donated by a fisherman, the fillets and scales had been removed. Age estimates were made according to the total length of fish (compared with growth charts – *pers. comm.* Graham Pickett) and looking at the banding visible in the otoliths. These produced very similar estimates of ages 4 or 5+. As the fish were caught in late May, some reading error is likely as the previous winter's band will have just been deposited in the otolith and consequently difficult to see. Thus, these individuals have been grouped and are referred to as 4/5+ bass.

Due to their small size, the tissue samples from invertebrates were also pooled across multiple individuals. Following consideration of the invertebrates collected in sufficient abundance and with sufficient geographical range to provide a spatial map of isotopic values at low trophic levels, the invertebrates processed were limited to common brown shrimp *Crangon crangon*, the prawns *Palaemon serratus* and *P. longirostris*, and polychaetes of the genera *Nereis*, *Nephtys* and *Aglaophamus*. These species are also recognised as important components in the diets of the fishes studied here (Molinero and Flos, 1991; Greenstreet, 1995; Cabral, 2000; Elliott *et al.*, 2002). For the shrimps and prawns, data were collected on carapace length, prior to the dissection of muscle tissue from their tails, being careful to avoid incorporation of any exoskeletal or gut tissues. Samples were then re-frozen in clean glass vials. Polychaetes were measured and rinsed of sedimentary material, followed by re-freezing of groups of pooled individuals. Amphipod samples and sediment samples were also processed at this stage, but were later excluded from the results due to analytical problems. Altogether, shrimp collected from 53 sites, prawns from 15 sites and polychaetes from 19 sites across seasons were prepared for stable isotope analysis (Chapter 4).

It should be noted that, due to logistical and financial limitations, sampling was not undertaken with the intention of a full food web study. Efforts were focused upon revealing the likely spatial variation in stable isotope signals available to the fishes, and the representation of this variation in their tissues.

2.3.2 Estuarine and coastal divisions

Although samples were collected at a multitude of sites along a large-scale estuarine to marine gradient, for many of the analyses it was necessary to categorise these sites to a particular region. The fact that these regions are not discrete later presents itself as a limitation in some analyses. As noted by Elliott and McLusky (2002), the nature of estuaries as spatial and temporal continua means that any scheme of regional classification is arbitrary and partially subjective. However, it is possible to divide the area of study into regions that make sense primarily in terms of physical processes and geographic features, but with these transcending into biological and geochemical patterns.

The most obvious indicator of physical change along the estuarine-marine gradient is salinity. Mean salinity data collected across all seasons for the last ten years by the Environment Agency was used subjectively to consider regions of relative marine impact on the estuary. East of approximately 51°29'53'N and 0°31'22'E the mean salinity rises above approximately 30. Although much of this eastern area is regularly referred to as 'outer' estuary, and is mostly very shallow (except the shipping channel), this salinity represents a largely marine source with a highly diluted contribution from the freshwater plume. This point also coincides with the geographical feature of the Thames estuary rapidly widening and a switch in the predominant intertidal substratum from soft estuarine mud to a firmer sandy substratum. Consequently, samples collected east of this point are considered 'coastal'. This division meets with the salinity-based classification scheme described by Elliott and McLusky (2002), in which water of salinity >30 is described as the estuary mouth and sea regions.

The estuarine region has been classified west of this point until Kew Bridge (51°29'15.23"N, 0°17'11.85"W). With a salinity range of approximately 0 to 30, the lower reaches of this estuarine area experience heavy mixing of fresh and marine waters. The estuarine region exhibits gradients in width (238m at London Bridge to 511m at Tilbury), maximum depth (9.2m at London Bridge to 19.2m at Tilbury) (Kinniburgh, 1998), substratum (hard stony substratum at Kew to soft muddy sediment in the lower estuary) and adjacent land use (residential areas, financial and business districts, and industrial regions). While geographic features may point toward division into three regions (upper estuary, lower estuary and coastal), for

effective analyses and application of the most appropriate mixing models, the classification of samples into just estuarine and coastal regions was considered best. However, as the results reveal, differential use of the upper and lower reaches of the estuary by the different fish species is evident. Although they may not be discerned in the mixing models (Chapter 5), these patterns are later described using simple graphical representations of the data, thus avoiding substantial loss of analytical resolution.

Section 3.1 discusses the options for regional divisions at greater length. This includes interpretation of discriminant function analyses (DFAs) performed to assess the accuracy with which invertebrates could be re-classified to their region, according to models with different regional divisions.

3. Development of Methodologies

This chapter considers some important methodological issues that were encountered. It is necessary to contemplate various technological and analytical options at this point, as these become important in later chapters.

3.1 Defining regions

In order to assess differential use of habitats and their food resources, it was necessary to divide the study area into distinguishable regions.

Section 2.3.2 described and justified the division of the study area into estuarine and coastal regions. While simplifying analysis and interpretation, and enabling consideration of broad-scale patterns, this division of the estuarine-marine continuum into two regions may be misleading. Division of the study area into three, four or five regions would highlight the gradual changes occurring and perhaps more closely reflect hydrographic patterns and landscape features. These options were ultimately rejected due to the need for a two-source model in later analyses (see section 3.2), but it is necessary to maintain an understanding of the smaller scale features to aid realistic interpretation of the role of estuaries. Table 3.1 describes the advantages and disadvantages of the different regional divisions. Included are the results from the discriminant function analyses (DFAs) from section 4.3.2, showing the accuracy with which the three isotopic indicators ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) can be used to re-classify invertebrate specimens to their region of collection. Models with more sources (i.e. more regional divisions) result in lower re-classification accuracy. This can partly be attributed to the reduction in sample sizes to include in the analyses, as the data are divided into successively smaller groups associated with successively smaller regions. More importantly, however, due to the nature of a continuum such as this, more regional divisions result in a larger area of overlap in stable isotope signatures, inhibiting the accurate re-classification of specimens. For example, the five source model specimens from the upper and upper-middle estuarine regions will show some overlap in stable isotope signals, limiting their ability to be accurately re-classified. Across all five regions, the collective overlap of source signals is considerable. Conversely, in the two-source model, the number of

specimens from estuarine and coastal regions showing source signal overlap is relatively small, and most (98.8%) of specimens are accurately re-classified.

In terms of the physical, chemical and biological representation of the estuarine continuum, division of the study area into three or four regions is desirable. However, re-classification accuracy of invertebrates is highest with the two-source model. Also, later application of mixing models deemed a two-source model the most applicable to this research (see section 3.2). In order to avoid overlooking the shortcomings of the two-source model, graphical analyses of raw data are used to discuss patterns in basal stable isotope signature and fish activity across the estuarine gradient (Chapters 4 and 5). This is favoured over arbitrarily and subjectively dividing the estuary into smaller regions.

Table 3.1 Summary of advantages and disadvantages of different regional divisions. The DFA (Discriminant Function Analysis) % result shows the accuracy with which the three isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) re-classified invertebrate specimens to their region of collection.

Regions	**DFA %**	**Advantages**	**Disadvantages**
Estuarine, Coastal	98.8%	Simplifies analysis/interpretation. Allows application of two-source IsoError model (see section 3.2).	Inhibits quantifying use of different parts of estuary.
Upper Estuary, Lower Estuary, Coastal	91.3%	Permits quantifying use of different parts of estuary. 'Lower Estuary' represents zone of heavy mixing and steepest gradients.	Requires 3-source IsoError or IsoSource model (see section 3.2). Smaller sample sizes, especially in upper estuary.
Upper Estuary, Middle Estuary, Lower Estuary, Coastal	83.8%	Permits quantifying use of different parts of estuary. Division of estuary into three parts is logical at the landscape scale.	Requires IsoSource model (see section 3.2). Small sample sizes.
Upper Estuary, Upper-Middle, Lower-Middle, Outer Estuary, Offshore	63.8%	Permits quantifying use of different parts of estuary. Maximising divisions improves representation of gradient.	Requires IsoSource model (see section 3.2). Samples sizes very small. 'Outer Estuary' and 'Offshore' signals similar

3.2 Mixing models

In stable isotope research, mixing models are often applied to data, in order to determine the relative contributions of different food sources to an organism's diet. Phillips (2001) provides evidence to show that the geometric models commonly used are flawed, providing a false impression of a unique solution. The isotopic ratios of a single element can be used to partition two sources; the isotopic ratios of two elements are necessary to partition three sources. However, while the visual clarity of the geometric model remains useful, the Euclidean distance equations do not scale the linear mixing of sources correctly (Phillips, 2001).

Phillips (2001) proposed the use of a linear mixing model which, unlike geometric methods, accurately estimates source proportions represented in an end-member, regardless of whether all sources are utilised. It is noted, however, that due to source variability and potential error propagation, source proportions from any mixing models should be presented as estimates with a measure of variation and/or confidence. The IsoError model (available at http://www.epa.gov) performs these calculations with linear mixing equations. Two variations of this model are available: (a) the *single-isotope two-source model*, and (b) the *dual-isotope three-source model*. Phillips and Gregg (2002) provide the equations used in the IsoError models.

Where the number of sources exceeds the number of isotope tracers by more than 1, it is not possible to calculate unique solutions. However, the linear mixing model can be extended for these circumstances, and results presented as the distribution of feasible solutions. This model (IsoSource; also available at http://www.epa.gov), described by Phillips and Greg (2003), operates by examination of all possible combinations of source contributions (0 to 100%) in small *increments* (e.g. 1%). These predicted mixture signatures are compared to the observed mixture signal, and those falling within a *tolerance* range (e.g. ± 0.2‰) are considered feasible solutions. The *increments* and *tolerance* levels are pre-determined by the user.

The following sections provide considerations of the advantages and disadvantages of the IsoSource and IsoError models in their application to the research presented in the following chapters. The model selected as the most appropriate is justified, and its role in supporting the previously defined regional divisions is recognised.

Note that, only stable isotopes of carbon and sulphur were used in the mixing model analyses of adult fishes. $\delta^{15}N$ data did not conform to the expected trophic patterns, likely due to confusion by anthropogenic N sources in the estuary that are largely absent in open coastal regions. This non-conformity confounds the use of $\delta^{15}N$ data as a source indicator. Section 5.2.3 discusses this matter further.

3.2.1 IsoSource

The use of the IsoSource model requires a minimum of three sources. As discussed in Section 3.1, the division of the estuary into different regions should enable different parts of the estuary to be quantified. Thus, this model would permit the use of the three-, four- or five-source models described in Section 3.1. The quantification of use of different parts of the estuary is relevant for this research, as juvenile bass (*Dicentrarchus labrax*) can be found in water of far lower salinity than are sole (*Solea solea*) and whiting (*Merlangius merlangus*). IsoSource also uses multiple isotopes simultaneously in partitioning sources. The application of multi-isotope models has been praised regularly in the scientific literature (e.g. Fry, 1983; Hesslein *et al.*, 1991; Newell *et al.*, 1995).

Unfortunately, the application of the IsoSource model to the data collected during this study led to several problems. First, the sources are not discrete, but were arbitrarily designated regions along the estuarine to marine continuum. Consequently, source signals have large standard deviations (see Figure 3.1). Although the model does permit the user to set the mass-balance tolerance as they see fit, it is not possible to account for the actual variation in source signals. Figure 3.1 shows the stable isotope data of adult fishes (all species and age groups) relative to the mean invertebrate signals from the different regions, according to the three-source model.

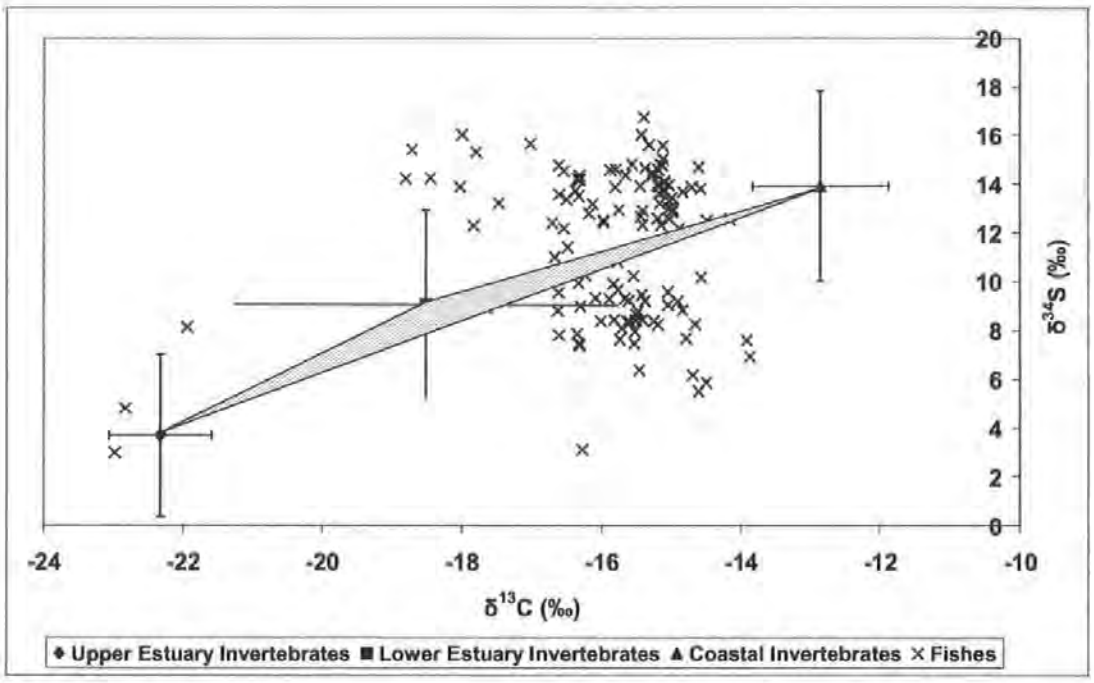


Figure 3.1 Plot of stable isotope values of fishes (all species and age groups), relative to source signals (± standard deviation) from invertebrate specimens, according to the three-source division of regions (Upper Estuary, Lower Estuary and Coastal regions). The shaded area is the mixing polygon connecting the three sources.

Secondly, and more importantly, is the seemingly erroneous weighting of source contributions calculated by the model when sources are part of a continuum. Fish stable isotope signatures that lie between that of the lower estuary and coastal region invertebrate signals, for example, often produce data suggesting a disproportionately large contribution from the upper estuary, relative to what would be expected. Applications of the IsoSource model documented in the literature have involved only discrete sources (e.g. Phillips and Gregg, 2003; Phillips *et al.*, 2005; Urton and Hobson, 2005; Benstead *et al*; 2006), and appear not to cope well with sources that are situated along a continuum.

A third problem relating to the non-discrete nature of the sources concerns the tolerance value applied. As can be seen in Figure 3.1, the mixing polygon connecting the three source signals is very narrow and very few fish data points fall within it. The inability to incorporate source variation data into the model exacerbates this problem. For source contribution data to be generated for samples that lie outside this mixing polygon, the model requires that the tolerance level is sufficiently large. As is the case for many samples in this application, where the tolerance value is so large that it

exceeds half the value of the difference between two sources, the model reports a range of feasible source contributions so wide as to be meaningless.

3.2.2 Dual-isotope three-source IsoError

The dual-isotope, three-source IsoError model maintains the ability to examine energetic contributions from different parts of the estuary. It also facilitates the simultaneous use of two isotopes, again meeting with multi-isotope recommendations (e.g. Fry, 1983; Hesslein *et al.*, 1991; Newell *et al.*, 1995). Furthermore, IsoError permits the incorporation of measures of source and mixture variation into source proportion calculations.

However, as in the IsoSource model, the application of the dual-isotope three-source model to the data presented here suffers from the non-discrete nature of the sources. The weighting of source contributions again appears to be skewed by the distribution of stable isotope source signals along a continuum. In addition, where sample signatures lie outside the mixing polygon, a negative contribution value is assigned to one of the sources. A negative source contribution is not possible, but when rounded to zero, source contributions for these specimens failed to sum to 1, thus rendering the results un-interpretable.

It is assumed that all possible sources and their signals have been accounted for. This research has aimed to investigate broad-scale patterns between estuarine and coastal regions. However, it is possible that sampling programs were not sufficiently extensive to capture all source signals from sub-habitats within estuarine and coastal regions. For example, although saltmarsh represents only a very small percentage (4.73%; Pihl *et al.*, 2002) of surface area in the Thames Estuary, specimens were not specifically targeted within saltmarsh habitat. More pertinent perhaps, is the lack of pelagic samples. Note that a lot of the fish samples have $\delta^{34}S$ above the mean for coastal invertebrates (Figure 3.1). Pelagic producers (and therefore invertebrates feeding upon them) tend to be enriched in $\delta^{34}S$, relative to their benthic counterparts. Consequently, inclusion of zooplankton in the coastal source signal may have raised the mean on the $\delta^{34}S$ axis. Whilst this may have increased the number of fish with stable isotope signals falling within the mixing polygon, it would not remedy the other problems associated with the three-source model.

3.2.3 Single-isotope two-source IsoError

The single-isotope two-source IsoError model avoids the mixing polygon problems experienced when applying the data presented here to three-source models. Where fish stable isotope signals do not lie between mean source signals, source contributions of <0 or >1 are produced. These can be rounded to 0 or 1 (equivalent to 0 or 100%), accordingly, and still produce interpretable results. The use of an IsoError model again permits incorporation of measures of variance in source signals. While this model does not permit the simultaneous use of two isotopes, application of this model has recognised an advantage in assessing source contributions independently for different isotopes. The results of the model outputs can be seen in section 5.3.3. The source contributions calculated by the model are markedly different according to whether $\delta^{13}C$ or $\delta^{34}S$ data are used. It is expected that this may be related to differences in the overall turnover rate of $\delta^{13}C$ and $\delta^{34}S$ in muscle tissue, due to the relative proportion of carbon and sulphur found in food items (*pers.comm.* Schimmelmann). The resultant lag in the $\delta^{34}S$ turnover would explain what appears to be the persistence of depleted $\delta^{34}S$ estuarine signals in the muscle tissue of some fishes (see Section 5.4.3 for further discussion of this). As such, it appears that independent consideration of the isotopic markers may be beneficial.

The primary criticism of the two-source IsoError model is that it does not permit quantification of energy contributions from different parts of the estuary. However, this was not a primary goal of this research: rather it was to quantify the overall estuarine contribution to coastal stocks. Nevertheless, interpretation of the differential use of portions of the estuary would be useful. Although not quantitative, such assessments are made from visual interpretation of graphs and raw data.

Following consideration of the relative benefits and flaws of the different models available, the single-isotope two-source IsoError model was selected as the most appropriate for this application. Phillips *et al.* (2005) describes studies that combined sources with only small differences between them, to successfully apply a two-source mixing model and make source contribution inferences at a larger scale. For example, Still *et al.* (2004) combined multiple species of C_3 and C_4 plants, respectively, in order to identify relative contributions to ecosystem respiration. Although differences in respiration may have been evident within the two groups, the study focused upon the difference between the groups and thus could apply a two-

source mixing model. Similarly, Hobson (1990) grouped species of fish that were prey to a bird species into marine and freshwater categories. Thus, rather than assessing relative contributions of many species of fish, a two-source model was used to differentiate between marine and freshwater food sources.

As in these examples, this work uses an *a priori* approach to combining sources, as opposed to the *a posteriori* approach, both described by Phillips *et al.* (2005). The *a posteriori* approach implements the IsoSource model and combines sources appropriately after analysis. The *a priori* approach, as used here, combines similar sources and then implements a simpler model to produce unique solutions. The favoured utility of the two-source IsoError model further supports the decision (as discussed in section 3.1) to divide the study area into two regions: estuarine and coastal.

3.3 Otolith chemistry technology

In the field of otolith chemistry, many different instruments and techniques have been developed to answer different research questions and utilise the potential offered by technological advances. Equipment has gradually become more accurate, precise, complex and expensive, and research has expanded to consider not only the chemistry of whole otoliths, but also of individual annuli. As the potential of otoliths in various aspects of fisheries biology research has been realised, equipment has been designed and built for more advanced research. However, otolith research is not solely responsible for these technological advances, with much of the equipment being designed in conjunction with the advancement of other disciplines, such as geology and medical science.

The different types of equipment utilised can initially be split into two categories: (1) electron microprobes, and (2) mass spectrometers. Electron microprobe analyses can be further categorised into wavelength dispersive electron microprobes (WD-EM), energy-dispersive electron microprobes (ED-EM), and some other closely related techniques, including the use of electron microscopes more commonly used to produce high resolution topographic electron images (Reed, 1993). Here, these electron microprobe techniques are considered collectively (further information can be found in Reed, 1993; de Pontual and Geffen, 2002).

Mass spectrometry has been utilised extensively in the field of otolith chemistry, with a vast range of mass analysers and sample introduction technologies being available. These include thermal ionisation mass spectrometry (TIMS), microwave-induced plasma mass spectrometry (MIPMS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and various forms of inductively coupled plasma mass spectrometry (ICPMS) such as isotope dilution ICPMS (ID-ICPMS), solution-based ICPMS (SB-ICPMS) and laser-ablation ICPMS (LA-ICPMS). The techniques most commonly encountered in the literature are those of the electron microprobe analyses, and the SB- and LA-ICPMS techniques. The technique most appropriate for a particular application largely depends on the hypothesis being tested as different studies require different resolutions and limits of detection (LOD). More practically, however, the technique used often depends on the size of the research budget. Table 3.2 summarises the relative advantages and disadvantages of electron microprobes, SB-ICPMS and LA-ICPMS, and provides a brief description of the operation of each. Further detail on the full range of available instrumentation can be found in Jarvis *et al.* (1992), Reed (1993), Montaser (1998), Thorrold and Shuttleworth (2000) and de Pontual and Geffen (2002).

As a result of financial and logistical restrictions on sampling and analytical activity, the techniques applied were chosen to optimise data output, giving priority to the principal research questions. Otoliths of 0+ fishes were analysed using SB-ICPMS, while adult otoliths were sectioned and analysed with LA-ICPMS (Chapter 6).

Table 3.2: Summary of advantages and disadvantages of electron microprobes and ICPMS techniques in otolith chemistry applications (Gunn *et al.*, 1992; Reed, 1993; Campana *et al.*, 1997; Chesney *et al.*, 1998; de Pontual and Geffen, 2002).

<table>
<tr><th></th><th>Process</th><th colspan="2">Advantages</th><th colspan="2">Disadvantages</th></tr>
<tr><td>Electron microprobe analysis</td><td>Bombardment of small region on sample with finely focused electron beam → X-ray spectrum generated → wavelength and intensity of spectrum allows elements present to be identified and their concentrations estimated.</td><td colspan="2">Can take advantage of chronological growth sequence in otoliths.
Cheap relative to more modern technologies.</td><td colspan="2">High limits of detection (LOD); therefore can analyse fewer elements.
More susceptible to measurement error – peak overlaps, spectral artefacts, background anomalies.
Correction for anomalies and background signals often required.
Requires highly polished and flat surface.</td></tr>
<tr><td rowspan="2">ICPMS</td><td rowspan="2">Sample introduction to inductively coupled plasma (ICP) → atomisation & ionisation → transportation to mass spectrometer.
In SB-ICPMS the otolith is dissolved in acid and introduced as a liquid.
In LA-ICPMS a small region of the otolith is ablated with a laser, vaporising the material, which is then transported to the ICP in an argon gas carrier.</td><td colspan="2">Lower LOD; therefore can analyse more elements
Higher sample throughput</td><td colspan="2">Expensive</td></tr>
<tr><td><u>LA-ICPMS</u>
Uses chronological growth sequence.
Less susceptible to sample contamination.</td><td><u>SB-ICPMS</u>
Good for whole otolith assays.
Less preparation required
Low LOD (rare-earth elements may be detected).</td><td><u>LA-ICPMS</u>
Extra expense of laser technology.
Sectioning & polishing necessary to access growth rings (time consuming and difficult with small otoliths).
Higher LOD than SB-ICPMS.</td><td><u>SB-ICPMS</u>
Difficult to utilise growth sequence (requires micro-milling).
More susceptible to sample contamination.</td></tr>
</table>

4. Variation in stable isotope signatures of the invertebrate prey of fishes.

4.1. Introduction

Dynamic estuarine systems develop where fresh and marine waters meet and mix. This physical mixing of the water, and dissolved and particulate matter, has many implications for the environment and the organisms that live in it. Estuaries are important stores and pathways for energy, being the discharge point for terrestrially produced energy, nutrients and minerals. Although the flora and fauna of estuaries must adapt to abrupt changes in physiological demands (i.e. as a result of salinity, temperature and oxygen parameters), they are highly productive in estuaries, and exploit the rich nutritional sources available.

The mixing of fresh and marine waters results in a declining representation of terrestrially-sourced material on moving seawards, and *vice versa*. This gradient may also be reflected in the relative use of marine and terrestrial energy sources by organisms inhabiting estuaries and adjacent coastal regions. In recent years, the use of naturally-occurring stable isotopes has become an established technique when seeking to describe the relative use of freshwater and marine derived energy sources.

Stable isotopes are forms of an element that possess different numbers of neutrons in their nucleus and therefore have different atomic masses. Stable isotopes are expressed in terms of a ratio of one isotope to the most common one (i.e. ^{13}C:^{12}C). They often vary predictably in the natural environment, which leads to different production sources possessing different isotopic signatures (Owens, 1987; Peterson and Fry, 1987). Although all isotopes of an element take part in the same reactions, mass differences mean that different isotopes do not react to synthesis processes at equal rates (Fry and Sherr, 1984). Consequently, isotopic ratios are depleted or enriched in their stable isotope ratio (expressed as δ-values in parts per thousand ‰ differences from a standard reference material – see Section 1.4.1) relative to source materials. For example, C_3 and C_4 plants have distinct isotopic signatures. Producers of terrestrial and marine origin are also usually distinguishable through analysis of their isotopic composition (Fry and Sherr, 1984; Owens, 1987). Furthermore, estuarine producers tend to show isotopic signatures particular to their position along an estuarine gradient. Since consumers tend to utilise organic matter from the region where they are found (Deegan and Garritt, 1997), the gradient of isotopic signals may

be revealed in the body tissues of consumers, especially if they are relatively sessile. Stable isotope analysis has been widely used to examine the relative use of marine and terrestrial production. Stephenson and Lyon (1982), for example, employed stable carbon isotope analysis to detect the use of marine and terrestrial food sources in an estuarine bivalve (*Chione stutchburyi*). The $\delta^{13}C$ data in bivalve muscle tissues ranged from -16.7 to -23.5‰, depending on their position in the estuary and the local hydrological regime. In keeping with the natural distributions, estuarine isotope ratios were depleted relative to those from sites at the seaward end of the estuary. Similarly, France (1995b) differentiated between littoral and pelagic consumers in a lake ecosystem using $\delta^{13}C$ signals. Incze *et al.* (1982) used the $\delta^{13}C$ of filter-feeding bivalves to compare two estuaries experiencing different levels of river input.

Although more often implemented in trophic studies, due to large fractionations of approximately 3‰ between trophic levels, nitrogen stable isotopes have also been used in tracing organic matter origin and spatial variation in isotopic signatures. Owens (1987) describes natural variation of $\delta^{15}N$ in the environment, including the tendency for gradually more enriched signals moving along an atmosphere-terrestrial-freshwater-estuarine-marine gradient. France (1994) compiled data in a literature survey indicating marine invertebrates to be enriched in $\delta^{15}N$ relative to their freshwater counterparts, while estuarine individuals carried signals indicative of their position along the gradient. Also, Jennings and Warr (2003) detected variation in $\delta^{15}N$ of filter-feeding bivalves (queen scallops *Aequipecten opercularis*) in coastal and offshore regions, successfully correlating it to gradients in salinity, temperature and depth. However, such underlying trends can easily be confounded by trophic interactions if organisms not feeding at the same trophic level are used. Consequently, $\delta^{15}N$ data are rarely used as an independent indicator of organic matter origin, and is more commonly applied to multi-isotope food-web studies. Furthermore, the normal trend in $\delta^{15}N$ is commonly perturbed by anthropogenic inputs to river and estuarine systems. Fertilisers and other components of agricultural run-off have distinct isotopic signatures: a number of studies have successfully identified $\delta^{15}N$ signals of these in rivers and estuaries, and in the organisms that live in them (e.g. Harrington *et al.*, 1998; Chang *et al.*, 2002; Anderson and Cabana, 2005). More pertinent though is the enriched $\delta^{15}N$ signals that occur as a result of urbanisation and discharge of human waste (treated and untreated). Numerous studies have demonstrated the techniques ability to assess

spatial and temporal variation in anthropogenic inputs (e.g. McClelland *et al.*, 1997; Gartner *et al.*, 2002; deBruyn *et al.*, 2003).

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses have frequently been used together to determine spatial features in the distribution and use of organic matter. Thornton and McManus (1994), for example, used $\delta^{13}C$ and $\delta^{15}N$ analyses in studying the provenance of organic matter sources at various sites in the Tay Estuary, Scotland. This combination of source indicators provided substantially more information than any single indicator.

More recently, the use of a third isotope has become incorporated in ecological research. Although the variability of sulphur isotope ratios ($\delta^{34}S$) can sometimes confound the clarity of spatial patterns (Oakes and Connolly, 2004), in many cases it can help to discriminate production sources (Connolly *et al.*, 2004). Multiple-isotope studies are heavily praised in the scientific literature as providing greater rigour and discriminatory power than can be achieved with individual isotopes (e.g. Peterson *et al.*, 1985; Deegan and Garritt, 1997; Peterson, 1999).

This study aimed to assess spatial variation in the stable isotopes signatures of selected invertebrate species from the Thames estuary and the adjacent coastal region. The extent to which the stable isotope data could be used to reclassify the invertebrates to their region of collection was also a key goal. Although interesting as a stand-alone study, this research was related to a subsequent study (Chapter 5 of this thesis) which relied on knowledge of the spatial variation in the stable isotope signatures of invertebrates to interpret signals in fish. The spatial isotope data for the invertebrates was used as a basal signature in mixing models, from which relative contributions of estuarine and coastal food sources to fish diets could be established. The invertebrate sampling was not designed as part of a full food-web study, but to represent the likely spatial variation in stable isotope signals available in the diets of the fishes studied.

4.2. Materials and Methods

The invertebrate samples for which stable isotope analyses were completed are listed in Table 4.1. The samples selected for analysis were limited by their availability, by the cost of analyses, and by the principal research goals.

4.2.1. Sample processing

The initial preparation of invertebrate tissue samples, as described in section 2.3.1 involved dissection and re-freezing. Samples were then freeze-dried, until no evidence of moisture remained. Clean lids were replaced and samples stored until ready for grinding, using pestles and mortars until they reached a flour-like consistency. To minimise potential contamination, clean conditions were maintained through the use of powder-free latex gloves, clean work surfaces and ethanol cleansing of forceps and spatulas between samples. In addition, pestles and mortars were brushed clean of sample debris, washed, scrubbed and rinsed with ethanol, wiped with lint-free lens tissues and dried thoroughly in an oven (~60°C) between samples. Between batches of replicate samples, pestles and mortars were left overnight in a 5% nitric acid bath, followed by two consecutive Milli-Q water rinsing baths and then thoroughly dried in an oven.

Following grinding, samples were returned to the glass vials and stored. Weighing into tin capsules was delayed until shortly before sample analysis, in order to limit the potential absorption of moisture. Carbon and nitrogen stable isotope analyses were conducted together, whereas sulphur isotopes require different calibration standards, equipment set-up and an oxidation aid. Thus, two samples were weighed from the glass sample vial. For carbon and nitrogen analyses, 0.7 mg (± 0.2) of each sample was weighed into 5 x 3.5 mm tin capsules using a micro-balance, folded to secure the material inside and stored in a multi-well plate until analysis. The weight of material weighed into each capsule was noted to three decimal places, for later use in mathematical corrections of stable isotope data output. Due to the lower concentration of sulphur in animal tissue samples, approximately 3.0 mg of sample plus 0.3 mg (± 0.03) of vanadium pentoxide (V_2O_5) as an oxidising agent were weighed into 6 x 4 mm tin capsules in preparation for analysis.

4.2.2. Sample analysis

Stable isotope analyses were carried out at the Scottish Universities Environmental Research Centre (SUERC) in East Kilbride, using a Thermo Finnigan Deltaplus XP, coupled with a Costech instruments elemental combustion system and a Thermo Finnegan Conflo III dilution (helium) system. For carbon and nitrogen

analyses, three internal calibration standards (gelatine, 14alanine, 15alanine) were interspersed among samples. The internal standards were checked periodically against secondary international standards (USGS 24, USGS 25, IAEA N1, IAEA N2, IAEA CH6 and IAEA CH7). For sulphur isotope analyses, internal calibration standards consisted of MET, HCYS, LCYS, and CP1. These were checked periodically against secondary international standards IAEA S1, IAEA S2 and NBS 123.[1]

The samples for which stable isotope data was obtained and used in subsequent analyses are summarised in Table 4.1. As collection of all species in all regions in all seasons was not possible, some groups of data have been pooled to improve sample size. Samples collected in the springs of 2004 and 2005 have been pooled. Therefore, a lack of annual variation must be assumed, or at least be sufficiently small to not mask the spatial variation of interest. The few early June 2004 samples are also included in the 'Spring' data set.

Table 4.1 Summary of invertebrate samples with stable isotope data used in later data analyses. Seasonal and species data are pooled later, increasing sample size.

Region of collection	Species	Season	n
Estuary	***C. crangon***	**Autumn**	**12**
Estuary	***C. crangon***	**Spring**	**1**
Estuary	***Palaemon* spp.**	**Autumn**	**10**
Estuary	***Palaemon* spp.**	**Spring**	**2**
Estuary	**Polychaete spp.**	**Autumn**	**6**
Estuary	**Polychaete spp.**	**Spring**	**4**
Coastal	***C. crangon***	**Autumn**	**12**
Coastal	***C. crangon***	**Spring**	**13**
Coastal	***Palaemon* spp.**	**Autumn**	**8**
Coastal	**Polychaete spp.**	**Autumn**	**2**
Coastal	**Polychaete spp.**	**Spring**	**12**

[1] USGS24 = graphite; USGS25, IAEA N1 and N2 = ammonium sulphate; IAEA CH6 = sucrose; IAEA CH7 = polythene; IAEA S1 and S2 = Ag_2S; NBS 123 = ZnS.

4.2.3. Data analysis

The invertebrate species used to provide insight into the basal spatial variation in stable isotope signals between estuarine and coastal habitats are split into three groups: (a) the common brown shrimp, *Crangon crangon*; (b) the prawns *Palaemon longirostris* (estuarine and freshwater margin) and *P. serratus* (shallow marine); and (c) polychaetes of the genera *Nereis*, *Nephthys* and *Aglaophamus*. Amphipod and sediment samples were also prepared for stable isotope analysis, but the data have not been included due to analytical problems. The two prawn species have been grouped, due to their functional similarities, as have the three polychaete genera. These species have previously been found to feature regularly in the diets of common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and whiting (*Merlangius merlangus*) (e.g. Costa, 1988; Molinero and Flos, 1991; Greenstreet, 1995; Cabral, 2000; Elliott *et al.*, 2002), and should therefore support later attempts to trace the feeding activity of these fishes (Chapter 5).

The stable isotope data for the different invertebrate groups (shrimps, prawns and polychaetes) were tested in Spearman's rank correlations, to determine any relationships between the body size of the individuals tested and $\delta^{15}N$. Any size-specific differences in the trophic levels (indicated by $\delta^{15}N$) of the invertebrates would necessitate separate consideration of different size-classes. As multiple individuals were used for each sample, the third quartile (75^{th} percentile) of body/carapace length was used as the measure of body size. This weighted the measurement towards the larger individuals, which constituted more of the homogenised sample. No significant relationships between $\delta^{15}N$ and body size were found. Consequently, invertebrate samples of all body sizes were included in the data analyses, considerably improving the sample sizes available.

Two-way crossed ANOSIM (Analysis of Similarity, using PRIMER 6) was used to test for differences in stable isotope signatures among species within regions, and among seasons within regions. ANOSIM acts as an analogue of ANOVA, testing permutation-based hypotheses about differences between groups of (multivariate) samples. Although some seasonal and species differences were revealed, data were pooled across seasons and invertebrate species, thus maximising sample sizes that could be used to map the spatial variation in isotopic signatures. The pooling of species and seasons is justified because any variation in isotope signatures arising as a

result of pooling ought to also be reflected in the diets (and therefore tissues) of the fish feeding on invertebrate prey. A 1-way ANOSIM was performed to assess the difference in isotopic signatures of estuarine and coastal samples when multi-species and seasonal data are pooled. In addition, 1-way SIMPER (Similarity Percentage analysis, using PRIMER 6) analyses were used to identify the relative strengths of the three stable isotopes driving the relationships inferred by the ANOSIMs. Isotope data for each element was plotted against distance from Kew Bridge and against each other to reveal further spatial patterns and clustering in the data.

Discriminant function analysis (using SPSS 11.5) was performed with a leave-one-out method to determine the accuracy with which the stable isotope data could be used to re-classify invertebrates to the region from which they were collected. This was repeated a number of times using different regional classifications for the observed values (using 2, 3, 4 and 5 source models) and different combinations of the three isotopes. The regional divisions comprising these different source models are described in section 3.1. A 2-source model (estuarine and coastal) with 3 isotopic tracers ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) was found to produce the best classification accuracy, hence the consideration of a two-source system in other analyses. Although univariate normality was not achieved for all variables, multivariate normality was monitored using Mahalanobis distances and homogeneity of variances with log determinants and Box's M test. Although desirable for discriminant function analysis, minor deviations from normality are not fatal. Furthermore, as the stable isotope data contain a mixture of positive and negative numbers, the potential for application of a single transformation across the data set was limited and therefore avoided.

4.3. Results

4.3.1. General patterns

ANOSIM results showed no differences in stable isotope signatures between *C. crangon* and *Palaemon* spp. in either estuarine or coastal regions. However, in the estuarine region, *C. crangon* and *Palaemon* spp. showed different isotopic signatures from polychaetes (Table 4.2). In the coastal region, only *C. crangon* showed a different isotopic signature from polychaetes (Figure 4.3). These differences appear to

be stronger in the estuarine region. While isotopic differences were found between autumn- and spring- collected invertebrates of coastal regions, there was no seasonal difference within the estuary (Tables 4.2 and 4.3).

Due to the expectation that seasonal variation, and variation between prey species, is incorporated in the diet of fishes, seasonal and cross-species data were pooled and included in the later analyses. A strong significant difference in the isotopic signatures of estuarine and coastal invertebrate samples was found under this procedure (Table 4.4).

SIMPER results (Tables 4.2, 4.3 and 4.4) show the relative strengths of the different stable isotopes in driving the difference inferred in the ANOSIMs. Generally, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ all make large contributions in the determination of similarities and differences assessed. In particular, the strong difference seen between estuarine and coastal regions using pooled seasonal and species data (Table 4.4), can be accounted for by 35%, 35% and 30% contributions from $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data, respectively.

Table 4.2 2-way crossed ANOSIM of species and seasonal differences within the estuary. R statistics in bold are significant to the 0.05 level. The results of SIMPER analysis are included, revealing the relative roles of the three isotopic tracers in the differences/similarities inferred by the ANOSIM.

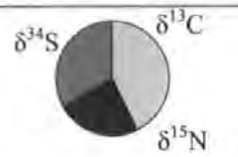
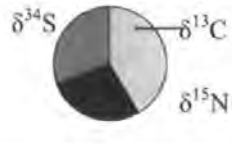
Region	Pairwise tests	R statistic	p value	SIMPER contributions
Estuarine	***C. crangon*** **Vs** ***Palaemon* spp.**	**0.105**	**0.07**	
	C. crangon **Vs** **polychaete spp.**	0.556	**0.001**	
	***Palaemon* spp.** **Vs** **polychaete spp.**	0.67	**0.001**	
Estuarine	**Autumn** **Vs** **Spring**	**-0.087**	**0.727**	

Table 4.3 2-way crossed ANOSIM of species and seasonal differences within the coastal region. R statistics in bold are significant to the 0.05 level. The results of SIMPER analysis are included, revealing the relative roles of the three isotopic tracers in the differences/similarities inferred by the ANOSIM.

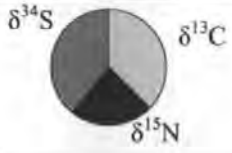
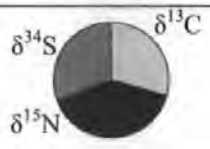
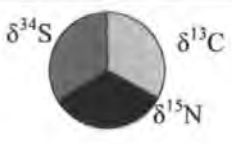
Region	Pairwise tests	R statistic	p value	SIMPER contributions
Coastal	***C. crangon*** **Vs** ***Palaemon* spp.**	**0.05**	**0.261**	
	C. crangon **Vs** **polychaete spp.**	0.209	**0.011**	
	***Palaemon* spp.** **Vs** **polychaete spp.**	**0.506**	**0.083**	
Coastal	**Autumn** **Vs** **Spring**	0.226	**0.01**	

Table 4.4 1-way ANOSIM of regional differences (seasons & species' pooled). R statistics in bold are significant to the 0.05 level. The results of a SIMPER analysis are included, revealing the relative roles of the three isotopic tracers in the difference inferred by the ANOSIM.

Pairwise test	R statistic	p value	SIMPER contributions
Estuarine **Vs** **Coastal**	0.89	**0.001**	

Many of the patterns exposed in the statistics may also be seen graphically in Figures 4.1 and 4.2. *C. crangon* and *Palaemon* spp. from both estuarine and coastal regions show very similar clustering using stable isotope values, while polychaetes in the estuary are enriched in $\delta^{13}C$ and $\delta^{34}S$ relative to the other species groups.

Overall, estuarine invertebrates show enriched $\delta^{15}N$ and depleted $\delta^{13}C$ and $\delta^{34}S$ signals, compared to their coastal counterparts. Mean (± standard deviation) values for estuarine invertebrates are: -22.07‰ (±2.95‰), 19.63‰ (±1.08‰) and 4.21‰ (±4.48‰) for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data, respectively. Mean (± standard deviation) values for coastal invertebrates are -15.11‰ (±0.97‰), 14.31‰ (±1.63‰) and 11.56‰ (±3.18‰) for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data, respectively. Large variation in the $\delta^{34}S$ signals is evident, causing weaker clustering of species and regional groups. However, its value as a discriminator of production sources is still evident from Figure 4.1, as well as the previous SIMPER analyses (Tables 4.2, 4.3 and 4.4) and the discriminant function analysis shown in Table 4.5.

For all three sets of stable isotope data, there is a clear gradient of enrichment ($\delta^{13}C$ and $\delta^{34}S$) or depletion ($\delta^{15}N$) (Figures 4.1 and 4.2) from the estuarine to coastal region. Stable isotope ratios are fairly constant in coastal regions. However, there are also clear within-estuary gradients in stable isotope values. Invertebrate specimens exhibited a gradual enrichment in all three stable isotope ratios in approximately the first 50 km moving downstream from Kew Bridge. In the lower reaches of the estuary the invertebrates then showed steeper gradients of enrichment in $\delta^{13}C$ and $\delta^{34}S$. In contrast, the $\delta^{15}N$ values of the invertebrates became increasingly depleted in the lower reaches of the estuary, until coastal levels were reached.

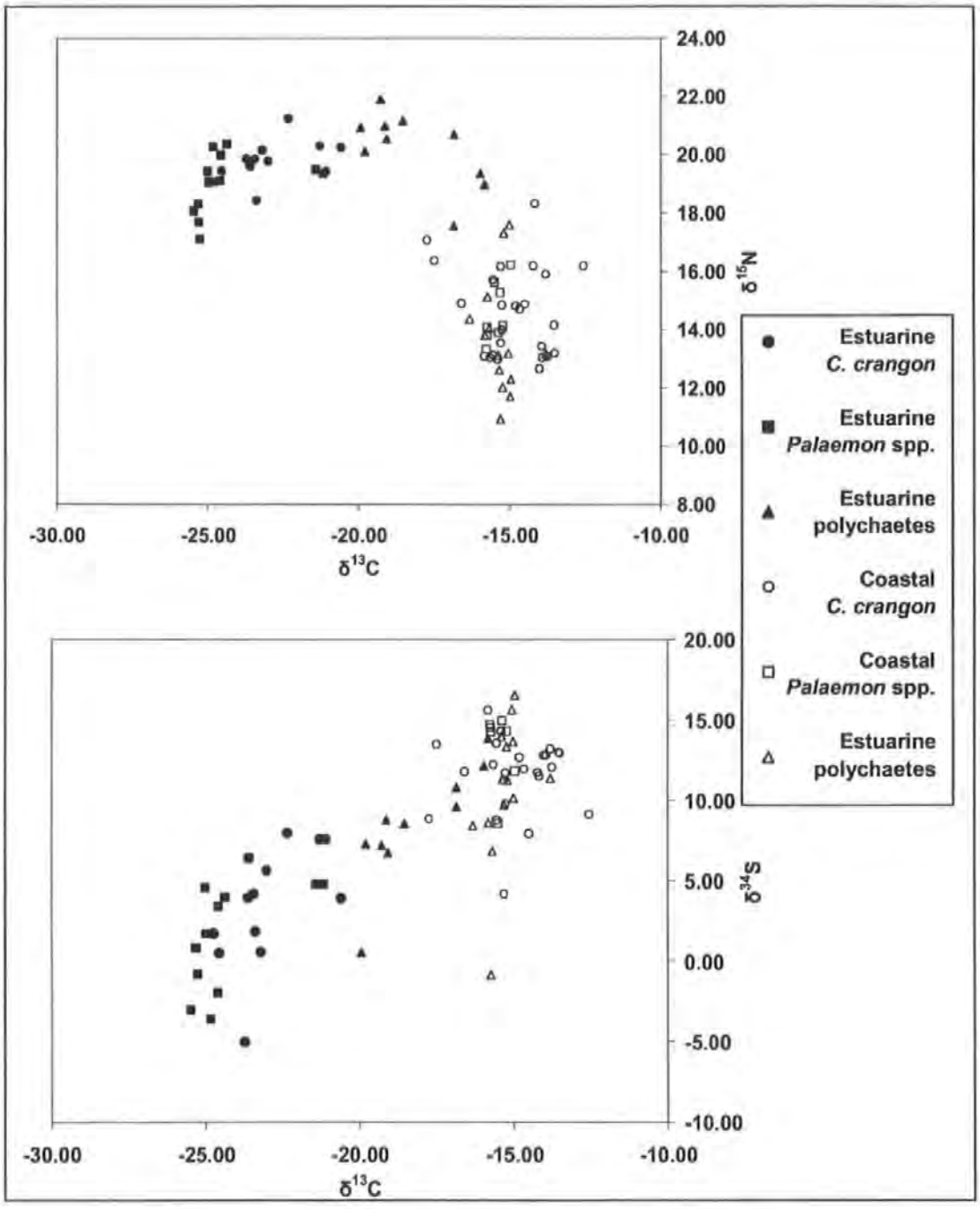


Figure 4.1 Stable isotope invertebrate data for the three species groups, classified into estuarine and coastally collected samples. Samples collected in different seasons have been pooled.

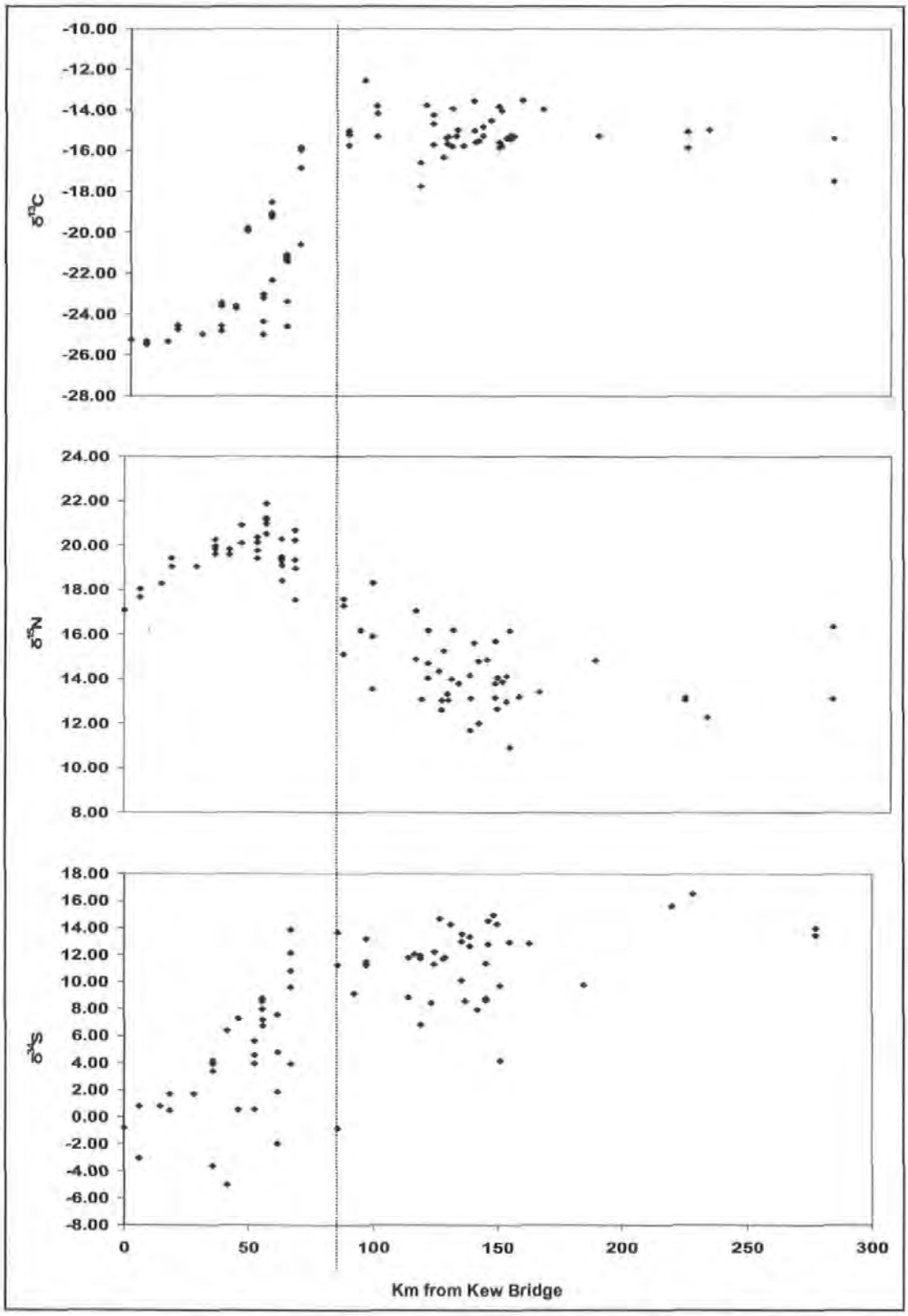


Figure 4.2 Stable isotope invertebrate data plotted against distance from Kew Bridge (km) independently for isotopes of carbon, nitrogen and sulphur. Samples from all seasons and of all three species groups (*C. crangon*, *Palaemon* spp., polychaete spp.) were included here. The dotted line represents the division between the estuarine and coastal regions used in the analyses (see section 2.3.2 for details).

4.3.2. Discriminant Function Analysis

In order that later (Chapter 5) analyses of fish tissues for interpretation of feeding patterns may be considered rigorous, it was necessary to use an invertebrate model with high re-classification accuracy. Invertebrate sample data were divided into different observed source categories (with 2, 3, 4 or 5 regional sources; see section 3.1 for description of these regional divisions) along the estuarine-marine gradient and run through discriminant function analysis. The predicted classifications produced by the model determined the accuracy with which the stable isotope data could re-classify invertebrate samples to their region of collection. Different combinations of the three isotopes were also tested to determine the most accurate re-classification system. Two, three and four source models were quite successful for all isotopic combinations, with the simultaneous use of all three isotopes always improving accuracy (Table 4.5). The preferred model is the 3 isotope-2 source (estuarine and coastal) model with 98.8% re-classification accuracy. This model misclassified only one estuarine sample.

Table 4.5 Summary of results from discriminant function analysis (DFA), using different combinations of sources and isotopes. % values describe the accuracy with which the isotopic tracers re-classified invertebrates to their region of collection. Cross-validated results presented.

Isotopes used	Number of sources specified and % accuracy of classification			
	2	3	4	5
$\delta^{13}C$	**93.9**	**87.8**	**50**	**48.8**
$\delta^{15}N$	**95.1**	**76.8**	**43.3**	**34.1**
$\delta^{34}S$	**87.5**	**75.0**	**50.0**	**51.3**
$\delta^{13}C$, $\delta^{15}N$	**97.6**	**91.5**	**70.0**	**62.2**
$\delta^{13}C$, $\delta^{34}S$	**93.8**	**87.5**	**73.3**	**60.0**
$\delta^{15}N$, $\delta^{34}S$	**96.3**	**85.0**	**73.3**	**58.8**
$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	**98.8**	**91.3**	**76.7**	**63.8**

4.4. Discussion

4.4.1. Species, seasonal and regional differences

The lack of any significant correlations between body size and $\delta^{15}N$ (indicative of trophic level) (data not shown) meant that it was not necessary to separate invertebrate data into size-specific groups (see section 4.2.3). Due to growth and increasing body mass with age, ontogenetic variation in food preference and hence trophic level is not uncommon among animals (France *et al.*, 1998; Jennings *et al.*, 2002a,b; Rossi *et al.*, 2004). However, for the size-ranges of shrimp, prawn and polychaetes used in this research, the lack of ontogenetic variation in $\delta^{15}N$ suggests that there is no intra-specific variation in trophic level. This allowed pooling of these samples and maximisation of sample size.

C. crangon and *Palaemon* spp. show very similar isotopic signatures (Tables 4.2 and 4.3), probably due to similarities in body form and feeding habit. These decapod crustaceans, however, show different stable isotope signals from polychaetes caught in the same region (with the exception of coastally caught *Palaemon* spp.). In the estuarine region (Table 4.2) the strength of these differences is misleading, due to the availability of the different species groups. Polychaetes were found only in the lower reaches of the estuary, where substantial sediment deposition forms suitable habitat. *C. crangon* were found as far upstream as South Bank (see map; Figure 2.1), whereas *Palaemon* spp. extended throughout the whole estuary. The gradient seen in the strength of the differences is therefore reflective of the spatial gradient in stable isotope signatures within the estuary. This gradient is displayed graphically in Figure 4.1. Polychaete spp. cluster separately from *C. crangon* and *Palaemon* spp, but still may be identified as part of an estuarine cluster. However, in the coastal region, there are still differences between the *C. crangon* and polychaete spp. (Table 4.3). Differences in feeding behaviour may partially explain this. Despite these differences, samples from the species groups were pooled to generate a basal spatial map of variation in stable isotope signals. For the application to which these data are later applied (Chapter 5), it is desirable that the variation in stable isotope signals in the diets of fishes is incorporated in this spatial map.

The same reasoning may be given in order to justify the pooling of seasonal data. As fishes feed across seasons, it is reasonable to expect that the stable isotope

signals of their tissues will have amalgamated any seasonal variation evident in their food sources. The expectation was that seasonal variation would be minimal in the coastal region, due to the relative constancy of the marine environment. Estuarine invertebrates were expected to reflect differences arising as a result of seasonal variations in rainfall and river input. Wissel and Fry (2005), for example, traced the response of $\delta^{13}C$ and $\delta^{15}N$ in invertebrates and small fish of the Breton Estuary, Louisiana, to seasonal variation in fluxes of freshwater input. However, Table 4.2 suggests that there is no seasonal variation in the stable isotope signals of Thames Estuary invertebrates. It is possible that this is an artefact of poor sample size for spring-caught estuarine invertebrates, in conjunction with the overwhelming spatial variation in stable isotope signals within the estuary. Nevertheless, there does appear to be some seasonal difference in stable isotope signals for coastally caught invertebrates ($R = 0.226$; $p = 0.01$; Table 4.3); this is weak relative to the overall difference between regions when seasons and species are pooled ($R = 0.89$; $p = 0.1$; Table 4.4), and therefore probably sufficiently overwhelmed by it for seasonal variation not to detract from broad-scale spatial differences. Some of this variation may relate to seasonal movements by shrimps and prawns. However, with the use of a two-source (estuarine-coastal) model, these organisms are less likely to move between regions, thus maintaining a more robust spatial model.

SIMPER results in Tables 4.2, 4.3 and 4.4 demonstrate substantial roles of isotopes of carbon, nitrogen and sulphur in partitioning the differences in stable isotope signals between invertebrates, in terms of species, seasonal and regional differences. The value of a multiple-isotope approach to such applications is also evident from Figure 4.1. Segregation between estuarine and coastal clusters is evident on axes of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, thus aiding in the partitioning of source signals.

4.4.2 Trends in $\delta^{13}C$

Natural variation in $\delta^{13}C$ shows an enrichment trend along the terrestrial-estuarine-marine gradient (Fry, 2002; Rubenstein and Hobson, 2004). This enrichment trend is evident along the estuarine-marine gradient, from near the upper tidal extent of the Thames Estuary (Kew Bridge), downstream to coastal regions adjacent to the estuary. $\delta^{13}C$ data points in the coastal region are relatively constant, indicative of the limited variation in the marine environment. Relative to their coastal

counterparts, the $\delta^{13}C$ of estuarine invertebrates is consistently depleted. However, $\delta^{13}C$ values are not constant throughout the estuary, but exhibit an enrichment trend from the upper to lower reaches of the estuary. The $\delta^{13}C$ gradient is steepest in the zone experiencing the heaviest mixing of marine and freshwaters, between approximately 45 and 60 km downstream from Kew Bridge (Figure 4.2).

Regardless of the within-estuary variation, visual examination of the spatial variation in the $\delta^{13}C$ of invertebrates suggests that $\delta^{13}C$ is a powerful discriminatory tool in source partitioning. As such, the data are later used in mixing models, in order to quantify the relative contributions of estuarine and coastal energy resources to the muscle tissue of fishes (Chapter 5).

Unlike the less favourable distributions of $\delta^{15}N$ and $\delta^{34}S$ data, the minimal variation in the $\delta^{13}C$ signals may also permit the accurate partitioning of sources (i.e. upper and lower regions) within the estuary. However, due to limitations incurred primarily by the mixing models applied later (Chapter 5), and the focus of this research on broad-scale patterns, this partitioning of sources within the estuary has not been attempted here.

4.4.3 Trends in $\delta^{15}N$

Previously, the application of $\delta^{15}N$ to ecological studies has usually involved determination of trophic relationships and food-web interactions. There are, however, a number of studies that have identified and successfully utilised variation in the $\delta^{15}N$ of food sources to support studies of resource use and migration. France (1994), for example, described differences in $\delta^{15}N$ between freshwater, estuarine and marine mussels, realising the potential of invertebrate $\delta^{15}N$ as a source indicator. However, where $\delta^{15}N$ is utilised in the partitioning of production sources, it tends to be in conjunction with $\delta^{34}S$ and/or $\delta^{13}C$ data, which usually possess stronger differences between these sources.

Previous descriptions of natural variation in $\delta^{15}N$ describe a weak trend of enrichment along an atmosphere-terrestrial-freshwater-estuarine-marine gradient (Owens, 1987). Here, however, the broad-scale difference between estuarine and coastal regions shows that coastally caught invertebrates are $\delta^{15}N$ depleted relative to their estuarine counterparts (Figure 4.2). The heavily urbanised nature of the Thames catchment area suggests that anthropogenic enrichment is a likely cause of this

pattern. Although sewage treatment facilities have massively improved the quality of wastewater, they primarily involve the removal of solid waste and remediation of extreme nutrient concentrations. Consequently, sewage treatment facilities discharge water enriched in nutrients, such as nitrogen. Numerous studies have used $\delta^{15}N$ data to assess spatial and temporal patterns in sewage discharges, assess the incorporation of sewage-derived material into organisms, and interpret the role of sewage discharge for secondary production (e.g. McClelland and Valiela, 1998a,b; Waldron *et al.*, 2001; deBruyn and Rasmussen, 2002; Gartner *et al.*, 2002; deBruyn *et al.*, 2003; Rogers, 2003; Gaston *et al.*, 2004; Schlacher *et al.*, 2005). Raw sewage is generally depleted in $\delta^{15}N$ relative to seawater (Owens, 1987), while treated sewage effluent tends to be enriched, due to microbial processes (Savage, 2005).

Closer examination of Figure 4.2 reveals patterns in $\delta^{15}N$ at a smaller spatial scale, likely relating to discharge of enriched waste water. The $\delta^{15}N$ signal peaks occurred between approximately 35 and 65 km downstream from Kew Bridge. Four of the principal sewage treatment facilities for the London area are located within this part of the Thames (Beckton, Crossness, Riverside and Longreach). The general depletion in the $\delta^{15}N$ signal with distance from this zone, both upstream and downstream, further supports the supposition that anthropogenic discharges are the causal factor. The degradation in the anthropogenic impact is most evident toward the marine environment, not levelling to a relatively constant level until approximately 120 km downstream from Kew Bridge. Two sewage treatment works are also situated slightly up-river of Kew Bridge (Mogden and Hampton), potentially explaining the $\delta^{15}N$ signals of invertebrates in the upper estuary, which are still enriched relative to coastal invertebrates.

Although variation in $\delta^{15}N$ of invertebrates does not follow patterns of natural variation, visual and statistical assessment of the data suggests that there is some potential for $\delta^{15}N$ as a discriminatory tool in source partitioning between the Thames Estuary and adjacent coastal regions. The ability of the stable isotope data to partition sources depends heavily on the position of the division between estuarine and coastal regions. As this division is highly subjective, it is important that it is well described and justified (see sections 2.3.2 and 3.1). Inconsistencies between studies in the criteria used to divide regions will influence results considerably. In this study, it would also hold for $\delta^{13}C$ and $\delta^{34}S$ data, but the effect would be particularly noticeable with $\delta^{15}N$, due to the distribution of data points seaward of the $\delta^{15}N$ peak (Figure 4.2).

4.4.4 Trends in $\delta^{34}S$

The application of $\delta^{34}S$ to tracer studies has been limited, due to the greater expense of $\delta^{34}S$ analysis, and a relatively poor understanding of the processes controlling its variation. However, to understand these processes, while beneficial, is not critical for the successful exploitation of the variation, provided that differences do exist between sources and are sufficiently stable. In addition, mass spectrometry has become cheaper, and in those studies that operate at a sufficiently large spatial scale and/or with sufficiently abundant replication, it has been possible to utilise $\delta^{34}S$ data to improve discriminatory power in source partitioning. Natural variations in $\delta^{34}S$ result in enriched values for marine sources, relative to terrestrial sources (Peterson, 1999; Rubenstein and Hobson, 2004). While mixing of fresh and seawaters in estuaries often result in intermediate $\delta^{34}S$ values in estuaries, where anaerobic sediments are a dominant substratum, $\delta^{34}S$ can be much reduced due to bacterial reduction of sedimentary sulphides (Peterson, 1999; Connolly *et al.*, 2004).

In this study, coastal invertebrates were enriched in $\delta^{34}S$ relative to those from the Thames Estuary (Figure 4.2). Examination of the gradient at a smaller spatial scale reveals a similar pattern to that seen for $\delta^{13}C$, only with greater variance. Coastally-caught invertebrate samples showed a fairly constant $\delta^{34}S$ signal, while invertebrates caught within the estuary reflected the gradient resulting from the mixing of marine and freshwaters. Although isotopically lighter than coastal invertebrates, $\delta^{34}S$ data of Thames Estuary invertebrates is not sufficiently depleted to be suggestive of major contributions by bacterially reduced sulphides of anaerobic sediments. Areas of anoxic sediment are present in parts of the lower estuary, however, and may be responsible for the greater variation in $\delta^{34}S$ signals observed in this portion of the estuary. The overall gradient in $\delta^{34}S$ signals in the estuary, however, is likely to be largely attributable to gradual trends in the availability of seawater sulphates and prominence of benthic detrital food webs. Due to the nature of the physical mixing process, the relationship between distance downstream and stable isotope ratios is, again, non-linear. The steepest gradient in $\delta^{34}S$ enrichment lies between approximately 35 and 55 km downstream from Kew Bridge (Figure 4.2), again coinciding with the zone of heaviest mixing of marine and freshwaters.

Despite the within-estuary variation in the $\delta^{34}S$ of invertebrates, visual examination of this trend suggests that $\delta^{34}S$ will act as a powerful additional

discriminatory tool in the partitioning of estuarine and coastal invertebrates. The utility of $\delta^{34}S$ data in this study is facilitated by the broad-scale nature of the research questions. Without larger sample sizes and discrimination of bacterially reduced sulphides, attempts to partition differences within the estuary would be compromised by the large variation in $\delta^{34}S$ values.

4.4.5 Discriminant function analyses (DFA)

DFAs of the stable isotope data of invertebrates (Table 4.5) contribute to the justification of the division of the study area into two regions. Although the three and four-source designs may make more sense in terms of hydrological, landscape and biological features, the two-source model provided the highest re-classification accuracies. Higher accuracy may have been achievable for the three and four source models if sample sizes were larger. Unfortunately, financial limitations on analysis made this impossible. Further discussion of the relative advantages and disadvantages in the different regional divisions may be found in section 3.1.

The results of the DFAs (Table 4.5) also justify the utility of a multiple-isotope approach in the partitioning of geographically separated material. The utility of multiple isotope tracers is valuable in improving the resolution with which end-members can be distinguished. Where single isotopes can fail to unambiguously separate source signals, simultaneous application of several isotopes often provide a solution (e.g. Newell *et* al., 1995; Peterson, 1999; Connolly *et al.*, 2004). Evidently, all three isotopes are independently powerful in their ability to re-classify the invertebrates back to their region of collection (Table 4.5).

4.4.6 Limitations and Suggestions

In this example, greater replication may have helped to further elucidate temporal trends in stable isotope signals of the invertebrates (seasonal and annual), the stability of which would add rigour to later interpretation of the stable isotope composition of muscle tissues of fishes (Chapter 5). Analysis of many more benthic invertebrate species, sediments, particulate organic matter and plankton samples would have introduced a food-web component to the study. Not only would this provide better representation of the variation in stable isotope signals available to

organisms, but would result in a fuller understanding of estuarine dynamics and the energetic pathways along which stable isotopes are transferred.

Other studies have assessed stable isotope patterns between estuarine sub-habitats. For example, Litvin and Weinstein (2003) used $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data to partition sources associated with benthic micro-algae, *Phragmites australis* red beds, *Spartina alterniflora* saltmarsh vegetation and suspended particulate matter, within the lower Delaware Bay, USA. Similarly, Newell *et al.* (1995) partitioned mangrove, planktonic and benthic micro-algal producers on the Malaysian coast, later determining the relative importance of these for panaeid prawn nutrition. Budgeting for more analyses, coupled with more extensive and intensive sampling regimes, may have revealed spatial differences in the stable isotope signatures of Thames Estuary invertebrates at a smaller scale. Although the Thames estuary consists of mostly soft substratum, subtidal and intertidal sub-habitats, including the small area of saltmarsh habitat in the outer estuarine reaches, may be distinguishable on the basis of their isotopic signatures.

Furthermore, extension of this study to consider gradients across multiple estuaries would be valuable. This study used the Thames Estuary as a proxy for other estuaries in the Southern North Sea. Confirmation of the assumptions this incurs would be of great benefit to the interpretation of results, facilitating generalisations with regard to estuarine function and dynamics.

The enriched $\delta^{15}N$ signals in the estuary and the degradation of this signal away from the region containing sewage treatment facilities led to the supposition that anthropogenic discharges are responsible. Analysis of discharge water could confirm this as the source of enrichment. Assessment of the persistence of this enrichment in the environment may also be of interest. Bedard-Haughn *et al.* (2003) discuss the potential uses of applying artificially-enriched ^{15}N tracers with isotopic signatures elevated far above natural levels, so as to be easily distinguishable, even following fractionation effects. Although expensive, ^{15}N-enriched tracers are reputed as the most reliable way to determine the flow and fate of N in a system (Hughes *et al.*, 2000; Bedard-Haughn *et al.*, 2003; Mutchler *et al.*, 2004). Enrichment of sewage discharge in this manner would allow determination of its persistence and spread through estuarine and coastal habitats.

Future research should also aim to develop standardised criteria to define regions constituting the estuarine-marine continuum. Although partially subjective

and arbitrary (see Elliott and McLusky, 2002), the definition of boundaries is necessary for studies attempting to differentiate different energetic sources. However, as discussed, the criteria used to define these boundaries can have a profound impact on the output of such research.

Finally, continued development in our understanding of factors controlling natural and human-induced variation in stable isotope signals, particularly that of $\delta^{34}S$, will aid experimental design and interpretation of data.

4.5 Conclusion

The analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data identified statistically significant differences in isotopic signatures between invertebrates caught in the estuary and those caught coastally. Estuarine invertebrates showed enriched $\delta^{15}N$ signals, but $\delta^{13}C$ and $\delta^{34}S$ signals were depleted relative to coastal invertebrates. $\delta^{13}C$ and $\delta^{34}S$ signals were fairly constant in the coastal region, but within-estuary signals exhibit enrichment with increasing salinity. Although further experiments are necessary for confirmation, enriched $\delta^{15}N$ signals within the estuary appear to be linked to sites of sewage discharge, with a gradual decrease in this signal toward the coastal region.

Discriminant function analyses support division of the continuum into a simple two-region model, re-classifying invertebrates to coastal and estuarine collection sites with 98.8% accuracy when $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data are applied simultaneously. Analyses also demonstrate the power of using multiple isotopic tracers in source partitioning applications.

5. Quantification of estuarine feeding activity by marine fishes.

5.1 Introduction

In the midst of a plethora of environmental issues, the conservation and management of estuaries is a serious and perhaps undervalued activity. Estuaries and their natural commodities support a vast number and diversity of organisms including wading birds, fishes and invertebrates, not to mention a multitude of human recreational and business ventures. Yet the majority of the world's large estuaries, and their adjoining watersheds and coastlines, continue to be the focus of urbanisation, contaminant discharge, canalisation and land reclamation.

The importance of estuarine habitat to juveniles of marine fishes has been a particular focus in research. Considerable qualitative evidence exists that suggests estuaries are important nursery habitats for many marine fishes (Pihl *et al.*, 2002). Estuarine nursery habitats are thought to provide an environment in which juvenile fishes can more easily avoid predation and feed upon a concentrated food supply (Nagelkerken *et al.*, 2000a,b). Invertebrate prey, such as polychaetes and copepods, are highly productive in estuaries and thought to support large numbers of juvenile fishes (Rogers *et al.*, 1998). However, it is also likely that estuaries are utilised as a thermal resource (Attrill and Power, 2002; 2004).

Estuaries may be important in supporting adult populations of marine fishes, which transport the estuarine organic matter that they assimilated into tissue mass in their migration from juvenile to adult habitat. This transport pathway for organic material is likely to represent a significant component in the flux of terrestrial material to the marine environment and, hence, the global cycling of carbon. The process also facilitates a useful application in understanding marine fish biology and the relative use of estuarine resources.

Previous attempts to quantify movement of marine fishes involved artificial tags and radio-telemetry (e.g. Symonds and Rogers, 1995; Metcalfe, 1997), but these were problematic and expensive. A cheaper and more promising approach may be based on stable isotope analysis. Stable isotopes are forms of an element with different atomic masses, expressed as ratios to the most commonly occurring isotope (i.e. ^{13}C:^{12}C). As described in section 4.1, the quantity in which these isotopes arrange themselves varies between different production sources (Owens, 1987; Peterson and

Fry, 1987). Thus, terrestrial, freshwater, estuarine and marine producers tend to have distinguishable isotopic signatures (Fry and Sherr, 1984; Owens, 1987; Peterson and Fry, 1987). Consequently, where these isotopic signals mix along the freshwater to marine gradient, gradual trends can be observed in organic matter originating from these sources. Although some fractionation occurs in the transfer of energy, these signals are passed along the food chain, becoming incorporated in the composition of tissues. If an organism switches to an isotopically distinct diet, usually through movement to a new vicinity or ontogenetic change in food choice, this signal will gradually be replaced with the new isotopic signal. The rate of this tissue turnover will be quicker in younger, faster growing individuals. Different body tissues (i.e. muscle, liver, heart, bone) also possess different turnover rates (Tieszen *et al.*, 1983; Lorrain *et al.*, 2002; Dattagupta, 2004). It is the exploitation of this delay in tissue turnover that allows biologists to use stable isotopes to infer use of multiple geographically and isotopically discrete food sources.

Hobson (1999) provides a review of how the origins and migration of wildlife can be traced using stable isotopes. Effective conservation, and in the case of marine fishes, effective fisheries management, usually requires an understanding of the connectivity of habitats and ecosystems through different life-history phases of a particular species. By exploiting the fact that food-web isotopic signals are reflected in the tissues of organisms, the relative importance of different sources can be assessed. For example, Fry *et al.* (2003) used isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to identify residency and movement of shrimp between different habitats in coastal marshes. Dittel *et al.* (2000) described the relative importance of marine detritus, benthic algae and phytoplankton in the diet of juvenile blue crabs (*Callinectes sapidus*) in coastal wetland habitats. The relative use of riverine, lacustrine and marine resources by salmonids was investigated using stable isotopes by Jardine *et al.* (2005). Correlations between fish length, $\delta^{13}C$ and $\delta^{15}N$ data were suggestive of energetic benefits from use of particular resources. Kline *et al.* (1998) found that stable isotopes could be used to discriminate between fish off the Alaskan Arctic coast that had fed recently in freshwater or marine environments.

For such studies to be achievable, it is first necessary to develop an understanding of the variation in the different source signals (as in chapter 4). Once these isotopic baseline signals have been established, organisms of higher trophic levels can be compared to them (following appropriate correction for trophic

fractionation) and the relative source contributions considered. While many studies have used mostly graphical analysis of source contributions, there are various models that allow values to be computed by inputting source and mixture isotope values. Although such models are subject to assumptions, they aid considerably in a biologist's endeavour to provide quantitative evidence. The most appropriate mixing model depends upon the particular application. Section 3.2 describes and discusses these options in detail and justifies the use of the single-isotope two-source IsoError model, as described by Phillips *et al.* (2005).

The primary aims of this study were to use previously collected stable isotope source data (chapter 4) to: (1) examine the reflection of source signals in 0+ fishes caught in the respective source sites, and (2) in conjunction with graphical interpretations, implement mixing models to quantify the relative contribution of estuarine food sources to the muscle tissue of adult common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and whiting (*Merlangius merlangus*) that were caught in the coastal region. An additional goal was to identify any energetic benefits arising as a result of particular feeding histories.

5.2. Materials and Methods

The fish samples for which stable isotope analyses were completed are summarised in Table 5.1. The samples selected for analysis were limited by their availability, by budgetary restriction on the number of analyses possible, and by the principal research goals.

5.2.1. Sample processing

The initial preparation of fish tissue samples, as described in section 2.3.1 involved dissection and re-freezing. Samples were then freeze-dried, until no evidence of moisture remained. Clean lids were replaced and the samples stored until ready for grinding, using pestles and mortars until they reached a flour-like consistency. To minimise potential contamination, clean conditions were maintained through the use of powder-free latex gloves, clean work surfaces and ethanol cleansing of forceps and spatulas between samples. In addition, pestles and mortars

were brushed clean of sample debris, washed, scrubbed and rinsed with ethanol, wiped with lint-free lens-tissues and dried thoroughly in an oven (~60°C) between samples. Between batches of replicate samples, pestles and mortars were left overnight in a 5% nitric acid bath, followed by two consecutive Milli-Q water rinsing baths and then thoroughly dried in an oven.

Once ground, samples were returned to the glass vials and stored. Weighing into tin capsules was delayed until shortly before sample analysis, in order to limit potential absorption of moisture. Carbon and nitrogen stable isotope analyses were measured together, whereas sulphur isotopes required different calibration standards, equipment set-up and an oxidation aid. Thus, two samples were weighed from each glass vial. For carbon and nitrogen 0.7 mg (+/- 0.2) of each sample was weighed into 5 x 3.5 mm tin capsules using a micro-balance, folded to secure the material inside and stored in a multi-well plate. The weight of material weighed into each capsule was noted to three decimal places for later use in mathematical corrections of stable isotope data output. Due to the lower concentration of sulphur in animal tissue samples, approximately 3 mg of sample plus 0.3 mg (+/- 0.03) of vanadium pentoxide (V_2O_5) as an oxidative aid were weighed into 6 x 4 mm tin capsules in preparation for analysis.

5.2.2. Sample analysis

Stable isotope analyses were carried out at SUERC, East Kilbride, using a Thermo Finnigan Deltaplus XP, coupled with a Costech instruments elemental combustion system and a Thermo Finnegan Conflo III dilution (helium) system. For carbon and nitrogen analyses, three internal calibration standards (gelatine, 14alanine, 15alanine) were interspersed among samples. The internal standards were checked periodically against secondary international standards (USGS 24, USGS 25, IAEA N1, IAEA N2, IAEA CH6 and IAEA CH7). For sulphur isotope analyses, internal calibration standards consisted of MET, HCYS, LCYS, and CP1. These were checked periodically against secondary international standards IAEA S1, IAEA S2 and NBS 123[2]

[2] USGS24 = graphite; USGS25, IAEA N1 and N2 = ammonium sulphate; IAEA CH6 = sucrose; IAEA CH7 = polythene; IAEA S1 and S2 = Ag2S; NBS 123 = ZnS.

Table 5.1 Summary of fish samples with stable isotope data used in later data analyses. Spring-caught fishes were left out of later analyses due to seasonal variation (except 4/5+ bass).

Region of collection	Species	Age	Season	n
Estuary	**Bass**	**0+**	**Autumn**	**27**
Estuary	**Bass**	**0+**	**Spring**	**7**
Estuary	**Sole**	**0+**	**Autumn**	**4**
Estuary	**Sole**	**0+**	**Spring**	**1**
Estuary	**Whiting**	**0+**	**Autumn**	**6**
Estuary	**Whiting**	**0+**	**Spring**	**5**
Coastal	**Bass**	**0+**	**Autumn**	**3**
Coastal	**Sole**	**0+**	**Autumn**	**16**
Coastal	**Sole**	**0+**	**Spring**	**6**
Coastal	**Whiting**	**0+**	**Autumn**	**24**
Coastal	**Whiting**	**0+**	**Spring**	**7**
Coastal	**Bass**	**4/5+**	**Spring**	**17**
Coastal	**Sole**	**1+**	**Autumn**	**26**
Coastal	**Sole**	**2+**	**Autumn**	**33**
Coastal	**Sole**	**3+**	**Autumn**	**24**
Coastal	**Whiting**	**1+**	**Autumn**	**17**
Coastal	**Whiting**	**2+**	**Autumn**	**9**

5.2.3. Data analysis

Prior to statistical tests, $\delta^{13}C$ data were corrected for lipid content. Lipids are ^{13}C-depleted relative to proteins by approximately 7‰ (Sweeting *et al.*, 2006). Variability in tissue lipid content can influence the interpretation of $\delta^{13}C$ data (Schmidt *et al.*, 2004). Lipid extraction techniques (e.g. Bligh and Dyer, 1959) have been found to alter $\delta^{15}N$ values, resulting in the development of arithmetic correction techniques. A mass-balance approach, originally described by Fry *et al.* (2003), was found to successfully estimate the lipid content of bass muscle tissue using the C:N data provided as part of mass spectrometry output during stable isotope analysis (Sweeting *et al.*, 2006). Here, the arithmetic calculations use a -7‰ isotopic offset of lipid relative to protein, as determined experimentally for bass (Sweeting *et al.*,

2006). It is assumed to also act a suitable approximation for sole and whiting. Differences between lipid-corrected and non-lipid-corrected $\delta^{13}C$ data are summarised in Table 5.2. Lipid bias is typically small and not expected to have a significant effect on analyses. However, for the sake of good practice, all analyses were performed using lipid-corrected data.

Table 5.2 Summary of $\delta^{13}C$ means and standard deviation comparing lipid-corrected and non-lipid-corrected data.

	Lipid-corrected		Non-lipid-corrected	
	$\delta^{13}C$ mean	$\delta^{13}C$ std. dev.	$\delta^{13}C$ mean	$\delta^{13}C$ std. dev.
Bass 0+	**-23.84**	**2.63**	**-24.29**	**2.58**
Sole 0+	**-16.58**	**2.67**	**-17.07**	**2.69**
Whiting 0+	**-17.00**	**1.38**	**-17.40**	**1.42**
Bass 4/5+	**-15.75**	**1.04**	**-16.40**	**1.03**
Sole 1+	**-15.48**	**0.49**	**-15.91**	**0.46**
Sole 2+	**-15.42**	**0.61**	**-15.82**	**0.57**
Sole 3+	**-15.35**	**0.57**	**-15.78**	**0.58**
Whiting 1+	**-16.80**	**0.93**	**-17.03**	**0.93**
Whiting 2+	**-17.12**	**1.02**	**-17.39**	**1.09**

The stable isotope values of the different 0+ fishes were explored for general patterns. ANOSIM (Analysis of Similarity, using PRIMER 6) was used to test the isotope composition of muscle tissues of young-of-the-year fishes (hereafter, 0+ fishes) for differences between seasons of capture for each species. Due to some variation in stable isotope data, the following analyses were restricted to fish collected in the autumn of 2004. This was by far the most successful sampling season, thus maximising replication. Further ANOSIM analyses were then used to test for differences between estuarine and coastally caught fish, and also between species within regions. SIMPER (Similarity Percentage analysis, using PRIMER 6) was also implemented to evaluate the relative contributions of the three stable isotopes in driving the differences between regions. These exploratory statistics were coupled with various plots to investigate clustering of groups of individuals and evaluate

likely feeding activity by 0+ fishes. The mean $\delta^{15}N$ data for each age group of each fish species were compared to that of invertebrates, to evaluate trophic relationships.

Figure 5.1 shows a summary of the $\delta^{15}N$ values of the various ages and species of fish, compared to the invertebrate source signals. Fractionation of $\delta^{15}N$ by approximately 3‰ between trophic levels is an established (though debated – e.g. Vanderklift and Ponsard, 2003) concept in stable isotope research (Owens, 1987). Therefore, this summary was produced to evaluate the relative trophic levels of the organisms so that the fish data could be corrected appropriately before further analyses.

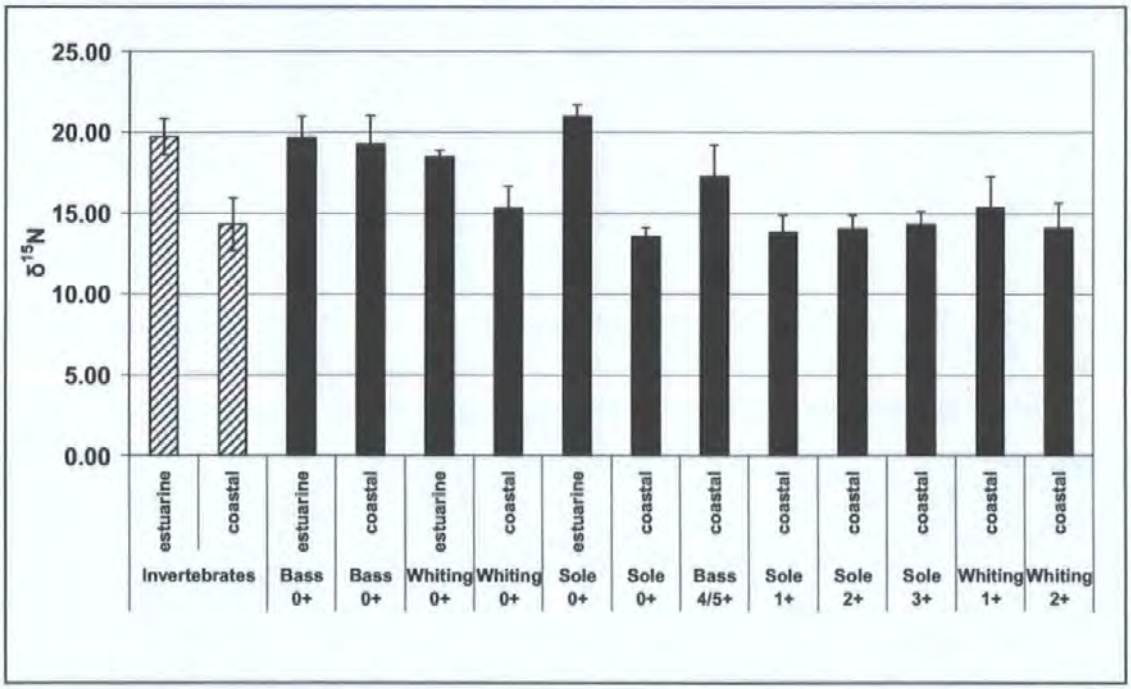


Figure 5.1 Mean $\delta^{15}N$ values (± standard deviation bars) for all fishes (filled bars), compared to that of invertebrates from estuarine and coastal regions (striped bars).

No corrections to account for trophic fractionation were applied to data for 0+ fishes. The $\delta^{15}N$ values (Figure 5.1) did not demonstrate any evidence of trophic differentiation between 0+ fishes and the invertebrates collected from the same region. Whilst trophic fractionation must be assumed for older and larger fishes, many of the 0+ fishes sampled are sufficiently small to be feeding upon very small food items of trophic levels lower than that of the invertebrates sampled in this study. The 0+ fishes were collected in the autumn, having settled out of their larval phase

only within the previous few months. Over the following four to six months 0+ fishes would grow rapidly and feed upon invertebrates of the size sampled in this study.

Discriminant function analysis (using SPSS 11.5) with a leave-one-out method was performed to classify 0+ fishes to source regions using their stable isotope values. This was done by including the 0+ fish data as ungrouped variables in the invertebrate (2 source-3 isotope) model. The regional classification for each individual sample was then compared to the actual region of capture. Although univariate normality was not achieved for all variables, and not all variances were homogenous, multivariate normality was supported using Mahalanobis distances. Although desirable for discriminant function analysis, minor deviations from normality and homogeneity are not fatal. Furthermore, as the stable isotope data contain a mixture of positive and negative numbers, the potential for application of a single transformation across the data set was limited and therefore avoided.

Spearman's rank correlations were implemented to identify relationships between estuarine contribution to muscle tissue mass (from the model output) and body mass and total length data (indicators of energetic benefit).

Although Figure 5.1 does not show $\delta^{15}N$ values indicative of trophic fractionation, it must be assumed and the data transformed for the older fishes studied here. The non-conformity of the $\delta^{15}N$ data to expected trophic responses is likely due to the mixing of several anthropogenic nutrient sources within the water column. Also, although $\delta^{15}N$ fractionation is conventionally assumed to be approximately 3‰ between trophic levels, considerable variance has been described (e.g. Pinnegar and Polunin, 1999; Vander Zanden and Rasmussen, 2001; McCuthcham *et al.*, 2003; Vanderklift and Ponsard, 2003; Yokoyama *et al.*, 2005), potentially confounding interpretation of data. Furthermore, the nature of this study involves investigation of different feeding strategies and movement between regions with different basal isotopic signatures. The utility of a stable isotope such as $\delta^{15}N$, which fractionates a lot between feeding events, could result in difficulties in differentiating between trophic effects and geochemical effects. Consequently, $\delta^{15}N$ data have been excluded from analyses of the older fishes, leaving $\delta^{13}C$ and $\delta^{34}S$ as tracers of feeding histories. The correction factors applied were based on a recent review of trophic fractionation by McCutchan *et al.* (2003) and experimental evidence from Sweeting *et al.* (in press). As the samples in the present study were of high-protein fish muscle tissues, correction factors of 1.5‰ and 1.9‰ were selected for $\delta^{13}C$ and $\delta^{34}S$ values,

respectively. $\delta^{13}C$ trophic fractionation levels have conventionally been thought to be very low, between 0 and 1‰. A compilation of published values by McCuthchan *et al.* (2003) and laboratory experiments on bass by Sweeting *et al.* (in press) support the use of 1.5‰ as a more suitable approximation of $\Delta\delta^{13}C$. This conversion was applied here and also assumed as a good approximation for sole and whiting.

The invertebrate data were corrected so as to be re-based to the fish trophic level. The invertebrates' trophic level was assumed to be 2.5, as it is unlikely they are feeding on pure phytoplankton (Jennings, *pers. comm.*). Also, the fish are assumed to be eating 1.5 trophic levels above the invertebrates, as it is likely that their diet includes small fishes (especially for bass and whiting). Hence, invertebrate data was corrected with the following equation:

$$\delta X_{\text{CORRECTED INVERTEBRATE}} = \delta X_{\text{INVERTEBRATE}} + [(2.5\text{-}1) * \Delta\delta X]$$

where δX is $\delta^{13}C$ or $\delta^{34}S$ data accordingly, and $\Delta\delta X$ is the fractionation of these over one trophic level (1.5 or 1.9‰, respectively).

An array of mixing models were applied to the data in order to quantitatively evaluate the feeding strategies of marine fishes in estuaries. A discussion of the models available and their appropriate applications is in section 3.2. The model considered most applicable to the data for this study was the IsoError single-isotope two-source model, as described by Phillips *et al.* (2001; 2005), as it is free of problems encountered non-discrete sources and allows explicit incorporation of source variability into mixing equations. Both $\delta^{13}C$ and $\delta^{34}S$ data were run independently in this model for individual fish. Using the mean and standard deviation values of the (invertebrate) source values, the output of the model provides the mean proportional contributions of the sources (estuarine and coastal) with standard error values. Scatterplots of individual fish stable isotope signatures, compared to invertebrate source signals, facilitates further interpretation of estuarine-coastal connectivity. Note, however, that where the value entered in the model does not lie between that of the sources, the model returns contribution values of <0 or >100% for the respective sources. In these cases, the source contributions were rounded to either 0 or 100% as appropriate.

While these figures show % estuarine contributions to tissues of individuals, it is also important to show the mean % contribution of estuarine signals deposited at

the population level. These data are presented, allowing comparison of relative estuarine signals persisting in the tissues of the different species and age groups.

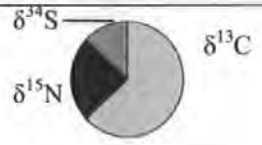
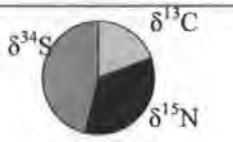
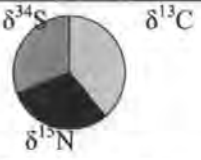
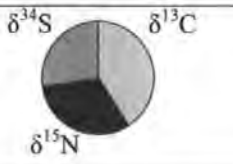
Although 1.5 and 1.9‰ correction factors were considered most appropriate for $\delta^{13}C$ and $\delta^{34}S$, respectively, in order to judge the effect of using different correction factors on the data, sensitivity analyses were implemented. For this, all model simulations were repeated multiple times using data sets that had been corrected differently. To accommodate the range of fractionations evident in the literature (McCutchan *et al.*, 2003), $\delta^{13}C$ data were corrected with factors of 0.0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1 and 2.4‰. $\delta^{34}S$ data were corrected with factors of 0.1, 0.4, 0.7, 1.0, 1.3, 1.6, 1.9, 2.2, 2.5‰.

5.3. Results

5.3.1. 0+ fishes general patterns

Seasonal differences were not found in all pair-wise comparisons, but autumn and spring samples were significantly different in the estuary for 0+ bass and whiting, and in the coastal region for 0+ sole (Table 5.3). SIMPER analyses show substantial roles of all three isotopic tracers in distinguishing similaritities and differences. It is expected that some of these results are artefacts of poor sample sizes in the spring, and that spring-caught 0+ fish may have settled from the larval phase too recently to provide a useful representation feeding activity (discussed further in section 5.4). Hence, all the subsequent analyses have had spring collected samples excluded, in order to simplify interpretation of the results, while maximising sample size.

Table 5.3 1-way ANOSIMs of seasonal differences within regions for 0+ fish. R statistics in bold are significant to the 0.05 level. The results of SIMPER analyses are included, revealing the relative roles of the three isotopic tracers in the differences/similarities inferred by the ANOSIM.

Species	Region	Pairwise test	R statistic	p value	SIMPER analysis
Bass	**Estuarine**	**Autumn Vs Spring**	0.292	**0.018**	
Sole	**Estuarine**	**Autumn Vs Spring**	**0.25**	**0.4**	
	Coastal	**Autumn Vs Spring**	0.332	**0.013**	
Whiting	**Estuarine**	**Autumn Vs Spring**	0.664	**0.02**	
	Coastal	**Autumn Vs Spring**	**0.093**	**0.221**	

Juvenile bass, sole and whiting all show significantly different stable isotope signatures between estuarine and coastally collected organisms. This difference is strongest for 0+ sole with a maximum R statistic of 1 (Table 5.4). SIMPER analyses demonstrate large contributions from all three isotopic tracers in driving this difference. $\delta^{13}C$ plays a disproportionately large role in identifying the difference between estuarine and coastal 0+ bass (Table 5.4).

Table 5.5 summarises differences and similarities between species within the estuarine and coastal regions, helping to distinguish feeding strategies of the 0+ fishes. All combinations of pairwise tests between species show significant differences, both in the estuarine and coastal regions. SIMPER analyses show that all three isotopic tracers generally play substantial roles in identifying species differences. Exceptions to this include a disproportionately small contribution by $\delta^{15}N$

in differentiating bass from whiting in the estuary, and by $\delta^{34}S$ in differentiating bass from sole in the coastal region (Table 5.5).

Table 5.4 1-way ANOSIMs of differences between regions for 0+ fish of each species. R statistics in bold are significant to the 0.05 level. The results of SIMPER analyses are included, revealing the relative roles of the three isotopic tracers in the differences/similarities inferred by the ANOSIM.

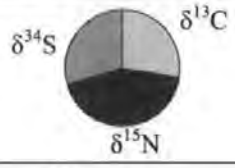
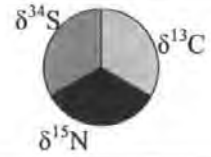
Species	Pairwise test	R statistic	Sig. level %	SIMPER analysis
Bass	**Estuarine Vs Coastal**	0.856	**0.001**	
Sole	**Estuarine Vs Coastal**	1	**0.001**	
Whiting	**Estuarine Vs Coastal**	0.456	**0.004**	

Table 5.5 1-way ANOSIMs of differences between species within regions for 0+ fishes. R statistics in bold are significant to the 0.05 level. The results of SIMPER analyses are included, revealing the relative roles of the three isotopic tracers in the differences/similarities inferred by the ANOSIM.

Region	Pairwise test	R statistic	p value	SIMPER analysis
Estuarine	**Bass Vs Whiting**	0.978	**0.001**	
	Bass Vs Sole	1	**0.001**	
	Whiting Vs Sole	1	**0.001**	
Coastal	**Bass Vs Whiting**	0.793	**0.002**	
	Bass Vs Sole	0.796	**0.029**	
	Whiting Vs Sole	0.987	**0.001**	

Many of the patterns determined by these statistics may also be seen graphically in Figure 5.2. Although estuarine sole sample size is relatively small, 0+ sole show the tightest clustering of stable isotope signatures, the most distinct signatures between estuarine and coastally caught fish, and the isotope values closest to that of their respective (invertebrate) source signals. Estuary caught 0+ bass show a very strong estuarine signal. Most coastally caught bass show intermediate isotopic signals, though sample size here is quite small. Conversely, most 0+ whiting caught coastally show quite strong coastal signals, while most of their estuary caught counterparts show relatively intermediate signals, although estuarine whiting sample size is also small.

Comparing the different species of 0+ fish within the regions with each other and the invertebrate signals also reveals some interesting patterns. Compared to bass

and whiting, variation in the signals of 0+ sole, in both the estuarine and coastal regions, is very small. Whilst showing a largely coastal signal, 0+ whiting from the coastal region show a slightly less coastal signal than that of 0+ sole, though this is not as close to an estuarine signal as 0+ bass from the coastal region (Figure 5.2).

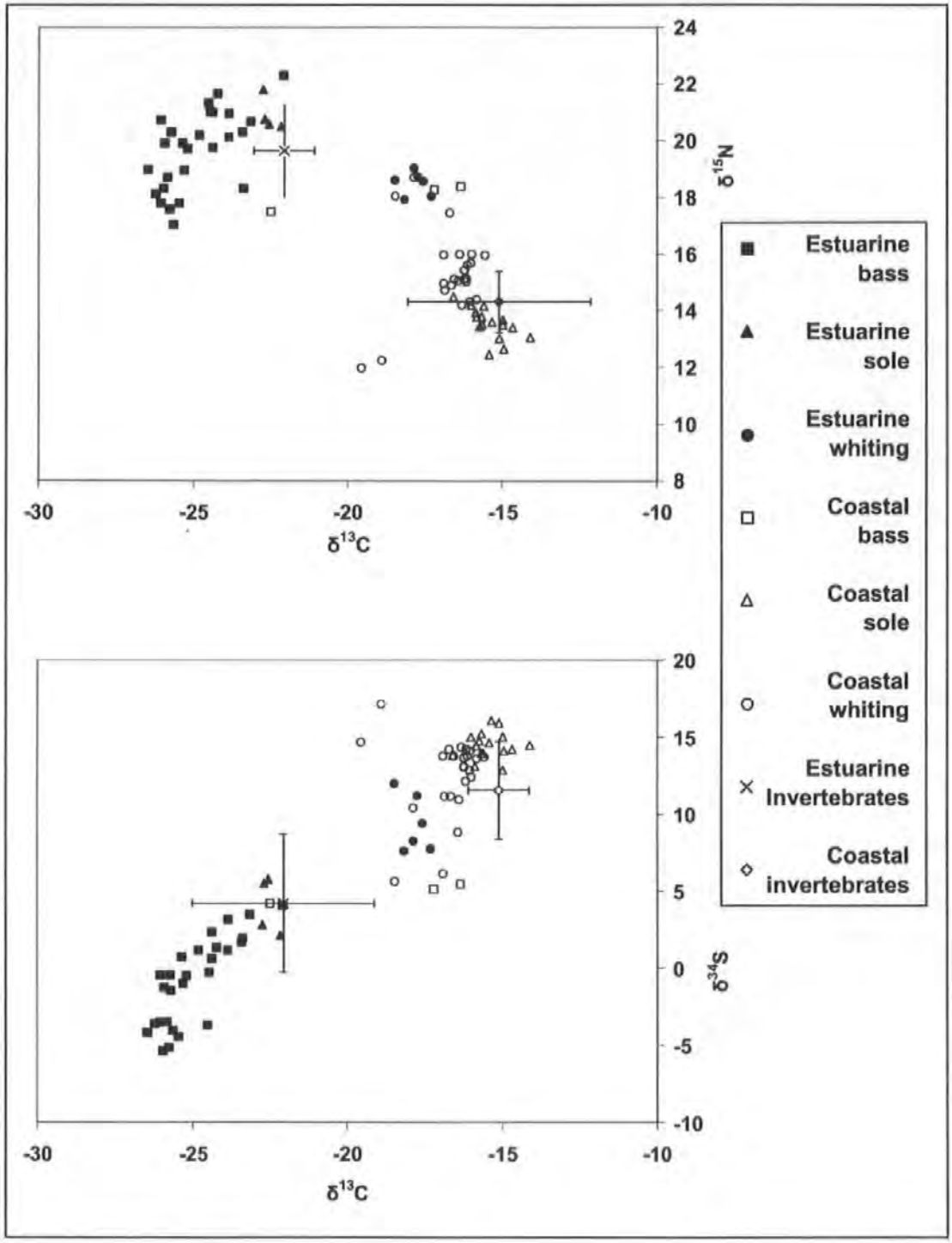


Figure 5.2 Stable isotope data for 0+ fishes caught in estuarine and coastal regions in autumn 2004. Mean values (± standard deviation) for invertebrates (from Chapter 4) from these regions are displayed.

5.3.2. 0+ fishes – Discriminant Function Analysis

Using the invertebrate 3-isotope 2-source discriminant function model described in Chapter 4, stable isotope data for 0+ fishes were run through the model as ungrouped cases, classifying them to the estuarine or coastal region according to their isotopic signatures. All 0+ sole had isotopic signatures indicative of their respective region of collection (Table 5.6, Figure 5.2). All estuarine caught 0+ bass were categorised as having estuarine isotopic signatures, as was 66.67% of those that were caught coastally, despite the largely intermediate signals seen in Figure 5.2. All estuary caught 0+ whiting were designated as having an estuarine signal, despite the apparently intermediate signals seen in Figure 5.2. Most (91.67%) coastally caught whiting individuals displayed coastal signals. Overall re-classification accuracies for bass, sole and whiting were 93.33%, 100% and 93.33%, respectively.

Table 5.6 Summary of 0+ fish classifications by discriminant function model to estuarine or coastal regions, according to their isotopic signatures. Cross-validated results presented.

Discriminant Function Classification ■ estuarine ▨ coastal		**Species**		
		Bass *D. labrax* 0+	**Sole** *S. solea* 0+	**Whiting** *M. merlangus* 0+
COLLECTION LOCATION	**Estuarine**	n = 27 fish classified to estuary = 27	n = 4 fish classified to estuary = 4	n = 6 fish classified to estuary = 6
	Coastal	n = 3 fish classified to coast = 1	n = 16 fish classified to coast = 16	n = 24 fish classified to coast = 22
% classification accuracy		**93.33%**	**100%**	**93.33%**

Spearman's rank correlations show significant positive relationships between sole's probability of estuarine reclassification and both fish body mass ($R = 0.694$; $p = 0.001$) and total length ($R = 0.591$; $p = 0.008$). Bass and whiting did not show any such relationships (Table 5.7).

Table 5.7 Summary of Spearman's rank correlations for juvenile fishes (variables: probability of assignment to estuarine group (from discriminant function); fish total length (mm) and body mass (g)). R statistics (correlation coefficients) in bold are significant to the 0.05 level.

		Bass	Sole	Whiting
Body mass	R	**-0.157**	0.694	**0.044**
	N	**30**	**20**	**30**
	p	**0.408**	**0.001**	**0.826**
Total length	R	**-0.252**	0.591	**0.055**
	N	**30**	**19**	**27**
	p	**0.179**	**0.008**	**0.786**

5.3.3. Adult fishes - Mixing models

Figure 5.3 shows the mean estuarine contributions in muscle tissue of adult fishes, calculated from the mixing models. The $\delta^{13}C$ model output identifies whiting as having the largest estuarine contributions to their muscle tissue composition, with 53.29% and 61.28% contributions for 1+ and 2+ whiting, respectively. 4/5+ Bass have a 48.44% contribution of $\delta^{13}C$ estuarine resources in their muscle tissue, while sole exhibit 40.03%, 36.40% and 35.58% contributions for 1+, 2+ and 3+ sole, respectively. Variation in $\delta^{13}C$ model outputs is relatively low. The $\delta^{34}S$ model outputs show much larger variation and different mean estuarine contributions to muscle tissues of fishes. Mean $\delta^{34}S$ estuarine contributions are largest in 4/5+ bass (86.17%), while 1+, 2+ and 3+ sole show lower estuarine contributions of 40.10%, 42.26% and 19.39%, respectively, and 1+ and 2+ whiting have 16.37% and 22.76% estuarine contributions, respectively.

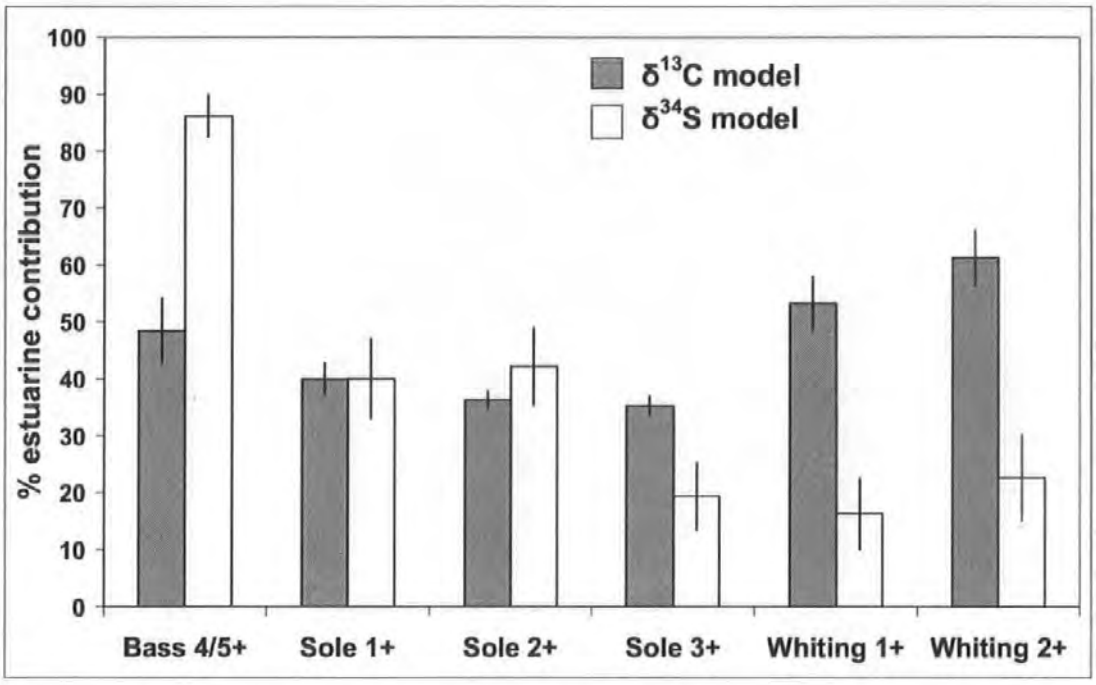


Figure 5.3 Mean percent estuarine contribution data for adult fishes (± standard error) for both $\delta^{13}C$ and $\delta^{34}S$ mixing model outputs.

Bass. From stable isotope data, bass of the 4/5+ age group that were caught coastally show a range of estuarine contributions to their muscle tissues. However, the contribution differs depending on the use of the $\delta^{13}C$ or $\delta^{34}S$ models. Firstly, there are two individuals which clearly have muscle tissue with highly estuarine isotopic signals; in this case, in terms of both $\delta^{13}C$ and $\delta^{34}S$ (Figures 5.4 and 5.5). The other specimens have $\delta^{13}C$ signals resulting in a range of 14 to 69% estuarine contribution from model data, and $\delta^{34}S$ signals resulting in estuarine contributions of 58 to 100% (Figure 5.5).

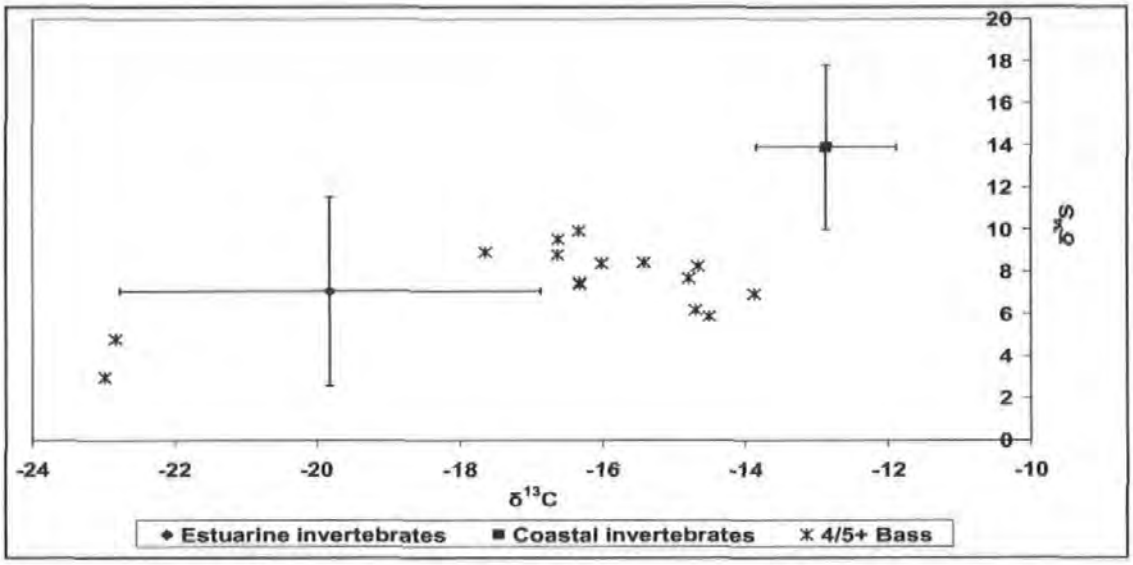


Figure 5.4 $\delta^{13}C$ and $\delta^{34}S$ data for individual 4/5+ bass compared with mean (± standard deviation) invertebrates signals. Invertebrate data corrected for trophic fractionation ($\Delta\delta^{13}C$ = 1.5‰; $\Delta\delta^{34}S$ = 1.9‰)

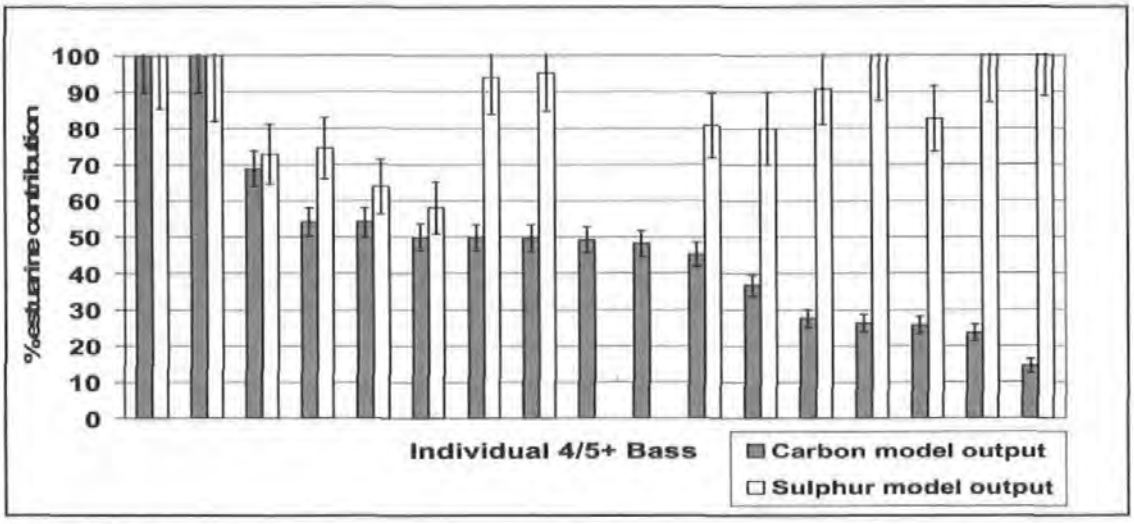


Figure 5.5 Output of single isotope IsoError models showing estuarine contributions for individual 4/5+ bass (± standard error), using $\delta^{13}C$ and $\delta^{34}S$ independently. Two samples do not have $\delta^{34}S$ data.

1+ Sole. One individual from the 26 1+ sole analysed has a strong estuarine contribution in terms of both $\delta^{13}C$ and $\delta^{34}S$. All others show relatively enriched $\delta^{13}C$. Whilst these 25 individuals are all within a $\delta^{13}C$ estuarine contribution range of 26 to 54%, they exhibit different levels of contribution in terms of $\delta^{34}S$. Some are relatively enriched (0 to 24% estuarine contribution) and others relatively depleted in $\delta^{34}S$ (71 to 92% estuarine contribution). Three individuals are relatively intermediate, with between 44 and 54% $\delta^{13}C$ estuarine contribution and between 45 and 62 % $\delta^{34}S$ estuarine contribution. As sex data were successfully acquired for all sole samples analysed, sex-specific data were plotted in Figure 5.6, although no differences between males and females were found (Mann-Whitney test, $p>0.05$; results not shown).

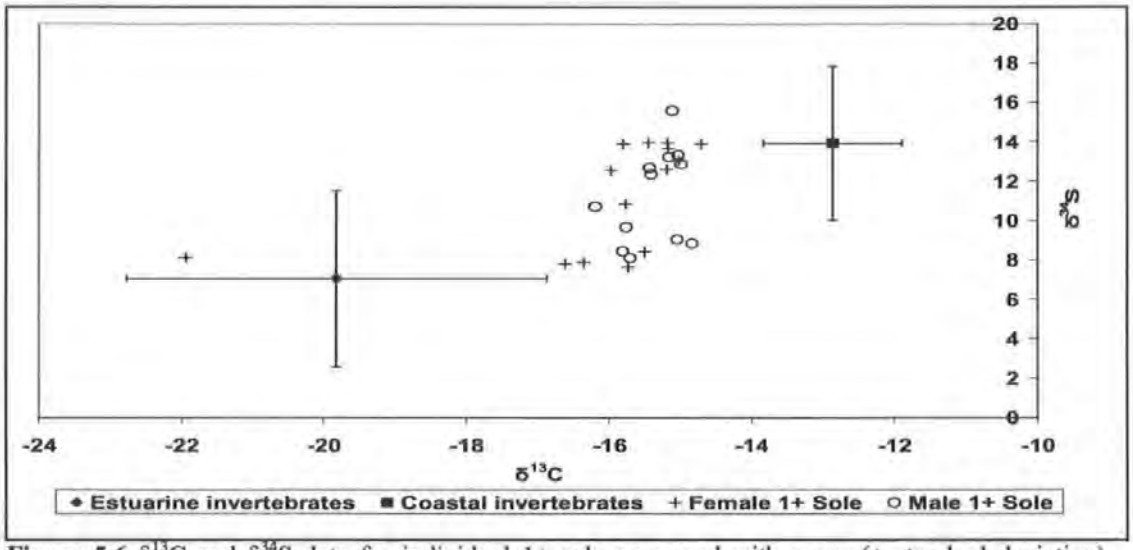


Figure 5.6 $\delta^{13}C$ and $\delta^{34}S$ data for individual 1+ sole compared with mean (± standard deviation) invertebrate signals. Invertebrate data corrected for trophic fractionation ($\Delta\delta^{13}C$ = 1.5‰; $\Delta\delta^{34}S$ = 1.9‰)

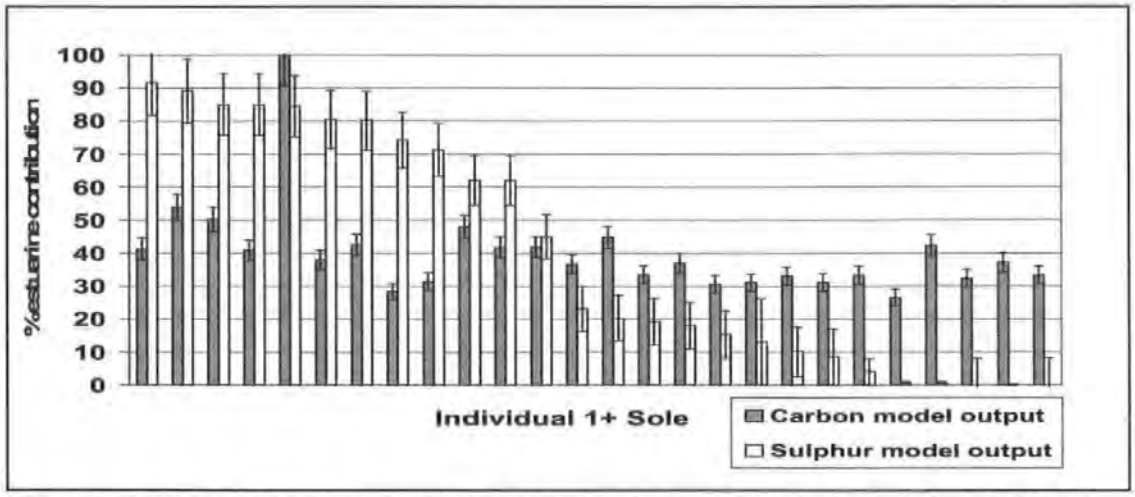


Figure 5.7 Output of single isotope IsoError models showing estuarine contributions for individual 1+ sole (± standard error), using $\delta^{13}C$ and $\delta^{34}S$ independently. One sample does not have $\delta^{34}S$ data.

2+ Sole. Most of the coastally caught 2+ sole can be categorised into two relatively discrete clusters (Figures 5.8 and 5.9), with the exception of just a few outliers. Again, both of these clusters have relatively enriched $\delta^{13}C$ (most individuals between 15 and 40% estuarine contribution). However, one group has relatively enriched $\delta^{34}S$ signals (0 to 20% estuarine contribution) and the other relatively depleted $\delta^{34}S$ signals (53 to 100% estuarine contribution, including outliers). As before, no differences were found between male and female 2+ sole (Mann-Whitney test, $p>0.05$; results not shown).

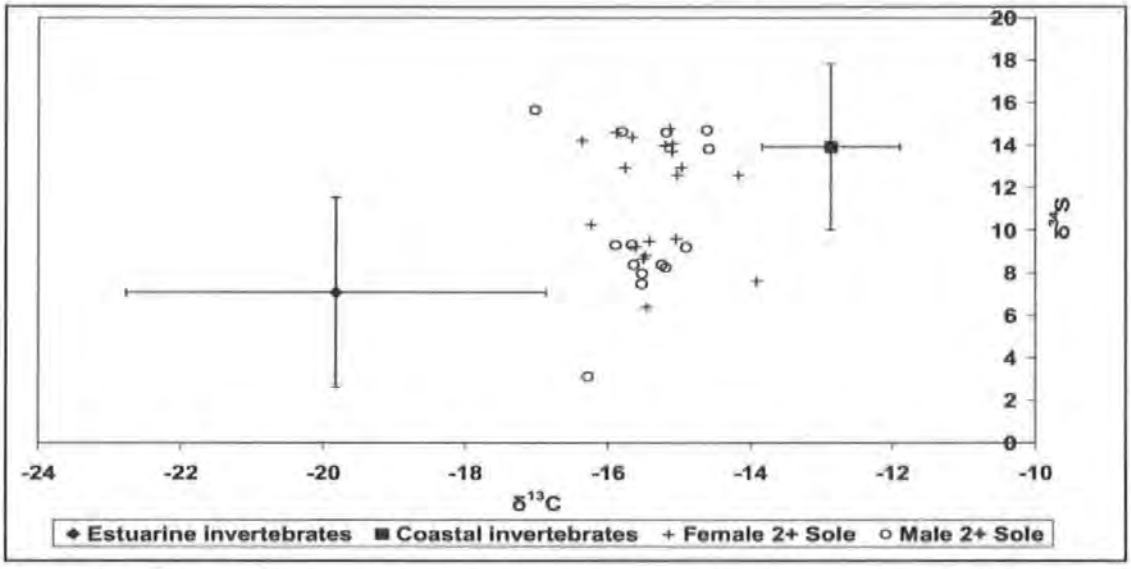


Figure 5.8 $\delta^{13}C$ and $\delta^{34}S$ data for individual 2+ sole compared with mean (± standard deviation) invertebrates signals. Invertebrate data corrected for trophic fractionation ($\Delta\delta^{13}C$ = 1.5‰; $\Delta\delta^{34}S$ = 1.9‰)

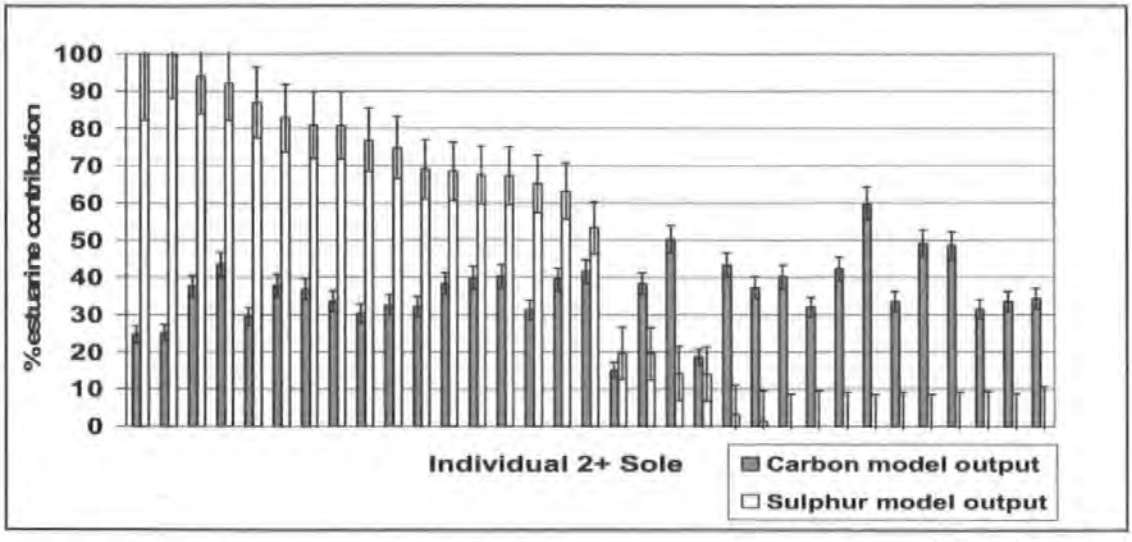


Figure 5.9 Output of single isotope IsoError models showing estuarine contributions for individual 2+ sole (± standard error), using $\delta^{13}C$ and $\delta^{34}S$ independently.

3+ Sole. The majority of the coastally caught 3+ sole show an estuarine contribution between 23 and 39% to their muscle tissues from the $\delta^{13}C$ model, and between 0 and 26% from the $\delta^{34}S$ model (Figures 5.10 and 5.11). A few individuals, however, show larger estuarine contributions. Only one individual shows similar outputs from $\delta^{13}C$ and $\delta^{34}S$ models. A weak but significant difference (Mann-Whitney tests, $p<0.05$; results not shown) was found in the $\delta^{34}S$ signals between male and female 3+ sole. Sample size is relatively small though, so this result should be interpreted cautiously.

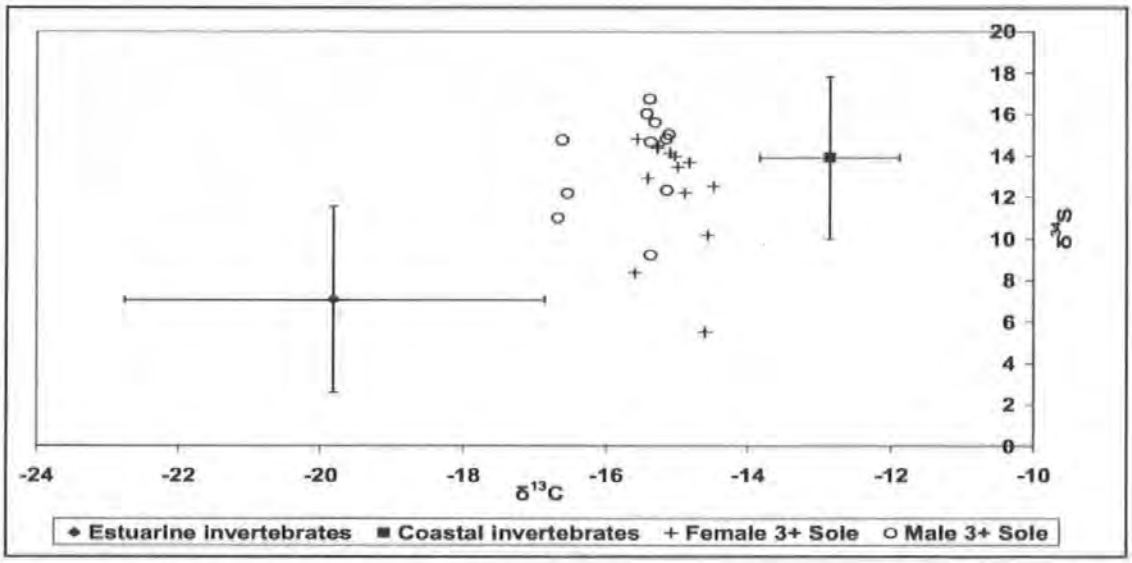


Figure 5.10 $\delta^{13}C$ and $\delta^{34}S$ data for individual 3+ sole compared with mean (± standard deviation) invertebrate signals. Invertebrate data corrected for trophic fractionation ($\Delta\delta^{13}C$ = 1.5‰; $\Delta\delta^{34}S$ = 1.9‰)

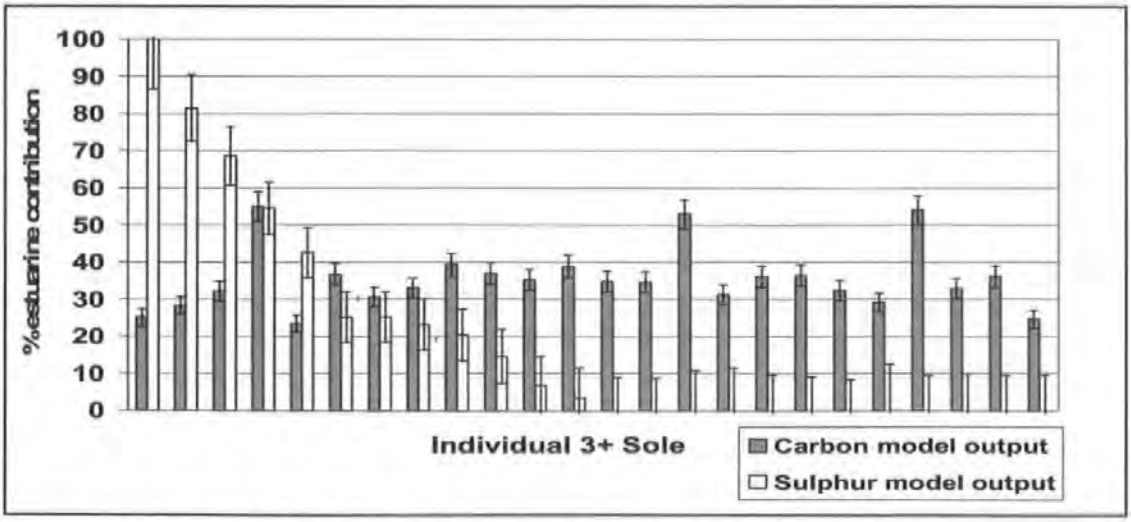


Figure 5.11 Output of single isotope IsoError models showing estuarine contributions for individual 3+ sole (± standard error), using $\delta^{13}C$ and $\delta^{34}S$ independently.

1+ Whiting. The majority of the coastally caught 1+ whiting exhibit $\delta^{34}S$ values similar to that of the coastal invertebrates, and as such have low estuarine contributions to their tissues in terms of $\delta^{34}S$ (0 to 22%) (Figures 5.12 and 5.13). Four of these have $\delta^{13}C$ values that are depleted relative to the other ten individuals. In terms of $\delta^{13}C$, these relatively depleted and enriched individuals have larger (66 to 85%) and smaller (44 to 54%) estuarine contributions to their signals, respectively. There are also three individuals which show relatively depleted $\delta^{34}S$ values and hence $\delta^{34}S$ estuarine contributions between 66 and 72%, across a $\delta^{13}C$ range of approximately 1.5‰ (Figures 5.12 and 5.13).

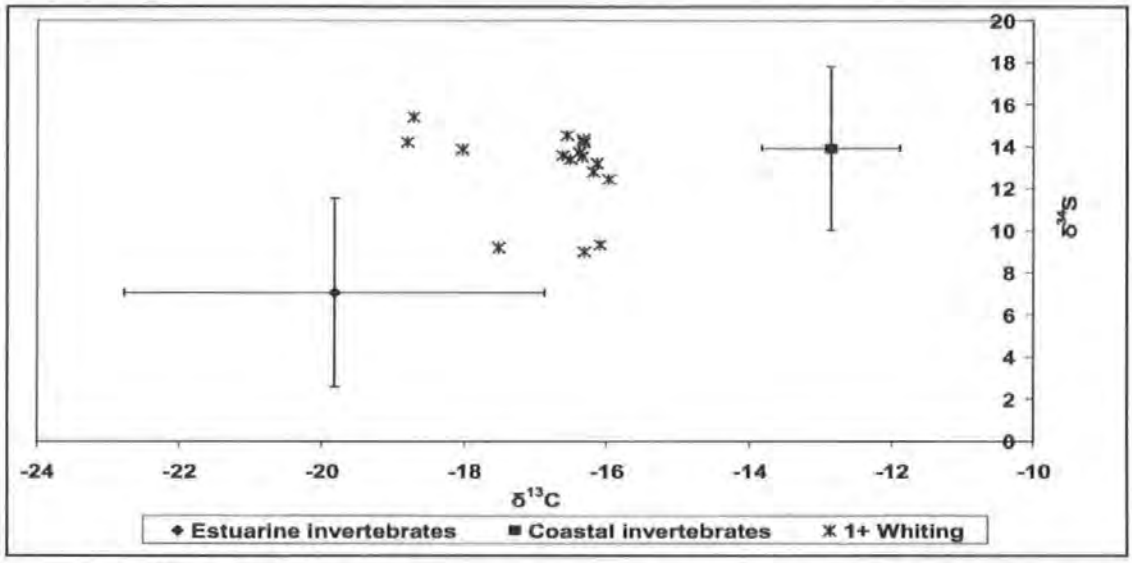


Figure 5.12 $\delta^{13}C$ and $\delta^{34}S$ data for individual 1+ whiting compared with mean (± standard deviation) invertebrates signals. Invertebrate data corrected for trophic fractionation ($\Delta\delta^{13}C$ = 1.5‰; $\Delta\delta^{34}S$ = 1.9‰).

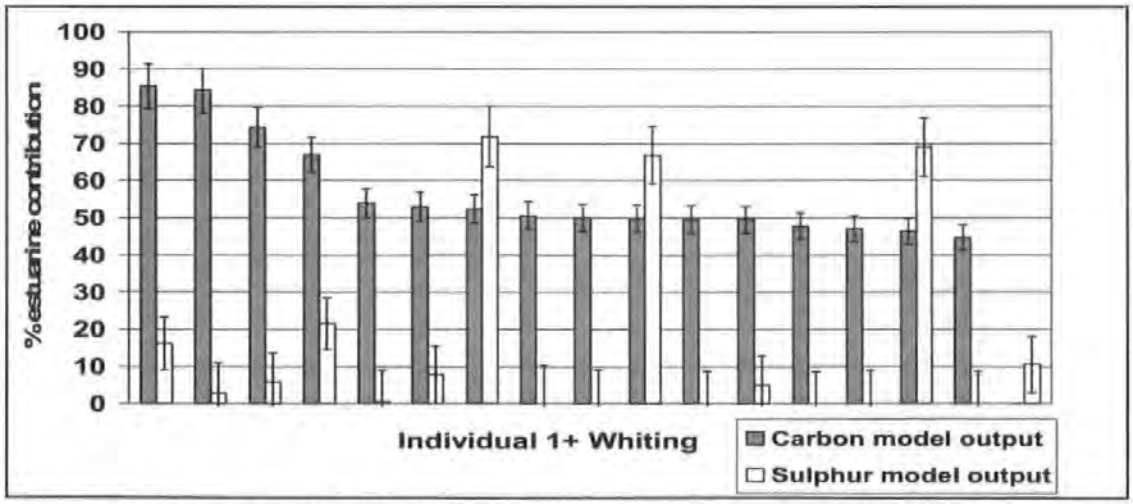


Figure 5.13 Output of single isotope IsoError models showing estuarine contributions for individual 1+ whiting (± standard error), using $\delta^{13}C$ and $\delta^{34}S$ independently. One sample does not have $\delta^{13}C$ data.

2+ Whiting. Coastally collected 2+ whiting show a range of isotopic values and estuarine contributions for both $\delta^{13}C$ and $\delta^{34}S$ data. Using $\delta^{13}C$ and $\delta^{34}S$ data, estuarine contributions range from 38 to 81% and 0 to 59%, respectively (Figures 5.14 and 5.15).

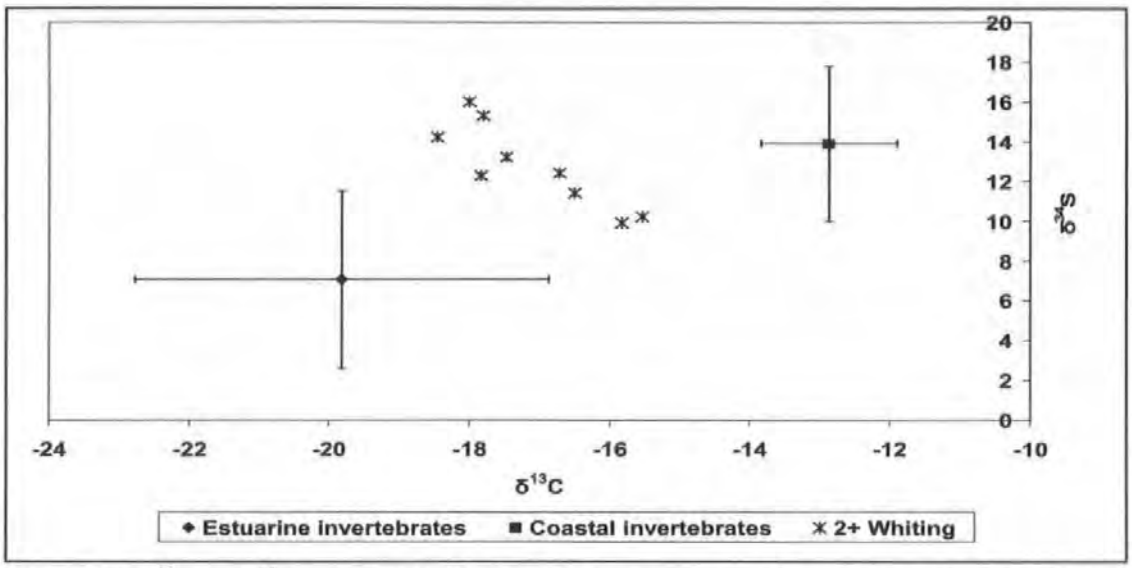


Figure 5.14 $\delta^{13}C$ and $\delta^{34}S$ data for individual 2+ whiting compared with mean (± standard deviation) invertebrates signals. Invertebrate data corrected for trophic fractionation ($\Delta\delta^{13}C$ = 1.5‰; $\Delta\delta^{15}N$ = 1.9‰)

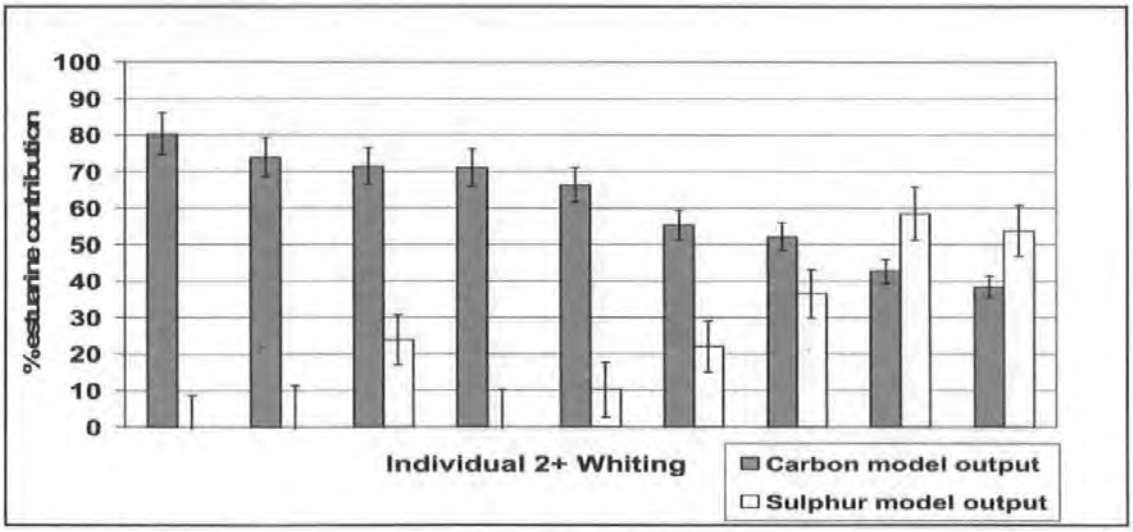


Figure 5.15 Output of single isotope IsoError models showing estuarine contributions for individual 2+ whiting (± standard error), using $\delta^{13}C$ and $\delta^{34}S$ independently.

Correlations with body size. Spearman's rank correlations (Table 5.8) show a few significant relationships between estuarine contribution (from mixing model output) and fish body mass or total length, though only with the $\delta^{13}C$ model data. There are significant positive correlations for male 1+ and 2+ sole with total length, and male 2+ sole with body mass. A positive relationship with body mass also exists for 2+ sole if gender is ignored.

Table 5.8 Summary of Spearman's rank correlations (variables: % estuarine contribution; fish total length (mm) and body mass (g)) run separately for results from $\delta^{13}C$ and $\delta^{34}S$ mixing models. R statistics (correlation coefficients) in bold are significant to the 0.05 level. (Body mass data for 4/5+ *bass* not available).

			Bass	Sole									Whiting	
	Age		4/5+	1+			2+			3+			1+	2+
	Sex		-	M+F	F	M	M+F	F	M	M+F	F	M	-	-
Body mass	$\delta^{13}C$	R	-	**-.007**	-.696	**.537**	**.366**	**.136**	.796	**.130**	**.349**	**.329**	**.074**	**.050**
		N	-	**25**	**13**	**12**	**30**	**17**	**13**	**22**	**12**	**10**	**17**	**9**
		p	-	**.974**	**.008**	**.072**	**.047**	**.602**	**.001**	**.566**	**.266**	**.353**	**.779**	**.898**
	$\delta^{34}S$	R	-	**-.124**	**-.022**	**-.243**	**-.103**	**.082**	**-.356**	**.076**	**-.321**	**.308**	**-.267**	**-.407**
		N	-	**25**	**13**	**12**	**30**	**17**	**13**	**22**	**12**	**10**	**17**	**9**
		p	-	**.555**	**.943**	**.448**	**.589**	**.753**	**.233**	**.736**	**.308**	**.387**	**.300**	**.277**
Total length	$\delta^{13}C$	R	**-.006**	**.165**	**-.340**	.598	**.156**	**-.063**	.630	**.070**	**.401**	**.142**	**.176**	**.176**
		N	**17**	**26**	**14**	**12**	**33**	**19**	**14**	**24**	**13**	**11**	**17**	**9**
		p	**.983**	**.420**	**.234**	**.040**	**.386**	**.797**	**.016**	**.744**	**.175**	**.677**	**.499**	**.651**
	$\delta^{34}S$	R	**-.048**	**-.104**	**.095**	**-.340**	**.046**	**.211**	**-.235**	**.039**	**-.281**	**.154**	**-.132**	**-.443**
		N	**16**	**26**	**14**	**12**	**33**	**19**	**14**	**24**	**13**	**11**	**17**	**9**
		p	**.860**	**.613**	**.747**	**.279**	**.800**	**.385**	**.420**	**.856**	**.352**	**.651**	**.614**	**.233**

Sensitivity Analyses. The sensitivity analyses show the responses of the $\delta^{13}C$ and $\delta^{34}S$ mixing models to their respective range of correction factors, using the mean response functions from fish considered individually in Figures 5.4 to 5.15. In the $\delta^{13}C$ model the difference between no (0‰) correction factor and that of 2.4‰ is a change in the model output of approximately 52% estuarine contribution. Every 0.3‰ increase in the correction factor results in approximately a 6% increase in the estuarine contribution calculated by the model (Figure 5.16).

Similarly, in the $\delta^{34}S$ model, every 0.3‰ increase in the correction factor results in an approximately 6% increase in estuarine contribution in the model output (Figure 5.17). Due to the highly enriched $\delta^{34}S$ signals of many of the samples (and hence the high frequency of low estuarine contributions), low correction factors result in a high frequency of 0% data points. It is of note that, where model outputs were <0% or >100%, values were rounded appropriately to 0% or 100%, hence the non-linear nature of some of these trends.

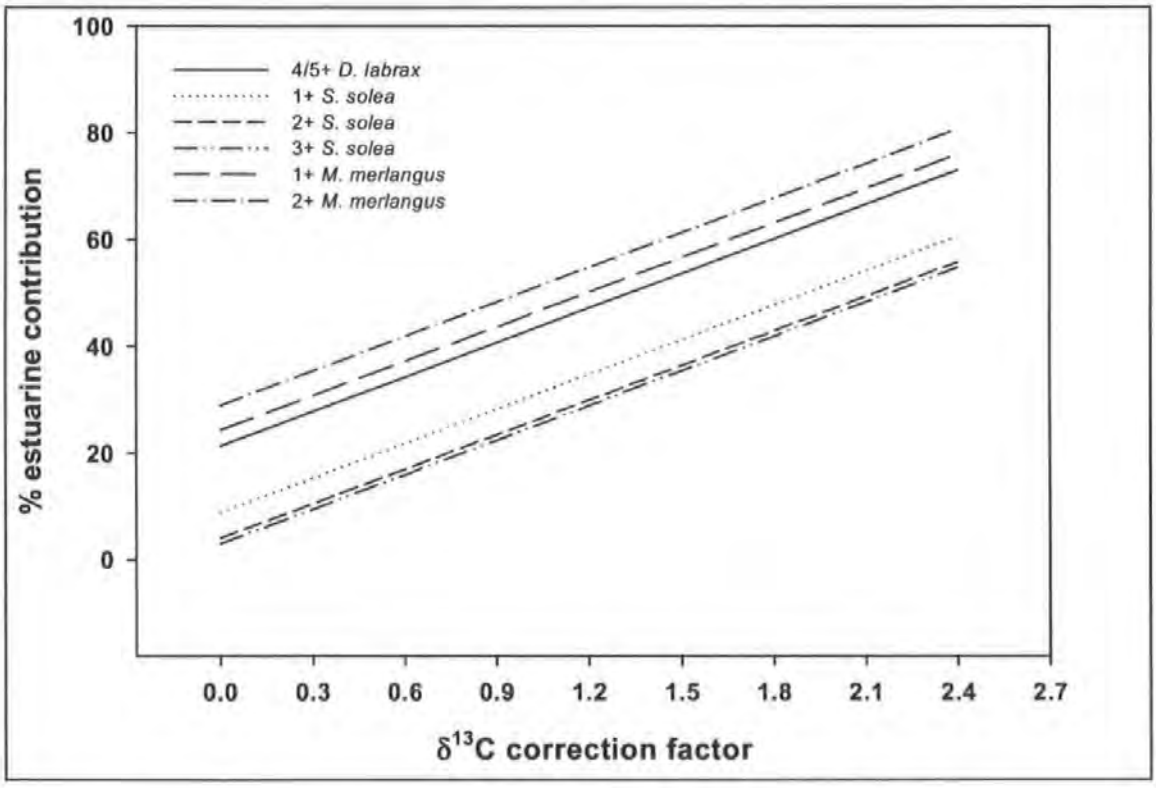


Figure 5.16 Response of IsoError mixing model output to application of different $\delta^{13}C$ trophic fractionation correction factors.

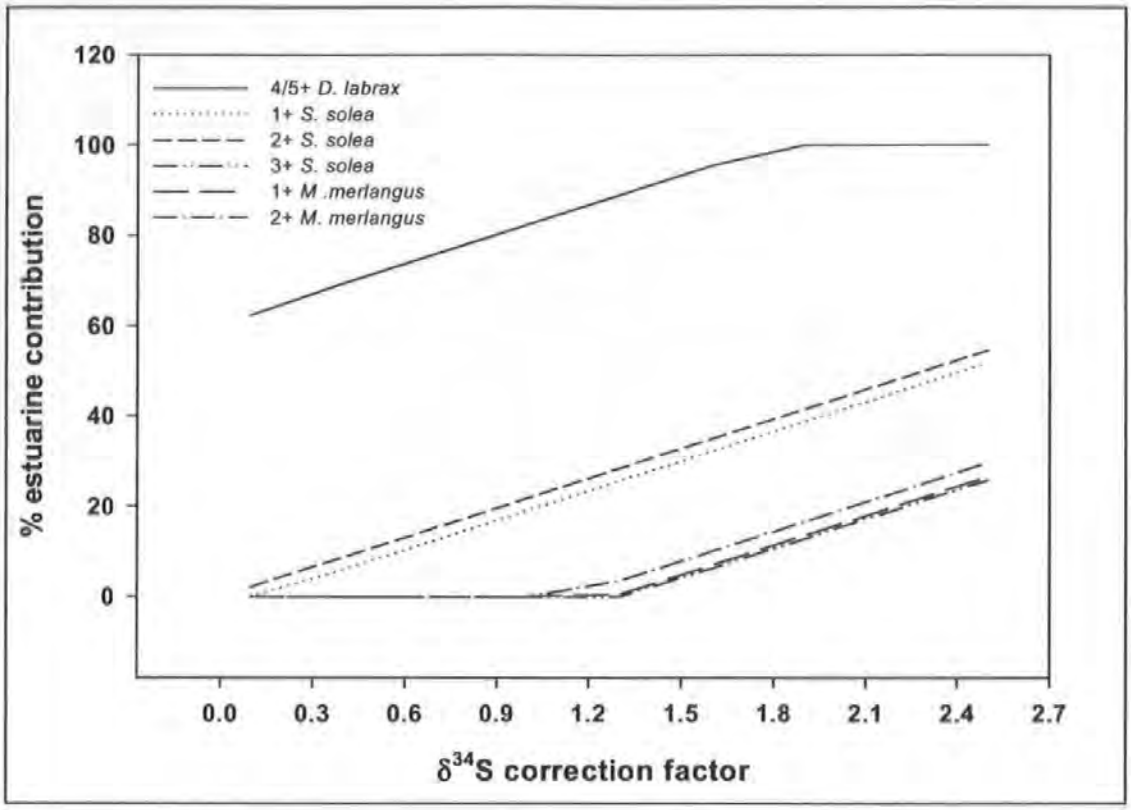


Figure 5.17 Response of IsoError mixing model output to application of different $\delta^{34}S$ trophic fractionation correction factors.

5.4 Discussion

5.4.1 0+ fishes - General patterns

Following tests for relationships between autumn and spring caught 0+ fishes, spring caught samples were excluded from further analyses. Significant differences occurred between seasonal samples for estuary caught bass and whiting, and coastally caught sole (Table 5.3). Some of the similarities and differences may be artefacts of small spring-time sample sizes. Furthermore, spring caught 0+ fishes are expected to have recently migrated from a larval phase. As the primary purpose of analysing 0+ fishes was to identify the reflection of invertebrate prey signals (Chapter 4) in 0+ fishes using the estuary or coastal region as a nursery ground, inclusion of these spring caught specimens would be inappropriate.

Analysis in PRIMER revealed strong differences between estuary and coastally caught 0+ fishes for all three species (Table 5.4). All three isotopic tracers played substantial roles in discriminating these differences. For bass, $\delta^{15}N$ was the weakest source tracer, probably due to the mid-estuarine peak in invertebrate prey signal (Figure 4.2), likely a consequence of anthropogenically enriched discharges from sewage treatment facilities (section 4.4.3). Sole and whiting were not found above Littlebrook and Barking power stations, respectively, and so are only influenced by lower estuary source signals. The differences between estuarine and coastal samples can be seen graphically in the clustering of individuals in Figure 5.2, helping to explain the relative power of the differences seen in Table 5.4. Sole show the strongest regional difference in stable isotope signature ($R = 1$; $p = 0.001$). Tight clustering of sole (Figure 5.2) demonstrates a very close relationship with mean invertebrate source signals. This is likely indicative of the lower mobility of this flatfish, and hence its inclination not to move between estuarine and coastal habitats. Limited movement between nursery grounds for young-of-the-year sole has also been described elsewhere (e.g. Coggan and Dando, 1988; Koutsikopoulos *et al.*, 1995; Riou *et al.*, 2001). It is proposed that, following their marine but relatively inshore larval phases, 0+ sole settle to the benthos, splitting into sub-populations, separated by their use of estuarine or coastal nursery habitats, with minimal mixing between these two zones.

Estuary caught bass cluster with $\delta^{13}C$ and $\delta^{34}S$ signals (more depleted than estuarine invertebrate means) that demonstrate their affinity with upper regions of the estuary (Figure 5.2). Although sample size is small, coastally caught bass appear to show relatively intermediate stable isotope signatures, suggesting that although caught coastally, some estuarine energy resources are utilised. This is likely facilitated by the greater mobility of bass and therefore their ability to move between regions.

Whiting are also highly mobile and may move easily between lower estuarine and coastal habitats. The majority of coastally caught whiting have stable isotope signals close to the mean coastal invertebrate signal, although considerable variation is seen in a number of outliers (Figure 5.2). Estuary caught whiting, however, show intermediate stable isotope signals. This suggests that, while whiting have the ability to feed in the estuary, they often exhibit a much more coastal existence. This weaker

differentiation between the use of estuarine and coastal habitats is also seen in the ANOSIM result (Table 5.4).

Inter-specific differences were also identified, within both the estuarine and coastal regions, reflecting differences in feeding preferences, mobility and physiological capabilities. Again, these differences may be seen graphically in Figure 5.2. The difference in stable isotope signals between estuarine bass and whiting may be explained by the ability of bass to utilise both upper and lower estuarine resources, and the relatively intermediate signals demonstrated by whiting caught in the lower estuary. SIMPER analyses show a limited discriminatory role for $\delta^{15}N$, likely due to the mid-estuarine peak in invertebrate prey stable isotope signals (Figure 4.2). Estuarine bass and sole also differ in their ability to cope with lower salinity regimes. It is also likely, however, that sole have a different diet, feeding largely on polychaetes and having a closer relationship with the benthic food-chain. The disproportionately large role of $\delta^{15}N$ in differentiating estuarine whiting and sole may relate to the persistence of highly enriched material deposited in soft sediments, to which sole are particularly exposed. Darnaude (2005) found that flatfish such as sole, which consume mostly deposit-feeding polychaetes, have a greater ability to exploit energy resources in terrestrial POM that has settled to the benthos.

The closer relationship of sole with the benthic food-chain partly explains differences seen in the coastal region, compared to feeding associated with the demersal and semi-pelagic habits of whiting and bass, respectively. Inter-specific differences with bass in the coastal region are partly attributed to the partial estuarine influence in the diets of these bass, although sample size for these is low and results should be interpreted cautiously.

5.4.2 0+ fishes - Discriminant Function Analyses

Discriminant function analyses classified the 0+ fishes to estuarine or coastal feeding groups, based upon their stable isotope signals relative to that of invertebrate signals described in Chapter 4. Sole were classified with 100% accuracy according to where they were caught (Table 5.6), suggesting very limited movement once settled in the nursery habitat of choice. This supports the hypothesis that two sub-populations of 0+ sole exist. To the best of my knowledge, a juvenile sole population has not previously been split into estuarine and coastal groups according to food resources.

However, the presence of 0+ sole in both estuarine and coastal habitats has been documented previously (e.g. Symonds and Rogers, 1995; Le Pape *et al.*, 2003a,b).

Despite the relatively intermediate signals seen in Figure 5.2, estuary caught whiting were all classified as having estuarine diets. Although a legitimate classification, to consider relative resource use it is beneficial to consider such analyses in conjunction with visual interpretation of the data, as in Figure 5.2. Some coastally caught whiting were classified as having estuarine diets, thus supporting the supposition that their higher mobility enables them to feed across regions. All estuary caught bass were classified with estuarine diets, demonstrating their propensity for feeding there. Despite small sample size, the coastally caught bass with an estuarine stable isotope classification (Table 5.6) is again indicative of the mobility of this species and its ability to feed across regions.

Correlations (Table 5.7) demonstrated strong energetic benefits for sole living in the estuary over those living coastally. Small sample size in the estuary requires that data should be viewed with caution. However, growth benefits from estuarine feeding are expected, due to the generally higher abundance of prey available (Darnaude, 2005). Similarly, Le Pape *et al.* (2003a) demonstrated higher growth rates of sole in estuarine than non-estuarine habitats around the Bay of Biscay in France.

Bass and whiting did not show any energetic benefits from use of estuarine energy resources. However, due to the weaker differentiation between signals discriminating resource use for these species, larger sample sizes may be necessary to reveal these effects. Alternatively, benefits arising from the use of estuaries may not be related to the energy acquired from estuarine feeding. These may include predator avoidance and reduced predator efficiency (Nagelkerken *et al.*, 2000a,b). The high turbidity of the Thames Estuary may provide 0+ fishes with the cover necessary to improve survival rates. Estuaries are also often considered to be thermal resources for fishes (Pickett and Pawson, 1994; Attrill and Power, 2002, 2004). Some evidence suggests facultative use of the Thames estuary as a thermal resource, rather than for exploitation of food resources (Attrill and Power, 2004). However, preferential use of warmer waters is expected to result in growth enhancement. Here, this is only seen in sole, not bass or whiting. An alternative suggestion is that some fish may use estuaries in the winter (when they can be colder than ocean waters) to reduce their basal metabolic rate during times of low food abundance. Hence, the benefits of estuarine use may vary between seasons.

5.4.3 Adults – Mixing models

The single-isotope two-source IsoError model applied to the data allows the generation of accurate and unique solutions to mixing applications, while providing a measure of variance and avoiding problems associated with the use of mixing polygons. Section 3.2 discussed the benefits and flaws of various mixing model options, justifying the use of the model applied here.

Although many studies (e.g. Newell *et al.*, 1995; Deegan and Garritt, 1997; Peterson, 1999; Connolly *et al.*, 2004), including Chapter 4, have encouraged the simultaneous application of multiple isotope tracers, it appears that independent consideration of isotopic tracers may be beneficial in some applications. Mixing model outputs vary greatly depending on the use of $\delta^{13}C$ or $\delta^{34}S$ data. These differences are expected to be a result of slower turnover of $\delta^{34}S$ in the tissues analysed. Isotopic turnover is defined as the isotopic change due to growth and metabolic tissue replacement associated with a change in diet (MacAvoy *et al.*, 2001). If it is the case that $\delta^{34}S$ experiences slower turnover in tissues than $\delta^{13}C$, then the isotopes may be used independently to reflect feeding activity at different time-scales. Experimental evidence for slower $\delta^{34}S$ turnover is scarce and weak (e.g. Hesslein *et al.*, 1993; MacAvoy *et al.*, 2001). However, the concentration of sulphur in the diet of most organisms is considerably lower than carbon. Due to this difference in the bulk representation of carbon and sulphur in the diet of fishes, it is reasonable to expect that overall turnover of $\delta^{34}S$ is slower (Schimmelmann, *pers. comm.*). Furthermore, Dattagupta *et al.* (2004) found that the $\delta^{34}S$ turnover of mussels growing in high sulphide environments is elevated. Thus, normal environmental conditions with low sulphur content are expected to show low $\delta^{34}S$ turnover rates. However, experiments testing this in a range of species and age/body mass classes are necessary in order to help optimise the potential of stable isotope research.

Variation in the predicted percentage contribution is also large between individuals, for both $\delta^{13}C$ and $\delta^{34}S$ models. To be able to interpret this variation as actual differences in feeding history, measures of $\delta^{13}C$ and $\delta^{34}S$ variation between individuals as a result of metabolic variability is necessary. Sweeting *et al.* (2005) assessed the standard deviation of $\delta^{13}C$ data of bass fed on a constant diet. From Sweeting *et al's* (2005) study, the value of 0.35‰ standard deviation is used to compare with variation between individuals in this study. A similar value is assumed

for sole and whiting, though laboratory experiments are necessary to confirm this. The standard deviation in $\delta^{13}C$ signals between individuals (excluding outliers) for the data are 1.04, 0.49, 0.61, 0.58, 0.93 and 1.02‰ for 4/5+ bass, 1+ sole, 2+ sole, 3+ sole, 1+ whiting and 2+ whiting, respectively. Consequently, much of the variation in $\delta^{13}C$ model output can be considered to be caused by different feeding histories.

Bass. Unfortunately, no 1+, 2+ or 3+ bass were caught during the course of this study. Bass use estuaries extensively, penetrating the upper reaches, being caught in large numbers as 0+ and 1+ fish, with catch rates declining for 2+, 3+ and 4+ fish (Claridge and Potter, 1983; Dando and Demir, 1985; Kelley, 1988; Jennings and Pawson, 1992; Potter *et al.*, 1997). It is expected that 1+ and 2+ bass would show particularly large contributions from estuarine energy resources in their muscle tissues. However, only 4/5+ bass were available for this study. Furthermore, these samples were acquired from an East Yorkshire fisherman, and therefore more likely to have resided in the Humber Estuary than the Thames. Thus, it is assumed that the basal spatial variation in stable isotope signatures seen in the Thames invertebrates is a suitable proxy for other estuaries.

Output from the $\delta^{13}C$ model suggests a mean estuarine contribution of 48.44% at the population level, while the $\delta^{34}S$ model gives a mean of 86.17%. This $\delta^{34}S$ measure is the highest for all the species and age groups studied here. If the proposed lag in $\delta^{34}S$ in fish muscle tissue is assumed, then while $\delta^{13}C$ data suggests extensive and relatively recent feeding in the estuary, $\delta^{34}S$ data demonstrates even more substantial estuarine feeding activity prior to that. Again, experimental work to develop our understanding of turnover rates of different isotopic tracers acquired through food, and in different ages or body mass classes of fishes, is necessary to optimise data interpretation.

At the individual level, variation in $\delta^{13}C$ model output is particularly evident, suggesting migratory and feeding plasticity in the recent life-history of these fishes. Variation between individuals is less extreme for $\delta^{34}S$ model output, inferring that although there is plasticity in recent migratory behaviours, early life-histories are less plastic (i.e. more consistently estuarine).

These interpretations concur with our current understanding of the life-history dynamics of European sea bass. Young of the year and 1+ bass are though to remain in the estuary, moving to deeper channels during cold winter months. The following

three years show a gradual progression to more coastal waters, visiting estuarine waters with diminishing regularity until maturity and adult life-histories are reached (Pickett and Pawson, 1994; Pawson, 1995).

Sole. Similar patterns of estuarine contribution are seen at both the individual and population level of 1 and 2+ sole. Mean estuarine contributions to the population are similar for $\delta^{13}C$ and $\delta^{34}S$ model outputs (40.03% and 40.10% for 1+ sole; 36.40% and 42.26% for 2+ sole), but variation in source contributions is considerably larger for $\delta^{34}S$ model outputs. With the exception of one extreme outlier, the limited variation between individuals from the $\delta^{13}C$ model suggests that there is not much plasticity in the recent life-histories of these fish. The very large variation in $\delta^{34}S$ model output, assuming the turnover lag theory proposed here, suggests greater plasticity in early life-history. The 0+ sole data revealed a distinct split in the nursery habitats used, with sub-populations separating into exclusive estuarine and coastally feeding groups. These mixing model data add power to the proposition that juvenile sole exhibit plasticity in nursery habitat use at a population level. Previous research has observed the presence of 0+ sole in both estuarine and non-estuarine coastal habitats (e.g. Rogers, 1993; Symonds and Rogers, 1995; Potter *et al.*, 1997; Le Pape *et al.*, 2003a,b). This is the first quantitative evidence, however, of plasticity in resource use through consumption of estuarine or coastally-sourced material. Sole are thought to stay within their nursery habitat for their first year. Most, but not all, move to coastal and offshore populations as 1+ fish (Costa *et al.*, 2002), and appear to adopt a less plastic diet, gradually replacing any estuarine isotopic signals with those derived from marine production sources.

The 3+ sole show mean estuarine contributions of 35.38% and 19.39% for $\delta^{13}C$ and $\delta^{34}S$ models, respectively. Although reduced in 3+ sole, the maintenance of a sizeable contribution of estuarine $\delta^{13}C$ signal in muscle tissue through age groups of sole suggests that turnover of structural carbon is very slow. As a slow-growing flatfish, this is a reasonable supposition. However, with the exception of a few outliers, which may represent individuals whose departure from estuarine nursery grounds were delayed, 3+ sole estuarine contributions in terms of the $\delta^{34}S$ model outputs are generally low. Unlike the 1+ and 2+ sole data, the samples no longer cluster into two groups along the $\delta^{34}S$ axis. As these fish are in their fourth year of

life, the lag in $\delta^{34}S$ signal has perhaps caught up as the estuarine signal has diminished.

Whiting. Young-of-the-year whiting have previously been described as having an affinity for estuaries (Pawson, 1995) and have been found to be the most common gadoid in the Thames Estuary (Thomas, 1998). From the end of their first year, whiting leave nurseries and adopt a relatively marine existence.

Model outputs show similar patterns for 1+ and 2+ whiting. Mean estuarine contributions to the population are 53.29% ($\delta^{13}C$) and 16.37% ($\delta^{34}S$) for 1+ whiting, and 61.28% ($\delta^{13}C$) and 22.76% ($\delta^{34}S$) for 2+ whiting. The $\delta^{13}C$ model outputs suggest considerable feeding on estuarine material, which is likely facilitated by their high mobility, regardless of how much time they actually spend in the estuary. Thus, qualitative data documenting whiting at particular sites may therefore be misleading in the interpretation of the use of estuaries. Although the mean estuarine contribution is low for $\delta^{34}S$ model output, variation is fairly large, as while most individuals appear to have largely coastal $\delta^{34}S$ signals, a few possess substantial quantities of estuarine $\delta^{34}S$ signal in their tissues. Guelinckx *et al.* (2006) suggest that individuals of amphidromous species may respond rapidly to environmental constraints or predation risk, shifting between coastal and estuarine areas in order to increase their individual fitness. Whiting may make use of their mobility, as well as tidal transport, in order to utilise estuarine habitat in this manner.

Without further experimentation, these $\delta^{34}S$ data are difficult to interpret. Due to a better understanding of $\delta^{13}C$ dynamics, the inclination is to quote $\delta^{13}C$ model output as the basis for reliably quantifying the incorporation of estuarine production into fish tissues. However, the discrepancies between isotopic tracers emphasise the need for experiments to determine turnover rates of isotopes in the tissues of fishes. In particular, development of our understanding of $\delta^{34}S$ dynamics, including variations with the physiology of different species and fish age-classes, is necessary.

Energetic benefits. Of the 0+ fishes, only sole showed energetic benefits of an estuarine stable isotope signal in muscle tissue. In the adult fishes, the only significant correlations were again for sole. However, correlations were not consistent for males and females, for $\delta^{34}S$ and $\delta^{13}C$ model data, or for body mass and total length. Significant positive correlations were found for male 1+ sole with total length ($\delta^{13}C$),

male 2+ sole with body mass and total length ($\delta^{13}C$) and for 2+ sole (males and females pooled) with body mass ($\delta^{13}C$ only), suggesting energetic benefits of estuarine feeding for these fish. Female 1+ sole showed a significant negative correlation with percent estuarine contribution, suggesting an energetic benefit of feeding coastally rather than in the estuary. It may be that the energetic benefits acquired by estuary use as juveniles are negated by the need to then migrate to marine sites as adults. However, sample sizes were too small to determine all energetic benefits with confidence. Furthermore, variation in stomach fullness could have skewed body mass data. Alternatively, benefits of utilising estuarine habitats as 0+ fish may relate to estuarine thermal properties (Attrill and Power, 2002; 2004) or predator avoidance (Nagelkerken *et al.*, 2000a,b), with estuarine feeding signals arising simply as an artefact of living there.

5.4.4 Sensitivity analyses

Until recently, isotopes of carbon and sulphur were thought to undergo sufficiently small fractionations between trophic levels as to be inconsequential. Therefore, correction factors have rarely been applied to $\delta^{13}C$ and $\delta^{34}S$ data. Recent research has highlighted many of the uncertainties and variability associated with trophic fractionation (e.g. McCutchan *et al.*, 2003; Vander Zanden and Rasmussen, 2001; Yokoyama *et al.*, 2005).

Evidently, the correction factor utilised to account for isotopic fractionation between trophic levels can have an important influence on the outcome of studies considering resource use and feeding histories (0.3‰ increase in correction factor = ~6% increase in model output). The original distribution of the data appears to play an important role in the response of the model to correction factors. In this example, much of the $\delta^{34}S$ data were highly enriched and close to the mean for the 'coastal source' signal. Consequently, different correction factors resulted in a shift in the coastal source mean that caused more or fewer of the fish data to fall outside the range of the source means. This problem may be partly related to a shortcoming in the acquisition of spatial data for the invertebrates (Chapter 4). In the coastal zone, invertebrates utilising pelagic production sources were not targeted and incorporated into the sample pool. As pelagic producers tend to be enriched in $\delta^{34}S$ relative to their benthic counterparts (Peterson, 1999), the mean coastal invertebrate $\delta^{34}S$ value may

have been set lower than it should have been to encompass the range available to the fishes. Although the deposition of pelagic material to the benthos, followed by assimilation into the benthic food-web, will have facilitated incorporation of some of the enriched signal, this is still likely to underestimate the mean coastal invertebrate $\delta^{34}S$ available in the diets of the fishes. Due to the broad-scale nature of this study, this potential flaw is not considered to seriously influence data interpretation.

Uncertainty in other manipulations of the data, for which sensitivity analyses could also be developed, would have a similar influence upon model outputs. For example, assumptions were made with regard to the trophic separation of adults from juveniles and from their invertebrate prey. Unlike bass and whiting, adult sole do not incorporate fish as a major dietary component, rather maintaining focus upon primary consumers (i.e. polychaetes and bivalves) (Darnaude *et al.*, 2004a,b; Darnaude, 2005). Therefore, adult sole may not experience as much increase in trophic level with growth. Consequently, trophic correction factors may be too large for this species, resulting in an underestimation of estuarine contribution from the mixing model.

5.4.5 Limitations and Suggestions

As is the case with all attempts to describe ecological dynamics at the population level, larger sample sizes would provide better representation of the population. In this study, data sets that would particularly benefit from further samples include 0+ sole and whiting from the estuary, 0+ bass from the coastal region, and adult bass and whiting. Sampling over a number of years, in order to account for any annual variation in stable isotope signals and their deposition along the food-chain, would also have been beneficial, but was financially and logistically unattainable. Aside from heightening the confidence with which generalisations about population dynamics can be made, improved replication may have benefited attempts to discern any energetic benefits of feeding upon estuarine or coastal nutrient sources.

In this study, there are two principal assumptions to consider. First, any annual variation in the stable isotope signatures of production sources must be assumed to be sufficiently small not to detract from the spatial variation studied here. Second, the variation in stable isotope signals of invertebrate tissues is assumed to be sufficient to encompass the variation incorporated in the diets of the juvenile fishes. It

is inevitable that these assumptions are not quite met, as other minor components of the fishes diets, such as mysids and zooplankton, were not included, and not all three invertebrate groups were available from all sampling locations. However, it is expected that they are adequately robust not to detract significantly from the rigour of the data.

The absence of pelagic invertebrates in the development of the basal map of spatial variation in stable isotope signals (Chapter 4) may have reduced the accuracy of mixing models, as pelagic producers tend to be $\delta^{34}S$ enriched relative to those in the benthos (Peterson, 1999). However, in a large-scale study such as this it is not considered as critical as it would when assessing variation in source signals at much smaller scales. Studies attempting to partition sub-habitats within the estuarine (e.g. saltmarsh, seagrass) or coastal (e.g. benthic, pelagic) regions would require much more careful targeting of known prey items. Furthermore, the high turbidity of the Thames Estuary and adjacent coastal regions is expected to limit the importance of pelagic production.

Due to tissue-specific variation in isotopic turnover rates, the analysis of multiple tissues can be beneficial in studies interested in identifying the time-scale of feeding-histories and migratory pathways (Tieszen *et al.*, 1983; Lorrain *et al.*, 2002). Where finances are not limiting, analysis of multiple tissues is recommended to help maximise the potential of stable isotope applications.

However, meaningful interpretation of such multiple-tissue data requires a quantitative understanding of the tissue-specific turnover rates. Even in single-tissue studies such as this one, to make inferences about the time-scale of feeding histories, it is necessary to know the rate at which stable isotopes are replaced following a diet-switch (Suzuki *et al.*, 2005; Guelinckx *et al.*, 2006). This study proposes that $\delta^{34}S$ signals experience considerably slower turnover, thus providing a means of partitioning the timing of changes in feeding activity with a single tissue type. Empirical testing of this should therefore be prioritised. Laboratory experiments are necessary for this, and should include analysis of species, age/size-class, tissue and isotopic tracer-specific variation in turnover rates. The interaction of turnover rates with physiological and environmental variables should also be investigated. Further reading on issues concerning isotopic turnover may be found in MacAvoy *et al.* (2001), Perga and Gerdeaux (2005) and Sweeting *et al.* (2005).

While it was possible to account for the likely metabolic variability in $\delta^{13}C$, experimental work is necessary to determine metabolically-driven differences in $\delta^{34}S$ between individuals, particularly as natural variation in $\delta^{34}S$ is greater. However, it is reasonable to assume that the particularly large magnitude differences in some of the $\delta^{34}S$ model output is a result of variation in feeding histories. It would also be of benefit to account for variability that may occur as a result of ontogeny or sex. Nevertheless, ontogenetic change in feeding habits is not anticipated for the age ranges tested here, and there does not appear to be an effect of sex for sole, for which such data was acquired.

Between-isotope variation in the degree of trophic fractionation has been realised for some time. However, recent research has accumulated evidence of substantial variation in fractionation of specific isotopic tracers. McCutchan *et al.* (2003) review variations in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ fractionations occurring with diet type, protein content of the analysed tissue, metabolic type (poikilotherm or homeotherm), nitrogenous waste type (ammonia or urea/uric acid), environment (terrestrial or aquatic), tissue-type and tissue preparatory procedures (i.e. lipid and carbonate removal). Consequently, previously assumed trophic fractionations may not be applicable across species, between individuals of a species, or even between tissues within an individual. As sensitivity analyses implemented here demonstrated, the correction factor applied to account for trophic fractionations can have a considerable bearing on mixing model results (Figures 5.16 and 5.17). Researchers should implement laboratory experiments testing diet-tissue fractionations in their study species. Effects of body size, age and environment should be included (e.g. Sweeting *et al.*, in press).

Although not considered to have a significant impact in this study, the variation imposed on $\delta^{13}C$ signals by lipids is a further issue requiring attention. Lipids are ^{13}C depleted relative to protein. The magnitude of this depletion can vary between seasons, individuals, species and tissues, potentially confounding data interpretation. The application of arithmetic correction factors has proved successful (e.g. Sweeting *et al.*, 2006) and more routine application of these should be implemented. Researchers should, however, endeavour to confirm the accuracy of these, using laboratory experiments to determine the taxon-specific lipid offsets used in the equations.

5.5 Conclusions

Young-of-the-year fishes caught in estuarine and coastal regions appear to reflect the spatial variation in stable isotope signals identified in invertebrates (Chapter 4) reasonably well. Differences between species appear to reflect feeding activity that may be related to ecological and functional differences. In particular, 0+ sole appear to split into two distinct sub-populations, separated by distinct clustering of stable isotope signatures. Sole are also the only species that appeared to receive significant energetic benefits from estuarine feeding, although bass and whiting analyses may have been limited by sample size.

In older fishes, overall estuarine contributions to muscle tissue composition are large for bass, sole and whiting. Variation is considerable, however, with large differences both within and between $\delta^{13}C$ and $\delta^{34}S$ model outputs. If the proposed lag in $\delta^{34}S$ turnover is assumed, some interesting evaluations can be made. Firstly, for bass, an affinity for estuarine nutrient sources as young juveniles is suggested, followed by greater plasticity (at the individual level) in their estuarine-coastal feeding as they grow, until adult migration patterns are adopted as approximately 5+ fish. Second, mixing model data suggest plasticity (at the population level) in the estuarine-coastal feeding of juvenile sole, reinforcing the idea of two sub-populations, followed by less plastic feeding behaviour as 1+ and 2+ fish, gradually becoming more marine. Whiting appear to exhibit plasticity (at the individual level) in their estuarine-coastal feeding habits as juvenile and adult fishes, likely owing to their high mobility. The variable plasticity of these species and age-groups of fishes is indicative of the range of habitats across the estuarine-marine gradient where they feed. Where plasticity is high, which is likely facilitated by high mobility, a fish may be more opportunistic in its feeding strategy, and may therefore be considered an estuarine-opportunist rather than an estuarine-dependent. Where this plasticity occurs at the population level, an individual may not feed at a wide range of salinity regimes, but the population does.

This study also emphasises the necessity for laboratory experiments to further our understanding of stable isotope dynamics and their utility in applications such as this. In particular, attention is required in the study of isotopic turnover rates and variation in trophic fractionation.

6. Otolith chemistry of juvenile and adult fishes.

6.1 Introduction

An ecotone is a narrow zone of rapid change between two relatively homogenous community types (e.g. lake littoral zones). In comparison, an ecocline is a large zone with a gradient of progressive change between two systems (Attrill and Rundle, 2002). A two-ecocline model, as described by Attrill and Rundle (2002), identifies estuaries as a continuum with two overlapping gradients: a declining freshwater gradient moving seaward, and a declining marine gradient moving inland. The zone where these two systems overlap is the estuary, possessing a gradient in its relative representation of its component parts: freshwater and marine water.

At its most fundamental level, the gradient from freshwater, through estuaries to fully marine habitat, is best represented by the chemical composition of the water. The marine environment has a relatively stable and predictable elemental composition. The elemental composition of freshwater, however, is heavily influenced by catchment features such as geology and land-use (i.e. agriculture, industry, residential). The influence of urbanisation and associated point and non-point source contamination is particularly evident around large estuaries.

The chemistry of a water body can become incorporated into the calcified hard parts of organisms inhabiting it (i.e. vertebrae, scales and otoliths). As such, the inhabitation of particular water bodies by fishes can be established by examination of these hard-parts. Otoliths are particularly useful in these applications as they chronologically sequence and maintain chemical signals in growth layers (see section 1.4.2). In the utility of these features, the investigation of diadromous behaviours has been common, facilitated by the large differences in otolith chemistry between end-members (freshwater and marine). Strontium (Sr), in particular, exhibits a strong gradient along the freshwater-marine continuum. For example, Secor (1982) used Sr signals to investigate anadromy in the striped bass (*Morone saxatilis*). Extensive research has been implemented on the diadromous migratory behaviours of various eel species, mostly using Sr signals in otoliths (e.g. Tsukamoto, 1998; Jessop *et al.*, 2002; Tzeng *et al.*, 2002).

More recent applications of otolith chemistry have utilised the chemical signals from particular habitats (e.g. estuaries) to identify and quantify their role as

nursery grounds and their connectivity with adult populations (e.g. Volk *et al.*, 2000; Yamashita *et al.*, 2000; Gillanders, 2002; Chang *et al.*, 2004). In order to be able to differentiate between chemical signatures in the juvenile portion of adult otoliths, it is first necessary to identify if these differences are present in juveniles residing in the various regions being studied. As well as this spatial consistency, it is also important that the chemical signals in the juvenile otoliths are temporally consistent, especially between years. Previous studies have demonstrated the potential for large fluctuations in the water chemistry of dynamic estuarine environments on scales of days and tidal cycles (Elsdon and Gillanders, 2006), as well as between years (Gillanders, 2002). These fluctuations have the potential to confound the spatial variation in some systems. In order to account for annual variation, it is recommended that juveniles be sampled over multiple years. Also, where differences exist, the adults whose juvenile residencies are assessed should be compared to juveniles of the same year-class (i.e. collected in the same year as the adults would have been juveniles) (Gillanders, 2002; Hamer *et al.*, 2003; Brown, 2006).

In this study, the time-scale of the research restricted the ability to sample over multiple years and seasons, as did various financial and logistical limitations. Thus, any temporal variation is assumed to be overwhelmed by spatial patterns, negating any discernable effects that the temporal variation may have. The large-scale of the area studied makes this expectation more reasonable, as does the location of the study in a temperate environment experiencing weaker extremes in rainfall patterns than most other similar studies. In a dynamic estuarine system such as the Thames, small-scale temporal variability in water chemistry (i.e. due to tidal cycle) may be large (e.g. Elsdon and Gillanders, 2006), but the period of time necessary for a fish to acquire a particular signal is greater than period over which such variation occurs (Elsdon and Gillanders, 2005b). Furthermore, it was not necessary for juvenile habitat tags to be unequivocally consistent, merely that they were sufficiently robust to allow accurate re-classification of individual juveniles to their region of collection. Similarly, another potential source of error comes from incomplete understanding of the manner and efficiency with which some elements are transferred from the water column to the otolith. The physiological interfaces through which the elements must travel to reach the otolith (gills, blood plasma and endolymph) may act as partial barriers. The extent to which they limit the transport of elements may vary between individuals and under different environmental conditions. However, as Thorrold *et al.*

(2001) noted, to use otolith geochemical signatures as natural tags, it is not always necessary to reconstruct the conditions exactly. Rather, it is only required that the signatures are sufficiently robust to permit the juvenile habitat of a fish to be identified. For further discussion of this topic, see Campana (1999) and de Pontual and Geffen (2002).

Investigation of juvenile habitat tags, with a view to quantification of nursery habitat use, has mostly been evident in Australian- and USA-based research. For example, Brown (2006) used the elemental composition of juvenile English sole (*Pleuronectes vetulus*) and speckled sanddab (*Citharichthys stigmaeus*) to reclassify them to the estuarine and coastal habitats they were collected from, with approximately 80% accuracy. Gillanders and Kingsford (1996) successfully reclassified blue groper (*Achoerodus viridis*) to estuarine seagrass and rocky reef habitats with 94.5% accuracy. Similarly, Forrester and Swearer (2002) reclassified juvenile California halibut (*Paralichthys californicus*) to bay and open-coast habitats with 83% accuracy, while Rosher *et al* (2001) used the same techniques to identify differences between otoliths of juvenile Pacific bluefin tuna (*Thunnus orientalis*), residing in three geographically-discrete regions, with 75-100% accuracy.

The principal aims of this study were, for common sole (*Solea solea*), European sea bass (*Dicentrarchus labrax*) and whiting (*Merlangius merlangus*), to: (1) develop techniques employed to be able to distinguish between estuarine and coastally-caught juveniles by identifying differences in otolith chemistry; (2) examine the accuracy with which multi-elemental signatures could be used to re-classify juveniles to their region of collection; (3) examine the potential of the juvenile chemical tags for each species to act as a proxy for the other species; and (4) assess the use of estuarine and coastal nursery habitats by fishes, through examination of the juvenile portion of adult otoliths.

6.2 Materials and methods

6.2.1 Sample preparation

Following dissection and removal from the fishes, otoliths were dried in multi-well plates (see section 2.3.1). Ageing of fishes from otoliths was not

undertaken for those assumed to be young-of-the-year, based upon size at age data (www.cefas.co.uk and *pers. comm.* Pickett). All the following preparation and analysis of 0+ fish otoliths was undertaken at the CQFE and LITER laboratories of Old Dominion University, Norfolk, Virginia, USA. All cleaning and preparation stages were undertaken in Class 100 metal-free clean rooms. All equipment was cleaned prior to use, maintaining stringent protocol. All probes, slides and vials were rinsed three times in Milli Q water, submersed for 24 hours in 20% nitric acid, rinsed again three times with Milli Q and allowed to dry thoroughly under a laminar flow hood.

Juveniles. For *S. solea* and *M. merlangus*, otoliths from 10 fish from both estuarine and coastal sites were selected for analysis. For *D. labrax*, otoliths from 13 estuarine and 5 coastal fish were analysed. Sample selection was restricted by fish availability and analytical costs, though samples from fish throughout the ranges of the respective regions was achieved, hence incorporating smaller-scale spatial variability. All otoliths were from fish collected in autumn 2004. Hence, although they had not yet deposited otolith material from their first winter, they had all settled to their nursery habitat of choice three to six months previously.

One otolith from each fish was cleaned of any remaining tissue using sharpened glass probes and a dissecting microscope. First, Milli Q was applied to loosen tissues, followed by 3-5 minutes of contact with ultrapure H_2O_2 solution (30% concentrate) while cleaning with the glass probes. Otoliths were then thoroughly rinsed with Milli Q to remove H_2O_2 and tissue fragments and left to dry under a laminar flow hood for 24 hours. They were then transferred to vials, which were half-filled with Milli Q and sonicated (with heat) for 5 minutes. New vials were labelled and their dry weight noted using a micro-balance. Using clean tips for each sample, most of the Milli Q was pippetted from the vials previously sonicated. The vials were then inverted to trap the otolith in the lid. Any remaining Milli Q was pipetted and the otolith transferred to the new vial without any need for contact with forceps or other potentially contaminating instruments. The vials were reweighed in order to calculate otolith weight. Two empty blank vials were also weighed for each species.

Otoliths were dissolved in concentrated, ultra-pure nitric acid and the resulting solutions diluted to 1 mL with 1% ultra-pure nitric acid. An Indium spike (internal standard) was added to give a final concentration of 2ppb. The same volumes of acid

and spike were added to the empty vials for the blanks. Prior to analysis, the vials were once again weighed, in order to calculate the solution weight and dilution factor for later mathematical corrections.

Adults. All adult *S. solea* and *M. merlangus* used in these analyses were caught in autumn 2004 from surveys trawling in coastal waters of the Southern North Sea, mostly adjacent to the Thames Estuary. Unfortunately, these sampling efforts were unsuccessful in the collection of adult *D. labrax*. Consequently, these fish were acquired through donation from a fisherman in Yorkshire. All *D. labrax* adults were collected from a coastal trawl near Bridlington, Yorkshire, in May 2005.

The number of otoliths of each species selected for analysis was restricted by their availability and financial limitations. 18 and 22 *S. solea* otoliths from age groups 1+ and 2+, respectively, were prepared for analysis. 30 *M. merlangus* otoliths from the 1+ age group, and 8 *D. labrax* from the 4/5+ age group were prepared for analysis. Unfortunately, as the bass used in this study were donated by a fisherman, the fillets and scales had been removed. Age estimates were made according to the total length of fish (compared with growth charts – *pers. comm.* Pickett, 2005) and looking at the banding visible in the otoliths. These produced very similar estimates of ages 4 or 5+. As the fish were caught in late May, some reading error is likely as the previous winter's band will have just been deposited in the otolith, making it difficult to see. Thus, these individuals have been grouped and are referred to as 4/5+ bass.

Using an Isomet saw, transverse sections (0.8 mm thick) were cut from each otolith, aligning the anterior side of the section closer to the core of the otolith. In a clean room, clean micro-slides were labelled and the sections transferred to these, embedding the posterior side of the section into clean crystalbond[3]. So as to ease accurate positioning of the laser, sections were polished using 30 and 9 μm lapping film, monitoring progress with a microscope, until the otolith core was clearly visible. The slide was then flushed with MilliQ water to remove loose fragments of otolith and crystalbond, followed by a 30 second soak in H_2O_2 and thorough rinsing with MilliQ water. Sections were then allowed to dry under a laminar flow hood. Petrographic slides were labelled on their undersides with glass-etchers. Sections

[3] Crystalbond is a mounting adhesive that melts upon heating and hardens quickly. Its chemical composition has been tested and does not contaminate the otolith.

were loosened from the crystalbond on a hotplate, transferred to petrographic slides with plastic-tipped forceps and glass probes (11 otolith sections per petrographic slide), and secured with clean crystalbond. Care was taken to ensure the side of the section for analysis (anterior side) was as flat as possible. Once the crystalbond had hardened, the petrographic slides were sonicated for 5 minutes and allowed to dry under a laminar flow hood prior to analysis.

6.2.2 Sample analysis

Juveniles. Samples were analysed by high-resolution solution-based inductively coupled plasma mass spectrometry (HR-SB-ICPMS), using an Aridus Desolvating Sample Introduction System and a Finnegan ELEMENT 2 HR-ICPMS. In order to assess instrument precision and drift, in-house calibration standards were prepared in addition to the two blanks. Elements measured were: Li, Mg, Mn, Rb, Y, Ba, La, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Pb, U, Na, P, Ca, Sc, V, Cr, Co, Ni, Cu, Zn and Sr. However, these were not all present in concentrations larger than their respective detection limits.

Adults. Samples were analysed by high-resolution laser-ablation inductively coupled plasma mass spectrometry (HR-LA-ICPMS), using an LUV266 Merchantek EO laser system and a Finnegan ELEMENT 2 HR-ICPMS. In order to assess instrument precision and drift, in-house calibration standards were prepared and introduced to the mass spectrometer in solution using an Aridus Desolvating Sample Introduction System. Elements measured were Mg, Ca, Mn, Sr, Ba, Pb, Zn, Y, Cu and Rb.

On each otolith the laser was used to ablate a line of material around the edge of the otolith, and a raster near the core of the otolith but within the first annual band (maintaining consistency in the position of the line between otoliths of each species). Rasters were positioned in an attempt to sample a region of the otolith approximately similar to layers analysed in the juvenile otoliths. Raster area dimensions were 600 x 100 μm (1900 μm long), with a 30 μm spot (diameter), 65% laser power and 16.3 μm sec^{-1} laser speed. For the ablation lines at the otolith edges this laser power and spot size were maintained. Laser speed was reduced to 6 μm sec^{-1} and the length of the line to 700 μm. Although variations may occur due to species and otolith-specific

microstructures, it is expected that these laser adjustments result in approximately equivalent sample sizes being introduced into the ICPMS (see Jones and Chen, 2003).

6.2.3 Data analysis

Juveniles. Following mathematical corrections to account for variation in dilution factors, elements found to be present at concentrations above detection limits for all samples were (detection limits in parentheses in $\mu g\ g^{-1}$): Li (0.0015), Zn (0.1985), Cu (0.0041), Ni (0.0105), Sc (0.0013), P (0.3), Na (9.41), Ba (0.003), Y (0.0001), Rb (0.0002), Mn (0.0023), Mg (0.01), Ca (0.069) and Sr (12.079). Other elements were excluded from subsequent data analyses. In order for the analyses to be consistent with most other otolith chemistry research, element concentrations were normalised to that of calcium and subsequent analyses performed using these element/Ca ratios.

Mann-Whitney tests were used to identify which element/Ca ratios had discriminatory power in distinguishing between otoliths of fish caught in estuarine and coastal regions. Although the study is in fact a continuum, it was necessary to assign a division of regions in order to quantitatively evaluate use of the estuary. This two-region model is in keeping with that used in previous work (Chapters 4 and 5 of this thesis) and is described and explained further in section 2.3.2. The element/Ca ratios demonstrating such discriminatory power were not consistent across species. In subsequent analyses, only element/Ca ratios identifying statistically significant differences between otoliths of estuarine and coastal fish were utilised. The varying levels of statistical significance provide an indication of the relative abilities of the different element/Ca ratios to discriminate between estuarine and coastal fishes.

One-way ANOSIM (analysis of similarity, in PRIMER 6) was used to assess multivariate differences in otolith chemistry (using the selected elements simultaneously) between juvenile fishes collected in the different regions. SIMPER (similarity percentage analysis, in PRIMER 6) was used to assess the relative contributions of the different elements to differences identified in the chemistry signatures.

Discriminant function analyses (DFA) with a leave-one-out method were performed to determine the accuracy with which the combinations of selected elements could be used to reclassify organisms to their region of collection. Although minor deviations from normality (univariate and multivariate) and homogeneity of

variances are not always important, in order to improve the rigour of the statistical output, data was natural log transformed. Only Mn (*M. merlangus* only), Rb (*S. solea and D. labrax*) and Sc (*D. labrax* only) still failed to acquire univariate normality. Multivariate normality was evaluated using Mahalanobis distances. Homogeneity of variances was evaluated with log determinants and Box's M statistics.

Further DFAs were conducted to test the ability of each species model to act as a proxy in reclassifying individuals of the other species. For each species model, the otolith chemistry data for the other species were entered as unclassified variables, using only element/Ca data showing significant differences between coastal and estuarine regions for both the species being applied to the model and the species used in the model design. The accuracy of the reclassifications were then compared to those from the previous DFA.

From the DFA output, the function describing the probability of a sample's assignment to the estuarine group (i.e. approximates to % estuarine signal) was entered into Spearman's rank correlations with body mass and total length data. These were performed in order to realise any relationships between measures of fish quality and inhabitation of estuarine habitat during the juvenile phase (i.e. energetic benefit).

Adults. Elements consistently found at concentrations higher than their detection limits were (detection limits in parentheses; in $\mu mol\ mol^{-1}$, except Mg/Ca and Sr/Ca in $mmol\ mol^{-1}$): Ba/Ca (0.018), Y/Ca (0.008), Rb/Ca (0.03), Mn/Ca (0.54), Mg/Ca (0.004), and Sr/Ca (0.002). Most Cu/Ca (0.7) data was above the detection limit; those below the limit have been excluded. Zn/Ca (3.1) and Pb/Ca (0.03) were consistently below the detection limits and not considered in further analyses.

Raster and line ablation data were charted for graphical interpretation of the differences between otolith chemistry of the juvenile portion of the otolith and recent growth at the otolith edge, at both the individual and population level. Mean and standard deviation data are presented and differences between otolith core and edge data examined using Wilcoxon sign rank tests.

The data ranges do not possess substantial overlap with those found in the juvenile otoliths, likely due to annual variation in water chemistry and/or differences between the techniques applied. Consequently, the juvenile data has not been used to assign adult fishes to either estuarine or coastal nursery habitats, as this would be

misleading. Hence, quantitative evaluation of estuarine use by the adult fish sampled is not achieved, but raw data is still interpretable by visual examination and some basic statistics.

6.3 Results

6.3.1 Juveniles

Mann-Whitney tests (Table 6.1) established which element/Ca ratios revealed differences in otolith chemistry between fish caught in estuarine and coastal regions. The elements possessing this discriminatory power were not consistent across species. Differences in *S. solea* were revealed with 9 elements (expressed as ratio to Ca): Sr, Sc, P, Na, Y, Rb, Mn, Mg, Li. For *M. merlangus*, 8 elements showed discriminatory power: Cu, Ni, Sc, Na, Y, Rb, Mn, Li. For *D. labrax*, only 5 elements showed differences: Sc, Ba, Rb, Mn, Li. These differences are also shown in Figure 6.1. Regrettably *D. labrax* sample size was small in coastal areas, hence the lack of tails on these box-plots.

In most cases, where differences exist between otoliths of estuarine and coastally caught fishes, the element/Ca ratio is higher in coastal than estuarine fish (Figure 6.1). An exception to this is Ba/Ca which, in *D. labrax*, is lower in coastally caught fish. For some elements, however, different species show different patterns. For Y/Ca, coastal *S. solea* show higher concentration ratios, while coastal *M. meralngus* show lower concentration ratios than their estuarine counterparts. For Rb/Ca, coastal *S. solea and M. merlangus* show higher concentration ratios than their estuarine counterparts, while *D. labrax* show the opposite.

Some other noteworthy patterns can be seen in Figure 6.1. Firstly, although Sr/Ca only shows differences between groups for *S. solea*, the median and 25th percentile values for estuarine *D. labrax* are noticeably lower than that for *S. solea* and *M. merlangus*. The same is true for Li/Ca ratios. Similarly for Ba/Ca and Rb/Ca, median and 75th percentile values for estuary *D. labrax* are noticeably higher than for *S. solea* and *M. merlangus*.

Table 6.1 Summary of Mann-Whitney tests for juvenile fishes. Grouping variable is region (estuarine (E) or coastal (C)), with N denoting sample size. p values in bold are significant to 0.05 level.

Element/Ca	*S. solea*				*D. labrax*				*M. merlangus*			
	U	N_E	N_C	p	U	N_E	N_C	p	U	N_E	N_C	p
Sr/Ca	2.0	10	10	**<0.001**	17.0	13	5	0.143	31.0	10	10	0.165
Zn/Ca	29.0	10	10	0.123	20.0	13	5	0.246	27.0	10	10	0.089
Cu/Ca	43.0	10	10	0.631	20.0	13	5	0.246	10.0	10	10	**0.002**
Ni/Ca	27.0	10	10	0.089	29.0	13	5	0.775	21.0	10	10	**0.029**
Sc/Ca	12.0	10	10	**0.003**	12.0	13	5	**0.046**	20.0	10	10	**0.023**
P/Ca	19.0	10	10	0.019	21.0	13	5	0.289	24.0	10	10	0.052
Na/Ca	0.0	10	10	**<0.001**	21.0	13	5	0.289	2.0	10	10	**0.000**
Ba/Ca	37.0	10	10	0.353	9.0	13	5	**0.019**	38.0	10	10	0.393
Y/Ca	1.0	10	10	**<0.001**	17.0	13	5	0.143	14.0	10	10	**0.005**
Rb/Ca	0.0	10	10	**<0.001**	0.0	13	5	**<0.001**	2.0	10	10	**0.000**
Mn/Ca	12.0	10	10	**0.003**	9.0	13	5	**0.019**	14.0	10	10	**0.005**
Mg/Ca	1.0	10	10	**<0.001**	16.0	13	5	.379	36.0	10	10	.315
Li/Ca	4.0	10	10	**<0.001**	5.0	13	5	**0.004**	3.0	10	10	**<0.001**

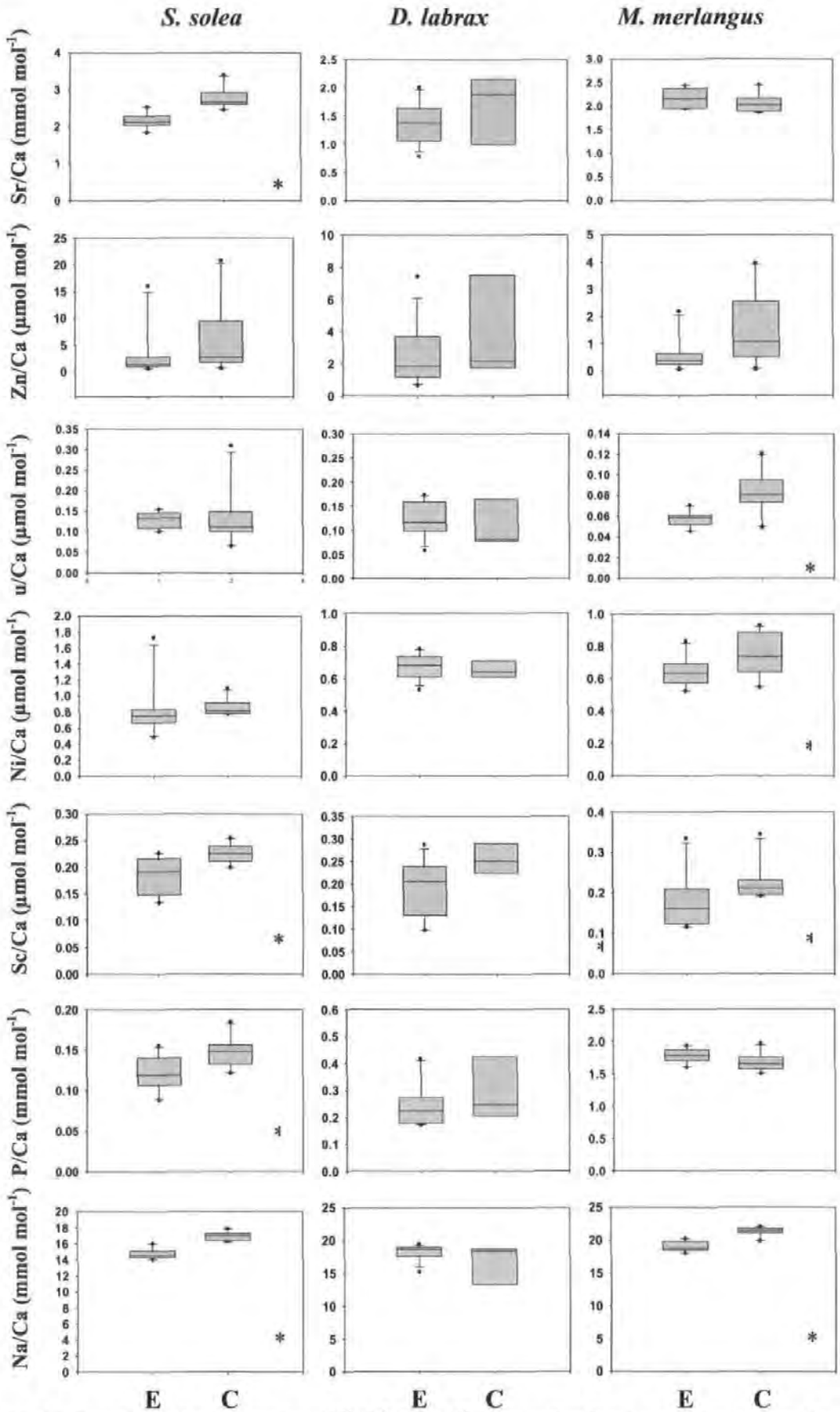


Figure 6.1. Box-plots (showing median, 10^{th},25^{th},75^{th}, 90^{th} %iles and outliers) comparing element/Ca data between fishes caught in estuarine (E) and coastal (C) regions. Asterisks indicate significance according to Mann-Whitney tests (see Table 6.1).

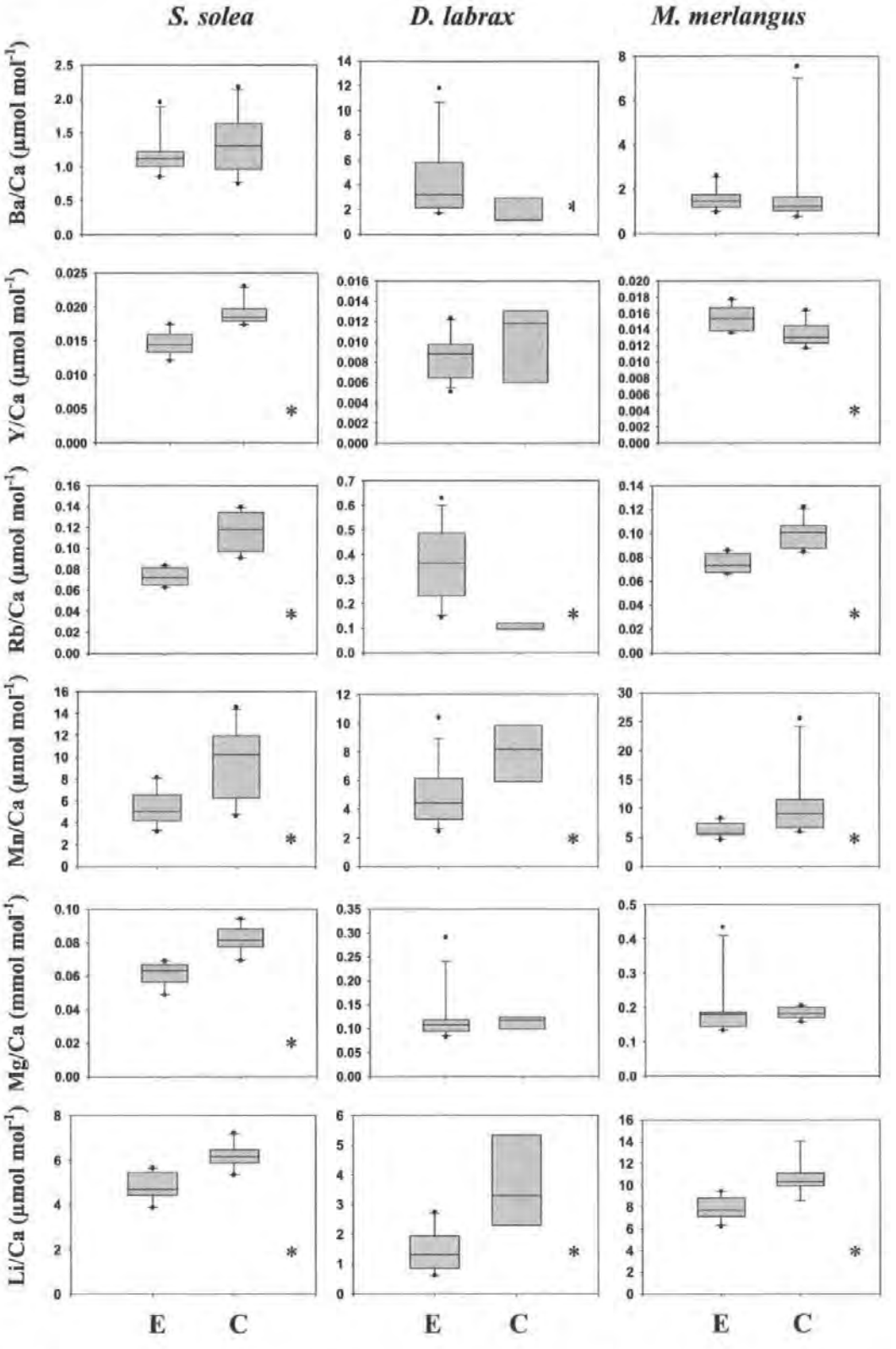


Figure 6.1 (continued). Box-plots (showing median, 10th,25th,75th, 90th %iles and outliers) comparing element/Ca data between fishes caught in estuarine (E) and coastal (C) regions. Asterisks indicate significance according to Mann-Whitney tests (see Table 6.1).

Figure 6.1 shows elemental differences between estuarine and coastally caught fishes on a case-by-case basis. ANOSIM implements the elemental data in a multivariate manner to identify the differences and their relative strengths in the elemental fingerprints of otoliths. For all three species the difference in otolith chemistry signature between estuarine and coastal fish is statistically significant. However, this difference was particularly strong in *S. solea* ($R = 0.97$; $p = 0.001$) and *M. merlangus* ($R = 0.776$; $p = 0.001\%$) (Table 6.2). SIMPER analysis assesses the relative role of different elements in identifying these differences, with all elements included in the analysis making substantial contributions to difference in chemical signatures (Table 6.2).

In DFA, the predicted classifications produced by the models determined the accuracy with which the otolith chemistry data could be used to reclassify the 0+ fishes to their region of collection (Table 6.3). All *S. solea* and *D. labrax* individuals were re-classified with 100% accuracy. Only one whiting was misclassified, giving the whiting model 95% reclassification accuracy.

Table 6.4 shows that the accuracy of the species models' was relatively poor when used as a proxy to reclassify the other species to estuarine and coastal regions.

Spearman's rank correlations (Table 6.5) show significant positive relationships between the probability of *S. solea* being reclassified to the estuarine group and both fish body mass ($R = 0.887$; $p = 0.000$) and total length ($R = 0.765$; $p = 0.000$). *D. labrax* show a statistically significant negative relationship between the probability of estuarine reclassification and fish total length ($R = -0.486$; $p = 0.041$). No other significant correlations were found.

Table 6.2 1-way ANOSIM results, assessing the power with which a combination of elements (those with significant outputs from Mann-Whitney tests only) can identify the differences between juveniles collected from estuarine and coastal sites. R statistics (*correlation coefficients*) in bold are significant to the 0.01 level.

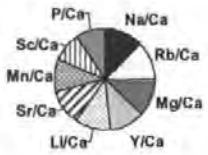
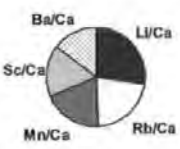
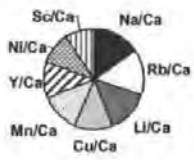
	Sole	**Bass**	**Whiting**
$N_{ESTUARINE}$	10	13	10
$N_{COASTAL}$	10	5	10
R	**0.97**	**0.459**	**0.776**
p	0.001	0.009	0.001
SIMPER analysis results			

Table 6.3 Summary of 0+fish reclassifications by discriminant function model to estuarine or coastal regions, according to their otolith chemistries. Cross-validated results are presented.

Discriminant Function Classification  **estuarine** **coastal**		**Species**		
		Sole ***S. solea*** **0+**	**Bass** ***D. labrax*** **0+**	**Whiting** ***M. merlangus*** **0+**
COLLECTION LOCATION	**Estuarine**	n = 10 fish classified to estuary = 10	n = 13 fish classified to estuary = 13	n = 10 fish classified to estuary = 10
	Coastal	n = 10 fish classified to coast = 10	n = 5 fish classified to estuary = 5	n = 10 fish classified to estuary = 9
% reclassification accuracy		**100%**	**100%**	**95%**

Table 6.4 Summary of 0+ reclassifications, testing the % accuracy with which each species model can reclassify the other species (i.e. as a proxy), compared to reclassifying its own species. Only variables (ln element:Ca data) showing significant differences between coastal and estuarine regions for both the species being applied to the model and the species used in the model design were used.

Model used	Species applied		
	Sole	**Bass**	**Whiting**
S. solea	100%	33.89%	65%
D. labrax	50%	100%	50%
M. merlangus	80%	22.22%	95%

Table 6.5 Summary of Spearman's rank correlations for 0+ fishes (variables: probability of assignment to estuarine group (from discriminant function); fish total length (mm) and body mass (g)). R statistics (correlation coefficients) in bold are significant to the 0.05 level.

		S. solea	***D. labrax***	***M. merlangus***
Body mass	**R**	**0.887**	-0.389	-0.089
	N	20	18	19
	Sig.	<0.001	0.111	0.716
Total length	**R**	**0.765**	**-0.486**	-0.020
	N	20	18	19
	Sig.	<0.001	0.041	0.935

6.3.2 Adults

Due to the more restrictive limits of detection associated with laser ablation applications, the suite of elemental data from adult otoliths is relatively limited. However, comparison of raster data from the juvenile portion of the otolith with ablation lines from near the otolith edge (recently deposited material) reveals some interesting trends, at both the population and individual level (Figure 6.2). Trends in elemental ratios are variable, both between and within species.

Wilcoxon sign rank tests (Table 6.6) identify element/Ca data sets that show a significant difference between the juvenile region of otoliths and the otolith edge. For

1+ and 2+ sole, significant differences exist with Mn/Ca, Cu/Ca, Y/Ca, Mg/Ca and Ba/Ca. 4/5+ bass show significant differences with Mn/Ca, Ba/Ca, Sr/Ca and Y/Ca data, while 1+ whiting show differences with all elements included in the analysis. Where the significant differences are found, the mean element/Ca concentration is generally lower at the edge than in the centre of the otolith, except for Sr/Ca, Cu/Ca and Y/Ca data.

It is also often the case that the portion of the otolith showing the larger mean element/Ca value also exhibits larger variation (Figure 6.2). Examination of individual data points shows that the degree of difference between otolith core and edge data is not consistent between individuals in a sample population. Some individuals show very small differences between the otolith growth layers, while others show very large differences (Figure 6.2).

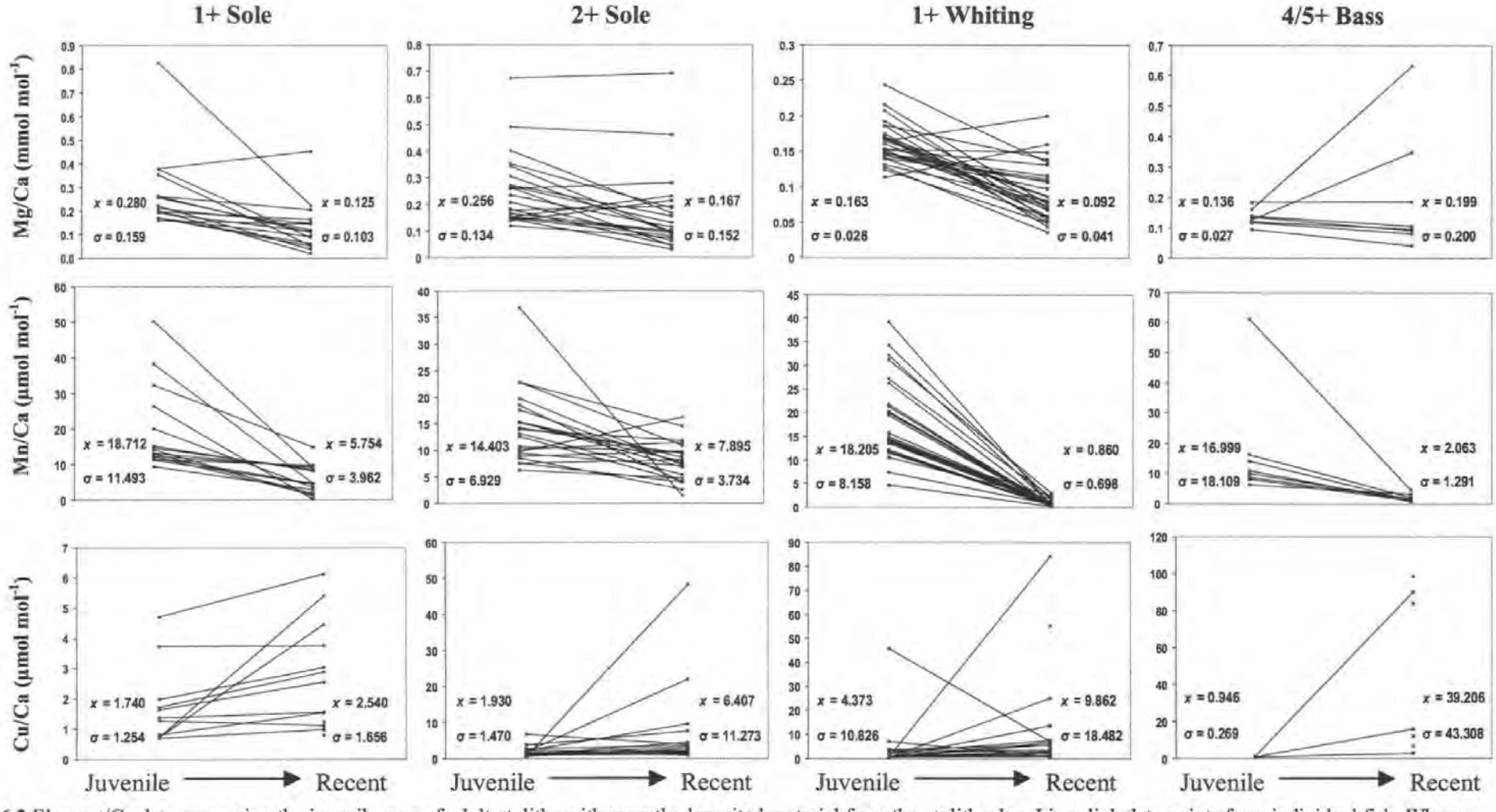


Figure 6.2 Element/Ca data comparing the juvenile core of adult otoliths with recently deposited material from the otolith edge. Lines link data points from individual fish. Where data points are not linked, the corresponding juvenile or recent data point was below the LOD and therefore removed from the data set (x = mean; σ = std. dev.)

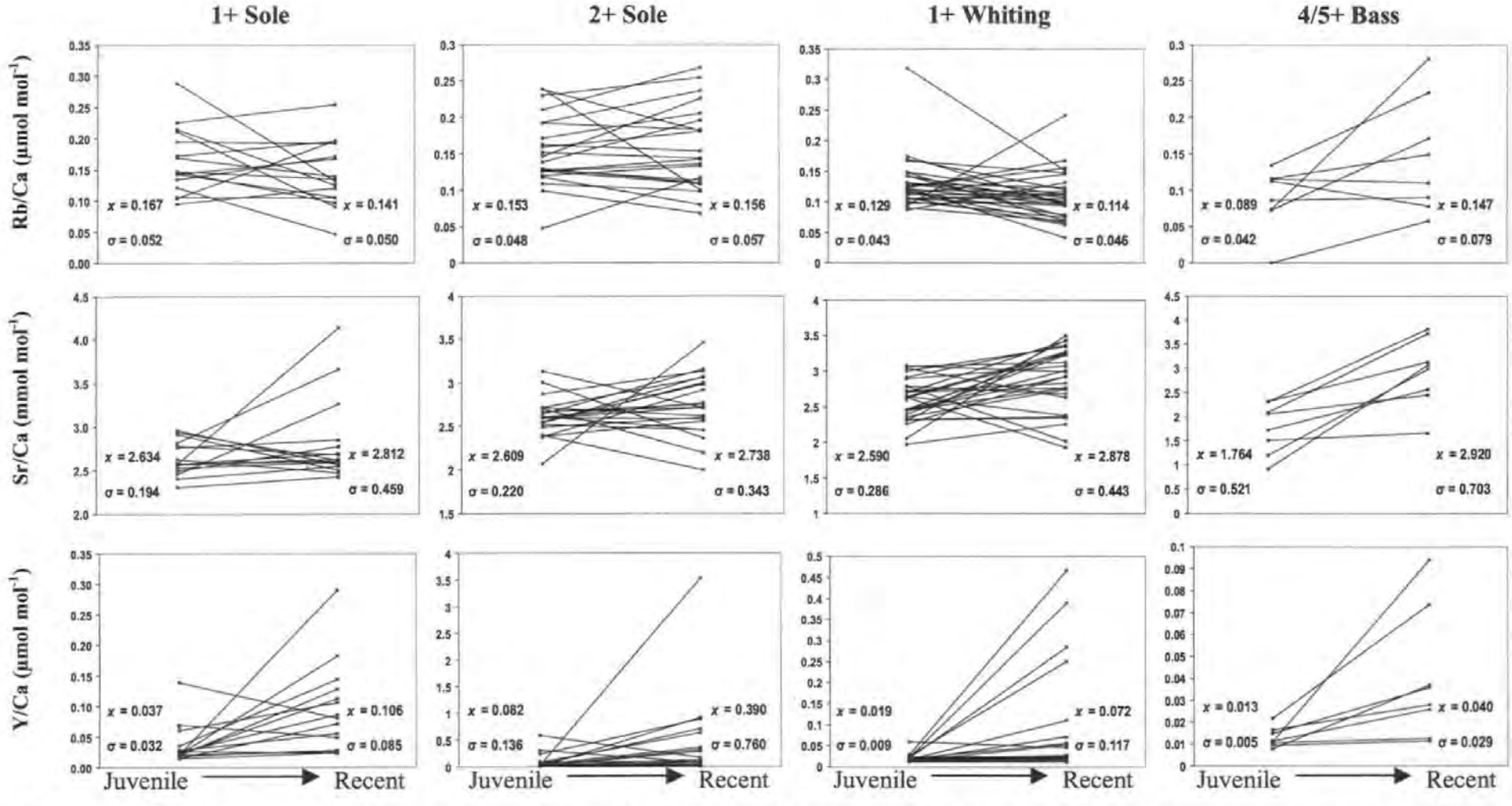


Figure 6.2 (cntd) Element/Ca data comparing the juvenile core of adult otoliths with recently deposited material from the otolith edge. Lines link data points from individual fish. Where data points are not linked, the corresponding juvenile or recent data point was below the LOD and therefore removed from the data set (x = mean; σ = std.dev.)

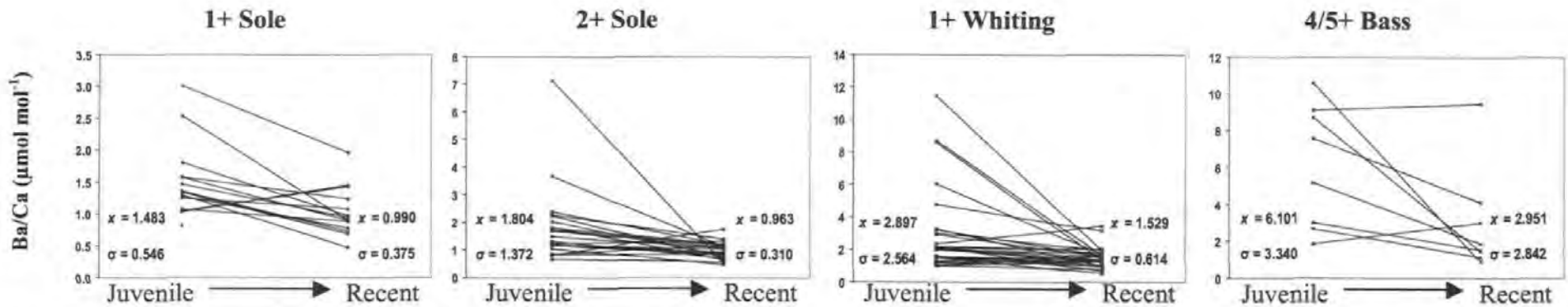


Figure 6.2 (cntd) Element/Ca data comparing the juvenile core of adult otoliths with recently deposited material from the otolith edge. Lines link data points from individual fish. Where data points are not linked, the corresponding juvenile or recent data point was below the LOD and therefore removed from the data set (x = mean; σ = std.dev.)

Table 6.6 Summary of Wilcoxon sign rank tests for adult otoliths. Variables refer to otolith portion: juvenile core (J) or otolith edge (E).
p values in bold are significant to the 0.05 level.

	1+ Sole				2+ Sole				1+ Whiting				4/5+ Bass			
Element/Ca	**Z**	**N_J**	**N_E**	**p**	**Z**	**N_J**	**N_E**	**p**	**Z**	**N_J**	**N_E**	**p**	**Z**	**N_J**	**N_E**	**p**
Mg/Ca	-3.385	17	17	**0.001**	-3.230	22	22	**0.001**	-4.453	30	30	**<0.001**	-0.280	8	8	0.779
Mn/Ca	-3.622	17	17	**<0.001**	-3.523	22	22	**<0.001**	-4.478	30	30	**<0.001**	0-2.521	8	8	**0.012**
Cu/Ca	-2.904	12	17	**0.004**	-2.482	17	19	**0.013**	-2.249	21	28	**0.025**	-1.604	3	8	0.109
Rb/Ca	-1.444	17	17	0.149	-0.666	22	22	0.506	-1.985	30	30	**0.047**	-1.680	8	8	0.093
Sr/Ca	-1.113	17	17	0.266	-1.445	22	22	0.149	-2.735	30	30	**0.006**	-2.521	8	8	**0.012**
Y/Ca	-2.741	17	17	**0.006**	-2.484	22	22	**0.013**	-3.630	30	30	**<0.001**	-2.521	8	8	**0.012**
Ba/Ca	-3.051	17	17	**0.002**	-3.295	22	22	**0.001**	-3.445	30	30	**0.001**	-2.100	8	8	**0.036**

6.4 Discussion

6.4.1 Juveniles - general patterns

Although considerable variation can occur between estuaries, and between seasons within estuaries, some global patterns in the chemical differences between riverine and marine end-members are evident. Table 6.7 summarises some of these differences, including those between dissolved and particulate elements.

Table 6.7 Summary of global dissolved and particulate concentrations of elements in riverine and oceanic waters ($\mu g\ l^{-1}$), expressed as means (adapted from Chester, 2003).

	Rivers		**Oceans**	
Element	**Dissolved**	**Particulate**	**Dissolved**	**Particulate**
Ba	60	600	20	1500
Ca	13300	21500	412000	10000
Cu	1.5	100	0.1	200
Li	12	25	180	45
Mg	3100	11800	$1.29x10^6$	18000
Mn	8.2	1050	0.2	6000
Na	5300	7100	$1.077x10^7$	20000
Ni	0.5	90	0.2	200
P	115	1150	60	1400
Pb	0.1	100	0.003	200
Rb	1.5	100	120	110
Sc	0.004	18	0.0006	20
Sr	60	150	8000	250
Y	-	30	0.0013	32
Zn	30	250	0.1	120

The estuary is where these end-member waters mix. Some elements may behave conservatively in this mixing process, while others become reactive and undergo sorption reactions, referred to as non-conservative behaviour. While the literature reveals that some patterns have been identified in these chemical

behaviours, these processes are difficult to predict, probably due to spatial and temporal variation both between and within estuaries. The biological reactivity of elements may be controlled by many physico-chemical factors, including pH, dissolved oxygen concentration, salinity and turbidity (Chester, 2003). Where mixing processes disturb sediments and re-suspend particulate matter, the addition of some elements previously locked up in the sediment is likely to increase. Thus, turbidity is considered a particularly important factor controlling estuarine biogeochemistry, such that sediments may be considered a third end-member in the mixing process (Chester, 2003).

Sr/Ca otolith data has been utilised extensively to distinguish fish inhabiting different salinity regimes (e.g. Secor, 1992; Jessop *et al.*, 2002; Chang *et al.*, 2004), with higher ratios common in marine waters. The discriminatory power of this indicator is, however, greater over larger salinity ranges (de Pontual and Geffen, 2002). Here, there were significantly different Sr/Ca data identified for sole caught in estuarine and coastal waters respectively (Figure 6.1), despite sole's estuarine range being restricted to the lower reaches. The significant pattern is likely aided by the low mobility of this flatfish, particularly as 0+ fish, as it is unlikely that they have moved between estuarine and coastal regions since settling from the larval stage. Bass and whiting are, however, far more mobile and likely to move between regions with ease, which may cause discrepancies between their location of capture and the chemical signals of their otoliths. This interpretation adds strength to the proposition that coastal bass still have a close association with the estuary, and that 0+ whiting demonstrate plasticity in their use of estuarine and coastal habitats, moving freely between them (Chapter 5).

The greater mobility of bass and whiting may also explain the lack of significant differences that are apparent for sole, where estuarine and coastal specimens differ (e.g. Mg/Ca, P/Ca; Figure 6.1). The Mg/Ca concentrations of 0+ sole meet the expectations derived from global Mg patterns (Table 6.7) and long term mean concentrations in the Thames, as monitored by the Environment Agency (Table 6.8). However, the large standard deviations in these data (Table 6.8), representing temporal variability at the scale of years, seasons and tidal state, likely weakens the ability to distinguish such signals in bass and whiting. Furthermore, sample size is low for coastal bass, perhaps limiting the power of the elemental tracers, despite the greater range of salinities to which bass are exposed. An exception to this is seen in

the Ba/Ca data, in which only bass show a significant pattern, with higher concentrations in the estuary-caught individuals (Figure 6.1). The elevated concentrations of dissolved Ba in low-salinity waters has been utilised previously in similar applications (e.g. Secor *et al.*, 2001; Thorrold and Shuttleworth, 2000). These elevated concentrations are reflected here, perhaps due to the physiological ability of bass to access upper estuarine areas.

Na/Ca data are distributed as expected in sole and whiting, with elevated concentrations in coastal specimens. The lack of a relationship in bass may be an artefact of poor sample size or of movement across regional boundaries. The power to discriminate between estuarine and coastal fish for all three species is seen with the elemental indicators of Sc/Ca, Rb/Ca, Mn/Ca and Li/Ca (Figure 6.1). Concentrations of lithium are, as expected (see Table 6.7), elevated in coastal fish. Other studies have successfully used Li in similar applications (e.g. Brown, 2006). Dissolved concentrations of Rb are typically higher in marine than riverine waters (Table 6.7). This pattern is, again, reflected well in sole and whiting otoliths (Figure 6.1). However, estuary-caught bass show highly elevated concentrations of Rb/Ca in their otoliths. Although Rb concentrations have not previously been monitored in the Thames estuary, bass may have been exposed to highly enriched anthropogenic sources of Rb in the upper estuary. Neither Mn/Ca nor Sc/Ca patterns from the otoliths (Figure 6.1) follow those expected from generalised global data of dissolved concentrations (Table 6.7). The elevated Mn/Ca in coastal samples may be explained by the elevated particulate Mn concentrations found in the marine environment (Table 6.7). Mn is predominantly associated with the particulate phase, but its behaviour is complicated by its redox-sensitivity (*pers. comm.*, Mark Fitzsimons). Under oxic conditions, Mn (IV) forms a precipitate with O. As sediments accumulate and become anoxic, Mn (IV) is reduced to Mn (II), which is soluble. However, the Mn will reprecipitate on contact with oxic water. Thus, while sediment resuspension might liberate pore-water Mn (II), the oxygenation of the sediment will restrict mobility of Mn. Thus, while much of the area described here as coastal habitat is sufficiently shallow for sediments to be easily disturbed, and for Mn to undergo addition back into a dissolved state, it is not clear how these conditions will be reflected in otoliths. Elemental addition and removal processes are also likely to be occurring in estuarine regions as part of erosion-deposition cycles. However, due to the larger surface area of sediment exposed to turbulence and the longer residence

time of the water, greater overall Mn concentrations may be expected in the coastal region.

The downstream flow of the Thames River may also be sufficiently rapid such that fluxes of Sc are flushed through the estuary, only becoming available for substantial biological-uptake when water flow has slowed in coastal regions. Short flushing times may result in insufficient residence time of water to allow heterogeneous chemical reactions to be completed (Millward, 1995). The point at which the division between estuarine and coastal regions has been designated (see Figure 2.1) approximately coincides with where the Thames channel rapidly widens. Flushing time may therefore decline rapidly downstream of this point, facilitating sorption reactions. As such, estuaries like the Thames (and the catchments from which their freshwater component drains) may be considered as the principal source of elements, while coastal regions play a larger role as sinks for elements.

In the Schelde Estuary, dissolved trace elements precipitate as sulphides in low salinity reaches during the summer, due to anoxic conditions (Millward, 1995; Millward and Turner, 1995; Zwolsman and van Eck, 1999). These are then transported downstream, oxidising and releasing elements into the dissolved phase in higher salinity waters. Parts of the Thames Estuary experience low dissolved oxygen concentrations, particularly during the summer (Kinniburgh, 1998), so similar processes may be at work here. The variety of factors controlling reactions resulting in the addition and removal of trace elements to and from solution is extensive. Millward (1995) provides a review of these processes in estuaries.

Such processes may also explain the lack of patterns and unexpected differences seen in the Zn/Ca, Cu/Ca and Ni/Ca data (Figure 6.1). Although concentrations have declined exponentially in recent decades (Power *et al.*, 1999), as an estuary still exposed to anthropogenic disturbance, these heavy metals were expected to be present at relatively high concentrations in the estuary. The EA Thames water chemistry data show an overall decline in concentration with distance downstream from Kew, but closer examination reveals upper-mid estuary maxima for Zn, Cu and Ni (Table 6.8). However, concentrations in otoliths do not necessarily accurately represent water concentrations (Campana, 1999; de Pontual and Geffen, 2002), and due to downstream flushing and the likely patchiness of contaminants, water chemistry data are difficult to link to concentrations in otoltihs. Also, the water quality of the Thames has improved substantially in recent years. Much of the

remaining heavy metal contamination may have settled in the lower estuarine and coastal sediments following gradual flushing downstream. This lends a potential explanation to some of the elevated heavy metal concentrations seen in the otoliths of coastal specimens (Figure 6.1). Sediment samples collected in 1989 (Attrill and Thomes, 1995) still possessed higher concentrations of these elements in estuarine than coastal waters. Data on current concentrations of heavy metals in Thames sediments would be useful. A final element that appears to hold some discriminator power is yttrium. Significant differences with Y/Ca are found for sole and whiting, but are not consistent in the direction of this difference (Figure 6.1). Due to the lesser mobility of sole, it is assumed that they better reflect the actual distribution of Y/Ca, with elevated concentrations in coastal waters. Compared to other rare earth elements (e.g. Moermond *et al.*, 2001; Elbaz-Poulichet and Dupey, 1999), literature searches reveal that relatively little is known about the behaviour of Y in estuaries.

ANOSIM was used to test the ability of simultaneous use of the multiple elemental indicators to identify differences in estuarine and coastal fishes. Sole showed a very strong difference, while those for bass and whiting, although still significant, were not as strong (Table 6.2). Again, this is indicative of the greater mobility of bass and whiting, allowing them to move relatively easily across estuarine and coastal margins, blending the regional chemical signals in their otoliths. Sample size is also low for coastal bass, statistically weakening the differences analysed.

Table 6.8 Summary of some Thames region Environment Agency water data, showing means and standard deviations from 1995 to 2003 sampling events (across multiple seasons and tidal states). Results from Spearman's rank correlations between mean concentrations and km downstream from Kew are shown (R values in bold are significant to 0.05 level).

Site	Km from Kew	Cu ($\mu g\ L^{-1}$)	Ni ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Mg ($\mu g\ L^{-1}$)	Mn ($\mu g\ L^{-1}$)
Barnes	3.50	8.93 ± 6.95	5.19 ± 2.08	25.68 ± 13.33	17.17 ± 78.53	48.63 ± 34.17
Hammersmith Br.	6.10	8.40 ± 4.87	4.80 ± 1.91	23.45 ± 5.82	-	-
Wandsworth Br.	12.00	13.44 ± 7.90	5.37 ± 2.00	29.89 ± 8.84	-	-
London Br.	20.75	11.12 ± 6.15	5.84 ± 2.47	33.44 ± 19.14	44.69 ± 78.56	52.91 ± 39.38
Greenwich	28.58	10.48 ± 10.68	5.89 ± 2.84	34.50 ± 21.15	78.97 ± 180.44	-
Victoria Dock	32.05	10.22 ± 11.07	6.64 ± 6.52	35.26 ± 27.07	118.16 ± 266.36	-
Woolwich	35.70	9.56 ± 6.89	5.99 ± 3.27	30.64 ± 14.82	138.49 ± 128.32	37.31 ± 34.69
Northern Outfall	38.00	8.00 ± 6.07	5.65 ± 3.26	28.56 ± 16.57	171.58 ± 155.35	-
Southern Outfall	41.00	11.39 ± 19.76	5.78 ± 2.91	29.93 ± 14.26	232.37 ± 172.83	-
Erithe	46.00	9.08 ± 5.79	5.80 ± 2.63	31.93 ± 14.27	349.70 ± 443.71	39.25 ± 36.64
Greenhithe	54.40	10.14 ± 6.42	5.63 ± 2.81	33.33 ± 17.97	527.24 ± 682.88	45.12 ± 54.39
Gravesend	61.80	9.63 ± 5.59	5.13 ± 3.68	32.25 ± 18.26	687.59 ± 857.43	-
Mucking	73.00	9.13 ± 4.93	4.89 ± 3.23	27.15 ± 15.19	837.39 ± 823.29	25.53 ± 25.28
Chapman Buoy	82.50	8.41 ± 4.46	3.98 ± 2.86	25.54 ± 60.20	982.52 ± 242.44	14.93 ± 9.97
Southend	89.70	7.16 ± 4.63	2.36 ± 1.38	16.59 ± 28.37	1119.53 ± 309.81	-
No2 Sea Reach	97.60	6.33 ± 4.40	1.71 ± 0.79	13.07 ± 29.87	1185.94 ± 185.80	6.18 ± 5.45
N Oaze Buoy	106.60	8.05 ± 6.69	1.21 ± 0.36	14.69 ± 18.91	1167.09 ± 178.19	7.45 ± 7.10
	R	**-5.05**	**-6.26**	-0.467	**0.997**	**-0.900**
	p	0.033	0.005	.050	<0.001	0.001

6.4.2 Discriminant Function Analyses

Discriminant function analyses were used to determine the accuracy with which the otolith chemistry data could be used to re-classify the 0+ fishes to their region of collection (Table 6.3). Note that this analysis does not aim to tease out variability in the relative inhabitation of different parts of the estuarine-coastal continuum. Rather, it aims to determine if the region in which the fish was caught (estuarine or coastal) can be identified as that in which the fish had spent most of its time, or if it had spent a disproportionate amount of time in the other region.

Sole were re-classified with 100% accuracy, showing that the fish had in fact been living in the same region in which they were caught. This strengthens the suggestion that 0+ sole exist in sub-populations divided by their use of estuarine or coastal nursery grounds (see Chapter 5). Despite the smaller differences in otolith chemistry signals, discriminant function analysis also re-classified 0+ bass with 100% accuracy. Therefore, although coastal bass maintain some association with the estuary (including feeding activity; see Chapter 5), they appear to spend a sufficiently large portion of their first three to six months in coastal habitats to acquire a coastal signal in their otoliths. Hence, 0+ bass may be considered capable of occupying coastal and estuarine habitats. Whiting were re-classified with 95% accuracy. This high level of accuracy suggests that 0+ whiting may occupy both estuarine and coastal regions. However, one of the ten coastal whiting possessed otoliths carrying chemical signals considered to be estuarine. If extrapolation of this proportion to the population level is assumed to be appropriate, then this represents substantial numbers of 0+ whiting moving between estuarine and coastal habitats in only the first three to six months since settling out of the larval phase. However, low sample size dictates that this data be interpreted cautiously.

Further discriminant function analyses were implemented in order to assess the ability of the 0+ data of each species to act as a proxy for the re-classification of the other species. Re-classification accuracies were poor where proxies were used (Table 6.4). In comparison to discriminant function analyses used elsewhere in the literature (e.g. Thorrold *et al.*, 1998; Brown 2006a,b), the 80% accuracy for sole re-classification using whiting data may be considered sufficient. However, this still involved a substantial margin of error and is not favoured here. The inability of these species to act as accurate proxies for one another likely relates to their differences in

function and life-history. Brown (2006) found that otolith chemistry data from English sole (*Pleuronectes vetulus*) and speckled sanddab (*Citharichthys stigmaeus*) could be used as proxies for one another with reasonable accuracy. However, these are both flatfish with similar life-histories. Sole, bass and whiting have benthic, semi-pelagic and demersal existences, respectively, and so may be exposed to different physiological and chemical limitations. This may partly explain the inconsistency between species in the element/Ca ratios successfully differentiating estuarine from coastal specimens. As a consequence of this, the multi-elemental potential of the models is limited for use of an individual species as a proxy for others.

Spearman's rank correlations (Table 6.5) identified energetic benefits of living in the estuary for sole, corroborating results from Chapter 5, which link estuarine feeding by sole to longer and heavier bodies. In terms of total length, bass show an energetic benefit of a coastal existence. Considering the abundance of 0+ bass inhabiting upper-estuarine regions, bass must acquire an alternative benefit from estuarine residency. Attrill and Power (2002; 2004) discuss the use of estuarine habitats as a thermal resource, though predator avoidance may provide a further benefit (Nagelkerken *et al.*, 2000a; b). Whiting do not reveal an energetic benefit of either estuarine or coastal living. As whiting have previously been described as having an affinity for estuaries (e.g. Pawson, 1995), those that reside in estuaries may also benefit from predator avoidance and/or thermal resource use. Alternatively, whiting may not acquire any distinguishable benefit from estuaries, with their residence as 0+ fish perhaps occurring as an artefact of the suitable depth of the water. Furthermore, estuaries and bays are more likely to intercept larvae than open coast habitats (Nagelkerken *et al.*, 2000a; b). Some 0+ fish, such as whiting, may become concentrated in and around estuaries for this reason alone.

6.4.3 Adults

Comparison between adult and juvenile otolith data may be misleading, due to possible annual variation in water chemistry (associated with rainfall patterns and pollution events) and differences between solution-based and laser-ablation analytical techniques. Although comparison of different layers within adult otoliths may also suffer from temporal variability (hence caution taken over data interpretation), the adult otoliths do reveal some interesting patterns. Permitting the assumption that

temporal variability in water chemistry only adds noise to otolith chemistry signals, comparison of elemental concentrations in the juvenile layers of adult otoliths with recently deposited material at the otoliths' edges permits inferences to be made regarding movement between waters of expected different chemical compositions.

Sole. Stable isotope analyses (Chapter 5) suggested that juvenile sole may utilise either estuarine or coastal nursery habitats, but exhibit very limited movement between these regions. Data (Chapter 5) suggest that by their second year (1+ fish) sole have a less plastic and relatively marine existence, being less likely to move back into an estuarine region. In the adult otoliths, this hypothesis is supported well by Mn/Ca and Ba/Ca data (Figure 6.2). Dissolved Mn and Ba are expected to be elevated in the estuary relative to the coastal region (Table 6.7) and have previously been identified as elements likely to reflect environmental conditions (Campana, 1999; de Pontual and Geffen, 2002). The juvenile portions of sole otoliths show minimum concentrations similar to those seen at otolith edges, but some show elevated concentrations, suggesting variability between individuals in the inhabitation of estuarine or coastal waters. Y/Ca data show the opposite pattern, with a greater range in concentrations and higher mean value from the otolith edges (Figure 6.2). Although relatively little is known about yttrium, its behaviour and its natural and anthropogenic variation, juvenile sole data suggested coastal concentrations are higher than in the estuary (Figure 6.1). Hence, the expectation of coastally elevated concentrations is met, but the larger variation in the juvenile portion of otoliths is not evident. It appears that some adult sole living in the coastal region are exposed to relatively elevated Y concentrations. Although the overall abundance of Y is low and therefore less likely to be osmoregulated during incorporation into otoliths (Campana, 1999), our poor understanding of Y dynamics limits the interpretation of this data.

Relative concentrations of Cu/Ca within sole otoliths also show unexpected patterns. In estuaries exposed to urbanisation and industrial development, such as the Thames, Cu concentrations should be higher than in coastal regions (i.e. Table 6.8). Forrester and Swearer (2002) identified elevated Cu and Pb in otoliths of California halibut (*Paralicthys californicus*) from bay habitats, relative to open coast. Here, the mean and standard deviations in Cu/Ca ratios are higher in recent otolith formations than juvenile layers. As discussed in section 6.4.1, a possible explanation for this may involve rapid downstream fluxing of dissolved or particulate Cu, becoming more

readily available for biological uptake in coastal regions with slower flow (Millward, 1995). Addition of Cu from re-suspended sediments in the wind and wave exposed coastal zone may also account for this, particularly as marine particulates tend to be Cu-enriched (Table 6.7). However, some evidence suggests that Cu distributions are physiologically-controlled, so otolith concentrations may not reflect ambient water composition (Campana, 1999; de Pontual and Geffen, 2002).

Campana (1999) also identifies Mg as an element that does not always occur in otoliths at concentrations proportional to its concentration in surrounding water. Variation in EA data (Table 6.8) is large for Mg, particularly in the lower estuary, making data interpretation difficult. Rb/Ca data do not show any significant differences between otolith edges and juvenile cores in sole. Although coastal concentrations were expected to be higher than estuarine (Table 6.7), a source of elevated Rb in the estuary could explain this pattern. However, monitoring of Rb has not previously been performed in the Thames Estuary.

Sr/Ca data do not show any significant patterns for sole either. It is expected that the salinity range across which sole exist is insufficient for this application. Laboratory experiments manipulating salinity over a narrow range of variation have failed to identify links between Sr/Ca and ambient salinity (de Pontual and Geffen, 2002).

Whiting. Otolith chemistry data from whiting shows very similar patterns to sole. Stable isotope data (Chapter 5) suggested plasticity in choice of feeding venue (estuary/coast) as juveniles, and the ability of individuals to move freely between regions. Due to their greater mobility, it is expected that some estuarine feeding and inhabitation may continue into young-adult stages. Large variation in the elemental concentrations of otolith edges with Cu/Ca, Mg/Ca, Sr/Ca, Rb/Ca and Y/Ca may be indicative of this. However, such patterns may also be confounded by sorption reactions in turbid water throughout the estuarine and coastal regions (see section 6.4.1; Millward, 1995; Millward and Turner, 1995), or by physiological regulation during otolith accretion (Campana, 1999).

Mn/Ca and Ba/Ca data, which present some of the most convincing trends in this study and are expected to reflect environmental conditions well (Campana, 1999; de Pontual and Geffen, 2002), show a wide range of concentrations in the juvenile portion of the whiting otoliths and a small range of low concentrations in recently

deposited edge material. While stable isotope data suggested some estuarine foraging as 1+ and 2+ fish, visits to the estuary for this purpose may not be sufficient for estuarine water chemistry signals to be acquired in otoliths. Elsdon and Gillanders (2005) suggest that, for reliable chemical signatures to be acquired in juvenile otoliths, individuals may have to occupy environments for more than 20 days. As the growth of adult otoliths is slower, this period may be longer for adults.

Bass. Previous evidence (Chapter 5) suggested that while bass may utilise both estuarine and coastal regions as juveniles, they exhibit extensive consumption of estuarine energy resources, later developing (i.e. by 4/5+) more plastic feeding behaviour, incorporating the coastal region as an additional feeding ground. Trends seen in the Mg/Ca, Cu/Ca and Y/Ca support this idea, though again must be interpreted cautiously due to potential sorption reactions in turbid water (see section 6.4.2; Millward, 1995; Millward and Turner, 1995), and physiological controls during otolith accretion (Campana, 1999). Although 4/5+ bass may spend portions of time in both estuarine and coastal environments, any visits to the upper estuary are likely to be occasional and brief. Therefore, 0+ bass, which may reside throughout the estuary, may still experience a wider range of chemical conditions. This justification may be used to explain patterns seen in the Mn/Ca, Sr/Ca and Ba/Ca data for bass (Figure 6.2). The ability to link these element/Ca ratios to expected behaviours for all species studied here strongly supports their use in tracing environmental histories (e.g. Campana, 1999; de Pontual and Geffen, 2002). This combination of elements has proved useful in many other studies (e.g. Gillanders and Kingsford, 2000; Thorrold and Shuttleworth, 2000; Gillanders, 2002b; Gillanders and Kingsford, 2003; Hamer *et al.*, 2003; Brown, 2006).

6.4.4 Limitations and Suggestions

The primary limitation of this study, attributed to logistical and financial constraints, was the inability to either (a) demonstrate a lack of temporal variation in the chemical composition of the Thames Estuary and adjacent coastal regions (between and within-year), or (b) compare adult otoliths with previously-collected juveniles from the same year class. Recent studies have stressed these as important stages in the study of connectivity between juvenile and adult habitats (e.g. Gillanders

and Kingsford, 2003; Hamer *et al.*, 2003; Gillanders, 2005; Brown, 206; Elsdon and Gillanders, 2006). Consequently, the differences identified between estuary and coastal 0+ fish cannot be used to classify the nursery habitat used previously by the adults. In many cases, the range of element/Ca concentrations seen in the juvenile core of the adult otoliths do not match those in the juvenile otoliths (Figures 6.1 and 6.2). Additional factors that may have influenced this arise from the positioning of ablation rasters in the juvenile portion of the adult otoliths, and analytical differences between the solution-based and laser ablation ICPMS techniques. Use of just one of these techniques for both juvenile and adult analyses would be preferential. However, juvenile sole otoliths were considered too small to section for laser ablation, and the micro-milling equipment necessary to isolate specific otolith layers to conduct SB-ICPMS on adult otoliths was unavailable.

The interpretation of results from studies such as this would be aided considerably by improved understanding of the processes that lead to chemical uptake in otoliths (Begg *et al.*, 2005). Relatively little is actually known about the biochemical processes influencing the deposition of most elements into otoliths (de Pontual and Geffen, 2002). Juvenile otoliths analysed here appear to exhibit more consistent significant differences for Group 1 and 2 elements of the periodic table (see Table 6.1), perhaps eluding to differences in barriers restricting incorporation into otoliths between elemental groups. Species-specific differences are likely and may be linked to physiological (including metabolic), morphological or behavioural traits (de Pontual and Geffen, 202; Moralis-Nin *et al.*, 2005; Campana, 2005; Elsdon and Gillanders, 2005). The species studied here are functionally different, with sole, bass and whiting possessing benthic, semi-pelagic and demersal existences, respectively. Controlled laboratory experiments are necessary for our understanding of these processes, and so that any variation they incur can be accounted for.

It is recommended that studies such as this are conducted in conjunction with biogeochemical surveys of temporal and spatial dynamics of water chemistry. To establish links between otolith chemistry and environmental variation, further laboratory experiments may also be needed. However, as noted by Campana (1999), the ambient concentration of an element in water does not necessarily match its availability to either the fish or the otolith. Discrepancies between water and otolith chemistries can be traced to a multitude of factors, but likely focus upon adsorption-desorption reactions occurring in turbid waters (see Millward, 1995; Millward and

Turner, 1995). Consequently, surveys of water chemistry should include measurements of dissolved and particulate concentrations, and sediments should be considered a third end-member in the freshwater-marine mixing process (Chester, 2003). Of relevance to this is the fact that the Thames is a very turbid estuary, throughout most of its tidal extent, as well as much of the adjacent coastal region into which the plumes extend. Therefore, addition and removal reactions may be abundant and complex, and may account for many of the unexpected chemical trends observed in otoliths in this study.

Overall, due to the limitations on interpretation incurred by the inability to account for any temporal variation, data analysis of adult otolith results was restricted to visual interpretation of graphs and simple statistics. Conclusive interpretation of much of these, however, was constrained. The combination of factors controlling heavy metal and trace element sorption reactions with processes controlling element and species-specific accretion of elements into otolith material makes such data interpretations very difficult.

6.5 Conclusion

Otolith chemistry differences between estuary and coastally-caught juvenile fish are most frequent and strongest for sole. This may be attributed to their low mobility during their first year. Bass and whiting are more mobile and more likely to move between regions. Many of the patterns seen in the otolith chemistry of 0+ fishes can be related to these behavioural and functional differences.

Long-term data sets belonging to the Environment Agency suggest that water chemistry throughout the Thames region is variable at short and long-term temporal scales. The highly turbid nature of much of the Thames Estuary and adjacent coastal regions is likely to contribute to this by facilitating addition/removal reactions between elements and the sediment. Consequently, many of the elemental concentrations in 0+ fish otoliths do not follow the patterns expected from total element concentrations in the water.

Despite this poor predictability of juvenile otolith signals, on a species-by-species basis, consistent differences do exist, allowing 95-100% accuracy in re-classification to the region of collection. Use of each species as a proxy for re-

classification of the others resulted in poor accuracy and is not recommended, especially between species that are functionally and behaviourally different.

The data suggest that 0+ sole received significant energetic benefits from estuarine residency, with elevated body mass and total lengths correlated with estuarine otolith chemistry signals. Bass and whiting did not receive distinguishable energetic benefits from estuarine or coastal residency, suggesting that they derive benefits from estuary use that are not associated with growth or feeding (i.e. refuge from predators).

Unfortunately, the inability to gather empirical data regarding temporal variability of chemical signals, or to sample across multiple years, impeded the comparison of adult otoliths with juvenile otoliths. However, comparison between the layers of adult otoliths were still of use. The elemental ratios of Mn/Ca, Ba/Ca and to an extent Sr/Ca, were the most revealing. Previous literature has often identified these as useful elements in biogeochemical studies across salinity regimes. Here, compared to other elements, they met with their expected distributions and with the most logical interpretations. As a result, it has been possible draw some conclusions with regard the movement of the fish species studied here. Sole and whiting appear to show plasticity in their use of estuarine and coastal regions as juveniles. It is expected, however, that this plasticity acts at the population level for sole, but at the individual level for whiting. As 1+ and 2+ fish, sole and whiting appear to adopt a more marine and less plastic existence. Bass evidently have access to a wide salinity range (and therefore larger range of water chemistries) as juveniles. As older fish, bass tend not to spend extended periods in the upper estuary but may frequent lower estuarine reaches and coastal regions. The variable plasticity of these species and age-groups of fishes is indicative of the range of habitats across the estuarine-marine gradient that they utilise. Where plasticity is high, which is likely facilitated by high mobility, a fish may be more opportunistic in its choice of habitat, and may therefore be considered an estuarine-opportunist rather than an estuarine-dependent. Where this plasticity occurs at the population level, an individual may not experience a wide range of salinity regimes, but the population does.

Mn, Ba and Sr provide the most easily interpreted patterns, partly due to being less reactive in the mixing of fresh and sea water, although the redox-sensitivity of Mn complicates our understanding of the bio-availability of dissolved Mn to otoliths. Although the patterns of other elements in otoliths are less predictable and harder to

link to water chemistry, they can still be of use in revealing fish movement and nursery habitat use. However, if the use of these other trace metals and rare-earth elements is to be optimised, it is recommended that experiments investigating their mixing behaviour in estuaries, and transfer from water into otolith are conducted.

7. Synthesis

7.1 Introduction

Knowledge of the connectivity between coastal and estuarine fish habitats has been emphasised as fundamental to our understanding of the population dynamics of fishes (e.g. Dame and Allen, 1996; Able, 2005; Herzka, 2005; Ray, 2005; Secor, 2005). The emergence of quantitative research on this topic can be attributed largely to the advancement of two analytical techniques. Firstly, soft-tissue stable isotope analysis has been revealed as a useful indicator of animal origins and movements (see Hobson, 1999; Rubenstein and Hobson, 2004). Secondly, otolith chemistry has developed rapidly in recent years, including applications for tracking fish movement between freshwater, estuarine and marine habitats (Gillanders, 2002b; Gillanders *et al.*, 2003; Begg *et al.*, 2005).

This chapter aims to tackle the principal questions and issues posed by the thesis, drawing upon previous knowledge and the key findings of this study. In particular, the coupling of soft-tissue stable isotope and otolith chemistry techniques is discussed. The simultaneous consideration of such data sets adds resolution to data interpretation, but also raises awareness of some analytical issues. The management implications of the main findings are also considered.

7.2 Sole, bass and whiting: summary of key findings so far

Prior to this study, the occurrence of juvenile sole (*Solea solea*), bass (*Dicentrarchus labrax*) and whiting (*Merlangius merlangus*) in estuaries, including the Thames Estuary, had been well documented (e.g. Thomas, 1998; Araújo *et al.*, 2000; Power *et al.*, 2000b). Bass are known to enter estuaries as post-larvae between June and August (Pickett and Pawson, 1994; Costa *et al.*, 2002). Bass have frequently been recorded in estuaries as 0+ and 1+ fish and may be found in estuaries as 2+ to 5+ fish during the summer months (Claridge and Potter, 1983; Kelley, 1988). Estuaries are considered to be an important habitat for bass (e.g. Kelley, 1988) which, as a euryhaline species, can tolerate freshwater and marine conditions at any age (Pickett and Pawson, 1994). Juvenile whiting are documented as having an affinity

for estuaries (Pawson, 1995), although their poor tolerance for freshwater restricts them to lower estuarine reaches. Large numbers have been reported in autumn and winter, with most appearing to leave the estuary by the following spring. Like whiting, sole are restricted to the lower estuary, but may be abundant there, arriving between May and August and leaving by their first or second birthday (Thomas, 1998; Costa *et al.*, 2002). Large numbers of juvenile sole have also been reported for inshore marine regions (e.g. Pawson, 1995; Symonds and Rogers, 1995; Le Pape *et al.*, 2003a;b). Other research upon sole has established links with sources of energy from the terrestrial environment (Salen-Picard *et al.*, 2002; Darnaude *et al.*, 2004a,b; Darnaude, 2005).

In this study, stable isotope analysis of soft tissues (Chapter 5) led to several principal conclusions. Firstly, bass juveniles appear to have a propensity for feeding in the estuary, although, to varying degrees, some do feed coastally. Bass of the 4/5+ age-group seem to exhibit greater plasticity (at the individual level) in their estuarine or coastal feeding habits. Secondly, sole appear to split into two sub-populations as 0+, separated by estuarine and coastal feeding and exhibiting no evidence of movement between these regions (see Chapter 5). Limited movement between nursery grounds has also been described elsewhere (e.g. Gilliers, 2006), and is likely a consequence of the low mobility of the species. However, the data suggest that site affinity diminishes in 1+ and 2+ fish. Sizeable estuarine signals are also seen in coastally-caught adult sole. Some of this signal is likely to be that persisting from feeding in the estuaries as juveniles ($\delta^{34}S$ mean model predictions: 19.39% to 42.26% estuarine contribution), while more recently acquired signals ($\delta^{13}C$ mean model predictions: 35.58% to 40.03% estuarine contribution) may result from exploitation of terrestrial POM transported in the river plume (see Darnaude *et al.*, 2004a,b; Darnaude, 2005). Whiting appear to have plastic feeding habits as juveniles and adults, consistent with expectations for highly mobile opportunistic predators.

Otolith chemistry analyses also allowed inferences to be made about the role of estuaries in supporting the study species. Juvenile sole showed partitioning in otolith chemistry signals between estuary- and coastally-caught individuals, supporting the non-mixing two-sub-population theory. While the strength of multi-element re-classification accuracies for juvenile bass and whiting were also remarkable, more subtle variations in the data were indicative of the wider salinity ranges inhabited by these more mobile species.

7.3 Does estuarine feeding follow from estuarine residency?

During the running of the discriminant function analyses performed in Chapters 5 and 6, the models produced a measure of the strength of the estuarine signal (on a scale of 0 to 1) for each juvenile fish soft-tissue (SIA) and otolith (elemental chemistry) sample, respectively. This data was compiled for individuals subjected to both types of analysis, and are displayed in Figure 7.1. Due to the overlap of many data points, the data are also presented in Table 7.1.

Of the juvenile bass caught in the estuary, all have strong estuarine inhabitation (otoliths) and feeding (muscle) signals. Those caught in coastal habitat, while possessing strong coastal residency signals in their otoliths, display a range of feeding signals, but with the majority showing intermediate estuarine-coastal stable isotope signals. Thus, it may be inferred that 0+ bass prefer to live and feed in the estuary, but due to their high mobility are capable of living coastally and migrating periodically to feed in the estuary. This would suggest a benefit of estuarine feeding, although bass did not appear to show any energetic benefits of estuarine feeding or residency.

Juvenile sole that have resided in the estuary since settling from the larval stage appear to have fed exclusively there, while those residing in the coastal region appear to have fed there exclusively. The estuarine juvenile sole were found to be both longer and heavier, suggesting an energetic benefit of an estuarine juvenile phase (see Tables 5.7 and 6.5). Due to the abundance of food in estuaries, density-dependent competition for such resources is unlikely. Given the abundance of juvenile sole also found in the coastal zone, there must be alternative benefits for juvenile sole using coastal nursery grounds (i.e. less energy expended on migrations). Further research investigating the persistence of individuals from each juvenile sub-population in successive years of a cohort's existence would be valuable.

While most of the juvenile whiting possessed otolith chemistry and stable isotope signals from the region in which they were caught, a number of individuals demonstrated greater plasticity. An individual with a strong coastal residency (otolith) signal had a strong estuarine feeding (soft-tissue) signal, while two with strong estuarine residency signals had intermediate feeding signals. This potential for individual plasticity is indicative of the highly motile and opportunistic nature of this

species. Thus, they do not appear to show a consistent preference for either estuarine or coastal living or feeding, and move freely between the habitats.

Spearman's rank correlations were performed to assess the relative consistency between results of discriminant function analyses derived from otolith chemistry and soft-tissue stable isotope data (Table 7.2). All three species show statistically significant positive correlations between the two techniques. These are particularly strong for juvenile sole, but less so for whiting and bass as their plasticity in feeding behaviours, enabled by their greater mobility, results in greater variability between individuals. These significant correlations exist, despite the variation in behaviours identified in Figure 7.1, thus indicating the value in using both statistical and graphical data interpretations.

The coupling of multiple techniques, such as soft-tissue stable isotope analysis and otolith chemistry, clearly has benefits when interpreting the finer details of resource use and habitat connectivity. Based on these techniques there are many avenues worthy of investigation, such as multiple-tissue studies and temporal dynamics, which would further improve the resolution and accuracy of data interpretation. It is also recommended that the applicability of other techniques, such as compound-specific stable isotope analysis, fatty acid analysis and genetic techniques (Hobson, 1999; Hobson, 2002; Meier-Augenstein, 2002; Rubenstein and Hobson, 2004), are explored further. These techniques may also be effectively implemented in conjunction with more traditional methods such as telemetry and mark-recapture (e.g. Cunjak *et al.*, 2005).

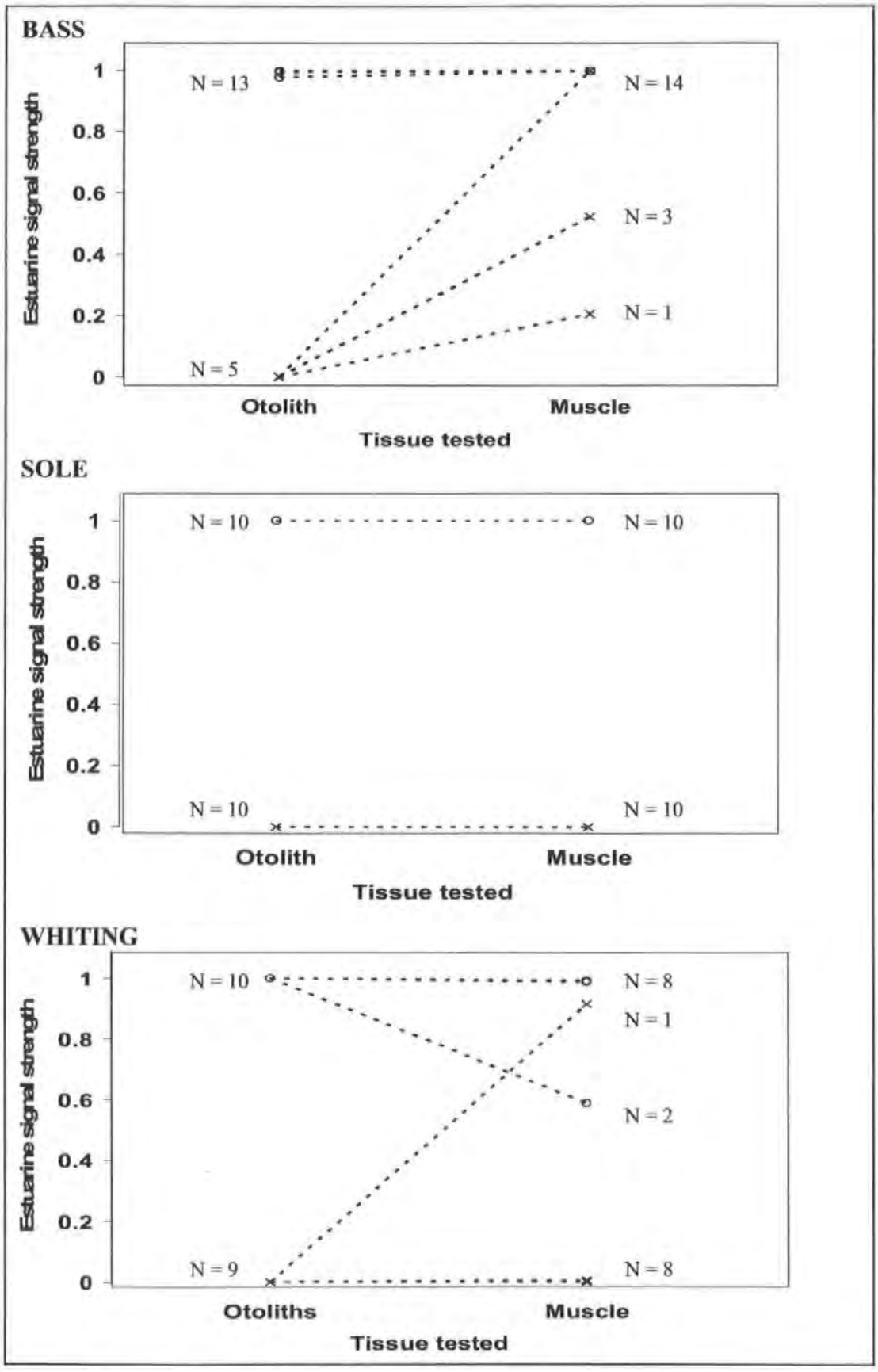


Figure 7.1 Comparing 0+ fish estuarine signal strengths in otoliths versus muscle tissue. Signal strength values acquired from discriminant function analyses. Circles and crosses represent estuary- and coastally-caught fish, respectively. Lines link samples from individual fish. The 'N' values indicate the number of samples represented at points where their signals overlap.

Table 7.1 Comparing the strength of estuarine signals from otolith chemistry and stable isotope (muscle) data for individual fish. Signal strength measured on scale of 0 (weak) to 1 (strong). Data were acquired from discriminant function analyses.

Species	Region of capture	Fish #	Strength of estuarine signal (0-1)	
			Otolith	Muscle
0+ Bass *D. labrax*	Estuary	BE1	1	1
		BE2	1	1
		BE3	1	1
		BE4	0.99987	1
		BE5	0.97789	1
		BE6	0.99482	1
		BE7	0.99978	1
		BE8	0.9972	1
		BE9	1	1
		BE10	0.9988	1
		BE11	1	1
		BE12	0.99706	1
		BE13	1	1
	Coastal	BC1	0.00002	0.99989
		BC2	0	0.52267
		BC3	0.00107	0.52267
		BC4	0.00004	0.20575
		BC5	0	0.52267
0+ Sole *S. solea*	Estuary	SE1	1	1
		SE2	1	1
		SE3	1	1
		SE4	1	1
		SE5	1	1
		SE6	1	1
		SE7	1	1
		SE8	1	1
		SE9	1	1
		SE10	1	1
	Coastal	SC1	0	0
		SC2	0	0
		SC3	0	0
		SC4	0	0
		SC5	0	0
		SC6	0	0
		SC7	0	0
		SC8	0	0
		SC9	0	0
		SC10	0	0
0+ Whiting *M. merlangus*	Estuary	WE1	1	0.99178
		WE2	1	0.99178
		WE3	1	0.99178
		WE4	1	0.99178
		WE5	1	0.98843
		WE6	1	0.98843
		WE7	1	0.98843
		WE8	1	0.98843
		WE9	1	0.5878
		WE10	0.99999	0.5878
	Coastal	WC1	0	0.00561
		WC2	0	0.00001
		WC3	0	0.00083
		WC4	0	0.00001
		WC5	0	0.00005
		WC6	0	0.00054
		WC7	0	0.91566
		WC8	0	0.00004
		WC9	0	0.00007

Table 7.2 Spearman's rank correlations between discriminant function values from stable isotope data and from otolith chemistry data. R statistics (correlation coefficients) in bold are significant.

Species	Bass	Sole	Whiting
N	18	20	20
R	**0.776**	**1.000**	**0.805**
P	<0.001	<0.001	<0.001

7.4 Issues of scale.

During the interpretation of soft-tissue stable isotope and otolith chemistry data, it is important to consider the temporal and spatial scales over which these natural tags become incorporated. As demonstrated in Section 7.3, there can be discrepancies between the strength of the estuarine signal from the otoliths (indicative primarily of residency) and muscle tissue (indicative of feeding history). The lack of such discrepancies for 0+ sole is expected to be caused by their lower mobility. Thus, the differences seen in some individual bass and whiting individuals may result from their higher mobility. When food is consumed and assimilated by a fish, the stable isotope signature of the prey item will be reflected (following trophic fractionation) in the soft-tissues of the fish. However, as noted by Elsdon and Gillanders (2005b), a fish may need to be resident in a particular water body for an extended period before their otoliths fully acquire the chemical signature from that vicinity. As such, sufficiently mobile fish may make brief foraging trips between regions without causing discernable changes in their otoliths. As a consequence, the results of studies aiming to reveal relative use of spatially discrete regions using otolith chemistry data alone may be misleading. Moreover, these data cannot always be used to infer use of resources, with otoliths reflecting only the region in which most time is spent. Stable isotope analysis of soft-tissues can aid substantially in this respect. However, unlike otoliths, muscles are subject to growth and replacement of their chemical composition. In this study, the turnover of signals means that analyses of adult fishes from the coastal region probably under-estimate the representation of estuarine energy resources in their diets as juveniles, and the subsequent offshore transport of this material. As discussed in Chapter 6, sulphur may be expected to show a lag in

turnover, relative to carbon, in soft-tissues, and so may be a more precise measure of the contribution of estuarine sources. This proposed lag in sulphur turnover has enabled consideration of plasticity between individuals on both recent and relatively historic time-scales (see Chapter 6).

A further important consideration relating to the spatial scale of natural chemical tags is the division of regions being compared. Elliott and McLusky (2002) recognised that because estuaries are, in their very nature, a spatial continuum, any scheme of regional classification is arbitrary and partially subjective. However, in order to assess the relative inhabitation and use of resources from estuarine and coastal regions, it is necessary to define boundaries. The positioning of the division between the estuary and coastal regions may have a considerable bearing on the findings of such studies. In the past, much of the region described here as coastal has been referred to as 'outer estuary' (e.g. Power *et* al., 1999; Power *et al.*, 2000a;b). However, salinity in this region is more than 30, and the stable isotope signals of invertebrates are at a level significantly different from invertebrates in the estuarine region, and no longer showing the mixing gradient evident across estuarine sites (see Chapter 4).

The extent to which the estuarine region is partitioned is also important. Although quantitative assessment of the importance of different parts of the estuary would be relevant, there are trade-offs in the benefits and restrictions of the approach used. Aside from limitations incurred by analytical techniques (see Section 3.2), it is necessary to consider sample size issues (Section 3.1) and the scale of likely fish movements within the estuary. In an estuary such as the Thames, which is under heavy tidal influence, the division of the estuary into multiple small regions could be misleading, as passive tidal transport across regions could confuse signals deposited in otoliths and soft tissues. Furthermore, strong tidal action would result in larger areas of overlap as stable isotope and otolith chemistry signals are incorporated across tidal cycles, further inhibiting the classification of specimens to specific geographic regions.

7.5 Management implications

The Millennium Ecosystem Assessment (Hassan *et al.*, 2005) identifies interactions between marine fisheries and coastal systems, and particularly estuaries,

as being of considerable importance in the provision of ecosystem services. The trade-offs between development and conservation are realised, with poor management of watersheds and estuaries often leading to the degradation of ecosystem services acting at both local and remote proximities relative to the estuary. The MEA (2005) states that the loss or reduction of nursery areas, such as estuaries, is implicated in the collapse of some fisheries. Also, the cross-habitat movement of organisms and non-living material plays an important role in sustaining communities of consumers. As such, it is recommended that coastal and estuarine habitats feature heavily in ecosystem-based fisheries management.

Regrettably, the application of management action in, often highly-urbanised, estuaries tends to require quantitative assessments with which to bargain for environmental goals over those offering more immediate financial gain. Studies such as this contribute to these requirements. Here, the results of Chapter 5 demonstrate sizeable energetic contributions from estuarine production sources in the tissues of fishes caught coastally. By processing stable isotope data through mixing models, 4/5+ bass possess, on average, between 48.44% and 86.17% contributions from estuarine energy resources. For 1+, 2+ and 3+ sole, mean estuarine contributions range from 19.39% to 42.26%, while for 1+ and 2+ whiting means range from 16.37% to 61.28%. These large ranges reflect differences in $\delta^{13}C$ and $\delta^{34}S$ data, which are expected to reflect relatively recent and older feeding activities, respectively. Experimental determination of turnover rates in these particular species would enable calculation of the time-span that these values represent.

Otolith chemistry data can also demonstrate use of estuarine habitat by marine fishes. The techniques applied here did not provide a fully quantitative assessment of the regions inhabited by fishes, but otolith chemistry does have the potential to do so where more extensive sample analysis is available. By providing a record of inhabitation, otolith data can provide valuable information on nursery habitat use for organisms utilising estuaries for resources other than food.

Further work with important implications for estuarine and fisheries management plans should include calculation of the economic value of particular habitats to ecosystem services. For example, Nickerson (1999) modelled projections of the value of estuarine mangrove habitat in the Lingayan Gulf of the Philippines, proposing a mean value of US$ 25,476 hectare^{-1} year^{-1} for commercial and municipal fisheries combined. Barbier (2000) discusses the valuation of the ecological function

of wetland habitats, including the application of the 'production function approach' in valuing environmental input to a marketed good. Such economic evaluations could also be applied to estuaries such as the Thames, allowing scientists to provide the leverage necessary for political action.

7.6 Conclusion

Independently, soft-tissue stable isotope analysis and otolith chemistry reveal useful information about the connectivity of estuarine and coastal habitats through the life-histories of marine fishes. Coupling of these techniques can provide further information on how fishes use resources at both the individual and population levels. Among the many potential caveats and complications that can exist with these techniques, it is particularly important to consider the spatial and temporal scales over which fishes move and feed, and the interactions of this with the scales at which isotopic and elemental signals vary and become incorporated into animal tissues.

Here, quantitative techniques determined that three species of marine fish utilise estuarine resources heavily. Unfortunately, the restrictions incurred by the budgets of most ecological research projects mean it is necessary to make some assumptions and prioritise research goals. However, through mindful design and an understanding of the limitations involved, it is possible to implement effective quantitative research into resource use and habitat connectivity. Due to functional differences, primarily influencing their relative mobility and salinity tolerances, the manner in which the species experienced the estuary and its resources were very different. Variability in estuarine resource use was also seen between individuals of the same species. This is particularly the case for sole, exhibiting either exclusive estuarine or coastal existences in their first year of life. Inhabitation of the estuary and the consumption of food therein, were identified for 0+ bass, sole and whiting. Importantly, estuarine energetic resources were very evident in the muscle tissue of many adult fishes, representing persisting signals from the juvenile phase, as well as more recently acquired signals from consumption of estuary-derived material.

Given the continued recognition of estuaries as important resources for the production of marine fishes, coupled with increasing pressure for policy decisions to facilitate sustainable fisheries, quantitative information such as that presented here

should be useful in bridging the gap between scientific knowledge and political priorities. Further research and recommendations have been highlighted (sections 4.4.6, 5.4.5 and 6.4.4), which can help in achieving this goal. The potential to apply such techniques to a vast array of species on a global scale makes these approaches a valuable commodity in the tool kits of researchers, conservationists and politicians.

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