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Hill, Jacqueline M

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Structure and flow of carbon and nitrogen to the western Irish Sea *Nephrops norvegicus* fishery: a stable isotope approach

by

Jacqueline M Hill

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School of Biological and Chemical Sciences

Queen Mary, University of London

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for the tea fairy

Abstract

Stable isotope analysis was used to describe the structure and flow of organic matter (carbon and nitrogen) from primary production in the water column to the benthos, and into secondary production that supports a fishery for the lobster *Nephrops norvegicus* in the western Irish Sea.

There was strong seasonal variation in the carbon and nitrogen stable isotopes of organic matter associated with the seasonal cycle of primary production in the surface waters. The seasonal isotope signal was used to trace the flow of organic matter from primary production through herbivorous zooplankton to the carnivorous pelagic larvae of *Nephrops norvegicus*. The production of larvae represented 0.2 % of spring bloom production.

Stable isotope analysis of organic matter throughout the water column was used to track the vertical flux of primary production from the surface to the benthos. The isotope signal was also used to quantify the input of carbon to the benthos, estimated to be 41 % of spring bloom primary production. The carbon input to the benthos supported a simple food chain of three higher trophic levels (TL2 – TL4). *Nephrops norvegicus* was positioned at TL3 where it accounted for 96 % of the total biomass at that level. The input of carbon to the benthos was equal to the removal of carbon from the benthos by the *Nephrops norvegicus* fishery assuming a transfer efficiency of 16 % between each trophic level.

Climate warming has caused a decline in primary production in some marine systems. Should similar impacts occur in the western Irish Sea, the subsequent decline in the flux of carbon to the benthos will result in a shortfall in the supply of carbon needed to support the current catch rate of the *Nephrops norvegicus* fishery.

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Chapter One

General Introduction

Coastal seas are amongst the most productive regions of the world's oceans and the fisheries they support have traditionally been economically important to rural communities. However, the margins of coastal seas are now amongst the most densely populated regions of the world and coastal waters are being increasingly used as a source of natural resources such as oil, gas and aggregates and as important areas for shipping, recreation, leisure and tourism. Such resources, together with the natural environment, collectively termed *'ecosystem services'*, are under threat because many areas of the marine environment are being modified by human activity. Over-fishing, coastal pollution and climate change have implications for the long-term sustainable use of coastal and shelf seas and have the potential to impact on ecosystem health defined in terms of 'system organisation, resilience and vigour, as well as the absence of signs of ecosystem distress' (Constanza, 1992 cited in Rapport et al., 1998).

In response to this wide range of impacts on the marine environment, the need for an inclusive or 'ecosystem approach' to managing ecosystem services has been widely recognised at both the national and international level. The UK Government, for example, in recognition of the economic and social importance of coastal resources, has set out a clear framework for an ecosystem-based management approach to sustainable development of "healthy, safe, productive and biologically diverse oceans and seas" (DEFRA, 2002). This strategy is set within the broader framework of the European Union Marine Strategy to protect Europe's Oceans and Seas (EC, 2006). Similarly, the Food and Agricultural Organisation of the United Nations (UN-FAO) Reykjavik 'Declaration on

Responsible Fishing in the Marine Environment' (2001) noted the importance of introducing ecosystem considerations into fisheries management. The UN-FAO has placed a responsibility on signatory countries to "identify and describe the structure, components and functioning of relevant marine ecosystems, diet composition and food webs, species interactions and predator-prey relationships, the role of habitat and the biological, physical and oceanographic factors affecting ecosystem stability and resilience" in order to develop ecosystem considerations in fisheries management.

The Irish Sea exemplifies the situation in many coastal seas, where there is a need to develop flexible management strategies to balance resource provision and the consequences of human activities. It has an abundance of natural resources that are of considerable commercial importance and, as a small (2430 km^3) semi-enclosed sea, it is potentially vulnerable to human impacts. There are commercially important fisheries, aquaculture and aggregate extraction, and increasingly, oil and gas extraction, recreational fishing and tourism are making a significant contribution to the overall value of Irish Sea resources (Vincent et al., 2004). The waters of the Irish Sea also support diverse marine life and its coastal margins are highly regarded for their wildlife and natural beauty (Roberts et al., 2003). In the Irish Sea, human-mediated impacts such as changes in nutrient concentrations (Allen et al., 1998; Gowen et al., 2002), increasing water temperature (Alcock and Rickards, 2001) and a decline in zooplankton density (Kennington and Rowlands, 2004) have all been observed. For example, sea surface temperature (SST) measurements undertaken by the Port Erin Marine Laboratory since 1904, show that SST in this region have increased by approximately 0.7 °C in the last 70-100 years (Shammon and Hartnoll, 2003). Allen et al (1998) show increases in background levels of dissolved inorganic forms of nitrogen and phosphorus in the Irish Sea, most likely

from anthropogenic inputs, since the 1960s. More recent analysis of Irish Sea nutrient data, however, indicates that increases in nutrients were mainly in the 1960s and 70s, and that levels have remained stable for the last quarter of the 19th Century (Gowen et al., 2002). In the last few years, however, there is some evidence for a decline in nutrients (Evans et al., 2003) which may be a return to 'normal' concentrations as a result of decreases in anthropogenic inputs in response to the demands of legislation on riverine and coastal water quality. It has also been suggested that primary production in the Irish Sea may have declined in recent years (Shammon and Hartnoll, 2003).

The fisheries of the Irish Sea are of considerable economic importance (Briggs, 1997). In Northern Ireland for example, the 2003 landings of fish and shellfish were valued at £15.7 million (DEFRA, 2003). With an annual landing of some 8,000 tonnes (Fig. 1.1), and a first-sale value of around £8 m (Vincent et al., 2004), the single most valuable fishery is that of the burrowing lobster *Nephrops norvegicus* (L.), commonly known as the Norway lobster or Dublin Bay prawn. The Irish Sea fishery for *N. norvegicus* is concentrated in the western Irish Sea and '*has been sustained for over 20 years with high levels of fishing effort*' (ICES, 2006a). The latest ICES report on demersal stocks in the western Irish Sea notes that there is no evidence from trends in population data, such as mean size and sex ratio, to indicate a problem in this geographical area and at present the fishery is considered by ICES to be "*fished sustainably*" (ICES, 2006a). Stock levels, ascertained by trawl surveys from 1994 to 2005, reached a maximum in 2003 and have declined slightly in 2004 and 2005 (ICES, 2006b) (Fig. 1.2). The 2005 abundance is close to the longer term average. The recent advice for the fishery is that fishing effort should remain at 2003-2005 levels but, because of uncertainty about the accuracy of reported landings, programmes to

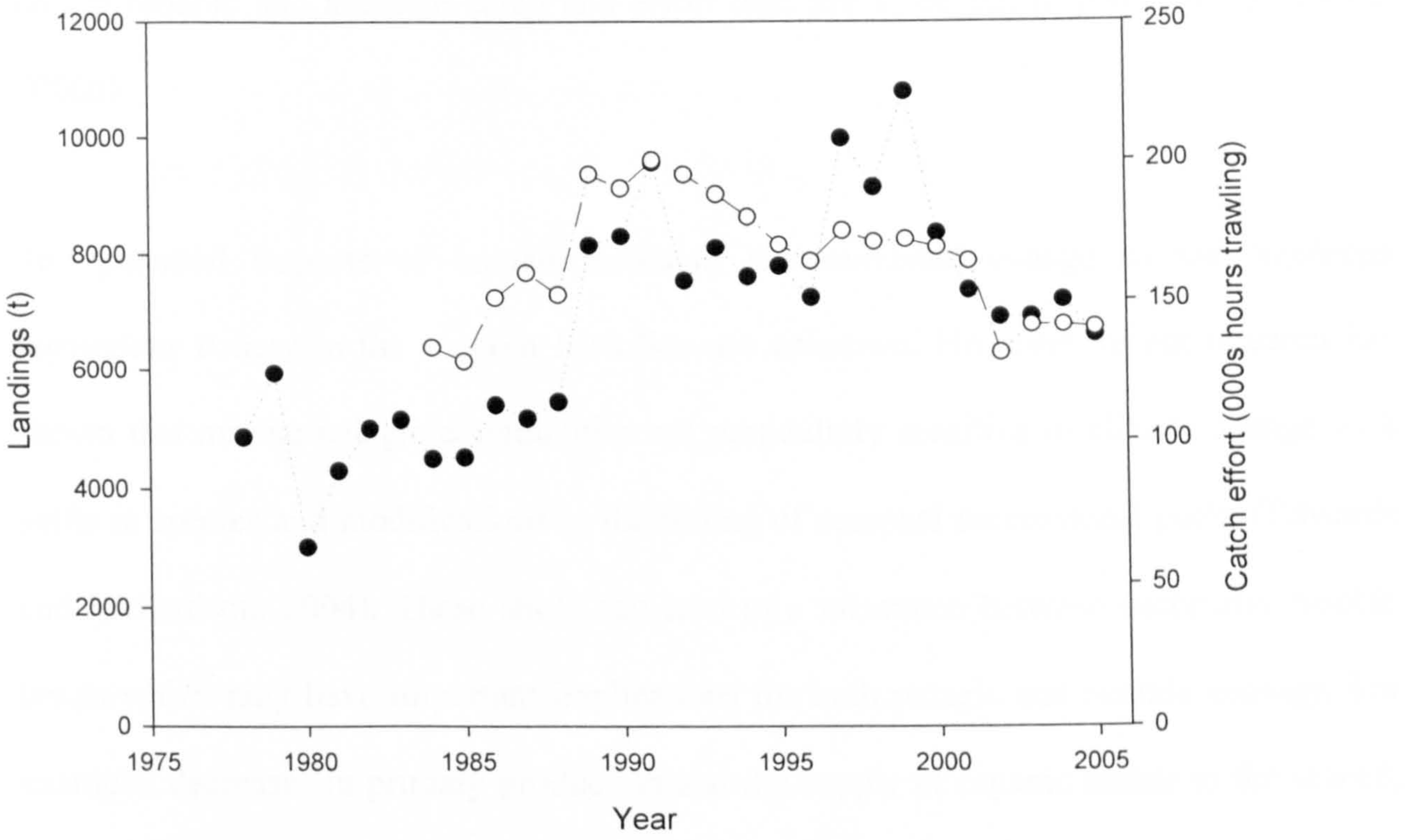

Fig. 1.1 Western Irish Sea (ICES Functional Unit 15) long-term trends in *Nephrops norvegicus* (•) landings and (○) catch effort (UK Northern Ireland trawlers). Data from ICES (2006a, 2006b).

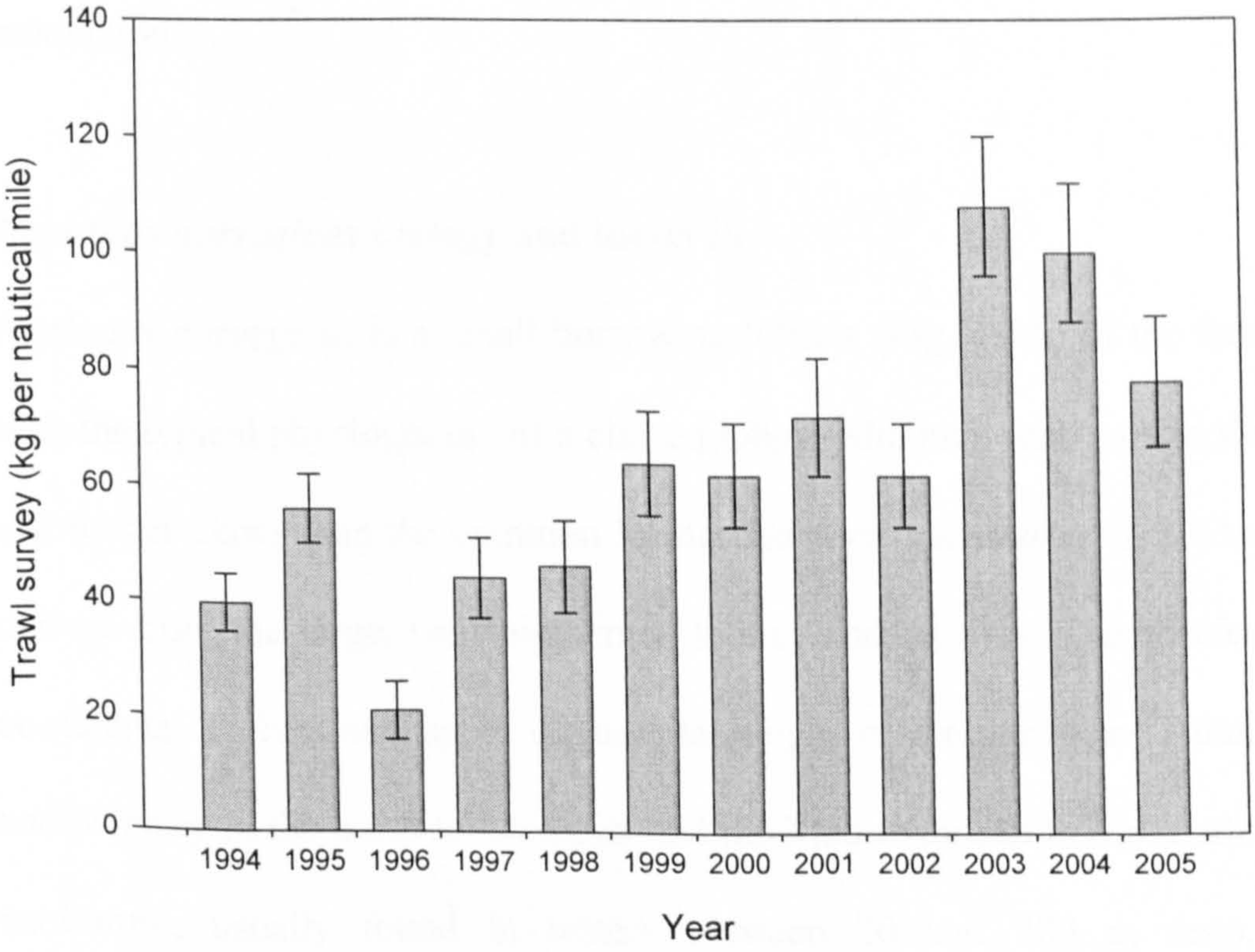

Fig. 1.2 *Nephrops norvegicus* index of stock biomass in the western Irish Sea (ICES Functional Unit 15). Data from ICES (2006b).

collect reliable and accurate catch and effort data are to be put in place (ICES, 2006a, 2006b).

The potential impacts of human mediated environmental change to the *Nephrops norvegicus* fishery in the western Irish Sea are unknown. However, recent research has shown that marine pelagic communities are particularly sensitive to climate change with shifts in species and modifications in the timing of seasonal successional peaks (Edwards and Richardson, 2004). These shifts can lead to a mismatch between successive trophic levels which may have important implications for both pelagic and benthic ecology. For example, decreases in primary production and the supply of organic matter to the seabed, in response to climate warming, has been observed in some areas (e.g. Fulweiler et al., 2007). The long term management of the *Nephrops norvegicus* fishery in the western Irish Sea will depend on a better understanding of the interactions between the environment and biology and in particular the food web linkages and energy pathways that lead to benthic production.

Nephrops norvegicus biology and life-cycle

Nephrops norvegicus is a small burrowing lobster (Fig. 1.3A) of the family Nephropidae with the typical physiognomy of a clawed lobster although with a more slender body shape and longer claws than the common lobster *Homarus gammarus* (L.). The name *Nephrops* derives from the large, well pigmented kidney shaped eyes. The species is found on the continental shelves and upper continental slopes of the north-east Atlantic, from Iceland and Norway in the north to Morocco and the Mediterranean in the south. *N. norvegicus* is sublittoral, usually found in waters between 20 and 800 m deep. Throughout its distribution, *N. norvegicus* is limited to cohesive mud, stable enough to support the

excavation of an extensive but shallow system of branching un-lined burrows (Fig. 1.3B and C) (Atkinson, 1974). The specific habitat requirement of *N. norvegicus* results in an overall geographical distribution that is highly discontinuous. *N. norvegicus* is also highly sedentary and rarely migrates over distances further than a few hundred metres (Chapman, 1982). With the exception of the planktonic larval stages, exchanges between neighbouring stock are virtually non-existent (Bell et al., 2006).

Reproduction in *N. norvegicus* shows significant latitudinal variation with the cycle of egg spawning through to hatching ranging from 6 months in the Mediterranean to more than 10 months in Iceland (Sarda, 1995). The size of sexual maturity also varies with latitude. In the UK waters age at sexual maturity is around 3 years for females and 4 years for males (Farmer, 1975; Bailey, 1984; Tuck et al., 2000). In the Irish Sea the size at sexual maturity was 23.5 – 26 mm carapace length (Hillis, 1979; Briggs, 1988). Mating in UK waters is generally annual with copulation taking place in the summer, when the females are still in a soft post-moult condition (Farmer, 1974; Bailey et al., 1986). Eggs are laid in late summer or early autumn and attached to the pleopods (swimming appendages) where they are brooded (Fig. 1.3D) for 9 months (Oakley, 1978). Immediately after spawning, berried females are thought to hide in their burrows, where they stay until the next hatching period in the spring (Bell et al., 2006), utilising their nutritional reserves and possibly feeding on more infaunal mollusc material than males (Oakley, 1978). Shortly after the eggs hatch the females moult and mate again.

Like most decapod crustaceans *N. norvegicus* has a planktonic larval stage that feeds and develops in the water column (Chapman et al., 1975) (Fig. 1.3E). In the western Irish Sea the larvae hatch into the water column in April and May (Dickey-Collas et al., 2000).

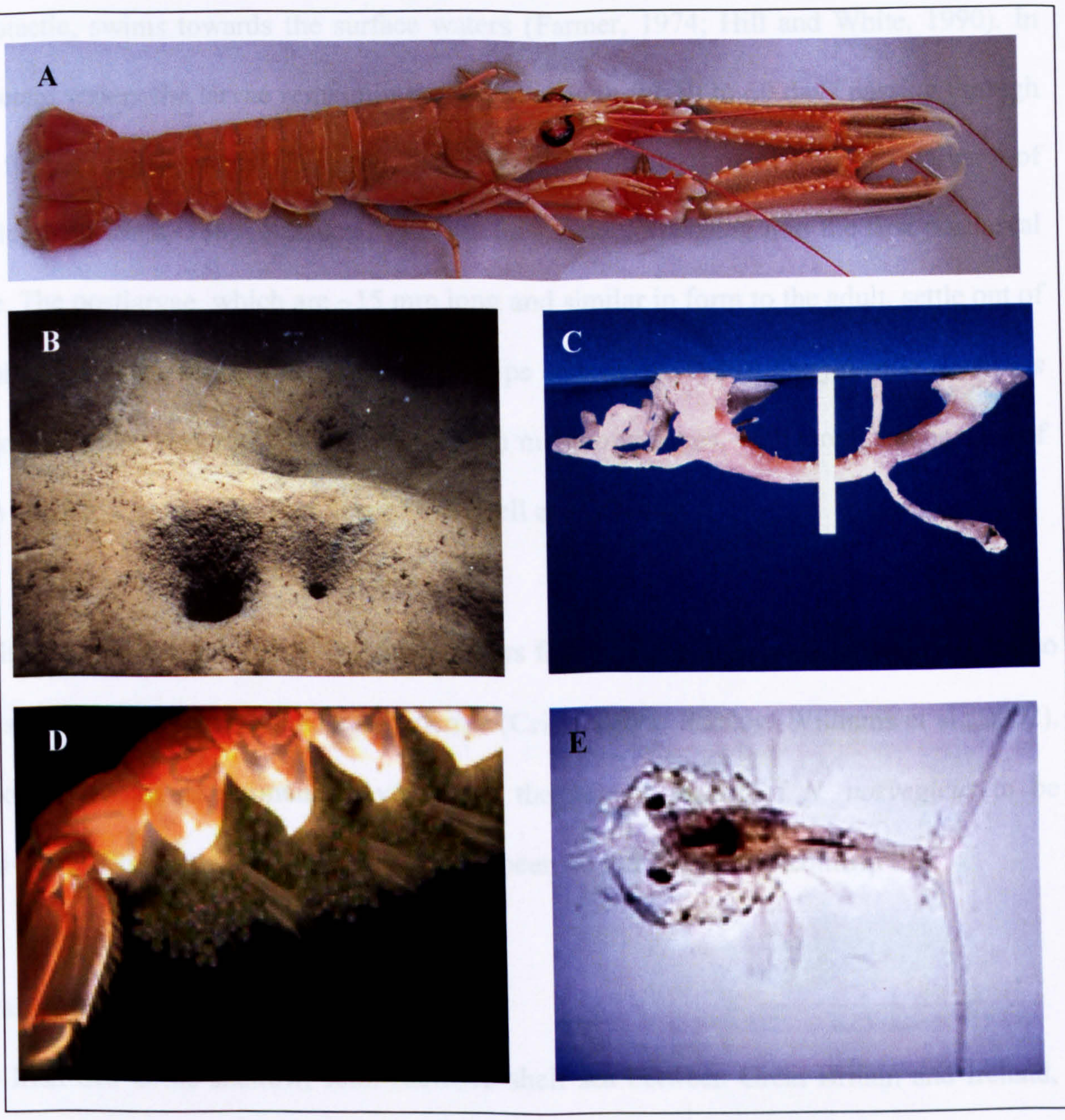

Fig. 1.3 *Nephrops norvegicus* and habitat: (**A**) a fully grown adult; (**B**) burrow opening in soft sediment in *N. norvegicus* grounds (C. Lumb, Loch Sween, Argyll); (**C**) resin cast of a *N. norvegicus* burrow with white 20 cm rule bar to show burrow depth (photograph from Prof. R.J.A. Atkinson); (**D**) brooded eggs on pleopods of female; and (E) stage III *N. norvegicus* larva.

Within a few minutes of hatching, and when clear of the egg membrane, the first free-swimming larval stage (first zoea, hereafter referred to as stage I larva), that is positively phototactic, swims towards the surface waters (Farmer, 1974; Hill and White, 1990). In temperate waters the larvae remain in the water column for 50 to 60 days passing through two further zoeal stages (hereafter referred to as stage II and stage III larvae) of development (Sars, 1889; Santucci, 1926) before metamorphosing into the first postlarval stage. The postlarvae, which are ~15 mm long and similar in form to the adult, settle out of the plankton to the benthos and burrow (Phillips and Sastry, 1980). The juvenile *Nephrops norvegicus* grow very rapidly, passing through numerous stages, with moult frequencies of up to one per month in the first year of life (Bell et al., 2006).

Adult *N. norvegicus* are opportunistic predators feeding mainly on small crustaceans and to a lesser extent polychaetes and echinoderms (Cristo, 1998; Parslow-Williams et al., 2002). Although feeding experiments have shown the pelagic larvae of *N. norvegicus* to be carnivorous (Rotllant et al., 2001) there have been no field studies to confirm this.

Physical oceanography of the Irish Sea

The Irish Sea is the shallow, semi-enclosed shelf sea between Great Britain and Ireland, connected to the Atlantic by the North Channel and St. Georges Channel (Fig. 1.4). Assuming the geographical boundaries of the Irish Sea to be the bottom of St. George's Channel and the midpoint of the North Channel (Fig. 1.4) the sea has a total volume of 2430 km^3, less than 10 % that of the North Sea, and an overall water residence time of about 12 months, although there is some regional variation (Dickson and Boelens, 1988). Two distinct hydrographic areas, the east and the west, result from regional differences in bathymetry, freshwater input and tidal amplitude (Bowden, 1955). The eastern Irish Sea is

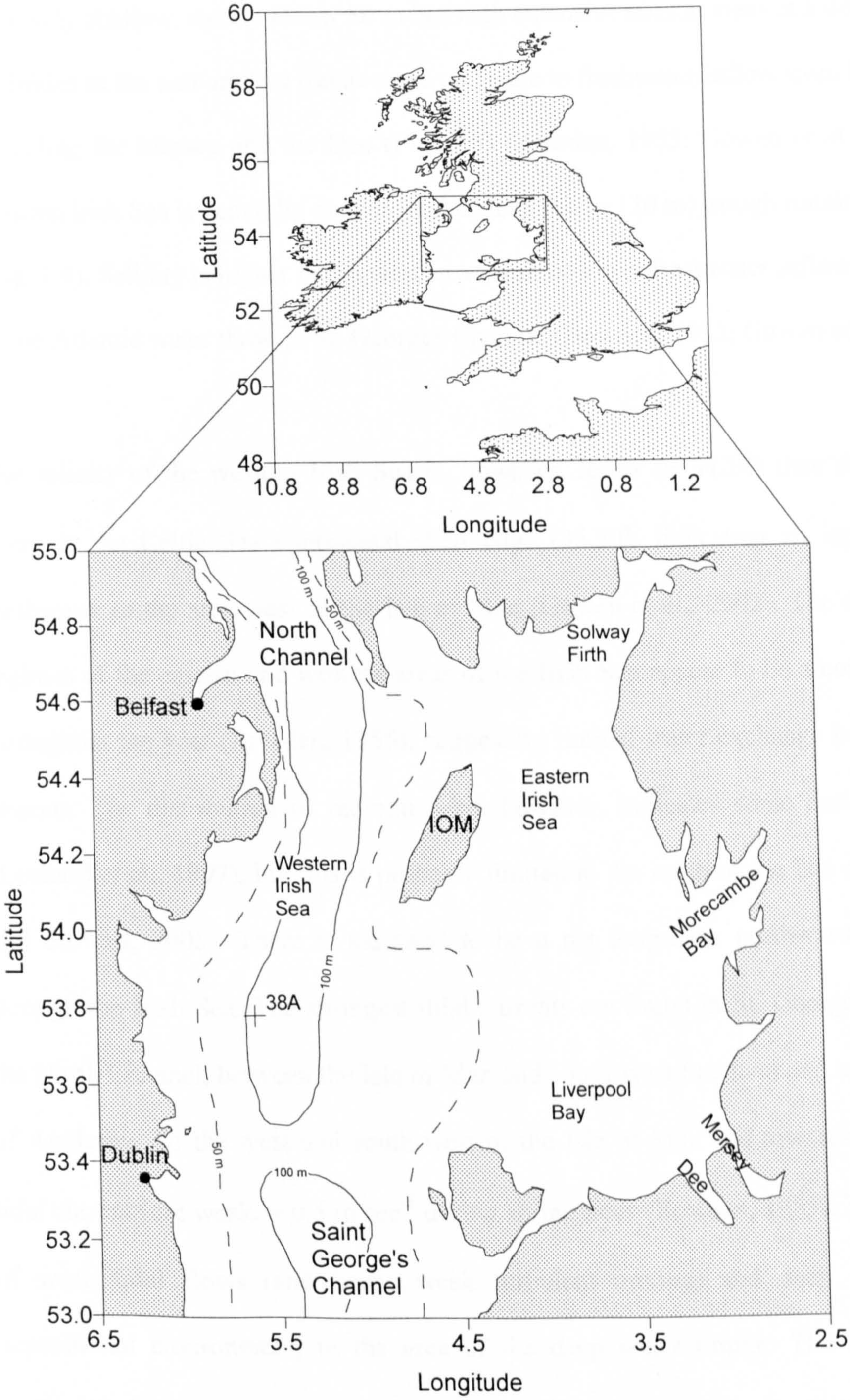

Fig. 1.4 Map of the British Isles and inset showing the Irish Sea with 50 m and 100 m contour lines and the location of the AFBI mooring 38A.

relatively shallow, mostly below 50 m but with extensive coastal areas at a depth of ~20 m. Salinities in the east are low (on average ~32) due to freshwater inflow from English rivers including the Mersey and the Dee (Fig. 1.5) (Bowden, 1955; Gowen et al., 2002). The western Irish Sea is generally deeper, with a deep (up to 130 m) trough running north-south (Fig. 1.4). Salinity is higher in the west as a result of lower freshwater inflow and inflow of saline Atlantic water through St. Georges Channel (Bowden, 1955; Gowen et al., 2002).

The salinity of the western Irish Sea is, however, lower (≈ 34.18) than that of Atlantic water at the Celtic Sea continental shelf edge (35.50), indicating an input of ~3.7 % freshwater to the area west of the Isle of Man (Gowen et al., 2002). The distinct salinity regimes of the eastern and western areas of the Irish Sea appear to be a consistent feature throughout the year (Bowden, 1955), suggesting limited water exchange between the two regions. The distribution of radionuclides, however, indicates some east-west transport (Leonard et al., 1997), but this is probably limited to the north of the Isle of Man (Gowen and Stewart, 2005). There is assumed to be a net long-term northward flow of water through the Irish Sea. The strongest tidal currents are found in St. George's Channel and the North Channel, between the Isle of Man and south-west Scotland and towards the coast of Anglesey. To the west and south-west of the Isle of Man and towards the Irish coast, tidal currents are weak, ≈ 0.5 m sec^{-1} during spring tides (Bowden, 1955). The combination of weak tidal flows (and hence weak turbulent mixing) and deep water creates a depositional environment, in the area of the deep water trough. The deposition helps maintain a large geographically isolated mud patch where *Nephrops norvegicus* is able to construct its burrows and it is this mud patch in the western Irish Sea that supports the Irish *N. norvegicus* fishery.

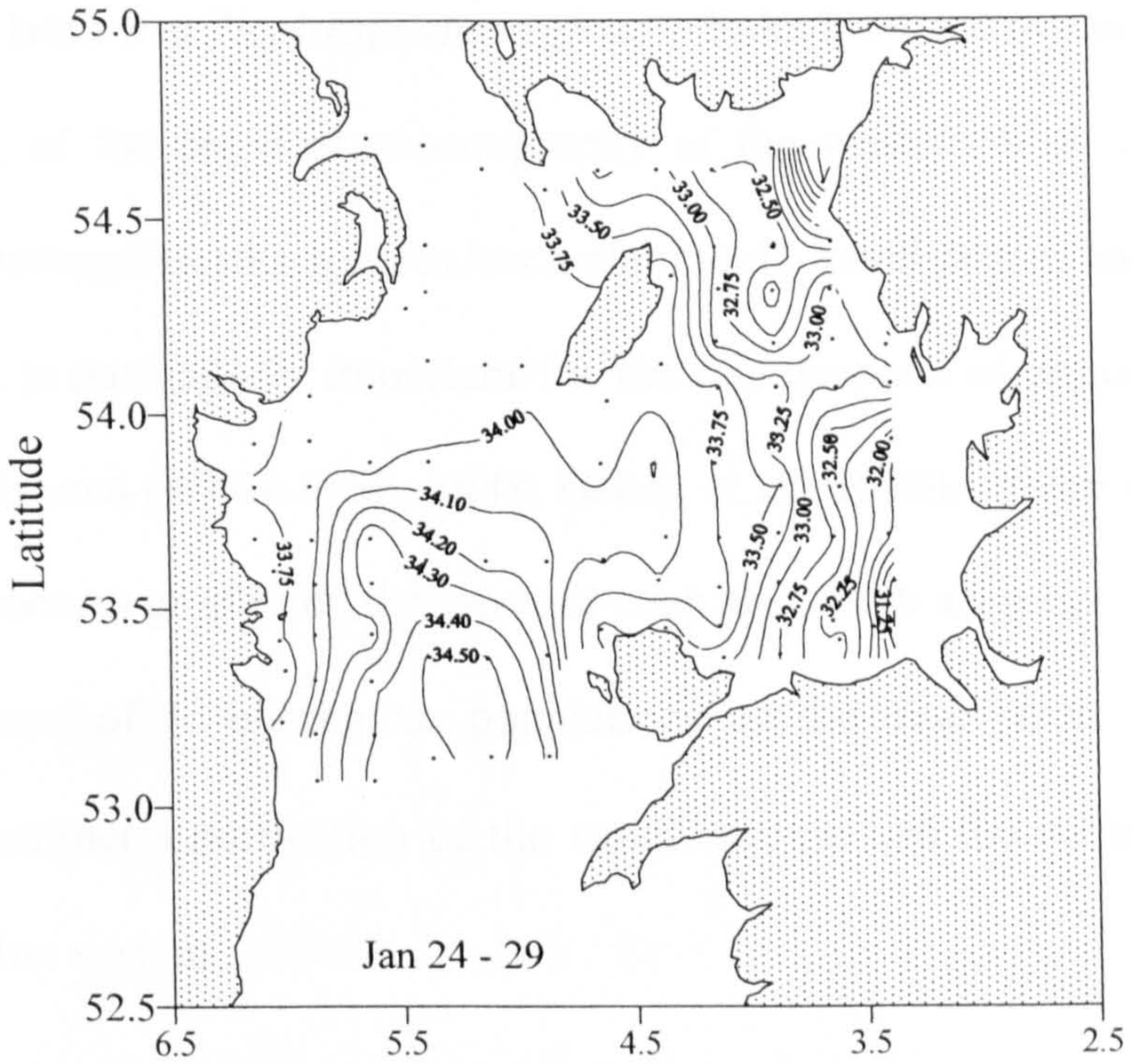

Fig. 1.5 The distribution of near surface salinity in the Irish Sea during January 2000. The contour intervals are 0.2 between 31 and 34 and 0.1 between 34 and 34.5.

The western Irish Sea *Nephrops norvegicus* – links between oceanography and biology

The coupling of the physical oceanography of the western Irish Sea and the life-cycle stages of *N. norvegicus* (the species has a planktonic larval phases and benthic juvenile and adult stages), is particularly important for the maintenance of populations, and hence the fishery, in this area (White et al., 1988; Bailey et al., 1995). There are two key aspects of the physical oceanography of the western Irish Sea, both seasonal, that are important for the maintenance of *N. norvegicus* populations on the mud patch. These aspects are the spring and summer stratification of the water column and the development of a cyclonic gyre circulation system.

In spring, thermal stratification of the deep waters of the western Irish Sea occurs as surface waters warm and there is insufficient tide and wind generated turbulent energy to maintain vertical mixing (Gowen et al., 1995; Gowen et al., 1999b). A dome of cold, nutrient rich bottom water in the deep trough becomes isolated from a surface mixed layer (SML) by the presence of a thermocline (Horsburgh et al., 2000), usually between 20 and 40 m depth. The presence of the cold water dome and associated horizontal bottom fronts are maintained by a cyclonic gyre, or closed current system, of near surface water. The gyre, a consistent feature from year to year, has a period of rotation of approximately 20 days and dominates the region during late spring and summer (Hill et al., 1994; Hill et al., 1997; Horsburgh et al., 2000).

Stratification of the water column during spring and summer and a supply of nutrients (which accumulate over the winter), in particular dissolved inorganic nitrogen, supports the spring growth or 'spring bloom' of phytoplankton, which is often dominated by diatoms (Gowen et al., 1995). The onset of the spring bloom is controlled by the availability of

light, as a function of solar irradiance, subsurface light attenuation and the depth of the surface mixed layer (Gowen et al., 1995). The western Irish Sea spring bloom usually starts in April and peaks sometime in May (Gowen and Bloomfield, 1996; Gowen et al., 1999a). The magnitude of the spring bloom, however, is determined by the availability of nutrients which rapidly become depleted in the thermally isolated surface mixed layer. Trimmer et al. (1999) estimated that ~70 % of the spring bloom was available to export to other trophic levels as new production. In the western Irish Sea there is generally an observable peak in mesozooplankton (mostly copepod) abundance (Gowen et al., 1998) which graze the spring bloom (Gowen et al., 1999a). There is also a pulse of organic matter to the seabed (Trimmer et al., 1999) shortly after the peak of the spring bloom. The input of organic matter to the seabed provides food for the benthic food web and ultimately supports production of *Nephrops norvegicus*.

The presence of the seasonal gyre in the western Irish Sea is likely to play a significant role in the recruitment of *Nephrops norvegicus* to the mud patch. The near-surface water circulation appears to act as a retention mechanism for organisms, including the pelagic larvae of *N. norvegicus* (White et al., 1988; Hill et al., 1996), so providing a better chance of recruitment to the adult grounds. Gyre systems supporting *N. norvegicus* stocks have been identified in other areas such as the North Sea Fladden Grounds, the Clyde Sea and the Adriatic (Bailey et al., 1995). The semi-enclosed nature of the gyre may also limit the import and export of primary production in the western Irish Sea during the spring and summer.

Thus, sinking detritus (which includes phytodetritus from diatoms, dinoflagellates and microflagellates and also copepod faecal pellets and dead copepods) settling on the benthos,

and ultimately supporting the *Nephrops norvegicus* fishery, may be predominantly derived from seasonal primary production within the gyre. If the import and export of seasonal production in the region is limited, the potential yield of benthic production from the western Irish Sea would depend largely on the level of seasonal production within the gyre, its deposition on the seabed and the efficiency with which the organic matter is converted to harvestable biomass through the food chain. Although the flow of energy from organic matter from the pelagic zone into secondary production in the benthos is not fully described in the western Irish Sea, it appears that in some years the impact of detrital carbon (from primary production) on the benthos is short lived, being rapidly mineralised and leaving little available for net secondary production (Trimmer et al., 1999). Thus, the food web linkages and energy pathways leading to benthic production, that supports the adult population of *N. norvegicus* and hence the fishery, are not entirely clear.

Thesis aims

The long term management of the *Nephrops norvegicus* fishery in the western Irish Sea will depend on a better understanding of the interactions between the environment and biology. In particular, there is very close coupling between the life cycle of *N. norvegicus* and the physical environment rendering the species potentially vulnerable to perturbations. For example, the seasonally retentive nature of the western Irish Sea, which probably limits the import and export of *N. norvegicus* larvae from other areas, means that the western Irish Sea populations are dependent on local recruitment. Thus, any changes in local oceanographic conditions, or phytoplankton and zooplankton dynamics, due to climate change for example, could affect the success of recruitment of the larvae to the benthos. In addition, the links between primary production in the pelagic zone and secondary

production of both pelagic larvae and benthic adult *N. norvegicus* are not fully described and there is an apparent shortfall in the supply of carbon to the benthos.

The aims of the thesis are to identify and describe the structure and flow of carbon and nitrogen and establish links between the pelagic and benthic elements of the marine ecosystem important to the production of *N. norvegicus*. The apparent retentive nature of the western Irish Sea makes it an ideal site for the investigation of trophic interactions, and the flow of energy from the pelagic to the benthic environment to support benthic production (Graf, 1992), and hence the fishery, of *Nephrops norvegicus*.

General approach

A fundamental requirement to understanding energy flow through complex marine systems, from carbon fixation through upper trophic-level consumers, is knowledge of trophic linkages between organisms. The analysis of gut contents, to ascertain the diets of particular species, has been a useful method for determining trophic interactions and the structure and functioning of food chains or webs (Hopkins, 1987; Hall and Raffaelli, 1991). However, although gut content analysis (GCA) provides vital information about the species consumed, it can only provide a snapshot in time of the diet spectrum, and since many animals are highly opportunistic feeders, their diet may vary substantially over time. The method is also biased in favour of hard bodied prey items which are likely to remain longer in the gut. In addition, while gut contents give a good indication of what has been consumed, such data can tell nothing about what food is actually assimilated. Also, a large investment in time and expertise is required to gain an understanding of temporal changes in diet.

More recently, stable isotope analysis (SIA) has been found to be a useful tool in food web studies (Kaehler et al., 2000; Jepsen and Winemiller, 2002; Jones and Waldron, 2003). The usefulness of the technique to ecologists arises from predictable fractionation between isotopes during physical, chemical and biological processes. For example, physical processes such as evaporation discriminate against heavy isotopes; and enzymatic fractionation can result in reaction products that are isotopically heavier or lighter than their precursor materials. The isotopic composition of a sample is measured as a ratio of the heavy to the light isotope, for example ^{13}C to ^{12}C in the case of carbon, in a sample compared with the same ratio in a known standard. A ratio, rather than absolute abundance, is used because differences are generally very small and any minor fluctuations, due to differences in sample preparation or day-to-day mass spectrometer measurements for example, are reflected in both the sample and the standard. The differences in the ratios are expressed in delta 'δ' notation and have units of per mil (‰) and all isotope ratios reported in the literature are referenced to primary standards which are set to 0 ‰. For carbon the primary standard is a marine limestone fossil, Pee Dee Belemnite (PDB) (Craig, 1953), which is rich in ^{13}C compared with other biological materials and so carbon isotope ratios in natural systems are usually negative (Fry and Sherr, 1989; Boutton, 1991). The primary standard for nitrogen is atmospheric N_2.

In natural systems, most of the variation in carbon isotopes results from biological fractionation, or discrimination against ^{13}C, during the process of photosynthesis (Farquhar et al., 1989 in Peterson and Fry, 1987). The three major photosynthetic pathways also have distinct carbon fractionation patterns, mainly because of the different properties of the CO_2 fixing enzymes in each pathway. For example, in the Calvin carbon fixation cycle of C_3 plants (most temperate terrestrial plants and marine algae), the enzyme RUBISCO

discriminates against $^{13}CO_2$, resulting in relatively low $\delta^{13}C$ values, usually between -32 ‰ and -20 ‰. In contrast, the carbon fixing enzymes of C_4 plants (primarily tropical and subtropical grassland plants) discriminate against ^{13}C much less, and so C_4 plants have higher values of $\delta^{13}C$, usually in the range -17 to -9 ‰. The third group, the CAM plants (Crassulacean acid metabolism, mostly Cactaceae and Euphorbiaceae) have carbon isotope values close to C_4 plants.

The stable isotope value of photosynthetically produced organic carbon remains relatively constant as organic matter moves through trophic levels (Peterson and Fry, 1987) and so $\delta^{13}C$ can be used to evaluate sources of diet, particularly where the ultimate sources for a consumer have different isotope values. In addition to C_3 and C_4 plants, $\delta^{13}C$ can also be used to discriminate between terrestrial and marine derived organic matter and between pelagic and benthic production (DeNiro and Epstein, 1978, 1981). For example, photosynthesis in the marine environment produces algae enriched in ^{13}C compared with terrestrial or freshwater plants, even though all are C_3 plants, probably because bicarbonate as a source of carbon for photosynthesis is substantially higher in ^{13}C than atmospheric CO_2. Thus, organic matter in marine systems is enriched, with an average $\delta^{13}C$ value of -22 ‰, compared with values in the range -30 ‰ to -24 ‰ in estuarine and riverine systems (Fry and Sherr, 1989).

In contrast, the stable isotope of nitrogen ($\delta^{15}N$) becomes enriched relative to diet, typically by about 3-4 ‰ (average 3.4 ‰) per trophic level, and so can be used to estimate trophic position (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984). The $\delta^{15}N$ value integrates the diet assimilated over time (which depends on the age, size and growth rate of the animal and the tissue type) and so is a useful tool for estimating trophic position

for organisms that frequently switch diet and where the diet is predominantly of prey that are difficult to identify in gut content studies. Natural variations in ^{15}N can also be employed as a natural tracer of the sources and sinks of nitrogen in natural conditions (Peterson, 1999).

Thus, the combined analysis of carbon and nitrogen stable isotope ratios of organisms can potentially determine trophic position and feeding links within ecosystems (Post, 2002), especially when used in conjunction with gut content analysis. The method can also provide a useful tracer of biological processes (Peterson, 1999) because factors such as growth rate and availability of nutrients, which vary seasonally, often cause temporal variation in stable isotope values. Isotopes cycle in biological systems through the alternative processes of fractionation, separating isotopes, and mixing that combines them. For example, in an estuarine food web, fractionation during plant photosynthetic carbon fixation results in different isotope signatures of seagrass and phytoplankton. Mixing of these different isotopes occurs when the food source of an estuarine consumer consists of both seagrass and phytoplankton. Thus, stable isotopes can be used as tracers for processes of fractionation and mixing in natural systems. In this way stable isotopes may be used to track the flow of energy in the western Irish Sea, from primary production in the water column, through the flux of organic matter to the benthos and the benthic organisms on which *Nephrops norvegicus*, and hence the fishery, depends.

Study site

Sampling took place at a single site within the *Nephrops norvegicus* grounds in the deep trough of the western Irish Sea (Fig. 1.4). The sampling site, station S38A, has a water depth of ~90 m and is the location of a permanent instrumented buoy (53°51′N 05°34′W)

deployed by AFBI (Agri-Food and Biosciences Institute[1]). The mooring is part of an AFBI long term monitoring programme to collect high resolution data on temperature, salinity, fluorescence, dissolved inorganic nutrients and phytoplankton. A large body of historical physical and biological data for S38A are, therefore, held by AFBI.

Sampling programme

The major sampling programme took place from February 2004 to January 2005 onboard the RV Lough Foyle. There were three further research cruises during the spring of 2006, onboard the RV Corystes, for additional sampling (see Table 1.1).

A variety of pelagic and benthic samples were collected using a range of sampling methods. These methods are explained in full in the appropriate chapters. However, a summary of the sampling programme, samples taken and the analysis carried out during the project is given in Table 1.2.

[1] In April 2006 AFBI was created from the amalgamation of the Department of Agriculture and Rural Development (DARD) Science Service and the Agricultural Research Institute of Northern Ireland (ARINI).

Table 1.1 Sampling dates for 2004/5 and 2006 field programmes at AFBI mooring station S38A in the western Irish Sea.

RV Lough Foyle	RV Corystes
23 February 2004	29 March 2006
02 April 2004	06 April 2006 *#
16 April 2004	25 May 2006
26 April 2004 *	
04 May 2004	
10 May 2004	
31 May 2004	
28 June 2004	
02 August 2004	
12 August 2004 *	
23 September 2004	
15 November 2004	
21 January 2005	

* CTD was unavailable and it was not possible to record profiles of temperature, salinity and chlorophyll fluorescence.

Water samples taken by AFBI staff on RV Corystes from the ship's clean seawater supply.

Table 1.2 Sampling schedule for 2004/5 and 2006 field programmes at AFBI mooring station S38A in the western Irish Sea.

Samples	Analysis	2004/2005 F	M	A	M	J	J	A	S	O	N	D	J	2006 M	A	M
Water column	Physical structure: temperature and salinity	←	—	—	—	—	—	—	—	—	—	—	→	↔		↔
Water column	Chlorophyll concentration, particulate organic matter carbon and nitrogen content and $\delta^{13}C$ and $\delta^{15}N$	←	—	—	—	—	—	—	—	—	—	—	→	←	—	→
Water column	Dissolved inorganic nitrogen	←	—	—	—	—	—	—	—	—	—	—	→			
Water column	Nitrate $\delta^{15}N$													←	—	→
Zooplankton	Carbon and nitrogen stable isotopes, community composition and biomass calculations	←	—	—	—	—	—	—	—	—	—	—	→			
Larval *Nephrops*	Isotope analysis and gut content analysis			↔	—	→										
Adult *Nephrops*	Carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$	←	—	—	—	—	—	—	—	—	—	—	→			
Adult *Nephrops*	Size/body mass analysis, isotope analysis			↔				↔						←	—	→
Benthic epifauna	Carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$	←	—	—	—	—	—	—	—	—	—	—	→			
Benthic infauna	Biomass, Carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$													←	—	→
Sediment	Chlorophyll concentration, Carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$	←	—	—	—	—	—	—	—	—	—	—	→			

Outline of the thesis

The major aims of the work presented in this thesis were to: describe the 2004 seasonal cycle of primary production and using stable isotope analysis investigate carbon and nitrogen flow through the planktonic food web to the larvae of *Nephrops norvegicus;* establish and quantify the linkages between the pelagic and benthic elements of the marine ecosystem that drive secondary production; and describe the trophic structure in the benthos and estimate the flow of carbon and nitrogen through the benthos that ultimately supports *N. norvegicus* populations and, hence, the fishery in the western Irish Sea.

Chapter Two

The overall aim of the work presented in Chapter Two was to describe and quantify and the seasonal dynamics of primary production, in particular the spring phytoplankton bloom, and to characterise the stable isotope dynamics of carbon and nitrogen during a seasonal cycle, in the western Irish Sea in 2004.

Chapter Three

The overall aim of the work presented in Chapter 3 was to investigate the links between primary and secondary production, to determine the diet of *N. norvegicus* larvae and investigate the trophic linkages between the larvae and zooplankton, via a combination of gut content and stable isotope analysis. Investigation of the composition of zooplankton populations, production and its stable isotope values were also undertaken to assess the links between *N. norvegicus* larvae and seasonal production of zooplankton and ultimately to the spring bloom.

Chapter Four

In this chapter the stable isotope values of particulate organic matter, produced during the spring bloom, are used to describe and estimate the vertical flux of organic matter from the euphotic zone to seabed in the western Irish Sea and together with estimates of production used to quantify the input of organic matter to the benthos. The highly retentive nature of the western Irish Sea during the stratified spring and summer, and high levels of secondary production, makes it an ideal region for investigating carbon input through pelagic-benthic coupling.

Chapter Five

The overall aim of the work presented in Chapter Five was to determine the abundance and biomass of organisms in the benthos at S38A in the western Irish Sea and together with carbon and stable isotopes model the flow of carbon through the benthos to the *Nephrops norvegicus* fishery. In particular, isotope analysis was used to establish the food sources and estimate the relative trophic position of *N. norvegicus* in order to determine the key organisms in the flow of energy to the fishery. The diet of *N. norvegicus* for different life cycle stages and any difference in males and females were also investigated.

Chapter Six

Chapter six presents a brief summary of the key findings of the research presented in the preceding chapters and a short discussion of the utility of stable isotope analysis in the context of this research.

All chapters were written as independent parts although some repetition, particularly of method description, has been unavoidable. Where possible these descriptions have been

reduced and reference has been made to the chapter where the detailed methods were first described.

Chapter Two

Seasonal dynamics of particulate organic matter in the western Irish Sea

Introduction

Marine phytoplankton are the base of the food chain on which zooplankton, fish, marine mammals, sea birds and the fishing industry all depend (Hardy, 1959). Pelagic production also regulates secondary production in the benthos (Mills, 1975; Graf, 1989) because in many areas it is the only form of organic input to the benthic marine food web (Graf, 1992). The overall productivity of the oceans is thus ultimately dependent on the patterns and magnitude of primary production in the water column.

In north temperate shelf seas that seasonally stratify, there is marked seasonality of production including a 'spring bloom' of phytoplankton (Margalef, 1958). The timing of the spring phytoplankton bloom is controlled by the availability of light and the depth of surface mixing (Gran and Braarud, 1935; Sverdrup, 1953; Smetacek and Passow, 1990; Tett, 1990). In the winter, low solar insolation coupled with vertical mixing of the water column means that phytoplankton receive insufficient light for net photosynthesis. In these conditions, little or no growth occurs and so phytoplankton must either subsist on accumulated reserves, form inactive resting stages or starve (Barnes and Hughes, 1999). In winter months, therefore, chlorophyll concentration (used as a measure of phytoplankton biomass) is at a minimum. In the spring, solar insolation increases so that fewer phytoplankton cells are light limited and growth slowly increases. Increased light levels also heat surface waters and in deep areas (where there is insufficient turbulence to vertically mix the water column) thermal stratification of the water column develops,

trapping phytoplankton in an illuminated 'surface mixed layer' (SML) called the euphotic zone (Tett, 1990). At typical spring irradiance levels, maximal phytoplankton cell division occurs when the surface mixed layer is 10-20 m and culminates in a rapid build-up of phytoplankton biomass, the 'spring bloom' (Smetacek and Passow, 1990). Thus, in deep, seasonally stratifying waters the length of the production season is dependent on the presence of a surface mixed layer, to retain phytoplankton in the euphotic zone where net production can take place, although stratification also limits nutrient availability (Barnes and Hughes, 1999). In shallow seas, where the depth of the euphotic zone exceeds the depth of the seabed, the production season is generally longer and potentially greater because growth is only dependent on light and nutrient availability, not stratification of the water column. In areas of higher energy, where stratification does not develop, production may still occur but not lead to a bloom.

While the level of sub-surface irradiance controls the onset and duration of the production season, it is the availability of dissolved inorganic nutrients (in particular nitrogen) that controls the overall amount of primary production (Parsons et al., 1984b). The accumulation of nutrients over the winter period fuels the spring growth of phytoplankton. However, in stratified systems dissolved inorganic nutrients are rapidly stripped from surface waters by growing phytoplankton and the thermocline limits replenishment from nutrient rich waters below. Thus, the period of rapidly increasing primary productivity at the beginning of the spring bloom is accompanied by a period of rapid depletion in nutrients and production becomes nutrient limited even when light levels are optimal (Parsons et al., 1984b). At the beginning of the spring bloom the biomass of algae is low but increases as the bloom begins and phytoplankton growth outstrips losses to higher trophic levels, in particular to grazing. As the bloom progresses, however, grazer biomass

increases, and the losses of phytoplankton due to grazing and sinking out of the euphotic zone, exceed the rate of accumulation by growth and the spring bloom declines. Zooplankton growth is temperature dependent and so the timing of peak zooplankton biomass, and therefore maximal grazing, will be determined by local temperature conditions (Gowen et al., 1999). Following the spring bloom, regenerated nitrogen, in the form of ammonium, is excreted by zooplankton and heterotrophic micro-organisms (Bode et al., 2004), which can be taken up by phytoplankton. Thus, production in near-surface waters of shelf seas may continue for several months, albeit at lower levels, beyond the initial spring bloom. The production season is, therefore, made up of 'new' production from the build-up of winter nitrogen that can be exported to higher trophic levels via grazing and sinking, and 're-generated' production that is not available for export (Dugdale and Goering, 1967).

In the western Irish Sea, primary production follows the pronounced seasonality described above, although some regional variation in timing and production, linked to differences in physical oceanography, does occur (Gowen et al., 1995). In the shallower coastal waters, for example, there is an early production season which starts in March or April and persists until early October. In the deeper offshore waters of the western Irish Sea, the sediments of which support the *Nephrops norvegicus* fishery, there is seasonal stratification and a shorter production season, often starting in April or May and lasting until August or September.

The Irish Sea supports several important fisheries and the primary productivity of the waters will ultimately determine secondary production and hence the success of the fisheries. In the water column primary production is transferred up the food chain via the grazing activity of zooplankton that are themselves an important food source for many

carnivores in the ocean. Primary production is also transferred to secondary production in the benthos via the flux of organic matter to the seabed. In the western Irish Sea secondary production in the benthos is assumed to be largely supported by primary production in the overlying water column. The first step to understanding the origins and pathways of organic matter from the water column into the *N. norvegicus* benthic fishery in the western Irish Sea, of the pelagic larvae and the benthic adult, is to determine the scale and isotopic signature of primary production in the waters overlying the fishery. These values can then be used to trace the flow of organic matter from primary production to higher trophic levels.

The overall aim of the work presented in Chapter Two was to describe and quantify the seasonal dynamics of primary production, in particular the spring phytoplankton bloom, and to characterise the stable isotope dynamics of carbon and nitrogen during a seasonal cycle, in the western Irish Sea in 2004. An understanding of the stable isotope dynamics of the production season is essential to the application of stable isotopes as a tracer of the flux of organic matter from primary production to higher trophic levels, in particular zooplankton in the water column and the flux of organic matter to the seabed to support secondary production in the benthos. In order to achieve this, the specific objectives were: (i) describe and quantify the seasonal cycle of dissolved inorganic nutrients and chlorophyll biomass and estimate seasonal primary production; and (ii) establish the carbon and nitrogen stable isotope seasonal dynamics of primary production.

Methods

Field programme

All sampling took place at station S38A in the western Irish Sea (Fig. 2.1 and see Chapter 1 for full site description) on ten occasions between February 2004 and January 2005 on board the RV 'Lough Foyle' and a further three occasions between March and June 2006 on board the RV 'Corystes'.

Water column characteristics

Sampling: During each cruise vertical profiles of temperature, salinity and fluorescence were recorded using a Falmouth Scientific Instruments (FSI) conductivity-temperature-depth (CTD) sensor and a Chelsea Instruments fluorometer, mounted on a rosette water sampler. The CTD was calibrated for temperature by FSI in 2003 and regular checks were made using an additional temperature sensor (Ocean Temperature Module) and reversing thermometers and corrections applied when necessary. The CTD was calibrated for salinity by salinometer measurements on discrete water samples (carried out at Ocean Scientific International Ltd using a Guildline Autosal 8400B). The depth of the surface mixed layer (SML) was defined as the depth where temperature was at least 0.5 °C lower than the temperature at 2 m (Talling, 1971; Gowen et al., 1995). The fluorometer was not calibrated, but the voltage output provided an estimate of chlorophyll fluorescence and, hence, an indication of the vertical distribution of chlorophyll through the water column. The depth of the euphotic zone (the depth at which irradiance was 1 % of surface irradiance (Smetacek and Passow, 1990)) was not measured directly, but on the basis of

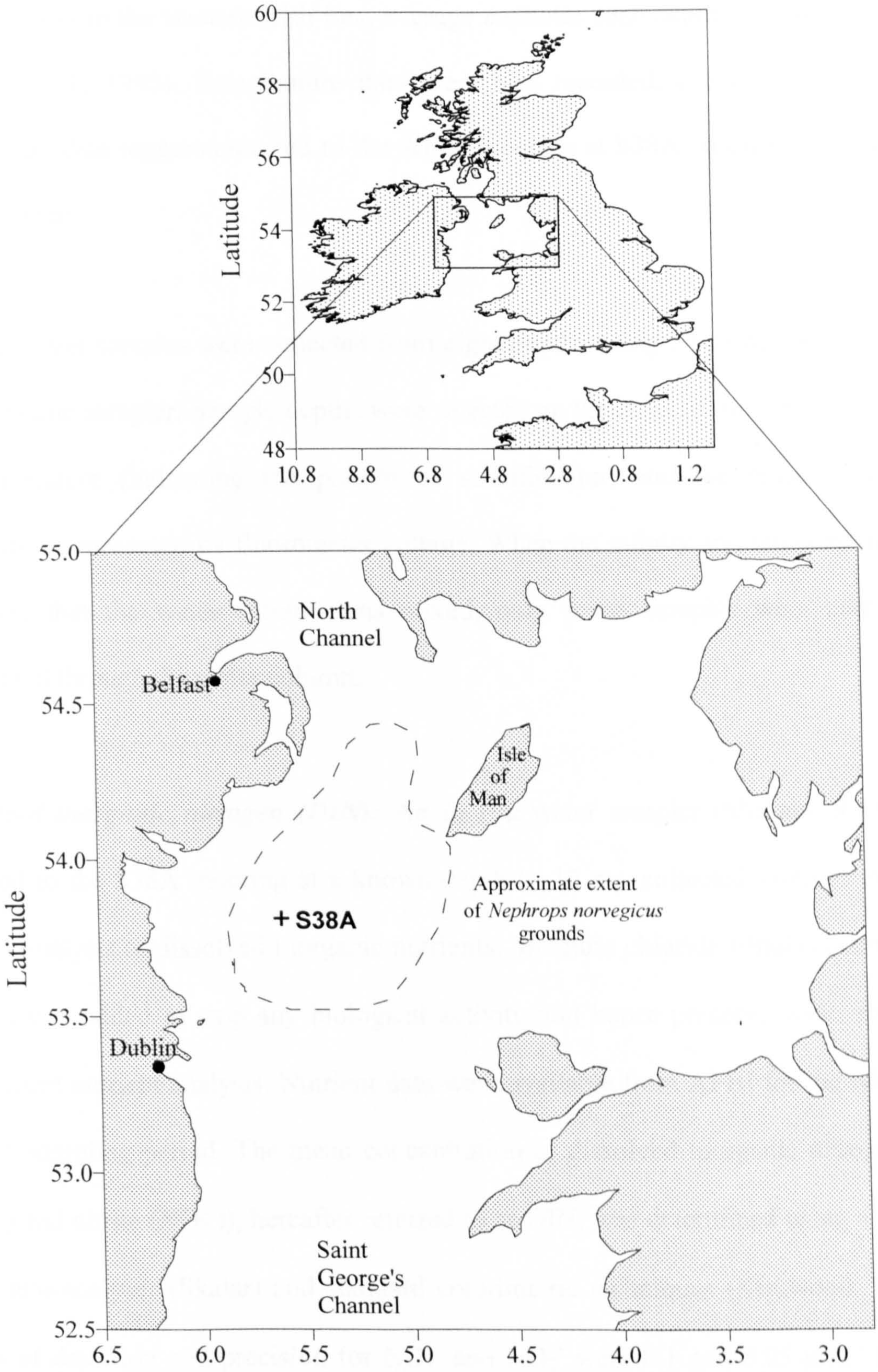

Fig 2.1 A map of the British Isles and inset showing the Irish Sea with the location of the AFBI mooring station S38A (+) (53°46'N, 05°38'W). The dashed line shows the approximate area of the spring stratified region and extent of the muddy *Nephrops norvegicus* ground.

previous work in the western Irish Sea, average euphotic zone depth was taken to be 23 m (Gowen et al., 1995). Temperature data were also recorded, every 3 hours, by seven temperature data loggers attached to the AFBI mooring at S38A, at different depths from ≈ 2 to ≈ 90 m.

Discrete water samples were collected from eight depths using 5 L water bottles, mounted on the rosette sampler. Sample depths were selected on the basis of the vertical distribution of temperature (indicating the pattern of stratification) and the relative chlorophyll fluorescence measured by fluorometer voltage. When the salinity and temperature profiles indicated that the water column was mixed, only seven samples were taken, evenly distributed through the water column.

Dissolved inorganic nitrogen (DIN): An *in situ* water sampler (McLane RAS 3-48N), attached to the S38A mooring at a known depth (≈ 12 m), collected samples every other day for analysis of dissolved inorganic nutrients. Mercuric chloride (final concentration 20 mg l^{-1}) was added to stop any biological activity and hence preserve water samples for subsequent nutrient analysis. Nutrient data were available from AFBI for the whole of the 2004/5 sampling period. The mean concentration of dissolved inorganic nitrogen (nitrate (NO_3^-) and nitrite (NO_2^-)), hereafter referred to as DIN, was determined using a segmented flow auto-analyser (Skalar) and standard colorimetric techniques (Kirkwood, 1996). The limits of detection and precision for NO_3^- and NO_2^- were 0.1 and 0.05 μmol L^{-1} ± 1 %, respectively.

Chlorophyll: Sub-samples (400 ml) of water from each water bottle were passed through a mesh (200 μm) to remove zooplankton (Omori and Ikeda, 1984) and filtered (Whatman

GF/F, nominal pore size 0.45 μM) under gentle vacuum (-0.35 bar). The filters were frozen to -20°C until later analysis. The chlorophyll concentration of water samples was determined by acetone extraction of algal pigments (90 %, 24 h at 4°C) after Tett (1987). Measurements of chlorophyll fluorescence were made (before and after acidification with 8 % hydrochloric acid to distinguish phaeopigments) using a Turner Designs Model 10 filter fluorometer calibrated against chlorophyll *a* (Sigma UK). Euphotic zone chlorophyll standing stock (mg m^{-2}) was estimated by interpolation between chlorophyll concentrations measured at discrete sampling depths in the upper 23 m of the water column. Gross primary production was estimated using the relationship between chlorophyll standing stock and water column production taken from published Irish Sea data (Gowen and Bloomfield, 1996):

$$\ln \text{production} = 0.637 \times (\ln \text{chlorophyll standing stock} + 3.625) \qquad (2.1)$$

Linear interpolation of daily primary production between sample dates was used to calculate spring bloom and seasonal production. The period of the spring bloom was defined by the period between the winter maximum and summer minimum of the total water column stock of DIN, and seasonal production by the period of stratification. The values of spring bloom and seasonal production are quoted without units of time but relate to a specific period of time as given in the results.

Phytoplankton identification samples: Water samples (200 ml) from each depth sampled were preserved with acidified Lugol's iodine (3 ml) (Parsons et al., 1984a). Sub-samples (50 ml) were settled in a sedimentation chamber over 24 h and counted using a Leitz DIAVERT inverted microscope (x 250 magnification). Identification was made to genus in most cases and to species where possible. Data on the carbon content of individual phytoplankton species, derived from measurements of cell dimensions and standard cell

volume carbon conversion factors, were provided by Dr B Kelly-Gerreyn (National Oceanography Centre, Southampton). Where identification was made to genus, mean carbon biomass values from several species were used. Phytoplankton carbon biomass per volume was then calculated for each sample. Euphotic zone samples, from 23 February to 10 May were analysed (see Table 2.5 in results for details of samples analysed).

Particulate organic matter carbon and nitrogen content and stable isotope analysis:

A sub-sample (1 L) of water was taken from each bottle on the rosette sampler and passed through a mesh (200 μm) to remove most zooplankton (heterotrophs such as tintinnids and ciliates, generally smaller than 200μm, would, however, remain) (Omori and Ikeda, 1984), collected on a pre-ashed (565°C annealing oven, 8 h) filter (Whatman GF/F), under gentle vacuum (-0.35 bar) and stored at -20°C until laboratory analysis. Filters were dried (60°C for 24 h) and cut into two halves. To remove inorganic carbonates for the measurement of organic carbon, one half of the filter paper was subjected to vapour acidification with concentrated HCl (12 M) and then re-dried (60°C for 24 h) prior to analysis (Hedges and Stern, 1984). The remaining untreated half of the filter paper was used for the measurement of total nitrogen content and nitrogen stable isotope values. Both halves of the filter paper were sub-sampled with a cork borer (diameter 5 mm) and 3 or 4 small discs placed in a tin capsule (ultra-clean 8 x 5 mm, Elemental Microanalysis) for elemental and stable isotope analysis. All equipment was cleaned with acetone and thoroughly dried between each sample.

Nitrate isotope analysis sample preparation: Water samples (approximately 3 L) were filtered (pre-ashed Whatman GF/F) to remove particulate matter and frozen to -20°C. On return to the laboratory, samples were rapidly defrosted and passed through a large

(200 ml) cadmium-copper reduction column to reduce nitrate to nitrite (Wood et al., 1967; Strickland and Parsons, 1972). The nitrite was then converted to an azo-dye, 1-phenylazo-2-naphthol (Sudan-1) which was concentrated from samples by reverse phase chromatography using C18 solid phase extraction (SPE) columns (6 ml, 500 mg) (Preston, 1992; Johnston et al., 1999). The Sudan-1 was then eluted from the SPE columns into small glass test tubes, with ethyl acetate (1 ml), and dried at 40 °C until solid. The Sudan-1 was then re-suspended in acetone (200 µl) and the solution placed in derivitization vials (300 µl) and left to evaporate until only 20 µl of the solution was remaining. The liquid concentrate of Sudan-1 in acetone was then pipetted onto a stack of 3 small discs (6 mm diameter) of pre-ashed GF/F filters, and when dry placed in tin capsules for isotope analysis. Solid standards of several different nitrate and nitrite standards (KNO_3, $NaNO_3$ and $NaNO_2$) were analysed for $\delta^{15}N$ and then solutions prepared in the same way as samples for preparation of a calibration curve of $\delta^{15}N$ signature of the azo-dye to the original solid (Johnston et al., 1999).

Carbon and nitrogen biomass and stable isotope analysis

Organic carbon, nitrogen content and stable isotope values were determined using an elemental analyser (ThermoFinnigan TC/EA Flash 1112, Bremen, Germany) coupled to a continuous flow isotope ratio mass spectrometer (CF-IRMS) (ThermoFinnigan Delta Plus, Bremen, Germany). Isotope values are reported using the standard delta (δ) notation (McKinney et al., 1950) in units of per mil (‰):

$$\delta X = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] \times 1000 \qquad (2.2)$$

where X is the heavy stable isotope of interest (e.g. ^{13}C or ^{15}N) and R is the ratio of the heavy to the lighter isotope. National Institute of Standards and Technology (NIST) standards of sucrose (for carbon) and ammonium sulphate (nitrogen) were used and all delta values are reported relative to Pee Dee Belemnite (PDB) and atmospheric N_2, respectively. Calibration with a range of urea standards for determination of carbon and nitrogen content was carried out for each sample run. A solid internal working standard, Cicloesanone-2,4-dinitrofenilidrazone ($C_{12}H_{14}N_4O_4$), was also run every 7 samples to monitor column performance, recovery and precision of C and N stable isotope values. Analysis of the internal standard indicated the internal precision of the mass spectrometer when the particulate organic carbon and nitrogen samples were being run was ± 0.15 ‰ for nitrogen and ± 0.13 ‰ for carbon (internal standards $n = 32$).

However, the accuracy and precision of nitrogen stable isotope values has been shown to be affected by small sample sizes (Avak and Fry, 1999). The size of samples of particulate organic matter (POM), collected on a filter, are often small because there are low concentrations of POM in the water column, and the time to filter water samples, particularly where there are high loadings of sediment particulate matter, limits the sample size. Also, as it is not possible to fit a whole filter paper into a tin capsule sub-samples are taken, further reducing the sample size.

Calibration of $\delta^{13}C$ and $\delta^{15}N$ with different mass amounts of urea were prepared to determine the impact of small sample size on both carbon and nitrogen stable isotope values. The calibration for carbon (Fig. 2.2A) showed increasing $\delta^{13}C$ and increasing variability with decreasing sample size. At the largest sample sizes of 200 µg ($\log_{10} = 2.3$) the $\delta^{13}C$ of the urea was -50 ‰. There was little change in $\delta^{13}C$ values of urea down to a

sample size of 42 μg ($\log_{10}$ = 1.6). For a sample size smaller than 42 μg there was an exponential enrichment in $\delta^{13}C$ values. The formula used to correct $\delta^{13}C$ values where sample sizes are small (< 42 μg), where x = $\log_{10}$ sample size, is:

$$\delta^{13}C = -36.78 + -17.82x + 6.7x^2 + -0.4x^3 \quad (2.3)$$

The same exercise with nitrogen showed there is a trend of decreasing $\delta^{15}N$ and increasing variability with decreasing sample size. The smallest samples in the calibration, 1.4 μg N, are up to 1.06 ‰ ± 1.88 ‰ lighter than the largest samples (196 μg N) (Fig. 2.2B). The formula to correct $\delta^{15}N$ values where sample sizes were small is:

$$\delta^{15}N = 0.503\,x - 1.1992 \quad (2.4)$$

Data analysis

Euphotic zone values presented are mean ± 1 standard error, except where stated. A *t*-test was used to test for differences between two means. Where there were more than two samples differences between means were tested using single-factor ANOVA and where significant followed by a Tukey test for pairwise comparisons (Zar, 1999). The strength of association between variables was determined by regression analysis: from the coefficient of determination (r^2) and analysis of variance to determine the significance of the regression line.

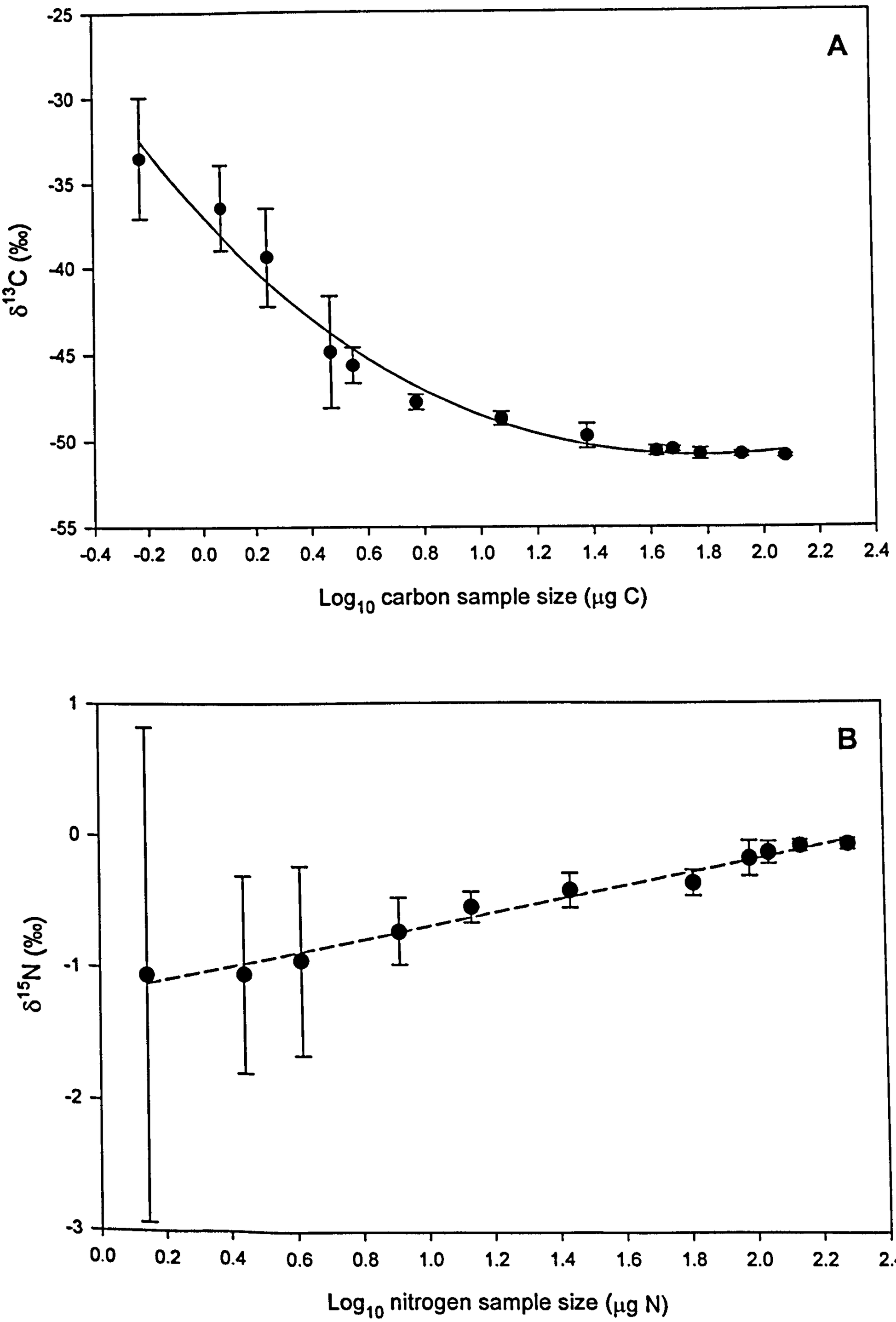

Fig 2.2 Stable isotope values as a function of amount of either **A**: carbon or **B**: nitrogen, in μg. Error bars are 95 % CI, $n = 46$.

Results

Water column characteristics

Stratification of the water column: At the time of first visit in February, 2004, the temperature of near surface (2 m) water at 38A was 8.5 °C and the water column was isothermal (Fig 2.3A). Intermittent warming of surface water, in comparison to bottom water, was evident between late March and late April. By mid April warming of the surface water by ~ 0.5 °C had occurred and stratification was fully established by 23 April (Fig. 2.4). At the beginning of May a surface mixed layer (SML), to a depth of 36 m, was evident (Fig. 2.3A and B). In subsequent weeks, thermohaline stratification intensified as the depth of the SML was reduced and the surface to bottom difference in temperature increased. In the middle of August the depth of the SML was ≈ 10 m and the surface to bottom temperature difference was 5.8 °C. A detailed picture of the seasonal pattern of stratification was obtained from the thermistors (temperature data loggers) deployed at selected depths on the AFBI mooring. It is evident from the near surface and bottom temperature data that stratification was eroded during September as surface waters cooled and bottom water warmed (Fig. 2.4). By September 22, the water column was isothermal, indicating that stratification had broken down. The production season, defined by the period of stratification was, therefore, the period from 23 April to 22 September (Fig. 2.4). There was a significant increase in salinity of the bottom water, corresponding with increasing DIN concentration, from 34.15 in September to 34.65 in November (Fig. 2.3B).

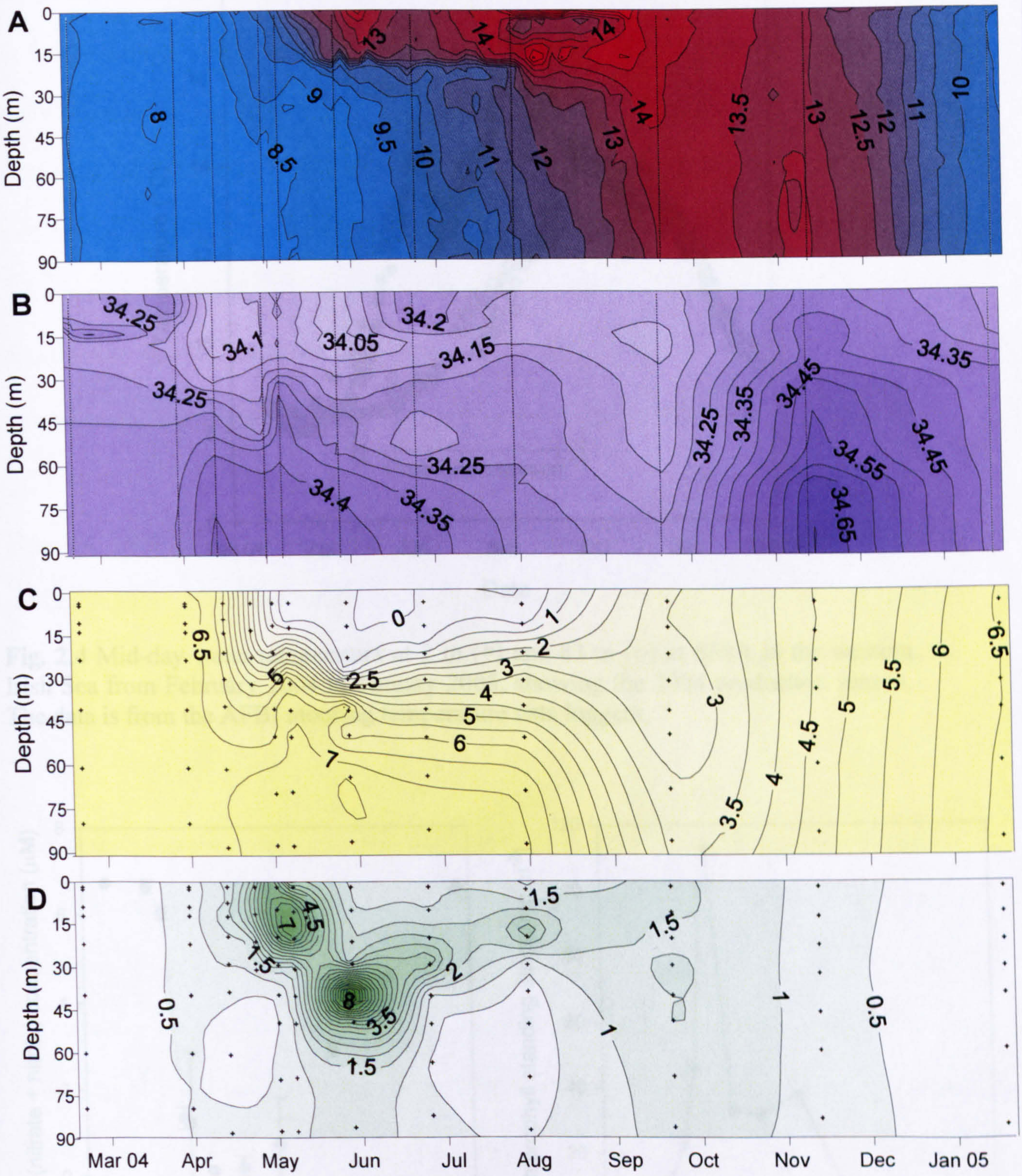

Fig. 2.3 Water column characteristics at the western Irish Sea station S38A between February 2004 and January 2005. **A:** temperature (°C); **B:** salinity; **C:** concentration of dissolved inorganic nitrogen (nitrate plus nitrite in μM) and **D:** concentration of chlorophyll (mg m^{-3}). Vertical lines indicate sampling dates (see Table 1.1) and each dot shows the point at which individual measurements were made. The contour interval is 0.5 for temperature, nutrients and chlorophyll and 0.05 for salinity. The dissolved inorganic nitrogen data were supplied by AFBI.

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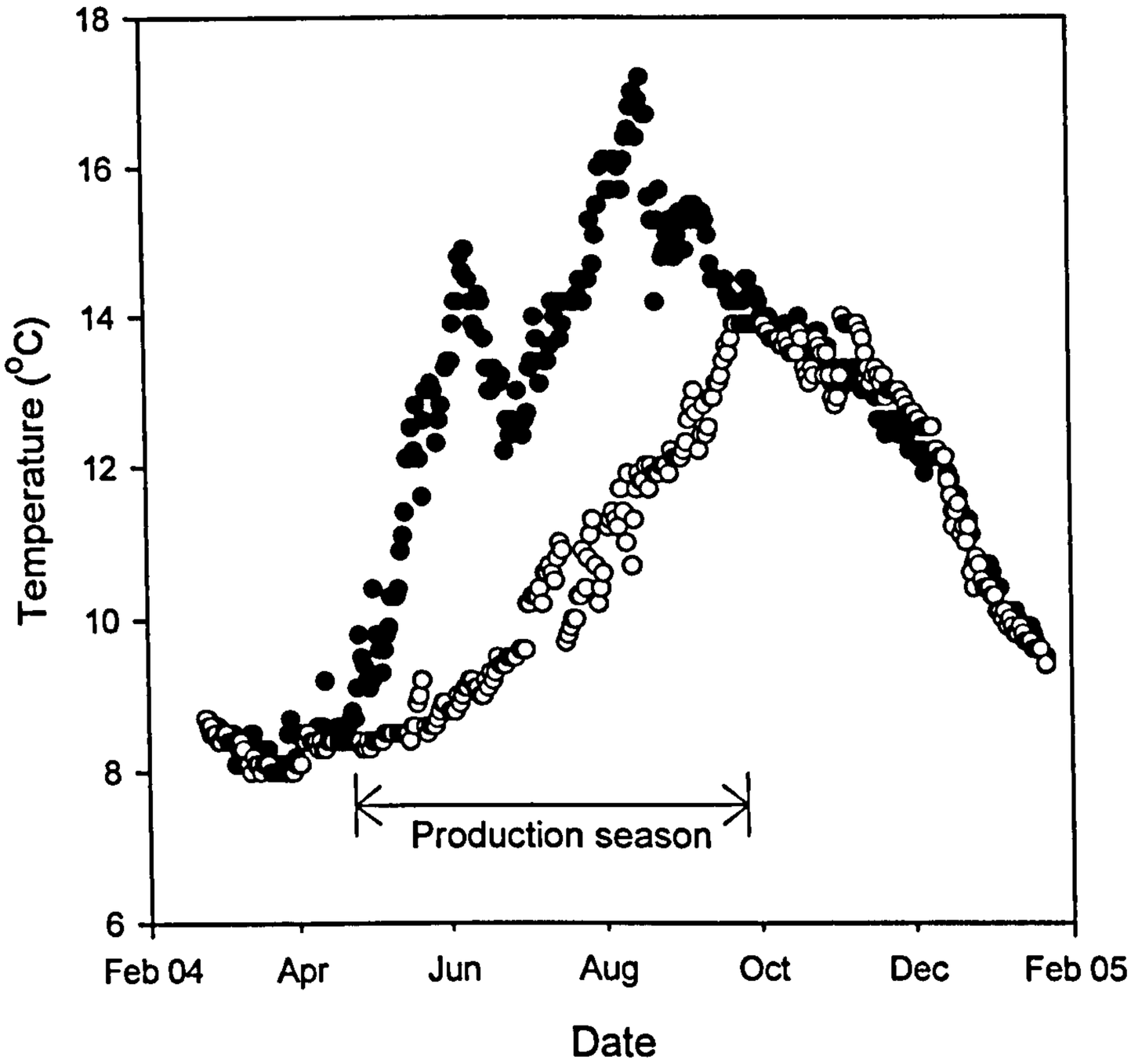


Fig. 2.4 Mid-day water temperature at 2 m (•) and 83 m (○) at S38A in the western Irish Sea from February 2004 to January 2005, showing the 2004 production season. The data is from the AFBI mooring temperature data loggers.

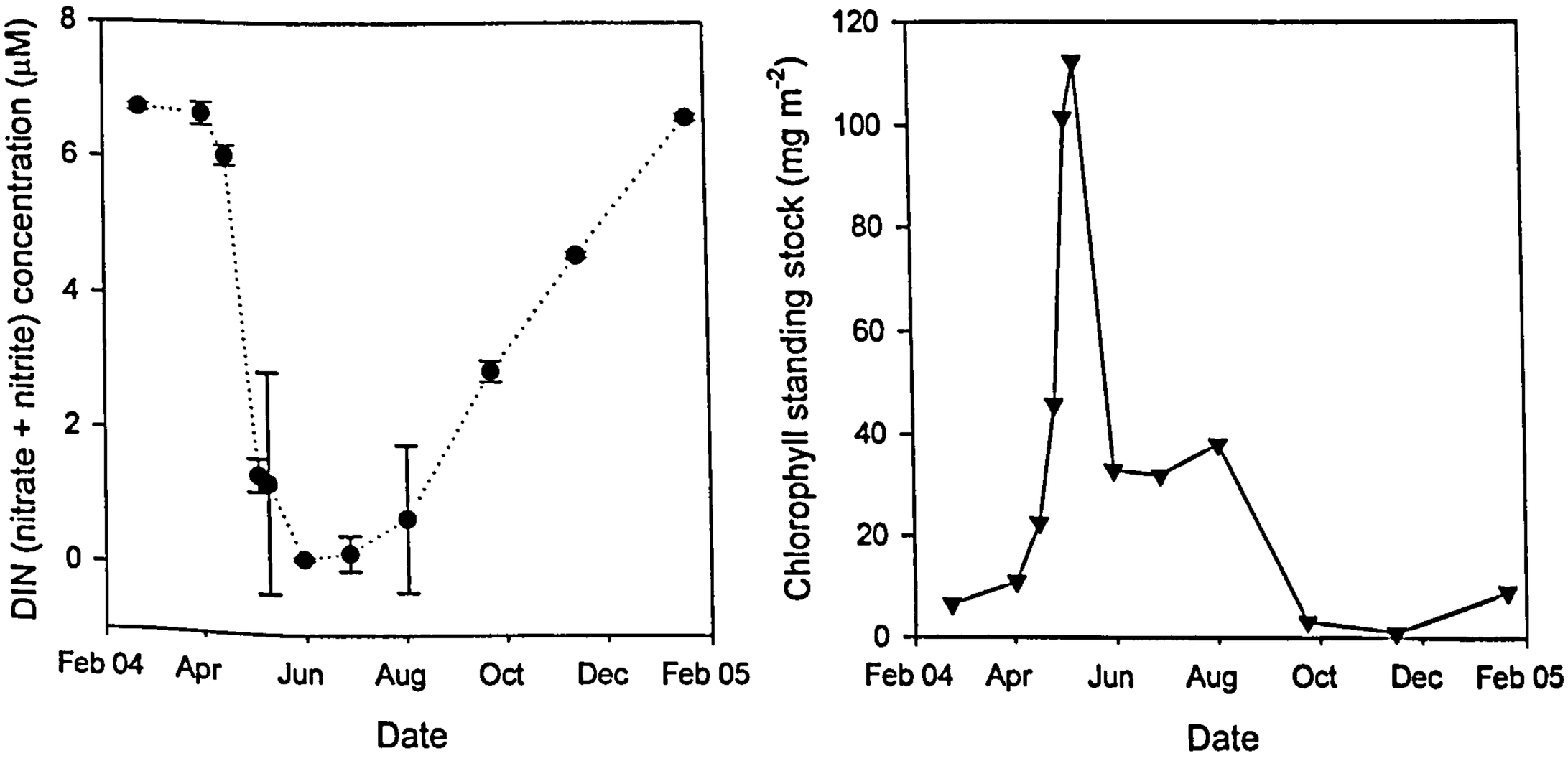


Fig. 2.5 A: Mean euphotic zone (0-23 m) concentration of dissolved inorganic nitrogen (DIN) and **B:** euphotic zone chlorophyll standing stock. DIN values are means ± SE, $n = 3$.

Dissolved inorganic nitrogen (DIN): The concentration of DIN at the end of February was at the winter maxima of 6.6 – 6.8 μM (AFBI data) and remained at this concentration until the beginning of April (Fig. 2.5A). A small decrease in DIN concentration, of ~0.6 μM, was evident in the top 30 m by mid April and by 4 May DIN had rapidly decreased to an average of 1.4 μM. At the end of May the euphotic zone was deplete of DIN (<0.1 μM) and remained so until early August (Fig. 2.5A). Throughout the spring and summer the bottom water concentration of DIN remained above 7 μM (Fig. 2.3C). By late September, stratification had broken down and DIN was ~3 μM throughout the water column. The concentration then steadily increased throughout the winter to 6.6 μM on 21 January 2005.

The total water column stock of DIN (calculated from interpolation of DIN concentrations at discrete depths) was at a maximum (625.9 mmol m^{-2}) at the beginning of April and fell to a minimum (409.5 mmol m^{-2}) at the end of May. This estimates usage during the spring bloom to be 216.4 mmol m^{-2}, 35 % of the total DIN available.

Chlorophyll concentration, standing stock and production: There was a distinct seasonal pattern in euphotic zone chlorophyll at station S38A with a winter minimum and spring maximum (Fig. 2.3D). During the winter, near surface (upper 20 m) chlorophyll concentration was ≤ 0.4 mg m^{-3}. There was a rapid increase in chlorophyll from the beginning of April to reach a maximum euphotic zone concentration of between 2.3 and 5.8 mg m^{-3} (mean 4.6 mg m^{-3}) by mid May. At the end of May, a high concentration (10.3 mg Chl m^{-3}) of chlorophyll was observed at 40 m. From the end of May to the beginning of August euphotic zone chlorophyll was ~ 1.5 mg m^{-3}. There was, however, evidence of a sub-surface (18 m) chlorophyll maximum (2.7 mg Chl m^{-3}) in early August.

By September the concentration of chlorophyll in surface waters had returned to winter values of ≤ 0.4 mg m^{-3}.

Maximum euphotic zone chlorophyll standing stock (determined by interpolation between chlorophyll measurements from discrete depths) was 112.5 mg Chl m^{-2} in mid May (Fig. 2.5, Table 2.1). For the whole water column (85 m), maximum chlorophyll standing stock was 231.8 mg m^{-2} at the end of May (Table 2.1). Estimates of daily production, based on standing stock are given in Table 2.1. Seasonal production (23 April – 22 September) was estimated to be 51.1 g C m^{-2}.

Particulate organic carbon and nitrogen: The concentration of euphotic zone particulate organic carbon (POC) and total particulate organic nitrogen (PON) exhibited a similar seasonal pattern to chlorophyll, with a winter minimum and a spring maximum (Fig. 2.6). The average for the winter (Feb, Nov and Jan) was 127 mg m^{-3} for POC and 12.7 mg m^{-3} for PON. Maximum concentrations of 370.0 mg m^{-3} of POC and 54.7 mg m^{-3} of PON were measured in early May (Table 2.2). The peak in POC and PON coincided with the peak in chlorophyll (Fig. 2.3D) and both POC and PON were significantly positively correlated with chlorophyll concentration (POC: $r^2 = 0.918$, $F_{0.05,11} = 112.3$, $P < 0.0001$; PON: $r^2 = 0.742$, $F_{0.05,11} = 28.7$, $P < 0.001$) for the whole sampling period. The average C:N ratio of particulate matter ranged from 5.0 to 10.1 with a spring minimum and winter maximum (Table 2.2). The lowest values occurred at the peak of the spring bloom in late April, early May (5.0 and 5.7 respectively) and the highest value recorded was 10.1 on 23 February 2004. Minimum average euphotic zone C:Chl ratios, of 49 and 72, were also observed during the bloom in May (Table 2.2).

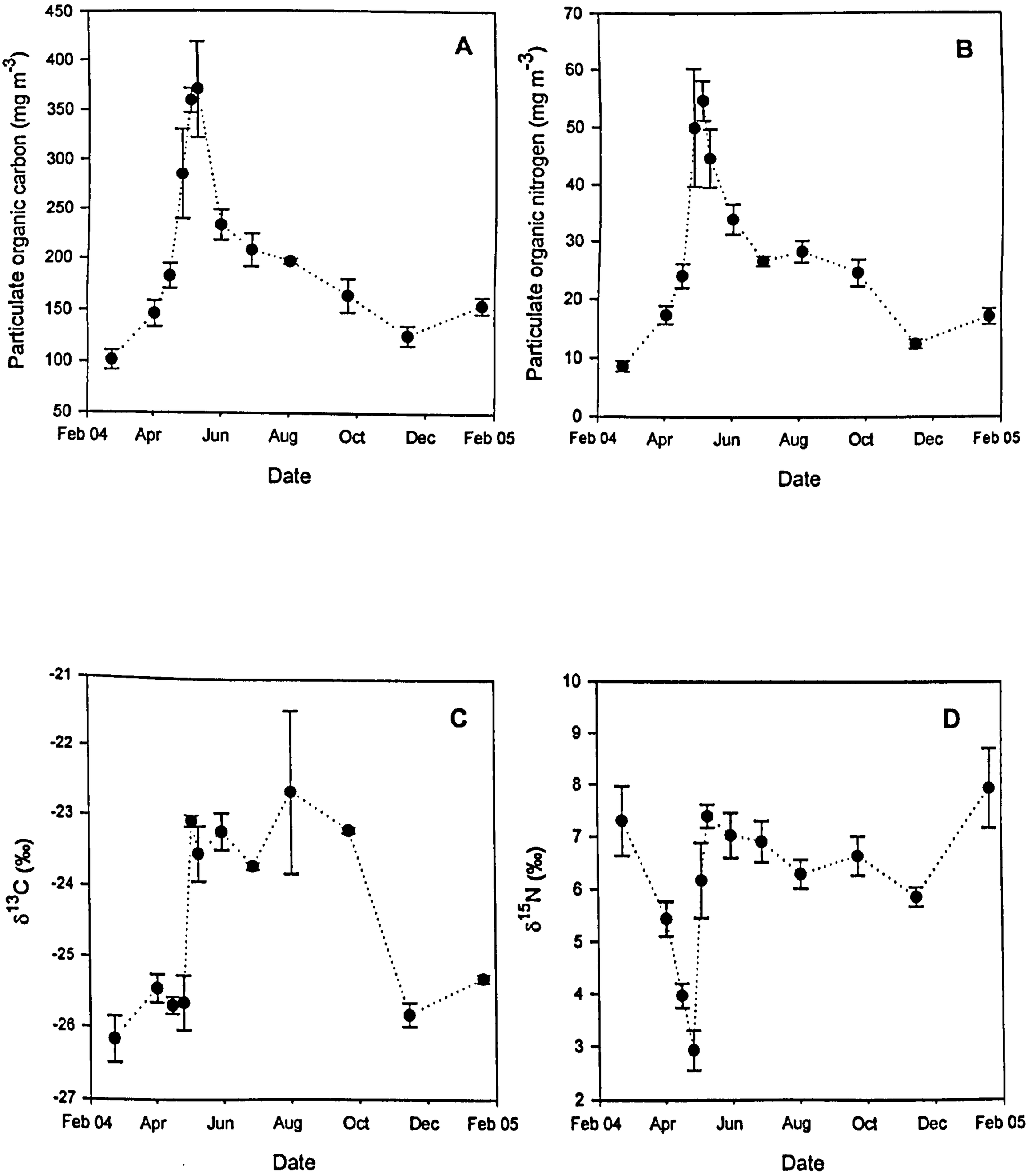


Fig. 2.6 Euphotic zone particulate organic matter at station S38A in the western Irish Sea: **A**: concentration of particulate organic carbon; **B**: concentration of particulate total nitrogen; **C**: carbon stable isotope ($\delta^{13}C$) and **D**: total nitrogen stable isotope ($\delta^{15}N$). All values are means ± standard error ($n = 3$).

Table 2.1 Euphotic zone (0-23 m) chlorophyll standing stock and daily production at S38A in the western Irish Sea, estimated from equation 2.1 (Gowen & Bloomfield, 1996).

Date	Euphotic zone standing stock ($mg\ m^{-2}$)	Estimated daily production ($mg\ C\ m^{-2}\ d^{-1}$)
23 February 2004	6.5	124.0
02 April	11.0	173.6
16 April	22.6	273.1
26 April	46.0	429.6
04 May	101.5	712.0
10 May	112.5	760.7
31 May	33.0	348.0
28 June	32.1	341.6
02 August	38.1	381.5
23 September	3.3	79.6
15 November	1.0	38.1
21 January 2005	9.1	153.4

Table 2.2 Average euphotic zone (0-23 m) values of particulate organic carbon (POC), particulate organic nitrogen (PON), stable isotope ratios of particulate organic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and characteristics of particulate organic matter at station S38A in the western Irish Sea. Standard errors in brackets (n = 3, except 2 April n = 5 and 16 April n = 4).

Date	POC ($mg\ m^{-3}$)	PON ($mg\ m^{-3}$)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:Chl	C:N
23 February 2004	101 (9.4)	8.7 (0.9)	-26.2 (0.3)	7.2 (0.7)	322	10.1
02 April	146 (12.7)	17.4 (1.5)	-25.4 (0.2)	5.4 (0.3)	307	7.2
16 April	183 (12.0)	24.1 (2.1)	-25.7 (0.1)	4.0 (0.2)	179	6.5
26 April	285 (45.4)	50.0 (10.3)	-25.6 (0.4)	2.9 (0.4)	144	5.0
04 May	359 (12.1)	54.7 (3.4)	-23.1 (0.1)	6.2 (0.7)	79	5.7
10 May	370 (48.1)	44.7 (5.1)	-23.5 (0.4)	7.4 (0.2)	81	7.2
31 May	233 (15.2)	34.0 (2.7)	-23.2 (0.3)	7.0 (0.4)	166	5.9
28 June	208 (16.4)	26.7 (0.9)	-23.7 (0.1)	6.9 (0.4)	160	6.7
02 August	197 (2.7)	28.3 (1.9)	-22.6 (1.2)	6.3 (0.3)	125	6.0
23 September	165 (16.2)	24.5 (2.3)	-23.2 (0.1)	6.6 (0.4)	1162	5.7
15 November	126 (9.5)	12.4 (0.8)	-25.8 (0.2)	5.9 (0.2)	2819	8.6
21 January 2005	155 (8.1)	17.1 (1.4)	-25.3 (0.1)	7.9 (0.8)	385	7.8

Particulate organic carbon and nitrogen stable isotope values: There were clear seasonal patterns in the stable isotope values for particulate organic carbon and nitrogen (Fig. 2.6C and 2.6D). The mean $\delta^{13}C$ for euphotic zone POC ranged from -26.2 to -22.6 ‰ with more depleted values in the winter and an average enrichment of 2.5 ‰ during the spring and summer (Fig. 2.6C). There was a significant difference between the highly stratified production season (April – September – determined by the period of stratification: see Fig. 2.4) and autumn/winter $\delta^{13}C$ values (t-test: $F_{0.05,26} = 138.4$, $P < 0.0001$). There was a significant positive relationship between POC $\delta^{13}C$ and the concentration of DIN ($r^2 = 0.81$, $F_{0.05,10} = 38.4$, $P < 0.001$) and a significant negative correlation with the C:N ratio ($r^2 = 0.340$, $F_{0.05,11} = 5.15$, $P = 0.04$).

The $\delta^{15}N$ of particulate organic nitrogen (PON) in the euphotic zone underwent significant changes early in the spring bloom (Fig. 2.6D). There was an initial period of depletion with the $\delta^{15}N$ falling from 8.1 ‰ at the end of February, to 2.9 ‰ by late April, followed by a rapid enrichment to 7.4 ‰ by 10 May. Thereafter, $\delta^{15}N$ of euphotic zone PON declined slowly to 5.9 ‰ by mid November followed by an increase to 7.9 ‰ in January 2005. There were, however, only significant differences in $\delta^{15}N$ values between 15 November and 10 May, and between 15 November and 21 January 2005 and so $\delta^{15}N$ values for the summer and autumn are stable.

Nitrate stable isotope analysis: Stratification of the water column in 2006 occurred between the end of March and the end of May, when a surface mixed layer was apparent (Table 2.3). There were no temperature profile data between these dates because of the unavailability of the AFBI mooring.

The concentration of dissolved inorganic nitrogen (DIN) in the surface water fell from 5.29 to 0.84 μM between the end of March and the end of May. Between 29 March and 6 April there was some depletion in the $\delta^{15}N$ of nitrate from -6.0 ‰ to -8.6 ‰. However, although the correlation between the solid and the sudan-1 phase of the standards was high (r^2 = 0.88) there was significant variability, of ± 2.7 ‰, between replicates of the sudan-1 phase of the solid standards. Therefore, the $\delta^{15}N$ values of the nitrate measured on these two dates are not significantly different.

However, by the end of May the nitrate was highly enriched with a $\delta^{15}N$ value of 27.3 ‰ (Table 2.3). There was therefore, an increase in $\delta^{15}N$ of about 34 ‰ from late March/early April to the end of May.

Table 2.3 Physico-chemical characteristics of surface waters (≈ 2 m) in the spring 2006 at station S38A in the western Irish Sea.

Date	29 March	6 April[#]	25 May
Surface water temperature (°C)	8.04	-	11.04
Depth of surface mixed layer (m)	None	-	27
Nitrate concentration (μM)	5.29	2.73	0.84
$\delta^{15}N$ Nitrate (‰)	-6.0	-8.6	27.3
POC (mg m^{-3})	215.2	233.4	145.8
PON (mg m^{-3})	24.0	28.6	17.1

[#]Water column profile data was not available for April 2006 because the CTD was unavailable and the mooring temperature logger data was not recorded for a period of 6 weeks because of the unavailability of the AFBI mooring. The April seawater sample was collected from the ship's clean seawater supply (≈ 5 m depth).

Discussion

The seasonal pattern of stratification in the western Irish Sea in 2004, with surface warming starting at the end of March and a clear surface to bottom difference maintained from late April to late September, is consistent with published accounts from previous years (Gowen et al., 1995; Gowen et al., 1998). The timing of the winter maximum of DIN at the beginning of February and the drawdown of nutrients from early April to the end of May is also fairly typical for the western Irish Sea. The winter maximum DIN concentration in the western Irish Sea in 2004 (6.7 μM), is well within the normal range observed over the last decade, although lower than the 1998 to 2002 average of 8.3 μM (Gowen and Stewart, 2005). In 2004, the peak concentration in euphotic zone chlorophyll of 4.59 mg m^{-3} in the middle of May is within the range of published values (Gowen et al., in press). The production season in 2004, which lasted from early April to mid September, corresponding to the period of stratification, had a distinct chlorophyll peak in mid-May and in timing and form is typical of other western Irish Sea observations (Gowen et al., 1995; Gowen and Stewart, 2005).

The accumulation of dissolved inorganic nitrogen over the winter fuels the new production of the spring bloom. Quantification of spring bloom production is, therefore, important for the estimation of new organic carbon and nitrogen available for export to higher trophic levels via grazing and sinking to the benthos. The two standard methods for measuring primary production are oxygen evolution and ^{14}C uptake (Strickland, 1960) and more recently ^{13}C methods have been used (e.g. Mateo et al., 2001). However, the time required to use these standard methods (approximately 12-14 hr incubation per sample for oxygen and 3 hr for ^{14}C measurements) was considered impractical for the current study. Instead,

an empirical relationship relating chlorophyll standing stock to gross primary production, derived from ^{14}C measurements in the Irish Sea in 1992 and 1993 was used (Gowen and Bloomfield, 1996). These authors found that variation in chlorophyll standing stock accounted for 71 % of the variation in daily production and had a goodness of fit similar to relationships developed in other areas such as the North Sea (Joint and Pomeroy, 1993). However, the relationship derived to fit the data does suggest that there is production when chlorophyll biomass is zero and, therefore, winter values for estimated production are likely to be an overestimation. However, for the production season (April to September) when chlorophyll biomass was present the relationship is considered a robust means of estimating primary production from 2004 western Irish Sea chlorophyll data.

Spring bloom production occurs during the period of draw down of winter nutrients (Dugdale and Goering, 1967). The spring bloom period is, therefore, taken to be the period between the winter maximum and the summer minimum of total water column stock of DIN. Wind-driven mixing events break down stratification for short periods, injecting bottom water DIN into the euphotic zone. Such a mixing event occurred in July, when the temperature of the surface water dropped from ~14 °C to ~12 °C (Fig. 2.4), which must have injected nutrients into the euphotic zone because there is a corresponding increase in euphotic zone DIN and chlorophyll concentration (Fig. 2.5, Table 2.7). The calculation of the drawdown of DIN has, therefore, been based on changes in water column totals, not the euphotic zone stock of DIN. The spring bloom for 2004 was estimated to be from 2 April to 31 May, from the maximum to the minimum total water column stock of DIN (see results section), and the primary production (from standing stock and equation 2.1) in this period was estimated to be 24.8 g C m^{-2}. Total annual production was calculated to be 79.5 g C m^{-2}, so the spring bloom accounted for 31 % of total annual production. For

comparison with other studies, seasonal production in 2004 (i.e. April to September) was 51.1 mg C m^{-2}.

Primary production data for the western Irish Sea, with which to compare the 2004 estimates is limited (Table 2.4). Comparisons, particularly of spring bloom production estimates, must also be treated with caution because of differences in methodology, in particular how the period of the spring bloom period is defined. In the current study, the spring bloom period was based on the timing of the drawdown of nutrients whereas in Trimmer et al. (1999) it was defined on the basis of the chlorophyll curve. There is no agreed method and the different methods may introduce differences. There is also inter-annual variability in the amount of winter nitrogen in the water column, often linked to the amount of rainfall and the consequent nutrient run-off from the land.

The 2004 estimate of spring bloom production is slightly lower, but similar to the estimates for 1997 and 1998 (Table 2.4) of 28.2 g C m^{-2} and 31.4 g C m^{-2} respectively (Gowen et al., 1999; Trimmer et al., 1999). The current estimate is, however, considerably lower than the spring bloom estimate for 1992/3, which will be related to the high concentration of winter DIN, a maximum of $\approx$ 9 μM (Gowen et al., 1995). Although there are inadequate data to determine if these estimates are indicative of a decline in primary production, the possibility is worth exploring further, particularly as a decline in primary production has been observed in recent years in western Irish Sea waters close to the Isle of Man (Shammon and Hartnoll, 2003). A long term decline in primary production would obviously have particular relevance for the western Irish Sea *Nephrops norvegicus* fishery because declines in annual primary production observed in other areas (e.g. Narangansett

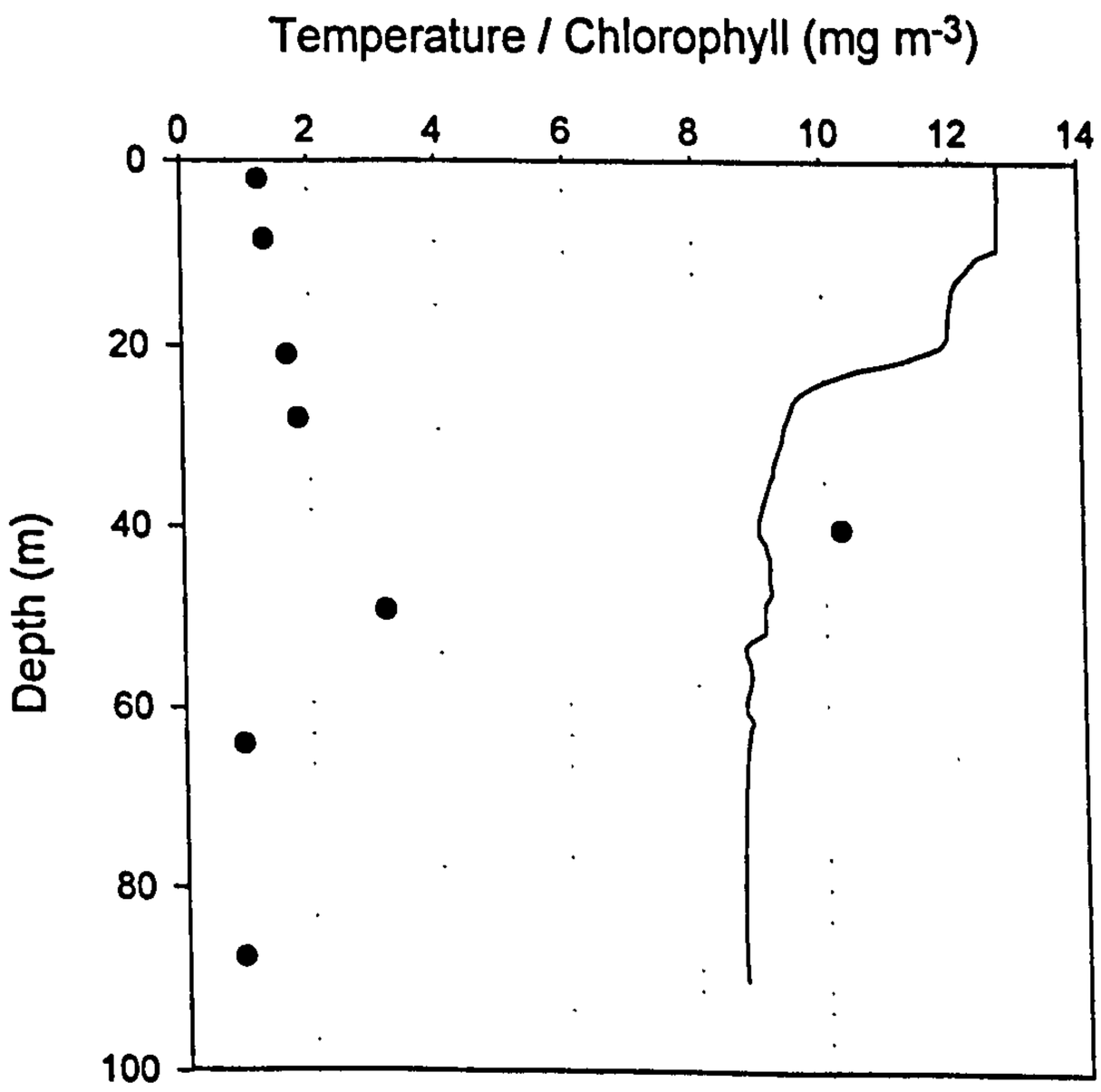
Table 2.4 Estimates of spring bloom and annual gross primary production in the western Irish Sea

Year	Spring bloom production[$] (g C m^{-2})	Spring bloom period	Annual production (g C m^{-2})	Reference
1992	51.3	Mar 25 – Jul 15	} 140	(Gowen and Bloomfield, 1996)
1993	38.9	Apr 15 – Jul 16		
1997	28.2	Apr 07 – Jun 04	89.5[#]	
1998	31.4	Mar 30 – Jun 22	99.7[#]	(Trimmer et al., 1999)
2004	24.8	Apr 02 – May 31	79.5	This study
Average	**34.9**		**102.2**	

$ Spring bloom production is defined as production that occurs during the period of nutrient drawdown. The timing of the spring bloom varies from year to year.

Annual production estimated from 1993/93 and 2004 ratio of 3.2:1 for total production:spring bloom production.

Fig 2.7 Depth profile of chlorophyll (•) and temperature (–) at station S38A in the western Irish Sea on 31 May 2004.



Bay, USA) have been seen to reduce the input of organic matter to the benthos (Fulweiler et al., 2007). In Narragansett Bay, the decline in primary production is thought to be due to increased grazing pressure in response to climate warming despite constant inputs of nitrate over the same period. Such impacts could, therefore, have implications for the western Irish Sea where the input of organic matter to the benthos supports a commercially important fishery.

As a comparison, new spring bloom production can be estimated from the drawdown of winter dissolved inorganic nutrients and the ratio of C:N for conversion of nitrogen assimilation to carbon production (Dugdale and Goering, 1967). From the period 2 April to 31 May the total water column stock of DIN fell by 216.3 mmol m^{-2} and the average euphotic zone C:N ratio was 6.3 (see Table 2.7), close to Redfield, which estimates total new production to be 20.0 g C m^{-2} compared with the estimate of 24.8 g C m^{-2} for total production based on chlorophyll standing stock.

In a discussion of new and regenerated production, Eppley and Peterson (1979) used *f* to define the ratio of new to total primary production. According to these workers, if *f* is the probability that an atom of nitrogen is assimilated as new nitrogen and 1-*f* is the probability of assimilation of regenerated nitrogen then *r*, the number of times that a nutrient is recycled is given by:

$$r = (1-f)/f \qquad (2.5)$$

The 2004 western Irish Sea spring bloom data (20.0 g C m^{-2} new production, 24.8 g C m^{-2} total production), gives an *f* ratio of 0.80, close to the value ($f = 0.75$) given by Trimmer et al. (1999) for the western Irish Sea in 1998. The 2004 value for *r* is, thus, 0.25, so 25 % of the winter nitrogen is recycled during the spring bloom and 75 % is exported. According to

Eppley and Peterson (1979), the exported nitrogen is accounted for by intact phytoplankton cells sinking out of the water column, taking assimilated nitrogen to the benthos. Thus, they argue that a high *f* ratio provides qualitative information on the nature of the sinking material, indicating the loss of intact cells by sinking. However, in this model the export of nitrogen to higher trophic levels in the water column through grazing is ignored. There was a significant increase in the copepod population during the spring bloom (see Chapter Three) and there must, therefore, have been a substantial transfer of phytoplankton N to copepods. According to Gowen et al. (1999) copepods consumed up to 76 % of daily production and some 22 % of spring bloom production in the western Irish Sea.

The calculation of euphotic zone gross production was based on the chlorophyll standing stock – primary production relationship of Gowen and Bloomfield (1996) and assumes that the bottom of the euphotic zone was the depth at which 1 % of photosynthetically active radiation (PAR) remains, which is widely believed to be the compensation depth, i.e. the point at which algal photosynthesis is balanced by algal respiration. However, this value, based on a single study of compensation illumination in the English Channel by Jenkins (1937) and later used by Sverdrup (1953) does not appear to hold true in all cases (Tett, 1990). More recent studies have shown that compensation illuminations for oceanic phytoplankton may be closer to 0.1 % than 1 % of PAR (Marshall and Orr, 1928; Tett, 1990). This helps to explain the occurrence of midwater chlorophyll maxima below 1 % of surface irradience in many marine systems (e.g. Fasham et al., 1985) and is suggested to be due to nutrient replete phytoplankton near the compensation point respiring at the lowest possible rate (Tett, 1990).

The estimation of spring production in the western Irish Sea in 2004, using the 1 % PAR euphotic zone, has excluded the large biomass of chlorophyll, with a concentration of 10.3 mg Chl m^{-3}, at 40 m at the end of May. The C:N ratio of 5.6, close to the Redfield ratio of 6.6 (Redfield, 1934), and a C:Chl ratio of 51 for this organic matter, are both suggestive of recently produced material. The high concentration of chlorophyll was measured below the thermocline (Fig. 2.7).

It is possible to estimate the depth at which 0.1 % PAR is available according to:

$$I_z = I_0 \cdot e^{-kdz} \qquad (2.6)$$

Where I_z and I_0 are irradiance at depth z and 0 respectively and the attenuation coefficient is K_d. From previous work, the depth of the 1 % light level in the western Irish Sea is 23 m (Gowen et al., 1995), which gives a value of K_d of 0.2. If the bottom of the euphotic zone is the 0.1 % light level, then using a K_d value of 0.2 and re-arranging the equation gives a depth of 35 m (ln $0.001/K_d$ or $6.91/0.20 = 35$). This would suggest that even using 0.1 % for the base of the euphotic zone, at 40 m the high biomass of chlorophyll observed, is just below the euphotic zone and is not, therefore, 'new' production.

Herman and Platt (1986) used an alternative method to calculate production, taking the compensation depth to be depth where the addition of another metre adds less than 1 % to the overall amount of production. This method estimates production occurring to a depth of 64 m on 31 May and increases standing stock of chlorophyll on this date alone from 34.7 to 207.8 mg Chl m^{-2}. Applying this method for the whole sampling period more than doubles the estimate of total spring bloom production from 24.8 to 53.1 g C m^{-2}. This gives gives an *f*-ratio of 0.38 which estimates 60 % of nutrients are recycled in the euphotic zone and only 40 % of production is exported to higher trophic levels. The export estimate is

lower than that expected for a spring bloom in temperate waters. Therefore, it seems more likely that the large biomass of chlorophyll, with low C:Chl and C:N ratios, at 40 m at the end of May is a mass of sinking, recently produced and therefore intact, phytoplankton.

In the winter months, when the water column is mixed, primary production is low. Production was at a minimum in September and November when standing stock of chlorophyll was at its lowest, 3.3 mg m^{-2} and 1.0 mg m^{-2} respectively, and this was reflected in the dramatic increase in the C:Chl ratio of particulate organic matter on these dates (Table 2.2). By January the standing stock of chlorophyll had increased to 9.1 mg m^{-2} and the C:Chl ratio had returned to pre-bloom values suggesting there was still little phytoplankton carbon in the bulk POM.

Stable isotope sample sizes

During the study, the sample size of carbon in euphotic zone POC ranged from 25 to 85 μg C (log_{10} values 1.4 to 1.9) (Fig. 2.2), with the smallest sample sizes occurring in the winter months. At the smallest sample size, $\delta^{13}C$ was artificially enriched, by an average of 0.6 ‰ ± 0.55 ‰, and so all POC $\delta^{13}C$ data has been corrected for sample size.

The size of nitrogen samples from euphotic zone PON, however, were much smaller, ranging from 3.0 to 8.4 μg (log_{10} values 0.5 to 0.9) (Fig. 2.2). At these sample sizes there is artificial depletion in $\delta^{15}N$ of between 1.00 ‰ ± 0.60 and 0.73 ‰ ± 0.45 (95 % confidence intervals calculated with t-distribution values for small sample sizes) from the smallest to the largest samples. Thus, all $\delta^{15}N$ data has been corrected for sample size.

POC stable isotope values

In the western Irish Sea in 2004, the $\delta^{13}C$ values of POC from February 2004 to January 2005 ranged from -26.2 ‰ to -22.6 ‰. The distinct seasonal differences, however, in the $\delta^{13}C$ of POC, observed in the current study, and others (for example see Wainwright and Fry, 1994; Rolff, 2000), means that for comparison with data from other areas this seasonality must be taken into account.

In the western Irish Sea there were two separate periods that had distinct $\delta^{13}C$ values. The first is the production season in the spring and summer, when the water column is seasonally stratified, phytoplankton production takes place and the gyre retains near surface waters. In this period (early May – September) $\delta^{13}C$ values for the POC were more enriched, ranging from -23.7 ‰ to -22.6 ‰ (average -23.2 ‰, see Table 2.2). The second period was autumn and winter, when the water column is vertically mixed and production is negligible. The $\delta^{13}C$ values during this period are much lower, ranging from -26.2 ‰ to -25.3 ‰ (average -25.8 ‰). The timing of the switch from low to high, and high to low, values coincide with the development and breakdown of stratification (Fig. 2.4). The importance of stratification is the development of the gyre circulation system that has an isolating effect on the waters of the western Irish Sea, preventing mixing with surrounding water bodies and, together with increased fractionation during production, is likely to be responsible for the distinct shift in carbon isotopic values of particulate organic matter. This shift was also seen in the cycling of carbon (and nitrogen) isotope values through the year (Fig. 2.12), and can be explained in terms of the balance between mixing and fractionation that drive changes in isotope values (Fry, 2006). In the production season fractionation by growing phytoplankton exceeds mixing with organic matter in mixed waters resulting in a shift to higher production $\delta^{13}C$ values. In the winter period (November

to March) there is very little production and the effect of mixing with detrital matter from the surrounding waters is greater than fractionation during microbial mineralisation of the detritus and so $\delta^{13}C$ values decline.

Causes of seasonal changes in $\delta^{13}C$ values

The rapid shift of ~2.5 ‰ to more positive POC values, during the highly stratified spring phytoplankton bloom, is typical of seasonal changes in $\delta^{13}C$ seen in other marine systems (Deuser, 1970; Fry and Wainright, 1991; Rolff, 2000). Several mechanisms have been proposed to explain the seasonal increase in $\delta^{13}C$ including: the exhaustion of inorganic carbon ($CO_{2(aq)}$) by vigorous phytoplankton growth; changes in the composition of bulk particulate organic matter; changes in phytoplankton species composition; and the dominance of ^{13}C heavy diatoms in spring blooms. Each of these will be discussed in turn.

As inorganic carbon becomes limiting is it thought that the ability of the phytoplankton to discriminate between the two isotopes is reduced and incorporation of ^{13}C increases (Falkowski and Raven, 1997). Although some diatoms have been shown to be limited by the supply of CO_2, under optimal light and nutrient conditions (Riebesell et al., 1993), the potential for inorganic carbon limitation of phytoplankton growth in the sea is still a contentious issue (Geider and MacIntyre, 2002). Falkowski and Raven (1997) believe that under 'natural' conditions most marine phytoplankton are not carbon limited. There have been several investigations of the relationship between $\delta^{13}C$ of POC and the concentration of $CO^{2}_{(aq)}$. Rau et al. (1992) found a highly significant negative correlation between measurements of POC $\delta^{13}C$ and $[CO_{2(aq)}]$ during a spring bloom in the North Atlantic when an increase from -22.9 ‰ to -18.1 ‰ in $\delta^{13}C$ was correlated with a fall in $[CO_{2(aq)}]$ from 13.2 to 10.2 μM. Another study in the North Atlantic (from 40 to 50 °N) by Goericke and

Fry (1994), however, found that variation of POC $\delta^{13}C$ at a single latitude could be as high as latitudinal variations (the amount of CO_2 dissolved in seawater varies with latitude because of temperature effects) and concluded that biological effects may have a larger impact on $\delta^{13}C$ than the concentration of $CO_{2(aq)}$. Similarly, Francois et al. (1993) observed a 6 ‰ variation in POC $\delta^{13}C$ across the sub-tropical convergence in the Indian Ocean without any variation in $[CO_2(_{aq})]$ and noted that high growth rates of phytoplankton seemed a better explanation for changes in $\delta^{13}C$. Fry and Wainright (1991) also suggested changes in growth rates may be the reason for differences in carbon fractionation with an increase in ^{13}C occurring during rapid growth.

Particulate organic matter in the sea is a mixture of living and dead phytoplankton, bacteria, detritus, and zooplankton faecal pellets, rather than pure phytoplankton. As such, seasonal changes in the composition may also drive changes in $\delta^{13}C$. Although there are methods to separate individual phytoplankton cells from bulk particulate organic matter (Price et al., 1974; Heaney and Jaworski, 1977; Hamilton et al., 2005), these are generally used for algal culturing and it is difficult to isolate sufficient material, within the short time scale before phytoplankton die and start to break down (thereby changing their isotope values), for isotope analysis. However, in the spring and summer, during the production season, particulate organic matter is mostly phytoplankton as indicated by a decrease in C:Chl ratio from a winter average of ~300 to ~80 at the peak of the spring bloom and a decrease in C:N ratios from ~8 to ~5. Previous comparisons between bulk particulate organic matter and net phytoplankton (mostly large centric diatoms), for example from the Georges Bank (Fry and Wainright, 1991), indicated that $\delta^{13}C$ values were not significantly different during the production season (Fry and Wainright, 1991) and were, therefore, representative of phytoplankton. At other times, however, particularly during the winter,

there will be a very high proportion of detrital material. Thus, a shift in the composition of bulk POM may be the most important factor determining $\delta^{13}C$ values. The C:Chl and C:N ratios of particulate organic matter provide a useful guide to the general composition of particulate organic matter (Parsons et al., 1984b) and can aid interpretation of stable isotope values. For example, the C:Chl and C:N ratio of an actively growing population of phytoplankton are quoted to be close to 30:1 and 6.6:1 respectively (Riemann et al., 1989). In the western Irish Sea, the C:N ratios (Table 2.2) are generally lower in the spring and summer period, indicating a shift in composition, with higher phytoplankton in the spring and summer and higher detrital material in the winter. Thus, there is enrichment in $\delta^{13}C$ during the bloom, associated with the increase in the phytoplankton component of POM, also indicated by the relationship between $\delta^{13}C$ and chlorophyll concentration.

Species composition of the phytoplankton population may be an important factor controlling carbon stable isotope values because specific groups of marine phytoplankton have been found to have different $\delta^{13}C$ values. In a study by Gearing et al (1984) in Narragansett Bay (Rhode Island) centric diatoms, with a mean $\delta^{13}C$ of -20.3 ‰ were found to be much heavier than micro-flagellates (<10μm) with a mean $\delta^{13}C$ of -22.2 ‰. Although diatoms appear to dominate the phytoplankton in the Irish Sea in most years, micro-flagellates may dominate in others, as they did in 1997 and 2001 (Gowen and Stewart, 2005). Observations of phytoplankton species composition in the western Irish Sea in samples collected between 23 February and 10 May indicate that micro-flagellates accounted for a significant proportion of carbon biomass, ranging from 38 to 63 % in April (Table 2.5). By the peak of the spring bloom in May, however, diatoms were dominant and so micro-flagellates do not appear to be an important determinant of low $\delta^{13}C$ values.

Enriched $\delta^{13}C$ values of organic matter, during spring blooms dominated by diatoms, has been observed because diatoms are a ^{13}C rich source of carbon in marine food webs (Fry and Wainright, 1991). Nutrient uptake has been suggested to influence diatom $\delta^{13}C$ as rapid N assimilation in algae increases uptake of ^{13}C rich bicarbonate by C_4 enzymes (Fry and Wainright, 1991). The significant correlation between POC $\delta^{13}C$ and the concentration of DIN in the western Irish Sea, and the cycling of carbon isotope values with DIN concentration (Fig. 2.12) supports this idea. Diatoms are an important component of the spring bloom in the western Irish Sea (Gowen and Stewart, 2005) and in 2004 carbon biomass from diatoms increased from around 5 % in February to between 60-90 % in May (Table 2.5). Therefore, an increase in heavy diatom biomass in the spring and summer, and the reduction in the importance of detrital matter, explains the increase in $\delta^{13}C$ POC during this period. The importance of the seasonal cycle of production on $\delta^{13}C$ values is also seen in the cycling of $\delta^{13}C$ with the seasonal changes in the concentration of DIN, chlorophyll and POC (Fig. 2.12).

Table 2.5 Estimates of euphotic zone phytoplankton carbon biomass by major phytoplankton group. Data is the average of euphotic zone samples (n=3), except where noted.

Date	Carbon biomass ($\mu g\ C\ m^{-3}$)	Diatoms	Dinoflagellates	Micro-flagellates[&]
23/02/2004	8.45	36.7%	58.6%	4.6%
02/04/2004	14.56	29.8%	31.8%	38.4%
16/04/2004	25.95	3.3%	33.4%	63.3%
26/04/2004[*]	67.98	1.7%	54.6%	43.7%
04/05/2004[#]	479.97	94.3%	3.2%	2.5%
10/05/2004[$]	122.45	60.4%	8.8%	30.8%

[&] Microflagellates in size range ~5-20 μm, [*] Data based on one sample (0-1 m), [#] Data from single sample from 18.2 m, [$] Data from one sample (0 m).

Origin of particulate organic carbon

A comparison with data from other marine studies shows western Irish Sea POC $\delta^{13}C$ values, even the higher values during the production period (Apr – Sep), to be at the lower end of the literature values (Table 2.6, Fig. 2.8). For example, spring $\delta^{13}C$ POC values in the North Atlantic (47°N, 20°W, the Joint Global Ocean Flux Study (JGOFS) North Atlantic Bloom Experiment (NABE)) ranged from -22.9 to -18.1 ‰ (Rau et al., 1992) and in the north-eastern Atlantic (Porcupine Abyssal Plain 48°50'N, 16°30'W) in July and September, within the main production season, the $\delta^{13}C$ of POC at 40 m was -21.9 ‰ (Iken et al., 2001). However, the western Irish Sea values are similar to the lowest POC values during the summer in the Celtic Sea and the English Channel (Dauby et al., 1994).

Outside the highly stratified production season the $\delta^{13}C$ values for POC are significantly lower and similar to other studies in coastal and estuarine areas where there is some freshwater influence (Table 2.6, Fig. 2.8). For example, Fry and Wainwright (1991) observed marine POC from Georges Bank and the Gulf of Maine in the US with $\delta^{13}C$ values as low as -26 ‰. There is a significant freshwater influence in these coastal waters, resulting in salinities of 32 to 33 in most months and analysis of oxygen stable isotopes have established the water sources to be freshwater from high latitudes (Houghton and Fairbanks, 2001). Similarly, in Woods Hole Harbour where the salinity is ~32, POC $\delta^{13}C$ values were between -25 ‰ and -19.8 ‰ (Wainwright and Fry, 1994). Rau et al. (1990) report POC $\delta^{13}C$ values from a 13 month period in the Port of Monaco (Mediterranean) ranging from -25.3 ‰ for the smallest particles (<3 μm) to -19.8 ‰ for the largest (> 150 μm), which are similar to the western Irish Sea values. Although the $\delta^{13}C$ values are low, the authors discounted the possibility that the close proximity of the sampling site to a well-populated shoreline increased the chance that at least some of the suspended

particulate matter was non-marine, because C:N ratios were low and so, they concluded, not typical of land-derived material. The authors did not, however, present salinity data so it is not possible to discount the possible contribution of freshwater and associated particulate organic matter as a reason for the low carbon isotope values.

One obvious source of low $\delta^{13}C$ to the western Irish Sea is particulate organic matter is from freshwater or estuarine input. Estuarine and riverine POC $\delta^{13}C$ values are characteristically low, where terrestrial C_3 detritus, with a $\delta^{13}C$ of -26 to -27 ‰, and river-estuarine phytoplankton between -30 ‰ and -24 ‰, contribute to more negative $\delta^{13}C$ values (Cifuentes et al., 1988; Fry and Sherr, 1989). The source and contribution that terrestrial and/or estuarine carbon might make is, however, difficult to determine without further study. The supply of freshwater from riverine discharge into the western Irish Sea from the Irish coast is limited, with some input from Dundalk Bay, and much smaller than riverine discharge into the east (Gowen and Stewart, 2005). The near surface distribution of salinity in the winter argues against extensive exchange between the eastern and western Irish Sea (Gowen et al., 2002 and references cited therein). During the annual solar heating cycle, tidal mixing fronts develop approximately in a line between the bottom of the Isle of Man and the Irish coast off Dublin (Simpson and Hunter, 1974). These fronts are thought to limit the exchange of water between the east and the west, and the flow of freshwater into the western Irish Sea from St George's Channel. Futhermore, as the near surface gyre becomes established, the waters of the western Irish Sea might be expected to become further isolated from other water sources.

However, surface waters in the western Irish Sea have a salinity of ≈ 34.2, indicating that there is some mixing or input of freshwater. This entrainment may occur as the gyre is

Table 2.6 Carbon stable isotope ($\delta^{13}C$) values of particulate organic matter (POM) and phytoplankton from marine systems in the Northern Hemisphere reported in the literature.

Location	Position	Salinity	Time	Sample	Size (μm)	Acid	$\delta^{13}C$ range		Ref[$]
Western Irish Sea: total sampling period	53° 51'N, 05° 34'W	34.2	Full year	POM	0.45 - 200	Y	-26.2	-22.6	This
Western Irish Sea: spring/summer							-25.7	-22.6	study
Western Irish Sea: autumn/winter							-26.2	-25.3	
Oceanic									
North Atlantic[1]	47° N, 20° W	35.5	Apr - May	POM	>3	Y	-22.9	-18.1	1
North Atlantic[2]	20° to 30° N	35		POM		?	-23.5	-19.5	2
Porcupine Abyssal Plain, NE Atlantic	48°50' N, 16°30' W	35.5[#]	Jul and Sep	POM	>1.2	Y	-21.9		3
North Sea	52° N, 02° W	33.9*	October	POM	>0.45	Y	-22.4	-18.7	4
English Channel		35.1*	Jun/Jul	POM	>0.45	Y	-23.3	-17.6	4
Celtic Sea		35.5*	Jun/Jul	POM	>0.45	Y	-24.5	-20.3	4
Pacific Ocean	5–33°N, 119-140°W	35	Feb-Mar	POM	0.1 - 60	Y	-23.5	-22.2	5
Coastal / estuarine									
Gulf of Maine (south)	42°N, 69°W	32.9[#]	Mar - Apr	POM	1.2 - 100	Y	-27	-22	6
Gulf of Maine (south)	42°N, 69°W	32.9[#]	Mar - Apr	Phyto	40 - 110	Y	-22	-21	6
Georges Bank (central/mixed)	42°N, 67–68°W	32 – 33.5	Mar - Apr	POM	1.2 - 100	Y	-26	-20	6
Georges Bank (south)	40°N, 69-70°W	32 – 33.5	Mar - Apr	Phyto	40 - 110	Y	-22	-15	6
Missippi River plume	28°50' N, 89°40' W		Mar - Apr	POM	1.2 - 100	Y	-24	-18	6
Narraganset Bay, Rhode Island	41° N, 71° W	-	Apr - Nov	Phyto	-	N	-23.2	-19.7	7
Woods Hole Harbour	-	32	Full year	POM	20 - 110	Y	-25	-19	8
Saanich Inlet, BC, Canada		31.2	Summer	POM	>1.2	Y	-20.3	-17.6	9
Port of Monaco, Mediterranean	43° 42' N, 07° 29' W	Coastal	Full year	POM	3 - 150	Y	-25.3	-19.8	10
Baltic Sea		7	Mar - Jan	POM			-26.1	- 21.7	11

Table 2.6 continued

Location	Position	Salinity	Time	Sample	Size (μm)	Acid	$\delta^{13}C$ range	Ref[$]
Coastal/estuarine continued....								
Bering Sea, Pacific	Eastern Bering Sea		Spring/summer	Phyto		N	-24.4	12
Maine, USA	44°N, 69°W	7km offshore	Spring/summer	POM	>1.0	N	-21.2	13
Maine, USA	44°N, 69°W	Estuary mouth	Spring/summer	POM	>1.0	N	-23.8	13
Maine, USA	44°N, 69°W	Estuary head	Spring/summer	POM	>1.0	N	-25.3	13
Marennes-Oleron Bay, France		34.7	Sep	POM	0.46 - 63	Y	-22.2 -21.0	14
Marennes-Oleron Bay, France		25.7	March	POM	0.46 - 63	Y	-24.7 -20.0	14

[$] References: 1 - (Rau et al., 1992), 2 - (Sackett et al., 1965), 3 - (Iken et al., 2001), 4 - (Dauby et al., 1994), 5 - (Benner et al., 1997), 6 - (Fry and Wainright, 1991), 7 - (Gearing et al., 1984), 8 - (Wainwright and Fry, 1994), 9 -(Nakatsuka et al., 1992), 10 - (Rau et al., 1990), 11 - (Rolff, 2000), 12 - (McConnaughey and McRoy, 1979), 13 - (Incze et al., 1982), 14 - (Kang et al., 1999).

[#] Salinity data from (Houghton and Fairbanks, 2001).

* Average values.

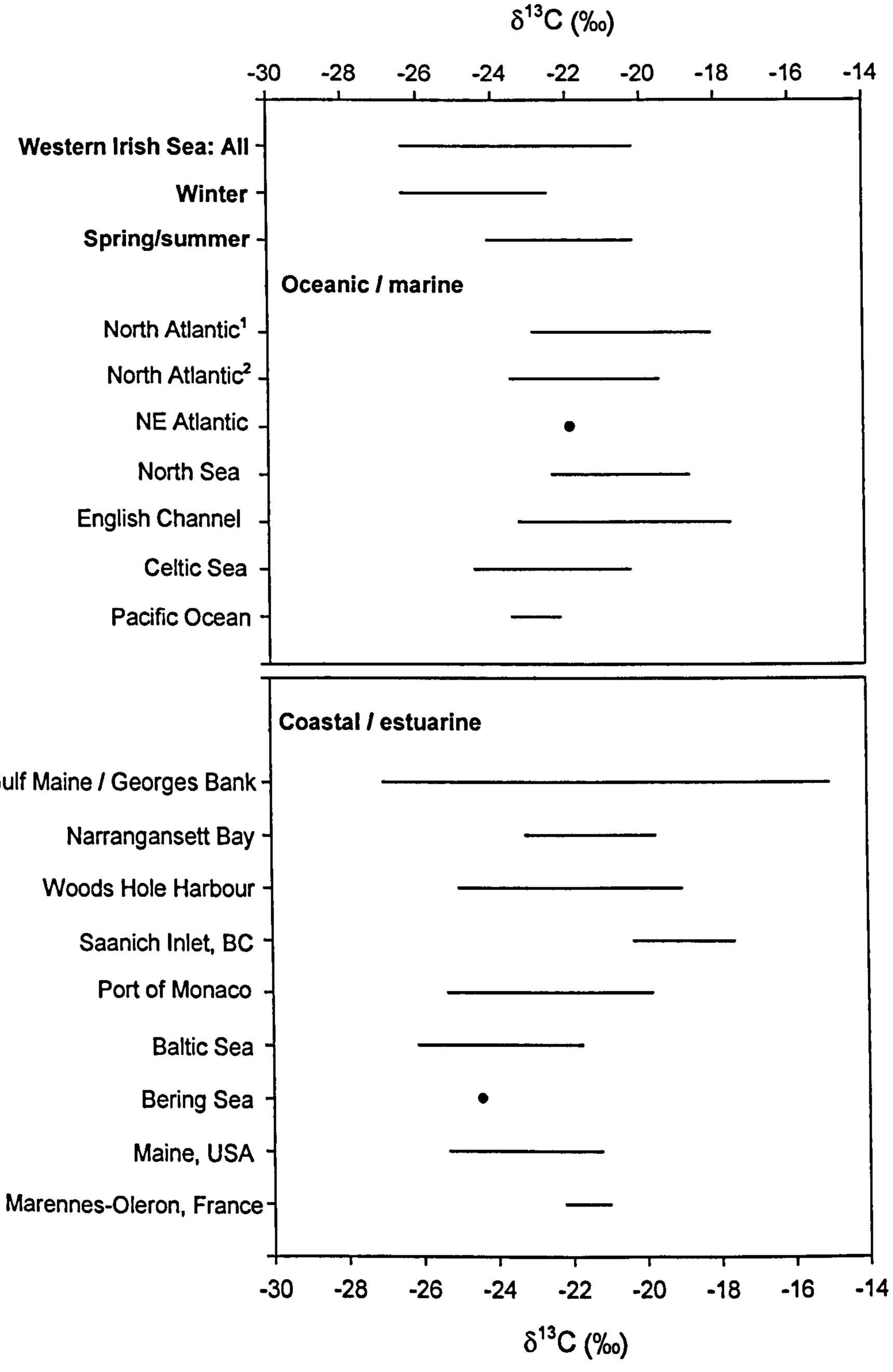


Fig. 2.8 Comparison of $\delta^{13}C$ of Western Irish Sea particulate organic matter with literature values from the Northern Hemisphere. References provided in Table 2.6

developing. For example, the movement of fish larvae from coastal waters to the western Irish Sea gyre during May, when stratification is usually established, may indicate some entrainment of Dundalk coastal waters into the gyre (Dickey-Collas et al., 1996; Dickey-Collas et al., 1997). Holt et al. (2004) undertook a modelling study and argued that nutrient rich water could be advected into the gyre from the North Channel. This advected water could also bring POM but it is unlikely to be of freshwater origin.

Simple mixing models (Fig. 2.9) with two sources - marine particulate matter and terrestrially/estuarine derived particulate matter for example – are often used to estimate the contribution of the two sources to particulate organic carbon in the area under study:

$$\delta_{sample} = (\delta_{source1}) * f_1 + (\delta_{source2}) * f_2 \qquad (2.7)$$

rearranges to give:

$$f_1 = (\delta_{sample} - \delta_{source2}) / (\delta_{source1} - \delta_{source2}) \qquad (2.8)$$

where there are two sources consisting of two fractions that sum to 1, fraction (f_1) for source 1 and fraction (f_2) for source 2 (Fry, 2006).

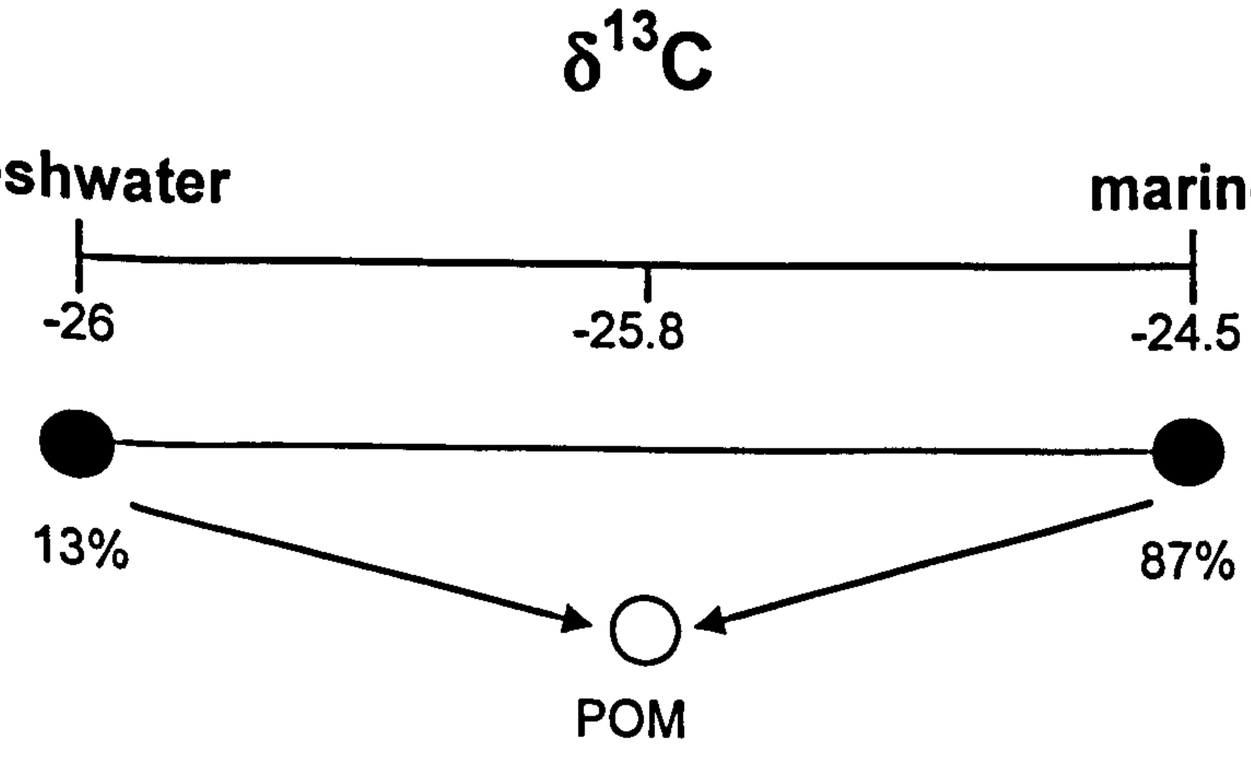


Fig. 2.9 Conceptual mixing model for carbon stable isotopes for particulate organic matter (○) with two sources (●), terrestrial and marine. After Fry (2006).

Application of such mixing models, however, is dependent on the values assigned to the sources in the model being distinct and as the data in Table 2.6 show, the values for oceanic and freshwater end members are quite variable. A mixing model using the lowest value of $\delta^{13}C$ for Celtic Sea Atlantic water POC found in the literature (-24.5 ‰, see Table 2.6) and the often quoted literature value of -26 ‰ for terrestrial/estuarine organic matter (Fry and Sherr, 1989) to give a $\delta^{13}C$ average of 25.8 ‰ outside the production season in the western Irish Sea, suggests a contribution of non-marine organic matter of 87 %, a highly unlikely figure. Thus, the usefulness of mixing models is highly dependent on the source data that is used to generate them. The general conclusion of many studies investigating the gradient of $\delta^{13}C$ POC values from riverine through to offshore areas, is that only where there is a large volume of freshwater inflow does the influence of freshwater derived organic matter extend beyond the freshwater and brackish regions of the estuaries (Tan and Strain, 1979; Gearing et al., 1984; Fry and Sherr, 1989). However, with 3.7 % freshwater input and long water residence times in the western Irish Sea, some influence of terrestrial or estuarine derived organic matter in the western Irish Sea might be expected. Terrestrial-marine carbon mixing models can also be complicated by isotopic changes, of up to 2 ‰, in organic material due to heterotrophic metabolism during decomposition processes (Fry and Sherr, 1989). Several marine studies show a depletion of ^{13}C in POC samples with depth (Eadie and Jeffrey, 1973; Tan and Strain, 1979), an indication of either decomposition of organic material as it sinks to the benthos, or the inclusion of other sources of organic carbon in the ecosystem. Thus, low $\delta^{13}C$ POC values in the winter months may reflect either decomposition processes affecting particulate matter or terrestrial input, or indeed a combination of the two, making estimates of any contribution made by terrestrial sources particularly difficult. An investigation of the

gradient of stable isotopes from coastal to offshore areas in the Irish Sea would be needed to determine sources of organic matter and explore any mixing models further.

PON stable isotope values

The $\delta^{15}N$ values for PON in the western Irish Sea ranged from 2.9 to 8.1 ‰, and are well within the range of values reported for other marine systems (Owens, 1987; Altabet et al., 1991; Nakatsuka et al., 1992; Holmes et al., 2002). In euphotic waters which lack any detectable nitrate, the $\delta^{15}N$ of suspended particles varies from -3 ‰ to +13 ‰ (Saino and Hattori, 1980; Wada, 1980; Saino and Hattori, 1985). In 2004, there were strong seasonal changes in $\delta^{15}N$ with the most dramatic taking place during the spring bloom when there was a period of depletion in ^{15}N from late winter (February) to early spring (early May), from 8.1 to 2.9 ‰, followed by rapid enrichment to 7.4 ‰ by the end of May. The rapid changes in $\delta^{15}N$ are greater than the level of fractionation (~3.4 ‰) observed between trophic levels (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984). This marked seasonality is typical of nitrogen isotope dynamics in temperate waters with a marked spring bloom (Mariotti et al., 1984; Altabet et al., 1991; Wainwright and Fry, 1994; Rolff, 2000).

Early work by Wada and Hattori (1976) showed that variations in the $\delta^{15}N$ of particulate organic matter in the sea depended on the concentration and forms of inorganic nitrogen. Studies have since shown that changes in the $\delta^{15}N$ of oceanic phytoplankton can be explained by nitrogen dynamics because of preferential uptake of ^{14}N which leaves the remaining pool of nitrate enriched in ^{15}N (Altabet and Francois, 1994; Benner et al., 1997; Granger et al., 2004). As nitrate concentration declines, the $\delta^{15}N$ of suspended particles

increases. This trend has been attributed to fractionation by light-limited phytoplankton during nitrate assimilation, preferentially taking up ^{14}N, resulting in an increase in the $\delta^{15}N$ of the remaining nitrate and, ultimately, of the particulate matter formed from it (Wada and Hattori, 1978; Wada, 1980).

The data from the 2004 study in the western Irish Sea conform to this model. The decrease in PON $\delta^{15}N$ from the end of February to the middle of the spring bloom (mid May) coincided with an increase in chlorophyll concentration (and also POC and PON that are correlated with chlorophyll) and a reduction in dissolved inorganic nitrogen (DIN) (see summary Fig. 2.10). The winter accumulation of DIN fuels the spring bloom and phytoplankton derived carbon and nitrogen content of particulate organic matter increases. The decrease in the C:N and C:Chl ratios from winter to spring also indicate that there was a shift away from a detrital dominated PON, which is likely to be heavy in ^{15}N following microbial decomposition (Owens, 1985; Saino and Hattori, 1985) over the winter, to a phytoplankton dominated PON in spring. The change in the composition of particulate organic matter during this time, and the preferential uptake of ^{14}N nitrate by the rapidly growing phytoplankton, is reflected in falling $\delta^{15}N$ values of the PON (Fig. 2.10). A similar shift, from high to low $\delta^{15}N$ PON, was observed in Chesapeake Bay by Montoya et al (1991) during a wind mixing event. Wind driven turbulence resulted in a short-term break down of thermal stratification and mixing of new DIN from the bottom water into the euphotic zone. Thus, it appears that when DIN is not limiting, there is preferential uptake of ^{14}N by phytoplankton and a subsequent drop in the $\delta^{15}N$ of PON.

As ^{14}N DIN is taken up by the phytoplankton, however, the pool of nitrate in the euphotic zone becomes increasingly enriched in ^{15}N, resulting in increasing $\delta^{15}N$ of phytoplankton,

and hence PON, as phytoplankton take up the residual ^{15}N. The western Irish Sea PON $\delta^{15}N$ increased rapidly in May when the concentration of DIN decreased (Fig. 2.10). Similar increases in PON $\delta^{15}N$, of between 4 and 5 ‰, corresponding to falling DIN concentration, were seen by Rolff (2000) in his study of particulate organic matter in the Baltic. The speed of increase may reflect short phytoplankton generation times of a few days (Parsons et al., 1984b) and the removal of 'light' phytoplankton from the particulate fraction via export to higher trophic levels through grazing and sinking to the benthos. An estimate of phytoplankton generation time was determined from the doubling time of chlorophyll biomass (a measure of phytoplankton biomass and, therefore, number of cells) during the period of exponential growth in chlorophyll concentration. During this period the doubling, or generation time, was 10 days. Significant and rapid increases in $\delta^{15}N$ of PON in the early stages of a spring bloom have also been observed in other marine systems. For example, in the North Atlantic Altabet et al. (1991) observed that PON $\delta^{15}N$ values increased by 7 ‰ over a 25-day period during a spring bloom and increases in PON $\delta^{15}N$ following nitrate depletion during phytoplankton blooms have also been reported for the North Sea (Mariotti et al., 1984) and the Pacific Ocean (Saino and Hattori, 1985; Goering et al., 1990). The pattern of PON $\delta^{15}N$ values in the western Irish Sea in 2004 is also similar to that observed during a phytoplankton bloom induced by nutrient addition in a controlled ecosystem enclosure (70 m^3) experiment in Sannich Inlet (British Columbia, Canada) (Nakatsuka et al., 1992). In this experiment, low PON $\delta^{15}N$ values occurred soon after the beginning of the bloom and rapidly became enriched as production increased and DIN was taken up.

The corollary of discriminatory uptake of ^{14}N from the DIN supply by phytoplankton during the spring bloom is that the remaining pool of nitrate becomes enriched in ^{15}N (see Fig. 2.11 for conceptual model). This is because the availability of nitrate in stratified

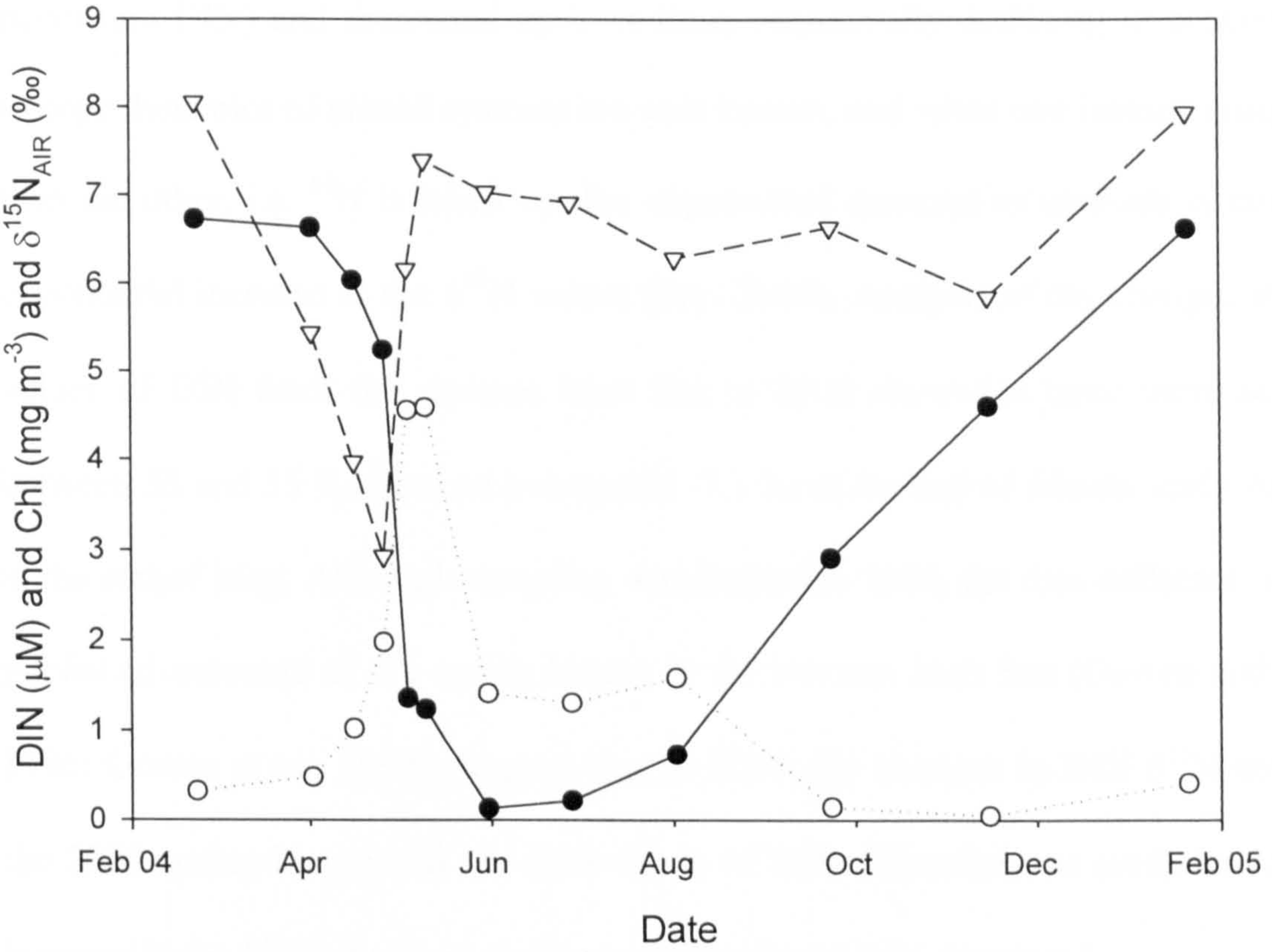


Fig. 2.10 Seasonal changes in concentrations of chlorophyll (○), dissolved inorganic nitrogen (●) and the $\delta^{15}N$ of PON (▽) from February 2004 to January 2005 at station S38A in the western Irish Sea.

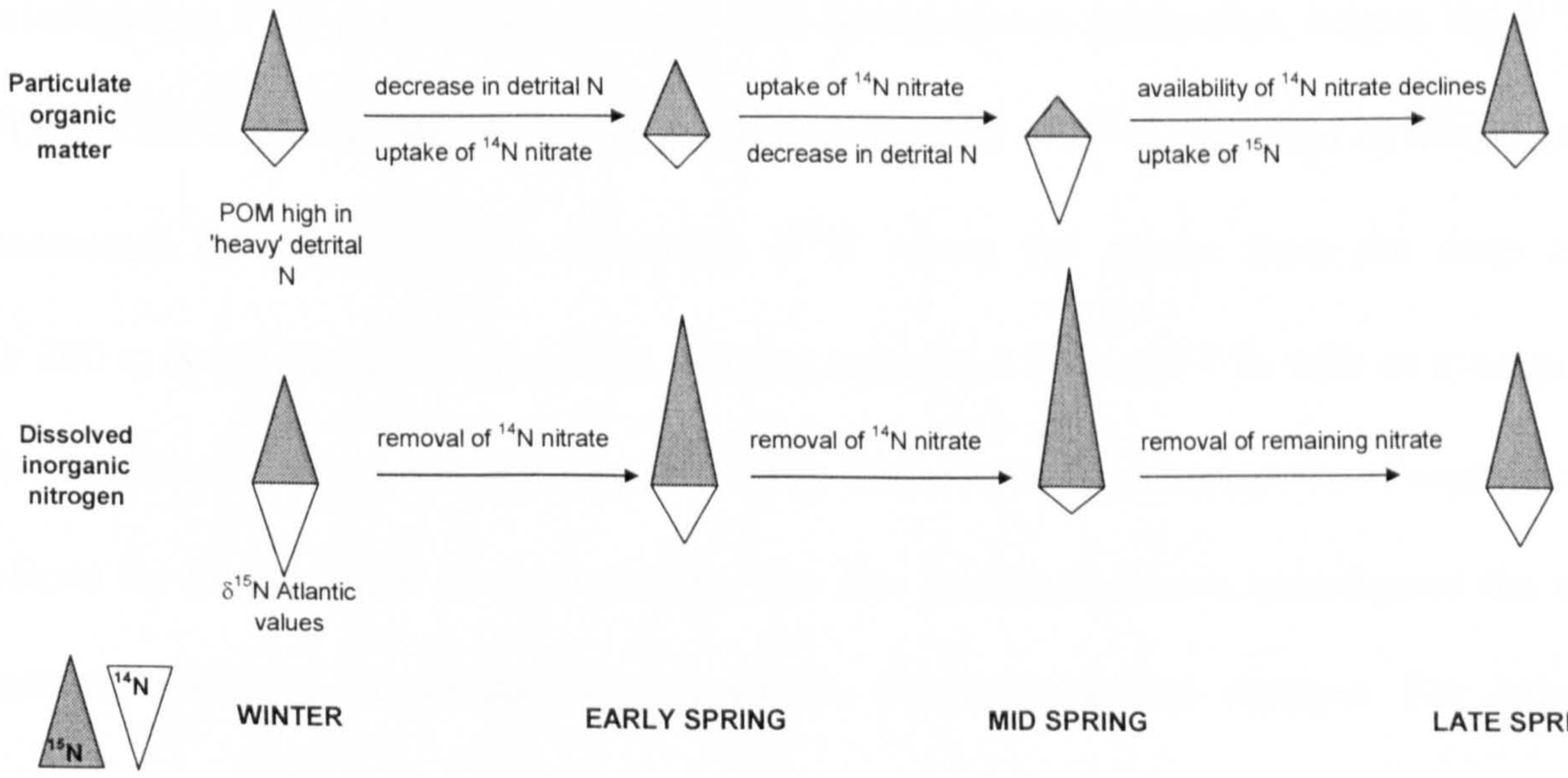


Fig. 2.11 Conceptual model to show changes in the balance between ^{15}N and ^{14}N in particulate organic matter and DIN during the progress of a spring bloom in stratified waters.

waters is an example of a 'closed' system, where a substrate is added once (i.e. the winter maximum DIN) and then used up over time, sequentially declining in concentration. The isotope dynamics of closed systems are well known, and when one isotope disappears faster than the other, i.e. ^{14}N is taken up, the exponential decrease in amounts is mirrored by an exponential increase in the $\delta^{15}N$ values (Fry, 2006). Analysis of the nitrogen stable isotope values of DIN from the western Irish Sea in 2006 showed a large increase in $\delta^{15}N$, of between 33 and 35 ‰ from an average of -7.3 ‰ at the end of March/ early April to 27 ‰ at the end of May. Although sampling was limited in 2006, the data collected, together with published accounts of the spring bloom in the western Irish Sea (Gowen and Bloomfield, 1996; Gowen et al., 1999), suggest that in 2006, the increase in DIN $\delta^{15}N$ coincided with the 2006 spring bloom and the draw-down of DIN. Therefore, as predicted, there was an increase in the $\delta^{15}N$ of nitrate as the concentration of DIN decreased.

There have, however, been few other studies of the dynamics of nitrate $\delta^{15}N$ values in the surface waters of the ocean with which to compare the Irish Sea data. Most research investigating $\delta^{15}N$ dynamics associated with spring bloom production, relates the $\delta^{15}N$ of PON to the concentration of DIN without measurement of $\delta^{15}N$ dynamics of nitrate as it is consumed by phytoplankton. Literature $\delta^{15}N$ values for nitrate from the deep ocean (> 200 m deep) are highly consistent, ranging between 4.5 ‰ and 7 ‰ with an average of 5 ‰ (Wada et al., 1975; Liu and Kaplan, 1989). There have been no reports of negative $\delta^{15}N$ values for nitrate found in the literature. The few studies that have investigated the stable isotope dynamics of nitrate have, however, observed similar changes. For example, Montoya et al. (1990) observed an increase in $\delta^{15}N$ of nitrate, from 6 ‰ to 13 ‰ as the concentration decreased during the spring bloom in Chesapeake Bay and, in their

controlled ecosystem enclosure experiment, Nakatsuka et al. (1992) calculated that an increase of 30 ‰ in the $\delta^{15}N$ of nitrate would explain the nitrogen isotope dynamics of particulate matter. Most studies, however, show that the $^{15}N/^{14}N$ of nitrate in the upper ocean typically suggests an isotope effect of 5-10 ‰ for nitrate assimilation (Sigman et al., 1997; Altabet, 2001). Altabet (2001), however, does note that there have been no reliable estimates of the $\delta^{15}N$ of nitrate at very low concentrations. Large enrichment in ^{15}N of nitrate is usually associated with denitrification. For example, nitrate $\delta^{15}N$ values up to 18 ‰ have been observed in the oxygen-depleted waters of the eastern tropical North Pacific Ocean by Cline and Kaplan (1975) and by Liu and Kaplan (1989). On balance the western Irish Sea data are suggestive of an increase in the ^{15}N pool of DIN as the spring bloom progresses and DIN is taken up. However, the negative winter $\delta^{15}N$ value and uncertainties about the scale of the increase in values means that further work is required to substantiate the results of this study.

For the remainder of the production season (May to September) the euphotic zone concentration of DIN was very low (<0.1 μM until early August and increasing to ~3 μM by September), particularly in early summer, and primary production is likely to have shifted to regenerated nitrogen in the form of ammonium excreted by zooplankton and micro-heterotrophs. Ammonium excreted by copepods is deplete in ^{15}N, by about 3 ‰, in relation to the animal and may explain some of the gradual decline in PON $\delta^{15}N$, from 7.4 to 5.9 ‰, during this period. At the beginning of August, there was a slight increase in the concentration of both DIN and chlorophyll in the euphotic zone, at ~19 m (Table 2.7). The sudden decline in the temperature of the surface water in July, by ~2 °C (Fig. 2.4) showed there must have been a mixing event which brought nutrient rich bottom water into the euphotic zone, fuelling phytoplankton growth and resulting in an increase in the $\delta^{13}C$ value

of POM at 19 m. This may also have resulted in the decline in PON $\delta^{15}N$ values as more ^{14}N in nitrate became available for uptake.

There was clear cycling of stable isotopes of particulate organic matter over the sampling period (February – January) (Fig. 2.12 and Fig. 2.13). Carbon and nitrogen stable isotopes cycle in relation to the seasonal cycle of production because of a shift from fractionation dominating the stratified production season and mixing dominating the mixed waters non-production season.

There are two general frameworks, closed and open systems, for understanding isotope changes in biological systems. The closed system is distinguished by the lack of new inputs and progression of reactions over time that consume the substrate that was present in the beginning. In closed systems, isotope values for substrates follow exponential trajectories over time (Fry, 2006). The production season in the western Irish Sea is a closed system because nutrient uptake greatly exceeds new input during the bloom (as the input of new nutrients is limited by vertical stratification) and nutrients fall to zero. During this period fractionation dominates the isotope dynamics. Phytoplankton $\delta^{15}N$ values fall rapidly with increasing production (chlorophyll and POC/PON increasing) and declining nutrients as DIN declines and then as nutrients become limiting the $\delta^{15}N$ values rapidly increase. After the peak of the spring bloom, when there is little production occurring, stable isotope values are stable and only change once stratification of the water column breaks down. Open system dynamics are now more important and although fractionation is still occurring, in the form of microbial mineralisation of organic matter, any isotopic changes are exceeded by the effects of mixing with organic matter from outside the closed system.

Thus, cycling can be seen to result from a shift from a mixed open to a cyclonic gyre driven closed system, and then back again, i.e. from low production to high production and back again, in the western Irish Sea.

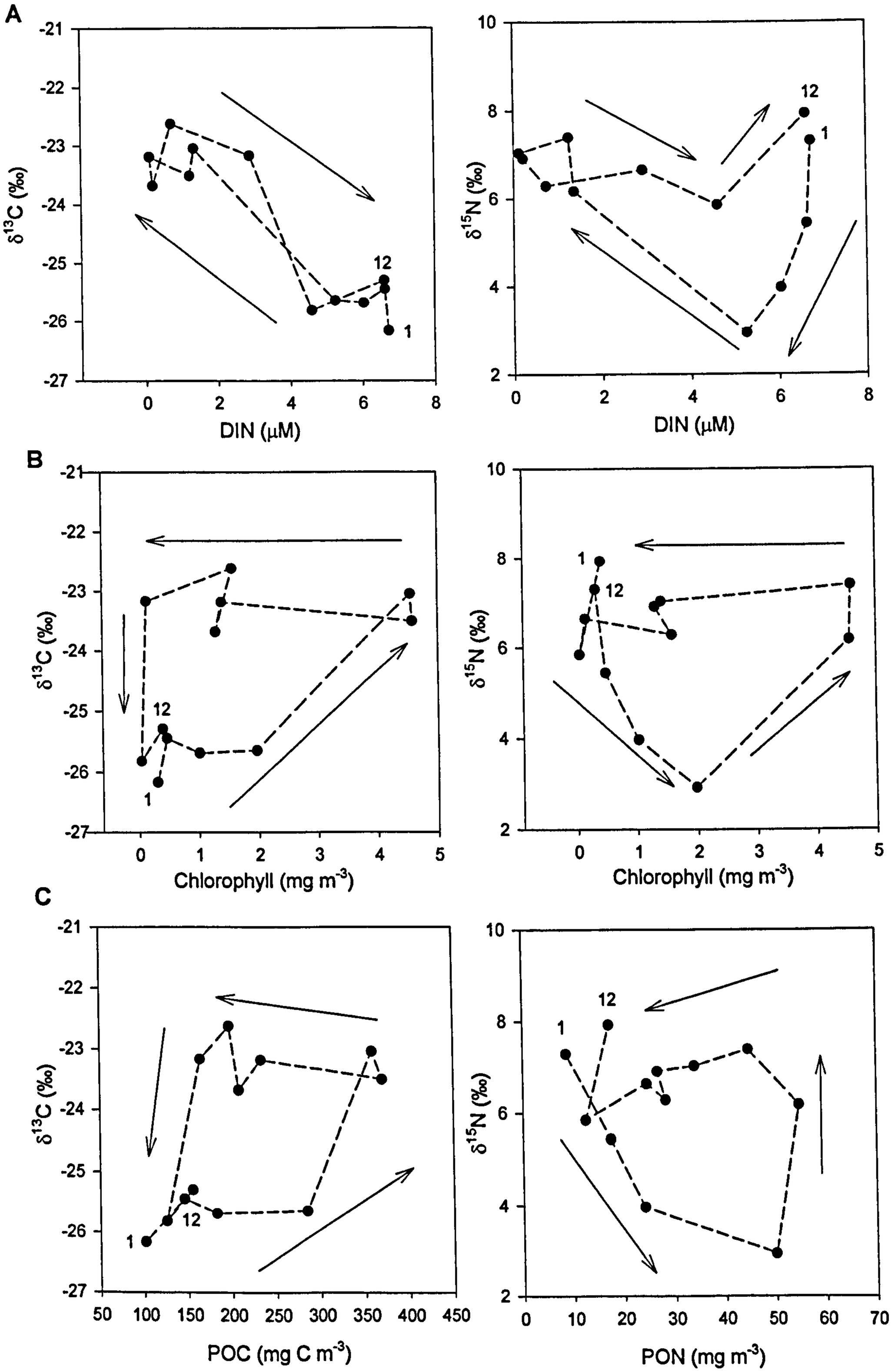


Fig. 2.12 $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter vs **A**: DIN; **B**: concentration of chlorophyll and **C**: concentration of POC in the euphotic zone at station S38A in the western Irish Sea from (1) 23 February 2004 to (12) 21 January 2005 with arrows showing direction of changes over time.

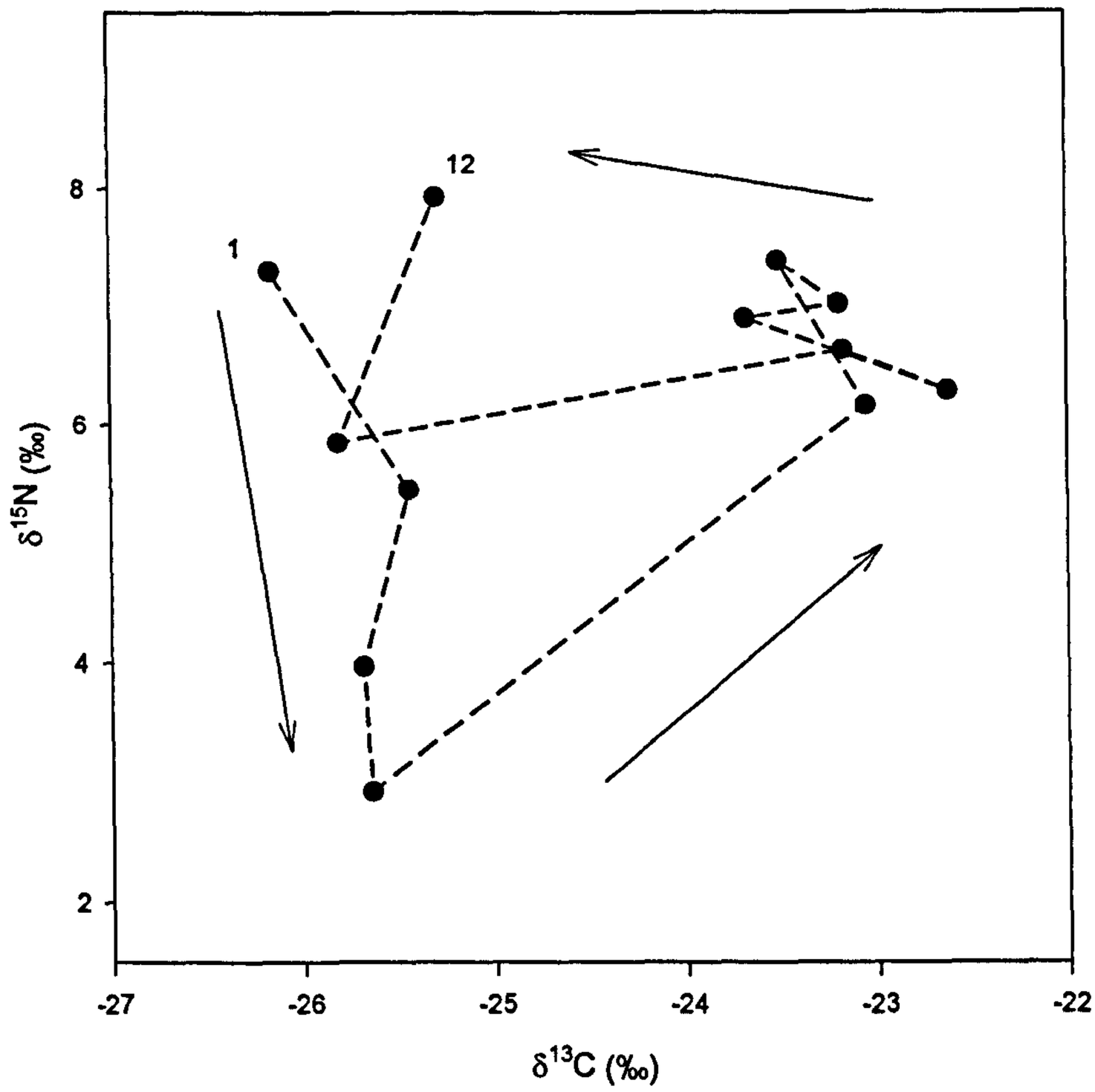


Fig. 2.13 Euphotic zone particulate organic matter isotope map (average $\delta^{13}C$ vs $\delta^{15}N$) for the western Irish Sea S38A from 23 February 2004 (1) to 21 January 2005 (12).

Conclusion

In 2004 seasonal stratification, nutrient and production dynamics were typical for the western Irish Sea. Spring bloom production in 2004, of 24.8 g C m^{-2}, was slightly lower than some previous estimates and data from other studies suggests there may have been a decline in production in the western Irish Sea in recent years.

A new finding is that the $\delta^{13}C$ of particulate organic carbon in the western Irish Sea is lower, particularly in the winter months, than that from other oceanic marine systems and this may reflect freshwater influence and long water residence times in the Irish Sea. There is significant input of freshwater from English rivers such as the Mersey and the Dee. It is currently not possible to estimate the proportion of terrestrially derived organic matter in western Irish Sea as there are no data to provide isotopic signatures for the potential terrestrial or estuarine source material. Further observations of $\delta^{13}C$ values of organic matter in the western Irish Sea, from a range of areas including coastal and shelf waters, are required to determine if there is a contribution of organic matter derived from non-marine sources to the western Irish Sea.

The seasonal pattern of $\delta^{13}C$ can be divided into a mixed non-production and a stratified production season. The mixed non-production season is characterised by low $\delta^{13}C$ indicating the presence of detrital matter, some potentially from freshwater or estuarine sources. The increase in $\delta^{13}C$ values during the growing season reflect the higher proportion of phytoplankton derived carbon, which is heavier because of the dominance of ^{13}C rich diatoms.

The seasonal cycle of PON $\delta^{15}N$ in the western Irish Sea was typical for temperate seasonally stratifying waters with a pronounced spring bloom of primary production. There was a strong seasonal signal because isotope dynamics are determined by nutrient availability and DIN becomes limited during the spring bloom. Early bloom values fell as ^{14}N was preferentially taken up by phytoplankton and the PON became dominated by new phytoplankton production. However, as dissolved inorganic nitrogen was taken up the remaining pool of DIN became enriched in ^{15}N and resulted in a subsequent rise in $\delta^{15}N$ value of particulate organic matter as phytoplankton assimilated ^{15}N.

The large temporal changes in the $\delta^{13}C$ and $\delta^{15}N$ of near-surface particulate organic matter provide a strong source signal that may be used to trace the flow of organic matter to the next trophic level, in particular to grazing planktonic zooplankton in the water column and the flux of particles to the benthos.

Table 2.7 All euphotic zone (0-23 m) values of particulate organic carbon (POC), particulate organic nitrogen (PON), stable isotope ratios of particulate organic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and characteristics of particulate organic matter at station S38A in the western Irish Sea from February 2004 to January 2005. (- data not available).

Date	Depth (m)	DIN (μM)	Chl (mg m^{-3})	POC (mg m^{-3})	PON (mg m^{-3})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:Chl	C:N
23 February 04	6.3	6.79	0.28	85.1	7.0	-26.0	6.9	306	10.4
	2.1	6.70	0.30	101.7	10.1	-25.8	8.1	341	8.6
	0.0	6.71	0.37	117.5	8.9	-26.8	6.8	320	11.3
02 April	20.9	6.55	0.45	128.1	16.2	-25.9	4.8	286	6.8
	10.6	6.56	0.52	189.7	22.7	-25.3	5.5	363	7.2
	7.3	6.91	0.50	118.6	14.0	-25.4	4.3	237	7.3
	0.9	6.55	0.45	135.2	15.5	-25.7	6.0	301	7.5
	0.0	6.60	0.45	158.3	18.7	-25.0	5.8	349	7.3
16 April	18.2	6.19	0.88	185.9	22.5	-25.7	4.4	212	7.1
	10.9	5.94	1.25	205.0	24.6	-25.6	3.6	164	7.1
	5.9	5.90	0.82	148.5	19.8	-25.5	3.0	181	6.4
	0.0	6.15	1.13	190.7	29.5	-26.0	4.6	169	5.5
26 April	20.0	-	1.11	198.6	31.8	-25.3	3.5	179	5.4
	10.0	-	2.63	303.1	50.7	-25.2	2.2	115	5.1
	0.0	-	2.20	352.7	67.4	-26.4	2.8	161	4.5
04 May	18.0	1.64	4.32	364.3	50.1	-23.1	5.9	84	6.2
	8.3	1.24	4.72	376.3	61.4	-23.2	7.5	80	5.3
	0.0	1.20	4.63	335.5	52.6	-22.9	5.1	72	5.5
10 May	18.9	3.13	5.77	285.6	46.1	-23.3	7.4	49	5.3
	9.1	0.34	5.70	452.1	52.6	-23.0	7.7	79	7.4
	0.0	0.24	2.29	372.2	35.3	-24.3	7.0	163	9.0
31 May	20.1	0.14	1.66	214.1	33.7	-22.7	6.8	129	5.4
	8.6	0.10	1.32	222.4	29.5	-23.6	6.5	168	6.5
	0.0	0.14	1.24	263.4	38.8	-23.3	7.8	213	5.8
28 June	18.8	0.51	1.73	237.0	28.4	-23.7	6.1	137	7.2
	8.6	0.06	1.02	207.7	25.8	-23.8	7.1	204	6.9
	0.0	0.07	1.15	180.4	25.7	-23.6	7.5	156	6.0
02 August	18.4	1.99	2.73	192.5	27.0	-20.3	6.7	70	6.1
	8.0	0.20	1.29	198.1	31.9	-23.4	5.9	153	5.3
	0.9	0.02	0.72	202.0	25.8	-24.2	6.1	280	6.7
23 September	18.9	2.78	0.20	142.3	21.4	-23.2	7.0	729	5.7
	9.2	3.08	0.11	196.1	29.0	-23.1	5.9	1809	5.8
	0.0	2.88	0.12	155.2	23.2	-23.2	7.2	1282	5.7
15 November	21.1	4.63	0.03	142.4	13.6	-26.1	5.9	4379	9.0
	10.6	4.55	0.05	109.4	11.0	-25.9	5.6	2243	8.5
	0.0	4.63	0.05	125.1	12.7	-25.5	6.2	2387	8.4
21 January 05	19.0	6.56	0.38	145.7	14.5	-25.3	-	380	8.6
	9.0	6.64	0.40	148.8	18.2	-25.2	7.2	374	7.0
	0.0	6.65	0.43	171.3	18.7	-25.4	8.7	398	7.9

Chapter Three

The flow of carbon from primary to secondary production and links to *Nephrops norvegicus* larvae

Introduction

Primary production in the sea is transferred through the food chain by the grazing activity of herbivores. In all the world's oceans these herbivores are dominated by small crustaceans, the copepods, which often account for 70-90 % of total zooplankton biomass (e.g. Nielsen and Sabatini, 1996). Copepods, and other herbivores such as tintinnids and ciliates, are vital to the productivity of marine ecosystems because they provide the important link between primary production and pelagic carnivores in the ocean such as larval fish. They also provide links to secondary production in the benthos as the food for the larvae of benthic organisms. The timing of zooplankton cycles may also determine the proportion of primary production that can be grazed, thereby affecting the amount of organic matter that ultimately reaches the seabed (Wassmann, 1998).

Recent research has shown that marine pelagic communities are particularly sensitive to climate change with shifts in species composition and modifications to the timing of seasonal successional peaks observed in North Sea zooplankton (Edwards and Richardson, 2004). In particular, the seasonality of taxa associated with low turbulence conditions were found to have moved forward in time, and the benthic larval component of the zooplankton had shown particularly large shifts forward. Such changes in marine pelagic communities can lead to a mismatch between successive trophic levels, with important implications for both pelagic and benthic ecology. In Naragansett Bay, USA, for example, an increase in year round grazing by zooplankton, in response to climate warming, is thought to be an

important factor in the observed loss of the spring diatom bloom, a decrease in mean annual chlorophyll production and a decrease in the deposition of organic matter deposition to the benthos (Fulweiler et al., 2007).

In the western Irish Sea, the pelagic larva of the commercially important benthic crustacean, *Nephrops norvegicus* is an important component of the zooplankton community. However, the coupling of the life cycle of *N. norvegicus*, particularly the pelagic larval stages, to the physical environment (Smith, 1987; Tuck, 1993) may mean the fishery is vulnerable to changes in the pelagic ecosystem driven by climate variation and changes in anthropogenic nutrient enrichment. The development of seasonal stratification and a cyclonic gyre of near surface water, which may be affected by climatic changes, is thought to be particularly important to the recruitment of *N. norvegicus* larvae in the western Irish Sea. The gyre acts as a retention mechanism, keeping the larvae over the deep muddy trough, corresponding closely to the distribution of adults (Hill et al., 1994; Gowen et al., 1995; Hill et al., 1996; Dickey-Collas et al., 2000a; Horsburgh et al., 2000). The western Irish Sea gyre is also thought to limit the exchange of water from mixed waters, limiting the import of *N. norvegicus* larvae from populations outside the western Irish Sea. Thus, recruitment to the local adult population appears particularly dependent on local larval production and recruitment and the *N. norvegicus* fishery, may be therefore, potentially vulnerable to any changes in the pelagic ecosystem.

In the Irish Sea, human-mediated impacts such as changes in nutrient concentrations and increasing water temperature have been observed (Alcock and Rickards, 2001; Gowen and Stewart, 2005). Changes in water temperature may lead to changes in the seasonal pattern of water column structure, important for the development of the spring bloom. Changes in

zooplankton communities have also been observed in the Irish Sea, with a decline in zooplankton density in recent years (Kennington and Rowlands, 2004). Such changes obviously have important implications for fisheries in general (Townsend and Cammen, 1988) and may have implications for recruitment of the larvae of the *Nephrops norvegicus* and the supply of organic matter to the benthos.

In the Irish Sea *Nephrops norvegicus* larvae hatch between April and June, (Nichols et al., 1983; Dickey-Collas et al., 2000a). The newly hatched larvae pass through three pelagic larval stages (I, II and III), remaining in the water column for 50 - 60 days, before metamorphosing to the juvenile stage that settles on the benthos (Farmer, 1975; Smith, 1987). While much is known about the life history of *Nephrops norvegicus*, understanding of the coupling between the seasonal cycle of pelagic production and the larvae is limited. The timing of the larvae in the water column coincides with the spring bloom and peak abundance of zooplankton, and presumably food availability. Laboratory studies have shown the larvae to be carnivorous, capturing prey by active swimming movements (Farmer, 1975), feeding on: mixed live plankton (Farmer, 1972); *Artemia salina* nauplii (Figueiredo, 1971; Farmer, 1972; Figueiredo and Vilela, 1972; Hillis, 1972) and the living eggs of the shrimp *Crangon crangon* (Figueiredo and Vilela, 1972). On the basis of his laboratory feeding experiments Farmer (1975) suggested that *N. norvegicus* larvae probably feed on copepods, small mysid shrimps, decapod larvae and small arrow worms (e.g. *Sagitta* spp.). Thus, although it appears that *N. norvegicus* larvae populations are dependent on the availability of zooplankton for their diet there have been no field studies or gut content analyses to confirm this.

The overall aim of the work presented in Chapter Three was to investigate the links between primary and secondary production in the water column and to investigate the importance of these production cycles to the larvae of *N. norvegicus*. Using stable isotope analysis, the strong seasonal signal from primary production (Chapter Two) can be used to track the flow of carbon from primary to secondary production and establish the trophic position of the larvae. The western Irish Sea gyre, which appears to retain planktonic animals, thereby keeping the larvae of *N. norvegicus* in the region above the mud-patch of the adults (Hill et al., 1996), provides a suitable location to study the trophic dynamics of *N. norvegicus* larvae.

The specific objectives were to: (i) describe the seasonal pattern in density and biomass of zooplankton; (ii) determine the transfer of carbon and nitrogen from primary production to the zooplankton; (iii) determine the species composition of the zooplankton community; and (iv) trophic level of the zooplankton using nitrogen stable isotope analysis; v) determine the density of *Nephrops norvegicus* larvae during the production season; vi) analyse the trophic position of the larvae using a combined approach of gut content and stable isotope analysis in order to vii) to determine the importance of the zooplankton bloom to *N. norvegicus* larvae.

Methods

Field programme

All sampling was conducted at the AFBI mooring station S38A in the western Irish Sea (53°46'N, 05°38'W) onboard RV Lough Foyle (see Chapter Two for full details). Zooplankton were collected between February 2004 and January 2005 and *Nephrops norvegicus* larvae from April to June 2004.

Zooplankton

Sampling: Zooplankton samples were collected by vertical hauls, with a 0.6 m-diameter ring net (200 μm mesh size), from approximately 8 m above the sea bed to the surface. The tows were carried out at constant speed to ensure a set volume of the water column was sampled. Three samples were collected on each occasion, two for isotope analysis and one for determination of species composition, density and biomass. Any animals larger than 1 cm (mostly ctenophores and euphausids) were removed. Samples for isotope analysis were placed in filtered seawater (pore size 22 μm) in a cool box for 6-8 h to allow for gut clearance. Samples were then fractionated into two size classes: 'small' (200-500 μm) and 'large' (500-1000 μm) and frozen at -20 ° C prior to analysis. Samples for identification and enumeration were fixed and preserved in 4 % buffered formaldehyde for approximately two months and then transferred to Steedman's solution (Steedman, 1976), a long term preservative for zooplankton.

Species composition and density: The small and large zooplankton were analysed differently because the density of the larger zooplankton sample was low and the whole sample could be sorted, identified and counted. All zooplankton were sorted into broad

taxonomic groups such as copepods and crustacean larvae (see Table 3.5) and identified to genus and species where possible (Todd et al., 1996; Mauchline, 1998; ICES, 2007). The density of each genus/group was calculated assuming a vertical net sampling profile. The smaller size fraction (200-500 μm) was sub-sampled by volume (1-5 ml depending on abundance) with a Hansen plunger pipette from a cylindrical flask placed on a magnetic stirrer. Zooplankton species composition and number of individuals per m^2 were estimated from counts of animals in the sub-samples.

In early spring and late winter the zooplankton samples were very green and there were observed to be significant numbers of large net phytoplankton, predominantly *Odontella* spp. and *Coscinodiscus* spp., caught up in the samples. Most of the phytoplankton cells captured with the zooplankton (200 μm mesh) were smaller than 500 μm, and so when the zooplankton samples were separated into the two size fractions (200-500 and 500-1000) they ended up in the small size samples. To estimate the abundance of cells in the samples, after zooplankton had been sorted and removed the remaining water sample was checked for the presence of phytoplankton cells by settling the water in an Utermohl (Utermöhl, 1931) sedimentation chamber. Where present, phytoplankton cells were identified to genus (Tomas, 1997) and counted. Data on phytoplankton species carbon content, derived from measurements of cell dimensions and standard cell volume carbon conversion factors, were provided by Dr B Kelly-Gerreyn (SOC) and used to calculate total phytoplankton carbon biomass (from phytoplankton >200μm that were inadvertently sampled by the zooplankton net) present in each zooplankton sample.

Each zooplankton group was assigned to one of three trophic levels: primary consumer (herbivore); secondary consumer (predator); or omnivore; on the basis of descriptions of

feeding in the literature (Table 3.5). Where information regarding feeding of the larvae could not be found the trophic level of the adult of the species had to be applied. Where phytoplankton biomass was present it was assigned a trophic level of autotroph = 1. Each trophic level, as described above, was assigned a score, 1, 2, 3 or 2.5 respectively, and the overall trophic level of each bulk zooplankton sample was estimated using the formula:

$$\text{Trophic level (TL)} = \frac{\Sigma(t_i \times b_i)}{\Sigma b} \quad (3.1)$$

where t is the estimated trophic level and b is carbon biomass for species i.

The production of zooplankton, in g C m^{-2}, in the western Irish Sea was calculated using the increment-summation method described by Benke (1996).

Carbon and nitrogen content and isotope analysis sample preparation: Bulk zooplankton samples were oven dried (60 °C for 24 h) to a constant weight, ground to a homogenous powder with an agate pestle and mortar and stored until required in acid-washed eppendorf tubes. All equipment was cleaned in acetone and thoroughly dried between samples. Sub-samples of ground zooplankton (~1.2 mg) from each sample were weighed into a tin capsule (ultra-clean 8 x 5 mm, Elemental Microanalysis) for elemental and stable isotope analysis.

The organic carbon (C) and nitrogen (N) content of each individual genus of adult organism (copepods, cladocerans, arrow worms, etc.) and larval group (e.g. polychaetes, echinoderms, etc.) was determined using between 30 and 150 individuals (depending on size) from each taxonomic group. The C and N values were used to calculate the biomass

of each genus and group and then all values were added to give the total C and N biomass for each zooplankton sample to investigate seasonal patterns of zooplankton biomass. The biomass was also used in the estimation of the overall trophic level of the zooplankton samples.

Nephrops norvegicus larvae

Sampling: On the basis of the findings in previous studies of larval abundance in the western Irish Sea (Dickey-Collas et al., 2000a), sampling started at the beginning of April. Plankton samples were collected with a high-speed 'Gulf III' sampler (mesh size 280 μm) deployed in a double oblique trajectory to within 5 m of the seabed (Nash et al., 1998). The volume of water filtered and clogging of the net were monitored by internal and external flow meters. Three tows of approximately 10 minutes were made on each sampling occasion. Larvae from the first two tows were placed in filtered (22 μm) seawater in a cool box for 6-8 hours to allow for gut clearance. The larvae were then identified to developmental stage (I, II or III) according to Santucci (1926) and frozen to -20 °C for subsequent isotope analysis. Up to 15 (depending on the numbers available) individual larvae of the same stage were pooled for isotope analysis to provide between 1 and 4 pooled samples (see Table 3.4). The contents of the third tow were treated with carbonated water to prevent gut evacuation by the larvae, either by defecation or stress-regurgitation during manipulations (Kleppel et al., 1988). Larvae were then removed, identified to stage and fixed in a 4 % buffered formaldehyde solution for subsequent gut content analysis.

Biomass and production: Approximately 50 individuals of each stage of *N. norvegicus* larvae were weighed before and after drying and the data used, in conjunction with the elemental data, to calculate the carbon biomass of larvae. The total seasonal production of

larvae, in g C m^{-2}, in the western Irish Sea was calculated using the increment-summation method described by Benke (1996).

Gut content analysis: Samples of larvae preserved in formaldehyde solution were transferred to ethanol (100 %) prior to dissection. The foregut was removed, placed in Euparal mounting fluid on a microscope slide and broken up to release the gut contents. The dissected parts were examined for identifiable prey items at x200 magnification using an Olympus BX50 microscope.

Isotope sample preparation: Larvae samples were dried to constant weight (60 °C for 24 h) in acid-washed glass vials, ground to a homogenous powder with an agate pestle and mortar and stored in acid-washed eppendorf tubes. All equipment was cleaned with acetone and thoroughly dried between samples. Sub-samples (~1 mg) were weighed into a tin capsule for elemental and isotope analysis.

Carbon and nitrogen content and stable isotope analysis

The method for isotope analysis is described in full in Chapter 2.

Data analysis and statistics

Isotope and density values for *Nephrops norvegicus* larvae are mean ± standard error (SE). The numbers of replicates are given in the tables. Zooplankton samples were pooled and there are, therefore, no replicates. The strength of association between variables was determined by regression analysis: from the coefficient of determination (r^2) and analysis of variance to determine the significance of the regression line.

Results

Zooplankton

Density and species composition: There was a clear seasonal pattern in density for both zooplankton size fractions with a spring peak and a winter minimum. The density of small zooplankton (200-500 μm) was higher than that of the large zooplankton (500-1000 μm) throughout the sampling period (Fig. 3.1). The maximum density of small zooplankton was just over 455,000 individuals m^{-2} on 31 May, and almost 31,000 individuals m^{-2} on the 10 May for the large.

All zooplankton samples were dominated by adult and development stages (copepodites) of copepods, which accounted for, on average, 83 % of total density in the small and 85 % in the large size fractions (Fig. 3.1). The most abundant copepods were the cyclopoid *Oithona similis* Claus and calanoids *Paracalanus* sp., *Acartia clausii* Giesbrecht, *Calanus* spp. and *Temora longicornis* Muller. Copepod nauplii, categorised in the larvae group, were also numerically important in the 200 – 500 μm zooplankton samples.

Biomass: The seasonal pattern of zooplankton carbon biomass (both size fractions) was similar to that for density with a winter minimum and a summer maximum (Fig. 3.2). The biomass of the small zooplankton peaked on 31 May (0.72 g C m^{-2}) and the large at the end of June (0.73 g C m^{-2}). Primary consumers (i.e. herbivores), in particular copepods (including copepodites), accounted for most of the carbon biomass of the small zooplankton, 99 % on average. Grazers (mostly copepods) were also important to the biomass of the larger zooplankton (on average 56 % of total carbon). The large peak in biomass of large zooplankton, at the end of June, however, was due to a significant increase in arrow worms *Sagitta* spp., that accounted for almost 50% of biomass (Fig. 3.2).

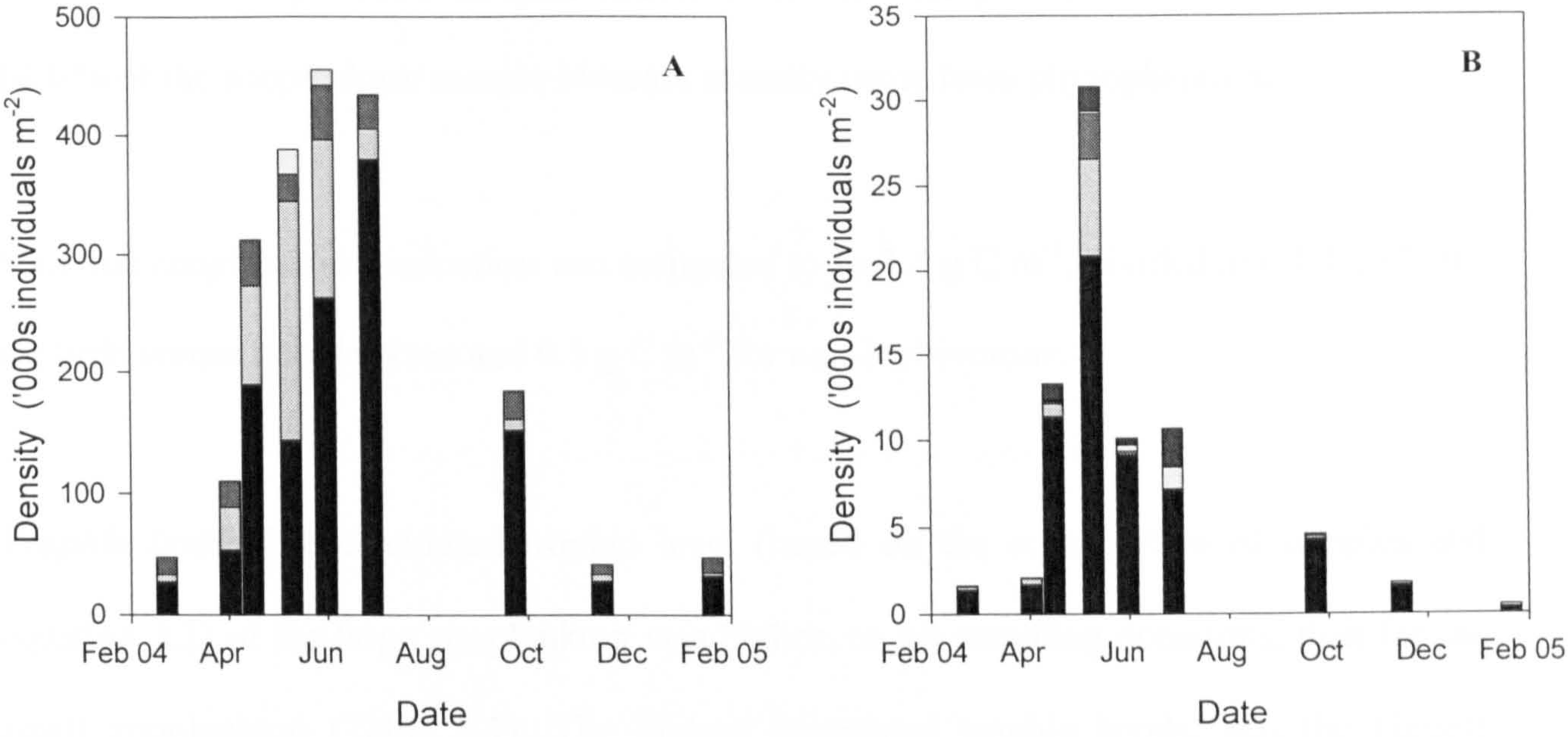


Fig 3.1 Density of zooplankton by group A: small zooplankton size fraction (200 – 500 μm); B: large zooplankton size fraction (500-1000 μm) at station S38A in the western Irish Sea.

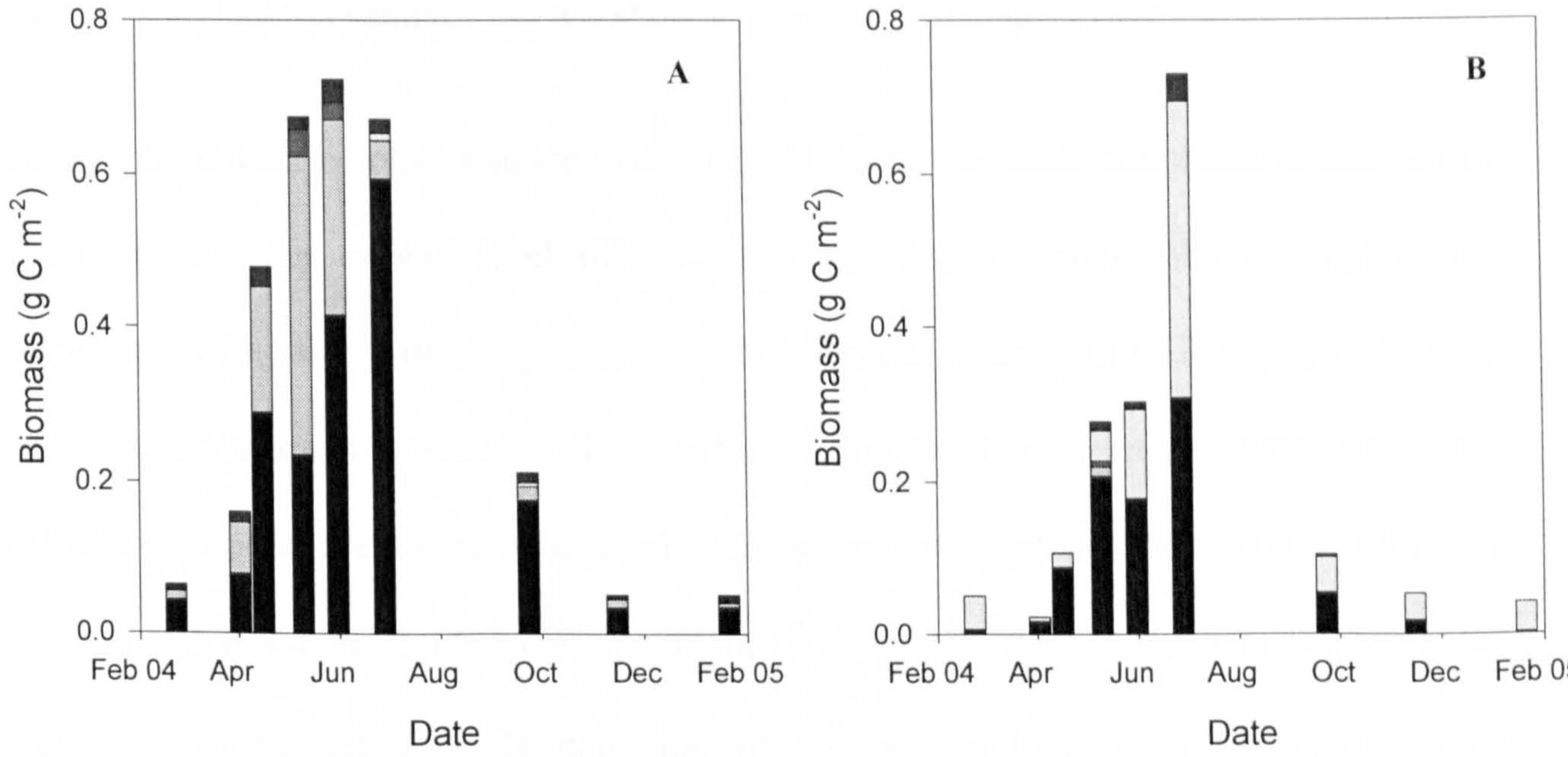


Fig 3.2 Carbon biomass of zooplankton by group A: small zooplankton size fraction (200 – 500 μm); B: large zooplankton size fraction (500-1000 μm) at station S38A in the western Irish Sea.

There were significant numbers of phytoplankton cells caught in the February 2004 and January 2005 zooplankton samples (Table 3.1). For example, in February, an estimated 14.6 % of the zooplankton sample biomass actually came from phytoplankton.

Seasonal zooplankton production was estimated to be 1.4 g C m^{-2}, divided into 1.1 g C m^{-2} for herbivorous zooplankton and 0.3 g C m^{-2} for non-herbivorous.

Trophic level: The calculated trophic level (based on the composition of samples and equation 3.1) of the large zooplankton was higher, on all sampling occasions, than for the small zooplankton (Table 3.1). The highest calculated trophic levels, and the biggest difference between the small and large size-fractions, were in February 2004 and January 2005. This was due to the higher proportion of adult zooplankton other than copepods, in particular the predatory arrow worm *Sagitta* spp. and polychaete *Tomopteris* spp. in the large zooplankton samples (Table 3.5). The calculated trophic level of the small size-fraction zooplankton samples did not show any seasonal changes (Table 3.1).

To test the impact of phytoplankton cells caught up in the small zooplankton samples in some months, the trophic level of the small size-fraction zooplankton samples was calculated with and without phytoplankton biomass (Table 3.1). The phytoplankton biomass had a small impact on the overall trophic level in February 2004, when the calculated trophic level of the bulk zooplankton sample was reduced from 2.09 to 1.98. On the basis of a 3.4 ‰ trophic enrichment in $\delta^{15}N$ values, this decrease in trophic level equates to a theoretical $\delta^{15}N$ depletion of 0.4 ‰ resulting from the inclusion of phytoplankton cells in the zooplankton samples.

Table 3.1 Calculated trophic level of small (200-500μm) and large (500-1000 μm) zooplankton samples based on biomass weighted species composition (see Table 3.5 for detailed breakdown of values by genus and taxonomic group). Trophic level key: 1 = autotroph (primary producer), 2 = primary consumer (herbivore), 2.5 = omnivore, 3 = secondary consumer (predator). Trophic level of small zooplankton calculated with (+P) and without (-P) phytoplankton.

	Phytoplankton carbon biomass (%)		**Biomass weighted trophic level* of zooplankton sample**			**Relative# $\delta^{15}N$ of total zooplankton sample**		
Date	**Small**	**Large**	**Small +P**	**Small -P**	**Large**	**Small +P**	**Small -P**	**Large**
23/02/04	14.6	0.05	1.98	2.09	3.08	6.7	7.1	10.5
02/04/04	4.2	0.01	2.05	2.08	2.34	7.0	7.1	8.1
16/04/04	0.5	0.01	2.07	2.08	2.29	7.0	7.1	7.8
10/05/04	0.2	<0.01	2.12	2.12	2.31	7.2	7.2	8.0
31/05/04	0.0	<0.01	2.09	2.09	2.42	7.1	7.1	8.3
28/06/04	0.0	<0.01	2.09	2.09	2.58	7.1	7.1	8.9
23/09/04	0.1	<0.01	2.05	2.05	2.49	7.0	7.0	8.6
15/11/04	3.4	0.01	2.01	2.04	2.63	6.8	6.9	8.9
21/01/05	8.2	<0.01	2.07	2.13	3.28	7.0	7.2	11.2

* Biomass weighted trophic level calculated from Equation 3.1

Relative $\delta^{15}N$ calculation based on an enrichment of 3.4 ‰ per trophic level (DeNiro and Epstein, 1981) and an assumed base trophic level (autotrophs) $\delta^{15}N$ of 1.0 ‰.

Carbon and nitrogen stable isotopes: The $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for zooplankton, of both size fractions, showed a significant seasonal pattern with a spring minimum and an autumn/winter maximum (Fig. 3.3 and Table 3.2).

There was rapid depletion in the $\delta^{13}C$ of both zooplankton size fractions at the beginning of the spring bloom (Table 3.2, Fig. 3.3). The $\delta^{13}C$ of the small zooplankton decreased from -21.3 ‰ in February to a minimum of -24.5 ‰ at the end of May. The $\delta^{13}C$ of the large zooplankton decreased to a minimum of -23.1 ‰ by mid April. Thereafter, the $\delta^{13}C$ of both small and large zooplankton increased and by August had returned close to pre-bloom values of ~-20 ‰ and remained so for the rest of the sampling period.

The seasonal patterns for $\delta^{15}N$ for both zooplankton size fractions were similar, with a period of depletion in early spring followed by enrichment to a peak in late summer (Fig. 3.3, Table 3.2). Minimum values of 4.1 ‰ and 4.9 ‰, for small and large zooplankton respectively, occurred in mid April. After September, the $\delta^{15}N$ for the small zooplankton increased close to a pre-bloom value of ~8 ‰. There was, however, significant enrichment in $\delta^{15}N$ of the large zooplankton in January 2005 which correlates with the higher calculated trophic level because of the increase in predatory animals.

The $\delta^{15}N$ of the large size-fraction zooplankton was higher than the small size-fraction in all samples except one (23 September). The average difference in the nitrogen isotope value from the small to the larger zooplankton was an increase of 1.1 ‰.

Stable isotope maps (Fig. 3.4) showed a general cycling of carbon and nitrogen zooplankton stable isotopes throughout the year. Both carbon and nitrogen became

Table 3.2 Carbon and nitrogen stable isotope values and C:N ratio of small zooplankton (200 – 500 μm) and large zooplankton (500 – 1000 μm) for the period February 2004 to January 2005 at S38A in the western Irish Sea.

	$\delta^{13}C$		$\delta^{15}N$		C:N ratio (atomic)	
Date	**Small**	**Large**	**Small**	**Large**	**Small**	**Large**
23-Feb-04	-20.4	-20.4	7.0	7.0	4.3	4.3
02-Apr-04	-21.2	-21.5	4.2	5.6	4.1	4.7
16-Apr-04	-22.6	-22.7	4.1	4.9	4.1	4.0
26-Apr-04	-23.3	-23.1	4.1	5.2	4.5	3.5
04-May-04	-23.9	-22.3	5.2	6.6	4.4	3.6
10-May-04	-24.5	-22.5	5.3	7.1	4.2	3.7
31-May-04	-22.9	-22.2	7.4	8.5	4.5	4.0
28-Jun-04	-20.9	-21.4	8.9	10.1	3.5	3.5
02-Aug-04	-21.4	-20.7	9.7	10.8	3.5	3.3
23-Sep-04	-20.5	-20.2	11.0	10.0	3.9	3.3
15-Nov-04	-20.3	-20.2	7.2	10.2	3.7	3.7
21-Jan-05	-19.8	-20.2	7.9	12.2	4.0	3.5

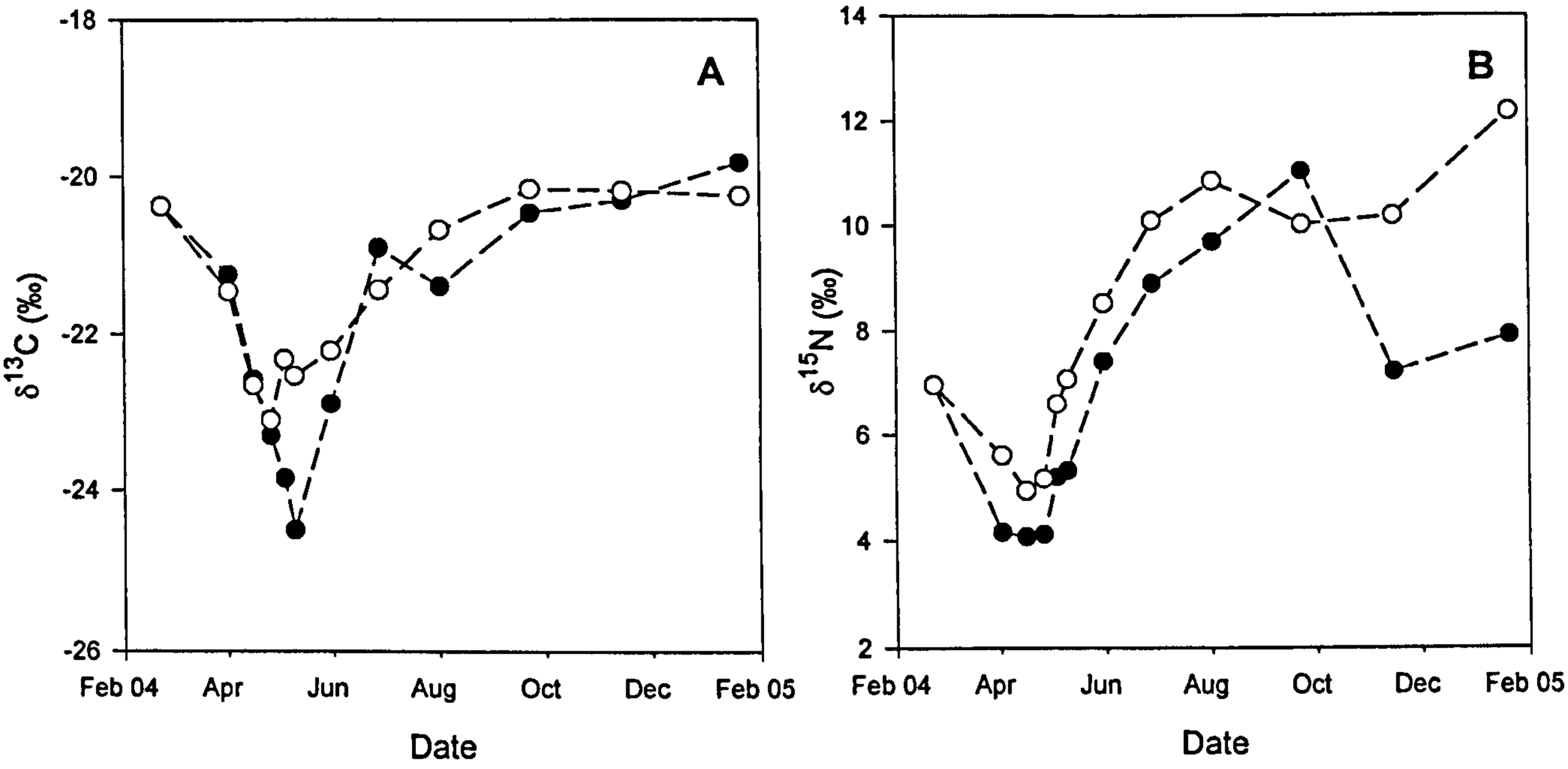


Fig. 3.3 Stable isotope values for zooplankton for the period February 2004 to January 2005 at station S38A in the western Irish Sea. **A**: carbon and **B**: nitrogen for size fractions (•) 200 – 500 μm and (○) 500 – 1000 μm.

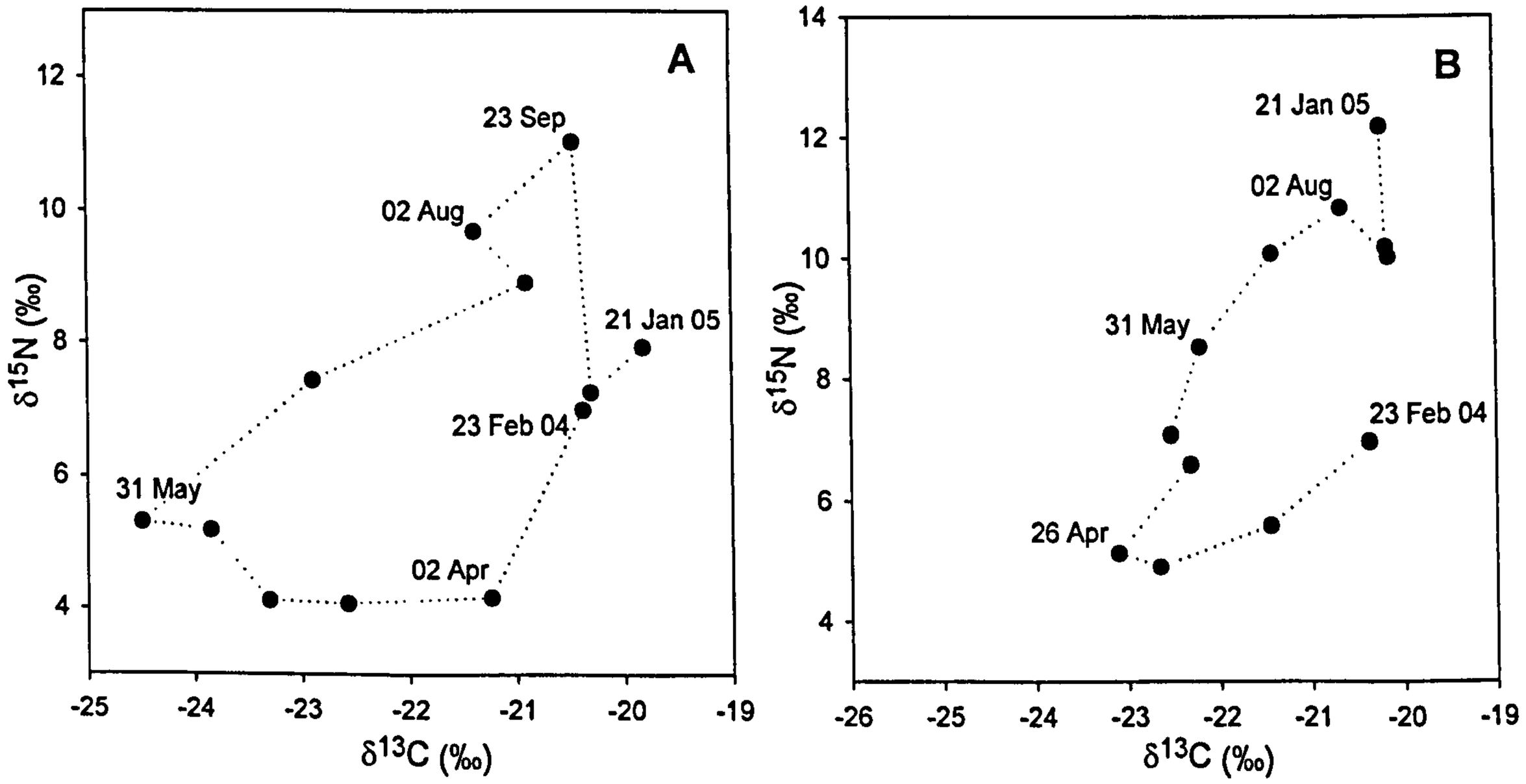


Fig. 3.4 Carbon and nitrogen stable isotope maps for zooplankton size fractions **A:** 200 – 500 μm and **B:** 500 – 1000 μm at station S38A in the western Irish Sea from February 2004 to January 2005.

depleted in the heavy isotope in the early stages of the spring bloom and then enriched later in the year.

Nephrops norvegicus larvae

Density: The first deployment of the high-speed plankton sampler on the 2 April 2004 collected a total of 8 stage I larvae giving a larval density of 1.5 larvae m^{-2} (Fig 3.5). The density of larvae was measured on a further six occasions. The sample with maximum density of all three stages of larvae, of 27 m^{-2}, was the sample at the beginning of May. At the end of June no further larvae were caught.

The highest density of stage I and II larvae were observed in samples from 4 May (Fig. 3.5). The density of stage I larvae declined in later samples and by the end of June no larvae were caught. The density of stage II larvae was also lower on 10 May and then increased again to a second, smaller peak at the end of May. The density of all three stages of larvae was lower for the 10 May samples. It was only possible to take a single sample on this sampling date due to poor weather conditions. Stage III larval density was highest in the sample from the end of May.

Gut content analysis: Analysis of the gut contents of 51 larvae from different stages and sampling occasions showed, on average, 24 % of larvae guts were empty (Table 3.3). Food items identified in the remaining guts were, almost without exception, parts of crustaceans. Calanoid copepods were the most common crustacean prey but there were also body parts from the cladocerans *Podon* spp. and *Evadne* spp. Copepod species

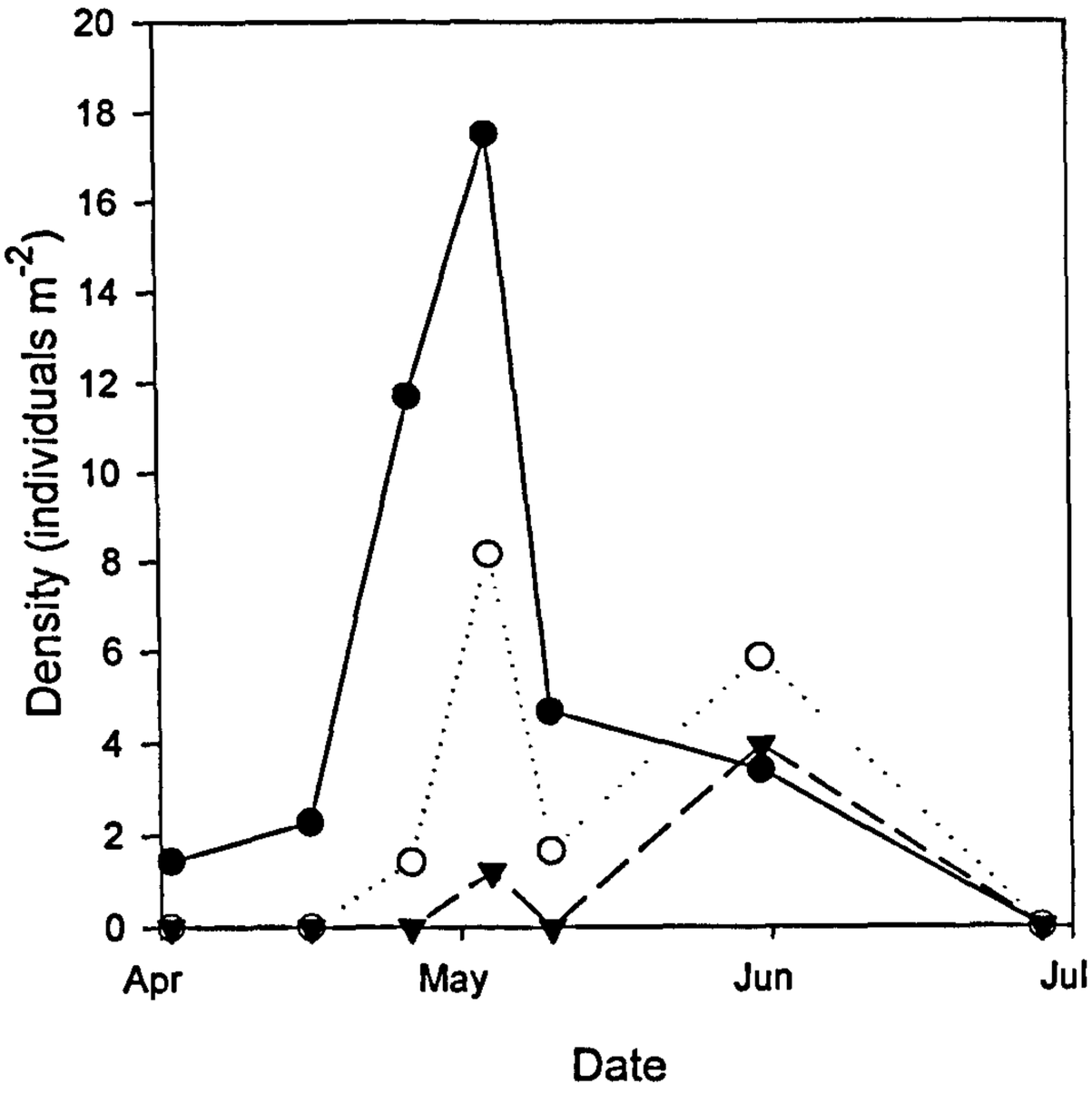


Fig. 3.5 Density of *Nephrops norvegicus* larvae: stage I (•), stage II (○) and stage III larvae (▼) at S38A in the western Irish Sea in the spring/summer of 2004.

Table 3.3 Summary of gut contents analysis of *N. norvegicus* larvae, by date and stage, from S38A in the western Irish Sea from April to June 2004.

	Stage I		Stage II		Stage III	
Date	**Full**	**Empty**	**Full**	**Empty**	**Full**	**Empty**
16/04/04	3	2				
26/04/04	5	2	4	2		
10/05/04	6	2	5	1		
24/05/04	2		5	1	2	0
31/05/04			5	0	2	2
Total	16	6	19	4	4	2
Stage Total	22		23		6	

identified include the omnivorous *Centropages hamatus* Giesbrecht and *Acartia* spp.. It was not possible to identify many gut contents to anything more than broad crustacean group. A single phytoplankton cell was found in each of only 4 (8 %) of the guts analysed and, with the exception of a single cell of the dinoflagellate *Ceratium furca* (250 μm) in the gut of a stage II larvae, were very small centric diatoms (approximately 20 μm in diameter).

Stable isotope analysis: The nitrogen content of larvae samples were small (10.5 μg N to 72.8 μg N or $\log_{10}$ sample size of 1.0 to 1.9), and within the size range where there is depletion in $\delta^{15}N$ (up to 0.8 ‰) (see Fig. 2.2 and calibration in Chapter Two). Although this made only small differences to the overall data the $\delta^{15}N$ values for all larval samples were corrected for sample size. The carbon content of larvae samples was high enough so that it was not necessary to adjust $\delta^{13}C$ values. See Chapter 2 for full details of small sample size effects and corrections.

The carbon and nitrogen stable isotope values of stage I larvae showed an initial decrease in the first few weeks after appearance in the water column (Fig. 3.6, Table 3.4). The $\delta^{13}C$ of stage I larvae declined from -18.9 ‰ to -21.3 ‰ in the first few weeks and then increased over the next few weeks to -19.3 ‰ at the end of May. The $\delta^{15}N$ values decreased from 10.7 ‰ to 9.3 ‰ and then increased to 10.8 ‰ by the end of May. The $\delta^{13}C$ and $\delta^{15}N$ of late stage I larvae were similar to early stage I larvae.

There was a drop in $\delta^{13}C$ and $\delta^{15}N$ from stage I to stage II larvae, by an average of 1.8 ‰ and 1.4 ‰ respectively (Fig. 3.6, Table 3.4). In contrast, stage III $\delta^{15}N$ values were similar to larvae from stage II.

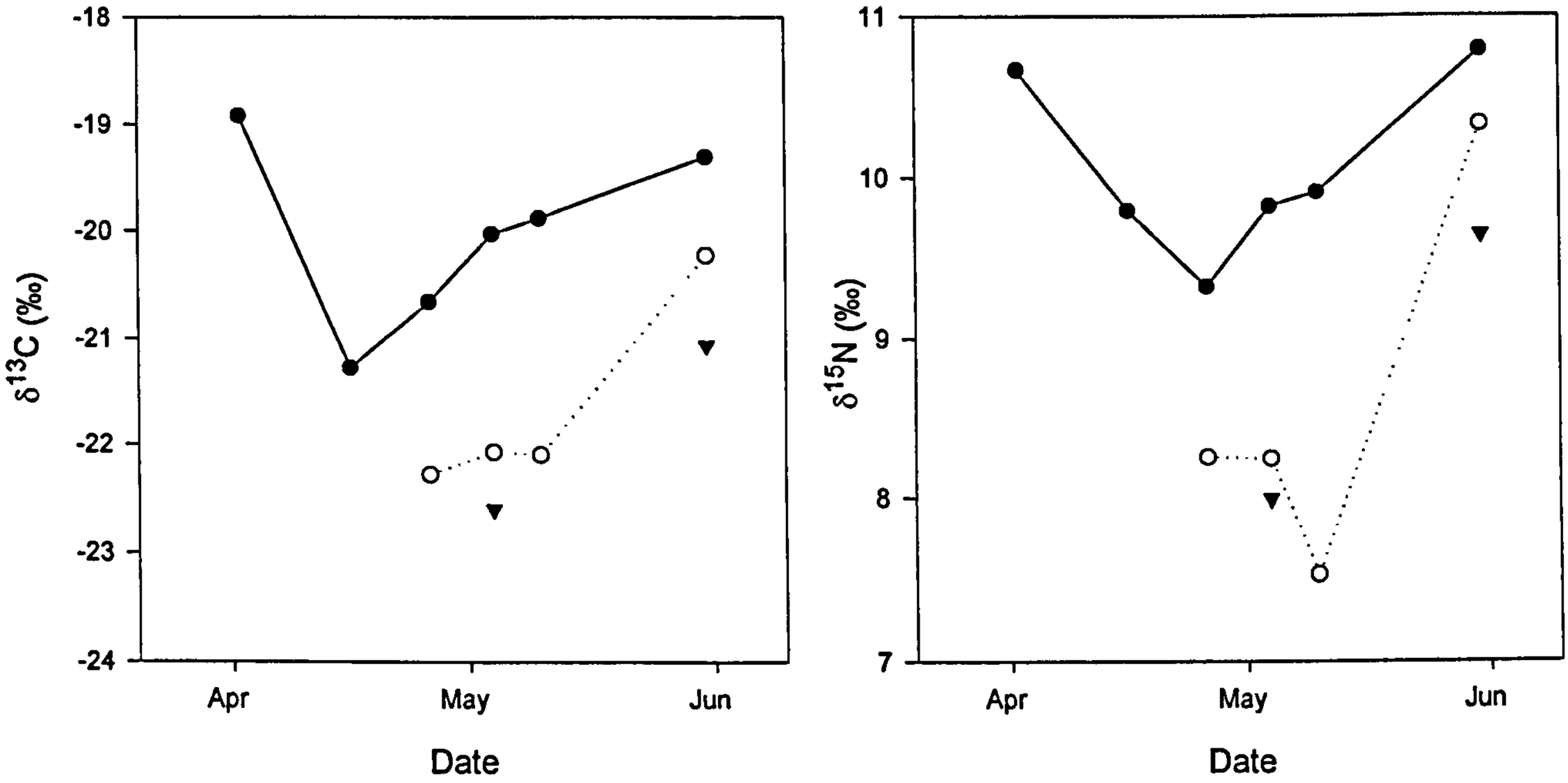


Fig. 3.6 Mean carbon and nitrogen stable isotope values for *Nephrops norvegicus* larvae stage 1 (•), stage 2 (○) and stage 3 (▼) from S38A in the western Irish Sea in 2004. Lines included to aid interpretation of data only. See Table 3.4 for *n* and SE.

Table 3.4 Carbon and nitrogen stable isotope values of *Nephrops norvegicus* larval stages I, II and III, from April to May 2004 at S38A in the western Irish Sea.

Stage	Date	No. of larvae analysed	No. of pooled samples (*n*)	$\delta^{13}C$ (‰) Mean (± SE)	$\delta^{15}N$ (‰) Mean (± SE)
I	02-Apr	8	1	-18.9	10.7
	16-Apr	6	2	-21.3 (0.4)	9.8 (0.3)
	26-Apr	43	3	-20.7 (0.1)	9.3 (0.1)
	04-May	42	4	-20.0 (0.2)	9.8 (0.3)
	10-May	15	1	-19.9	9.9
	31-May	23	2	-19.3 (0.04)	10.8 (0.01)
II	26-Apr	4	1	-22.3	8.3
	04-May	41	3	-22.0 (0.1)	8.2 (0.2)
	10-May	15	1	-22.1	7.5
	31-May	37	3	-20.2 (0.01)	10.3 (0.1)
III	04-May	4	1	-22.6	8.0
	31-May	20	2	-21.1 (0.1)	9.6 (0.02)

Discussion

Zooplankton

The seasonal pattern in density and biomass of zooplankton in the western Irish Sea, with a summer peak and winter minimum, is typical of zooplankton cycles often observed in northern temperate coastal systems (Heinrich, 1962). The close coupling of primary production to zooplankton abundance, with peak zooplankton density and biomass, several weeks after the observed peak in chlorophyll standing stock, is also well established (Gowen et al., 1998; Gowen et al., 1999). The dominance of copepods, both adults and developmental stages, in the zooplankton of the western Irish Sea is also typical of north temperate coastal systems (Nielsen and Sabatini, 1996).

Secondary production during the spring bloom arising from primary production (calculated from the biomass of herbivores) was estimated to be 1.1 g C m^{-2}, and the production of larger non-herbivorous zooplankton was 0.32 g C m^{-2}, 30 % of the grazer biomass. The amount of spring bloom primary production estimated to be eaten by zooplankton was 4.1 g C m^{-2}, which equals a transfer efficiency of 27 %. At the phytoplankton to herbivore level, transfer efficiency is thought by some to be close to 20 % (Barnes and Hughes, 1999). Pauly and Chistensen (1995) synthesised data from 48 trophic models of aquatic ecosystems, and found that transfer efficiencies ranged from 2 to 24 % but that the mean of a total of 140 estimates was 10 %. The transfer efficiency between phytoplankton and herbivores is thought to vary depending on the availability of food and the growth rate of organisms (Ryther, 1969). In young, actively growing organisms Ryther (1969) considered 30 % efficiency a biological potential and so the transfer efficiency estimated for the 2004 spring bloom in the western Irish Sea, when zooplankton are growing rapidly in a high food availability environment, seems reasonable.

The calculated trophic level (TL) for the small zooplankton remained at ~2, reflecting the dominance of copepods in the samples throughout the year (Table 3.1, Table 3.5). The calculated trophic level (TL) of the large zooplankton samples, on the other hand, showed significant seasonal variation (Table 3.1, Table 3.5). These seasonal changes were the result of changes in species composition, with more predatory species in the winter (particularly the arrow worms *Sagitta* spp. and pelagic polychaetes *Tomopteris* spp. (TL >3) and more copepods in the spring and summer (TL 2.3 – 2.6). Such a shift to more predatory species would be expected to have an impact on the $\delta^{15}N$ values of the samples and so composition will be important for the interpretation of the large zooplankton isotope data. Thus, the difference in trophic level between the small and the large zooplankton changes over the sampling period and is only equivalent to a complete trophic level in the winter.

The zooplankton sampling method (200 μm bongo net) resulted in the inclusion of some large phytoplankton, in particular diatoms of the genus *Odontella* and *Coscinodiscus,* particularly in the winter months of February 2004 and January 2005. When the zooplankton samples were size fractionated, most of the net phytoplankton passed through the 500 μm mesh and were retained in the small size-fraction samples. Although the winter density of phytoplankton in the western Irish Sea was low, as indicated by low chlorophyll (Chapter Two), there were so many phytoplankton cells caught in the zooplankton net to make some of the samples green. There was some concern that the inclusion of primary producers would have a significant influence on the $\delta^{15}N$ value of the bulk zooplankton samples. The overall trophic level of the zooplankton samples was, therefore, estimated with and without the inclusion of phytoplankton biomass to assess the potential impact on $\delta^{15}N$. The phytoplankton content within the small zooplankton samples was high only in

February 2004 (14.6 % of small zooplankton sample biomass). Calculation of the overall sample trophic level in February, based on species composition, showed that the inclusion of primary producers had a negligible impact, reducing the trophic level by only 0.1, a theoretical reduction in $\delta^{15}N$ values by 0.4 ‰. From mid April to the end of September, however, there were very few phytoplankton cells included and $\delta^{15}N$ values were not affected.

Nitrogen stable isotopes of zooplankton

From February through to the peak of the spring bloom in May there was close coupling between the $\delta^{15}N$ of zooplankton and its putative diet, PON (Fig. 3.7). The decline and subsequent increase in the $\delta^{15}N$ values of PON from February to 10 May is mirrored in the $\delta^{15}N$ values of both size fractions of zooplankton. The $\delta^{15}N$ values of POM and zooplankton are closely correlated for this period (small zooplankton: $r^2 = 0.62$, $F_{0.05,4} = 6.6$, $P = 0.06$; large zooplankton: $r^2 = 0.88$, $F_{0.05,4} = 31.2$, $P = 0.005$). The inclusion of phytoplankton cells in the February sample, did not change the overall strong seasonality of nitrogen isotope values of the small zooplankton and can be ignored.

There was a slight time lag between the changes in the $\delta^{15}N$ values in PON and zooplankton (Fig. 3.7). This reflects the longer tissue turnover times of zooplankton in comparison to phytoplankton and, hence, the integration of diet over a longer period of time. The time averaging of the dietary signature would also explain why the $\delta^{15}N$ values of zooplankton do not decline by the same magnitude as PON during the spring bloom and differences in growth rates may explain why the smaller zooplankton response is greater than the large. Zooplankton $\delta^{15}N$ values respond to PON changes, seen on 26 April, by 4 May, which estimates the zooplankton tissue turnover time to be about 8 days.

There is, however, a general lack of information regarding the rate of turnover of isotopes, and hence their expression, in tissues in natural systems (Grey, 2006), making it difficult to infer dietary changes from stable isotope values. Nevertheless, there have been a few estimates of tissue turnover times in crustaceans. For example, the $\delta^{15}N$ half-life (the time taken for the isotope of the consumer to reach half-way between two diets) of brine shrimp was estimated to be between 4 and 18 days (Fry and Arnold, 1982). Tissue turnover was also found to be faster in organisms that are growing or developing (Fry and Arnold, 1982). The biomass data were used to estimate a doubling or generation time for zooplankton, during the period of exponential increase, of 28 days. This estimate compares well to laboratory observations of copepod generation times. For example, at a temperature of 9.7 °C Thompson (1982) observed the generation time of *Pseudocalanus* sp. to be 37 days. Since generation times decrease with increasing temperature, a faster generation time in the western Irish Sea, when surface waters increased in temperature from ~8 °C to ~13 °C, would be expected. During each generation copepods are losing tissue through frequent moulting (6 times from first stage copepodite to adult) and so are likely to respond quickly to changes in diet. An estimated turnover time of 8 days is, therefore, believed to be robust.

There was a general enrichment of ^{15}N from POM to zooplankton (Fig. 3.7), as predicted by the general pattern of increasing ^{15}N with trophic level (DeNiro and Epstein, 1981). During the spring bloom the trophic fractionation in $\delta^{15}N$ between POM and zooplankton ranges from 0 to 2.2 ‰ for the small zooplankton and between 0.3 and 3.2 ‰ for the large. It will be low at the beginning of the bloom as the isotope values of zooplankton take time to equilibrate. Towards the end of the spring bloom, when zooplankton should be in equilibrium with the diet, fractionation is lower than the average 3.4 ‰ per trophic

level expected (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). However, the extent of ^{15}N fractionation between trophic levels has been found to vary depending on whether consumers were maintained on an invertebrate (1.4 ‰), plant (2.2 ‰) or a high protein diet (3.3 ‰) (McCutchan Jr. et al., 2003) and this may explain the lower fractionation between POM and the small zooplankton. The fractionation for the large zooplankton is higher, as expected, but difficult to interpret because the trophic enrichment can only be strictly applied when dealing with distinct trophic levels, and the large zooplankton samples are a mixture of different trophic levels.

There was an average enrichment of 1.2 ‰ in $\delta^{15}N$ from the small to the large size zooplankton fraction over the productive season of April to August. This indicates larger zooplankton feeding at a higher trophic level and is consistent with the hypothesis that larger organisms feed further up the food chain than small ones (Cousins, 1980). The increase from small to large zooplankton in this study is equivalent to 0.35 of a trophic level (DeNiro and Epstein, 1981). These findings are similar to trophic enrichment between similar size fractions of plankton observed in a study in the Baltic Sea (Rolff, 2000).

After the peak of the spring bloom in May, the close coupling between the $\delta^{15}N$ values for zooplankton and the putative diet appears to break down. Zooplankton $\delta^{15}N$ values become progressively enriched (up to 5 ‰ higher than PON) over the summer and autumn, at a time when $\delta^{15}N$ values for PON are static or in slight decline. This enrichment in zooplankton $\delta^{15}N$ values, by an amount greater than that predicted by trophic enrichment has several possible explanations. These explanations include: a significant change in the species composition of zooplankton samples, to more predatory

species, causing overall trophic level and, hence, $\delta^{15}N$ values to rise; a switch in the diet of zooplankton from a plant (diatoms for example) to an animal (heterotrophs such as tintinnids and ciliates for example) based diet; or starvation of zooplankton as algal biomass falls resulting in an increase in $\delta^{15}N$ values (Fry, 2006). Each of these explanations is discussed in turn.

A change in zooplankton sample species composition, from herbivorous to more predatory and/or omnivorous species would explain increasing $\delta^{15}N$ values of zooplankton. This was certainly the case for the large zooplankton, where the biomass of predatory species increased in the autumn and winter months (Fig. 3.8). Not all of the increase in $\delta^{15}N$ over the summer months can, however, be explained by composition indicating the importance of other factors as well. There was no corresponding change in the composition of the small zooplankton samples to explain the increase in $\delta^{15}N$ values, which can be seen by the lack of a correlation between predicted and actual $\delta^{15}N$ (Fig. 3.8). There must, therefore, be another explanation for the rise in $\delta^{15}N$ values.

The $\delta^{15}N$ values of zooplankton may rise as a result of a shift in diet from algae to animals. Copepods are described as opportunistic omnivores, feeding on both phytoplankton and heterotrophic protists (Kleppel, 1993), and can switch diet depending on availability of prey items (Sommer et al., 2005). By the summer months, algal biomass had declined significantly and so copepods, and other zooplankton, may be eating other organisms. Copepods have been seen to feed selectively on dinoflagellates and microzooplankton, such as ciliates, when given the choice (Kleppel et al., 1991) and the

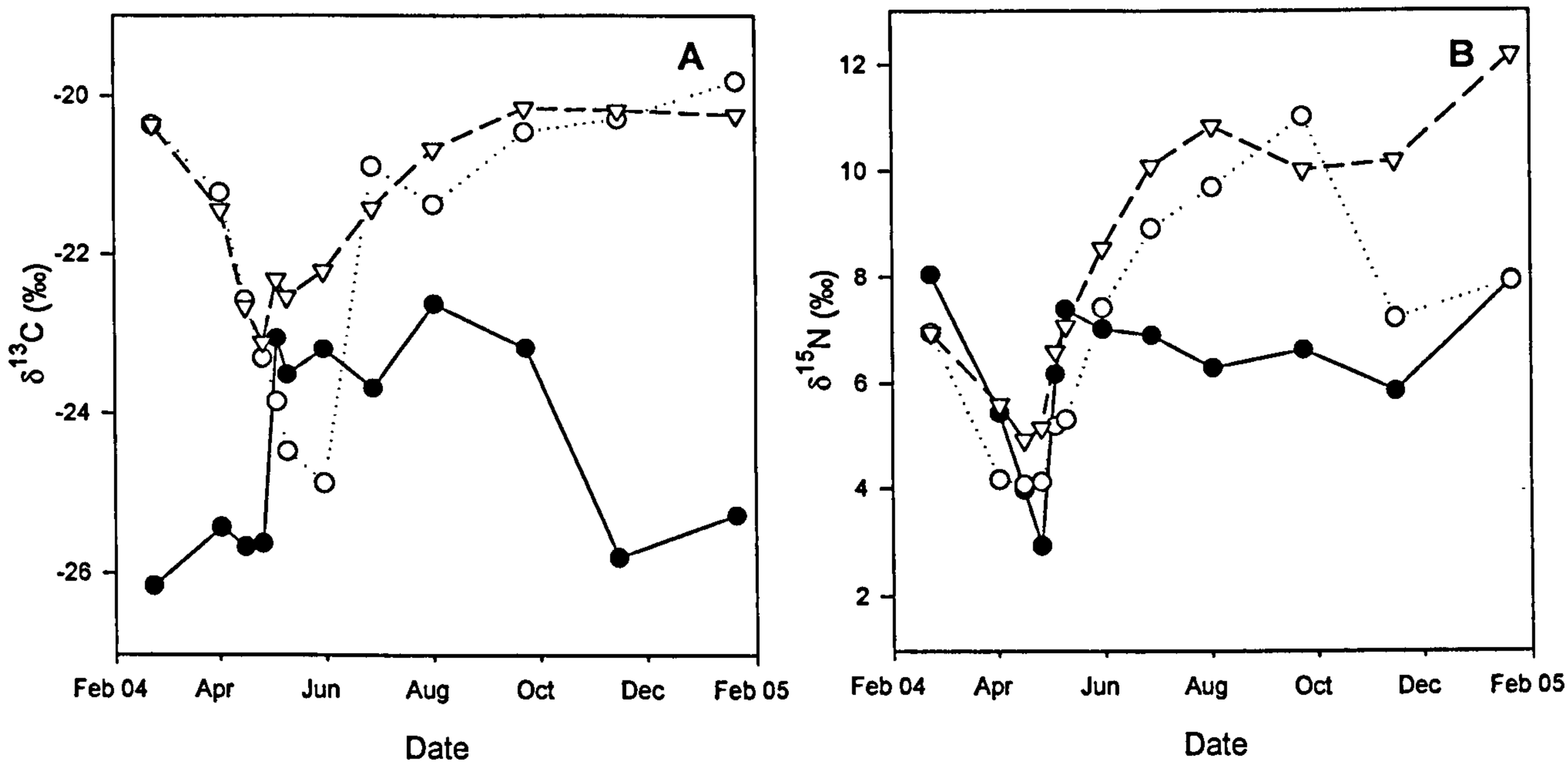


Fig. 3.7 Summary of stable isotope values of particulate organic matter (•), small zooplankton (○) and large zooplankton (▽) at station S38A in the western Irish Sea from February 2004 to January 2005. **A**: carbon and **B**: nitrogen. Lines joining data points included to aid interpretation.

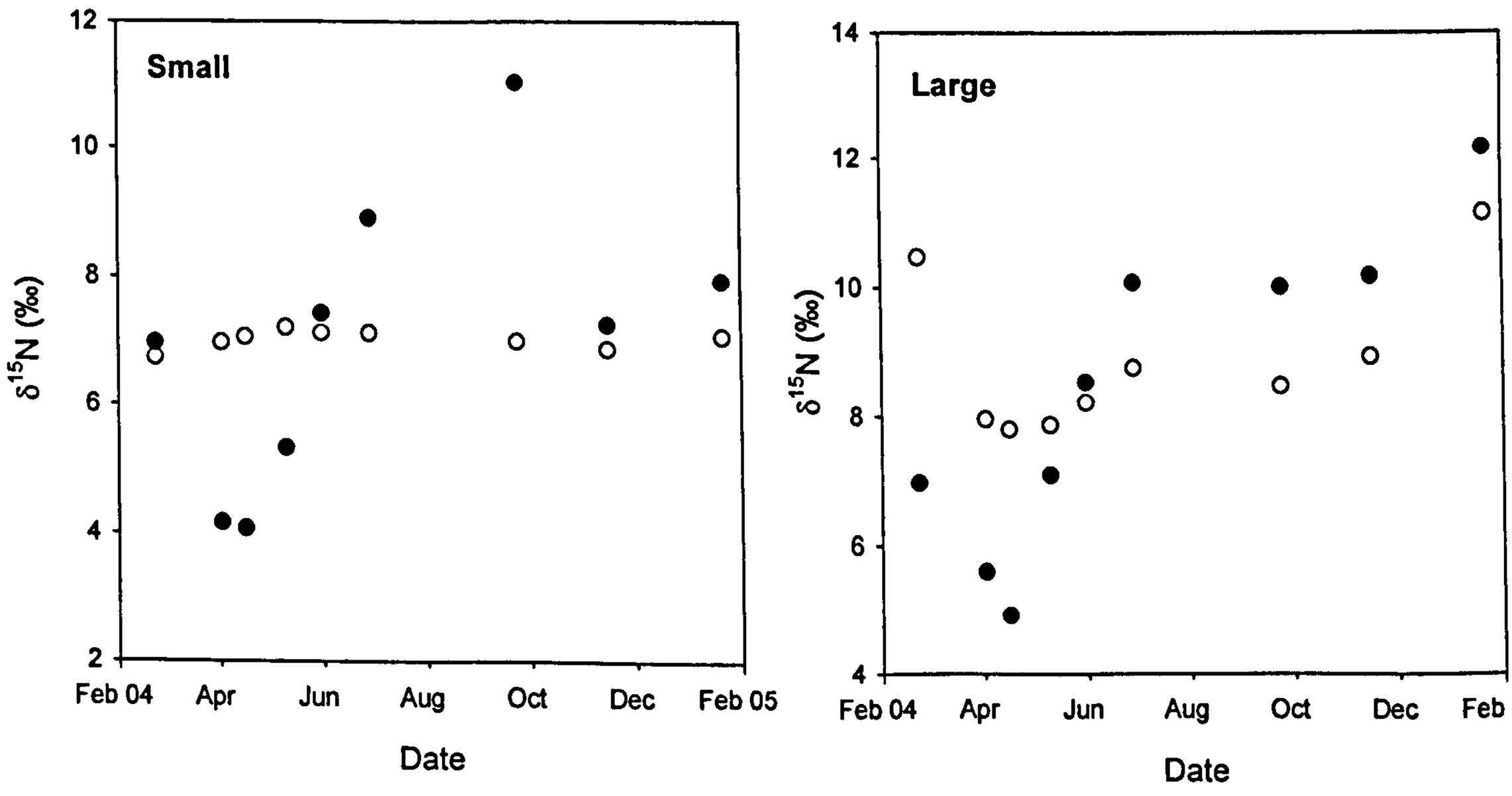


Fig. 3.8 Calculated $\delta^{15}N$ (○) and observed $\delta^{15}N$ (•) of large and small zooplankton samples.

$\delta^{15}N$ value of copepods has been seen to increase in response to a dietary switch to ciliates (Sommer et al., 2005). Also, Schmidt et al. (2003) observed increases in $\delta^{15}N$ of 3 ‰ from copepodite to adult, pointing to ontogenetic shifts in diet. Although feeding experiments were not carried out to test this hypothesis directly, a succession of species during the summer to a heterotrophic loop with mixotrophic species has been observed for the western Irish Sea in other years (R. Gowen pers. comm.). Thus, the increase in the proportion of adult copepods, feeding on an increasingly heterotrophic diet over the summer would explain the large increases in $\delta^{15}N$ for the small zooplankton and the most likely explanation.

Another reason for increases in $\delta^{15}N$ values could be starvation, which has been seen to cause $\delta^{15}N$ values to rise as ^{14}N is preferentially excreted. For example, in starvation experiments Adams and Sterner (2000) observed an increase of 0.4 ‰ in *Daphnia* sp. over 5 days. The 'starvation response' is, as above, often small and has not always been found to be the case (for example Gorokhova and Hansson, 1999). It is unlikely, therefore, that starvation would be responsible for the large increases in the $\delta^{15}N$ values for the small zooplankton.

To conclude, a change in the diet of zooplankton, as the composition of the phytoplankton and microplankton changes, and a maturing of the copepod population, is the best explanation for the seasonal increase in $\delta^{15}N$ of zooplankton in the small zooplankton. For the large zooplankton, a change in species composition to more predatory species is responsible for a large part of the change in $\delta^{15}N$, but changes in diet and development of copepods are also likely to be important.

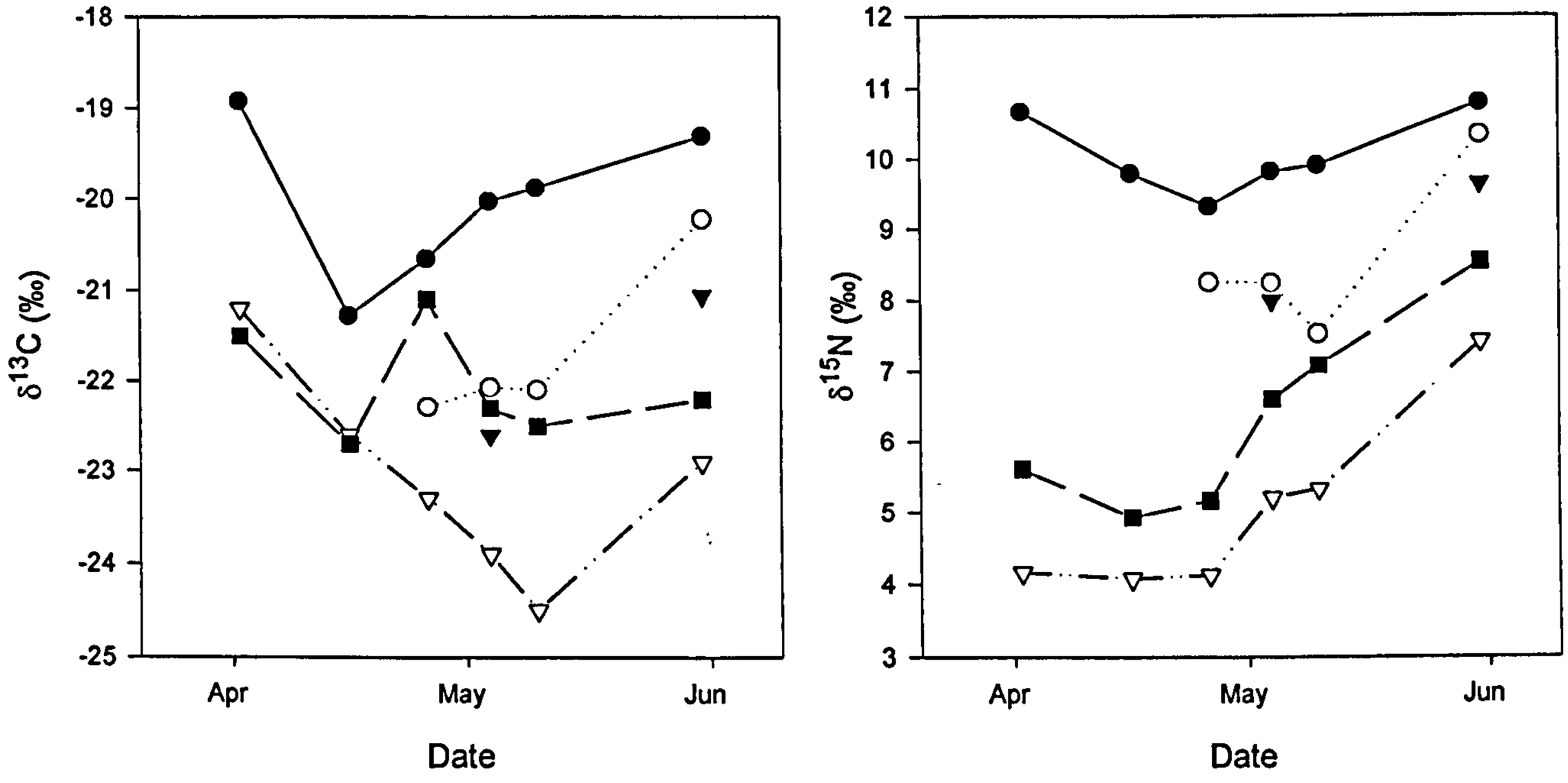


Fig. 3.9 Summary of carbon and nitrogen stable isotope values for zooplankton: (■) large and (▽) small; and *Nephrops norvegicus* larvae: (●) stage I, (○) stage II; and (▼) stage III; at station S38A in the western Irish Sea from February 2004 to January 2005. **A**: carbon and **B**: nitrogen. Lines joining data points included to aid interpretation.

After the end of September the $\delta^{15}N$ values for small zooplankton decline and return to values similar to that observed in February 2004. The decrease in $\delta^{15}N$ is a result of the loss of adult copepods and an increase in developmental stages, nauplii and copepodites, which represents the next cohort of copepods.

Carbon stable isotopes of zooplankton

Comparison of the stable isotope values of zooplankton carbon versus its putative diet, particulate organic carbon (POC), indicated seasonal changes were not closely coupled (Fig. 3.7). For example, the rapid decline in the $\delta^{13}C$ of zooplankton (of both size fractions) from winter to the spring bloom occurs at a time when the $\delta^{13}C$ of particulate organic matter was increasing. Three hypotheses may account for the differences in $\delta^{13}C$ values between zooplankton and POC: (i) POC, and therefore the $\delta^{13}C$ of POC, is not representative of the diet of zooplankton; (ii) there is another, unidentified source of carbon that zooplankton are feeding on; and (iii) there are factors other than the $\delta^{13}C$ of the diet that are important to the carbon isotope dynamics of zooplankton. Each of these hypotheses is discussed in turn.

Most of the copepods in the western Irish Sea are herbivorous (Table 3.5). As $\delta^{13}C$ does not usually change significantly between trophic levels (DeNiro and Epstein, 1978) copepod $\delta^{13}C$ values should reflect those of the diet. Bulk particulate organic matter in the water column, taken to represent diet, actually consists of a mixture of detritus, phytoplankton and zooplankton faecal pellets. Therefore, the POC samples may not be representative of a largely phytoplankton diet. However, during the spring bloom the POC samples largely consist of algal cells, as indicated by the decrease in the C:Chl and C:N ratios of organic matter. For example, from February to the beginning of May the C:Chl

ratio decreased from 322 to 79, and the C:N ratio from 10.1 to 5.7 (see Table 2.2). Also the increase in the $\delta^{13}C$ of the POC at this time is due to the presence of diatoms, which are known to be enriched in $\delta^{13}C$ (Fry and Wainright, 1991). Thus, it appears that POC, and thereby the $\delta^{13}C$ of POC, is representative of diet, particularly during the spring and summer.

The second hypothesis is that the zooplankton were feeding on another source of carbon. Although many species of copepods are recorded as herbivores there is generally some omnivory in most species in response to food availability (Kleppel, 1993) and so a switch in diet may be responsible. Copepods feeding on heterotrophic organisms, rather than algae, may result in changes in $\delta^{13}C$ values. However, a switch from an herbivorous diet, by omnivorous copepods, during the spring when algal cells are highly abundant seems unlikely. In addition there is very close coupling of the $\delta^{15}N$ values of PON and zooplankton which indicates the importance of POM in the diet of zooplankton and so the presence of another source of carbon is not thought likely.

The final explanation is that a factor other than diet is a more important determinant of zooplankton $\delta^{13}C$ values. In this instance, the biochemistry of marine copepods, which dominate the zooplankton, seems the most likely reason for the absence of a close coupling of POC and zooplankton $\delta^{13}C$ values. Copepods, in particular calanoids such as *Calanus* spp., accumulate large quantities of lipids from an algal diet, for provision of a food reserve, as an aid to buoyancy and to provide energy to go into egg production (Mauchline, 1998). The accumulation of lipids in copepods is known to be seasonal (Marshall and Orr, 1955; Lee, 1974; Hopkins et al., 1984) and can be at least two-fold, from phytoplankton to zooplankton (Sargent and Henderson, 1986). As lipids are deplete

in ^{13}C (DeNiro and Epstein, 1977), the seasonal accumulation in copepods results in lower $\delta^{13}C$ values during the spring and summer when copepods are developing and growing. There is a larger decline in the $\delta^{13}C$ values of the small zooplankton than the large because the small has higher copepod, and therefore, more lipid biomass, as also indicated by the higher C:N ratio of the small zooplankton. An increase in copepod lipids, therefore, is the most likely reason for the decline in $\delta^{13}C$ of zooplankton in the spring. From June, however, there is a rapid increase in $\delta^{13}C$ values, and this increase may be a response to the seasonal enrichment in POM $\delta^{13}C$, which increases by ~2 ‰ over the production season. The increase may also be responding to the utilization of lipid reserves as food supplies decline.

The chemical extraction of lipids can be carried out before carbon isotope analysis. However, this procedure tends to alter stable nitrogen isotopes (Sotiropoulos et al., 2004) and since both $\delta^{13}C$ and $\delta^{15}N$ are routinely derived from one sample, lipid removal effectively doubles the number of samples that have to be analysed. The significant increase in time and cost precluded lipid extraction in the current study, so changes in lipid concentration of zooplankton have not been assessed. In addition, the sample sizes of *Nephrops norvegicus* larvae were too small to be able to remove an aliquot for lipid extraction.

The $\delta^{13}C$ values of zooplankton in the western Irish Sea ranged from -24.5 ‰ to -19.8 ‰ for the small zooplankton and from -23.7 ‰ to -20.2 ‰ for the large. Zooplankton $\delta^{13}C$ measured in other studies are -20.2 ‰ ± 1.4 from the Gulf of Mexico, -20.9 ‰ in Malaysia, -19.6 ‰ ± 1.8 in the Torres Straight, Australia, -21.5 ‰ ± 1.1 from the Scotian Shelf and between -23.8 ‰ and -18.5 ‰ for a similar size fraction from Georges Bank

and the Gulf of Maine (Fry and Quinones, 1994). Marine zooplankton are generally thought to have a $\delta^{13}C$ values of -20 ‰ (in comparison to phytoplankton -22 ‰) (Boutton, 1991). The lowest carbon isotope values for western Irish Sea zooplankton probably reflect the low values of western Irish Sea POM, from -26.2 to -22.6 ‰ as described in the previous chapter. However, the reasons for depleted values of POM require further study. There is clear cycling of the stable isotope values for both size fractions of zooplankton (Fig. 3.4), which is very similar to the cycling seen in the particulate organic matter in the water column (see Chapter Two, Figs. 2.11 and 2.12). This cycling reflects the strong seasonal change from a 'closed' production season from April to September, where fractionation is the most significant isotope dynamic to an 'open' non-production season where production and fractionation is low and isotope dynamics are dominated by the process of mixing in the water column.

***Nephrops norvegicus* larvae**

In 2004, the time of hatching of *Nephrops norvegicus* larvae is likely to have been at the end of March or beginning of April because there was a very low density of larvae, of 1.5 larvae m^{-2} when sampling started on 2 April (Fig. 3.5). This timing is consistent with previous studies in the western Irish Sea (Dickey-Collas et al., 2000a; Dickey-Collas et al., 2000b). Stage II larvae were first collected at the end of April, which provides an estimate of around 24 days for development from stage I to II larvae, very similar to previous estimates in the western Irish Sea (Dickey-Collas et al., 2000b). The maximum density of stage I and stage II larvae occurred at the same time at the beginning of May. In previous studies the peak of stage II normally occurs 2-3 weeks after stage I (Dickey-Collas et al., 2000a). There was, however, a further smaller peak in stage II density at the end of May. There may be some variation in density estimates as a result of significant

patchiness in the distribution of *N. norvegicus* larvae over the spatial scales sampled (900 – 1200 m) (Pepin and Anderson, 1997), although the data are the average for three separate tows. Stage III larvae were apparent from the beginning of May. The abundance of stage III larvae peaked at the end of May and were absent by the end of June which is consistent with previously observed stage III larvae duration of about three weeks in the western Irish Sea (Dickey-Collas et al., 2000b). The interval between peaks of stage I and stage III larvae is also similar to previous studies (Dickey-Collas et al., 2000a). At the end of June, only a single post-larva was captured from a total of 193 m^{-3} of water sampled. These results show that timing and density of *N. norvegicus* larvae in the western Irish Sea in 2004 was similar to the seasonal pattern observed in previous years (Hillis, 1974; Dickey-Collas et al., 2000a).

The total net production of *N. norvegicus* larvae during the spring and summer of 2004 was estimated to be 2.7 mg C m^{-2}, 1.8 mg C m^{-2} and 0.6 mg C m^{-2} for stages I, II and III respectively, giving a total of 5.1 mg C m^{-2}. The peak biomass of *Nephrops norvegicus* larvae in the water column coincided more closely to peak biomass of small rather than large zooplankton (Fig. 3.10) and this suggests that prey size may be important. The total production of zooplankton over the seasonal period was 1.4 g C m^{-2} so total larval production is equivalent to 0.4 % of the zooplankton production. This suggests that larvae, which the gut content analysis showed feed on copepods and cladocerans, are unlikely to be prey limited at any time during the larval season, although the extent of competition with other species for zooplankton food is unknown. This does assume, however, that the larvae are not highly selective of particular prey species and further gut content analysis is required to confirm this. The $\delta^{15}N$ values for larvae do, however, track those of the bulk small zooplankton samples and so it is suggested that the bulk sample is representative of

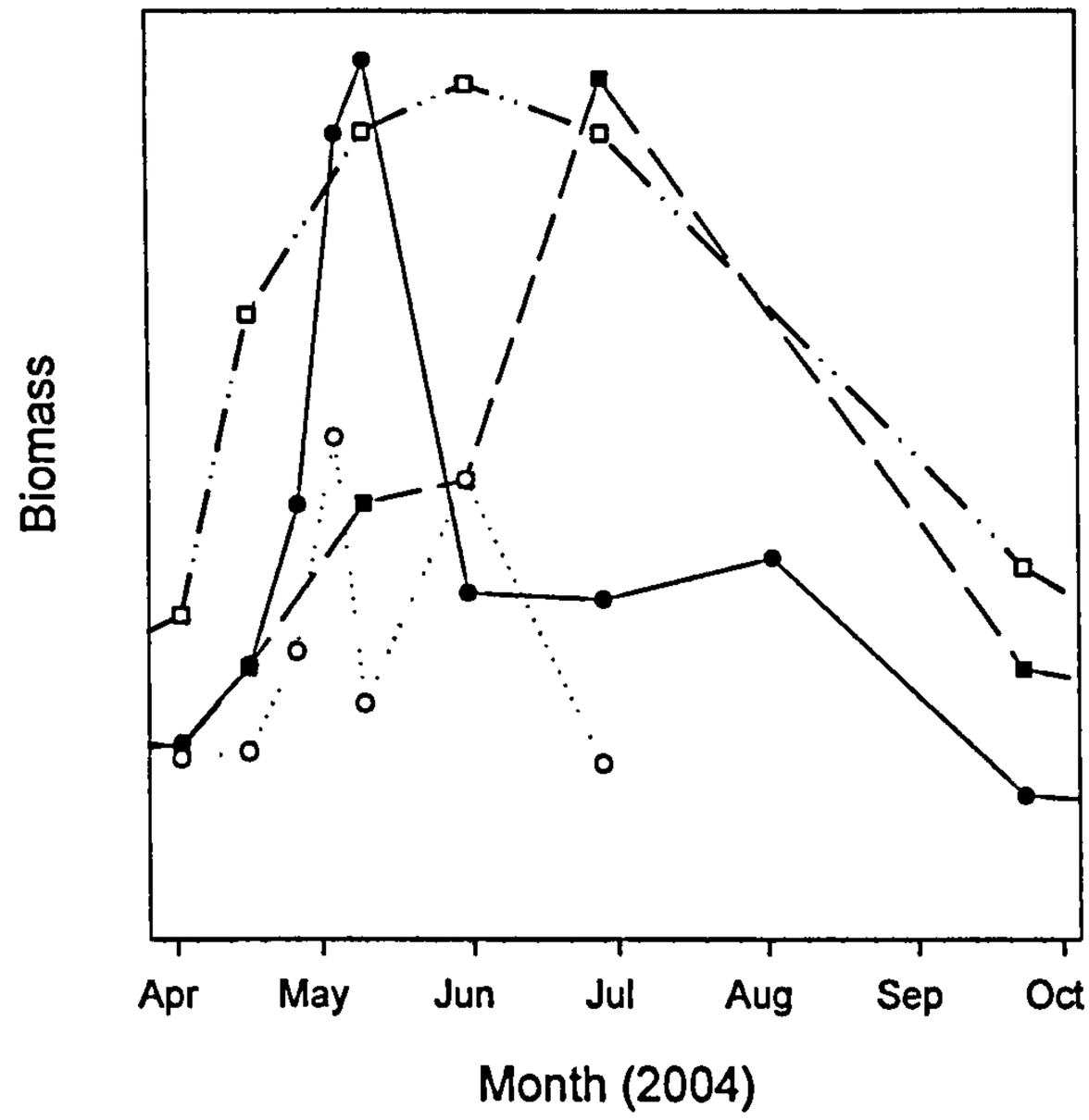


Fig. 3.10 Comparison of seasonal patterns in biomass of (•) chlorophyll, (□) small zooplankton, (■) large zooplankton, and (○) *Nephrops norvegicus* larvae. See Figs. 3.2 and 3.5 for biomass data. The data are not to scale and is presented to show seasonal trends only.

diet suggesting that larvae are not highly selective of species. The larvae may, however, be selective of prey size as might be expected. The abundance of all zooplankton is so high in comparison to the larvae, however, that it does not seem likely that *Nephrops norvegicus* larvae would be particularly vulnerable to any changes in the timing of the seasonal cycles in zooplankton, as observed in the North Sea (Edwards and Richardson, 2004).

Nephrops norvegicus larvae gut contents

Analysis of the gut contents of *N. norvegicus* larvae showed that the larvae are highly carnivorous, feeding almost exclusively on a diet of copepods and cladocerans. It was only possible to identify two genera of copepod, the herbivorous *Acartia* spp. and omnivorous *Centropages hamatus*, because most of the crustacean exoskeletons in the gut were very fragmented making identification difficult. One of the constraints of gut content analysis is that recalcitrant parts, such as crustacean exoskeletons, will remain in the gut for longer than soft bodied prey biasing gut contents in favour of crustaceans. If soft bodied animals present in the western Irish Sea, such as chaetognaths, were also common prey items, some remaining hard parts, such as the 'spine jaws' from which they derive their name, would have been visible in the guts. In addition, diatoms and dinoflagellates, that have readily identifiable parts were found in only a few individual larvae and, in most cases, were very small and probably incidental to ingestion of other dietary items. Nevertheless, some soft bodied organisms such as naked dinoflagellates, microheterotrophs and medusae may be under-represented. A significant proportion of larvae had empty guts (24 %) and, as such, provided no dietary information (Table 3.2). However, because stable isotope analysis measures diet assimilated over a period of time, gut contents at any one time of sampling is unimportant. Therefore, isotope analysis can

be particularly useful in trophic studies for organisms that have high rates of starvation or where there are particular difficulties in undertaking gut content analysis. Gut content analysis confirms the carnivorous feeding nature of *Nephrops norvegicus* larvae, with a preference for crustacean prey, in particular copepods.

Carbon stable isotopes of larvae

The $\delta^{13}C$ of stage I *Nephrops norvegicus* larvae declined rapidly from -18.9 ‰ to -21.3 ‰ in the first two weeks of sampling. The $\delta^{13}C$ value of -18.9 ‰ measured at the beginning of April, is the $\delta^{13}C$ of newly hatched larvae, and to a large extent therefore, reflects the $\delta^{13}C$ of the material derived from maternal investment in the eggs. However, the larvae start feeding immediately after hatching (Farmer, 1975; Rotllant et al., 2001) and as organic matter is assimilated into growing tissues larval $\delta^{13}C$ values start to reflect that of the diet. The $\delta^{13}C$ of the April zooplankton sample, that was dominated by copepods and so representative of diet, was -25 ‰ and assimilation of this low $\delta^{13}C$ diet into larval tissues explains the initial and rapid decline of larval values. The speed at which dietary $\delta^{13}C$ is reflected in an organisms tissues is dependent on how quickly tissues are turned over. Determination of isotopic turnover rates of tissues normally requires lengthy diet switching laboratory experiments and has been carried out in only a few studies. There are also significant difficulties in rearing *Nephrops norvegicus* larvae in the laboratory (Dickey-Collas et al., 2000b; Rotllant et al., 2001). In the current case, however, a diet switch has occurred naturally in the field because the larva only starts to feed once it has hatched, prior to this the isotope value is derived from maternal investment. Thus, tissue turnover time can be estimated from the time taken for the larval isotope value to equilibrium with the diet. The $\delta^{13}C$ and $\delta^{15}N$ values of the larvae initially decline in response to feeding on zooplankton. The $\delta^{13}C$ values reach a minimum on 16 April but the

values are not closely coupled to those of the diet. The $\delta^{15}N$ values reach a minimum after 22 days and track the zooplankton values thereafter (Fig. 3.9) indicating the larvae have come to isotopic equilibrium with the diet and the turnover time is estimated to be 3 weeks. This estimate can be compared with data from tissue turnover studies of similar organisms. For example, tissue turnover studies of juvenile mysids observed rapid assimilation of diet into exoskeleton (2-3 weeks) but not in muscle tissue (6-8 weeks) (Gorokhova and Hansson, 1999). Rapid tissue turnover rates have been observed in other crustaceans. For example, in a feeding experiment, juveniles of the shrimp *Litopanaeus vannamei* attained equilibrium with a new low $\delta^{13}C$ diet after 3 weeks and an increase in weight of 300 % (Parker et al., 1988) and in a diet switching experiment the freshwater water flea, *Daphnia hyaline,* reached isotopic equilibrium within 2 weeks (Grey et al., 2001). Fry & Arnold (1982) found that tissue turnover was related to growth, and was primarily a function of weight gained with tissue isotopes reflecting diet after a four-fold increase in weight. In a laboratory study of *Nephrops norvegicus* larval growth, there were significant increases in absolute and percentage biomass of carbon and nitrogen between moults. In particular, there were significant increases in the percentage biomass of carbon and nitrogen in the first few days after hatching of stage I larvae, from 34 – 38 % and 8 - 9.4 % respectively (Anger and Puschel, 1986). These data, together with estimates of tissue turnover times from other studies, supports the assimilation of dietary isotope values from zooplankton as the explanation for the rapid depletion in $\delta^{13}C$ at the beginning of the larval season.

After the initial decline in the $\delta^{13}C$ value of stage I larvae, there is an increase, of 2 ‰ by the end of May, so that values are similar to those at the beginning of the season. This increase does not appear to reflect changes in the $\delta^{13}C$ of the small size-fraction of

zooplankton during the same period, which continue to decline (because of increasing lipid rich copepod biomass), reaching a minimum of -26.4 ‰ by early May (Fig. 3.9). This difference may reflect changes in biochemistry, particularly of lipids, or feeding selectively on predatory copepods that have low lipid content and therefore higher $\delta^{13}C$ values. *Nephrops norvegicus* eggs are known to have a high lipid content (Narciso, 1998) and it is likely that as lipids remaining in the larval tissues are utilised, the lighter ^{12}C is preferentially excreted and $\delta^{13}C$ rises as a consequence (Fry and Arnold, 1982).

Between stage I and stage II larvae there is a step-wise decline in $\delta^{13}C$, of ~1.7 ‰ on average, but no further change from stage II to III. A very similar change between stage I and II is also seen for $\delta^{15}N$ values and this is strongly suggestive of the removal of maternally derived isotopic signature in the carapace during the first moult. Studies on laboratory reared *Nephrops norvegicus* larvae show that between 9 and 16 % of carbon, and 5.6 to 7.5 % of nitrogen are lost with the moulted exoskeleton (Anger and Puschel, 1986). However, before the exoskeleton is shed there is re-absorption of material into the newly forming layer (Lockwood, 1968) and, at this point, there may be some fractionation of isotopes. In a study of mysids (possum shrimps) Gorokhova and Hasson (1999) found that the $\delta^{13}C$ of exuviae were, on average 1.2 ‰ heavier than the whole body value. Thus, moulting in *N. norvegicus* larvae would leave the remaining tissue lighter and may explain the decline in $\delta^{13}C$ values. In addition, the turnover and assimilation of nitrogen and carbon from the diet directly into new tissue is particularly high during periods of rapid growth just after moulting (Gorokhova and Hansson, 1999), and in some crustaceans new material from the diet is rapidly incorporated into the exoskeleton (Gorokhova and Hansson, 1999; Schmidt et al., 2003). These changes during moulting explain the step-wise shift towards the lower $\delta^{13}C$ dietary signature between stage I and stage II. For

example, a loss of 10 % of carbon during moulting, plus an increase in body weight by 16 % due to rapid growth from assimilation of a zooplankton diet with a $\delta^{13}C$ of -25 ‰, would account for the observed decline in $\delta^{13}C$ from -20.7 ‰ to -22.3 ‰ between stage I to stage II larvae. Thus, the combination of moulting and rapid growth explains the rapid shift in larvae $\delta^{13}C$ values.

Nitrogen stable isotopes of larvae

The initial decline in $\delta^{15}N$ values of stage I larvae, from 10.7 ‰ in early April to 9.3 ‰ on 26 April, is similar to the rapid early changes seen in $\delta^{13}C$ values. Recently hatched larvae start feeding in the water column and begin to reflect the isotopic signature of the diet. The $\delta^{15}N$ of the larvae then reach a minimum and track the zooplankton values closely indicating equilibration of larval isotopes with diet. There was a weak non-significant correlation between *N. norvegicus* larval and zooplankton $\delta^{15}N$ of both size fractions for the period of the investigation. However, when the initial 'maternal' signature of the early larvae on April 2 was excluded the correlation between the larvae and the zooplankton prey was strong and significant (small size fraction: $r^2 = 0.96$, d.f. = 4, $P = 0.003$; large size fraction: $r^2 = 0.85$, d.f. = 4, $P = 0.03$). By the end of May, larval $\delta^{15}N$ was 3.4 ‰ higher than the small, and 2.3 ‰ higher than the large zooplankton fraction. The $\delta^{15}N$ values for stage II and III were lower and this indicates that the small zooplankton fraction, mostly copepods, was the most important diet and is consistent with the gut content analysis.

Conclusion

The strong seasonal nitrogen isotope signal from primary production was clearly traced through two successive trophic levels of secondary pelagic production, from phytoplankton to herbivorous zooplankton and from zooplankton to predatory *Nephrops norvegicus* larvae.

The production of herbivorous zooplankton, arising directly from spring bloom production, was 1.1 mg C m^{-2}, giving an estimated transfer efficiency of 27 % between primary and secondary production in the water column. The biomass of the large zooplankton fraction was 0.3 g C m^{-2} which suggests a transfer efficiency also of 27 % between these two size fractions. However, the large zooplankton was not a discrete trophic level, being made up of a mixture of herbivores, omnivores and predatory organisms.

Analysis of the $\delta^{15}N$ values of zooplankton and *N. norvegicus* larvae and the gut content analysis confirm that *N. norvegicus* larvae are carnivorous, with a preference for crustacean prey, in particular copepods. The larvae feed on the second trophic level in the water column, the same trophic level as the adult (see Chapter Five).

The production of larvae represented only 0.4 % of zooplankton production and shows that larvae are not food limited during their development. Any species preferences are unknown. The larvae do, however, appear to be selective of prey size. The abundance of all zooplankton is so high in comparison to the larvae, however, that it does not seem likely that *Nephrops norvegicus* larvae would be particularly vulnerable to any changes in

the timing of the seasonal cycles in zooplankton, in response to climate change as observed in the North Sea (Edwards and Richardson, 2004).

Table 3.5 Composition of small zooplankton (200 – 500 μm) sample by carbon biomass with calculated trophic level and $\delta^{15}N$. Trophic level key: 1 = autotroph (primary producer); 2 = primary consumer (herbivore); 2.5 = omnivore; 3 = secondary consumer (predator).

Zooplankton description	Trophic position[1]	NITROGEN Biomass per individual (μg N)	CARBON Biomass per individual (μg C)	TOTAL CARBON BIOMASS %								
				23/02/04	02/04/04	16/04/04	10/05/04	31/05/04	28/06/04	23/09/04	15/11/04	21/01/05
Copepods												
Calanus	2	0.30	1.27		5.14	0.37	0.33	2.73	1.07	2 41	15 48	7.37
Temora	2.5	0.39	1.68		2.58	5.75	6.22	2.79	3.71	0.37		
Acartia	2	0.50	2.22		2.48	0.98	2 43	5.52	15.17	5 69	2.35	1.68
Candacia armata	3											
Centropages hamatus	2.5	0.42	2 00			0.20				0.22		
Metridia lucens	2.5	0.72	3 43				0.18					
Oithona	2	0.17	0.96	14.18	14.62	18.31	8.02	14.10	26.34	57.25	28.24	42.64
Pseudocalanus	2	0.60	3.07	4.01	7.28	7.49	7.04	4.19	10 63	5.81	4 87	
Paracalanus	2.5	0.42	2.00	10.10	3.91	3 91	10 96	12.59	10.09	3.34	4.24	
Anomalocera	2											
Microcalanus	2	0.42	2.00		3.07	3.72		3.22	4 00	0.45		10.60
Unidentified	2	0.42	2.00	31.05	8.38	19 56		12.30	17.45	7.80	11.66	2.52
Large gammand	2.5											
Copepodites	2	0.27	1.92	14.72	39.69	33.51	57.01	35.23	7.27	8 13	19 34	8.72
Cladocerans												
Evadne	2	0.31	1.75			0 09	5.11	2.73		0.20		
Podon	2	0.35	2.00				0.21	0.49	0 21			
Other adult organisms												
Sagitta sp. adult	3											
Sagitta sp. juvenile	3	0.75	3.00						0.95			
Tomopteris	3											
Medusa	2.5	1.00	3.57						0.38	2.39		
Larvae												
Echinoderm larvae (Ophioroids)	2	0.10	0.37							1.10		
Copepod nauplii	2	0.16	0.67	6.27	2.90	2.82	1.75	3.01	1.83	1.34	6.39	1.69
Polychaete larvae	3	0.29	1.50	2.24	3.56	1.69	0.39	0.37	0.16	1.00	1.19	12.11
Veliger larvae	2	0.10	0.37	1.37	0.10			0.02	0.08	0.41	0.19	0.64
Urochordate larvae	2	0.10	0.37				0.08					
Crab zoea	2											
Euphasid larvae	2											
Barnacle nauplius	2	0.10	0.37		0.31	0.12	0.02	0.04				
Cnidarian larvae	2	0.10	0.37				0.08			0.57		
Larvacean	2	0.19	0.73			0.21		0.28	0 54	1.14	1.35	0.37
Bryozoan larvae	2	0.10	0.37	1.50	1.43	0.57		0 20		0.08		1.66
Bivalve larvae	2	0.10	0.37		0.36	0.16		0.18	0.12	0.20	1.26	1.84
TOTAL BIOMASS (g C m^{-2})				**0.08**	**0.17**	**0.48**	**0.67**	**0.72**	**0.67**	**0.21**	**0.05**	**0.06**
ESTIMATED TROPHIC LEVEL[3]				**2.09**	**2.08**	**2.08**	**2.12**	**2.09**	**2.09**	**2.05**	**2.04**	**2.13**
ESTIMATED $\delta^{15}N$ [4]				**7.1**	**7.1**	**7.1**	**7.2**	**7.1**	**7.1**	**7.0**	**6.9**	**7.2**

Table 3.5 Composition of large zooplankton (500 – 1000 μm) sample by carbon biomass.

Zooplankton description	Trophic position[1]	NITROGEN Biomass per individual (μg N)	CARBON Biomass per individual (μg C)	TOTAL CARBON BIOMASS %								
				23/02/04	02/04/04	16/04/04	10/05/04	31/05/04	28/06/04	23/09/04	15/11/04	21/01/05
Copepods												
Calanus	2	4.77	25.64	2.01	38.97	26.10	30 08	48.46	41.65	35.26	23.77	2 62
Temora	2.5	1.20	5.20	0.07	7.43	5.10	4.17	1.21	0.03	0 91		0.04
Acartia	2	1.11	4.25	1 68	8.59	2.78	2 27	2.30	0.10	5.89	6.73	1.66
Candacia armata	3	11.18	59.85			2.61	3 20	0.07	0.35	0.40	0.39	0.51
Centropages hamatus	2.5	2.50	10.09		3.07	1.32	1.62	0.26		0 91	0 20	
Metridia lucens	2 5	2.87	13.73		0.63	1.80		0.90		1.75	0 09	
Oithona	2	0.17	0.96	0.30		1.13	0.05		0.00	0.05	0.03	
Pseudocalanus	2	1.19	6.13	5.39	6.80	24.34	18.69	4.68		5 92	2.42	0.89
Paracalanus	2.5	1.19	6.13	0.38		15 91	15.09	0.67			1.90	
Anomalocera	2	15.97	114.97		5.24			0.27				
Unidentified	2	0.82	4.47	4.73	3.94			0.01		0.76	0 68	1.14
Large gammarid	2.5	15.97	114.97							0.39		
Copepodites	2	0.27	1.92		2.77	1.26	3.90	0.01				
Cladocerans												
Evadne	2	0.59	3.07			0.40	2.79	0.01	0 00			
Podon	2	0.96	4.24			0.09	0.23	0.33	0 03	0.03		
Other adult organisms												
Sagitta sp. adult	3	41.84	221.09	30 63		2.17	8.45	33.90	49.46	43.02	61.22	18 81
Sagitta sp. juvenile	3	1.70	7.15			0.31				0.05		
Tomopteris	3.5	89.55	544.48	52.81	16.55	5.34	4.16	1.27	1.32			74.13
Medusa	2.5	5.04	21.41	1.04	1.63	8 22	1.17	2.63	2.22	0 65	2.54	0.18
Larvae												
Echinoderm larvae (Ophioroids)	2	0.16	0.67							0.00		
Copepod nauplii	2	0.16	0.67	0.01		0.42				0.00		
Polychaete larvae	3	0.42	2.01	0.06	0.03	0.26	0.11	0.01	0.16	0.01		
Veliger larvae	2	0.16	0.67	0.05		0 01		0 00				0.01
Urochordate larvae	2	0.16	0.67	0.00								
Crab zoea	3	8.14	40.23	0.56	3 67		0 61	0.99	3 55	3 78		
Euphasid larvae	3	5.17	21.59	0.15	0.66		1.82	1.92	0.71	0.22		
Nephrops larvae	3	8.14	40.23					0.09				
Larvacean	2	1.15	4.20			0.37	1.57		0.42			
TOTAL BIOMASS (g C m^{-2})				**0.05**	**0.02**	**0.11**	**0.28**	**0.30**	**0.73**	**0.11**	**0.05**	**0.04**
ESTIMATED TROPHIC LEVEL[3]				**3.08**	**2.34**	**2.29**	**2.31**	**2.42**	**2.58**	**2.49**	**2.63**	**3.28**
ESTIMATED $\delta^{15}N$ [4]				**10.5**	**8.0**	**7.8**	**7.9**	**8.2**	**8.8**	**8.5**	**8.9**	**11.2**

[1]Trophic level assigned on the basis of diet reported in literature (Jagger et al., 1988; Kleppel, 1993; Nybakken, 1997; Mauchline, 1998; Tonnesson and Tiselius, 2005).

[2]Phytoplankton biomass estimated from carbon biomass values provided by Strathman (1967)

[4]Calculation based on average enrichment in $\delta^{15}N$ of 3.4 ‰ per trophic level (DeNiro and Epstein, 1981)

Chapter Four

Pelagic – benthic coupling: estimating inputs of organic carbon to the benthos in the western Irish Sea

Introduction

In benthic habitats of coastal and shelf seas, where light does not penetrate and primary production cannot occur, the quantity and quality of the supply of organic matter reaching the sediment has been shown to be an important factor influencing benthic community structure, abundance and biomass (Graf et al., 1982; Grebmeier et al., 1989; Josefson and Conley, 1997). In temperate regions, the flux of organic particles to the benthos is often seasonal and linked to the timing of planktonic production in surface waters (McCave, 1975; Davies and Payne, 1984; Tamelaṇder and Heiskanen, 2004). The speed of deposition of pelagic production, as well as the amount, is important because rapidly deposited seasonal production may arrive at the sediment surface in a less decomposed and higher quality state than material sedimented at other times of the year (Smetacek, 1980; Davies and Payne, 1984). Where seasonal production is dominated by diatoms, deposition to the bottom can be rapid and can represent a large proportion of the total input to the benthos (Smetacek et al., 1978; Smetacek, 1980; Parsons et al., 1984; Thornton, 2002). The biogeochemical significance of diatom aggregates as a means of transporting carbon and other nutrients from the euphotic zone to the seabed is well established (Thornton, 2002).

The sediments of the western Irish Sea (WIS) support a substantial fishery for the benthic lobster *Nephrops norvegicus.* The supply of organic matter to these sediments is assumed to come from seasonal production in the overlying water column. The waters in this area are too deep for benthic primary production and advection of matter from other areas

appears limited (as discussed in Chapter Two). In the spring and summer the deep water of the western Irish Sea becomes thermally stratified, and is characterised by a spring bloom of phytoplankton production (Gowen et al., 1995; Gowen and Bloomfield, 1996). Density gradients associated with stratification create a cyclonic gyre of near-surface water which is thought to retain planktonic organisms (White et al., 1988) and may also limit the import and export of organic material between the gyre and surrounding mixed water. The weak tidal flows in the gyre also result in a highly depositional environment which creates the muddy sediments *N. norvegicus* needs to build its burrows.

The seasonal deposition of pelagic production on the benthos in the western Irish Sea was observed by Trimmer et al. (1999), with an increase in sediment phytodetritus and a pulsed increase in benthic oxygen consumption soon after the peak of the spring bloom. It is this seasonal input of organic matter that is believed to support secondary production in the benthos, and hence the fishery. The authors estimated, however, that the organic matter deposited on the benthos was remineralised very quickly and that in some years little carbon would be available for net secondary production. Thus, further work to estimate the flux of organic matter to the benthos, that ultimately supports the *Nephrops norvegicus* fishery in the western Irish Sea, is needed.

The stable isotopes of carbon and nitrogen have been used to determine the source and fate of organic matter in biological systems (Peterson, 1999; Post, 2002). In marine systems, the isotopes of carbon have proved to be useful in describing near-shore food webs where organic matter originates from a mixture of isotopically distinct sources such as terrestrial, estuarine or marine plant material (e.g. see Fry and Parker, 1979; Dittel et al., 2000; de la Moriniere et al., 2003). Nitrogen isotopes are usually used to determine trophic position and relationships but have also proved useful in detecting anthropogenic input of organic

matter, such as sewage and land run-off (e.g. Spies et al., 1989; Tucker et al., 1999). In addition, carbon and nitrogen isotope values often vary seasonally, especially during periods of high productivity (e.g. Montoya et al., 1991; Rolff, 2000). In recent years, the isotopic composition of marine suspended particulate organic nitrogen (PON) has been used to study seasonal dynamics and as an indicator of the sources and sinks of organic matter in planktonic systems (e.g. see Voss et al., 1997). In the western Irish Sea the $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter in the productive zone of the water column have been shown to change significantly during the spring bloom (Chapter Two) and this temporal variation can be used to track the flux of production in the pelagic zone to the benthos.

In this chapter the stable isotope values of particulate organic matter, produced during the spring bloom, are used to describe and estimate the vertical flux of organic matter from the euphotic zone to seabed in the western Irish Sea and together with estimates of production used to quantify the input of organic matter to the benthos. The highly retentive nature of the western Irish Sea during the stratified spring and summer, and high levels of secondary production, makes it an ideal region for investigating carbon input through pelagic-benthic coupling.

The specific aims of the work presented in this chapter were to: (i) use the stable isotope values of pelagic production during the spring bloom (as discussed in Chapter Two) to investigate the vertical flux of organic matter; (ii) track the input of spring bloom organic material to the benthos using surficial sediment chlorophyll, organic carbon and total nitrogen content; and (iii) estimate the input of pelagic carbon to the benthos during the 2004 spring bloom in the western Irish Sea.

Methods

Sample site. Sampling took place at the AFBI permanent mooring station, S38A, on ten occasions between February 2004 and January 2005 on board the RV 'Lough Foyle'. Full details of the sampling site can be found in Chapter Two.

Water column characteristics

The methods for sampling of water column characteristics (salinity, temperature), chlorophyll, C and N content and isotope sample preparation are as described in full in Chapter 2. On each occasion a sample of bottom water, depth 85 m, was taken.

Sediment characteristics

Sampling: Sediment was sampled with a multicorer consisting of 4 tubes (6 cm diameter, 30 cm length). The multicorer was generally deployed twice (depending on sea-state), and four to five replicate samples that did not have burrow holes, were selected on each occasion. The samples had lots of flocculent material and undisturbed sediment surface indicating limited scouring of surface sediments as the sampler arrived at the seabed. Intact sediment cores in their tubes were placed in a fridge (4 ^{o}C) and left to settle overnight. The surface water was siphoned off, the sediment slowly extruded and the top 1 cm was carefully cut from the top of the core in 4 x 2.5 mm sections. Samples were stored at -20 ^{o}C for later analysis.

Chlorophyll: The chlorophyll concentration in a measured volume of sediment (1-2 cm^3) was determined by acetone extraction after the method of Tett (1987) and as described in full for water samples in Chapter 2.

Sample preparation for isotope analysis: Approximately 2 cm^3 of sediment from each sample was placed in an acid-washed glass scintillation vial (20 ml) and oven dried to a constant weight (24 h at 60 °C). Samples were then homogenised in an acid-washed pestle and mortar. For the determination of organic carbon content and carbon stable isotope value half the sample was subjected to aqueous acidification with concentrated HCl (12 M) to remove inorganic carbonates and re-dried prior to analysis (Hedges and Stern, 1984) (Boutton, 1991). The remaining half of the sample was untreated for measurement of total nitrogen content and nitrogen stable isotope value. Sub-samples (~12 mg) of each treatment, acidified and non-acidified, were weighed into a tin cap for elemental and isotope analysis.

Carbon and nitrogen stable isotope value analysis

Full details of the method are described in full in Chapter Two.

Data analysis and statistics

Sediment values are mean ± 1 standard error. However, water column data are single values per depth as it was not feasible to take replicate samples at each depth. Analysis of variance (ANOVA) was used to test for differences between mean values. The strength of association between variables was determined by regression analysis: from the coefficient of determination (r^2) and analysis of variance to determine the significance of the regression line. In the regression analysis of water column characteristics (Y) versus depth (X) there was an increase in the standard deviation of Y with decreasing X and so all Y variables were $\log_{10}$ transformed (see Fig. 4.4) (Zar, 1999).

Results

Euphotic zone

The spring bloom in the western Irish Sea in 2004 was between 2 April and 31 May, and gross spring bloom production was estimated to be 24.8 g C m^{-2}. The peak of the spring bloom was on 10 May when there was a maximum euphotic zone chlorophyll concentration of 4.6 mg Chl m^{-3} (Fig. 4.1). Full details of the production season are found in Chapter Two.

Bottom water

There was an increase in the concentration of chlorophyll in the bottom water from mid May to the end of June, with a peak of 0.9 mg Chl m^{-3} on 31 May (Fig. 4.1). Outside this period the background concentration was $\leq$ 0.3 mg Chl m^{-3}. There was also an increase in particulate organic carbon (POC) and nitrogen (PON) from late April to the end of May with maximum concentrations (carbon: ~200 g C m^{-2}, nitrogen: 23 mg N m^{-2}) in May (Fig. 4.2, Table 4.4). The C:N ratio of bottom water particulate organic matter was variable, ranging from 6.6 to 10.2 with no seasonal pattern (see Table 4.4, bottom water = deepest depth sampled on each occasion: ~85 m).

The $\delta^{13}C$ values of POC in the bottom water increased during the spring and summer, with a maximum of -23.3 ‰ at the beginning of August (Fig. 4.3). The $\delta^{15}N$ values of PON were variable, ranging between 6.9 ‰ and 8.2 ‰, except for a rapid decline to 5.8 ‰ and 6.0 ‰ in mid April and early May (Fig. 4.3). There was no relationship between $\delta^{13}C$ and POC or between $\delta^{15}N$ and PON in the bottom water.

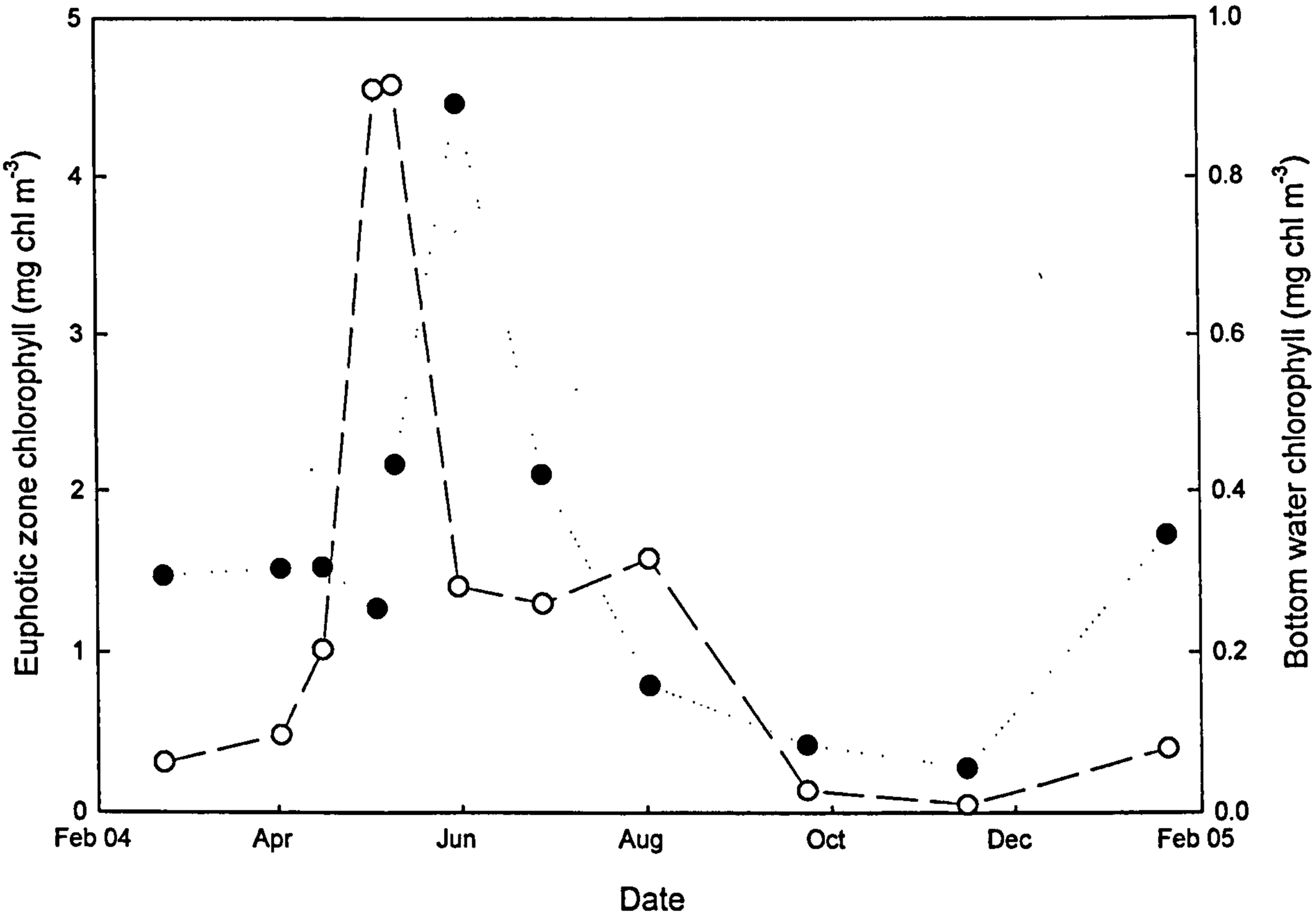


Fig. 4.1 Chlorophyll concentration in bottom water (~85 m) (closed circles) and euphotic zone (0-23 m) (open circles) at Station S38A in the western Irish Sea from February 2004 to January 2005.

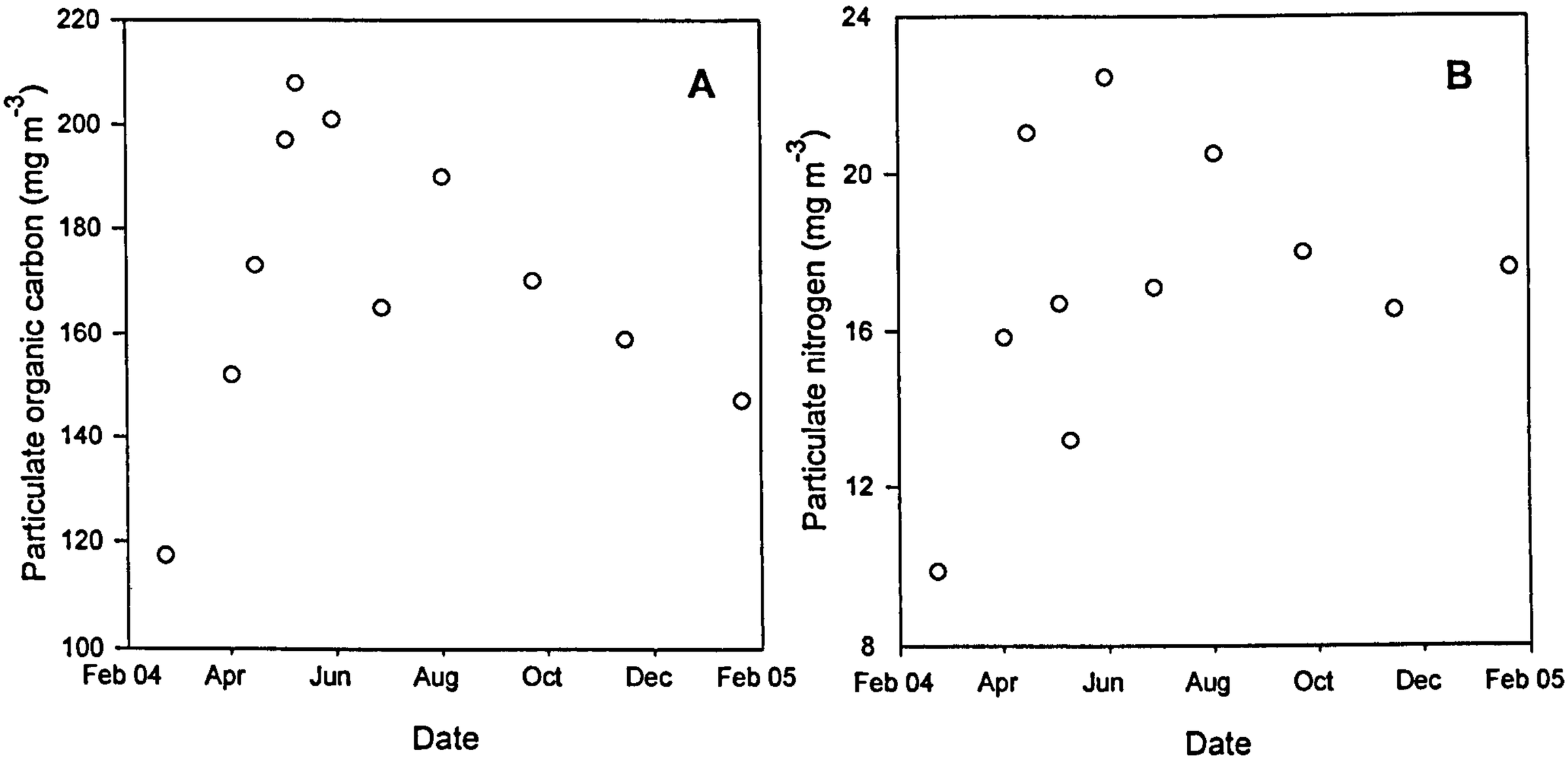


Fig. 4.2 Concentration of **A**: particticulate organic carbon and **B**: particulate organic nitrogen in bottom water (~85 m) at S38A in the western Irish Sea from February 2004 to January 2005.

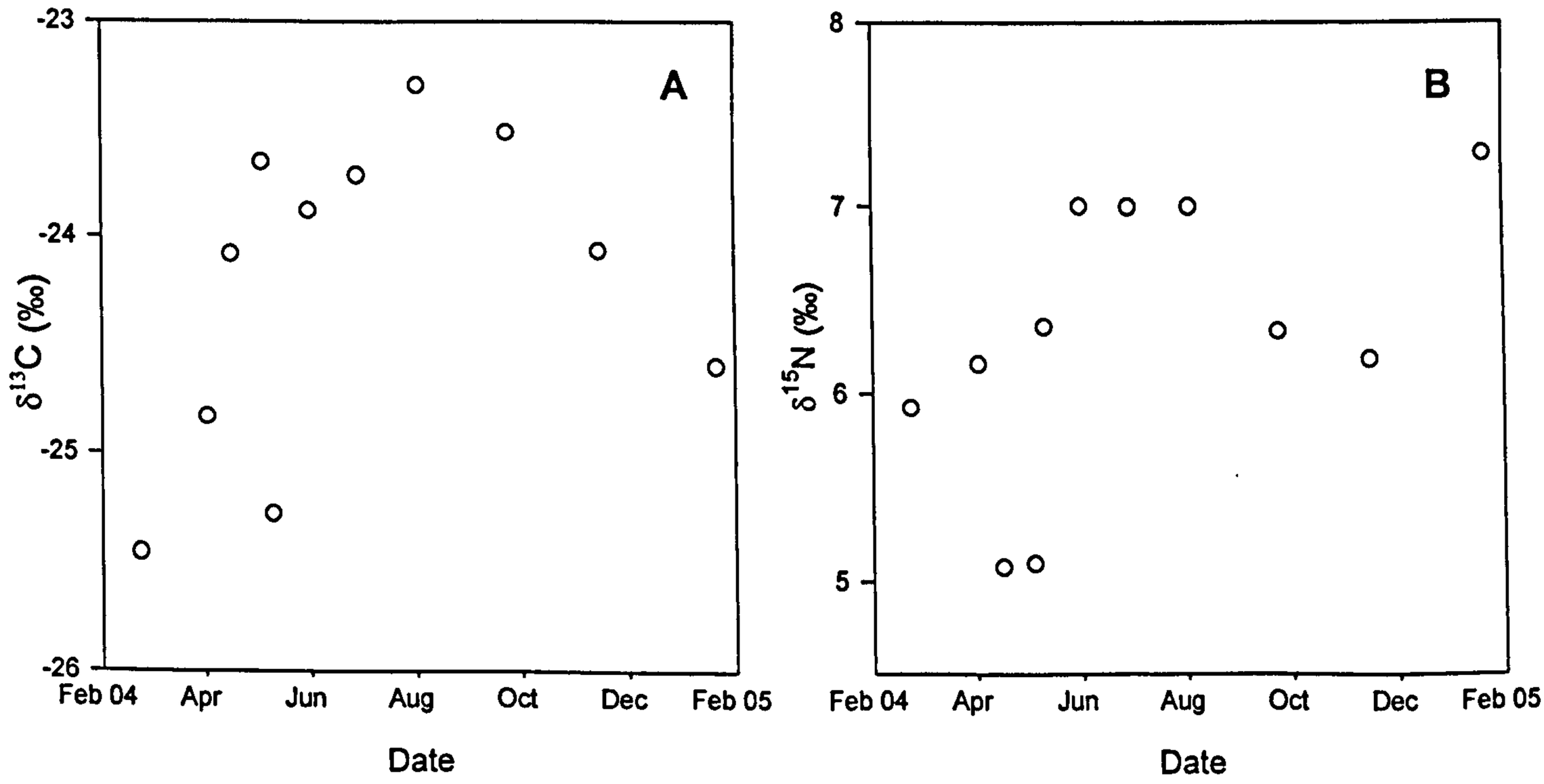


Fig. 4.3 Stable isotope values of **A**: carbon ($\delta^{13}C$) and **B**: nitrogen ($\delta^{15}N$) of particulate organic matter in bottom water (~85 m) at S38A in the western Irish Sea from February 2004 to January 2005.

Changes in particulate organic matter with depth

There were changes in particulate organic matter with depth (Fig. 4.4), with a decline in the carbon and nitrogen content (as a percentage of particulate organic matter). Further analysis of the data, however, revealed that the significant, although moderate ($r^2 \leq 0.4$), relationships only applied to data from 16 April to 23 September (Table 4.1), which corresponds closely to the timing of the production season (see Chapter Two). The carbon content declined from an average of 2.7 % in the euphotic zone to 1.6 % in the bottom water and the nitrogen from 0.38 % to 0.18 %. Outside of the production season, when the water column was mixed, there were no changes in these variables with depth.

There was no change in the C:N and C:Chl ratios or in the $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter with depth at any time of the year (Fig. 4.4, Table 4.1).

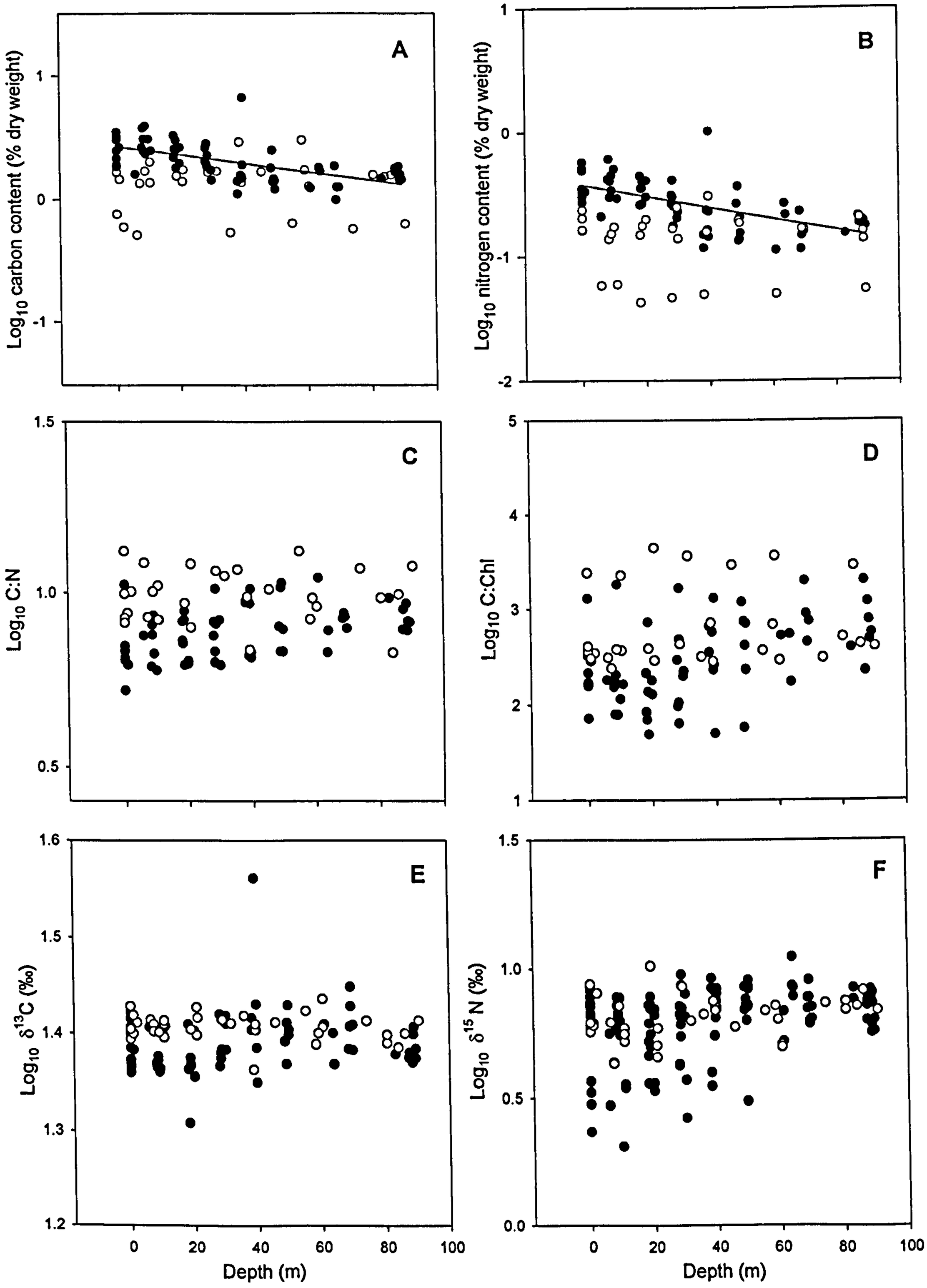


Fig. 4.4 Characteristics of particulate organic matter with depth: **A**: percentage carbon; **B**: percentage nitrogen; **C**: C:N ratio; **D**: carbon to chlorophyll (C:Chl) ratio; **E**: $\delta^{13}C$; and **F**: $\delta^{15}N$ at S38A in the western Irish Sea from February 2004 to January 2005. All y-axis variables to $\log_{10}$. Regression lines added where signficant. Production season (black circles), non-production season (open circles).

Table 4.1 Parameters and test statistics for linear relationships between water column particulate organic matter characteristics (log_{10} transformed) and depth (m) from station S38A in the western Irish Sea. The production season is 23 April to 22 September.

Characteristic of particulate organic matter	Dates	n	r^2	$F_{0.05, n}$	P
Carbon content (% dry weight)	Production season	58	0.37	33.6	<0.0001
Nitrogen content (% dry weight)	Production season	58	0.40	37.4	<0.0001
C:N ratio	Production season	58	0.24	17.9	<0.0001
C:Chl ratio	Production season	57	0.17	11.5	0.0013
$\delta^{15}N$ (‰)	Production season	152	0.08	8.9	0.003
$\delta^{13}C$ (‰)	Production season	100	0.09	6.3	0.01

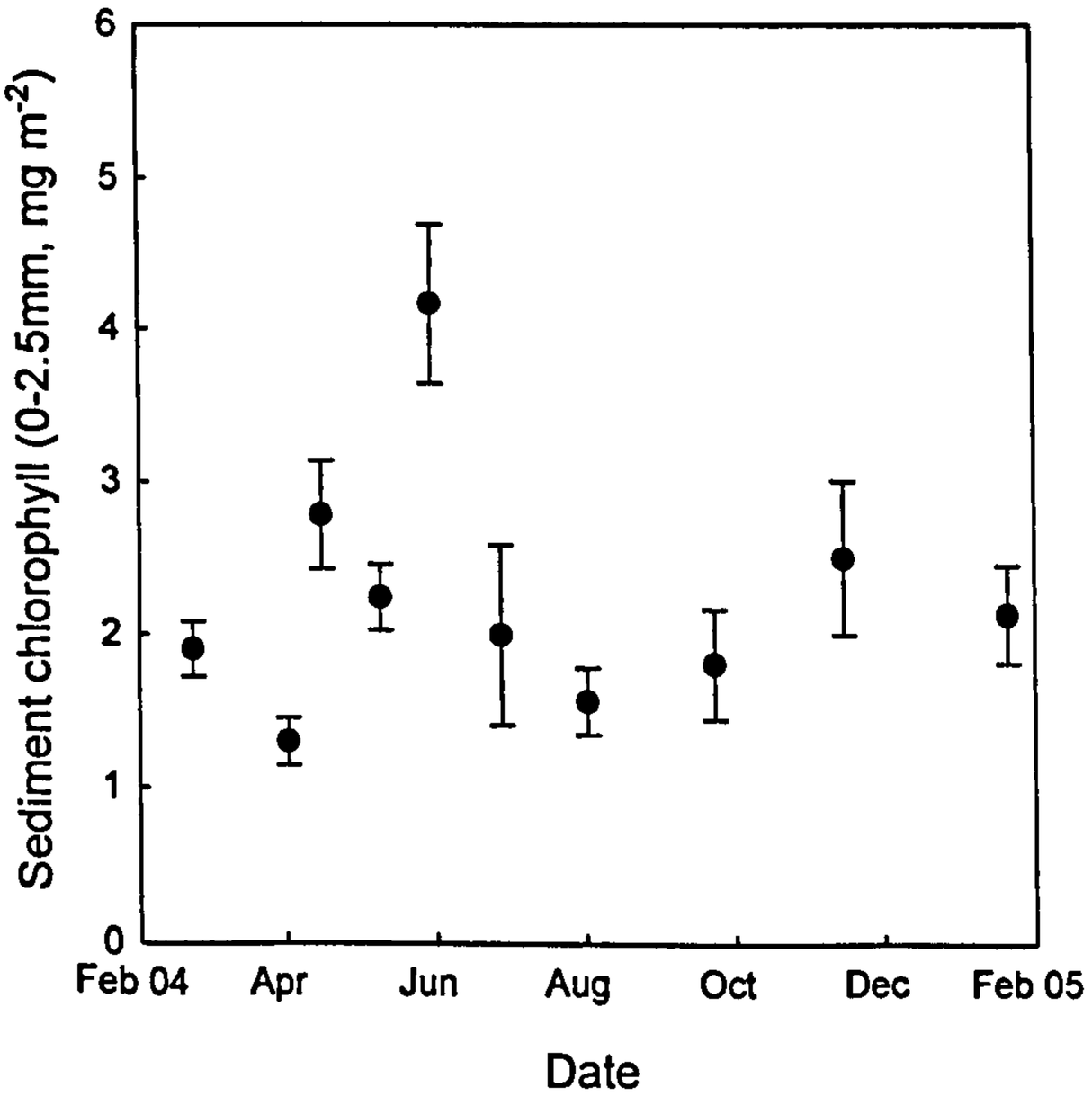


Fig. 4.5 Mean (± SE) chlorophyll concentration in top 2.5 mm of sediment at S38A in the western Irish Sea from February 2004 to January 2005.

Sediment

There was an increase in the concentration of chlorophyll in the surface (2.5 mm) sediment, from ~2 mg m^{-2}, to a peak of 4.2 mg m^{-2} at the end of May (Fig. 4.5), which coincided with the maximum concentration of chlorophyll in the bottom water (Fig. 4.1).

There was temporal variation, but no clear seasonal trend, in the organic carbon (average 10.6 mg C g^{-1}), nitrogen content (average 2.0 mg N g^{-1}) (Fig. 4.6) or the C:N ratio of organic matter in the sediment (Fig. 4.7). There were also no obvious seasonal changes in the $\delta^{13}C$ and $\delta^{15}N$ of organic matter in the sediment. The $\delta^{13}C$ ranged from -23.5 ‰ to -21.8 ‰ and $\delta^{15}N$ values from 6.4 ‰ to 7.3 ‰ (Fig. 4.8).

Comparison of bottom water and sediment isotope values

There was no apparent coupling of the $\delta^{13}C$ and $\delta^{15}N$ values of organic matter in the bottom water and the sediment (Fig. 4.9). There was however, significant enrichment in $\delta^{13}C$ and $\delta^{15}N$ from the bottom water to the sediment ($t_{0.05,12} = 8.0$, $P < 0.001$) (Fig. 4.10). The average isotope value for carbon in bottom water was -24.5 ‰ (range: -25.9 ‰ to -23.5 ‰) compared with -22.7 ‰ (range: -23.5 ‰ to -21.8 ‰) in the sediment. For particulate nitrogen $\delta^{15}N$ values were 6.2 ‰ (5.0 - 7.3 ‰) in the bottom water compared with 6.8 ‰ (6.4 to 7.3 ‰) in the sediment.

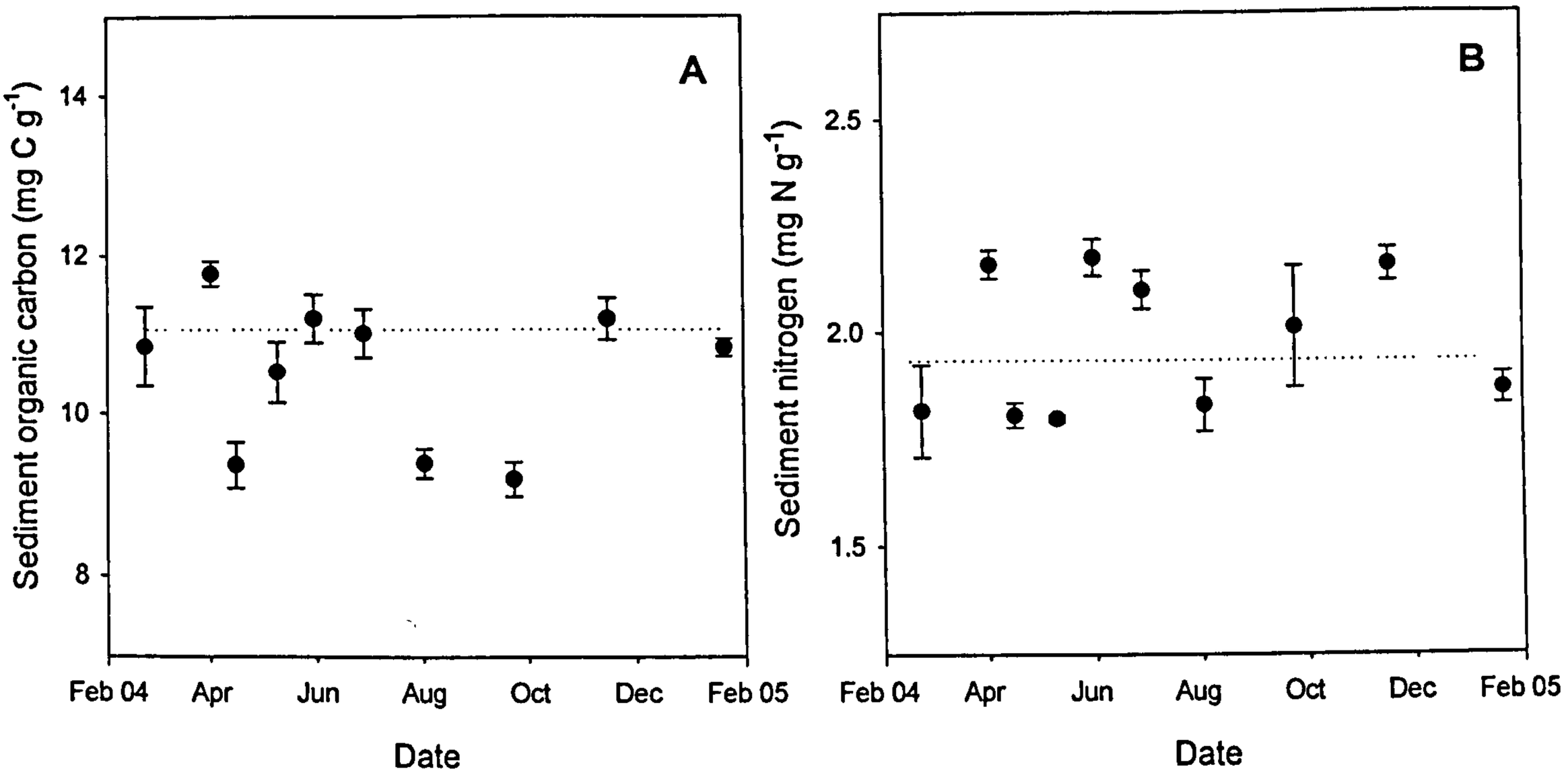


Fig. 4.6 Concentration (w/w) of **A**: organic carbon and **B**: nitrogen in surface sediment (top 2.5 mm) at S38A in the western Irish Sea from February 2004 to January 2005. Dotted line shows average value for sampling period.

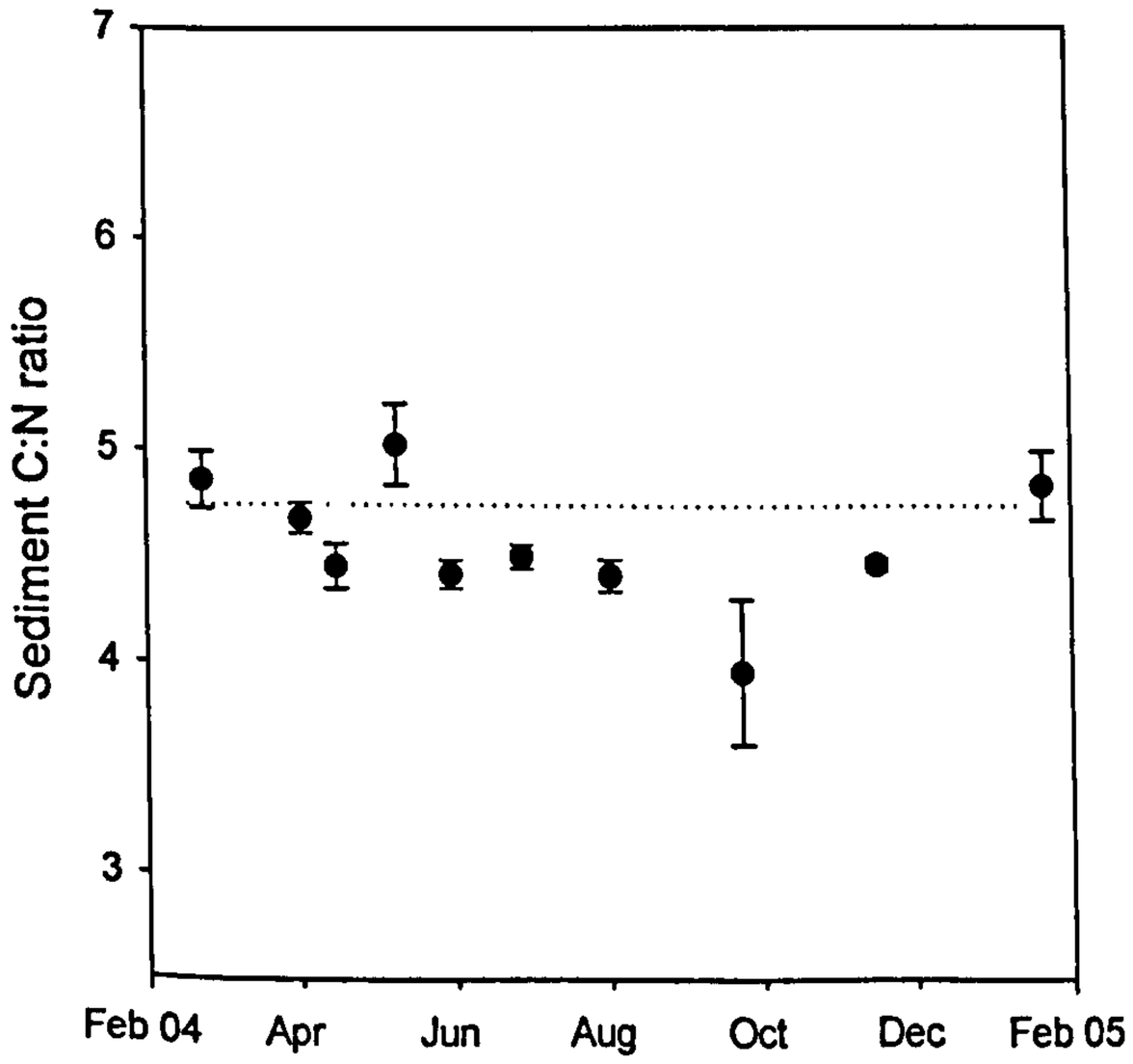


Fig. 4.7 Carbon to nitrogen (C:N) ratio of organic matter in surface (top 2.5 mm) sediment at S38A in the western Irish Sea from February 2004 to January 2005. Dotted line shows average value for sampling period.

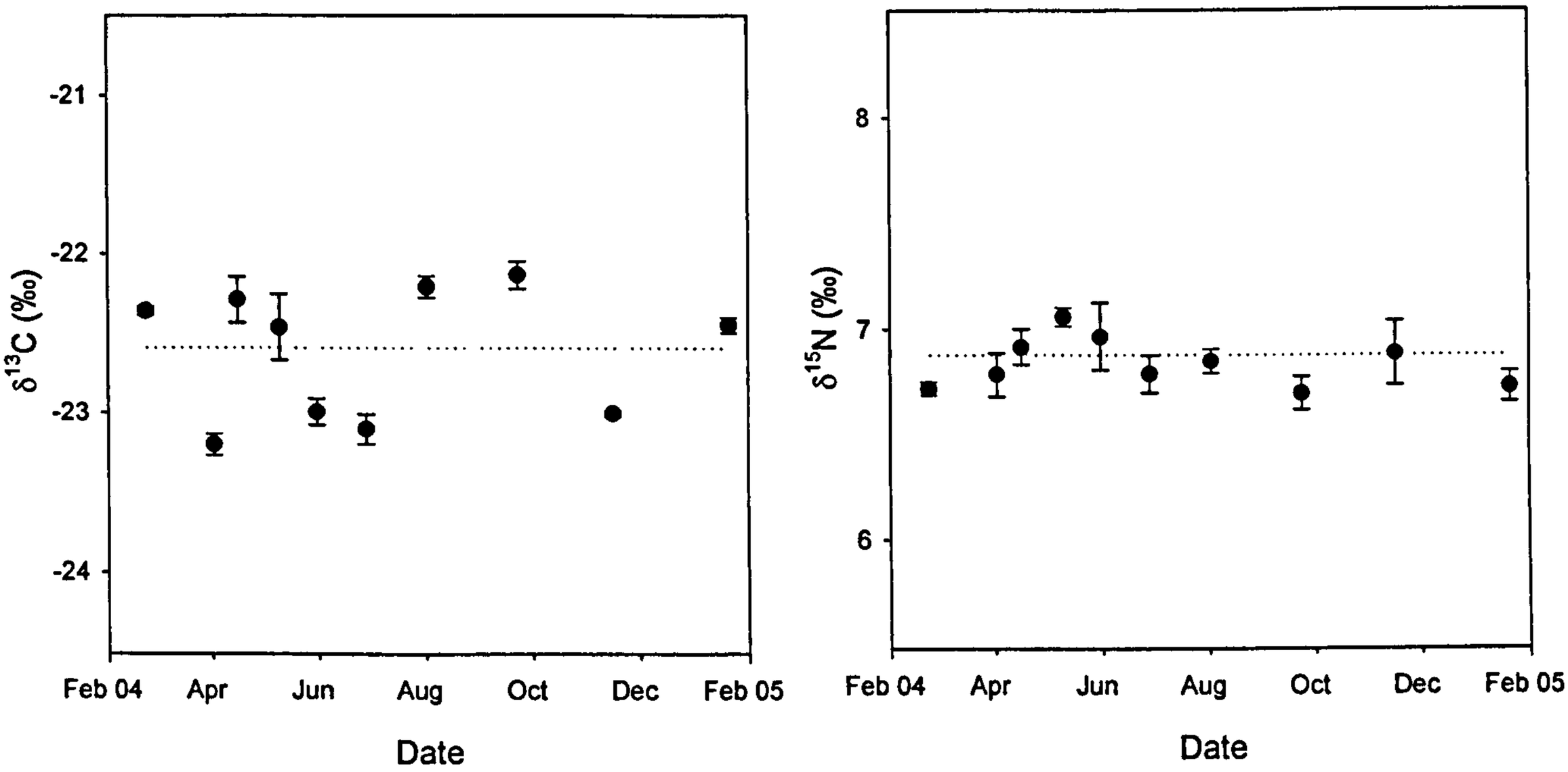


Fig. 4.8 Carbon and nitrogen stable isotope values (mean ± SE) of organic matter in surface (top 2.5 mm) sediment at S38A in the western Irish Sea from February 2004 to January 2005. Dotted lines show average for sampling period.

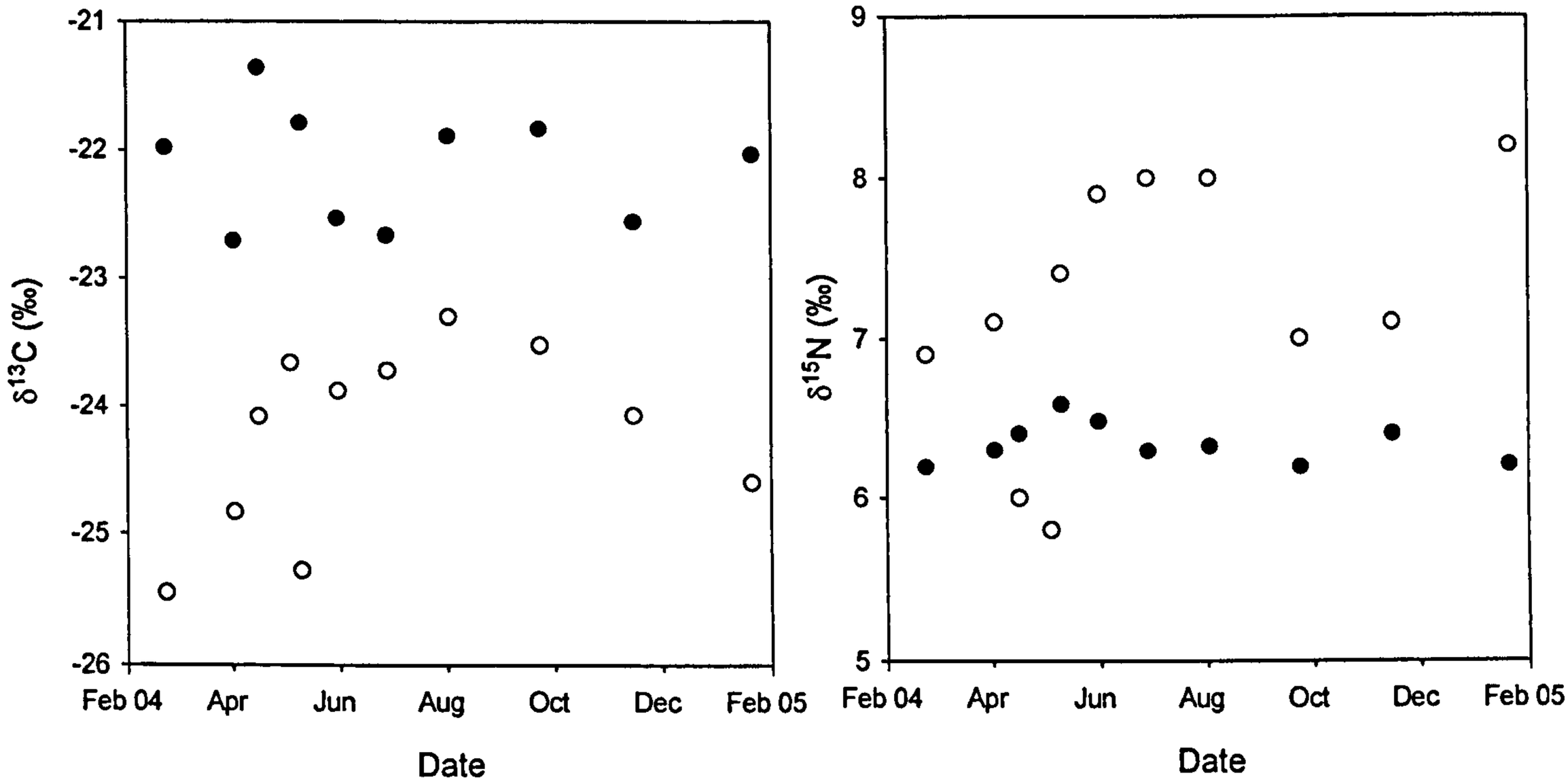


Fig. 4.9 Carbon and nitrogen stable isotope values of organic matter in sediment (closed circles) and bottom water (open circles) at S38A in the western Irish Sea from February 2004 to January 2005.

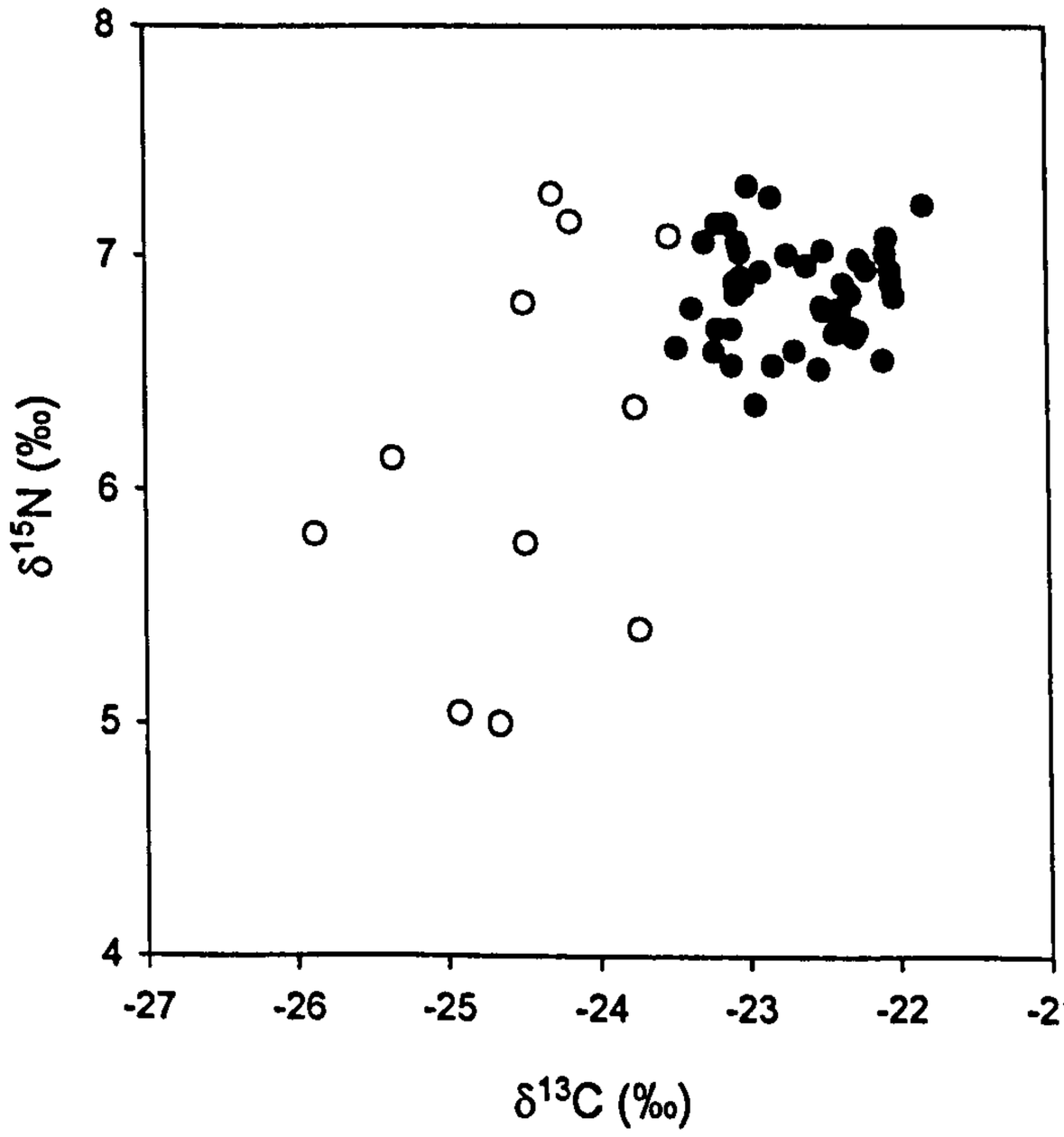


Fig. 4.10 Carbon and nitrogen stable isotope plot for particulate organic matter in bottom water (open circles) and organic matter in surface sediment (closed circles) at S38A in the western Irish Sea. All data for February 2004 to January 2005.

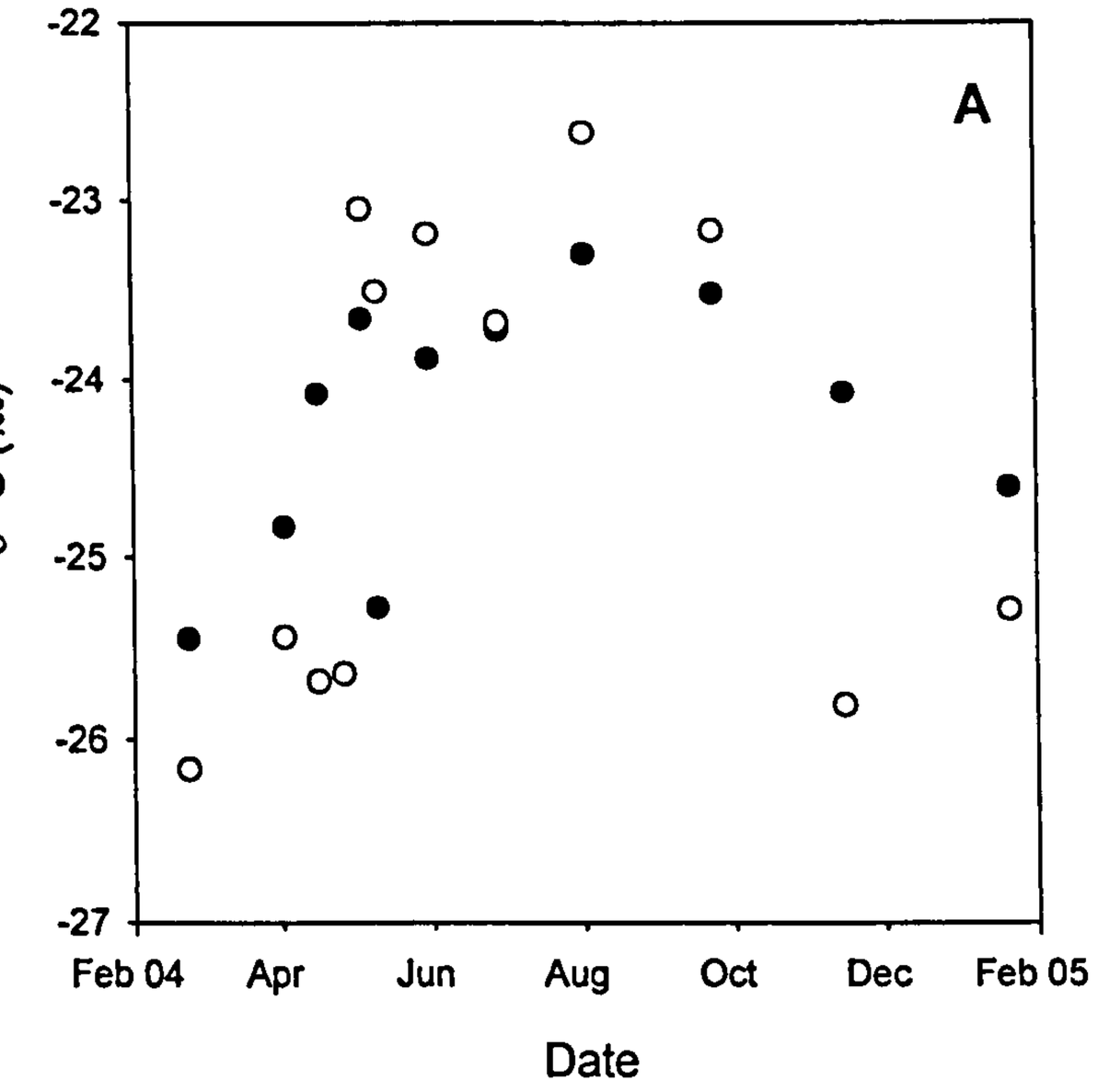
Discussion

Vertical flux of euphotic zone production

The vertical flux of pelagic production from the euphotic zone to the benthos is seen in the close coupling of chlorophyll and stable isotope values of particulate organic matter between the euphotic zone and the bottom water (~85 m) (Figs. 4.11).

Chlorophyll

The increase in the chlorophyll concentration in the bottom water (from ~0.3 to a peak of 0.9 mg Chl m^{-3}) measured at the end of May closely mirrored the seasonal pattern of chlorophyll in the euphotic zone. The input of pelagic production was also observed in the large increase in chlorophyll in the sediment at the same time showing that the flux of material to the benthos in the western Irish Sea is linked to production in the overlying waters. The seasonal cycle of chlorophyll in the surface and the bottom water are separated by a time lag that can provide an estimate of the sinking rate of organic matter. For example, the large increase in euphotic chlorophyll on 4 May is first detected in the bottom water on 10 May, indicating a minimum transfer time of 6 days. The peak chlorophyll concentrations in the euphotic zone and bottom water, however, were on the 10 and 31 May, respectively, suggesting a transfer time of 21 days. Thus, the transfer of organic matter to the benthos is estimated to be between these values, giving an average sinking time of 13 days. The speed of the flux of pelagic organic biomass, from the surface to the bottom water, is thus estimated by dividing the distance travelled (from middle of euphotic zone: 11.5 m to 85 m bottom water = 74 m) by the sinking time (13 days) to give a sinking rate of 5.7 m d^{-1}.



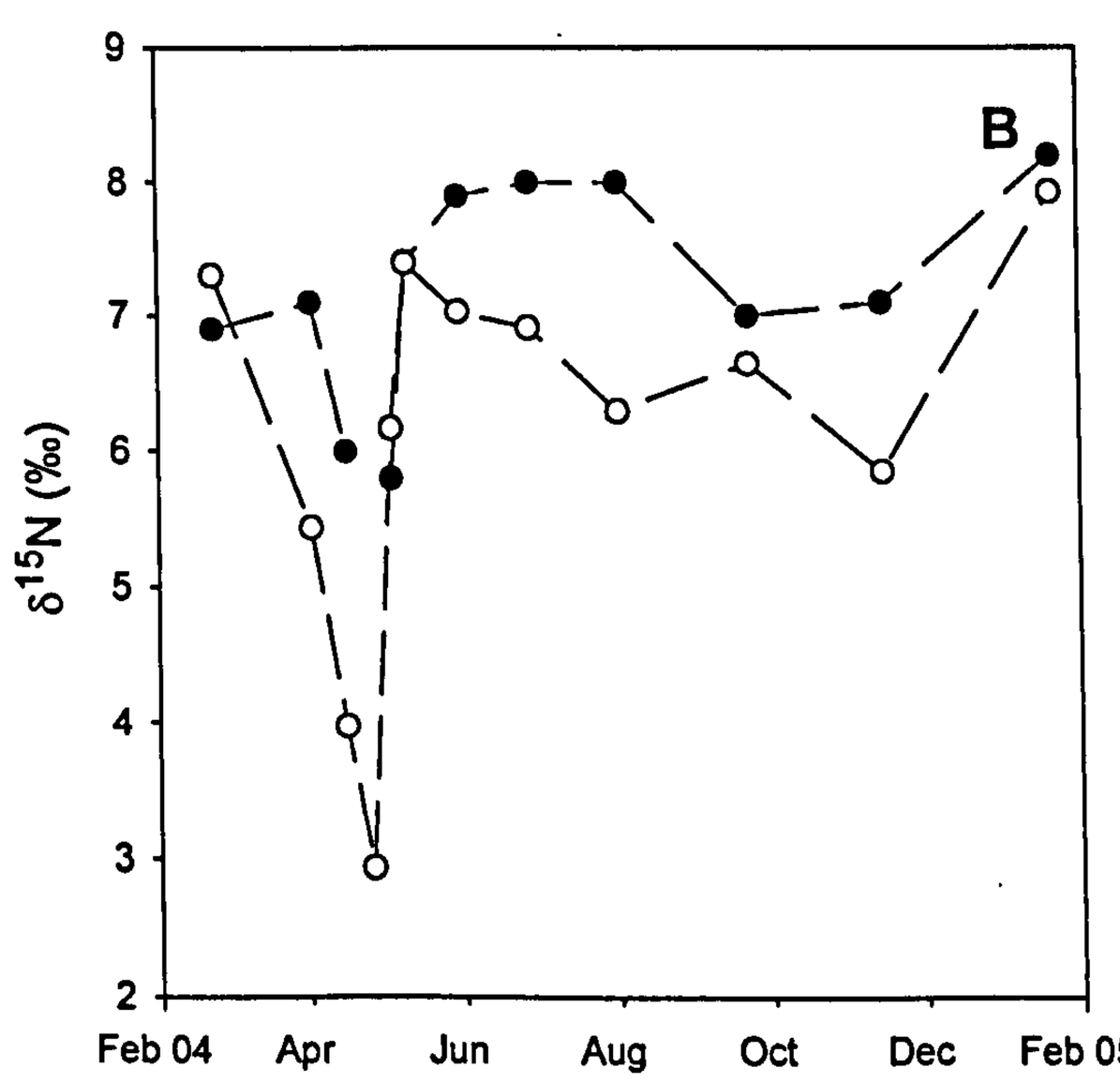


Fig. 4.11 Comparison of **A**: carbon and **B**: nitrogen stable isotope values of particulate organic matter in the euphotic zone (open circles) and bottom water (closed circles) at S38A in the western Irish Sea from February 2004 to January 2005.

Carbon and nitrogen content

The carbon and nitrogen content of the POM in the bottom water increased over the spring bloom, although the pattern was not as distinct as it was for chlorophyll. This is probably because of the larger background pool of particulate organic matter present in the bottom water and the much larger relative increase in chlorophyll concentration.

Stable isotopes

There were distinct seasonal changes in the $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter in the euphotic zone during the production season (as discussed in Chapter Two) that were clearly observed at 85 m (Fig. 4.11). The biggest changes in the isotope values in the bottom water were due to changes in the seasonal production signal, and not changes with depth, indicating close coupling of surface water production to the benthos. Thus, the isotope values can be used to track the movement and processing of organic matter through the water column. For example, in the euphotic zone there was a distinct increase in the $\delta^{13}C$ values of POM in the period April – September, and $\delta^{15}N$ values undergo rapid depletion and subsequent enrichment, in April and May (full details in Chapter Two and see Table 4.4). These changes in the $\delta^{13}C$ and $\delta^{15}N$ values of POM in surface waters, particularly in the early stages of the spring bloom, are rapidly mirrored in particulate material at 85 m. The initial decline in euphotic zone $\delta^{15}N$ seen on 2 April is observed in the bottom water on 16 April, suggesting POM produced in the euphotic zone reached the benthos at least 14 days later, giving an estimated transfer time of 14 days. The subsequent increase in $\delta^{15}N$ values of euphotic zone POM from 26 April to 4 May, was observed in the bottom water on 10 May, suggesting a downward flux of organic matter, from the euphotic zone to bottom water, taking between 6 and 14 days. Taking an average of the above estimates (12 days) gives a sinking rate of 6.2 m day^{-1}, similar to the value estimated

from the flux of chlorophyll. The $\delta^{13}C$ values of POM from the euphotic zone and bottom water are also closely coupled, with the characteristic productive season enrichment observed in both surface and bottom water. Thus, the flux of material from surface waters to bottom water during the bloom, as indicated by the transfer of chlorophyll and stable isotope values of particulate organic matter, is rapid with an estimated sinking time of 12 days and an average sinking rate of 6.0 m d^{-1}.

As a comparison, in a study of sinking rates of phytoplankton using a technique based upon measurement of the transit time of radioactively (^{14}C) labelled cells, Bienfang (1981) found the sinking rate of a diatom dominated (76 % of biomass) phytoplankton bloom assemblage to be much lower, at 1.69 ± 0.38 m day^{-1}. However, the diatoms in that study were generally smaller (diameter ≈30 μm) and the biomass of cells (as indicated by chlorophyll) was lower than that measured here. Thus, the difference in the estimated sinking rates between these studies may be due to the nature of the sedimenting particles. Diatoms, in particular, often aggregate into larger particles with higher sinking rates so that the flux of organic material during a spring bloom may be more rapid (Smetacek, 1985; Thornton, 2002), and a distinct pulse of phytoplankton sedimentation is often evident (Smetacek et al., 1978). Studies in the Kiel Bight (Smetacek et al., 1978) and the Baltic Sea (Bianchi et al., 2002; Tamelander and Heiskanen, 2004) for example, have shown that diatom dominated spring bloom matter can be rapidly transferred to the benthos, although these areas were shallower (20 – 40 m) than the western Irish Sea study site.

The peak of the 2004 spring bloom in the western Irish Sea was, however, dominated by diatoms (see Chapter Two) and there was a distinct pulse of chlorophyll detected in the sediments, suggestive of the rapid deposition of surface material to the benthos. The clear

transfer of isotope values of particulate organic matter from the euphotic zone to bottom water also indicates that there was rapid deposition of bloom production to the benthos. The biogeochemical significance of diatom aggregates as a means of transporting carbon and other nutrients from the euphotic zone to the seabed is well established (Thornton, 2002) and the rapid flux in the Irish Sea indicates the deposition of a potentially high quality food supply for benthic organisms.

Changes in particulate organic matter with depth

The carbon and nitrogen content of the particulate organic matter declined with depth only during the production season, which indicates the mineralisation of seasonal production as it moves towards the benthos. Outside of the production season the carbon and nitrogen content was the same throughout the whole depth of the water column, indicative of a well mixed water body. There was no change in the C:Chl and C:N ratios with depth during this time because production was low and most of the organic matter was likely to be refractory in nature.

In the production season the carbon and nitrogen content of particulate organic matter declined by about 40 % from the surface to the bottom water, so that only 60 % of the organic matter that sinks reaches the bottom water and hence the sediment. This loss of organic matter with depth is well established and is due to bacterial mineralisation as material moves through the water column and may also be due to leaching of soluble compounds (Karl et al., 1988). There was, however, no change in the C:N and C:Chl ratios of the particulate organic matter as it sank suggesting that there was none of the preferential consumption of nitrogen-rich components often seen in marine systems (Heip et al., 1995) indicating that there may have been limited mineralisation during vertical flux

because of the speed of deposition. This also suggests that the organic matter reaching the benthos would have bee readily available to benthic organisms.

The $\delta^{13}C$ and $\delta^{15}N$ values of organic matter do not change with depth at any time during the year. They do however, change in response to the seasonal changes in the benthos because of the close coupling of euphotic zone production and input to the benthos. Thus, the $\delta^{13}C$ and $\delta^{15}N$ values provide a good source signal indicating that the input of organic matter to the benthos is from production in the overlying water and there is no indication of another source of carbon to the benthos.

Sediment response to vertical flux

Although there was a doubling of chlorophyll (from 2.0 to 4.2 mg Chl m^{-2}) in the surface sediment (0-2.5 mm) at the end of May, the carbon and nitrogen content and the $\delta^{13}C$ and $\delta^{15}N$ values of sediment organic matter did not change in response to the flux of seasonal production to the bottom water. The average carbon content of surface sediment was 10.6 mg C g^{-1} and nitrogen content was 1.97 mg N g^{-1}. The absence of a detectable increase in the organic carbon or nitrogen content of the sediment, or in the C:N ratios, in response to the clear downward flux of surface POM, is best explained by the large 'background' of organic matter in comparison to the smaller flux from the water column. The carbon content in the sediment surface at the end of May was 11.2 mg C g^{-1} (of dry weight of sediment). The sediment at S38A had an average porosity of 0.7 (v/v) (Trimmer et al., 1999) giving a carbon content of 7.8 mg C cm^{-3} (or 7.8 x 10^5 mg m^{-3}) of wet sediment. This equates to 19,600 mg C m^{-2} in the top 2.5 mm layer of sediment, compared with the maximum concentration of POC in the bottom water of 200 mg C m^{-3}. The background carbon content is, therefore, almost 100 times greater than the concentration of matter

arriving, which may explain why there were no seasonal changes observed in the organic carbon in the sediment. The C:N ratio of the sediment was on average 4.6 and although there are some differences between months they did not indicate a clear seasonal change (Fig. 4.6). There was, however, a decline in sediment C:N ratios from the end of May to the beginning of August, which could be a response to the input of fresh material. This seems unlikely, however, as the C:N ratios of organic matter in the bottom water are much higher than the sediment.

Isotope enrichment in the sediment

The carbon and nitrogen stable isotope values of organic matter in the sediment were higher than the values for organic matter in the bottom water. In particular, there was significant enrichment in ^{13}C in the organic matter of the sediment in comparison to bottom POC, by an average of 1.8 ‰. Such enrichment is generally explained by isotopic fractionation by bacteria in the sediment during decomposition, because of the preferential loss of ^{12}C during respiration (McConnaughey and McRoy, 1979). There is, however, also evidence to suggest that CO_2 respired by bacteria is more deplete in ^{13}C than other organisms (Hagy et al., 2005) and this may explain the large observed fractionation in carbon. There does appear to be significant loss of carbon (in relation to nitrogen) from the sediments, because there was a significant decrease in C:N ratio of organic matter between bottom water and sediment (from ~7:1 to 4.6:1), probably due to respiration and may be associated with the significant enrichment in $\delta^{13}C$ values. Enrichment between POC and the sediment in the marine environment has been observed in several other areas including the Bering Sea (McConnaughey and McRoy, 1979), the Arctic (Hobson et al., 1995), and the western North Pacific (Japan) (Nakanishi and Minagawa, 2003; Usui et al., 2006). The carbon and nitrogen stable isotope values of organic matter in western Irish Sea sediments are similar to the values found in other marine systems (Table 4.2).

Table 4.2 Carbon and nitrogen stable isotope values of sediment organic matter from a range of marine habitats.

Location	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Notes	Reference
Pacific, SE Alaska	-20.8	5.9		(Goering et al., 1990)
Saanich Inlet, Canada		7.0		(Nakatsuka et al., 1992)
California coast	-21.4	7.8		(Peters et al., 1978)
East Bering Sea	-21.6	8.0		(Peters et al., 1978)
Gulf of Alaska	-23.4	5.7		(Peters et al., 1978)
Atlantic	-22.8	5.5		(Huon et al., 2002)
Seto Inland Sea, Japan	-20.0	6.0		(Mishima et al., 1999)
Equatorial Pacific		7.0	$[NO_3^-] = 8\mu M$	(Altabet and Francois, 1994)
Average	**-21.7**	**6.6**		
Western Irish Sea	-22.7	6.8		This study

Estimating inputs of carbon to the benthos

The peak chlorophyll concentration in the bottom water was 0.9 mg Chl m^{-3}, compared with a peak euphotic zone concentration (euphotic zone average, see data in Chapter Two) of 4.6 mg Chl m^{-3}. The peak concentration of chlorophyll in the bottom is 20 % that of the peak in the euphotic zone, which gives an estimate of 20 % for the flux of organic matter from the surface to the bottom. Spring bloom production (which is defined as production in the period of nutrient drawdown, 2 April to 31 May 2004, in the euphotic zone as described in Chapter Two) in the western Irish in 2004 was estimated to be 24.8 g C m^{-2} (see Chapter Two for full details) giving an estimate of flux of 5.0 g C m^{-2} to the benthos over the same period (Estimate 1). For the whole of the production season (23 April to 22 September, the period of stratification of the water column as defined in Chapter Two) the estimated flux would be 10.2 g C m^{-2} (i.e. 20 % of 51.1 g C m^{-2}).

The transfer of material to the bottom water, and consequently to the sediment (particularly as there is an absence of suspension feeders in this region of the Irish Sea), can also be estimated by the downward flux of spring bloom production indicated by stable isotope values. In particular, the decline in bottom water $\delta^{15}N$ values was closely coupled to the decline in the euphotic zone, and so the nitrogen isotope values can be used to estimate the contribution of pelagic production to the bottom water using a simple mixing model:

$$f_1 = (\delta_{SAMPLE} - \delta_{SOURCE2}) / (\delta_{SOURCE1} - \delta_{SOURCE2}) \quad \text{and} \quad f_2 = 1 - f_1 \qquad (4.1)$$

where f is the fractional contribution of two sources. The sample is the $\delta^{15}N$ value of the final product of interest, in this instance the organic matter in the bottom water that shows a decrease in $\delta^{15}N$ because of euphotic zone input. There was a decline in bottom water

$\delta^{15}N$ to an average value of 5.9 ‰ between 16 April and 4 May. This material is a mixture of euphotic zone production (source1) and organic matter already in the bottom water (source2). The average $\delta^{15}N$ of particulate matter from early April to late April (the material that would be sinking to the benthos) was 4.1 ‰ (see Table 4.4 for data) and the $\delta^{15}N$ of bottom water organic matter is, on average, 7.5 ‰, outside of the period of the spring bloom. There was, however, an increase of 0.017 ‰ m^{-1} in $\delta^{15}N$ values with depth (Fig 4.4F) that must be factored in to the calculation. From the euphotic zone (0-23 m) to the bottom water (~85 m) the $\delta^{15}N$ value of the organic matter will increase by 1.1 ‰, from 4.1 ‰ to 5.2 ‰. Thus, with $\delta^{15}N$ values of the two sources to be 5.2 ‰ and 7.5 ‰, a contribution of 70 % euphotic zone organic nitrogen and 30 % bottom water organic nitrogen will give a final $\delta^{15}N$ value of 5.9 ‰. So, 70 % of the nitrogen in the bottom water comes from the euphotic zone.

This estimate, however, is only concerned with the actual contribution of the nitrogen element in the mixture and not the total mass of material of interest. The concentration of nitrogen in the euphotic zone is higher than the concentration of nitrogen in the bottom water and so a mass balance model (Fry, 2006) can be used to calculate the relative contributions of the total organic matter from the two sources using the following formula:

$$f_{TOTAL1} = f_1 * W_2 / (f_1 * W_2 + f_2 * W_1) \quad \text{and} \quad f_{TOTAL1} = 1 - f_{TOTAL2} \tag{4.2}$$

where W is the weighting factor, such as concentration, to infer total amounts of material involved.

The average concentration of PON in the euphotic zone at the end of April was 50 mg m^{-3} compared with a bottom water concentration of 25.4 mg m^{-3} on 4 May (Table 4.4). Thus, the contribution of euphotic zone material is 54 %, which suggests that 13.4 g C m^{-2} (i.e. 54 % of 24.8 g C m^{-2} spring bloom production) is deposited on the benthos during the spring bloom (estimate 2). This method provides an estimate of the proportion of material reaching the benthos, rather than having to track amounts, and provides a useful method for the studying the flux of organic material to the benthos.

From Chapter Two it was estimated that 75 % of 2004 spring bloom production was available for export to higher trophic levels: i.e. grazing by zooplankton and flux to the benthos for secondary production. The significant increase in the copepod population during the spring bloom, with a maximum abundance and biomass in May and June (see Chapter Three for full details) results from the transfer of phytoplankton carbon to copepods. According to Gowen et al. (1999) copepods consumed up to 76 % of daily production and 22 % of spring bloom production in the western Irish Sea.

The 2004 spring bloom production was estimated to be 24.8 g C m^{-2}, giving 18.6 g C m^{-2} (75 %) available for export. Zooplankton grazing removes 22 %, or 4.1 g C m^{-2}, of that exportable production. The production of herbivorous zooplankton (Chapter Three) was estimated to be 1.1 g C m^{-2}, which estimates the transfer efficiency from primary to secondary production to be 27 %. Thus, after zooplankton grazing, 16.6 g C m^{-2} of the exportable spring bloom production is estimated to be available for vertical flux to the benthos. There is, however, mineralisation of organic matter as it sinks to the benthos. The carbon and nitrogen content of the particulate organic matter has been shown to decline with depth during the productive season. Carbon declines by 40 % (and nitrogen by 52 %)

(Fig. 4.4), so that of the 16.6 g C m^{-2} in the euphotic zone, 10.0 g C m^{-2} would remain for input to the benthos (estimate 3).

As a comparison an estimation of the flux of detritus to the benthos can also be determined using an empirical relationship that relates production and depth. Such models generally estimate the proportion of organic matter arriving on the benthos as a function of the magnitude of primary production in the surface water and the depth of the water column. For example, Suess and Müller (1980) found a relationship between primary production and the depth of water column to describe the flux of organic carbon to the benthos which is described by the formula:

$$\text{Flux } C_{org} = 5.9 \times \text{depth}^{-0.616} \times \text{production} \qquad (4.3)$$

At a depth of 93 m at S38A, and spring production of 24.8 g C m^{-2}, the carbon input to the benthos is estimated to be 9.7 g C m^{-2} (estimate 4).

Many models developed to provide quantitative descriptions of pelagic-benthic coupling assume that particle flux to the sediment depends primarily on vertical deposition. However, in many marine systems the quantity of organic matter reaching the sediment is also determined by lateral, mostly near-bottom processes and may also need to be considered. The western Irish Sea is characterised by low tidal currents in the bottom water and so advection into the area is expected to be limited particularly during the spring and summer, when gyre currents isolate the bottom water and appear to retain planktonic organisms, and maybe particles, in the area. Simpson and Bowers (1984) found that stratification in the Irish Sea can be successfully simulated without including the effects of advection because currents are generally small.

The input of spring bloom production, as an average of the different estimates and data from 1997 (which had similar level of spring bloom production), is therefore, estimated to be 9.9 g C m^{-2}.

Table 4.3 Summary of estimates of spring bloom input to the benthos in the western Irish Sea in 2004 and other literature values.

Method of estimation	**Input of spring bloom production (g C m^{-2})**	**Notes**
1. Chlorophyll transfer	5.0	This study
2. Isotope transfer	13.4	This study
3. Grazing/mineralisation loss	10.0	This study
4. Suess & Muller	9.7	This study
5. 1997	11.3	Trimmer et al. (1999)
Average	**9.9**	

Conclusion

There were seasonal differences in the flux of particulate matter to the benthos, with the timing of deposition of organic matter linked to the timing of planktonic production in surface waters. A pulse of input of organic matter was observed by an increase in chlorophyll concentration in the sediment. Outside of the production season, when the water column was well mixed, there was no evidence of input of organic matter to the benthos.

There was close coupling between the stable isotope values of production in the euphotic zone and the flux of organic matter to the benthos. The spring bloom was dominated by diatoms resulting in the rapid flux of production to the benthos, in an estimated 12 days during the peak of the spring bloom. The loss of carbon and nitrogen, as organic matter travelled down the water column was estimated to be 40 %. The carbon stable isotope values indicate that the supply of organic matter to the benthos is of euphotic zone origin and the absence of changes in the $\delta^{13}C$, $\delta^{15}N$ and C:N ratio of organic matter suggest an input of a high quality supply of organic matter, as expected when deposition is rapid. The estimate of the input of carbon to the benthos during the 2004 spring bloom, usin a number of different methods, was 9.9 g C m^{-2}.

Table 4.4 Total data set for particulate organic matter in the water column at station S38A in the western Irish Sea from February 2004 to January 2005. Highlighted rows show the data for bottom water (~85 m).

Date	Depth (m)	DIN (μM)	Chl (mg m^{-3})	POC (mg m^{-3})	PON (mg m^{-3})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:Chl	C:N
23-Feb-04	0	6.7	0.37	117.5	8.9	-26.8	6.8	320	11.3
	2.1	6.7	0.30	101.7	10.1	-25.8	8.1	341	8.6
	6.3	6.8	0.28	85.1	7.0	-25.9	6.9	306	10.4
	35.7	6.7	0.27	85.0	7.3	-26.2	6.6	312	10.0
	55.2	6.6	0.31	111.8	8.5	-26.5	6.8	362	11.3
	74.2	6.6	0.33	100.8	8.6	-25.8	7.3	302	10.1
	90.5	6.7	0.29	117.4	9.9	-25.9	6.9	399	10.2
02-Apr	0	6.6	0.45	158.3	18.7	-25.0	5.8	349	7.3
	0.9	6.6	0.45	135.2	15.5	-25.7	6.0	301	7.5
	7.3	6.9	0.50	118.6	14.0	-25.4	4.3	237	7.3
	10.6	6.6	0.52	189.7	22.7	-25.3	5.5	363	7.2
	20.9	6.6	0.45	128.1	16.2	-25.9	4.8	286	6.8
	39.3	6.8	0.42	117.1	17.1	-25.5	7.0	279	5.9
	60.5	6.8	0.37	106.6	11.7	-26.4	5.0	285	7.8
	80.6	6.8	0.30	152.6	15.8	-24.8	7.1	503	8.3
16-Apr	0	6.2	1.13	190.7	29.5	-26.0	4.6	169	5.5
	5.9	5.9	0.82	148.5	19.8	-25.5	3.0	181	6.4
	10.9	5.9	1.25	205.0	24.6	-25.6	3.6	164	7.1
	18.2	6.2	0.88	185.9	22.5	-25.7	4.4	212	7.1
	28	6.2	0.59	171.2	22.8	-26.3	4.2	290	6.4
	38.1	6.2	0.31	108.1	11.5	-26.0	3.7	348	8.1
	61	6.7	0.23	121.6	11.0	-25.6	6.0	522	9.4
	89.5	6.7	0.31	172.8	21.0	-24.0	6.0	566	7.0
26-Apr	0	-	2.20	352.7	67.4	-26.4	2.8	161	4.5
	10	-	2.63	303.1	50.7	-25.2	2.2	115	5.1
	20	-	1.11	198.6	31.8	-25.3	3.5	179	5.4
	30	-	0.69	152.2	24.6	-24.9	3.2	222	5.3
04-May	0	1.2	4.63	335.5	52.6	-22.9	5.1	72	5.5
	8.3	1.2	4.72	376.3	61.4	-23.2	7.5	80	5.3
	18	1.6	4.32	364.3	50.1	-23.1	5.9	84	6.2
	28.4	3.9	1.88	197.9	31.4	-23.6	5.8	105	5.4
	39.1	6.0	0.26	149.4	22.7	-	6.8	570	5.6
	49.2	6.1	0.19	140.5	13.5	-25.7	6.3	733	8.9
	69.9	7.5	0.22	160.2	20.3	-24.9	6.7	745	6.8
	88.7	7.4	0.25	196.9	25.4	-24.4	5.8	773	6.6
10-May	0	0.2	2.29	372.2	35.3	-24.3	7.0	163	9.0
	9.1	0.3	5.70	452.1	52.6	-23.0	7.7	79	7.4
	18.9	3.1	5.77	285.6	46.1	-23.3	7.4	49	5.3
	29.8	6.2	0.93	185.5	22.2	-26.2	7.3	198	7.2
	39.6	7.0	0.65	166.7	16.3	-26.9	7.3	257	8.8
	49.5	7.1	0.44	180.9	17.0	-26.8	5.1	410	9.1
	69.3	7.0	0.39	171.8	20.2	-26.8	6.1	444	7.3
	88.9	7.0	0.43	208.3	25.2	-25.5	7.7	480	7.1

Table 4.4 continued

Date	Depth (m)	DIN (μM)	Chl (mg m^{-3})	POC (mg m^{-3})	PON (mg m^{-3})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:Chl	C:N
31-May	0	0.1	1.24	263.4	38.8	-23.3	7.8	213	5.8
	8.6	0.1	1.32	222.4	29.5	-23.6	6.5	168	6.5
	20.1	0.1	1.66	214.1	33.7	-22.7	6.8	129	5.4
	28.1	0.5	1.82	176.7	21.4	-23.2	6.9	97	7.1
	39.7	7.1	10.26	518.5	79.7	-22.4	8.4	51	5.6
	49.0	5.5	3.16	184.4	27.3	-23.3	7.4	58	5.8
	64.1	7.6	0.93	159.6	20.6	-23.3	8.1	172	6.6
	87.5	7.4	0.89	201.1	22.5	-24.0	7.9	225	7.7
28-Jun	0	0.1	1.15	180.4	25.7	-23.6	7.5	156	6.0
	8.6	0.1	1.02	207.7	25.8	-23.8	7.1	204	6.9
	18.8	0.5	1.73	237.0	28.4	-23.7	6.1	137	7.2
	28.4	3.1	3.23	207.9	30.8	-24.0	6.6	64	5.8
	39.4	4.4	0.52	119.5	12.8	-25.3	7.0	229	8.0
	49.7	5.8	0.51	117.5	17.4	-25.0	7.9	230	5.8
	63.7	6.8	0.26	140.5	21.0	-25.1	8.6	540	5.7
	83.1	7.5	0.42	164.7	17.1	-23.9	8.0	391	8.3
02-Aug	0.9	0.0	0.72	202.0	25.8	-24.2	6.1	280	6.7
	8	0.2	1.29	198.1	31.9	-23.4	5.9	153	5.3
	18.4	2.0	2.73	192.5	27.0	-20.3	6.7	70	6.1
	28.6	3.7	0.28	131.7	12.8	-26.0	9.1	476	8.8
	38.1	4.1	0.16	107.8	11.4	-25.3	8.8	655	8.1
	49.8	5.6	0.14	100.4	12.8	-25.3	8.7	703	6.7
	69	6.9	0.12	108.9	12.5	-25.5	8.4	886	7.5
	88.4	7.1	0.16	190.4	20.5	-23.4	8.0	1197	8.0
23-Sep	0	2.9	0.12	155.2	23.2	-23.2	7.2	1282	5.7
	9.2	3.1	0.11	196.1	29.0	-23.1	5.9	1809	5.8
	18.9	2.8	0.20	142.3	21.4	-23.2	7.0	729	5.7
	28.7	2.7	0.11	178.6	20.2	-24.2	7.0	1647	7.6
	39.7	2.5	0.11	144.5	17.8	-24.2	7.5	1290	7.0
	48.6	2.6	0.11	134.1	19.9	-24.6	7.7	1178	5.8
	68.6	3.1	0.08	156.6	19.6	-24.2	7.3	1970	6.8
	87.3	3.4	0.08	170.0	20.2	-23.7	7.0	2002	7.2
15-Nov	0	4.6	0.05	125.1	12.7	-25.5	6.2	2387	8.4
	10.6	4.6	0.05	109.4	11.0	-25.9	5.6	2243	8.5
	21.1	4.6	0.03	142.4	13.6	-26.1	5.9	4379	9.0
	31.6	4.7	0.03	116.7	9.6	-25.7	6.3	3588	10.4
	45.6	4.7	0.05	130.9	11.7	-25.7	5.9	2896	9.6
	59.2	4.8	0.04	155.8	15.2	-25.1	6.3	3592	8.8
	84.1	4.9	0.06	159.0	16.5	-24.3	7.1	2838	8.2
21-Jan-05	0	6.7	0.43	171.3	18.7	-25.4	8.7	398	7.9
	9	6.6	0.40	148.8	18.2	-25.2	7.2	374	7.0
	19	6.6	0.38	145.7	14.5	-25.3	-	380	8.6
	28.9	6.6	0.34	142.4	15.3	-25.9	8.5	424	8.0
	38.7	6.6	0.40	282.5	24.4	-23.0	7.5	707	9.9
	58.4	6.6	0.41	280.0	28.7	-24.4	7.2	680	8.3
	86.1	6.7	0.35	147.2	17.6	-25.1	8.2	426	7.2

Chapter Five

Trophic structure and carbon transfer in the benthos of the western Irish Sea

Introduction

The soft muddy sediment of the western Irish Sea supports an economically important fishery for the languoustine *Nephrops norvegicus*. The annual catch rate of around 8000 t, which has a first sale value of £8 m (Vincent et al., 2004), has been sustained for over 20 years (ICES, 2006). There is, however, little information regarding the structure of the benthic food web and the flow of energy to the *N. norvegicus* fishery. The few studies of benthic species assemblages in the western Irish Sea have found the *N. norvegicus* grounds to have low diversity, abundance and biomass of macroinvertebrates in comparison to other areas (Hensley, 1996; Ellis et al., 2000). The secondary production of the benthos is supported by the organic detritus in the sediment, which is ultimately dependent on production in the overlying water and which may be vulnerable to anthropogenic impacts such as climate change and nutrient availability. The vulnerability of fisheries to changes in benthic food webs has been observed in other areas. For example, in the northwest Atlantic Ocean, the collapse of Atlantic cod stocks has been attributed to a progressive decline in the nature and extent of the energy flow through the benthic system attributed to commercial fishing (Choi et al., 2004). In Narragansett Bay in the USA, long term data suggest that a climate-induced decrease in primary production has led to a decrease in organic matter deposition to the benthos, thought to be due to climate-induced increases in zooplankton grazing (Fulweiler et al., 2007). Thus, a fundamental requirement to understanding the robustness of the *N. norvegicus* fishery to

possible anthropogenic impacts, is to improve the knowledge of the transfer of energy, through the benthic system to the fishery. In particular, there is a need to understand the trophic structure and identify the key species involved in the transfer of energy through the benthic system to the *Nephrops norvegicus* fishery.

Investigation of the diet of *Nephrops norvegicus*, from a range of habitats including Scotland (Thomas and Davidson, 1962; Parslow-Williams et al., 2002), the Atlantic (Cristo, 1998; Cristo and Cartes, 1998), the Mediterreanean (Gual-Frau and Gallardo-Cabello, 1988) and the Irish Sea (Oakley, 1978), has shown the species to be an opportunistic predator. *Nephrops norvegicus* feeds preferentially on crustaceans but also a range of molluscs, echinoderms and polychaetes (Cristo, 1998; Cristo and Cartes, 1998; Parslow-Williams et al., 2002). These studies, from a range of different geographical areas, show that diet is usually dependent on availability of prey items. Although primarily active predators studies in some areas have shown that *N. norvegicus* will scavenge on freshly dead food if available (Oakley, 1978) so that in areas of trawling activity discards may represent an important increase in food availability.

The study of trophic relationships often involves the use of gut content analysis, to establish trophic links between organisms (e.g. Kleppel et al., 1991; Pinn et al., 1998a). There are, however, some shortcomings of the method. It can often be a time-intensive exercise because predator-prey interactions change in time and space (e.g. see Kingsford, 1992) requiring intensive data collection. Also, analysis may be biased because of differential rates of digestion of prey items, organisms with hard body parts, crustaceans for example, are likely to remain in the gut longer and may be biased. Extensive taxonomic knowledge is often required, and prey items may be broken down (in the

gastric mill of a crustacean for example), making material very difficult to identify. Gut content studies are an excellent means of ascertaining what prey are ingested; however, they only provide a snapshot in time which may not always take into account temporal variation in diet.

Stable isotope analysis, particularly of nitrogen, can be used to assess the trophic level of organisms because ^{15}N in the tissues of a consumer is typically enriched by 3-4 ‰ relative to prey (Minagawa and Wada, 1984; Peterson and Fry, 1987; Fry and Sherr, 1989). The $\delta^{15}N$ of a consumer also reflects diet assimilated over time (DeNiro and Epstein, 1981) and so can often identify the most important prey items over a period of time. The realised trophic position, measured by $\delta^{15}N$ analysis, can give a more accurate picture of trophic relationships than gut content analysis (Vander Zanden and Rasmussen, 1999; Post, 2002). Stable isotope analysis does, however, lack taxonomic resolution and in many studies the two techniques are combined (e.g. Grey et al., 2002).

The overall aim of the study presented in this chapter was to determine the abundance and biomass of organisms in the benthos of the western Irish Sea and together with carbon and nitrogen stable isotopes determine the number of trophic levels and position of *Nephrops norvegicus* in the benthic food chain. The structure of the benthic food web can then be used to estimate the input of carbon to the benthos required to support the *N. norvegicus* fishery and compare this to the actual estimates of input discussed in Chapter Four. The results presented in Chapter Three indicate that the pelagic larval stage of the life cycle of *N. norvegicus* is not food (carbon) limited. In the benthos it is important to determine the balance between the input of carbon to the sediment and the benthic food chain and the output of carbon from the benthic food chain to the fishery in the western Irish Sea. The

balance between the input and output of carbon from this food chain will indicate the potential vulnerability of the benthic stage to changes in ecosystem function that have been observed, in the Irish Sea and other marine systems, to reduce the input of organic carbon to the benthos.

The specific aims of the work presented here were to: i) identify the assemblage of benthic macrofauna, including estimates of density and biomass; ii) analyse the trophic structure and linkages of the organisms in the benthos using carbon and nitrogen stable isotope analysis; iii) investigate food sources and trophic position of *Nephrops norvegicus* in relation to life cycle stage, sex and size; and iv) use the data to quantify the carbon input into the benthic food chain and to estimate the input of organic matter needed to support the production of *N. norvegicus* in the benthos.

Methods

Field programme

All sampling was conducted at station S38A, the site of the permanent AFBI mooring, in the western Irish Sea (53°46'N, 05°38'W, Fig. 2.1 and see Chapter 1 for a full site description) on ten occasions between February 2004 and January 2005 onboard the RV 'Lough Foyle'. A further visit was made in May 2006 onboard the RV 'Corystes'.

Sampling

Nephrops norvegicus: Animals on the surface of the seabed were collected on an approximate monthly basis, using a small (2 m) beam trawl (10 mm mesh to limit clogging by sediment) towed for 10 min. The largest *Nephrops norvegicus* caught by this equipment, in sufficient numbers for regular isotope analysis, had a carapace length of 25 mm (approximate age class 3 years). On each sampling occasion approximately 24 animals (12 males and 12 females where possible) were selected for isotope analysis and frozen at -20 °C. On return to the laboratory animals were defrosted and dissected for abdominal muscle tissue, carapace and fore-gut contents. In November 2004 and April 2005 eggs were removed from berried females and frozen at -20 °C for subsequent isotope analysis.

Individuals of *Nephrops norvegicus* from a broader size range (~13-45 mm carapace length) were collected during an AFBI '*Nephrops* and by-catch' research cruise in August 2004, when a large otter trawl (mesh size 70 mm) was deployed. Six individuals, three males and three females, from each millimetre size class, were selected and frozen at -20 °C for subsequent isotope analysis.

Benthic macroinvertebrates: Benthic infauna were sampled with a box corer (area 0.1 m^2, depth 50 cm) in May 2006 at S38A. Sediment from the core was washed through a metal sieve (500 μm) to retain animals. Samples were stored at -20 °C prior to analysis of benthic biomass and stable isotopes. Unfortunately, it was not possible to take more than two box cores in 2006, because damage to the box core precluded further use. Animal abundance and biomass data from samples collected with a Day grab, (as part of the National Marine Monitoring Programme (NMMP), at S38A from 1998 to 2002 (supplied by Dr M. Service, AFBI) have been included to supplement the field box core data. Thus, all abundance and biomass data is an average of the box core data and the Day grab samples. There was no difference in the abundance, diversity and biomass between the box cores and the Day grabs.

Benthic epifauna, for analysis of stable isotope values, were also sampled on an approximate monthly basis, from February 2004 to January 2005, using a small (2 m) beam trawl (10 mm mesh) towed for approximately 10 min. Animals were washed free of sediment and sorted into taxonomic groups. Bivalves and polychaetes were left in filtered (22 µm mesh) seawater for 8 hr for clearance of gut contents. All animals were frozen at - 20 °C prior to isotope analysis. In May 2006, five replicate beam trawls (2 m), of known distance, were made for quantitative analysis of the abundance and biomass of benthic epifauna. All animals were sorted, counted and weighed before and after drying.

The authorities for organisms are found in Hayward and Ryland (1996) and have not been quoted in the text.

Stable isotope sample preparation

Macroinvertebrate infauna and epifauna were dissected for muscle tissue, except for polychaetes where a section of the whole body was evaluated. With the exception of *Nucula sulcata*, where muscle tissue from 5 animals was pooled, all samples were individual organisms. All samples were oven dried to constant weight (24 h at 60 °C) in acid-washed glass vials, homogenised with an agate pestle and mortar and stored in acid-washed eppendorf tubes. Sub-samples (~1 mg) were weighed into a tin capsule (ultra-clean 8 x 5 mm, Elemental Microanalysis) for isotopic analysis.

Carbon and nitrogen content and stable isotope analysis

The method for isotope analysis is described in full in Chapter 2. The trophic level (TL) of a consumer can be calculated using $\delta^{15}N$ values, where 3.4 ‰ is the assumed trophic enrichment according to Minagawa and Wada (1984). The following formula can be used to estimate trophic level:

$$TL = (\delta^{15}N_{consumer} - \delta^{15}N_{base\ resource}) / 3.4 + 1 \qquad (5.1)$$

Data analysis

Values presented are mean ± 1 standard error. Where the standard error is not given it is because there were no replicates. The number of replicates varied between sampling dates and so details of numbers are given in the tables. A *t*-test was used to test for differences between two means. Where there were more than two samples differences between means were tested using single-factor ANOVA and where significant followed by a Tukey test for pairwise comparisons (Zar, 1999). To test for differences in the relationship between body mass and $\delta^{15}N$ between male and female *Nephrops norvegicus* an ANCOVA test was used.

The strength of association between variables was determined by regression analysis: from the coefficient of determination (r^2) and analysis of variance to determine the significance of the regression line.

Results

Benthic macroinvertebrates

Abundance and biomass: The biomass of benthic infauna was dominated by three species, *Nephrops norvegicus*, the burrowing thalassinidean shrimp *Calocaris macandreae* and the bivalve *Nucula sulcata* plus a number of predatory polychaetes (Table 5.1). *C. macandreae* also dominated the epifauna, together with small numbers of bottom or near-bottom dwelling species such as brown shrimp of the genera *Crangon* (which may include *C. crangon* or *C. allmanni* or a mixture of both) and the prawns *Dichelopandalus bonnieri* and *Pasiphaea sivado* (Table 5.1). Only two deposit feeding species, *C. macandreae* and *N. sulcata,* were found in the soft mud at S38A (see Table 5.2 for description of trophic position and diet of all fauna). There were no suspension feeders collected in any of the samples. The total wet weight, calculated from the sum of infaunal and epifaunal biomass, was 20.4 g and total carbon biomass was 1.3 g C m^{-2} (Table 5.1). Abundance data by individual species is provided in Table 5.9 at the end of the chapter.

Carbon and nitrogen stable isotopes: The $\delta^{13}C$ of all benthic and bottom dwelling species collected from S38A was significantly enriched compared with the $\delta^{13}C$ of organic matter (OM) in the sediment (Fig. 5.1). There was a 5 ‰ increase in $\delta^{13}C$ between OM and deposit feeding organisms, *C. macandreae* and *N. sulcata*, both with a $\delta^{13}C$ value close to -17 ‰ (Fig. 5.1 and Table 5.2). *Pasiphaea sivado*, which is a benthic and pelagic feeder was the organism least enriched in $\delta^{13}C$ compared with sediment OM. The $\delta^{13}C$ of the sigalionid polychaetes was also low. The remaining organisms, mostly predators (see Table 5.2 for full details) had $\delta^{13}C$ values of ~16 ‰, an enrichment of ~1 ‰ over the deposit feeders.

Table 5.1 Mean abundance and carbon and nitrogen biomass of benthic fauna at station S38A in the western Irish Sea. Infaunal organisms sampled with a box corer ($n = 2$) and a Day grab ($n = 25$, NMMP data supplied by Dr. M. Service). Epifaunal organisms ($n = 5$) sampled with a 2 m beam trawl (mesh size 10 mm). (Other crustaceans = amphipods, copepods and tanaids).

Species/faunal group	TL	Abundance mean ± SE (m^{-2})	Wet biomass mean ($g\ m^{-2}$)	Dry biomass mean ($g\ m^{-2}$)	Carbon biomass ($g\ m^{-2}$)	Nitrogen biomass ($g\ m^{-2}$)
Infauna						
Calocaris macandreae	2	8.9 ± 0.15	5.6	1.5	0.4553	0.0988
Echinoderms		0.4	0.006	0.001	0.0003	0.0001
Nematodes		31.9 ± 5.5	0.013	0.0003	0.0001	0.0000
Nemerteans		3.3 ± 0.2	0.013	0.003	0.0013	0.0004
Nephrops norvegicus	3	1.54 ± 0.15	5.8	1.3	0.4394	0.1198
*Nucula sulcata**	2	20.4 ± 1.04	5.9	0.3	0.1146	0.0252
Oligochaetes		10.7 ± 0.76	0.001	0.0001	0.0001	0.0000
Other crustaceans		6.3 ± 0.02	0.003	0.0008	0.0003	0.0001
Polychaetes	3/4	97.4 ± 0.09	2.8	0.624	0.2673	0.0743
Total			**20.14**	**3.80**	**1.279**	**0.319**
Epifauna						
Asterias rubens	2.5	0.0003 ± 0.0001	0.003 ± 0.0014		0.0003	0.00006
Calocaris macandreae	2	0.049 ± 0.008	0.046 ± 0.007		0.0037	0.00080
Crangon sp(p).	3	0.013 ± 0.006	0.014 ± 0.007		0.0014	0.00035
Dichelopandalus bonnieri	3	0.098 ± 0.014	0.250 ± 0.035		0.0240	0.00597
Goneplax rhomboides	3	0.002 ± 0.001	0.004 ± 0.002		0.0003	0.00006
Liocarcinus depurator	3	0.0003 ± 0.0002	0.001 ± 0.0004		0.0001	0.00001
*Nucula sulcata**	2	0.016 ± 0.005	0.013 ± 0.004		0.0005	0.0001
Pagarus bernhardus	3	0.001 ± 0.0004	0.001 ± 0.0007		0.0001	0.00003
Pasiphaea sivado	2.5	0.038 ± 0.023	0.030 ± 0.018		0.0032	0.0008
Polychaetes	3/4	0.003 ± 0.001	0.005 ± 0.002		0.0005	0.0001
Total			**0.367**	**0.09**	**0.034**	**0.008**
Grand total			**20.41**	**3.89**	**1.313**	**0.327**

* wet weight includes shell

Table 5.2 Mean $\delta^{13}C$ and $\delta^{15}N$ values of infaunal and epifaunal invertebrates from station S38A in the western Irish Sea during the sampling period February 2004 to January 2005 and literature descriptions of trophic position and gut contents. Key to gut content analysis (GCA) trophic level (TL): D – deposit feeder, P – predator, S – scavenger. * Different sample numbers carbon/nitrogen .

Species	*n*	$\delta^{13}C$ Mean (± SE)	$\delta^{15}N$ Mean (± SE)	$\delta^{15}N$ TL	GCA TL	Prey items observed in previous studies	Reference
Sediment organic matter	88	-22.2 ± 0.07	6.8 ± 0.03	1.0		Base resource for benthic food web	
Calocaris macandreae	50	-17.5 ± 0.03	9.7 ± 0.04	1.9	D	Organic matter	(Pinn et al., 1998a, 1998b)
Nucula sulcata	23/45*	-17.3 ± 0.12	9.9 ± 0.06	1.9	D	Organic matter	
Goneplax rhomboides	38	-15.5 ± 0.28	12.8 ± 0.13	2.8	P		
Liocarcinus depurator	12	-16.3 ± 0.49	12.5 ± 0.19	2.7	P	Crustaceans, molluscs, polychaetes, fish	(Hall et al., 1990)
Pagarus bernhardus	8	-16.4 ± 0.33	12.6 ± 0.31	2.7	S	Scavenging omnivore	(Fish and Fish, 1996)
Crangon sp(p).	3	-16.0 ± 0.16	12.9 ± 0.20	2.8	P	Crustaceans, molluscs, polychaetes	(Oh et al., 2001; Taylor, 2005)
Dichelopandalus bonnieri	5	-16.6 ± 0.14	12.8 ± 0.10	2.8	P	Crustaceans, echinoderms, polychaetes, molluscs	(Lagardere, 1973)
Pasiphaea sivado	6	-18.2 ± 0.14	10.6 ± 0.43	2.1	P	Night: benthic e.g. *Calocaris macandreae*, day: pelagic e.g. chaetognaths, fishes	(Cartes, 1993)
Nephrops norvegicus	216/311*	-16.3 ± 0.04	12.3 ± 0.03	2.6	P/S	*Calocaris macandreae*, molluscs, echinoderms	(Cristo and Cartes, 1998)
Asterias rubens	2	-16.3 ± 0.63	11.1 ± 0.15	2.3	P	Molluscs, polychaetes, echinoderms	(Fish and Fish, 1996)
Glycera sp.	23	-16.4 ± 0.08	15.7 ± 0.10	3.6	P	Small invertebrates	(Fish and Fish, 1996)
Nephtys sp.	19	-15.9 ± 0.16	13.4 ± 0.06	2.9	P	Crustaceans, molluscs, polychaeates, protozoa	(Caron et al., 2004)
Eunicid polychaete	5	-16.1 ± 0.40	16.7 ± 0.22	3.9	P		(Fish and Fish, 1996)
Nemerteans	16	-16.6 ± 0.16	12.4 ± 0.10	2.6	P/S	Crustaceans and annelids plus some molluscs, fish	(McDermott and Roe, 1985)
Notomastus sp.	2	-15.9 ± 0.61	13.0 ± 0.14	2.8	S/D		(Fish and Fish, 1996)
Sigalionidae	3	-17.2 ± 0.75	13.9 ± 0.26	2.1	P		(Fish and Fish, 1996)

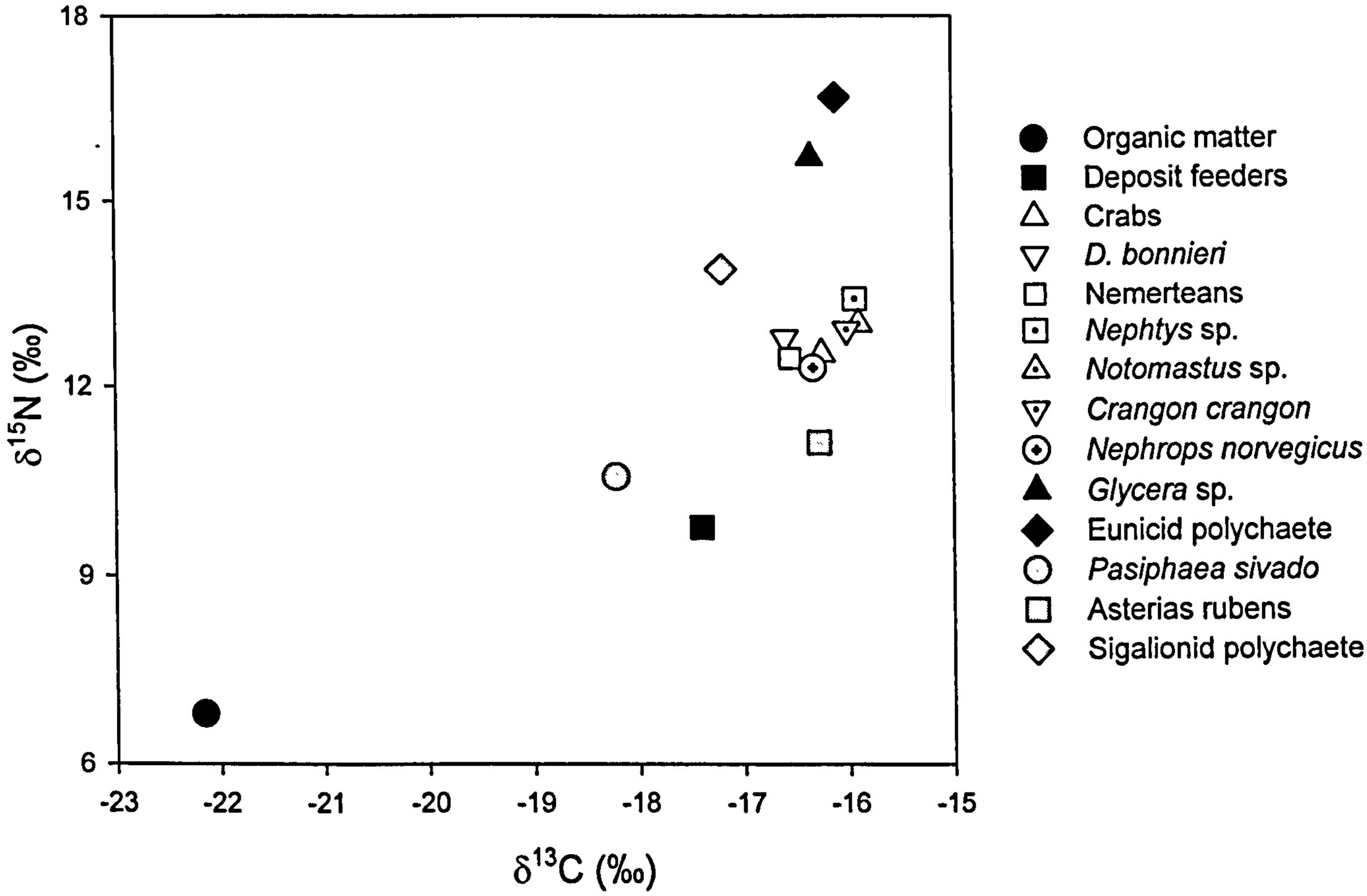


Figure 5.1 Mean $\delta^{13}C$ and $\delta^{15}N$ values for benthic macroinvertebrates at station S38A in the western Irish Sea. Value for deposit feeders is the average of *C. macandrae* and *N. sulcata*. Value for Crabs is average of *G. rhomoboides*, *L. depurator* and *P. bernhardus*. See Table 5.7 for sample sizes and isotope values, with standard error, of these organisms.

The deposit feeders, *C. macandreae* and *N. sulcata* had similar $\delta^{15}N$ values of 9.7 ‰ and 9.8 ‰. This is 3 ‰ higher, equal to almost one trophic level (see Equation 5.1), above sediment OM $\delta^{15}N$ (Fig. 5.1, Table 5.2). *Pasiphaea sivado* occupied a slightly higher, but similar trophic position with a $\delta^{15}N$ of 10.6 ‰. There were a large number of animals, including *Nephrops norvegicus*, crabs, *Crangon sp(p).*, *Dichelopandalus bonnieri* and a number of unsegmented acoelomate worms called nemerteans (that were not identified to anything more than phylum), that had very similar $\delta^{15}N$ values. For this group, $\delta^{15}N$ values ranged from 12.3 ‰ to 12.9 ‰, placing them all approximately 1 trophic level above the deposit feeders and 2 trophic levels above sediment OM. The starfish *Asterias rubens* was positioned between between trophic levels 1 and 2. The organisms with the highest $\delta^{15}N$ values were predatory polychaetes of the genus *Glycera* and family Eunicidae, with values a further 3-4 ‰ higher at 15.7 ‰ and 16.7 ‰ respectively, placing them at a fourth trophic level in the benthos.

Several benthic species were collected on enough sampling occasions to investigate seasonal trends in stable isotope values. There was temporal variation in both carbon and nitrogen isotopes for most of the species analysed (Fig. 5.2). For the two deposit feeding species, temporal variation was much greater, particularly for carbon, in *Nucula sulcata* than *Calocaris macandreae*. There was a decline in $\delta^{13}C$ of *N. sulcata* during the spring bloom, from -17.4 ‰ to -18.6 ‰. It was not possible to test for differences in $\delta^{13}C$ values of *N. sulcata* muscle tissue over time because there were no replicates for some sampling dates. However, each *N. sulcata* sample is a pooled sample of 5 animals and so is an average values. The $\delta^{15}N$ values of *N. sulcata* varied by only 0.6 ‰, from 9.6 ‰ to 10.2 ‰, during the whole sampling period. The isotope values for *C. macandreae* ranged from

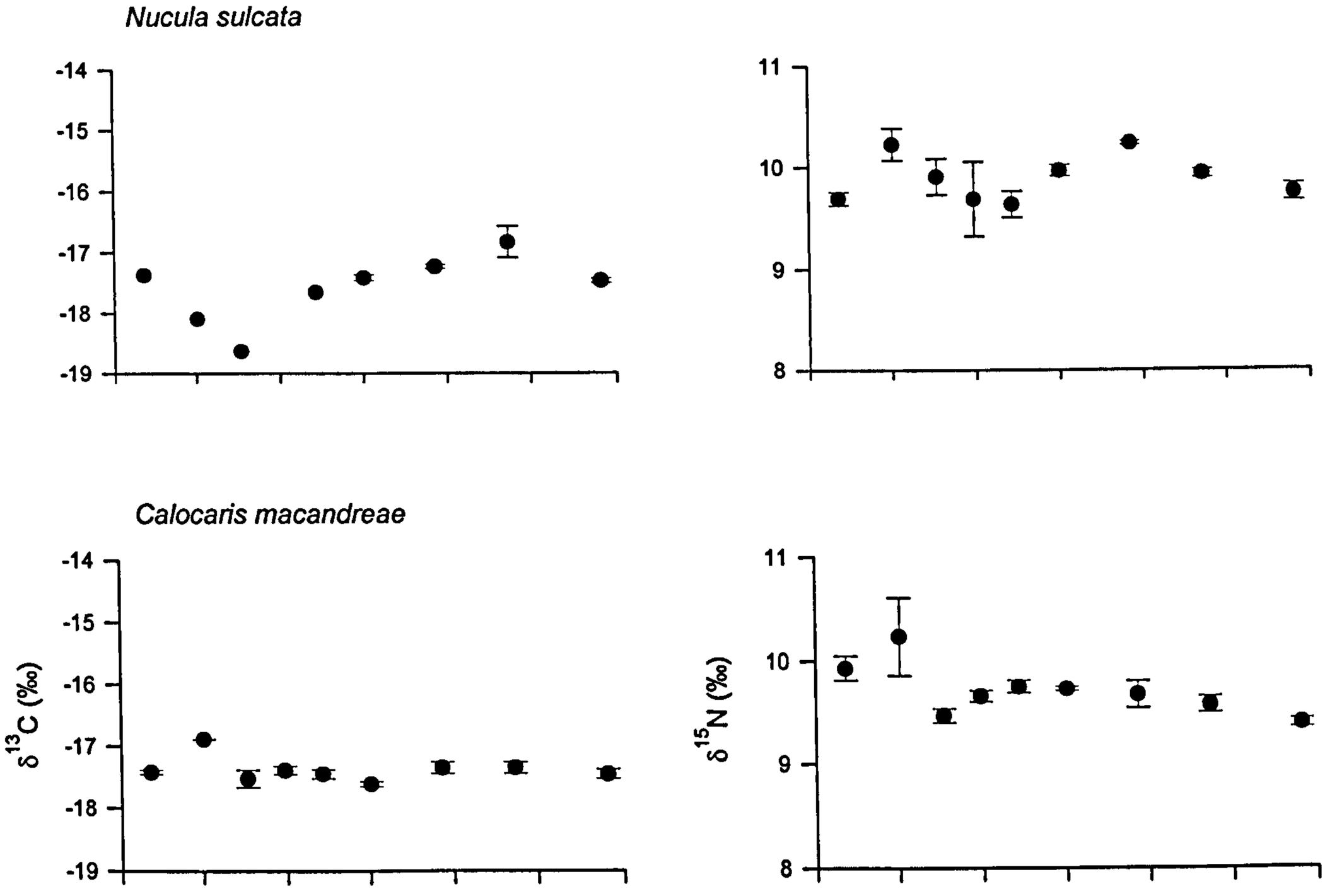


Figure 5.2 Carbon and nitrogen stable isotope values of benthic invertebrate taxa from February 2004 to January 2005. Values are mean (± SE). Where error bars are missing only a single organism was collected or several small individuals were pooled.

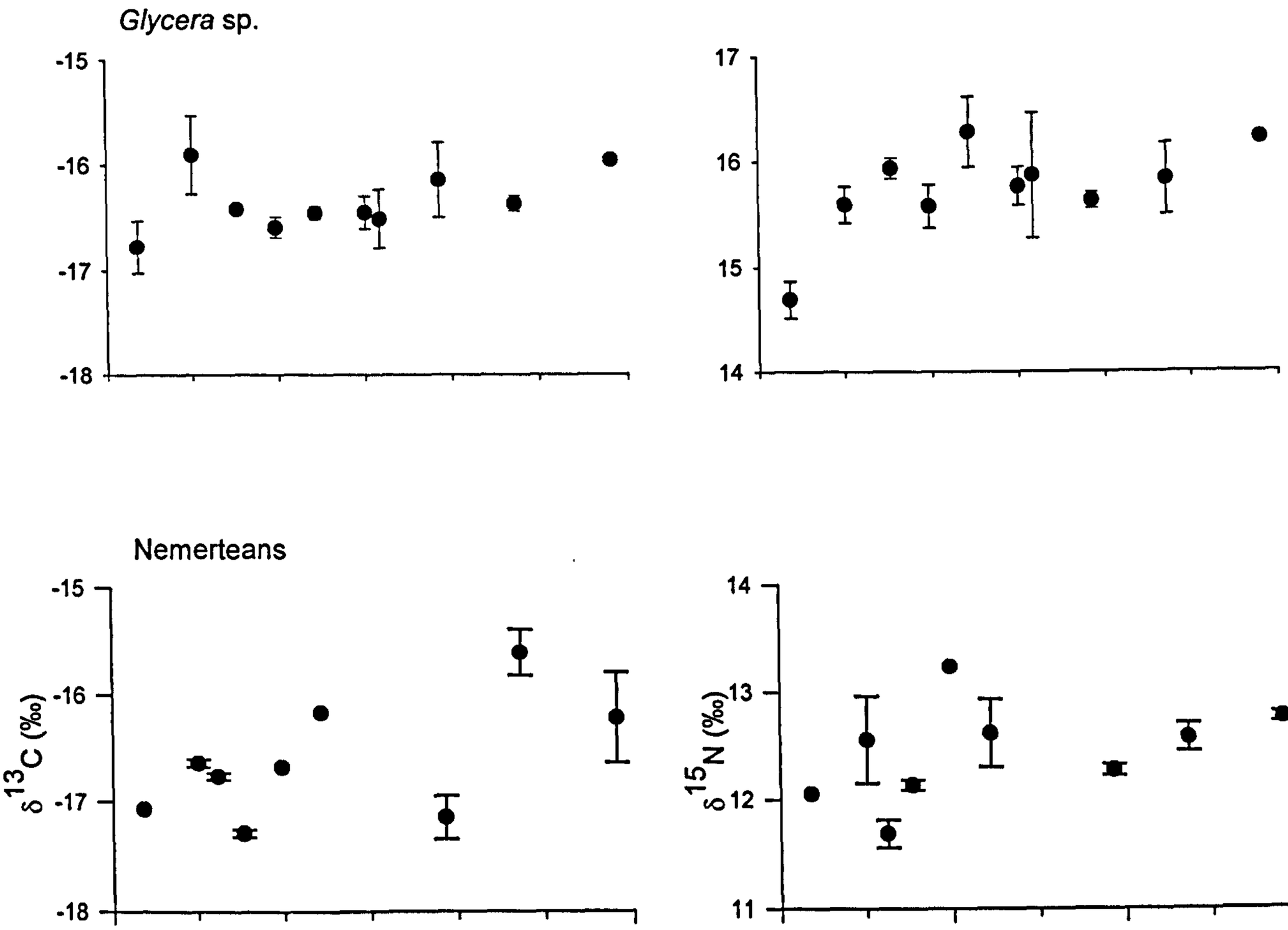


Figure 5.2 continued.

-17.6 ‰ to -16.9 ‰ for carbon and from 9.4 ‰ to 10.2 ‰ for nitrogen. Temporal variation in $\delta^{13}C$ and $\delta^{15}N$ was also much higher in the crab *Goneplax rhomboides*, the polychaetes *Glycera* spp. and *Nephtys* spp. and nemerteans. There were, however, no obvious seasonal patterns. There was also more inter-sample variation in the predatory animals.

Nephrops norvegicus stable isotopes

***Life cycle stages*:** The stable isotope values of *Nephrops norvegicus*, of both carbon and nitrogen, declined with life cycle stage from adult to egg to larvae and then increased from larvae to juvenile and back to adult (Fig. 5.3, Table 5.3). Carbon stable isotope values for *N. norvegicus* ranged from -16.3 ‰ (adults) to -21.6 ‰ (stage III larvae) and $\delta^{15}N$ from 12.3 ‰ (adults) to 8.9 ‰ (stage II larvae) with significant differences between all life cycle stages (except larvae stages II and III which were similar).

The average $\delta^{13}C$ and $\delta^{15}N$ values for adult *N. norvegicus* muscle tissue, for the whole of the sampling period, were -16.3 ‰ and 12.3 ‰ respectively. There was no difference between isotope values for eggs collected in November and April and so the data were pooled. The pooled carbon and nitrogen isotope values for *N. norvegicus* eggs were -19.3 ‰ and 10.9 ‰ respectively, significantly lighter than adult muscle tissue. The $\delta^{13}C$ and $\delta^{15}N$ of Stage I larvae were lower than that of the eggs at -20.2 ‰ and 9.9 ‰. The $\delta^{13}C$ values for Stage II (-21.4 ‰) and Stage III (-21.6 ‰) were similar and lower than the value for Stage I. The $\delta^{15}N$ values for Stages II and III were also similar (8.9 ‰ and 9.1 ‰ respectively) and lower than for Stage I. Juvenile *N. norvegicus*, carapace size ~11 mm, had carbon and nitrogen isotope values of -17.2 ‰ and 11.5 ‰. From April to the end of May (when the larvae were in the water column) the average $\delta^{13}C$ and $\delta^{15}N$ values for the putative diet of larvae,

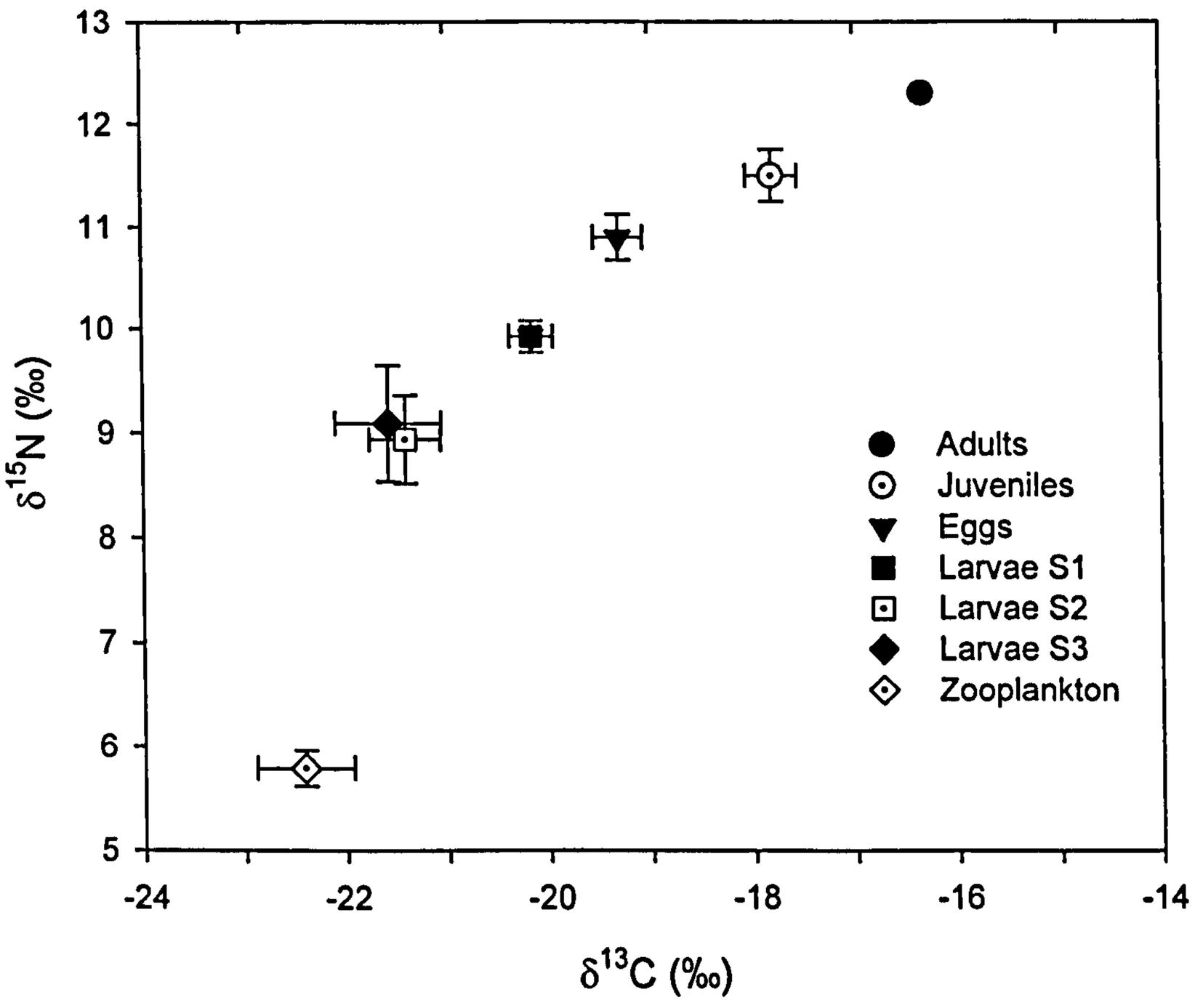


Figure 5.3 Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ for life cycle stages of *Nephrops norvegicus* and zooplankton from station S38A in the western Irish Sea in 2004. All error bars were plotted, where they are not visible the error is very small. Samples sizes (n) given in Table 5.3.

zooplankton (see Chapter 3 for full details), were -22.4 ‰ and 5.8 ‰, respectively (Fig. 5.3).

***Body mass*:** The relationships between body mass and stable isotope values were explored using simple correlation. There were significant positive relationships between wet body mass (hereafter referred to as body mass) and $\delta^{15}N$ of muscle tissue of *Nephrops norvegicus* (Fig. 5.4, Table 5.4). The enrichment in $\delta^{15}N$ of muscle tissue, from the smallest to largest animals (body mass 1 to 54 g, carapace size 11 – 49 mm), was 1.2 ‰, from 11.9 ‰ to 13.1 ‰. There was no relationship between body mass and $\delta^{13}C$. There was no difference in values of $\delta^{15}N$ and $\delta^{13}C$ with body mass between males and females.

There was a significant positive relationship between body mass and $\delta^{15}N$ of *N. norvegicus* gut contents with a larger enrichment of 1.9 ‰ over a similar size range (Fig. 5.4, Table 5.4). There were no differences in $\delta^{13}C$ or $\delta^{15}N$ between the gut contents of male and female *N. norvegicus*.

***Tissue types*:** There were significant differences ($p < 0.0001$) in the $\delta^{13}C$ and $\delta^{15}N$ values for muscle tissue, carapace and gut contents of *N. norvegicus* (Table 5.5). In particular, carapace was significantly enriched in ^{13}C and deplete in ^{15}N, with average values of -13.0 ‰ and 7.2 ‰ compared with -16.3 ‰ and 12.3 ‰ for muscle tissue and -17.1 ‰ and 11.4 ‰ for gut contents. Stable isotope values for whole body samples were similar to muscle tissue at -16.4 ‰ and 11.9 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

***Temporal variation*:** There was significant variation in stable isotope values for all the tissue types, over the sampling period. However, the variation was lower in muscle tissue

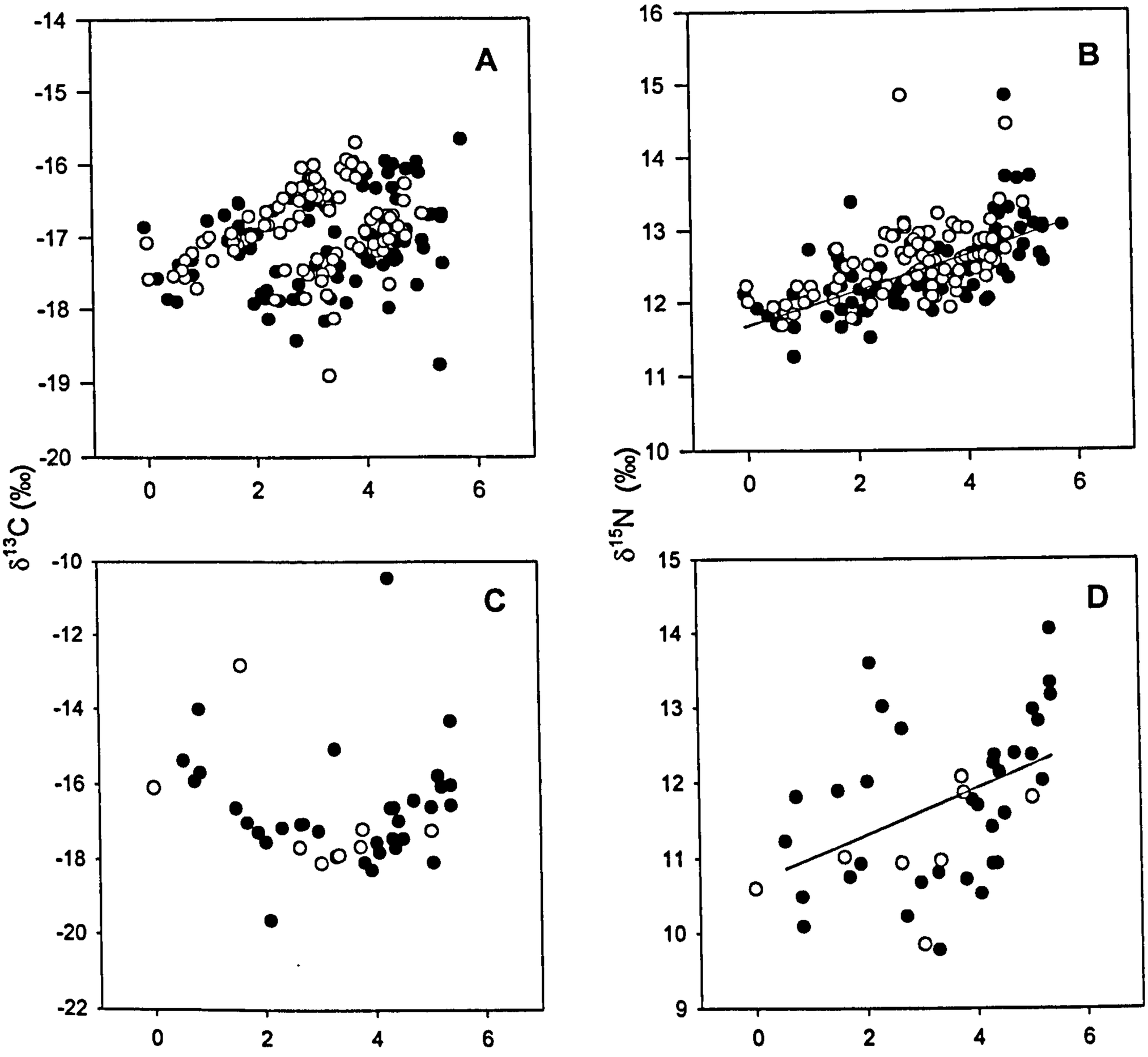


Figure 5.4 Relationship between $\log_2$ body mass and *Nephrops norvegicus* muscle tissue **A**: $\delta^{13}C$ and **B**: $\delta^{15}N$ and between $\log_2$ body mass and *Nephrops norvegicus* gut contents **C**: $\delta^{13}C$ and **D**: $\delta^{15}N$. Closed circles are males, open circles are females. Line of best fit applies to the whole data set (males and females) and are included only where significant.

Table 5.3 Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of life cycle stages of *Nephrops norvegicus* and zooplankton from station S38A in the western Irish Sea in 2004.

Description	*n*	$\delta^{13}C$		$\delta^{15}N$	
		Mean	SE	Mean	SE
Benthic – adults[$]	216/311[#]	-16.3	0.04	12.3	0.03
Benthic – juvenile[∞]	10	-17.8	0.25	11.2	0.23
Eggs	12	-19.3	0.24	10.9	0.22
Larvae Stage I	13[*]	-20.2	0.20	9.9	0.16
Larvae Stage II	8[*]	-21.4	0.35	8.9	0.42
Larvae Stage III	3[*]	-21.6	0.52	9.1	0.55
Zooplankton (Apr – Jun)	57[*]	-22.4	0.48	5.8	0.17

[$] Muscle tissue from adults of carapace sizes 24-26 mm
Different sample numbers for carbon/nitrogen
[∞] Juvenile carapace size 4-6 mm (< 1 yr old)
* Pooled samples (see Chapter 3)

Table 5.4 Parameters and test statistics for linear relationships between total wet body weight (BW; grams $\log_2$-transformed) and $\delta^{15}N$ and $\delta^{13}C$ of muscle tissue and gut contents of *Nephrops norvegicus* from station S38A in the western Irish Sea for animals collected in April and August 2004. The form of the fitted relationships is $\delta^{15}N$ or $\delta^{13}C$ (‰) = a + b ($\log_2$ BW)

Date	Tissue type	Isotope	a	b	*n*	r^2	*P*
August 2004	Muscle	$\delta^{15}N$	+11.9	0.211	184	0.238	<0.0001
August 2004	Gut contents	$\delta^{15}N$	+10.6	0.314	41	0.209	0.002

Table 5.5 Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ for different tissue types of *Nephrops norvegicus* from station S38A in the western Irish Sea from February 2004 to January 2005. [#] Different sample numbers for carbon and nitrogen isotope analysis.

Description	*n*	$\delta^{13}C$		$\delta^{15}N$	
		Mean	SE	Mean	SE
Muscle tissue	216/311[#]	-16.3	0.04	12.3	0.03
Whole organism	2	-16.4		11.9	
Carapace	46	-13.0	0.17	7.2	0.12
Gut contents	192	-17.1	0.13	11.4	0.08

than carapace or gut contents, with variation of only 0.7 ‰, from -16.4 ‰ to -15.7 ‰, in the carbon isotope values of *Nephrops norvegicus* muscle tissue (Fig. 5.5). The highest $\delta^{13}C$ occurred at the end May and the lowest in September, although there does not appear to be a clear seasonal pattern. The $\delta^{13}C$ for May was significantly higher than all other months (F_{181} = 12.01, $P < 0.001$). The temporal variation in $\delta^{15}N$ of muscle tissue was also low, ranging from a maximum of 12.8 ‰ in February, followed by a steady decline to a minimum of 11 % at the end of June (Fig. 5.5). Thereafter, $\delta^{15}N$ values increased but were variable. There were significant differences in muscle tissue $\delta^{15}N$ values between some months ($F_{0.05,301}$=17.47, $P < 0.0001$). There were also some significant, although small, differences in isotope values between males and females but not in all months (Table 5.6). The $\delta^{13}C$ values for males and females were different for samples taken in February, May, September and November but there was no obvious seasonal trend. Male and female $\delta^{15}N$ values were significantly different in February, late April and May.

Carapace isotope values show some temporal variation although there was no clear seasonal pattern for either $\delta^{13}C$ or $\delta^{15}N$ (Fig. 5.6). Carapace $\delta^{13}C$ was significantly lower ($P < 0.04$) in February compared with the rest of the sampling period. The range of carapace isotope values was larger than for muscle tissue: $\delta^{13}C$ varied from -14.5 ‰ to -12.2 ‰ and $\delta^{15}N$ from 6.5 ‰ to 7.8 ‰. There was also considerably greater within sample variation in both $\delta^{13}C$ and $\delta^{15}N$. It was not possible to test for differences between males and females for each sampling occasion because of the limited number of samples run (only 3 on some occasions). However, across the whole data set, for the period April 2004 to January 2005, there were significant differences in the $\delta^{13}C$ and C:N ratio of male and female carapace.

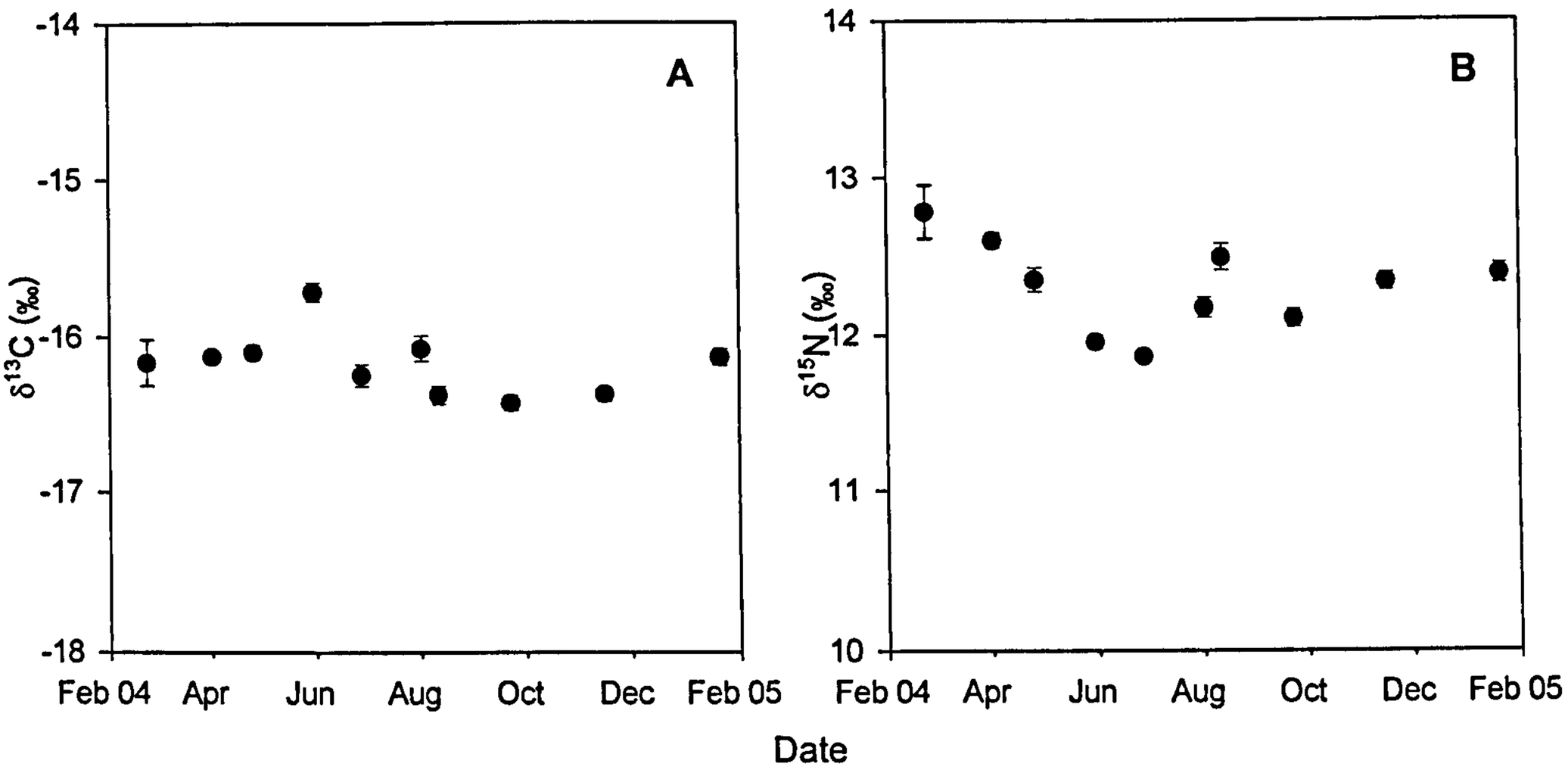


Figure 5.5 Mean (± SE) values of **A:** $\delta^{13}C$ and **B:** $\delta^{15}N$ of muscle tissue of *Nephrops norvegicus* from station S38A in the western Irish Sea from February 2004 to January 2005.

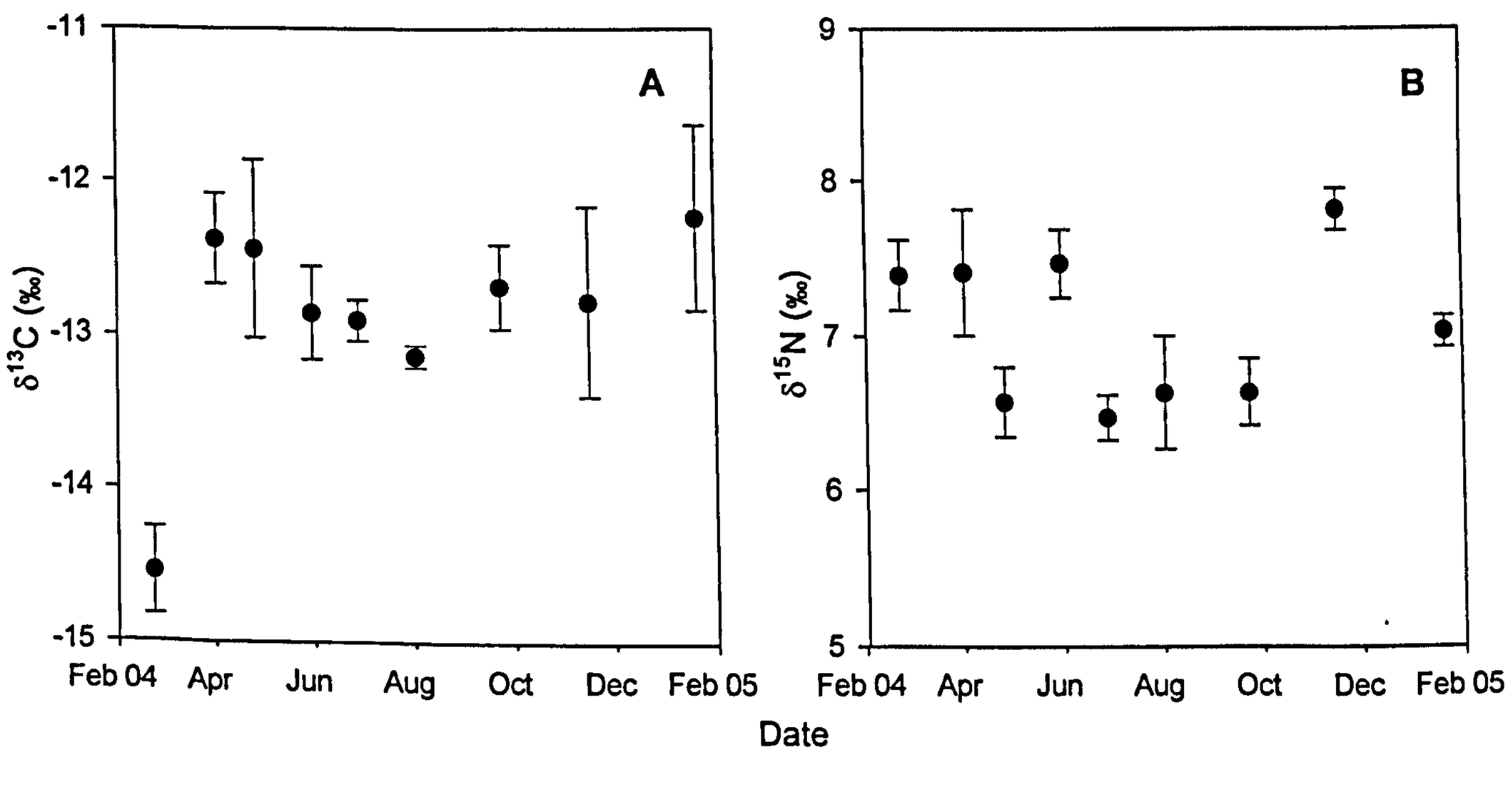


Figure 5.6 Mean (± SE) values of **A:** $\delta^{13}C$ and **B:** $\delta^{15}N$ of carapace tissue for *Nephrops norvegicus* from station S38A in the western Irish Sea from February 2004 to January 2005.

Table 5.6 Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of male and female *Nephrops norvegicus* muscle tissue from station S38A in the western Irish Sea for the sampling period February 2004 to January 2005. n_1 = males, n_2 = females. *P* value from *t*-test of male vs female values.

Date	n_1/n_2	$\delta^{13}C$		*P*	$\delta^{15}N$		*P*
		Male	Female		Male	Female	
23 February 04	8/22	-16.5 ± 0.09	-15.7 ± 0.19	**	13.0 ± 0.28	12.1 ± 0.21	*
2 April	30/20	-16.2 ± 0.05	-16.1 ± 0.05		12.6 ± 0.05	12.6 ± 0.08	
26 April	14/14	-16.2 ± 0.06	-16.0 ± 0.05		12.5 ± 0.09	12.2 ± 0.11	*
31 May	22/24	-15.8 ± 0.06	-15.6 ± 0.09	*	12.1 ± 0.07	11.8 ± 0.05	**
28 June	22/22	-16.4 ± 0.07	-16.2 ± 0.10		11.9 ± 0.04	11.8 ± 0.06	
2 August	12/12	-16.1 ± 0.09	-16.0 ± 0.14		12.1 ± 0.09	12.2 ± 0.10	
12 August	11/9	-16.5 ± 0.12	-16.3 ± 0.06		12.5 ± 0.11	12.6 ± 0.10	
23 September	11/12	-16.5 ± 0.04	-16.2 ± 0.07	*	12.1 ± 0.10	12.1 ± 0.05	
16 November	11/11	-16.5 ± 0.05	-16.2 ± 0.04	***	12.4 ± 0.08	12.3 ± 0.07	
21 January 05	12/11	-16.1 ± 0.07	-16.1 ± 0.08		12.4 ± 0.06	12.4 ± 0.11	

* $P = 0.05 - 0.01$ ** $P = 0.01 - 0.001$ *** $P < 0.001$

The $\delta^{13}C$ was significantly enriched in males (-12.6 ‰) compared with females (-13.5 ‰) ($F_{0.05,44} = 8.74$, $P = 0.005$). The C:N ratio was higher in males than females; 8.08 versus 7.13 ($F_{1,44} = 14.28$, $P < 0.001$). There was also a significant positive relationship between the $\delta^{13}C$ of carapace and the C:N ratio ($r^2 = 0.740$, d.f. = 45, $P < 0.001$), which applied to both males and females (Fig. 5.8). There was a weak but significant negative relationship between $\delta^{15}N$ and the C:N ratio of carapace ($r^2 = 0.270$, d.f. = 45 , $P < 0.001$), although the strength of this relationship was significantly affected by two values (out of 45) that had much lower C:N ratios and higher $\delta^{15}N$ values than all other samples (Fig. 5.8). There was no difference in the $\delta^{15}N$ values between male and female carapace.

There were no differences between months for $\delta^{13}C$ values of *Nephrops norvegicus* gut contents and very high within sampling variability (Fig. 5.7). There was a difference in $\delta^{13}C$ values between males and females only for the August sample when only a few animals were analysed (Table 5.7). With the exception of 28 June, the $\delta^{15}N$ values of gut contents from April to September were significantly lower than winter values (ANOVA: $F_{8,183} = 8.2$, $P<0.001$) (Fig. 5.7). Within sample variation for both $\delta^{13}C$ and $\delta^{15}N$ was high compared with muscle tissue.

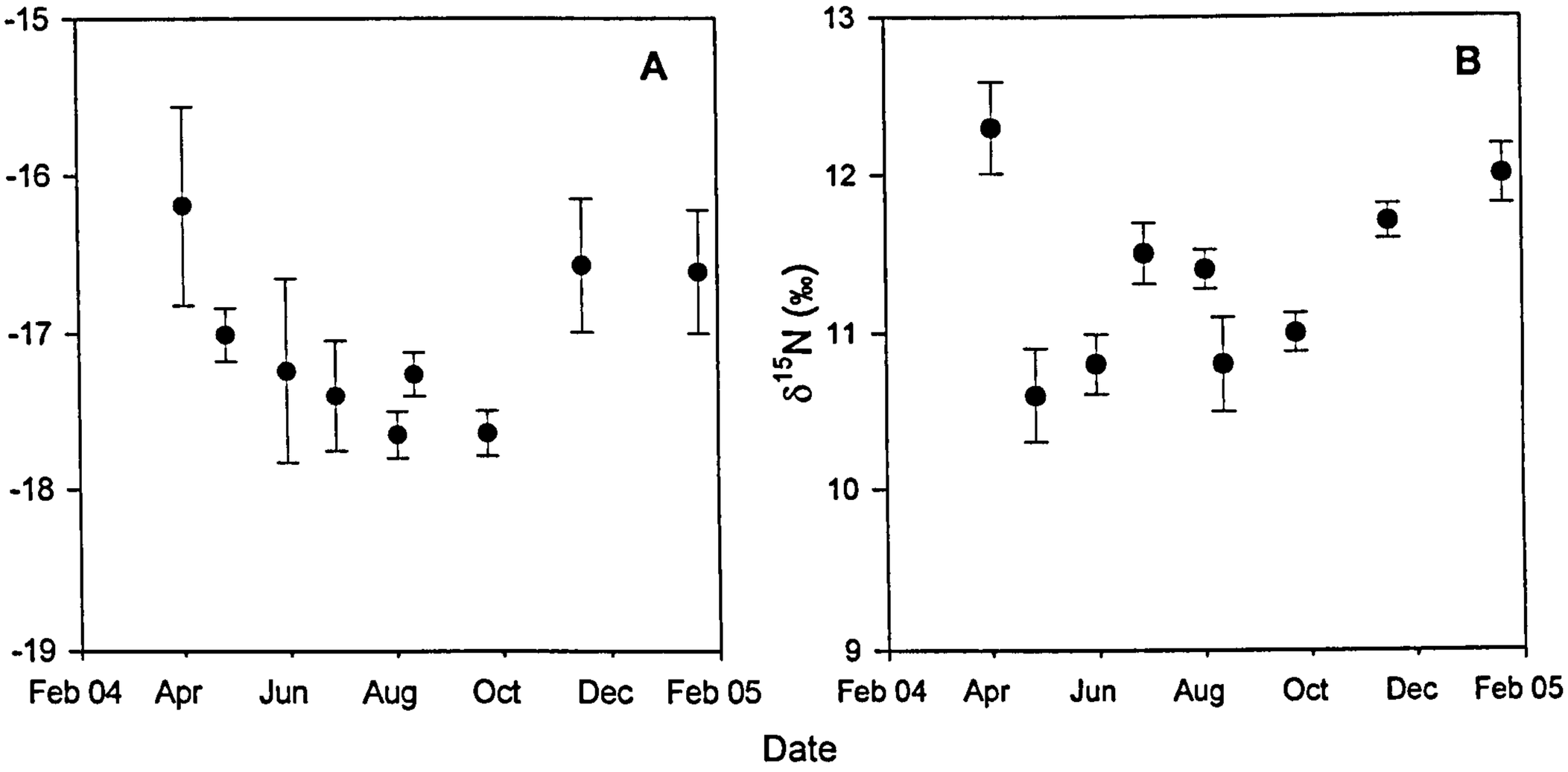


Figure 5.7 Mean (± SE, n = 42) values of **A:** $\delta^{13}C$ and **B:** $\delta^{15}N$ of gut contents of *Nephrops norvegicus* from station S38A in the western Irish Sea from April 2004 to January 2005.

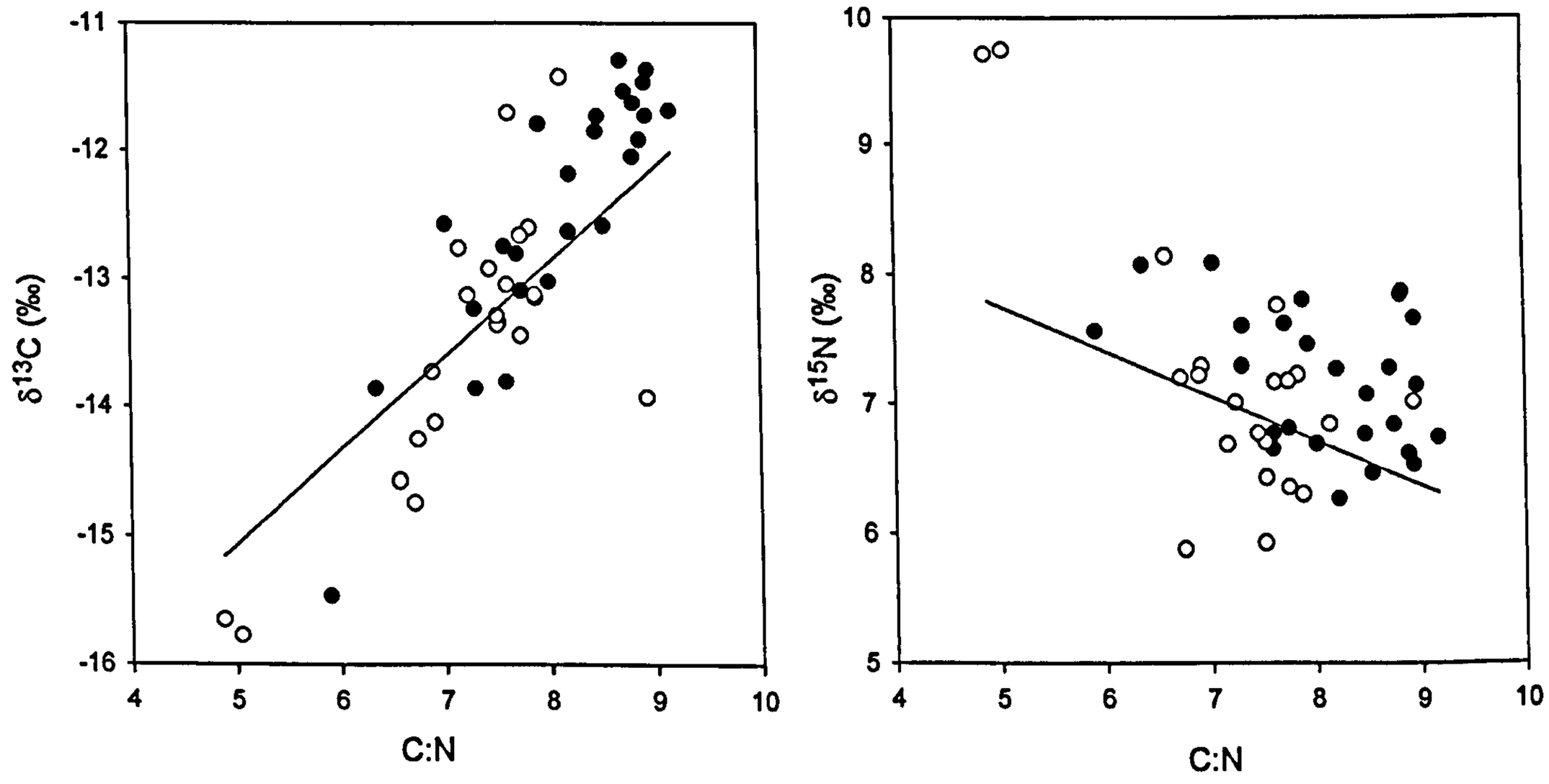


Figure 5.8 Relationship between C:N ratio and stable isotope values of *Nephrops norvegicus* carapace tissue a) carbon and b) nitrogen. Closed circles are males and open circles are females. Line of best fit applied to whole data set (males + females).

Table 5.7 Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of male and female *Nephrops norvegicus* gut contents from station S38A in the western Irish Sea for the sampling period April 2004 to January 2005. n_1 = males, n_2 = females. *P* value from t-test of male vs female values.

Date	n_1/n_2	$\delta^{13}C$ Male	$\delta^{13}C$ Female	*P*	$\delta^{15}N$ Male	$\delta^{15}N$ Female	*P*
23 February 04	-	-	-	-	-	-	-
2 April	8/10	-17.4 ± 1.29	-18.7 ± 0.64		11.9 ± 0.44	12.3 ± 0.44	
26 April	10/5	-18.9 ± 0.30	-18.9 ± 0.28		11.0 ± 0.28	10.7 ± 0.54	
31 May	11/7	-19.9 ± 0.65	-19.0 ± 1.37		10.7 ± 0.29	10.5 ± 0.37	
28 June	11/12	-19.1 ± 0.59	-19.6 ± 0.42		11.4 ± 0.30	11.1 ± 0.25	
2 August	12/13	-19.6 ± 0.18	-19.7 ± 0.24		11.1 ± 0.09	11.2 ± 0.22	
12 August	4/3	-19.1 ± 0.04	-19.8 ± 0.12	**	11.5 ± 0.70	10.4 ± 0.36	
23 September	12/11	-19.6 ± 0.18	-19.6 ± 0.24		10.6 ± 0.16	11.1 ± 0.17	
16 November	11/11	-18.1 ± 0.79	-18.9 ± 0.30		11.4 ± 0.13	11.5 ± 0.18	
21 January 05	12/15	-18.9 ± 0.49	-18.1 ± 0.59		11.9 ± 0.34	11.7 ± 0.21	

* $P = 0.05 - 0.01$ ** $P = 0.01 - 0.001$ *** $P < 0.001$

Discussion

Abundance and biomass of benthic macrofauna

The abundance and diversity of benthic infauna at station S38A in the western Irish Sea (WIS) was dominated by polychaete worms, typical of sediment habitats that are subject to frequent trawling disturbance (Kaiser and Spencer, 1996; Jennings et al., 2001). In a broader scale survey of the *Nephrops norvegicus* grounds in the WIS, Hensley (1996) also found a dominance of polychaetes, which constituted almost 80 % of all taxa recorded. The cohesive nature of the sediment, however, provides a habitat for burrowing organisms, in particular *N. norvegicus* and the burrowing thalassinidean shrimp *Calocaris macandreae* and these species are more important than polychaetes in terms of biomass. Nevertheless, the overall diversity of organisms at S38A was low, with a total of 33 species, all but 13 of which (mostly crustaceans) were polychaetes (Table 5.9). Ellis et al. (2000) found considerably less diversity in *N. norvegicus* dominated areas, with around 30 taxa in muddy sediments compared with 91 to 140 taxa in areas from the Bristol Channel, St George's Channel and Carmarthen Bay. Hensley (1996) also noted an apparent lack of fauna in the centre of the mud basin. The macrofaunal catch per unit effort, measured in kilograms per hour, in the *N. norvegicus* grounds, was the lowest of all the areas surveyed in the western Irish Sea by Ellis et al. (2000). There were only two deposit feeding species, the bivalve *Nucula sulcata* and *C. macandreae* recorded in this study of benthic fauna at S38A. There was also an absence of suspension feeding animals at S38A, which may be due to the nature of the sediment or may reflect high levels of disturbance from trawling activity (Kaiser et al., 2006; de Juan et al., 2007). Most of the other species recorded during this study were predators including the starfish *Asterias rubens* and several crab species. The total wet

weight and dry weight of benthic macrofauna at S38A were 20.4 g m^{-2} and 3.8 g m^{-2}, respectively.

Considering the importance of the mud patch for the *N. norvegicus* fishery, it is evident that there have been few studies of the macrofaunal community of the benthos in the western Irish Sea. There is, for example, no information on seasonal changes in the abundance or biomass of the species present. However, with the data available, from this and other studies, it can be concluded that the muddy sediments that support *N. norvegicus* have low invertebrate biodiversity and biomass, particularly when compared with other, less muddy, areas of the western Irish Sea (Hensley, 1996; Ellis et al., 2000).

Trophic structure and relationships in the benthos

The trophic structure of the benthic system was explored using nitrogen stable isotopes. Values of $\delta^{15}N$ typically show fractionation of about 3.4 ‰ per trophic level, and so can be used to estimate trophic position of organisms and elucidate food web structure (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). The $\delta^{15}N$ data show clear trophic enrichment from organic matter to deposit feeders to predators with 4 apparent trophic levels (Fig. 5.1). From the discussion on the depth of the euphotic zone in Chapter Two, it is evident that the depth of the water column at S38A (~93 m) rules out benthic primary production and so the base resource for the benthic food chain, particularly in the absence of suspension feeders, is identified as a single source: the organic matter (derived from several sources including primary production in surface waters, microorganisms and meiofauna in the sediment involved in the processing of the organic detritus) in the sediment. The $\delta^{15}N$ of the organic matter appears to be subject to very little temporal variation, and remained close to 6.8 ‰ for the whole year, and so provides a reliable $\delta^{15}N$ 'isotope baseline' for the benthic food

chain. The trophic position of all other benthic invertebrates can then be determined in relation to this baseline.

The $\delta^{15}N$ value of the deposit feeding bivalve *Nucula sulcata*, at 9.9 ‰, is consistent with the expected position of one trophic level (TL) above the $\delta^{15}N$ of sediment organic matter on which the species feeds, with enrichment of 3.1 ‰. The $\delta^{15}N$ of *Calocaris macandreae* is similar, at 9.7 ‰ but the exact diet of this species is still unclear. Gut content studies have shown *C. macandreae* is predominantly a deposit feeder but the species may employ other feeding strategies such as scavenging, suspension feeding and cannibalism (Pinn et al., 1998a, 1998b; Atkinson and Taylor, 2005). A $\delta^{15}N$ value of 9.7 ‰ for *Calocaris macandreae*, similar to that of *Nucula sulcata*, also equates to an increase of one trophic level above the sediment and suggests that in this area of the western Irish Sea the species relies on deposit feeding. The average enrichment of 3 ‰ for these two deposit feeders indicates they may be more efficient at utilizing nitrogen than the average trophic enrichment factor of 3.4 ‰ seen between many organisms and their prey.

With $\delta^{15}N$ values 2.5 – 3.1 ‰ above the deposit feeders, there is a large group of mostly predatory organisms at a third trophic level (TL3), with $\delta^{15}N$ values ranging from 12.3 ‰ to 12.9 ‰. The $\delta^{13}C$ values of this group are ~1 ‰ higher than the deposit feeders, consistent with the trophic enrichment in ^{13}C predicted between a consumer and its diet (DeNiro and Epstein, 1978). The trophic enrichment factor below the 'standard' 3.4 ‰ suggest either the efficient assimilation of nitrogenous material, slight variation in the enrichment factor depending on food source (McCutchan Jr. et al., 2003) or feeding on organisms with low $\delta^{15}N$ values that have not been sampled. The TL3 group includes all the crustaceans that were sampled: *Nephrops norvegicus*, the crabs, *Crangon sp(p).*, *Dichelopandalus bonnieri*,

and the nemerteans. The $\delta^{15}N$ analysis indicates that the diet of these organisms is likely to be the deposit feeders and the results of gut content studies from the literature confirms these organisms are known prey items (Table 5.2). Although there are several species in TL3, *N. norvegicus* accounts for 94 % of the biomass (0.44 g C m^{-2}) at this trophic level, so competition for food with other species is likely to be low.

A fourth trophic level, with $\delta^{15}N$ values close to 16 ‰, was also identified and places predatory polychaetes such as *Glycera* spp. and those from the family Eunicidae at a higher trophic level than *N. norvegicus*. High $\delta^{15}N$ values for benthic polychaetes are not uncommon. In the benthos near Greenland, for example, the polychaetes *Lumbrineris* sp. and *Phyllodoce mucosa* were found to have $\delta^{15}N$ values of 14.1 ‰ and 12.3 ‰ respectively, which gave derived trophic levels of 3.6 and 3.2, higher than the majority of other invertebrates in the system (Hobson et al., 2002) and in a north Pacific Bay polychaetes were in the highest trophic group (Goering et al., 1990). In the Bay of Biscay, however, the $\delta^{15}N$ value of the predatory polychaete *Glycera rouxii* was only ~2 ‰ enriched compared with *Nephrops norvegicus*, approximately half the increase seen in the current study (Le Loc'h and Hily, 2005), which may reflect a more mixed diet because of differences in the availability of prey species. Many polychaetes are known to feed on smaller polychaetes (e.g. see Beukema, 1987) which may result in high $\delta^{15}N$ values. For example, in a study on the Georges Bank in the northwestern Atlantic, Fry (1988) found a 3.2 ‰ increase between small and large polychaetes, but did not provide details of species. Despite the high abundance of small polychaetes found in other studies of the western Irish Sea benthos (Hensley, 1996), there were no small polychaetes sampled in the current study, and so further research is required to determine their importance to the benthic food web and position in the trophic hierarchy. The trophic level of fish and octopods, which may

represent a further trophic level above the invertebrates, have not been investigated in this study.

There were a few organisms - *Pasiphaea sivado*, *Asterias rubens* and sigalionid polychaetes - that did not fit into the well defined trophic levels suggested by the $\delta^{15}N$ of the other organisms. The shrimp *P. sivado* feeds on both pelagic and benthic prey including *Calocaris macandreae* (Cartes, 1993). The $\delta^{15}N$ value of *P. sivado*, at only 1.3 ‰ higher (i.e. less than a full trophic level) than *C. macandreae*, clearly reflects the importance of other prey (that have not been identified), and in particular the importance of pelagic animals as indicated by a low $\delta^{13}C$ value, of around -18 ‰. The lower $\delta^{13}C$ of pelagic feeders reflects the lower $\delta^{13}C$ of zooplankton and other pelagic species, the production of which ultimately results from primary production which has low $\delta^{13}C$ values. For example, in the current study primary production had a $\delta^{13}C$ value of ~23 ‰. The contribution of pelagic and benthic prey can be estimated by a simple mixing model. Assuming a $\delta^{13}C$ value for pelagic organisms to be -21.4 ‰ (estimated from the average $\delta^{13}C$ for large zooplankton, Chapter Three), and an average $\delta^{13}C$ value of -16.2 ‰ for benthic infauna, estimates the pelagic contribution to diet to be 24 %. *Asterias rubens* is a predator of molluscs, polychaetes and other echinoderms and a $\delta^{15}N$ value of 11.1 ‰ suggests the deposit feeders may also be an important, but not dominant, component of the diet. No other organisms measured had $\delta^{15}N$ values lower than the deposit feeders so it has not been possible to estimate the contribution of deposit feeders to the diet of *Asterias rubens*. The realised trophic position of the sigalionid polychaetes is between the large TL3 group and the TL4 group of predatory polychaetes. The sigalionids also had deplete $\delta^{13}C$ values (-17.2 ‰) compared with an average of -16.2 ‰ for other benthic infauna, indicating consumption of pelagic prey.

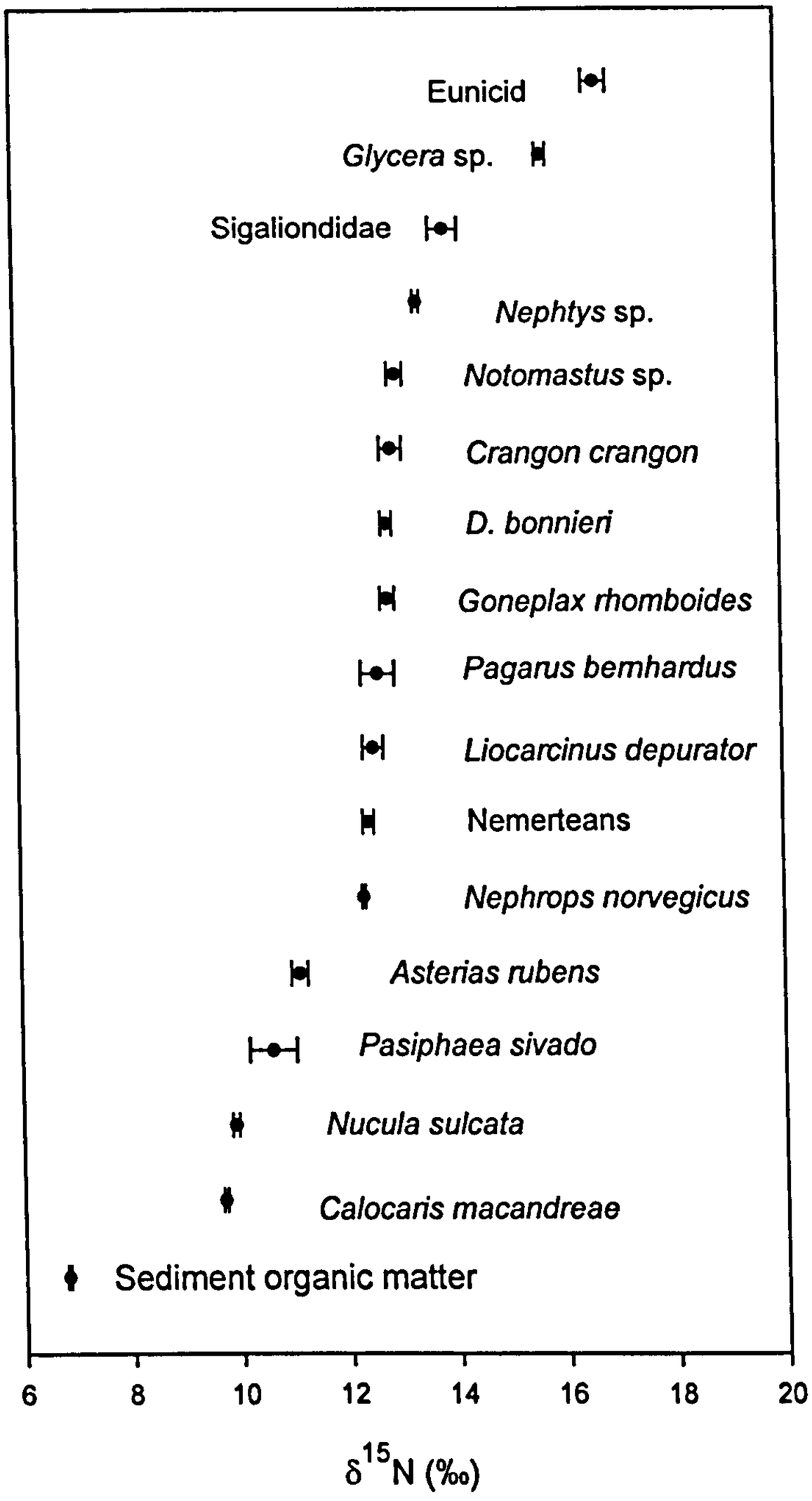


Figure 5.9 Trophic continuum of benthic organisms from S38A in the western Irish Sea from nitrogen stable isotope analysis (means ± SE).

The $\delta^{15}N$ and realised trophic levels of the organisms collected from S38A are consistent with the literature findings of prey items of the species of interest and the known availability of prey at S38A. The idea of animals fitting into rigidly defined trophic levels, however, has been questioned, particularly because of the prevalence of omnivory in many organisms (e.g. France et al., 1998). Although there was a continuum of trophic enrichment, with a range of $\delta^{15}N$ values from 9.7 ‰ to 16.7 ‰, there does appear to be a distinct trophic structure with three trophic levels (Fig. 5.9). For example, there are several species in TL3, with slightly different $\delta^{15}N$ values suggesting small differences in diet. In particular, however, there was a distinct shift in $\delta^{15}N$ values between benthic deposit feeders and the large group of benthic predators. Thus, it appears that the benthic food chain in this region of the western Irish Sea, where species diversity is low, is fairly simple and some distinct trophic levels are evident.

Temporal variation in stable isotope values of organisms may reflect changes in diet, physiological condition or ontogenetic changes. For example (and as previously discussed), the rapid decline in the $\delta^{13}C$ values of *Nucula sulcata* during the spring bloom, probably reflects assimilation of recently deposited low $\delta^{13}C$ phytoplankton detritus. There were, however, no clear seasonal patterns for any of the other species observed. In particular, the absence of a similar change in the $\delta^{13}C$ of the muscle tissue of *Calocaris macandreae,* despite the same diet as *N. sulcata*, is interesting. This may be due to differences in tissue turnover time so that short-term changes in diet are not reflected in the muscle tissue of *C. macandreae*. The much higher ratio of carbon to total wet biomass, of 0.08 in *C. macandreae* (compared with 0.02 for *N. sulcata*) suggests this may be the case.

Similarly, temporal variation in isotope values for the other organisms may be because of the difference in tissue turnover times between animals. Further research into the seasonal patterns in isotope values for individual species will need to consider the sampling time scale required to reflect tissue turnover times, selection of the tissue type for investigation and a better understanding of the temporal changes in the abundance of prey items.

Carbon in the benthos

The stable isotope values of particulate organic carbon in the water column, as presented in Chapter Four, indicated that the supply of carbon to the benthos in the western Irish Sea comes from production in the overlying water column. The carbon stable isotope values of the sediment organic matter are fairly typical for marine sediments (see Table 4.3 in Chapter Four). The fractionation in $\delta^{13}C$ values of ~2 ‰ between the particulate organic matter reaching the benthos and the organic matter in the sediment was also found to be fairly typical for marine sediments and thought to be due to the contribution of bacterial and meiofaunal biomass and in particular enrichment during bacterial respiration.

Carbon isotopes generally show little fractionation (0-1 ‰) between consumers and their diets, and so can be used to test the idea that the organic carbon in the benthos supplies the benthic food web. There was, however, a significant enrichment in ^{13}C between the organic matter (OM) in the sediment and the benthic macrofauna. For example, the deposit feeders *Nucula sulcata* and *Calocaris macandreae* were almost 5 ‰ enriched compared with the sediment OM on which they feed, which significantly exceeds the average ~1.0 ‰ enrichment usually observed between trophic levels (DeNiro and Epstein, 1978). This enrichment in $\delta^{13}C$ from sediment organic matter to benthic organisms appears to be common in marine (and freshwater) systems (Table 5.8). For example, in the Bering Sea, the

Table 5.8 Literature values of carbon stable isotope values for pelagic particulate organic matter (POM), sediment organic matter (SOM) and benthic deposit feeders from a variety of marine studies to show the magnitude of enrichment in $\delta^{13}C$ from pelagic to benthic (P - B) systems.

Location	$\delta^{13}C$ POM (‰)	$\delta^{13}C$ SOM (‰)	$\delta^{13}C$ deposit feeders (‰)	Deposit feeding organisms	$\delta^{13}C$ enrichment P – B (‰)	Reference
Western Irish Sea	-24.5	-22.7	-17.5	Bivalve – *Nucula sulcata* Crustacean – *Calocaris macandreae*	7.1	This study
Bay of Biscay	-23.8		-16.0	Bivalve – *Nucula sulcata*	7.8	(Le Loc'h and Hily, 2005)
Arctic, (NEW Polynya)	-27.7	-23.5	-21.3 to -18.0	Bivalve – *Astarte crenata* Echinoderms – brittle stars	6.4 to 9.7	(Hobson et al., 1995)
Estuarine mud flats, Netherlands	-22 to - 20	-23.0	-16.5	Polychaete – *Arenicola* sp.	3.5 to 5.5	(Herman et al., 2000)
Porcupine Abyssal Plain	-21.9		-16.5	Average of several phyla	5.4	(Iken et al., 2001)
Bering Sea	-24.4	-22.9	-18.7 to -17.3	Bivalves – *Macoma* sp. and *Yoldia* sp.	5.7 to 7.1	(McConnaughey and McRoy, 1979)
Pacific, SE Alaska	-20.3	-20.8	-17.7	Bivalves – *Macoma* sp. and *Yoldia* sp.	2.6	(Goering et al., 1990)

$\delta^{13}C$ of the deposit feeding bivalve *Yoldia limatulata* was 4 to 5 ‰ enriched over sediment OM (McConnaughey and McRoy, 1979). There were similarly large shifts observed in the *Nephrops norvegicus/Merluccius merluccius* fishing grounds of the Bay of Biscay (Le Loc'h and Hily, 2005), the Arctic (Hobson et al., 1995; Hobson et al., 2002), the Georges Bank (Fry, 1988), and estuarine mud flats in the Netherlands (Herman et al., 2000). This enrichment is generally seen in detrital systems and so is often attributed to the presence of a strong microbial loop, maybe with larger fractionation by bacteria, in which carbon is first partially broken down by microorganisms and meiofauna before being consumed by deposit feeders. Stronger isotope fractionation by deposit feeders has also been suggested as a possible mechanism. These ideas are discussed in more detail below.

Detrital food for marine deposit feeders, such as *C. macandreae* and *N. sulcata*, is generally considered of poor quality because it is bound up in a matrix of sediment and has a large component of indigestible refractory organic carbon (Lopez and Levinton, 1987). The refractory nature of the organic matter is generally believed to be the result of the leaching of soluble organic compounds (Karl et al., 1988) and microbial mineralisation as particulate organic matter settles through the water column (Heip et al., 1995 and references cited therein), reducing both the quantity and quality of the carbon. Thus, by the time organic matter reaches the benthos much of the labile fraction has been removed leaving refractory components such as cellulose and chitin that are of little direct food value to benthic animals (e.g. Levin et al., 1999).

Several studies have shown that benthic marine detritus provides a poor quality food supply for benthic organisms. Nedwell (1987), for example, used a microbial assay to

estimate that a maximum of 1 % of the total organic carbon in the top 1 cm of sediment in a salt-marsh was available for microbial breakdown. Other studies have measured the composition of organic matter, and in particular the proportion of hydrolysed proteins and carbohydrates that are believed to be available to deposit feeders (Mayer et al., 1995). Results from such studies include 14 % of carbon in an intertidal mud-flat (George, 1964) and 10 % of detritus in the organically rich sediments of the Porcupine Abyssal Plain (Danovaro et al., 2001) were estimated to be available as food for deposit feeders. In the western Irish Sea, however, there is no indication of a change in the quality of organic matter as it travels through the water column. Although the overall percentage of carbon and nitrogen in organic matter declines with depth (Chapter Four), the C:N ratio of the organic matter increased only marginally between the surface and the bottom water and the $\delta^{13}C$ value did not change with sinking. Heip et al. (1995) report that C:N ratios of particulate material are usually lower than those of bottom sediments due to preferential consumption of nitrogen-rich components. In the Irish Sea, however, the opposite is the case, with lower C:N values in the sediment compared with particulate organic matter. A decrease in the C:N ratio may indicate the accumulation of nitrogen in bacteria (Suess and Mueller, 1980 and vanDuyl et al., 1993 both in Heip et al., 1995). However, further analysis of the composition of the organic matter in the sediment in the western Irish Sea would be needed to determine how much is available to the deposit feeders.

The scarcity of bioavailable carbon for benthic organisms has led to much discussion regarding the potential food sources for deposit feeders, including microbes (bacteria, microalgae, protozoans, fungi), meiofauna, and nonliving detrital organic matter (Lopez and Levinton, 1987). The assimilation efficiencies of deposit feeders feeding on bacteria were found to be much higher than for nonliving fraction of organic matter (Lopez and

Levinton, 1987 and references cited therein) which led to a view that microbial biomass was a more important food source than the non-living organic matter (e.g. Sokolova, 1997). However, although shelf sediments may have high densities of bacteria (up to 10^{11} ml^{-1}, Barnes and Hughes, 1999) bacterial carbon accounts for less than 2 % of total carbon in typical muddy sediments and is insufficient to meet the energetic needs of most deposit feeders (Cammen, 1980; Rublee, 1982; Lopez and Levinton, 1987; Barnes and Hughes, 1999). For example, in an intertidal study in the Wadden Sea the biomass of bacteria ranged from 70 - 350 mg C m^{-2}. In an *in-situ* ^{13}C labelling study in marine intertidal sediments van Oevelen et al. (2006) found that no more than 10 to 15 % of total carbon demands of benthic fauna was met by bacterial carbon. Work by Cammen (1989) on the energetic needs of deposit feeders showed that assimilation efficiencies of between 3.2 % and 5.5 % of ingested detritus was enough to satisfy the needs for respiration of most deposit feeders and 4-13 % would allow for growth. Lopez and Levinton (1987) concluded that most deposit feeders require both living and non-living organic fractions, and that, whilst microbes may be an important source of protein, they are not present in sufficient biomass to meet the total calorific needs of benthic organisms. However, in species such as *Calocaris macandreae* bacterial carbon may be more important because burrow walls of a number of species have been shown to have increased microbial populations (Atkinson and Taylor, 2005).

Several researchers have shown the importance of freshly deposited organic material for the growth of benthic deposit feeders and the direct incorporation of this material into animal tissue (Heip et al., 1995 and references cited therein). In many organisms, reproductive cycles are timed to benefit from the seasonal supply of fresh detritus arriving on the seabed. However, deposit feeders are in direct competition for food with the microbial community and so recently arrived fresh material is rapidly assimilated (Graf et

al., 1982). The rapid decline in the $\delta^{13}C$ values for *Nucula sulcata* in April and May (from around -17 ‰ to a minimum of -18.7 ‰), suggests rapid assimilation of organic matter recently arrived from the pelagic zone, which had a $\delta^{13}C$ value of ~-24 ‰ (see Table 4.5 in Chapter Four), into bivalve tissues. The incorporation of ^{13}C labelled algae into the tissues of small bivalves *Macoma baltica*, within a few days, in an *in-situ* ^{13}C labelling experiment in the Westerschelde estuary (Herman et al., 2000) indicates that rapid diet-induced changes in $\delta^{13}C$ are possible. Further evidence of rapid assimilation of recently arrived detritus, comes from the observed increase in the glycogen and lipid content in the tissues of a surface feeding clam, in response to a phytoplankton bloom (Graf et al., 1982; Graf et al., 1983). Thus, the rapid decrease in *N. sulcata* $\delta^{13}C$ values is likely to be a response to the short-lived flux of primary production from the overlying water column.

However, fresh detrital matter is utilised very rapidly, often accompanied by the rapid increase in sediment oxygen demand (e.g. the post-bloom response in the western Irish Sea oberved by Trimmer et al., 1999) as fresh input is utilised by microbes, meiofauna and macrofauna. The shortlived decrease in $\delta^{13}C$ values of *N. sulcata*, in response to the flux of pelagic production is a reflection of this. The food supply that remains after the utilisation of fresh input is more refractory carbon.

Although this refractory, non-living detrital matter is considered the primary food source for deposit feeders, it is the microbial catalysis, including partial digestion of detritus, which makes it more readily available to deposit feeders (Lopez and Levinton, 1987), that may explain the shift in $\delta^{13}C$ values from detritus to deposit feeders. During respiration, the loss of ^{13}C deplete CO_2 leaves tissues enriched in ^{13}C, increasing the $\delta^{13}C$ value of the carbon detritus. There is also evidence to suggest that the CO_2 respired by organisms such

as bacteria and foraminifera is more deplete in ^{13}C than that respired by metazoan animals (McConnaughey and McRoy, 1979; Moodley et al., 2002) which would result in a greater difference in $\delta^{13}C$ between organic matter and bacteria.

Thus, when fresh detrital supplies have been depleted, the carbon assimilated by deposit feeders is likely to be enriched in ^{13}C by more than the expected ~1 ‰ seen between a consumer and its diet resulting in the large shift in $\delta^{13}C$ values from the sediment to benthic organisms. Selective feeding, often based on particle size, may also be a means by which deposit feeders preferentially ingest ^{13}C enriched, less refractory carbon. The bivalves *Yoldia limatula* for example, rejects approximately 95 % of the sediment collected by the palp tentacles (Bender and Davis, 1984). The ^{13}C enriched carbon of the deposit feeders is then transferred to the remainder of the benthic food web leading to the clear separation between pelagic and benthic carbon isotopes in detrital systems.

Quantifying carbon transfer to the benthos

One of the aims of the study was to estimate carbon flux from primary production to the benthos and then the flux of carbon through secondary production in the benthos. Species were analysed for their total carbon content and accordingly the carbon content of all the faunal biomass calculated. The total macrofaunal carbon biomass at S38A was estimated to be 1.3 g C m^{-2}. Using empirical relationships from other benthic studies it is possible to provide some comparative estimates of benthic macrofaunal biomass based on the magnitude of primary production and the depth of the water column. For example, in a meta-analysis of data from a range of well-mixed estuarine systems Herman et al. (1999) found a linear relationship ($r^2 = 0.77$) between benthic biomass (B) and pelagic primary

production (P) given by:

$$B = -1.5 + 0.105P \tag{5.2}$$

where biomass is measured as grams of ash-free dry weight m^{-2} (g AFDW m^{-2}) and primary production as g C m^{-2}. In the western Irish Sea the 2004 spring bloom production was estimated to be 24.8 g C m^{-2} and for the whole production season (April - September) was 51.1 g C m^{-2}. Although flux to the benthos, discussed in Chapter Four, was calculated as a result of vertical flux during the spring bloom there is likely to have been deposition at other times and so seasonal production is included to give an upper estimate. Using equation 5.2, for the spring bloom and seasonal estimates, the biomass (AFDW) of benthic macrofauna was estimated to be between 2.2 and 3.9 g m^{-2}. This relationship is, however, based on data from shallow estuarine systems and as the work in Chapter Four showed the carbon content of particulate organic matter declines with depth. It is necessary, therefore, to adjust the benthic macrofauna estimate to reflect the depth of the water column (93 m) at S38A. There was a loss of 40 % of the organic carbon as organic matter travelled from the middle of the euphotic zone (11 m) to the bottom water (85 m). Assuming an average depth in the Herman *et al.* meta-analysis (a mixture of estuaries and coastal bays) to be 30 m, organic matter in the western Irish Sea has to sink an additional ~60 m to the benthos, during which time carbon loss is estimated to be ~30 % (see Fig. 4.4). Thus, after sinking an additional 63 m, only 70 % of the original organic matter would reach the sediment, reducing the estimate of benthic biomass to 1.5 – 2.7 g m^{-2}. Taking a carbon: AFDW ratio of 0.5 (Heip et al., 1995) gives a final carbon macrofaunal biomass of 0.8 – 1.4 g C m^{-2} compared with the estimate of 1.3 g C m^{-2} from measurement of the actual biomass of macrofauna. Therefore, the actual faunal biomass in the benthos

is similar to what would be predicted on the basis of previously established links between primary production in surface waters and depth of the water column. The value is also similar to that measured in the North Sea, of 0.6 – 1.6 g C m^{-2} measured by Steele (1974).

The flux of energy in the benthos can also be explored by considering the biomass, in terms of carbon, that is removed from the benthos by the *Nephrops norvegicus* fishery each year and using the concept of the Eltonian pyramid and transfer efficiencies to estimate the input of carbon required to sustain it. The latest ICES estimate of the total annual landings (including Northern Ireland (~8000 t), other UK and International landings but excluding undeclared 'black' landings) from the western Irish Sea is 15,242 t (15.2 x 10^6 kg) (ICES, 2006). Applying a factor of 0.23 for conversion of wet to dry weight and a factor of 0.4 from dry weight to carbon (established from analysis of *N. norvegicus* individuals of carapace size 25 mm in the current study) gives a total carbon biomass of 1.4 x 10^6 kg (15.2 x 10^6 x 0.23 x 0.40) of *N. norvegicus* removed from the western Irish Sea each year. The area of the western Irish Sea that supports the *N. norvegicus* fishery is 5,791 km^2 (Irish Marine Institute web site: www.marine.ie). Thus, the fishery removes 242 kg C km^{-2} (1.4 x 10^6 kg C divided by an area of 5791 km^2) or 0.24 g C m^{-2} y^{-1}.

The nitrogen stable isotope analysis showed that *Nephrops norvegicus* is in the third trophic level in the benthos, feeding on the second trophic level of deposit feeders that utilise the base resource (TL1) of organic matter in the sediment. To estimate the carbon needed to support the removal of 0.24 g C m^{-2} y^{-1} of *Nephrops norvegicus* the transfer of energy between these trophic levels must be estimated. Transfer efficiencies between trophic levels take into account the loss of energy or organic matter because some food is

ingested and not assimilated, and most assimilated carbon is lost through respiration and excretion. Pauly and Christensen (1995 and the references cited therein) synthesised data from 48 trophic models of aquatic ecosystems, and found that transfer efficiencies ranged from 2 to 24 % but that the mean of a total of 140 estimates was 10 %.

Using transfer efficiencies it is, therefore, possible to consider the carbon required at each trophic level need to support the annual removal of *Nephrops norvegicus* biomass. For example, at 10 % transfer efficiency 0.24 g C m^{-2} y^{-1} of *N. norvegicus* (the amount removed by the fishery) would have resulted from the transfer of energy from 2.4 g C m^{-2} y^{-1} of deposit feeders. Of this 90 % would be lost to respiration and excretion leaving only 10 % or 0.24 g C m^{-2} y^{-1} for growth of *N. norvegicus* biomass. Similarly, at a transfer efficiency of 10 % the 2.4 g of deposit feeders would result from eating 24 g C m^{-2} y^{-1} from the sediment. From the range of transfer efficiencies found in aquatic systems by Pauly and Christensen (1995), it is possible to consider the carbon input required to support the removal of 0.24 g C m^{-2} y^{-1} of *N. norvegicus* by the fishery for a range of different transfer scenarios (Table 5.10). Only at a transfer efficiency between each trophic level, of 11 % to 16 %, would the input of carbon to the benthos be enough to support removal by the fishery. For the deposition of spring bloom production only (for which we have evidence of deposition – see Chapter Four) the transfer efficiency is the higher estimate. The $\delta^{15}N$ trophic enrichment found in the benthic food chain was closer to 3 ‰ than to the 3.4 ‰ average from many previous studies. Although this may represent some omnivory, which is expected even though the diversity of prey items appears limited, lower trophic fractionations can result from higher assimilation efficiencies. Lower trophic fractionations have been noted for invertebrates (McCutchan Jr. et al., 2003) so transfer efficiencies in this system may be as high as 16 %. Such high

Table 5.10 Estimates of carbon flux to the benthos required to support 0.24 g C m^{-2} y^{-1} of *Nephrops norvegicus* biomass removed by the fishery at different transfer efficiencies and estimates of actual primary production and flux to the benthos. Full details of spring bloom and seasonal production (April – September) found in Chapter Two.

	Transfer efficiency			
Carbon (g C m^{-2} y^{-1})	**3 %**	**11 %**	**16 %**	**20 %**
Flux to benthos required	267	19.8	9.9	6
Estimated flux to benthos				
From spring bloom production		9.9		
From seasonal production		17.9		
Estimated primary production				
Spring bloom primary production		24.8		
Seasonal primary production		51.1		

transfer efficiencies were also estimated by Steele (1974) in the North Sea who concluded that the yield of commercial fish was high in terms of the food web on which it was based.

The data suggest that the carbon input to and carbon output from the western Irish Sea benthos is similar indicating that production of *Nephrops norvegicus* may be vulnerable to a decline in the supply of carbon. Climate warming has caused a decline in primary production and the flux of organic matter to the benthos in some marine systems (Fulweiler et al., 2007) and should similar impacts occur in the western Irish Sea, a subsequent decline in the flux of carbon to the benthos will result in a shortfall in the supply of carbon needed to support the current catch rate of the *Nephrops norvegicus* fishery. An understanding of this balance between the carbon input and output may be important to the long term management of the *N. norvegicus* fishery in the western Irish Sea.

Using realised trophic levels, determined by the $\delta^{15}N$ analysis, it is also possible to investigate the trophic patterns of macrofaunal biomass in the western Irish Sea. Macrofaunal biomass data were aggregated to trophic level: deposit feeders (TL2), primary predators (TL3), and secondary predators (TL4), to give a total carbon biomass for each trophic level of 0.57 g C m^{-2}, 0.47 g C m^{-2} and 0.27 g C m^{-2} respectively. The carbon biomass decreases with increasing trophic level as expected from the Elton pyramid but the transfer efficiencies estimated with this data are very high: 82 % between TL2 and TL3 and 57 % between TL3 and TL4.

The general relationship between trophic levels and community structure was clarified by Elton (1927), who recognised the importance of the abundance, biomass and size of animals in an ecosystem. In particular, the description of food webs or ecological systems

in energetic terms has been useful for ecological research. This loss of energy in the transfer of organic matter from one trophic level to the next, dictates that the weight of all predators must always be much lower than that of the prey items and the total weight of the prey items much lower than that of plant (or detrital) production. The resulting arrangement of biomass (and indeed sizes and abundance) is commonly known as the 'Eltonian pyramid' (Elton, 1927).

The high transfer efficiencies estimated from biomass of benthic organisms indicates there may be some bias and under-sampling introduced by the sampling methods used. In particular, the burrowing species *N. norvegicus* and *Calocaris macandreae*, are probably under-sampled. The sampling depth of the Day grab, on which most of the benthic biomass data is based, is only 10 cm. The burrows of *N. norvegicus* are known to extend to at least 30 cm (Rice and Chapman, 1971) and bioturbation in the mud channel of the western Irish Sea has been observed to extend down to 55 cm (Kershaw, 1986). Grabs can also be ineffective for fast moving infaunal organisms, such as *N. norvegicus* and *C. macandreae*, and the small sample size requires many replicates to be taken. Also, the absence of small polychaetes collected in the current study suggests there may be incomplete sampling of the macro-invertebrate community. It is possible that small surface dwelling polychaetes were scoured away by bow waves created from the landing of the box corer on the sediment surface. The box corer, which penetrates to a depth of 50 cm is likely to sample burrowing organisms more effectively. Unfortunately, there were only two box cores collected. Small beam trawls are an often used sampling method for benthic studies to sample the upper layer of sediment, collecting slow-moving or shallow burrowers. However, to limit clogging of the net, particularly in muddy habitats as in much of the western Irish Sea, the mesh size was a minimum of 10 mm, which may

have under-sampled the smaller organisms. For example, *Calocaris macandreae* is up to 30 mm in length but is only about ~5 mm in diameter, and the bivalve, *Nucula sulcata*, is also small (10 mm x 10 mm) and so losses from the cod end of the trawl net may be high. There also may be some sampling bias. For example, *N. sulcata* is a sub-surface deposit feeder and it is possible that some buried animals are not captured using the beam trawl. There was a distinct bias in the number of live to dead shells in the samples (at least one to fifty) that may represent sampling bias of only those shells on the surface, although it may also be due to the persistence of dead shells in the sediment. However, a beam trawl can undertake sampling over a much wide area than grabs and also reduces the effects of small-scale heterogeneity and so the results are more representative of general patterns. Nevertheless, the sampling methods employed in the current study (and as used in other studies) appears to have undersampled, particularly the deposit feeders which would account for the low biomass (in comparison to *N. norvegicus*) at this trophic level, and burrowing organisms such as *N. norvegicus*. A more intensive sampling programme would be needed to improve the estimates of benthic biomass made here.

Ontogenetic shift in the diet of *Nephrops norvegicus*

Many species change their diet as they develop and grow. These ontogenetic changes are often discrete and may coincide with metamorphosis (e.g. amphibians), but more gradual shifts in diet also occur during growth (e.g. see Hentschel, 1998). The life cycle of *Nephrops norvegicus* is characterised by increasing body size throughout its life, but there is also a significant two-way shift between a pelagic and a benthic phase with the release of a pelagic larva and the metamorphosis of the pelagic larvae to a benthic juvenile and eventually to a benthic adult. Thus, in addition to changes in prey determined by body size the diet of *N. norvegicus* naturally shifts between pelagic and benthic prey during its

development. This shift is clearly reflected in the significant changes in stable isotope values between life cycle stages (Fig. 5.1).

The high $\delta^{13}C$ values of benthic adult *N. norvegicus* compared with the pelagic larvae stage is typical of the enrichment in ^{13}C seen from pelagic to benthic consumers, as previously discussed. The adult $\delta^{13}C$ value of -16.3 ‰ reflects its benthic existence and the dependence of the benthic food web on a detrital energy source. The eggs of *N. norvegicus*, which are carried on the abdomen of the female (for a period which depends on latitude and in Irish Sea populations is 9 months (Oakley, 1978)) before the larvae are released into the water column, have a $\delta^{13}C$ value of -19.3 ‰, half way between the benthic and pelagic phases of *N. norvigcus*' life cycle. This depletion of ^{13}C, however, is a reflection of difference in biochemical composition between adults and eggs rather than a shift in diet. *N. norvegicus* produces large eggs containing a high level of lipids, necessary for embryonic development (Rosa et al., 2003; Rosa and Nunes, 2003). Lipids are known to be deplete in ^{13}C, by ~6 ‰ relative to protein (McConnaughey and McRoy, 1979) and ~3 ‰ relative to muscle tissue (Tieszen et al., 1983) and so the eggs have a lower $\delta^{13}C$ value compared with the parent. The stable isotope values of the early larvae are similar to the egg but rapidly start to reflect the $\delta^{15}N$ values of the diet when the larvae start feeding (see Chapter 3).

The larvae of *N. norvegicus* are carnivorous, feeding predominantly on copepods and other pelagic crustacean zooplankton (see gut content analysis Chapter 3). Assimilation of the low $\delta^{13}C$ zooplankton diet, into the tissues of the rapidly developing larvae results in falling $\delta^{13}C$ values as the larvae grow. There was a further drop in $\delta^{13}C$ values of the larvae after the first moult when organic matter containing the isotope signature of

maternally derived tissue is lost. Thus, by the final stage of larval development, stage III, the larvae had been feeding in the water column for about 6 weeks and were likely to be in equilibrium with their diet. The $\delta^{13}C$ value was low (-21.6 ‰), reflecting a pelagic existence, with an 0.8 ‰ enrichment in $\delta^{13}C$ from prey (-22.4 ‰) to consumer.

The carbon isotope value of the smallest juvenile *Nephrops norvegicus* collected, of carapace length (CL) 11 mm, was -17.8 ‰, significantly heavier than larval values and almost back to the benthic adult values. After settlement to the benthos, *N. norvegicus* juveniles grow rapidly and attain a mean CL of 14 mm after one year, during which time they may have moulted up to ten times (Farmer, 1973). The change in diet on settlement, from pelagic to benthic prey, is quickly reflected in juvenile tissues, due to rapid growth coupled with loss of tissue through repeated moultings, so that within a year the juvenile $\delta^{13}C$ is very close to that of the adult. The capture of very small juvenile *N. norvegicus* from the field has proved extremely difficult in this and other studies (e.g. Spicer and Eriksson, 2003) and so there are no data for *N. norvegicus* in the under 11 mm carapace length size range. The small catch numbers and the general absence of juveniles in the gut contents of fish known to prey on *N. norvegicus* indicates that they rarely emerge from their burrows in the first year of life (Chapman, 1980). Polyester resin casts of *N. norvegicus* burrows have revealed often complex systems, with different sized ventilated tunnels, suggesting the juveniles inhabit small side branches in the adult burrows (Rice and Chapman, 1971). It is during this time that the enrichment in juvenile $\delta^{13}C$ values, from pelagic to benthic 'signature' would have occurred.

The nitrogen stable isotope values of *N. norvegicus* show similar changes between life cycle stages, which become increasingly deplete in ^{15}N from adults to eggs and from eggs

to larvae. The biochemical differences in the composition of eggs compared with muscle tissue, responsible for low $\delta^{13}C$ values, is probably also responsible for the shift in $\delta^{15}N$ from 12.3 ‰ of adults to 10.9 ‰ of eggs. The absence of a change in the isotope values of eggs between November and April, when the larvae are developing and growing, indicates that the original composition of the eggs determined the carbon and nitrogen isotope values.

The 3.2 ‰ decline in $\delta^{15}N$ from adults to stage III larvae is indicative of an ontogenetic change in the diet between adults and larvae. On the basis of the generally accepted ^{15}N enrichment of 3.4 ‰ between prey and consumer (Minagawa and Wada, 1984) this appears to represent a decrease of 1 trophic level from adults to larvae. However, gut content analysis has revealed that the larvae occupy the third trophic level (feeding on primary consumers, i.e. herbivores), the same trophic position as the adults in the benthos. The difference in the $\delta^{15}N$ values of the same trophic level in the two phases of the marine environment, pelagic and benthic, results from a much smaller enrichment in ^{15}N in the pelagic zone, particularly between the base resource (POM) and zooplankton. Although the trophic enrichment factor of 3.4 ‰ has proved fairly robust for a range of consumers McCutchan et al (2003) analysed data from a range of studies where consumers were raised on controlled diets. They found significant variation in $\delta^{15}N$ depending on diet of consumers with lower enrichment (2.2 ‰) on an algal diet compared with a high protein diet (3.3 ‰). Therefore, an understanding of the potential for variation in ^{15}N fractionation and 'baseline' values is required for the successful interpretation of isotope values in assigning trophic levels. It also shows the importance of combining gut content analysis and stable isotope analysis for the elucidation of food web relationships. Combining both techniques has shown that *N. norvegicus* is a secondary consumer in both the larval and

adult phase of its life cycle. Thus, when the post-larvae adopts the benthic habit, juvenile $\delta^{15}N$ values increase, reflecting the difference in the isotope structure between the pelagic and benthic system rather than an actual shift in the trophic level of prey items.

Analysis of isotope values of *Nephrops norvegicus* adults also indicates that diet changes with size. Both $\delta^{13}C$ and $\delta^{15}N$ increase as *N. norvegicus* gets larger. The diet of many species changes through their life cycle, particularly as body size determines potential prey (Cohen et al., 1993) and so larger individuals are expected to feed at higher trophic levels. From juveniles to the largest animals (11 – 49 mm) $\delta^{13}C$ increased from ~-18 ‰ to ~-16 ‰ and reflects a move away from the low $\delta^{13}C$ of juvenile tissues assimilated from pelagic prey towards benthic food sources. A similar increase in $\delta^{13}C$ values of *N. norvegicus* with size was found in a study of the fishing grounds in the Bay of Biscay (Le Loc'h and Hily, 2005), which the authors attributed solely to changes in diet from a mixture of pelagic and benthic prey in juveniles to a strictly benthic diet in adults. The juveniles are, however, thought to lead a predominantly burrow bound existence in the first year so juveniles may switch to a fully benthic diet as soon as they settle on the seabed. In this case, the decline in $\delta^{13}C$ values may reflect the continued loss of the maternal signature, first observed in the larvae. However, $\delta^{13}C$ values do increase across the whole size range of animals observed so there may be an increasing dependence on benthic animals with increasing size.

The increase in $\delta^{15}N$, and therefore trophic level, with body mass for *Nephrops norvegicus* is consistent with the idea that body size determines potential prey (Cohen et al., 1993). However, the increase in $\delta^{15}N$ from 11.9 ‰ to 13.1 ‰ for the smallest to the largest animals observed is fairly small, indicating *N. norvegicus* at S38A remains close to the

third trophic level (secondary consumer) throughout its life. The increase of 1.2 ‰ from juvenile to the largest adults, which are probably 6-7 years old, is an increase of only a third of a trophic level and does not represent a large shift in prey items. Studies of the gut content of *N. norvegicus*, from a range of different habitats, have shown the species to be an opportunistic predator and that diet generally reflects prey availability (Gual-Frau and Gallardo-Cabello, 1988; Cristo, 1998; Cristo and Cartes, 1998; Parslow-Williams et al., 2002). This suggests that the range of prey items available to *N. norvegicus* of all sizes, in sufficient biomass to be an important component of the diet, is fairly low. The macrofauna biomass data from S38A supports this idea, with only the deposit feeders *Calocaris macandreae* and *Nucula sulcata*, plus various polychaetes, present in any significant biomass. The $\delta^{15}N$ of *N. norvegicus*, at 3 ‰ above that of the deposit feeders, is a strong indication that these species are particularly important prey items throughout the benthic stage, regardless of size. It also discounts the suggestion of Loo et al. (1993) that *N. norvegicus* may directly utilise spring bloom production. In the Bay of Biscay *N. norvegicus* was also found to remain at the third trophic level, with an enrichment in $\delta^{15}N$ of only 0.53 ‰ from smaller to larger animals (size range 14 – 42 mm) (Le Loc'h and Hily, 2005). Although the authors do not present data on the biomass of macroinvertebrates at their study site, there were very few benthic species at a lower trophic level than *N. norvegicus*. Thus, at this site *N. norvegicus* was dependent on a similarly diversity-poor diet of *N. sulcata* and another burrowing shrimp species, *Alpheus glaber*. In contrast, *N. norvegicus* from the Silver Pit area of the North Sea appear to have a much wider range of potential prey items available and the increase in $\delta^{15}N$ with body mass, over a slightly smaller range of body mass (up to 32 g compared with 49 g in the current study) (Jennings et al., 2002), is more than double that seen at the western Irish Sea station. At the North Sea site, several species of decapod crustaceans, echinoderms

and bivalves were present in adequate biomass and with $\delta^{15}N$ values significantly lower than *N. norvegicus* to suggest that they were potential prey items. Thus, the relationship between size and trophic level can, for opportunistic predators like *N. norvegicus*, be a reflection of species diversity, and hence prey availability, in the surrounding habitat.

The significant decline in the $\delta^{15}N$ of *N. norvegicus* muscle tissue during the spring and summer, of ~1 ‰, was not correlated with changes in the values of the putative prey items, *C. macandreae* or *N. sulcata*. The tissue turnover time for the size of animals tested (3 yr old individuals, wet body weight 9-10 g) is unknown, but will be much longer than rapidly growing larvae and juveniles and is probably measured in months rather than weeks. Thus, the temporal changes in $\delta^{15}N$ are not expected to be the result of recent seasonal changes in the $\delta^{15}N$ of the food supply. Investigation of $\delta^{15}N$ values over greater temporal and spatial scales is needed to determine possible sources of variation. Isotope values of muscle tissue are the least variable in comparison to carapace and gut contents because of the longer time averaged assimilation of dietary carbon into muscle tissue. There was, however, a decline in the $\delta^{15}N$ value of gut contents that corresponded to the timing of the production season and a decrease in the $\delta^{15}N$ values of *Nucula sulcata*, which may also be evidence of the importance of this species as a food source for *N. norvegicus*. Several investigations of the diet of *N. norvegicus* noted that there were few changes in diet according to season (Gual-Frau and Gallardo-Cabello, 1988; Cristo, 1998; Cristo and Cartes, 1998).

For most months there was no difference between males and females for either the $\delta^{13}C$ or $\delta^{15}N$ values of muscle tissue and gut contents. Low catch rates of female *N. norvegicus* during the months when they are carrying eggs (Aug/Sep to Apr/May), indicating a

burrow based existence, has led to the suggestion that females are not eating during this time (Rice and Chapman, 1971) or feeding on a more infaunal diet (Oakley, 1978). Starvation is thought to cause $\delta^{15}N$ to rise as ^{14}N is preferentially excreted leaving enrichment of ^{15}N, although the effect will depend on turnover times and so in larger reproductively mature females a response may not be detected in muscle tissue. An increase in $\delta^{15}N$ in starving animals has been observed but is often small. For example, the $\delta^{15}N$ of *Daphnia* sp. starved for 5 days increased by 0.4 ‰ (Adams and Sterner, 2000). Loo et al. (1993) proposed suspension feeding as an explanation for how berried females can survive in their burrows for about 8 months without emerging. If these explanations were correct, however, an observable difference in male and female muscle tissue or gut contents might be expected. Analysis of $\delta^{15}N$ of muscle tissue has shown that *N. sulcata* and *C. macandreae* are important prey for *N. norvegicus* and the burrowing *C. macandreae* in particular may still be eaten by females without them spending a significant amount of time outside their burrows. Several dietary studies of *N. norvegicus* found no difference in gut content between males and females (Gual-Frau and Gallardo-Cabello, 1988). In the Irish Sea Oakley (1978) found that as females mature they appeared to eat more infaunal mollusc material than mobile crustaceans, which fits with the idea that they remain close to their burrows when berried (Bell et al., 2006). Even with such a shift in diet, females will be feeding at the same trophic level as the males and explain why there is no difference in the $\delta^{15}N$ values of males and females.

There were however, differences between the isotope values of carapace between males and females. The $\delta^{13}C$ of female carapace is significantly lower, by 1.1 ‰, than the males. This appears to have no relation to diet. The C:N ratio is also significantly lower in

females and so isotope differences may be related to differences in the biochemical composition of the carapace in males and females.

White muscle tissue is the animal tissue type most commonly used in isotope based studies of food webs, prey items and trophic levels (Harrigan et al., 1989; Tucker et al., 1999; Kaehler et al., 2000). Muscle tissue was selected on the basis of laboratory experiments, in particular with fish, that found it most closely reflected the isotope value of the diet (Pinnegar and Polunin, 1999). In the current study, isotope analysis of *Nephrops norvegicus* muscle tissue and the whole organism showed them to be similar (Table 5.5) and so muscle tissue was considered representative. However, the validity of only using muscle tissue for isotope studies has been questioned by many researchers. In particular, the long tissue turnover times of muscle tissue means that it cannot reflect short term changes in diet. A tissue type such as the hepatopancreas may be more useful to track short-term changes in diet although the high lipid content may obscure the coupling of $\delta^{13}C$ values between diet and tissue. However, stomach content analysis would have shown changes if there had been a significant shift in diet. The inclusion of a fast tissue type, such as the hepatopancreas, may have been useful. For many organisms in the current study, however, it was not possible to just take muscle tissue, either because of small size or the difficulty of just removing muscle tissue (e.g. polychaetes).

Conclusion

The diversity and abundance of organisms at S38A is low compared with less muddy areas in the western Irish Sea. There is a simple food chain in the benthos, with 4 trophic levels. The presence of these relatively distinct trophic levels, in particular the deposit feeders (TL2), and the group of predatory crustaceans including *Nephrops norvegicus* (TL3), indicates the reliance on the deposit feeders as the primary food source of *N. norvegicus*. The difference in $\delta^{15}N$ values of organisms within each TL suggests some variation in diet amongst animals within each trophic level. The large shift in $\delta^{13}C$ from the organic matter in the sediment to the benthic fauna seem common to many detrital based marine food webs. Microbial processing and selective feeding by bivalves is thought to be the reason for the enrichment in $\delta^{13}C$.

Nephrops norvegicus occupies the same trophic level throughout its life, feeding on primary consumers, despite a switch between the pelagic and the benthic marine ecosystem during its development. The larvae are carnivorous, feeding on other crustaceans such as copepods and cladocerans. In the western Irish Sea there appears to be a very low diversity of prey items for adult *N. norvegicus*, and during the benthic stage the diet is dominated by the deposit feeders *Calocaris macandreae* and *Nucula sulcata*. There is only a slight increase in the trophic level of *N. norvegicus* with increasing size, further emphasising the importance of deposit feeders throughout its life and the lack of other species in sufficient biomass to be important prey items. There is no evidence, from the stable isotope analysis, to suggest that the diet of male and female *N. norvegicus* are different, despite apparent differences in behaviour.

The analysis of carbon input, trophic structure in the benthos and fishing levels suggests that the carbon input to and carbon output from the western Irish Sea benthos is similar. This suggests that production of *Nephrops norvegicus* may be vulnerable to a decline in the supply of carbon. Climate warming has caused a decline in primary production and the flux of organic matter to the benthos in some marine systems (Fulweiler et al., 2007) and should similar impacts occur in the western Irish Sea, a subsequent decline in the flux of carbon to the benthos will result in a shortfall in the supply of carbon needed to support the current catch rate of the *Nephrops norvegicus* fishery. An understanding of this balance between the carbon input and output may be important to the long term management of the *N. norvegicus* fishery in the western Irish Sea.

Table 5.9 Diversity and mean abundance of infaunal species at S38A. Abundance data is the average of five NMMP samples from each year from 1999 to 2002 and two samples from 2004 current study. Where the SE is missing there was only a single individual.

Species	Abundance m^{-2} (mean ± SE)			No. of species
Crustaceans				10
Alteutha sp.	0.4	±	0.04	
Calocaris macandreae	8.9	±	0.15	
Cumella pygmaea	0.4	±	0.14	
Eriopisa elongata	0.4	±	0.04	
Eudorella emarginata	2.6	±	0.11	
Jassa sp.	0.4	±	0.04	
Maera loveni	1.1	±	0.13	
Mysidacea	0.4			
Nephrops norvegicus	1.5	±	0.15	
Pseudocuma longicornis	0.7			
Total crustaceans	16.7			
Echinoderms				1
Labidoplax buski	0.4			
Nematodoes	31.9	±	5.47	
Nemerteans	3.3	±	0.20	
Bivalves				1
Nucula sulcata	20.4	±	1.04	
Oligochaetes				1
Tubificoides amplivasatus	10.7	±	0.76	
Polychaetes				20
Abyssoninoe hibernica	1.1			
Ancistrosyllis groenlandica	2.2	±	0.26	
Aricidea laubieri	1.5	±	0.15	
Dasybranchus sp.	1.1	±	0.13	
Eumida sanguinea	0.4			
Glycera sp.	10.7	±	0.48	
Gyptis rosea	1.5	±	0.19	
Glyphohesione klatti	0.4			
Harmothoe impar	0.7	±	0.11	
Levinsenia gracilis	33.0	±	3.08	
Litocorsa stremma	3.3	±	0.28	
Lumbrineris hibernica	2.2	±	0.28	
Mediomastus fragilis	17.8	±	1.09	
Minuspio multibranchiata	1.5	±	0.25	
Monticellina dorsobranchialis	2.2	±	0.17	
Nephtys incisa	4.8	±	0.33	
Ophiodromus flexuosus	1.1	±	0.13	
Panthalis oerstedi	1.1	±	0.13	
Poecilochaetus serpens	0.4			
Prionospio sp.	10.4	±	0.71	
Total polychaetes	97.4			
Total number of animals	**181**			

Chapter Six

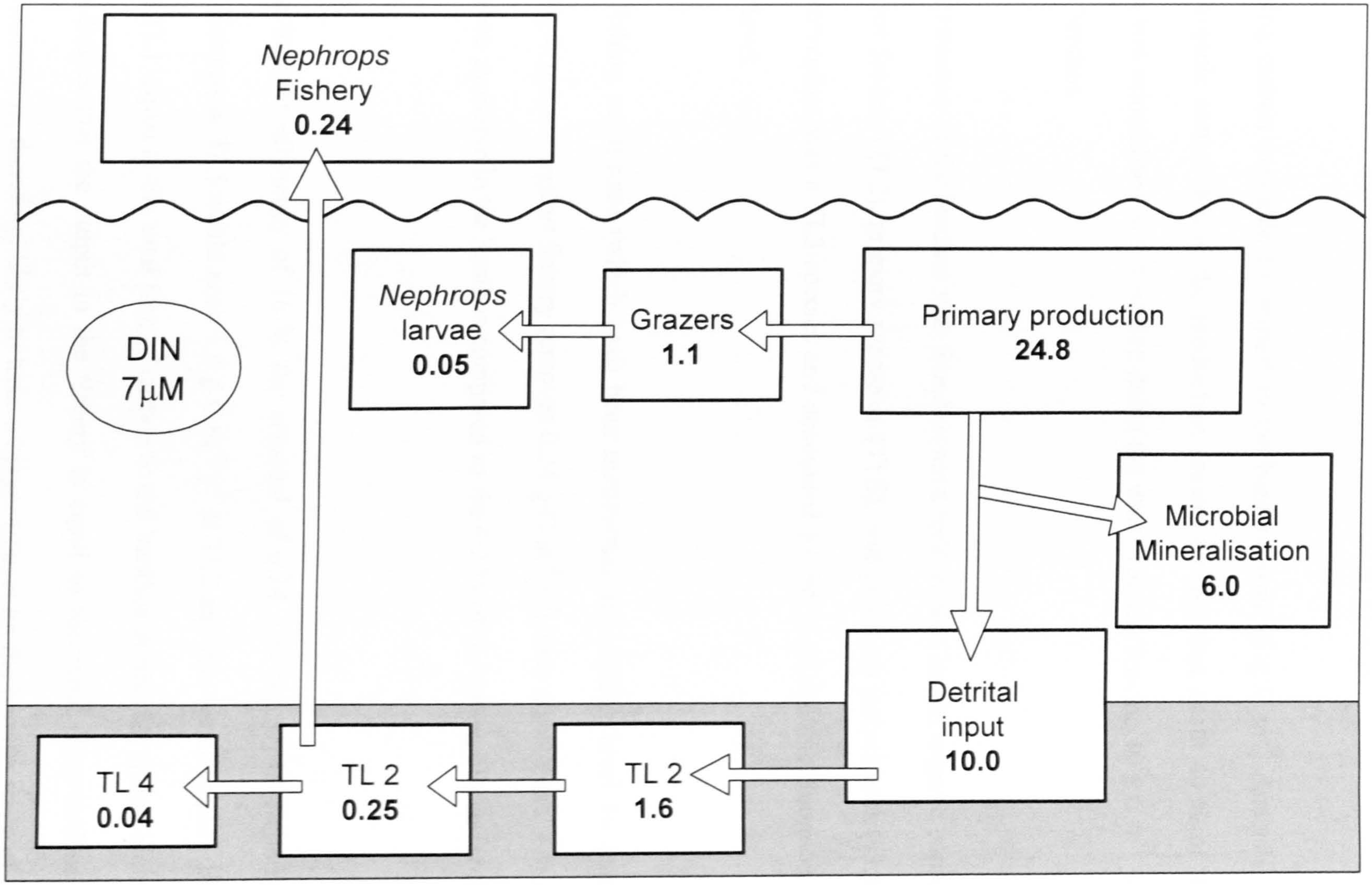
General discussion

The aim of the research presented here was to use stable isotope analysis to describe the structure and flow of carbon and nitrogen to the *Nephrops norvegicus* fishery in the western Irish Sea. The most important finding from this research is that the benthic stage of the *Nephrops norvegicus* life cycle is potentially vulnerable to changes in ecosystem processes that impact on primary production and the supply of organic matter to the benthos. At current fishing levels for *N. norvegicus* the carbon biomass removed from the western Irish Sea benthos is just balanced by the inputs of carbon from primary production in the overlying waters. The findings are summarised below and in Fig. 6.1.

Primary production during the spring bloom of 2004 was estimated to be 24.8 g C m^{-2}, similar to previous estimates. Of this production 80 %, 20 g C m^{-2} was available for export to higher trophic levels, in particular zooplankton grazing and the flux of organic matter to the benthos to fuel secondary production.

The production of zooplankton, calculated from the biomass of zooplankton in the water column during the spring bloom, was estimated to be 1.1 g C m^{-2}. Assuming grazing removed 22 % of the spring bloom production, i.e. 4.1 g C m^{-2}, the transfer efficiency from phytoplankton to herbivorous zooplankton was 27 %. The pelagic larvae of *Nephrops norvegicus* hatched into the water column to coincide with earlier stages of the zooplankton production cycle to feed on a diet of mostly copepods. Total production of the

Figure 6.1 Summary of the structure and flow of carbon to the *Nephrops norvegicus* fishery in the western Irish Sea. All units are g C m^{-2}. Values for primary production, grazers and *N. norvegicus* larvae are production estimates for the spring bloom. Detrital input is the flux of carbon to the benthos from spring bloom production and trophic level (TL) values in the benthos are annual flux of carbon needed to support carbon removal by the fishery assuming a trophic transfer efficiency of 16 %.



larvae was estimated to be 0.05 g C m^{-2}, which represents 4 % of the total available diet and only 0.2 % of spring bloom production.

The remaining carbon available for export to the benthos was 16 g C m^{-2}. Analysis of particulate organic matter during the production season showed that about 40 % of the organic flux was mineralised as it travelled down the water column leaving 10 g C m^{-2} for input to the benthos.

The trophic structure of the benthos was found to have four trophic levels: organic carbon (TL1), deposit feeders (TL2), primary predators (TL3), and predatory polychaetes (TL4). *Nephrops norvegicus* was a TL3 species and accounted for 96 % of the total biomass at this trophic level.

At current fishing catch rates (which have been maintained at a similar level for many years) the *Nephrops norvegicus* fishery removes 0.24 g C m^{-2} y^{-1}. This represents 2.4 % of the total carbon available in the benthos compared to the 0.2 % of the spring bloom for the larvae.

Assuming a transfer efficiency of 16 % the removal of 0.24 g C m^{-2} y^{-1} of *Nephrops norvegicus* biomass at TL3 would need 1.6 g C m^{-2} y^{-1} at TL2 and this would require 10 g C m^{-2} y^{-1} at TL1, equal to the total flux of carbon to the benthos during the spring bloom. The data indicates that the output to the fishery is equal to the input. Any decline in primary production is, therefore, likely to have a bigger impact on the benthic stage of the life cycle than the pelagic.

Climate warming has caused a decline in primary production and the flux of organic matter to the benthos in some marine systems (Fulweiler et al., 2007) and should similar impacts occur in the western Irish Sea, a subsequent decline in the flux of carbon to the benthos will result in a shortfall in the supply of carbon needed to support the current catch rate of the *Nephrops norvegicus* fishery.

This study has shown that stable isotope analysis can be a powerful tool for tracking the flow of organic matter in ecological systems and in describing trophic structure and linkages in both the pelagic and the benthic environment. The high temporal variation in the $\delta^{13}C$ and $\delta^{15}N$ values of organic matter associated with cycles of production is well established in aquatic systems. Although such variation may complicate their direct use as indicators of the base resources in food web studies, in the current work temporal variation has provided a powerful means of tracking the flow of carbon from primary production to higher trophic levels in the water column and the flux of organic matter to the benthos. These seasonal changes in the isotope values of particulate organic matter were large and rapid enough to be able to trace the flow of production through two trophic levels in the water column. The coupling of stable isotopes of production in the water column and flux to the benthos has also provided a means to estimate the speed of deposition and quantify the input of carbon to the benthos.

In contrast, the absence of a temporal change in the stable isotope values of organic matter in the benthos has provided a reliable indicator of the base resource in the benthos of the western Irish Sea. It has, therefore, been possible to determine the trophic structure in the

benthos and hence, the flow of energy through the food chain to *Nephrops norvegicus* and its fishery.

This is the first time that the isotopic signature of primary production in the western Irish Sea have been reported. An interesting finding is that the $\delta^{13}C$ of particulate organic carbon in the western Irish Sea is lower, particularly in the winter months, than that from other oceanic marine systems and this may reflect freshwater influence and long water residence times in the Irish Sea. However, further observations of $\delta^{13}C$ values of organic matter in the western Irish Sea, from a range of areas from coastal to shelf waters, are required to determine if low $\delta^{13}C$ values are due to the contribution of organic matter derived from non-marine sources waters to the western Irish Sea.

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