A thesis entitled

EVALUATING THE ORIGINS AND TRANSFORMATIONS OF ORGANIC MATTER AND DISSOLVED INORGANIC NITROGEN IN TWO CONTRASTING NORTH SEA ESTUARIES

by

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DECLARATION

I certify that the work described in this thesis is my own, except where otherwise stated, and has not been previously submitted for a degree at this, or any other university.

Jason M. E. Ahad

ABSTRACT

In order to delineate the potential sources and to understand the main controls on the biogeochemical cycling of dissolved and particulate organic matter (DOM, POM) and dissolved inorganic nitrogen (DIN) during estuarine mixing, comprehensive seasonal geochemical and isotopic and surveys across the freshwater-tidal interface were carried out in the Tyne and Tweed Estuaries, NE UK. This study provided a contrast between a relatively pristine system (Tweed) with one that is heavily influenced by anthropogenic activity (Tyne).

Geochemical and isotopic (^{13}C, ^{14}C and ^{15}N) analyses demonstrated the predominance of terrigenous organic matter in both these estuaries, with elevated river discharges leading to enhanced terrestrial loading. High pCO_2 values in the Tyne (summer) and Tweed (winter) suggested that a significant fraction of this terrestrially-derived organic matter (both DOM and POM pools) is relatively labile and can potentially undergo significant mineralization during estuarine mixing.

In both estuaries *in situ* processing of DIN was relatively minor, with mixing between different sources being the main factor in controlling the distribution of nitrate and ammonium across the salinity gradient. However, anthropogenic ammonium discharges in the Tyne were found to have an enormous direct and indirect impact on estuarine nitrogen cycling.

Large, concave removals of terrigenous high molecular weight (HMW) DOC caused by flocculation, biodegradation, and/or photochemical oxidation were associated with a non-conservative ^{13}C-enrichment in δ^{13}C signatures. Radiocarbon dates showed an export of young (modern) HMW DOC and old (100-1000s of years), terrigenous POC to the North Sea. ^{14}C-enriched values in coastal North Sea HMW DOC were attributed to anthropogenic discharges originating from within the coastal North Sea environment. In the Tweed, seasonal changes in soil characteristics resulted in an older age for POM during the summer. In the Tyne, decreases in POC% with increasing salinity sometimes coincided with an increase in POC age. This was attributed to mixing with older sediment and to the possible preferential loss of the younger, more labile POC fraction during mineralization.

This study has shown that land use patterns, sewage inputs, and freshwater flushing time are the main influences in determining the behaviour and origin of organic matter and DIN entering the coastal North Sea in these two systems.

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This thesis is dedicated to my dad, Emile, and to my mom, Martha, whom I know would have been very proud to see me complete a PhD.

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CHAPTER 1: Evaluating the sources and biogeochemical cycling of dissolved inorganic nitrogen in two temperate North Sea estuaries using stable isotopes

ABSTRACT

Concentrations and nitrogen isotope ratios of nitrate ($\delta^{15}NO_3^-$) and ammonium ($\delta^{15}NH_4^+$) from the Tyne and Tweed Estuaries, NE England, were analysed during winter and summer periods. During both winter and summer $\delta^{15}NH_4^+$ signatures in the Tyne Estuary were dominated by a source originating from a sewage works located in the lower part of the estuary. While the magnitude of this ammonium plume varied considerably on a temporal basis (32.1-149.2 μM), the $\delta^{15}NH_4^+$ signature of this sewage-derived discharge was remarkably constant (+10.6 ±0.5 ‰) during all four surveys to the Tyne. This sewage signal could be tracked across the estuary and explained much of the variability in $\delta^{15}NH_4^+$ signatures. In the Tweed, ammonium concentrations were relatively low (<7 μM) and $\delta^{15}NH_4^+$ values closely followed concentration weighted mixing between riverine and marine sources. In both the Tyne and Tweed Estuaries most of the variability in nitrate concentrations and $\delta^{15}NO_3^-$ signatures across the salinity gradient could also be explained by mixing between riverine and Coastal North Sea sources. The ability of both isotopic and concentration weighted mixing curves to describe the changes seen in $\delta^{15}NO_3^-$ and $\delta^{15}NH_4^+$ signatures demonstrated very little estuarine processing of dissolved inorganic nitrogen (DIN). Large seasonal differences in the isotopic signature of riverine nitrate were attributed to changes in the relative proportions of different sources. In the Tweed increased contributions from agricultural soil, atmospheric and fertiliser sources during the winter led to a higher nitrate flux and more ^{15}N-depleted $\delta^{15}NO_3^-$ values. In the Tyne, where agricultural inputs are less important, isotopically light nitrate entering the estuary during one winter survey demonstrated the importance of atmospherically-derived sources during periods of high river discharge associated with winter storm events. This study highlights the important role that hydrological conditions, seasonal variability and large estuarial inputs of sewage-derived ammonium can have in controlling the biogeochemical cycling of DIN in temperate estuaries.

INTRODUCTION

Nitrogen (N) is generally considered to be a limiting nutrient in both freshwater and marine ecosystems (Downing, 1997; Hecky and Kilham, 1988; Schindler, 1977; Vitousek and Howarth, 1991). And since the advent of the industrial era, human population increases have lead to significant changes in the amount of terrestrially-derived dissolved inorganic nitrogen (DIN; nitrate, nitrite and ammonium) that enters rivers and estuaries, and this trend is expected to continue in the near future (Galloway et al., 1995). These elevated N inputs enter estuaries either through either riverine transport or via atmospheric deposition, which includes both direct deposition to the surface of estuaries and indirect watershed runoff (Castro and Driscoll, 2002). Anthropogenic N sources carried by overland flow include fertiliser, sewage and industrial discharges, whereas fossil fuel combustion and agricultural production are main contributors to atmospheric N (Galloway et al., 1995). It has been estimated that atmospheric N accounts for at least 26% of the terrestrial input into the North Sea (Rendell et al., 1993). And across the UK, DIN concentrations in wet deposition are relatively high, with precipitation-weighted mean concentrations of nitrate and ammonium approaching levels of up to 40 and 60 μM, respectively (Irwin et al., 2002).

Increased DIN input from anthropogenic sources along the land-sea margin has led to coastal eutrophication, whereby overall increases in primary production are accompanied by major shifts in the dominant flora (Duarte, 1995) and fauna (Heip, 1995) living in estuarine environments. Furthermore, enhanced nitrogen loading has led to a substantial increase in harmful algal blooms along the coastal regions of the U.S. and other parts of the world (Anderson, 1989; Cosper et al., 1989; Hallegraeff, 1993; Smayda, 1990) leading to such phenomena as ‘brown’ or ‘red’ tides. Moreover, elevated ammonium inputs may lead to enhanced N_2O generation in estuarine and coastal zones generated via nitrification (de Wilde and de Bie, 2000; Hashimoto et al., 1999). Increases in the atmospheric concentration of N_2O, which is a very effective greenhouse gas, could have tremendous implications for global climate change (Galloway et al., 1995; Lashof and Ahuja, 1990). Since biogeochemical processes within estuaries will transform, remove and add to the overall DIN pool, understanding the main controls on these processes is therefore essential in determining the extent to which these highly dynamic systems regulate DIN inputs to marine systems.

In order to delineate biogeochemical processes within an estuary, concentrations of DIN are traditionally plotted versus salinity. Assuming steady state, tidal averaged conditions, a negative deviation from conservative mixing (i.e., a straight line relationship between generally higher riverine inputs and lower marine background marine concentrations) implies the loss of a particular dissolved constituent during estuarine mixing (Officer, 1979). Conversely, positive deviations from conservative mixing suggest a supplementary estuarine source. For example, denitrification or algal and microbial uptake will result in non-conservative concave removal of nitrate across as estuary, whereas nitrification will result in non-conservative convex addition of nitrate and depletion of ammonium. However, in cases where there is an approximate balance between sources and sinks, concentration versus salinity plots may result in apparent conservative behaviour which can be falsely interpreted as a lack of biological activity (Middelburg and Nieuwenhuize, 2001). In such cases the role of estuarine biogeochemical processing will be greatly underestimated.

The simultaneous use of concentration data with stable isotope signatures of DIN can provide further insight into DIN attenuation caused by biogeochemical processing within an estuarine environment. Utilising stable isotope signatures to examine biological modification and cycling of reactive N relies on the extent to which δ^{15}N signatures are altered during a particular process. This alteration results from isotope fractionation caused by kinetic effects, which occur as the result of differences in activation energies caused by mass differences (Galimov, 1985). This typically results in the residual substrate becoming more enriched in the heavier isotope, ^{15}N (i.e., a less negative δ^{15}N value). For example, during denitrification the residual nitrate will become increasingly more enriched in ^{15}N due to the preferential use of isotopically lighter nitrate (Cline and Kaplan, 1975; Liu and Kaplan, 1989; Mariotti et al., 1981), whereas nitrification results in the production of nitrate which is relatively ^{15}N-depleted (Mariotti et al., 1981; Miyake and Wada, 1971; Sutka et al., 2004). Laboratory and field studies have also shown distinct nitrogen isotope fractionation signals during microbial and algal uptake of DIN (Altabet, 2001; Mariotti et al., 1984; McCusker et al., 1999; Ostrom et al., 1997; Sigman et al., 1999;

Velinsky and Fogel, 1999; Waser et al., 1998). Consequently, even in cases where nitrate concentrations across an estuary exhibit conservative behaviour, $\delta^{15}NO_3^-$ may point to significant biogeochemical processing. For instance, in a study carried out by Middelburg and Nieuwenhuize (2001) in the Loire Estuary, while nitrate concentrations generally exhibited conservative mixing, $\delta^{15}NO_3^-$ signatures showed substantial non-conservative ^{15}N-enrichment. While the authors were not able to identify the exact mechanisms involved with this non-conservative ^{15}N-enrichment, it was suggested that nitrate being consumed was replenished with an isotopically heavier nitrate, perhaps from a partially denitrified sediment derived source.

Due to isotope fractionations in the precursor nitrogen species, stable nitrogen isotope signatures ($\delta^{15}N$) can also provide a valuable means to elucidate the origin of DIN. For example, it has been shown that fertiliser-derived nitrate, which is relatively more ^{15}N-depleted, can be successfully separated from septic tank / animal waste, which typically has heavy $\delta^{15}N$ values > 7‰ (Kendall, 1998). In addition, the average isotopic signature of both ammonium (~-10 to +2 ‰) and nitrate (~-4 to +6‰) in precipitation is normally isotopically lighter than DIN found in soils, which typically ranges between 0 to 10‰ (Fogg et al., 1998; Garten Jr., 1992; Moore, 1977; Paerl and Fogel, 1994). Within an estuarine environment, $\delta^{15}N$ signatures have demonstrated their usefulness as accurate indicators of anthropogenic pollution. Relying on isotopic differences between nitrate in groundwater derived from natural soils and that originating from wastewater (which is generally more ^{15}N-enriched), McClelland and Valiela (1998) were able to identify the effects of increasing wastewater inputs on the $\delta^{15}N$ values of estuarine producers in Waquoit Bay, MA, US. However, the use of $\delta^{15}N$ signatures as a tool to delineate DIN origin can often be complicated by significant source signal overlap. For instance, nitrate derived from sewage sources will often possess the same $\delta^{15}NO_3^-$ signature as nitrate originating from animal waste (Kendall, 1998). Since stable isotope signatures of DIN in estuaries will reflect both the source of the material and the degree of biological transformation, only in cases where there is minimal signal overlap and relatively minor subsequent isotope fractionation effects can $\delta^{15}N$ values be used successfully as provenance indicators.

Utilising concentrations and $\delta^{15}N$ ratios, the main objectives of this study were to:

1) Determine whether $\delta^{15}N$ signatures of ammonium and nitrate could help us understand the main controls on the biogeochemical cycling of DIN in two contrasting northeastern UK estuaries, the Tyne and Tweed (Figure 1).
2) Determine whether $\delta^{15}NH_4^+$ signatures in the Tyne could be used to trace ammonium discharges originating from the Howdon sewage works, one of the UK's largest estuarial secondary treatment facilities
3) Determine whether $\delta^{15}NO_3^-$ signatures in both estuaries could discriminate between different nitrate sources, despite the potential for signal overlap and the natural complexity associated with estuarine environments.

In order to properly assess the potential nitrogen isotope variability within these two estuaries, samples were collected during different seasonal and hydrological conditions.

MATERIALS AND METHODS

Description of Study Sites

Tyne Estuary

The River Tyne, which flows through the densely populated city of Newcastle, has a total drainage area of approximately 2900 km^2 and an average freshwater flow of ~48 m^3/s. Its two main tributaries are the North Tyne, which receives humic-rich waters draining areas of blanket peat afforestation, and the South Tyne, which drains relatively pristine moorland (Baker and Spencer, 2004). The North and South tributaries converge downstream to form the River Tyne, which supplies more than 90% of the total river discharge into the Tyne Estuary (Figure 1). Most of the remaining freshwater input is derived from the River Derwent. The maximum extent of the tidal estuary is approximately 33 km inland from the North Sea, and the residence time is approximately 5-20 days (A. P. Stubbins, private communication). The Tyne is a partially mixed mesotidal estuary (Baker and Spencer, 2004). Although industrial fluxes to the lower part of the estuary are in decline, it continues to receive significant amounts of urban waste, particularly from the Howdon sewage works (HSW). Located approximately 5.9 km upriver from the seawall, HSW is one of the UK's largest secondary treatment facilities (Baker and Spencer, 2004; Upstill-Goddard et al., 2000). However, the water quality in the Tyne upstream of Newcastle has improved significantly over the past few decades.

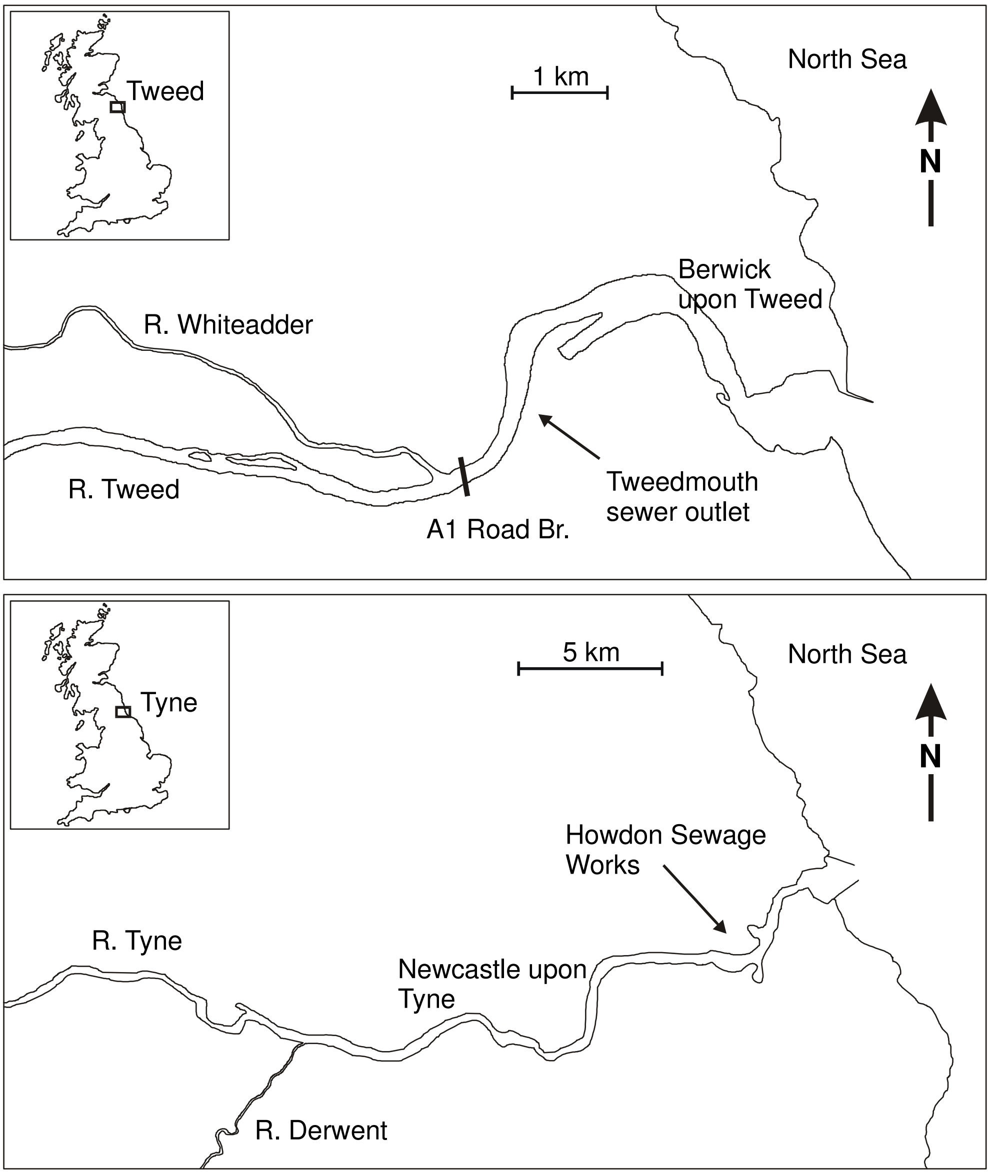

Figure 1. Map of the Tyne and Tweed Estuaries

Nitrate and nitrite inputs into the Tyne Estuary, which subsequently generally obey conservative mixing behaviour across the salinity gradient, range from 56.5-118.0 µM (mean 81.3 µM) and 0.3-3.6 µM (mean 1.3) respectively, and no correlation between nutrient input and river discharge has been observed (Kitidis, 2002). Ammonium concentrations in the Tyne River range from 1.3-26.2 µM (mean 10.3 µM), although axial profiles of the estuary have shown that ammonium levels in the Tyne are generally dominated by point source inputs originating from HSW (Figure 1), which can often comprise over 70% of the DIN pool in the lower part of the estuary (Kitidis, 2002). Although rates have not been adequately quantified, high levels of both ammonium and nitrifying bacteria imply that nitrification is an important process in the Tyne Estuary (J. Barnes, private communication). The Tyne Estuary is considered to be a net heterotrophic system during both the winter and summer, with low chlorophyll a concentrations (< 4.5 µg/L) in both seasons signifying that autochthonous material comprises a relatively minor component of the organic matter (Chapters 2-5).

Tweed Estuary

The Tweed River and its tributaries drain a rural, relatively sparsely populated region located in the border region between England and Scotland. Most of the Tweed's discharge originates from the main river, although approximately 10% of the freshwater input comes from the Whiteadder, which joins the Tweed approximately 6.5 km upriver from the North Sea. The combined catchment area of the Tweed and Whiteadder is approximately 4900 km^2 and the average freshwater input into the estuary is ~84 m^3/s (Fox and Johnson, 1997). The maximum length of the Tweed estuary (Figure 1) is around 13 km and the residence time is approximately 1 day (Uncles and Stephens, 1996). The Tweed is a partially mixed to stratified microtidal estuary (Uncles and Stephens, 1996). The Tweed catchment is dominated by agricultural activity, ranging from upland areas of moorland used for sheep grazing to more arable regions in the lowlands (Neal, 2002). The Tweed receives significantly less anthropogenic inputs than the Tyne, as reflected in its favourable water quality ratings (Robson and Neal, 1997).

Large seasonal variations in nitrate inputs have been reported in the Tweed Estuary, ranging from over 300 µM in winter to less than 30 µM in summer (Uncles et al., 2003). These seasonal variations in nitrate concentrations have been attributed to reduced biological processing and the additional release of stored terrestrial nutrients during the winter (Balls, 1994; Uncles et al., 2003). Although a sewage works is located within the tidal estuary (Figure 1), the smaller population base in the

Tweed watershed implies that sewage effluent should not significantly contribute to the DIN pool within the freshwater-tidal interface. Thus, compared to the Tyne ammonium in the Tweed is generally quite low (<10 µM; Balls, 1994). As in the Tyne, nutrients profiles in the Tweed Estuary normally reflect conservative mixing between relatively high river inputs and low North Sea concentrations (Balls, 1994; Uncles et al., 2003), although non-conservative behaviour for ammonium has also been reported (Shaw et al., 1998). While a study by Shaw et al (1998) has found that phytoplankton activity within the estuary can cause removal of DIN, uptake rates in the Tweed are generally low compared to other estuaries. The low chlorophyll a concentrations (<2 µg/L) typically found in the Tweed Estuary (Uncles et al., 2000, Chapter 2) have been attributed to rapid flushing which prevents the occurrence of repeated algal cell division. Because of the rapid flushing time and associated quasi-conservative mixing behaviour, little biogeochemical modification of nutrients is thought to occur in the Tweed Estuary (Uncles et al., 2002). While the Tweed is considered to be net heterotrophic system during the winter, photosynthesis becomes the dominant process during the summer (Chapter 2). Due to the relatively low chlorophyll a concentrations, photosynthetic activity in the Tweed is thought to be dominated by benthic macrophytes (Uncles et al., 2000, Chapter 2).

Sample Collection

Data reported here (Tables 1& 2) are from near-surface water samples collected on single day excursions to the Tyne (Feb-02, Jul-02, Mar-03, Jul-03) and Tweed (Jul-03, Dec-03) Estuaries. Site selection within the estuary was based on *in situ* salinity measurements (using a portable probe) in order to ensure adequate, high-resolution coverage of the full salinity gradient. Once back in the laboratory, salinity was analysed on a pre-calibrated Hanna (model 8633) conductivity meter. Samples were collected at a depth of 1-2 m from a small boat using a submersed pump. Water samples for concentration and isotopic analysis of DIN were collected by large volume pumping into pre-cleaned 4 L high density polyethylene (HDPE) containers. River discharge data is reported here as the two-day average incorporating the days prior to and day of sampling. Discharges for the Tyne (Q = 144.6 m^3/s, 27-28 February 2002; Q = 8.6 m^3/s, 10-11 July 2002; Q = 30.7 m^3/s, 03-04 March 2003; Q = 7.3 m^3/s, 22-23 July 2003) and Tweed (Q = 14.5 m^3/s, 07-08 July 2003; Q = 151.2

m^3/s, 02-03 December 2003) were obtained from the UK Environment Agency and from the Scottish Environment Protection Agency, respectively. Care was taken to sample the entire estuarine salinity range. However, flow conditions and tidal status

Site/Date	salinity	NH_4^+ [μM]	NO_3^- [μM]	$\delta^{15}NH_4^+$ ‰	$\delta^{15}NO_3^-$ ‰	chl-a (μg/L)
Tyne						
28/2/02	31.7	4.9	11.25	8.8	-0.5	NM*
28/2/02	17.7	6.5	30.89	8.3	-1.3	NM*
28/2/02	15.0	125.9	41.36	10.4	-0.3	NM*
28/2/02	9.9	14.4	52.45	10.1	-1.0	NM*
28/2/02	6.9	11.6	56.32	7.9	-0.3	NM*
28/2/02	3.0	11.4	65.19	7.3	1.7	NM*
28/2/02	2.1	11.0	69.73	8.5	-0.3	NM*
11/7/02	33.2	12.4	1.7	8.3	NM*	0.8
11/7/02	30.9	35.7	8.9	11.3	8.1	1.4
11/7/02	25.9	10.0	4.7	9.9	6.9	3.0
11/7/02	19.3	10.1	*20.4*	9.9	8.9	2.4
11/7/02	15.0	16.3	29.9	10.1	9.5	2.4
11/7/02	9.8	7.7	35.3	9.9	7.5	4.3
11/7/02	4.7	6.8	36.1	9.1	7.8	2.5
11/7/02	0.2	6.1	41.6	7.9	6.5	1.3
11/7/02	0.2	6.5	43.1	8.2	6.3	1.4
4/3/03	34.1	22.4	11.9	10.7	-0.9	0.3
4/3/03	28.7	32.1	17.4	11.1	1.6	0.2
4/3/03	19.0	23.9	31.6	9.9	5.1	0.2
4/3/03	14.8	20.8	36.8	9.3	2.6	0.2
4/3/03	14.1	21.2	36.6	9.6	4.8	0.4
4/3/03	10.5	19.5	39.8	9.4	5.3	0.6
4/3/03	5.3	17.2	43.0	8.0	8.1	1.2
4/3/03	1.3	14.0	47.6	6.4	7.5	0.6
4/3/03	0.1	13.8	45.4	5.6	7.7	0.6
4/3/03	0.1	13.4	45.8	5.4	7.6	0.7
23/7/03	32.5	14.7	6.6	8.5	NM*	1.0
23/7/03	27.1	33.2	16.8	10.4	5.8	2.2
23/7/03	25.1	19.7	36.0	10.5	10.7	0.9
23/7/03	24.2	26.1	26.6	10.1	7.1	1.4
23/7/03	21.6	25.4	30.2	10.1	9.1	2.7
23/7/03	20.6	25.6	40.6	10.7	9.6	0.7
23/7/03	20.1	26.7	37.3	10.0	10.3	1.4
23/7/03	18.3	31.0	39.5	10.3	9.3	2.8
23/7/03	16.4	32.3	46.8	10.5	10.5	1.4
23/7/03	13.9	26.2	47.3	10.7	10.1	1.4

*NM, not measured

Table 1. Concentrations and $\delta^{15}N$ signatures of ammonium ($\delta^{15}NH_4^+$) and nitrate ($\delta^{15}NO_3^-$), and concentrations of chlorophyll a (chl-a) measured in the Tyne Estuary (Feb & Jul-02, Mar & Jul-03).

restricted the accessible salinity range on some occasions. During the July 2003 transect in the Tyne Estuary, the combined effects of a spring tidal event with extremely low flow resulted in a low salinity end-member sampling point of 13.9 psu due to inaccessibility of the inner estuary. Conversely, during the December 2003 sampling trip to the Tweed Estuary high river flow resulted in a survey comprised predominantly of low salinity water samples. This strong dependence on surface salinity with freshwater runoff has been previously reported in the Tweed Estuary (Uncles et al., 2000).

Site/Date	salinity	NH_4^+ [µM]	NO_3^- [µM]	$\delta^{15}NH_4^+$ ‰	$\delta^{15}NO_3^-$ ‰	chl-a (µg/L)
Tweed						
8/7/03	33.2	2.4	7.3	7.8	NM[*]	0.2
8/7/03	32.4	3.1	6.3	8.5	7.4	0.3
8/7/03	29.5	3.1	21.9	9.6	9.2	0.6
8/7/03	27.4	3.3	26.5	9.9	8.9	1.2
8/7/03	17.9	4.5	79.4	10.1	10.7	1.4
8/7/03	8.6	3.9	83.1	9.4	10.6	1.4
8/7/03	4.2	2.9	92.6	10.0	9.8	1.4
8/7/03	1.4	2.6	97.9	9.4	10.2	1.5
8/7/03	0.1	3.2	103.2	9.9	10.2	1.6
8/7/03	0.1	3.0	103.1	9.4	10.1	1.7
3/12/03	31.30	2.1	17.4	5.3	6.1	1.8
3/12/03	19.38	4.2	150.8	6.8	5.6	NM[*]
3/12/03	4.51	6.1	228.2	7.2	6.1	NM[*]
3/12/03	1.90	6.1	226.8	7.1	6.3	3.9
3/12/03	1.27	5.4	231.6	7.3	6.0	3.1
3/12/03	0.97	5.9	231.3	7.0	6.2	3.4
3/12/03	0.90	5.8	227.3	6.9	5.7	2.1
3/12/03	0.19	5.5	235.5	7.1	6.2	4.6
3/12/03	0.16	5.5	227.4	7.3	6.5	4.0
3/12/03	0.12	5.7	230.0	6.3	6.0	3.8

[*] NM, not measured

Table 2. Concentrations and $\delta^{15}N$ signatures of ammonium ($\delta^{15}NH_4^+$) and nitrate ($\delta^{15}NO_3^-$), and concentrations of chlorophyll a (chl-a) measured in the Tweed Estuary (Jul-03, Dec-03).

Isolation of dissolved inorganic nitrogen (DIN)

After GF/F filtration, the water samples were acidified with 1 ml/L H_2SO_4 to prevent biological activity and to preserve volatile ammonia as ammonium. Isotopic analysis of ammonium was determined using a diffusion method adapted from Holmes et al. (1998), which involves trapping ammonium onto an acidified (4 N

H_3PO_4) GF/D disk wedged between two Teflon membranes. Depending on the ammonium concentration in the sample, between 100-400 ml of filtered water was transferred to 250-500 ml Wheaton glass bottles for these incubation experiments. All samples were then amended with NaCl to obtain identical salinity values of 35 (i.e., marine salinity) and subsequently brought back to their initial pH values using NaOH. After the GF/D filter packs were placed inside a bottle, MgO (300 mg/L) was added to increase the pH of the water to ~9.8 in order to convert ammonium to ammonia. The bottles were immediately capped tightly and placed on an orbital shaker at room temperature and incubated for three weeks. Afterwards, the filter packs were removed from the bottles, rinsed with MilliQ water, placed inside pre-combusted glass scintillation vials, and then dried and stored inside a dessicator containing silica gel and an open container of H_3PO_4 (to remove trace amounts of background NH_3) prior to $\delta^{15}N$ analysis.

Isotopic analysis of δNO_3^- values were determined using a method adapted from Sigman et al. (1997). This method involves reducing nitrate to ammonia using Devarda's alloy followed by its subsequent extraction onto an acidified GF/D disk in the same manner as described above for δNH_4^+ analysis. The nitrate extraction procedure is incapable of separating NO_3^- from NO_2^-; therefore, all $\delta^{15}N$ values reported here are combined isotopic signatures of both nitrate and nitrite. δNO_3^- incubations were carried out using water that had not been used for the δNH_4^+ incubations but had been purged of ammonia and labile DON prior to the addition of Devarda's alloy (BDH). This was done by adding MgO (300 mg/L) to raise the pH to ~9.8 followed by heating the samples at 75°C for ~6 days or until they evaporated down to dryness. Once dry, the samples were redissolved with MilliQ water until the volume was equal to 25% of the initial sample volume. Initial sample volumes ranged from 20-400 ml, with 250 ml bottles having been used for samples $\leq$ 200 ml and 500 ml bottles for the 400 ml samples. For samples with very high nitrate concentrations (e.g., Tweed, December 2003), the small initial volumes (20 ml) were diluted with MilliQ (to 100 ml total volume) in order to obtain a peak intensity within the range required for isotopic analysis.

In order to correct for potential isotopic fractionation caused by the DIN extraction procedures, isotopic standards containing nitrate and ammonia concentrations similar to those found in the Tyne Estuary were used. Isotopic standards for ammonium and nitrate analyses were prepared as aqueous solutions (in MilliQ) containing dissolved NH_4Cl (Fisher) or KNO_3 (Fisher) of a known isotopic signature and for at least two different concentrations. Differences between the aqueous standards and the $\delta^{15}N$ values of the NH_4Cl and KNO_3 salts were used to correct for isotopic fractionation associated with the incubation experiments. Additional standards were necessary for the nitrate incubations in order to determine and correct for the $\delta^{15}N$ signature of the Devarda's alloy blank. With the exclusion of the initial GF/F filtration step, all nitrate and ammonium standards were prepared and analysed using the same procedures as described for the samples. The $\delta^{15}N$ difference between standards and samples was ≤ 1.3‰.

Concentration and $\delta^{15}N$ analysis of DIN

Concentrations of nitrate and ammonium and $\delta^{15}NO_3^-$ and $\delta^{15}NH_4^+$ ratios were determined using a Carlo Erba Instruments NA2500 elemental analyser coupled with a Micromass PRISM III Isotope Ratio Mass Spectrometer (EA-IRMS) located at the University of Edinburgh. Ammonium and nitrate concentrations were determined using a Skaler San*plus* nutrient autoanalyser or a Bran and Luebbe Autoanalyser III employing standard colourimetric techniques (Strickland and Parsons, 1972). However, since ammonium concentrations measured via colourimetric methods were not obtained for the Mar-03 Tyne survey and for the two Tweed surveys, ammonium levels from these three transects were determined by comparing sample to standard peak intensities on the PRISM III Isotope Ratio Mass Spectrometer. This technique has been shown to be an acceptable means of obtaining reliable concentration data (Ward et al., 2000). As mentioned previously, since the diffusion extraction method is not able to separate nitrite from nitrate, $\delta^{15}NO_3^-$ values reported here are the combined nitrite/nitrate peak intensities and they will be referred to as simply $\delta^{15}NO_3^-$ throughout the rest of this paper.

Ratios of ^{14}N to ^{15}N are expressed in delta notation as per mil (‰) differences relative to an internationally recognised atmospheric nitrogen standard, where:

$$\delta^{15}N = [\{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{standard}\} / (^{15}N/^{14}N)_{standard}] \times 1000 \qquad (1)$$

Most $\delta^{15}N$ values (and concentrations) reported here for ammonium and nitrate are the result of duplicate sample analyses. The error associated with $\delta^{15}NO_3^-$ and $\delta^{15}NH_4^+$ values in this study incorporates both internal reproducibility and accuracy associated with EA-IRMS analysis, and was ≤1.0‰. Accuracy and reproducibility for ammonium concentrations determined via EA analysis was generally better than ±10%, and was better than ±5% using colourimetric techniques. The Devarda's alloy used in these experiments was determined to have an effective N concentration of 1.1 µM and a $\delta^{15}N$ signature of -5.7‰ ±0.8‰, which was similar to the nitrogen blank reported in the literature (Sigman et al., 1997).

RESULTS AND DISCUSSION

Behaviour of ammonium and nitrate concentrations

In Figure 2, concentrations of ammonium from the four surveys to the Tyne Estuary and from the two surveys to the Tweed Estuary are plotted versus salinity. As these figures demonstrate, during all six transects concentrations of ammonium were significantly higher in the Tyne than in the Tweed Estuary. In the Tweed, the concentrations of ammonium ranged between 2.4 µM at the coastal North Sea end-member to a maximum of 4.5 µM at a salinity of 17.9 during Jul-03 and between 2.1 µM (31.3 practical salinity units, psu) to 6.1 (1.90 psu) during Dec-03. During the winter ammonium concentrations in the Tweed showed a general decrease with increasing salinity defined by the higher riverine concentration relative to the North Sea end-member values. In the summer, riverine concentrations of ammonium were lower and only slightly higher than the North Sea values. In addition, there was a general trend of convexity across the salinity gradient indicating an estuarine source of ammonium. In the winter, when the riverine ammonium concentration and freshwater discharge was higher, estuarine sources were less distinct (although a slight positive deviation from conservative behaviour was still evident).

In the Tyne, the concentrations of ammonium ranged between 4.6-149.2 µM, 6.1-35.7 µM , 13.4-32.1 µM and 14.7-33.2 µM during Feb-02, Jul-02, Mar-03 and Jul-03, respectively. In Figure 2 the location of the Howdon sewage works (HSW) in the Tyne Estuary during each survey is marked in relation to the salinity gradient (located at salinities of 15.0, 30.9, 28.7 and 27.1 during Feb-02, Jul-02, Mar-03 and

Figure 2a

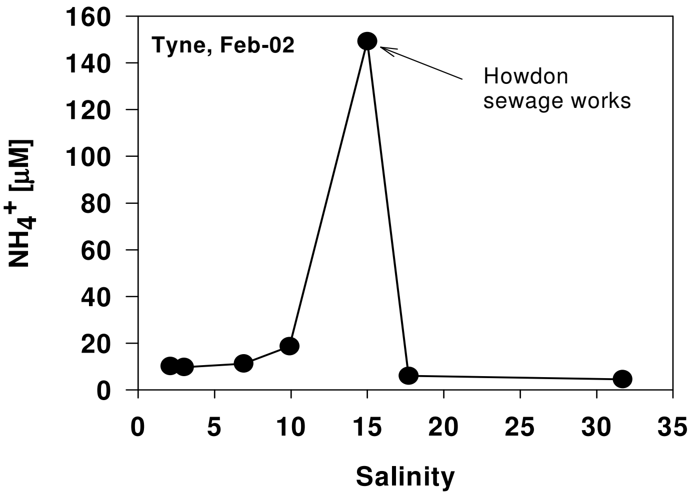

Figure 2b

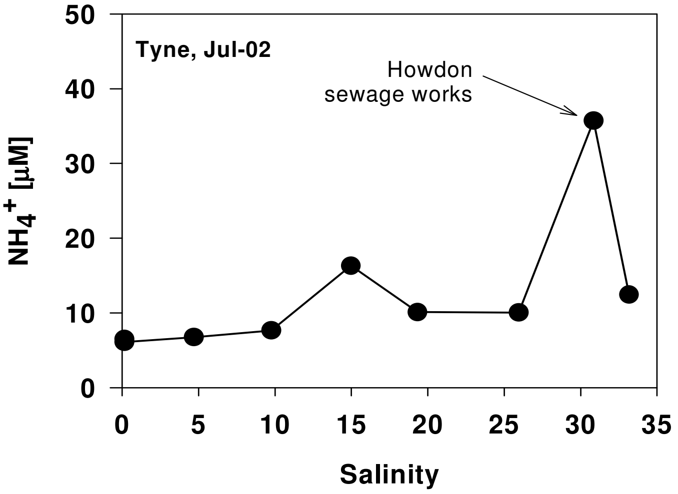

Figure 2c

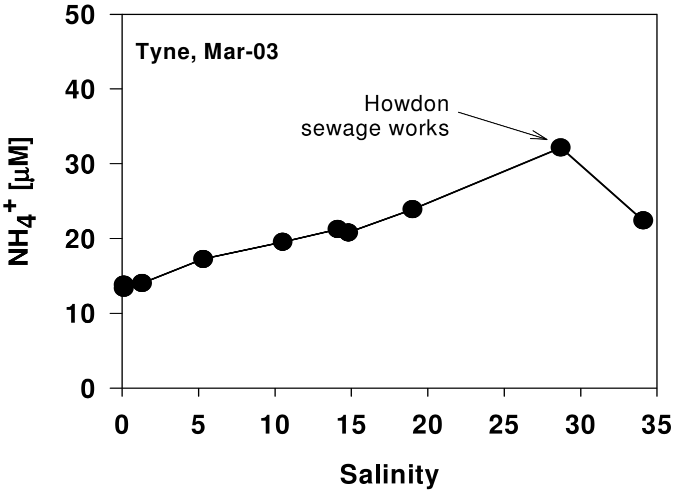

Figure 2. Concentrations of ammonium in the Tyne Estuary during (a) Feb-02, (b) Jul-02, (c) Mar-03, and (d) Jul-03 and in the Tweed Estuary during (e) Jul-03, and (f) Dec-03 plotted versus salinity.

Figure 2d

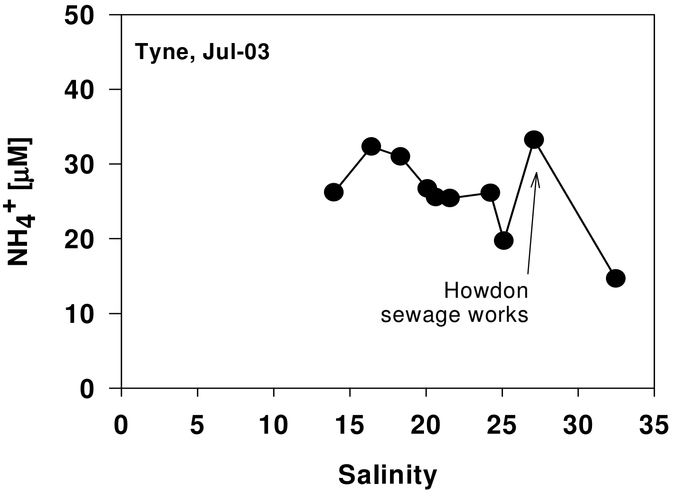

Figure 2e

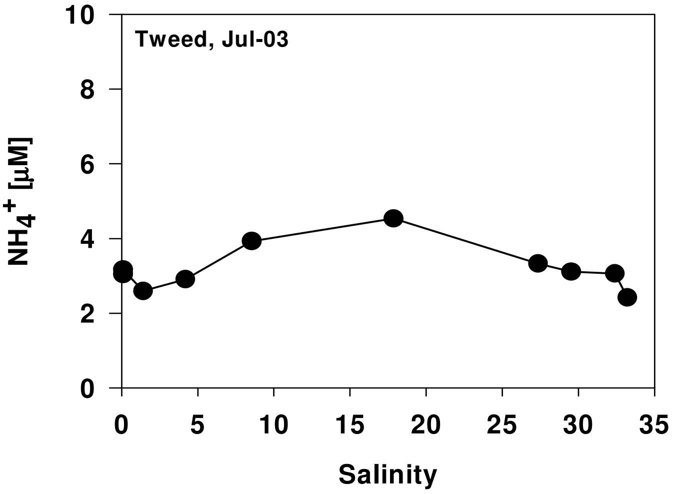

Figure 2f

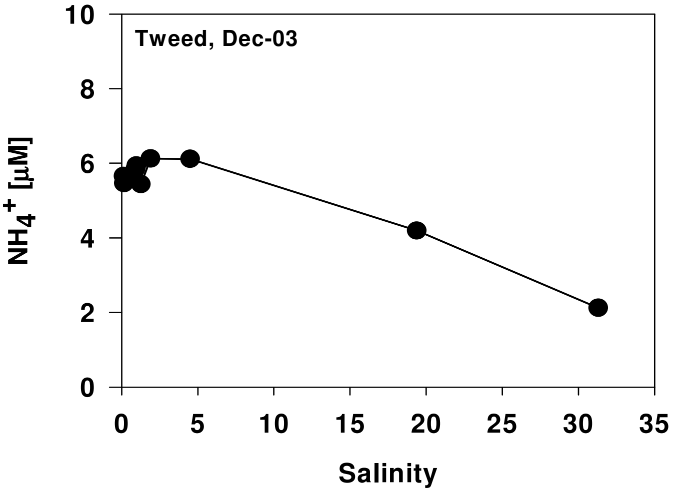

Figure 2. Concentrations of ammonium in the Tyne Estuary during (a) Feb-02, (b) Jul-02, (c) Mar-03, and (d) Jul-03 and in the Tweed Estuary during (e) Jul-03, and (f) Dec-03 plotted versus salinity.

Jul-03, respectively). As these graphs illustrate, HSW played an important role in contributing to the relatively elevated ammonium levels found throughout the Tyne Estuary. With the exception of the Jul-03 survey, this large ammonium plume could consistently be traced to its point of origin throughout the entire estuary. The HSW ammonium plume is well defined as a sharp peak during high river flow conditions when the estuary is being flushed rapidly (e.g., Figure 2a, Feb-02). With declining flow rates the residence time of water in the estuary increases and the effect of HSW discharge becomes more pervasive, influencing the ammonium levels across the salinity gradient. The extreme case is the Jul-03 survey (Figure 2d), which exhibits a very broad ammonium plume extending through much of the salinity range sampled. This reflects the very low river discharge ($Q = 7.3$ m^3/s) and spring tide conditions during this period. In addition, because HSW is located close to the mouth of the estuary, even the most saline samples collected (e.g., Figure 2c; salinity 34.1, 22.4 μM NH_4^+) have elevated ammonium values. This demonstrates that HSW discharge significantly impacts nutrient levels in the North Sea proximal to the mouth of the Tyne estuary.

In Figure 3, concentrations of nitrate from the four surveys to the Tyne Estuary and from the two surveys to the Tweed Estuary are plotted versus salinity. In the Tyne, the concentrations of nitrate ranged between 11.3-69.7 μM, 1.7-43.1 μM, 11.9-45.8 μM and 6.6-47.3 μM during Feb-02, Jul-02, Mar-03 and Jul-03, respectively. The highest riverine nitrate inputs observed during this study were found during the period of highest river discharge (Feb-02, $Q = 144.6$ m^3/s), and the riverine nitrate levels fell with declining discharge in Mar-03 and Jul-02. Thus, with the exception of the Jul-03 sampling period when riverine nitrate concentrations were not obtained due to extreme low flow conditions (7.3 m^3/s), higher river flows are also matched by higher nitrate levels. This observation contradicts (Kitidis, 2002), where the lack of a relationship between nitrate inputs and discharge has been reported.

The straight solid lines on these figures represent the expected concentration due to conservative mixing between marine and riverine sources. For the Tyne, the marine end-member values for nitrate concentrations cannot be considered as true North Sea values given the influence of HSW-derived nutrient inputs. Remarkably, the influence of HSW plume is not clearly expressed in the nitrate concentrations as

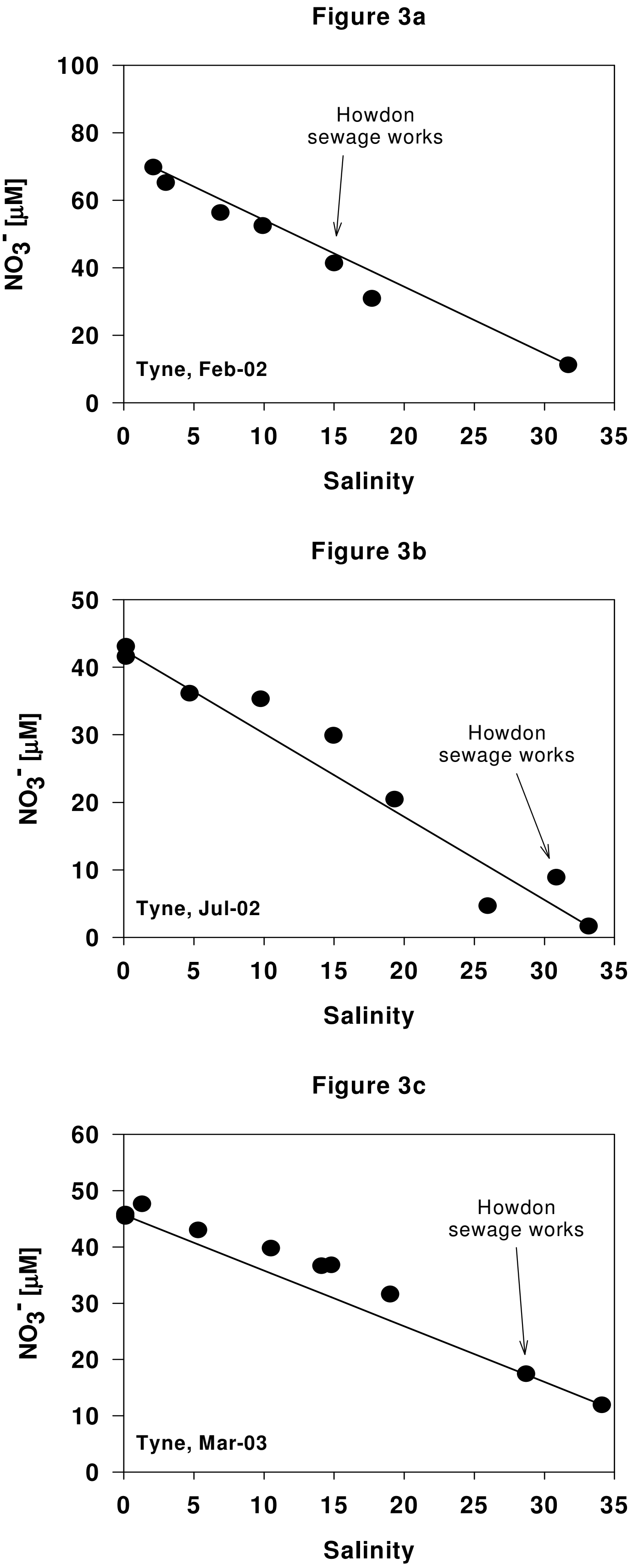

Figure 3. Concentrations of nitrate in the Tyne Estuary during (a) Feb-02, (b) Jul-02 and (c) Mar-03 plotted versus salinity. The straight solid lines represent the conservative concentration mixing curve between riverine and marine sources.

Figure 3d

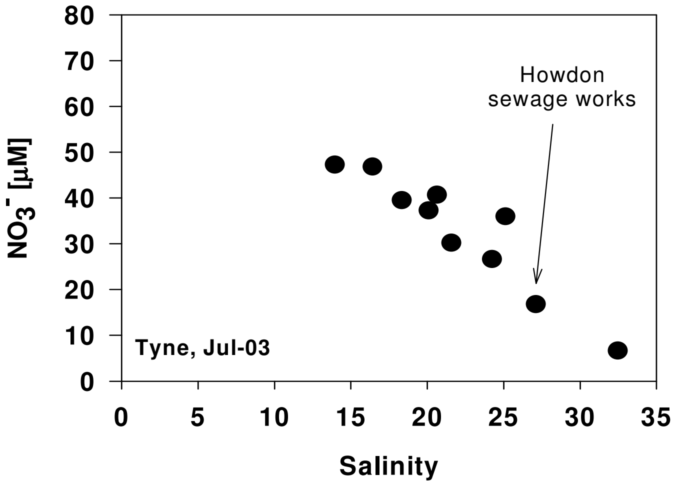

Figure 3e

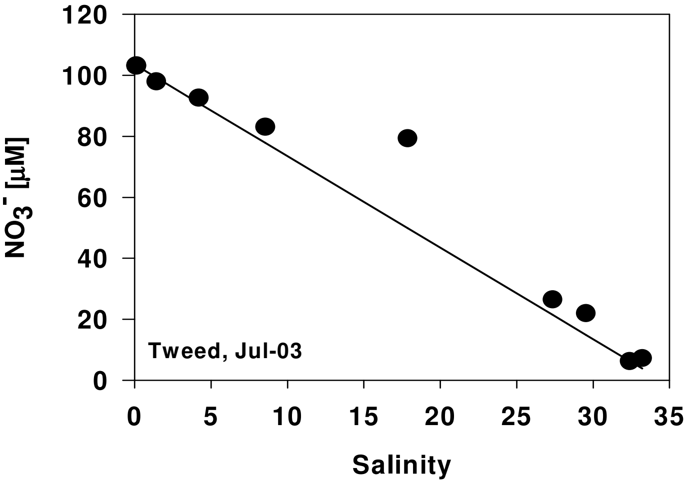

Figure 3f

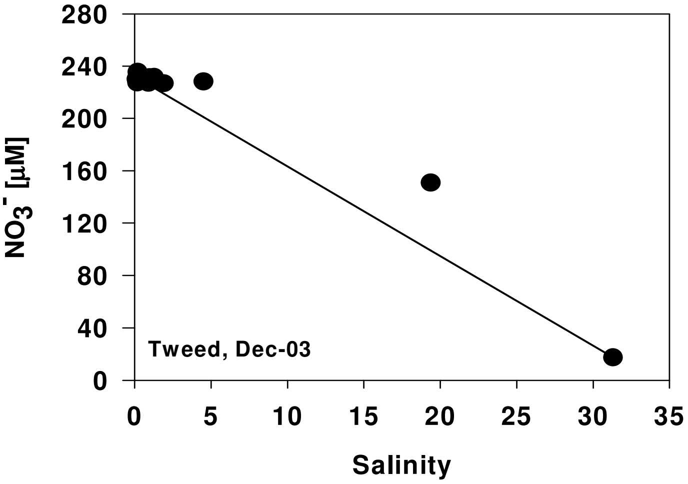

Figure 3. Concentrations of nitrate in the Tyne Estuary during (d) Jul-03 and in the Tweed Estuary during (e) Jul-03, and (f) Dec-03 plotted versus salinity. The straight solid lines represent the conservative concentration mixing curve between riverine and marine sources.

in the ammonium levels, which implies that direct nitrate inputs from HSW are small. Nitrate concentrations in the Tyne generally exhibited conservative mixing between relatively high riverine inputs and low nitrate levels in the seaward samples and deviations from the mixing curves were small (Figure 3). These include the small positive deviations during the Jul-02 and Mar-03 surveys and a single point negative deviation during the Jul-02 survey (25.9 psu). A freshwater end-member nitrate concentration was not determined for the Tyne during the July-03 survey, which precludes the evaluation of estuarine mixing during this survey.

Nitrate concentrations in the Tweed Estuary are plotted in Figures 3e and 3f. In general, riverine nitrate inputs in the Tweed were much higher than in the Tyne (103.1 µM, Jul-03; 231.0 µM, Dec-03). This fundamentally reflects the differences in landuse in the two river catchments. The higher nitrate inputs in the Tweed reflect the larger proportion of agricultural and grazing activity in its catchment relative to the Tyne. In the Tweed, the concentrations of nitrate ranged between 103.2 µM at the freshwater end-member to 7.3 µM at the coastal North Sea during Jul-03 and between 235.5 µM (freshwater) to 17.4 µM (31.3 psu) during Dec-03. This increase in riverine nitrate inputs into the Tweed Estuary during high river discharge in winter has been previously documented by others (Balls, 1994; Uncles et al., 2003). Both during summer and winter there were slight non-conservative nitrate peaks in the Tweed estuary. During the winter survey the large influx of freshwater necessitated closely spaced sampling locations near the mouth of the estuary in order to obtain higher salinity samples (see Introduction). Therefore, it is likely that this non-conservative behaviour was a function of the narrow salinity range and high riverine concentrations and not the result of an estuarine input. The non-conservative nitrate increase found in the summer (as observed for ammonium) may be related to discharges emanating from the Tweedmouth sewer outlet, which was located near this sampling site (Figure 1). Uncles et al. (2003) have attributed small mid-salinity nitrite increases to sewage-related discharge. However, since nitrate concentrations during Jul-03 were relatively high, it is unlikely that minor sewage inputs would have had a significant impact on the estuarine nitrate concentrations observed during this survey. Small nitrate inputs in the Tweed Estuary have been attributed to transient effects associated with rapidly changing runoff (Uncles et al., 2003). However, since the non-conservative nitrate behaviour reported in this study corresponded with a sampling location situated just downriver of the confluence with the River Whiteadder (Figure 1), it is also possible that this tributary was contributing a significant amount of nitrate to the Tweed Estuary. Unfortunately this cannot be directly verified since nitrate concentrations in the Whiteadder were not measured during this study.

$\delta^{15}NH_4^+$ signatures: General trends

In Figure 4, $\delta^{15}NH_4^+$ signatures from all six surveys to the Tyne and Tweed Estuaries are plotted versus salinity. In the Tweed, $\delta^{15}NH_4^+$ values were slightly lower in the winter than in the summer, having concentration weighted averages (after combining freshwater values; ≤ 0.2 psu) of +9.5±0.7‰ (n = 9) and +6.9 ±0.6‰ (n = 8) during Jul-03 and Dec-03, respectively. $\delta^{15}NH_4^+$ signatures ranged from 7.8‰ at the coastal North Sea to 10.1‰ at a salinity of 17.9 during July 2003 and from 5.3‰ (31.3 psu) to 7.3‰ (0.16 and 1.27 psu) during Dec-03. $\delta^{15}NH_4^+$ signatures in the Tyne were within the same range as those measured in the Tweed, ranging from 7.3-10.4‰, 7.9-11.3‰, 5.4-11.1‰ and 8.5-10.7‰ during Feb-02, Jul-02, Mar-03 and Jul-03, respectively. The seasonal contrast in $\delta^{15}NH_4^+$ values was slightly smaller in the Tyne, showing concentration weighted averages (after combining freshwater values; ≤ 0.2 psu) of 9.6 ±1.7‰ (n = 16) and 10.2 ±0.9‰ (n = 18) during winter and summer, respectively.

$\delta^{15}NH_4^+$ signatures and HSW input in the Tyne Estuary

As noted for ammonium concentration profiles, the most important factor in controlling $\delta^{15}NH_4^+$ signatures across the Tyne Estuary was the Howdon sewage works (HSW), which was the source of a consistently large ammonium plume that could be traced directly back to the sewage effluent. The location of HSW in the Tyne Estuary during each survey plotted in relation to the salinity gradient is marked in Figure 4. Although the magnitude of the ammonium plume emanating from HSW varied between each survey, the concentration weighted average $\delta^{15}NH_4^+$ signature of the effluent ($\delta^{15}NH_4^+$ = +10.6 ±0.5‰, n = 4) was remarkably consistent throughout the four seasonal transects reported in this study. This invariance in the sewage-derived $\delta^{15}NH_4^+$ signature was likely the main reason for the small seasonal

Figure 4a

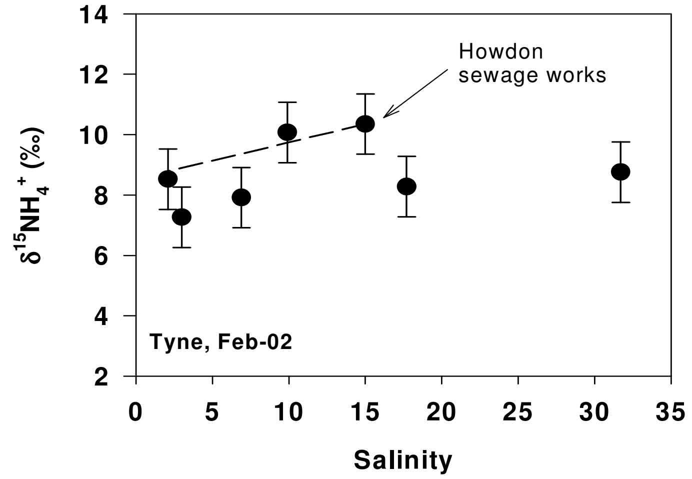

Figure 4b

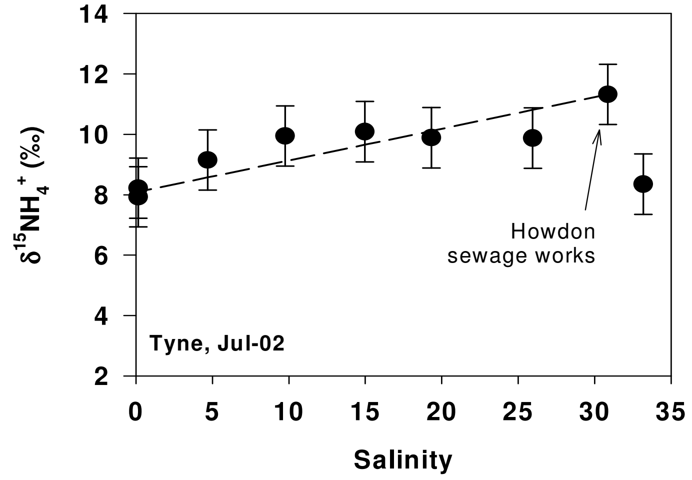

Figure 4c

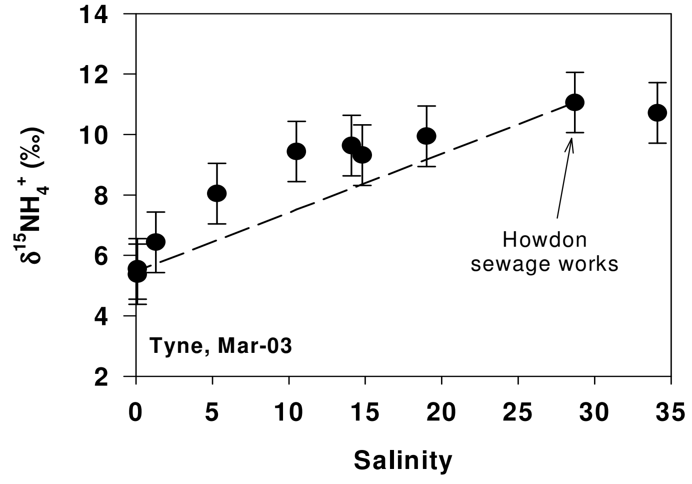

Figure 4. $\delta^{15}NH_4^+$ values in the Tyne Estuary during (a) Feb-02, (b) Jul-02 and (c) Mar-03, and in the Tweed Estuary during (e) Jul-03, and (f) Dec-03 plotted versus salinity. The dashed lines in Figures 4 a-c represent the sewage-riverine isotope mixing curve as calculated using Eq. 2 for the Tyne Estuary. Vertical error bars represent an accuracy and reproducibility of ±1‰ on $\delta^{15}NH_4^+$ values.

Figure 4d

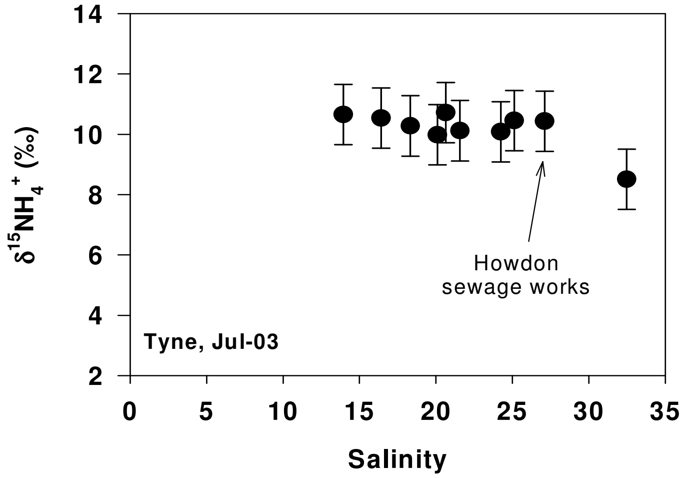

Figure 4e

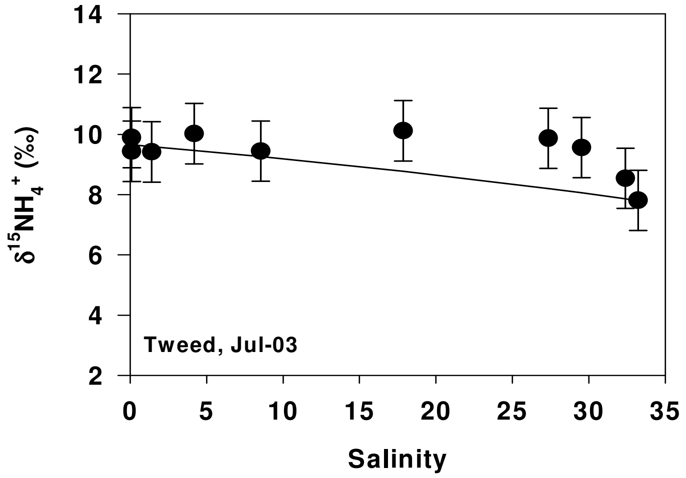

Figure 4f

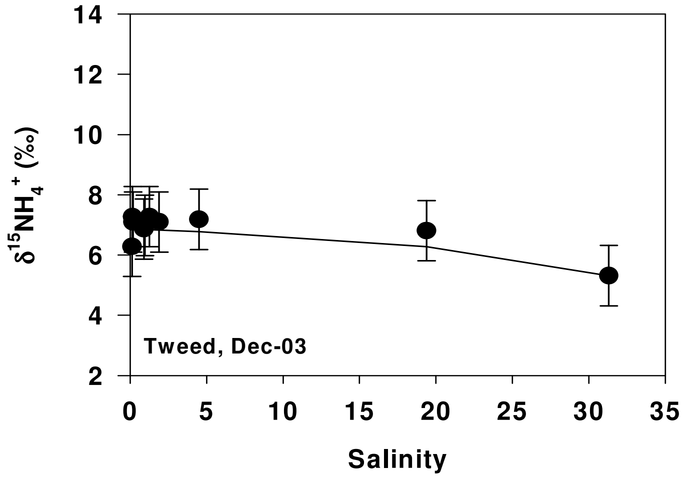

Figure 4. $\delta^{15}NH_4^+$ values in the Tyne Estuary during (d) Jul-03 and in the Tweed Estuary during (e) Jul-03, and (f) Dec-03 plotted versus salinity. The solid lines in Figures 4 e-f represent the conservative isotopic mixing curve as calculated using Eq. 3 for the Tweed Estuary. Vertical error bars represent an accuracy and reproducibility of ±1‰ on $\delta^{15}NH_4^+$ values.

variations in $\delta^{15}NH_4^+$ values found throughout the Tyne Estuary. The relatively isotopically enriched $\delta^{15}NH_4^+$ signature for HSW-derived ammonium reported here is consistent with other sewage-derived $\delta^{15}NH_4^+$ values reported in the literature (Kendall, 1998; Tucker et al., 1999). As demonstrated by Figure 4, the isotopic signature of the sewage-derived ammonium discharge could be traced across the entire estuary, generally showing the most ^{15}N-enriched $\delta^{15}NH_4^+$ value at the location closest to the point of origin.

In order to track the extent of the HSW ammonium plume across the Tyne Estuary, a simple a two end-member isotope mass balance equation using $\delta^{15}NH_4^+$ values was utilised:

$$\delta^{15}NH_4^+{}_{mix} = f_R(\delta^{15}NH_4^+{}_R) + f_S(\delta^{15}NH_4^+{}_S) \quad (2)$$

where f_R, f_S, $\delta^{15}NH_4^+{}_R$ and $\delta^{15}NH_4^+{}_S$ refer to the fraction and $\delta^{15}NH_4^+$ signatures of the riverine and sewage components during each particular survey, respectively. $\delta^{15}NH_4^+{}_{mix}$ is the isotopic signature of ammonium expected due to linear mixing between sewage and riverine sources, and $f_R + f_S = 1$. Since HSW is located in the lower part of the estuary, we can assume that marine ammonium inputs are insignificant compared to the overwhelming sewage signal. Indeed, the relatively high ammonium concentrations found in the marine sampling sites in the Tyne Estuary during the Jul-02 (12.4 μM), Mar-03 (22.4 μM) and Jul-03 (14.7 μM) surveys are undoubtedly related to the HSW plume. This is supported by the relatively minor (< 2.5 μM) ammonium concentrations found in the Coastal North Sea sampling sites in the Tweed Estuary, where anthropogenic ammonium inputs are considerably smaller. Therefore, the use of a two end-member mixing equation incorporating only riverine and sewage contributions is justified in assessing ammonium distributions across the Tyne Estuary.

In Figure 4 these riverine-sewage mixing lines are plotted as dashed lines together with the measured $\delta^{15}NH_4^+$ values. During Feb-02 (Figure 4a) some of the measured $\delta^{15}NH_4^+$ values fell slightly below this line, perhaps suggesting that this model overestimated the contribution from HSW. As Figure 4a shows, the large

HSW peak found during this survey (149.2 µM) seemed to be confined to a relatively narrow zone located in close proximately to the plume source. Thus, during this period of high river discharge (Q = 144.6 m^3/s) it was likely that HSW-derived ammonium did not greatly affect other parts of the estuary. During Mar-03 and Jul-02 (Figures 4b and 4c) much of the variability in measured $\delta^{15}NH_4^+$ trends could explained by simple mixing between the heavy ammonium source from Howden and the lighter riverine source. In the Jul-03 survey, the absence of a riverine end-member value precluded the evaluation of mixing trends. This period of unusual low river discharge may have also exhibited different source signatures from other survey periods. The good agreement between measured and predicted distributions for $\delta^{15}NH_4^+$ values based on the simple mixing model strengthens our conclusion that ammonium distributions across the Tyne Estuary were primarily controlled by discharges emanating from the Howdon sewage works.

Behaviour of $\delta^{15}NH_4^+$ signatures in the Tweed Estuary

In the Tweed (Figures 4 e-f), where ammonium concentrations were much lower, the influence of a point source $\delta^{15}NH_4^+$ value was relatively less important. Therefore, in the case of the Tweed we have used a concentration weighted mixing model that incorporates the riverine and the North Sea end-members (Middelburg and Nieuwenhuize, 2001):

$$\delta^{15}N_{pred} = \frac{(f_F N_F \delta^{15}N_F + f_M N_M \delta^{15}N_M)}{N_{pred}} \qquad (3)$$

Here f_F, f_M, N_F and N_M refer to the fraction (calculated from salinity data) and ammonium concentrations of the freshwater and the marine components, respectively, and N_{pred} is the amount of ammonium expected due to conservative mixing of the freshwater and marine end-members. $\delta^{15}N_F$ and $\delta^{15}N_M$ demote the $\delta^{15}NH_4^+$ signatures of the freshwater and marine end-members, respectively. Using this equation, we are able to predict the $\delta^{15}NH_4^+$ signature from conservative two end-member mixing ($\delta^{15}N_{pred}$) for a sample at a known salinity. In Figures 4e and 4f, concentration dependent $\delta^{15}NH_4^+$ mixing is plotted as a solid line. During the Dec-03 survey the measured variability in $\delta^{15}NH_4^+$ signatures can be explained by mixing between North Sea values (2.1 µM) and riverine values (5.6 µM). During Jul-03, however, there are positive deviations from the mixing model indicating a slightly heavier ammonium source with in the estuary. The highest ammonium concentration (4.5 µM) and most ^{15}N-enriched $\delta^{15}NH_4^+$ signature (+10.1‰) was measured at the same sampling site where the non-conservative input of nitrate was recorded (17.9 psu). This small increase in ammonium concentration and relatively more ^{15}N-enriched $\delta^{15}NH_4^+$ signature may be associated with sewage effluent originating from the Tweedmouth sewer outlet (Figure 1), as has been observed in nitrite and phosphate distributions (Uncles et al., 2003). However, considering the insignificant size of this ammonium 'spike' in comparison to the HSW signal, even if this increase in concentration was attributed to the Tweedmouth sewer outlet, it is clear that sewage-derived ammonium in the Tweed plays a much more minor role than it does in the Tyne Estuary.

In general, the riverine ammonia entering the two estuaries was more ^{15}N-enriched in the summer than during the winter, particularly considering the surveys where riverine end-member values were well-established by sampling low-salinity waters (e.g., Mar-03 and Jul-02 in the Tyne and the two Tweed surveys). It is conceivable that the heavy values in the summer can be explained by fractionation due to increased biological utilisation of ^{14}N-depleted ammonia during riverine transit. In the Tyne, chlorophyll levels (Table 1) were generally higher in the summer, which supports biological utilisation of ammonium in soils and/or during riverine transit. In contrast, the Tweed showed higher chlorophyll levels (Table 2) in the winter which has been mainly attributed to debris from benthic macrophytes (aquatic grass) washed in during high river discharge. Thus, it appears that in the Tweed the more ^{15}N-depleted winter values were most likely related to changes in the isotopic composition of soil-derived ammonium.

$\delta^{15}NO_3^-$ signatures: General trends

In Figure 5, $\delta^{15}NO_3^-$ signatures from all six surveys to the Tyne and Tweed Estuaries are plotted versus salinity. In order to test whether $\delta^{15}NO_3^-$ signatures obeyed conservative mixing between marine and riverine (i.e., freshwater) sources, a two end-member mass balance equation using both isotopic and concentration data

Figure 5a

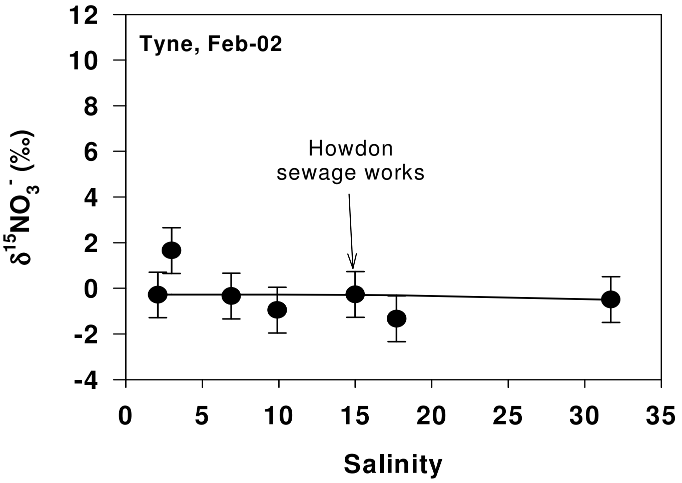

Figure 5b

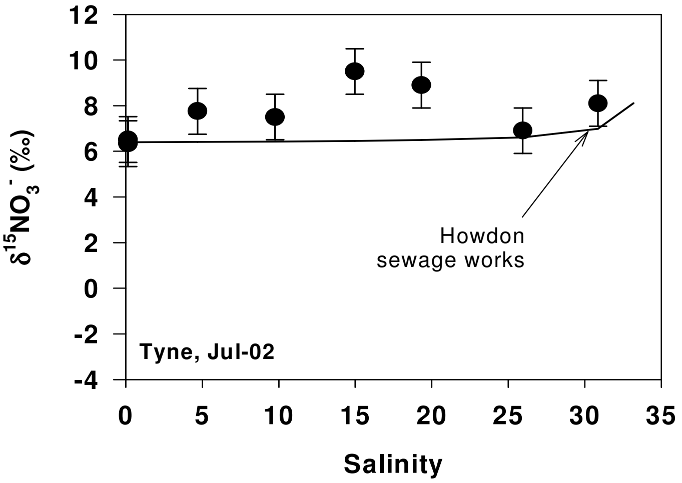

Figure 5c

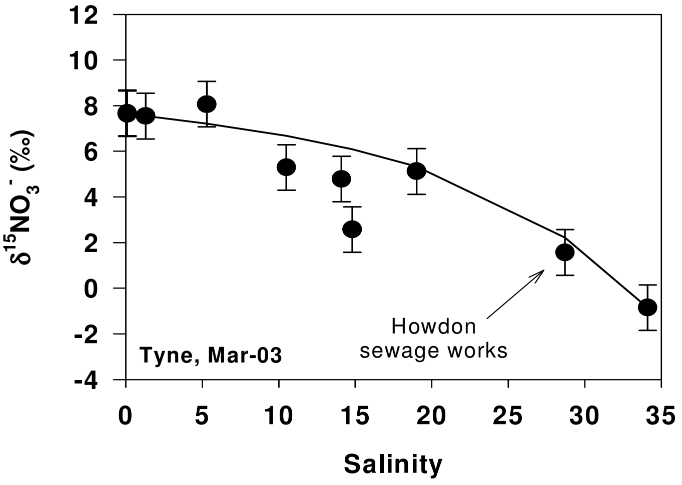

Figure 5. $\delta^{15}NO_3^-$ values in the Tyne Estuary during (a) Feb-02, (b) Jul-02 and (c) Mar-03 plotted versus salinity. The solid lines represent the conservative isotopic mixing curve as calculated using Eq. 3. Vertical error bars represent an accuracy and reproducibility of ±1‰ on $\delta^{15}NO_3^-$ values.

Figure 5d

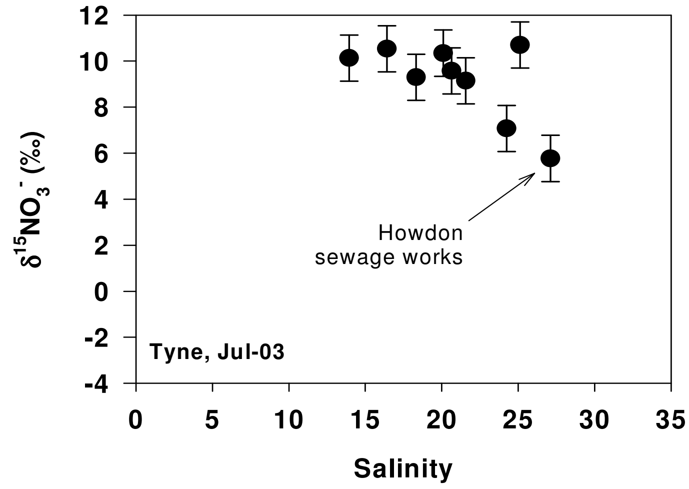

Figure 5e

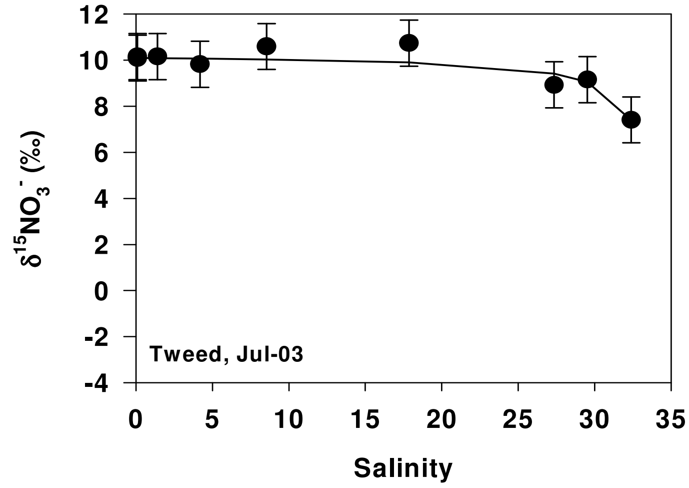

Figure 5f

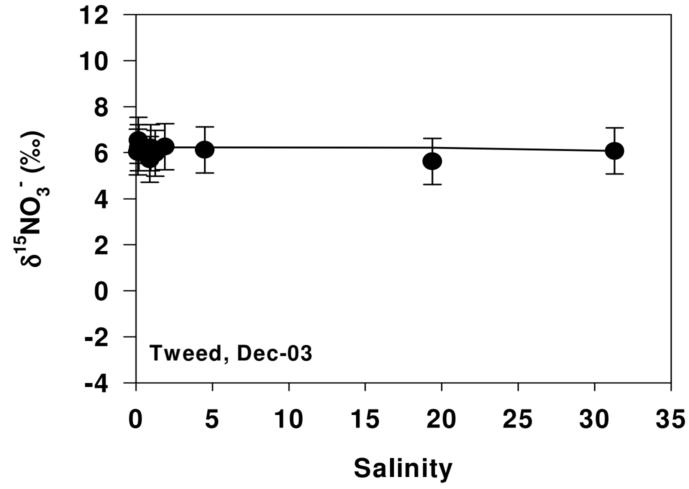

Figure 5. $\delta^{15}NO_3^-$ values in the Tyne Estuary during (d) Jul-03 and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. The solid lines represent the conservative isotopic mixing curve (Eq. 3). Error bars represent an accuracy and reproducibility of ±1‰ on $\delta^{15}NO_3^-$ values.

was utilised, as previously described for ammonium in the Tweed (Eq. 3). Here f_F, f_M, N_F and N_M refer to the fraction (calculated from salinity data) and nitrate concentrations of the freshwater and the marine components, respectively, and N_{pred} is the amount of nitrate expected due to conservative mixing of the freshwater and marine end-members. $\delta^{15}N_F$ and $\delta^{15}N_M$ denote the $\delta^{15}NO_3^-$ signatures of the freshwater and marine end-members, respectively. Using this equation, we are able to predict the $\delta^{15}NO_3^-$ signature from conservative two end-member mixing ($\delta^{15}N_{pred}$) for a sample at a known salinity. In Figure 5, concentration dependent $\delta^{15}NO_3^-$ mixing is plotted as a solid line. In Figure 5d, no mixing model was attempted because during this survey period low salinity samples were not obtained to ascertain a riverine end-member value.

In the Tweed, $\delta^{15}NO_3^-$ signatures were generally higher in the summer than in the winter, having a concentration weighted average (after combining freshwater values; ≤ 0.2 psu) of +10.1 ±1.1 (n = 8) and ranging from +7.4‰ (32.4 psu) to +10.7‰ (17.9 psu). In the winter, $\delta^{15}NO_3^-$ signatures were relatively invariant across the estuary, having a concentration weighted average of +6.1 ±0.2‰ (n = 8) and falling within a relatively narrow array of values ranging from +5.6‰ (19.38 psu) to +6.5‰ (0.16 psu). In the Tyne, a large contrast in $\delta^{15}NO_3^-$ values across the estuary was found between the two different surveys carried out in the late winter. During Feb-02, $\delta^{15}NO_3^-$ signatures were relatively ^{15}N-depleted, having a concentration weighted average of -0.1 ±0.9‰ (n = 7) and ranging between -1.3 to +1.7‰. During the Mar-03 survey, $\delta^{15}NO_3^-$ signatures showed a gradual decrease with increasing salinity, ranging between -0.9‰ (34.1 psu) to +8.1‰ (5.3 psu). In the two summer surveys, $\delta^{15}NO_3^-$ signatures were generally more ^{15}N-enriched across the estuary, with values ranging from +6.4‰ (freshwater) to +9.5 ‰ (15.0 psu) and +5.8‰ (27.1 psu) to +10.7‰ (25.1 psu) during Jul-02 and Jul-03, respectively.

The $\delta^{15}NO_3^-$ signatures of riverine inputs were highly variable with survey periods, probably indicating changes in source and upstream processing of nitrate. This was particularly evident during the Feb-02 survey, where the least saline value was highly depleted (-0.3‰) relative to all other sampling periods. Similarly, the seaward end-member values in the Tyne were also highly variable, with very depleted values found during the Feb-02 (-0.5 ‰, 31.7 psu) and Mar-03 (34.1 psu, -

0.9‰) sampling periods and more enriched values found during the summer surveys (Jul-02, 30.9 psu, 8.1 ‰; Jul-03, 27.1 psu, 5.8 ‰). The nitrate concentration distributions in both the Tyne and Tweed Estuaries generally followed conservative mixing between relatively high riverine inputs and low North Sea background levels (Figure 3), and this is more or less matched by concentrated weighted $\delta^{15}NO_3^-$ signatures with only minor deviations (Figure 5). The changes in nitrate source signals from river and sea and estuarine processing of nitrate will be considered separately in the following sections.

Source differentiation of nitrate entering the estuary

Riverine sources:

The isotopic composition of riverine nitrate entering the Tyne during Jul-02 and Mar-03 and the Tweed during Dec-03 was remarkably similar, with an average value of +6.7 ±0.7‰. The large deviations in source signatures was seen in the Feb-02 Tyne survey where low salinity values were extremely depleted (-0.3‰). In the Tweed, there was an approximately 4‰ seasonal contrast between summer (Jul-03; +10.2‰) and winter (+6.0 ‰) source nitrate signatures.

Nitrate derived from soil sources can cover a wide range of $\delta^{15}N$ signatures (~-2‰ to ~+12‰), with an average value of around +4‰ for natural soils ((Kendall, 1998), and references therein). However, it is expected that $\delta^{15}NO_3^-$ derived from deeper soils is more ^{15}N-enriched, reflecting the increase in $\delta^{15}N$ with depth of soil organic matter (Nadelhoffer and Fry, 1988; Novák et al., 1999). The more ^{15}N-enriched $\delta^{15}NO_3^-$ signature of the Tweed during summer (approximately 4‰ heavier than winter) may thus be related to changes in soil-derived nitrate. The soil-derived nitrate entering the Tweed catchment during the approximate baseflow conditions experienced during summer would presumably originate from a slightly greater depth, thus possessing a more ^{15}N-enriched signal (Ostrom et al., 1998). Tilling in summer months can also lead to the exposure of deeper soils and the nitrate-derived from this source is expected to be heavier. Such contrasts in the isotopic composition of soil-derived nitrate could be further accentuated because the high discharge rates in winter can wash out a greater proportion of nitrate derived from fertiliser and/or atmospheric sources with isotopically lighter nitrate (Kendall, 1998, and references therein). These additional sources in winter from fertiliser and atmospheric deposition are reflected in elevated NO_3^- levels during high discharge in Tweed during this winter and the corresponding lighter isotopic signatures (Figures 5e and 5f). Another factor that could lead to heavier $\delta^{15}NO_3^-$ signatures during summer can result from enhanced biological uptake of nitrate in the River Tweed. This is supported by net autotrophy in the River Tweed and its estuary during the summer months as indicated by supersaturation of O_2, low excess partial pressures of CO_2, and slightly depleted $\delta^{18}O$ values of DO (Howland et al., 2000; Neal et al., 1998, Chapter 2). Because the standing stock of algae generally remains low in the Tweed during both seasons (<2 µg/L, Chapter 2, Uncles et al., 2000), any enhanced nitrate uptake in the summer is not reflected in the chlorophyl-a contents.

Howland et al. (2000) has suggested that benthic macrophytes could contribute to significant productivity in the Tweed Estuary. Balls (1994) attributed summer declines in nitrate levels in the River Tweed to enhanced primary production. High spring and summer cholorophyl a levels have been recorded by others in the Tweed reflecting bloom condition (Uncles et al., 2000). Thus, it is unclear why higher nutrient uptake by riverine algae is not reflected in the chlorophyll a contents during our summer survey. Furthermore, denitrification related enrichment of $\delta^{15}NO_3^-$ during river transit is negated by documented DO supersaturation in the River Tweed (Scottish Environmental Protection Agency). Thus, the seasonal differences in δNO_3^- in the River Tweed river is mainly attributed to soil sources and changes in proportions of soil-derived versus fertiliser and atmospheric sourced nitrate.

The unusually depleted $\delta^{15}NO_3^-$ values (-0.3‰) in Tyne river during the Feb-02 survey is uniquely associated with very large river discharge rates following a winter storm (refer to introduction). The following year, the average discharge during Feb-03, normally one of the NE UK's wettest months, was only 41.2 m^3/s. In contrast, the discharge during the Feb-02 storm event was much higher than the average River Tyne discharge (average = 48 m^3/s; Feb-02 survey = 144.6 m^3/s). The lighter $\delta^{15}NO_3^-$ signal associated with this high flow event cannot be attributed to soil sources given the surface flow conditions during this period. Therefore, this signal is most likely to be associated with atmospheric sources, which in many studies has been shown to possess δNO_3^- values less than 0‰ (Garten Jr., 1992; Moore, 1977;

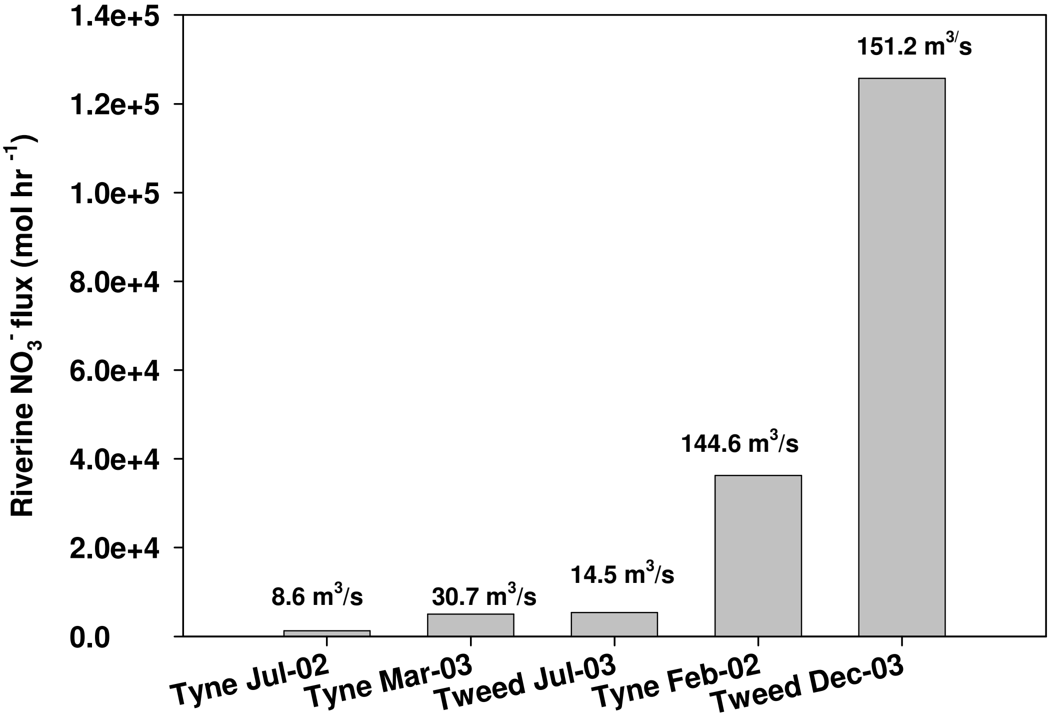

Figure 6. Riverine nitrate fluxes (mol hr^{-1}) and freshwater discharge (m^3/s) in the Tyne and Tweed.

Paerl and Fogel, 1994; Yeatman et al., 2001). In a study involving 10 estuaries located on the eastern coast of the U.S., Castro & Driscoll (2002) reported that total atmospheric inputs (watershed runoff plus direct deposition to the surface of the estuary) accounted for 15-42% of the total nitrogen input. In the UK, nitrate accounts for approximately one third of the acidifying effect of the combined wet deposition of non-sea-salt sulphate and nitrate, with precipitation-weighted mean concentrations of nitrate in precipitation over NE England approaching levels of up to 40 μM (Irwin et al., 2002). Thus, it is possible that the high levels of precipitation in the Tyne Watershed leading up to the Feb-02 sampling period (UK Met Office, 2002) may have contained a significant amount of atmospheric nitrate. In addition, flooding and surface flow conditions associated with this event would have washed into the river atmospheric nitrate that has remained accumulated in the Tyne catchment area. This would have lowered the overall estuarine δNO_3^- signal and sustained the relatively large nitrate concentrations (69.7μM) and fluxes (3.6×10^4 mol hr^{-1}) following the storm event (Figure 6). Therefore, prolonged periods of precipitation could lead to a significant accumulation of atmospheric-derived nitrate, resulting in a higher proportion of ^{15}N-depleted nitrate in the overall riverine $\delta^{15}NO_3^-$ signature.

In contrast to the isotopically light $\delta^{15}NO_3^-$ values found in the Tyne during the Feb-02 transect, during the other winter survey (Mar-03) the riverine $\delta^{15}NO_3^-$ signature was +7.7 ‰. However, in contrast to Feb-02, prior to the Mar-03 survey there was an unusually long period of dry, slightly milder weather (UK Met Office, 2003) which was associated with unseasonably low river discharge (UK Environment Agency). These unseasonably low flow conditions preceding the March 2003 survey therefore point to a limited contribution from relatively ^{15}N-depleted atmospheric inputs. The contrast between the two winter surveys highlights the important role that river flow conditions played in influencing the sources and hence the isotopic signature of nitrate in the Tyne Estuary.

Nitrification and nitrate uptake in the mouth of the Tyne Estuary:

The global average of marine $\delta^{15}NO_3^-$ is in the order of around 5-6‰ (Deutsch et al., 2004). However, the data presented in Figure 5 indicates a highly variable Coastal North Sea $\delta^{15}NO_3^-$ value for the high salinity samples. The highest salinity samples during the Mar-03 and Feb-02 Tyne surveys were -0.5 ‰ and -0.9 ‰, respectively. In contrast to the winter surveys the two summer surveys of the Tyne yielded more enriched values (Jul-02: 8.1‰ and Jul-03: 5.8‰). No such seasonal contrast in marine end-member values was evident in the Tweed, where the $\delta^{15}NO_3^-$ signatures of the highest salinity samples were 7.4 ‰ during Jul-03 and 6.4 ‰ during Dec-03. The isotopic composition of nitrate in temperate coastal waters generally ranges between the composition of freshly upwelled deep-sea nitrate (5-6‰) to slightly more enriched values due to preferential biological uptake of $^{14}NO_3^-$ (Horrigan et al., 1990; Middelburg and Nieuwenhuize, 2001). Thus, the highly depleted $\delta^{15}NO_3^-$ values of coastal sea water entering the Tyne Estuary during the winter surveys require an explanation.

Low δ^{15}N values of nitrate and organic matter in the coastal seas have been attributed to the nitrate sourced from atmospheric deposition (Fogel and Paerl, 1993; Ostrom et al., 1997; Paerl and Fogel, 1994; Paerl et al., 1993). Indeed, atmospheric wet deposition of nitrate in the coastal areas of NE England are expected to be relatively high, in the order of around 2-4 kg N ha^{-1} yr^{-1} (Irwin et al., 2002), and could provide the lighter $\delta^{15}NO_3^-$ signatures for costal waters entering the estuaries. However, the coastal water entering the Tweed did not exhibit any light $\delta^{15}NO_3^-$ even during the winter months. Thus, it is unlikely that that nitrate sourced from atmospheric deposition is the sole explanation for the depleted nitrate signatures of marine source waters in the Tyne during winter.

Instead, the depleted $\delta^{15}NO_3^-$ signatures for saline samples in the Tyne may have resulted from nitrification of ammonium (Mariotti et al., 1981; Sutka et al., 2004). Reported isotopic enrichment factors (ε) for nitrification range between -19‰ (Miyake and Wada, 1971) to approximately -35‰ (Mariotti et al., 1981). This should result in an initial $\delta^{15}NO_3^-$ value that is around -19 to -35‰ lighter than the original $\delta^{15}NH_4^+$ signature. Thus, ^{15}N-depleted nitrate in the mouth of the Tyne Estuary (Mariotti et al., 1981; Sutka et al., 2004) may have been produced by this process. Several lines of evidence support this contention. Firstly, the large amounts of HSW-derived ammonium discharged near the mouth of the Tyne Estuary could facilitate rapid flushing of ammonium into the coastal waters. Indeed, the highest salinity waters of the Tyne sampled during various surveys have large ammonium concentrations with enriched isotopic signatures reflecting the prevalence of HSW-derived ammonium in the coastal waters near the mouth of the Tyne (Figures 2 and 4). Secondly, relatively high N_2O saturations have been measured in the Tyne Estuary (up to 250% N_2O saturation), and the highest levels were generally found in the seaward end of the estuary, which has been attributed to the influence of HSW (Barnes, 2003). The generation of N_2O by bacterial nitrification in oxygenated waters is supported by relatively ^{15}N-depleted N_2O signatures (Ostrom et al., 2000). Barnes (2003) also found that in UK estuaries N_2O supersaturation does not show any consistent relationship to water temperature but shows a positive relationship to ammonium levels, indicating that nitrification and hence N_2O production is fuelled by ammonium levels in these estuaries. Thirdly, recent studies have documented the production of isotopically-depleted nitrate in cold coastal waters (annual average ca. 0.2‰ Ostrom et al., 1997). This study conducted in Conception Bay (off the SE coast of Newfoundland) attributed light $\delta^{15}NO_3^-$ values to the production of nitrate via nitrification. Therefore, the large ammonium inputs close to the mouth of the Tyne are thought to fuel nitrification in the adjoining coastal waters. The nitrate produced by nitrified coastal waters is then advected back into the Tyne Estuary with depleted $\delta^{15}NO_3^-$ signatures.

Since the advection of nitrate generated by coastal nitrification was inferred during the winter surveys (Mar-03 and Feb-02), and nitrification rates are expected to be even higher during warmer conditions (Berounsky and Nixon, 1993), one would also expect this process to occur in the summer. However, as shown in Figures 5b and 5d, nitrate entering the Tyne Estuary from offshore was enriched during the Jul-02 and Jul-03 surveys. However, it is possible that both nitrification followed by subsequent removal was taking place during the summer, resulting in a net ^{15}N-enrichment in $\delta^{15}NO_3^-$ values. For instance, in a study carried out in the Delaware Estuary, Cifuentes et al. (1989) found that $\delta^{15}NO_3^-$ signatures measured in October were 3‰ heavier than samples collect in January, despite more intensive nitrification during the autumn. These heavier values were explained by higher primary production rates during October, indicating that algal uptake was resulting in a subsequent ^{15}N-enrichment in nitrate generated via nitrification. Ostrom et al. (1997) found that in Conception Bay, where the annual average nitrate values were depleted due to nitrification, spring-bloom conditions produced the most enriched $\delta^{15}NO_3^-$ signatures (~8 ‰). Indeed, the chlorophyll a concentrations of waters entering the Tyne from offshore were relatively higher in the summer surveys (Jul-02 and Jul-03) than during Mar-03 (Table 1). Therefore, it is possible that algal uptake in coastal waters during the summer could have resulted in an overall ^{15}N-enrichment masking the depleted $\delta^{15}NO_3^-$ values produced by nitrification.

Estuarine processing of DIN:

The conservative isotopic mixing curves calculated using Eq. 3 are plotted together with the measured $\delta^{15}NO_3^-$ values in Figure 5 as solid lines. The fact that $\delta^{15}NO_3^-$ signatures in both the Tyne and Tweed closely followed conservative behaviour indicates that very little processing of nitrate takes place in both estuaries. One possible reason for the lack of any significant biological modification of nutrients in these two estuaries is their relatively short water residence times (~1 day in the Tweed and 5-20 days in the Tyne). For example, in a study carried out by Balls (1994) in nine Scottish rivers it was concluded that nutrients generally behaved conservatively in short, rapidly flushed estuaries (e.g., Tweed), whereas in large, slowly flushed systems internal processing played a more important role in controlling nutrient distributions within the estuarine zone. Therefore, considering the slightly longer residence time in the Tyne compared to the Tweed Estuary, and the longer residence time expected in the summer during low river flow conditions, it is not surprising to find instances in the Tyne where $\delta^{15}NO_3^-$ signatures deviate from conservative mixing (Figures 5b and 5c). In any case, the relatively minor levels of biological modification of nutrients observed in the Tyne during this study does not compare with other UK estuaries that have longer residence time (e.g., Thames), where up to 100% DIN attenuation has been observed during dryer periods (Trimmer et al., 2000).

In the Tyne, small but significant deviations from conservative mixing include the lighter $\delta^{15}NO_3^-$ values during Mar-03 (Figure 5c) and the slightly heavier $\delta^{15}NO_3^-$ values during Jul-02 (Figure 5b). In Mar-03 the Tyne exhibited high turbidity with a well pronounced turbidity maximum (Chapters 4 and 5). Well-oxygenated water containing elevated particle concentrations has been shown to support the growth of large numbers of attached nitrifiers (Billen, 1975; Soetaert and Herman, 1995). Thus the lighter excursion from conservative mixing in the Mar-03 survey could have resulted from nitrate produced by nitrification. The small shift towards heavy values in Jul-02 could be explained by the preferential uptake of ^{14}N nitrate by algae. This sampling period recorded some of the highest chlorophyll a concentrations found during all four surveys to the Tyne Estuary (up to 4.3 μg/L; Table 1). However, it is worth emphasizing that most of the variability in nitrate isotopes in Tyne could be explained by mixing rather than in estuarine processing.

In the Tweed, the mid-estuarine nitrate peaks seen in the nitrate concentrations (Figures 3e and 3f) were not replicated by the isotopic data (Figures 5e and 5f). This supports a riverine source for the winter profile and indicates that in the summer this input must also possess the same $\delta^{15}NO_3^-$ signature as found in the River Tweed. While it may have been the case for ammonium, it is unlikely that the non-conservative behaviour of nitrate in the Tweed Estuary during the summer was due to inputs from the Tweedmouth sewer outlet (Uncles et al., 2003). Instead, the isotopic data indicate nitrate discharge from the River Whiteadder (Figure 1) draining similar catchment characteristics to be responsible for the additional nitrate inputs.

SUMMARY

In the Tweed, enhanced agricultural activity in the watershed leads to elevated nitrate inputs into the estuary relative to the Tyne. Increased contributions from agricultural soil, atmospheric and fertiliser sources during the winter led to a higher nitrate flux and more ^{15}N-depleted $\delta^{15}NO_3^-$ values. In the Tyne, where agricultural inputs are less important, isotopically light nitrate entering the estuary during the Feb-02 survey demonstrated the importance of atmospherically-derived sources during periods of high river discharge associated with winter storm events. During these types of infrequently sampled periods of high runoff, river-dominated estuaries with relatively short residence times therefore can act as conduits for the delivery of ^{15}N-depleted atmospherically-derived nitrate to world's oceans. Under such conditions nitrate concentrations will also behave more conservatively, indicating a limited amount of estuarine processing.

In both the Tyne and Tweed estuaries most of the variability in nitrate and ammonium across the salinity gradient could be explained by mixing between different sources, with sewage-derived inputs originating from HSW the dominant source for ammonium in the Tyne. The ability of both isotopic and concentration weighted mixing curves to describe the changes seen in $\delta^{15}NO_3^-$ and $\delta^{15}NH_4^+$ signatures demonstrates very little estuarine processing of DIN in these two systems. In estuaries with relatively quick freshwater flushing times like the Tyne and Tweed it is therefore not surprising that the effects of high nutrient loading will be felt in the coastal zone rather than in the estuary itself, as has been suggested by others (Nedwell et al., 2002). For instance, the large inputs of sewage-derived ammonium in the Tyne lead to enhanced nitrification in adjacent North Sea waters, as evidenced by isotopically light $\delta^{15}NO_3^-$ values found near the mouth of the Tyne indicating a source of nitrification-generated nitrate. And as indicated by relatively high N_2O saturations measured in the Tyne Estuary (up to 250% N_2O saturation; (Barnes, 2003)), this sewage-derived ammonium also appears to be an indirect greenhouse gas point source.

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CHAPTER 2: Respiration-Photosynthesis dynamics in the Tyne and Tweed Estuaries, UK

ABSTRACT

In order to evaluate the respiration-photosynthesis dynamics in two contrasting North Sea estuaries, pH, temperature, alkalinity, chlorophyll a (chl-a), and isotopic ratios of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and dissolved oxygen ($\delta^{18}O_{DO}$) were measured in the Tyne (July 2003) and Tweed (Jul-03 and Dec-03) Estuaries. Using a concentration dependent isotope mixing line, $\delta^{13}C_{DIC}$ values in the Tweed demonstrated mostly conservative behaviour across the estuary, reflecting mixing between riverine and marine sources, although samples were slightly more ^{13}C-enriched than predicted $\delta^{13}C_{DIC}$ values. In the Tyne, measured $\delta^{13}C_{DIC}$ values were slightly more ^{13}C-depleted than those predicted by conservative mixing. The slightly ^{13}C-enriched $\delta^{13}C_{DIC}$ values, combined with low pCO_2 (<2 times atm. pres.) and ^{18}O signatures of dissolved oxygen ($\delta^{18}O_{DO}$) lower than expected for equilibrium with the atmosphere across the salinity gradient pointed to net autotrophy in the summer in the Tweed Estuary. Conversely, in the Tyne during the summer and in the Tweed during the winter higher pCO_2 (up to 6.5 and 14.4 times atm. pres. in the Tweed and Tyne, respectively), more ^{13}C-depleted $\delta^{13}C_{DIC}$ and ^{18}O-enriched $\delta^{18}O_{DO}$ indicated heterotrophy as the dominant process.

INTRODUCTION

Estuaries are generally considered to be net heterotrophic systems and sources of CO_2 to the earth's atmosphere (Frankignoulle et al., 1998; Smith and Hollibaugh, 1993). In Europe alone, it has been estimated that estuaries emit between 30-60 million tons of carbon per year to the atmosphere, which corresponds to roughly 5-10% of the anthropogenic emissions form Western Europe (Frankignoulle et al., 1998). The magnitude of this flux depends mainly on the balance between uptake of photosynthetic CO_2 and its release via respiration. In turn, the relative intensity of these two processes depends on a wide variety of factors, including enhanced allochthonous input of labile organic matter, water residence time, sunlight availability, rates of community metabolism, temperature and nutrient load (Abril et al., 2002; Howland et al., 2000; Ram et al., 2003; Smith and Hollibaugh, 1993; Wang and Veizer, 2000). In addition, several studies have shown that seasonal variations can lead to an estuary shifting from being net autotrophic to net heterotrophic (Howland et al., 2000; Kemp et al., 1992; Ram et al., 2003; Smith and Hollibaugh, 1997).

Understanding the relative importance of respiration versus photosynthesis within an estuary can therefore provide valuable insight into organic carbon cycling dynamics (i.e., production versus consumption) in these highly complex systems. Two useful parameters that can be used to evaluate these processes include the isotopic ratios of dissolved inorganic carbon (DIC) and dissolved oxygen (DO). DIC concentrations in an estuarine environment are mainly determined by the degree of mixing between marine and freshwater DIC, atmospheric efflux, the relative intensities of photosynthetic and oxidative processes, carbonate or atmospheric CO_2 dissolution, and sediment resuspension (Abril et al., 2004; Abril et al., 2003; Wang and Veizer, 2000). In a system where the soil CO_2 is primarily derived from the decomposition of C_3 plant organic matter ($\delta^{13}C$ ~-28‰), the CO_2 produced has the same $\delta^{13}C$ value as the initial substrate, undergoing little or no fractionation. The partial diffusion of this CO_2 gas results in a ^{13}C-enrichment of approximately +4.4‰ (Cerling et al., 1991), and its subsequent dissolution into water (temperature = 10-20°C, pH = 7.0-9.0) results in a fractionation of approximately +8‰ (Wigley et al., 1978), generating a $\delta^{13}C_{DIC}$ value of around -17 to -21‰. On the other hand, dissolution of atmospheric CO_2 produces $\delta^{13}C_{DIC}$ values of around -1‰, which explains the average value of ~0‰ found for DIC derived from the dissolution of marine limestone. DIC in river water during baseflow conditions should therefore have $\delta^{13}C$ signatures ranging from approximately -17 to -21‰ for systems dominated by silicate weathering caused by soil CO_2 to around -10 to +2‰ in areas where carbonate weathering is involved (Wang and Veizer, 2000). Photosynthesis and respiration can subsequently alter the DIC concentrations and $\delta^{13}C$ signatures, whereby the former will cause a positive isotopic shift due to preferential removal of the lighter isotope during DIC uptake and the latter will cause a negative isotopic shift due to the oxidation of organic matter with a more depleted ^{13}C signature (Mook and Tan, 1991).

Similarly, isotopes of dissolved oxygen ($\delta^{18}O_{DO}$) have also been employed to examine respiration-photosynthesis dynamics in aquatic systems (Quay et al., 1995;

Wang and Veizer, 2000). The air-water equilibrium value for dissolved O_2 is +24.5‰, with +23.8‰ derived from atmospheric oxygen (Coplen et al., 2002) and +0.7‰ resulting from oxygen fractionation during dissolution in the water (Benson and Krause, 1984). If photosynthesis is the dominant process, DO will become supersaturated and the resulting $\delta^{18}O$ value will be less than +24.5‰, due to the production of isotopically lighter O_2, which during aquatic photosynthesis is identical to the $\delta^{18}O$ of the source water (Wang and Veizer, 2000). On the other hand, respiration will result in DO undersaturation, and the resulting $\delta^{18}O$ will be greater than +24.5‰ due to the preferential utilization of the lighter isotope (Kiddon et al., 1993; Wang and Veizer, 2000). Combing $\delta^{13}C_{DIC}$ with $\delta^{18}O_{DO}$ isotopes therefore provides a powerful tool to delineate between isotopic shifts caused by atmospheric equilibration and estuarine mixing process with those produced by photosynthesis and respiration.

In this study we have measured pH, temperature, alkalinity, chlorophyll a (chl-a), $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ isotopes in order to qualitatively determine the relative importance of respiration versus photosynthesis in two contrasting North Sea estuaries, the Tyne and Tweed (NE UK). Surveys were carried out in summer low flow conditions (July 2003) in the Tyne and Tweed Estuaries and winter high flow conditions (December 2003) in the Tweed Estuary in order to further evaluate the influence of seasonal/hydrologic variability.

MATERIALS AND METHODS

Description of Study Sites

The River Tyne, which flows through the densely populated city of Newcastle, has a total drainage area of approximately 2900 km^2 and an average freshwater flow of ~48 m^3/s. Its two main tributaries are the North Tyne, which receives humic-rich waters draining areas of blanket peat afforestation, and the South Tyne, which drains relatively pristine moorland (Baker and Spencer, 2004). The North and South tributaries converge downstream to form the River Tyne, which supplies more than 90% of the total river discharge into the Tyne Estuary (Figure 1; Chapter 1). Most of the remaining freshwater input is derived from the River Derwent. The maximum extent of the tidal estuary is approximately 33 km inland from the North Sea, and the residence time is approximately 5-20 days (A. P. Stubbins, private communication). The Tyne is a partially mixed mesotidal estuary (Baker and Spencer, 2004). Although industrial fluxes to the lower part of the estuary are in decline, it continues to receive significant amounts of urban waste, particularly from sewage treatment facilities located at Howdon in the lower estuary (Baker and Spencer, 2004; Upstill-Goddard et al., 2000). However, the water quality in the Tyne upstream of Newcastle has improved significantly over the past few decades. The Tyne Estuary is fairly deep and gently sloping, with only a very minor extent of salt marshland across the tidal interface.

The Tweed River and its tributaries drain a rural, relatively sparsely populated region located in the border region between England and Scotland. Most of the Tweed's discharge originates from the main river, although approximately 10% of the freshwater input comes from the Whiteadder, which joins the Tweed approximately 6.5 km upriver from the North Sea. The combined catchment area of the Tweed and Whiteadder is approximately 4900 km^2 and the average freshwater input into the estuary is ~84 m^3/s (Fox and Johnson, 1997). The maximum length of the Tweed estuary (Figure 1; Chapter 1) is around 13 km and the residence time is approximately 1 day (Uncles and Stephens, 1996). The Tweed is a partially mixed to stratified microtidal estuary (Uncles and Stephens, 1996). The catchment is dominated by agricultural activity, ranging from upland areas of moorland used for sheep grazing to more arable regions in the lowlands (Neal, 2002). The Tweed receives significantly less anthropogenic inputs than the Tyne, as reflected in its favourable water quality ratings (Robson and Neal, 1997). The Tweed Estuary is relatively shallow, with large areas of exposed mudflats visible during low tide, and is fairly steeply rising (Uncles and Stephens, 1996) Salt marshes in the Tweed Estuary make up <10% of the tidal surface area (Joint Nature Conservation Committee, UK).

Analytical Procedures

Data reported here (Tables 1& 2) are from near-surface water samples collected on single day excursions to the Tyne and Tweed Estuaries in July 2003 and from the Tweed Estuary in December 2003. Site selection within the estuary was based on *in situ* salinity measurements (using a portable probe) in order to ensure adequate, high-resolution coverage of the full salinity gradient. Once back in the laboratory, salinity was analysed on a pre-calibrated Hanna (model 8633) conductivity meter. Samples were collected at a depth of 1-2 m from a small boat using a submersed pump. Water temperatures were measured in situ, whereas total alkalinity and pH were measured in the laboratory within 8 hours of sampling. Unfiltered water for these measurements was collected in 250 ml airtight plastic bottles and pre-poisoned with $HgCl_2$. The pH was determined using a Jenway 3051 pH meter with a precision of ±0.02 pH units based on the standard deviation of replicate samples. Total alkalinity was measured via HCl titration and replicate field samples yielded a relative standard deviation (%RSD) of better than ±9 percent. Total DIC (Appendix 1), partial pressures of CO_2 (ppm) and excess partial pressures of CO_2 (pCO_2 / atmospheric CO_2) were calculated from temperature, pH and alkalinity (Clark and Fritz, 1997) and are presented in Table 1. Unfiltered water samples for isotopic analysis of DIC and dissolved oxygen (DO) were collected in 30 ml airtight glass vials that were pre-poisoned with $HgCl_2$.

Suspended material for chlorophyll *a* (chl-a) analysis was collected on pre-combusted (440°C for 4 h) GF/F filters (Whatman). It has been observed that the retention capability of these filters, which have a nominal pore size of ~0.7 μm, is approximately equal to that of 0.2 μm Nuclepore filters (Chavez et al., 1995). Therefore, the GF/F filters used in this study should have retained the bulk of phytoplankton biomass, including the smaller nanoplankton (2 to 20 μm) and picoplankton (0.2 to 2 μm) size classes. The samples were filtered and then stored at -20°C prior to chl-a extraction. After soaking the filters for several hours in 90% acetone, chl-a in the leachate was determined by measuring fluorescence with a Turner Model 450 fluorometer (Parsons et al., 1984). Based on multiple analyses of standards and samples, the precision for the chl-a measurements was found to be ±10 % RSD.

$\delta^{13}C$ values for DIC were run on an Optima mass spectrometer that was equipped with a Gilson® autosampler and $\delta^{18}O$ values for DO were analysed using a Ap2003 mass spectrometer. For a further explanation of the method for isotope analysis of DO refer to Barth et al. (2004). $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios were expressed in the delta notation as per mil (‰) differences relative to the international VPDB and VSMOW standards, respectively. At several sites across both estuaries triplicate samples were collected for isotopic analysis of DO and DIC. Based on the standard deviation of replicate samples and standards the analytical precisions were ±0.3‰ for all $\delta^{13}C$ values and ±0.2‰ for $\delta^{18}O_{DO}$.

River discharge data are reported here as the two-day averages incorporating the days prior to and day of sampling. Discharges for the Tyne ($Q = 7.3$ m^3/s, 22-23 July 2003) and Tweed ($Q = 14.5$ m^3/s, 07-08 July 2003; $Q = 151.2$ m^3/s, 02-03 December 2003) were obtained from the UK Environment Agency and from the Scottish Environment Protection Agency, respectively. Care was taken to sample the entire estuarine salinity range. However, flow conditions and tidal status restricted the accessible salinity range on some occasions. During the July 2003 transect in the Tyne Estuary, the combined effects of a spring tidal event with extremely low flow resulted in a low salinity end-member sampling point of 13.9 psu due to inaccessibility of the inner estuary. Conversely, during the December 2003 sampling trip to the Tweed Estuary high river flow resulted in a survey comprised predominantly of low salinity water samples. This strong dependence on surface salinity with freshwater runoff has been previously reported in the Tweed Estuary (Uncles et al., 2000).

RESULTS AND DISCUSSION

Geochemical parameters reported here are listed in Table 1 and all isotope data are listed in Table 2. Chl-a concentrations during all surveys in both the estuaries were relatively small, ranging from 0.2 to 1.7 μg/L (July 2003) and from 2.1 to 4.6 μg/L (December 2003) in the Tweed Estuary and from 0.7 to 2.8 μg/L (July 2003) in the Tyne Estuary. Although comparisons in the literature are not available for the Tyne, chl-a values similar to those reported here have been observed previously in the Tweed (<2 μg/L; Uncles et al., 2000), who suggested these low values were due to rapid flushing which prevents the occurrence of repeated algal cell division within the tidal estuary. The slightly higher chl-a concentrations in the Tweed during December were probably related to higher levels of plant debris in the river during this period of high discharge. We therefore assume that autochthonous production in both the Tweed and Tyne estuaries should have only a minor impact on the carbon cycling of dissolved inorganic carbon.

Total DIC concentrations varied across the Tyne estuary from a high of 3.11 mM at a salinity of 20.1 to 2.39 mM in the coastal North Sea (Figure 2a). Unfortunately, due to the lack of a proper riverine end-member, it was impossible to determine from this sample set whether DIC distributions in the Tyne during the Jul-03 reflected a non-conservative estuarine input or a relatively high freshwater

Site/Date	salinity	T (°C)	pH	total alk [mM]	total DIC [mM]	pCO_2 (ppm)	excess pCO_2	chl-a (mg/L)
Tweed								
8/7/03	33.22	12.0	8.25	2.31	2.35	718	1.9	0.2
8/7/03	32.39	12.2	8.26	2.31	2.35	702	1.9	0.3
8/7/03	29.53	12.7	8.25	2.25	2.29	704	1.9	0.6
8/7/03	27.35	13.1	8.27	2.25	2.29	676	1.8	1.2
8/7/03	17.87	14.8	8.33	2.19	2.22	584	1.6	1.4
8/7/03	8.56	16.5	8.39	2.19	2.22	519	1.4	1.4
8/7/03	4.20	17.3	8.41	2.13	2.16	486	1.3	1.4
8/7/03	1.41	17.8	8.46	2.13	2.16	436	1.2	1.5
8/7/03	0.11	18.0	8.77	2.13	2.16	213	0.6	1.6
8/7/03	0.11	18.0	8.76	2.13	2.16	218	0.6	1.7
3/12/03	31.30	8.0	8.10	2.22	2.28	933	2.5	NM[*]
3/12/03	19.38	8.2	7.85	2.13	2.22	1597	4.3	NM[*]
3/12/03	4.51	8.1	7.68	1.86	1.98	2061	5.5	NM[*]
3/12/03	1.90	8.4	7.45	0.97	1.08	1841	4.9	3.9
3/12/03	1.27	8.5	7.57	1.44	1.56	2065	5.5	3.1
3/12/03	0.97	8.5	7.46	1.24	1.38	2300	6.1	3.4
3/12/03	0.90	8.7	7.42	1.02	1.14	2071	5.5	2.1
3/12/03	0.19	8.9	7.34	0.90	1.02	2201	5.9	4.6
3/12/03	0.16	9.0	7.32	0.94	1.08	2423	6.5	4.0
3/12/03	0.12	9.0	7.34	0.94	1.08	2314	6.2	3.8
Tyne								
23/7/03	32.47	14.7	8.17	2.34	2.39	902	2.4	1.0
23/7/03	27.11	16.2	7.85	2.79	2.89	2292	6.1	2.2
23/7/03	25.11	16.6	7.81	2.73	2.84	2471	6.6	0.9
23/7/03	24.24	17.9	7.68	2.67	2.81	3314	8.8	0.7
23/7/03	21.57	18.8	7.57	2.76	2.94	4464	11.9	1.4
23/7/03	20.64	15.5	7.64	2.79	2.96	3687	9.8	2.8
23/7/03	20.10	19.2	7.55	2.91	3.11	4954	13.2	2.7
23/7/03	18.32	18.4	7.50	2.85	3.07	5389	14.4	1.4
23/7/03	16.42	19.3	7.52	2.73	2.93	4986	13.3	1.4
23/7/03	13.94	19.3	7.51	2.73	2.94	5103	13.6	1.4

[*]NM, not measured

Table 1. Geochemical parameters of water samples from the Tyne and Tweed Estuaries, including salinity, temperature (T), pH, total alkalinity (alk), total dissolved inorganic carbon (DIC), partial pressure of CO_2 (pCO_2), excess pCO_2 (relative to atm. pres.), and chlorophyll a (chl-a).

concentration. $\delta^{13}C_{DIC}$ values in the Tyne changed in a near linear manner from -5.9‰ at the least saline sampling location (13.9 psu) to +0.1‰ at the marine end-member (Figure 2b), showing a good linear correlation with salinity ($r^2 = 0.92$, n = 9). The values reported here for total DIC at the mouths of the Tyne and Tweed Estuaries are similar to those reported in the literature for the coastal North Sea (Bakker et al., 1996; Hellings et al., 1999; Howland et al., 2000). Measured pH

Site/Date	salinity	$\delta^{18}O_{DO}$ ‰	$\delta^{13}C_{DIC}$ ‰	$\delta^{13}C_{DIC-pred}$ ‰	$\delta^{13}C_{DIC}$ - $\delta^{13}C_{DIC-pred}$ ‰
Tweed					
8/7/03	33.22	25.2	0.6	0.0	0.6
8/7/03	32.39	24.9	0.9	-0.2	1.1
8/7/03	29.53	NM*	NM*	-	-
8/7/03	27.35	24.3	-1.2	-1.7	0.5
8/7/03	17.87	23.8	-4.6	-4.6	-0.1
8/7/03	8.56	23.6	-8.0	-7.5	-0.5
8/7/03	4.20	23.0	-8.2	-9.0	0.8
8/7/03	1.41	22.5	-9.9	-9.9	0.0
8/7/03	0.11	NM*	NM*	-	-
8/7/03	0.11	NM*	NM*	-	-
3/12/03	31.30	24.9	NM*	-	-
3/12/03	19.38	24.9	NM*	-	-
3/12/03	4.51	25.0	NM*	-	-
3/12/03	1.90	25.0	NM*	-	-
3/12/03	1.27	24.5	NM*	-	-
3/12/03	0.97	25.1	NM*	-	-
3/12/03	0.90	24.9	NM*	-	-
3/12/03	0.19	25.0	NM*	-	-
3/12/03	0.16	25.0	NM*	-	-
3/12/03	0.12	25.0	NM*	-	-
Tyne					
23/7/03	32.47	25.4	0.1	-0.1	0.2
23/7/03	27.11	25.3	-2.7	-2.0	-0.7
23/7/03	25.11	25.6	-2.6	-2.7	0.1
23/7/03	24.24	26.2	-3.8	-3.0	-0.8
23/7/03	21.57	26.1	-4.5	-3.8	-0.7
23/7/03	20.64	25.5	-4.1	-4.1	0.0
23/7/03	20.10	26.6	-5.1	-4.3	-0.9
23/7/03	18.32	26.1	-5.6	-4.8	-0.8
23/7/03	16.42	NM*	NM*	-	-
23/7/03	13.94	26.1	-5.9	-5.9	0.1

*NM, not measured

Table 2. Isotopic parameters of water samples from the Tyne and Tweed Estuaries, including, $\delta^{18}O$ of dissolved oxygen ($\delta^{18}O_{DO}$), $\delta^{13}C$ of dissolved inorganic carbon ($\delta^{13}C_{DIC}$), $\delta^{13}C_{DIC}$ predicted by conservative mixing ($\delta^{13}C_{DIC-Pred}$), and differences between measured $\delta^{13}C_{DIC}$ values and $\delta^{13}C_{DIC-pred}$ ($\delta^{13}C_{DIC}$ - $\delta^{13}C_{DIC-pred}$).

values in the Tyne ranged from 7.51 to 8.17, and in general increased toward the North Sea (Figure 2c). Partial pressures of CO_2 decreased toward the North Sea (Figure 2d). Using an atmospheric CO_2 concentration of 375 ppm, excess pCO_2 values ranged from 2.4 to 14.4, with the highest value found at a salinity of 18.3.

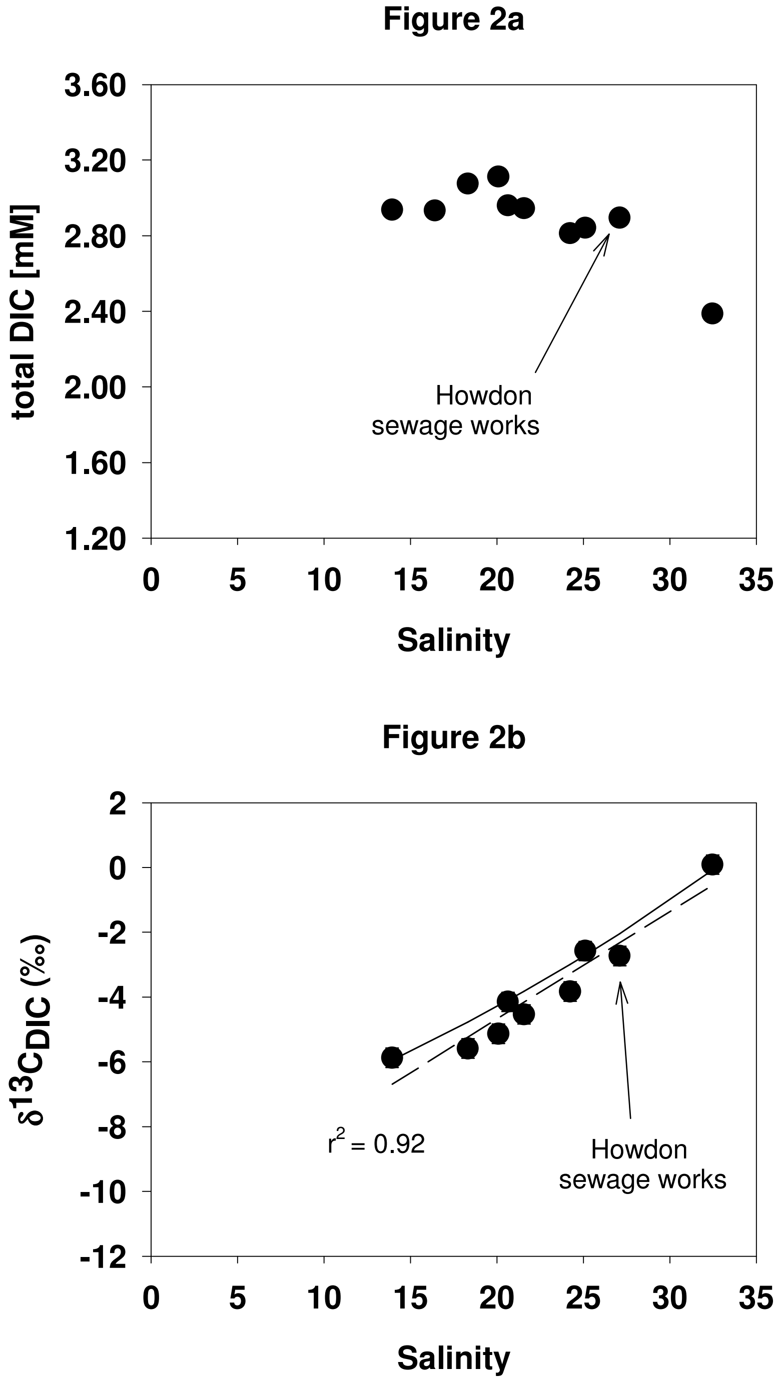

Figure 2. (a) Concentrations of total DIC [mM] and (b) $\delta^{13}C$ values (‰) for DIC ($\delta^{13}C_{DIC}$) in the Tyne Estuary during Jul-03 plotted versus salinity. The dashed line in Figure 2b represents the linear relationship between $\delta^{13}C$ and salinity ($r^2 = 0.92$, $n = 9$). The solid line in Figure 2b represents the conservative isotopic mixing curve (Eq. 1). Accuracy and reproducibility was ±0.3‰ for $\delta^{13}C$ values.

The total DIC in the Tweed during July 2003 ranged from 2.16 (freshwater) to 2.35 mM (marine) and behaved more linearly across the salinity gradient than in the Tyne (Figure 3a). The very good linear relationship between total DIC and salinity (not shown, $r^2 = 0.95$, n = 10) implied conservative mixing between riverine

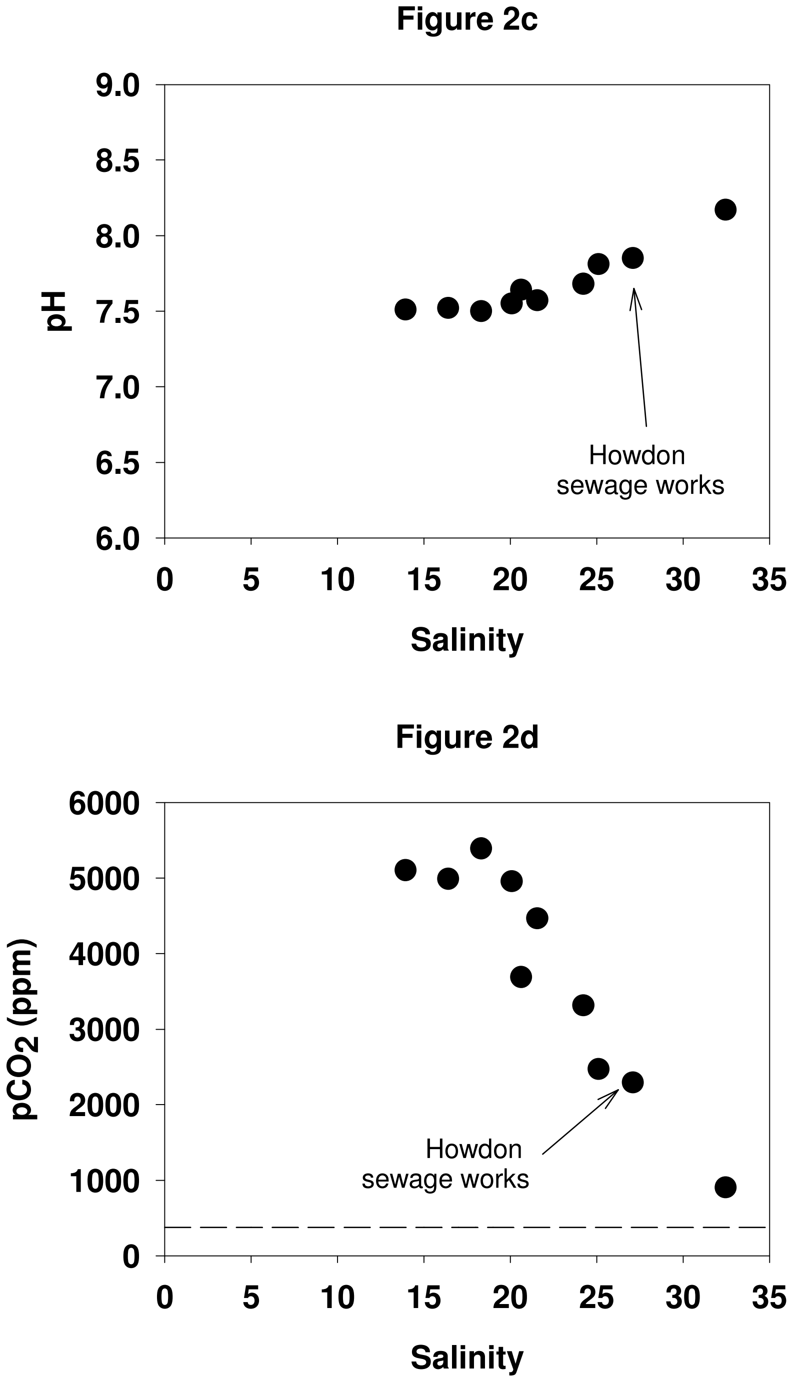
Figure 2c

Figure 2d

Figure 2. (c) pH and (d) partial pressures of CO_2 (pCO_2) in the Tyne Estuary during July 2003 plotted versus salinity. The vertical dashed line in Figure 2d represents the atmospheric CO_2 concentration (375 ppm).

and coastal North Sea end-members. $\delta^{13}C_{DIC}$ signatures (Figure 3b) ranged from -9.9‰ (1.4 psu) to +0.6‰ (33.2 psu) in the coastal North Sea. The strong linear relationship between $\delta^{13}C_{DIC}$ values and salinity ($r^2 = 0.99$, n = 7) in the Tweed

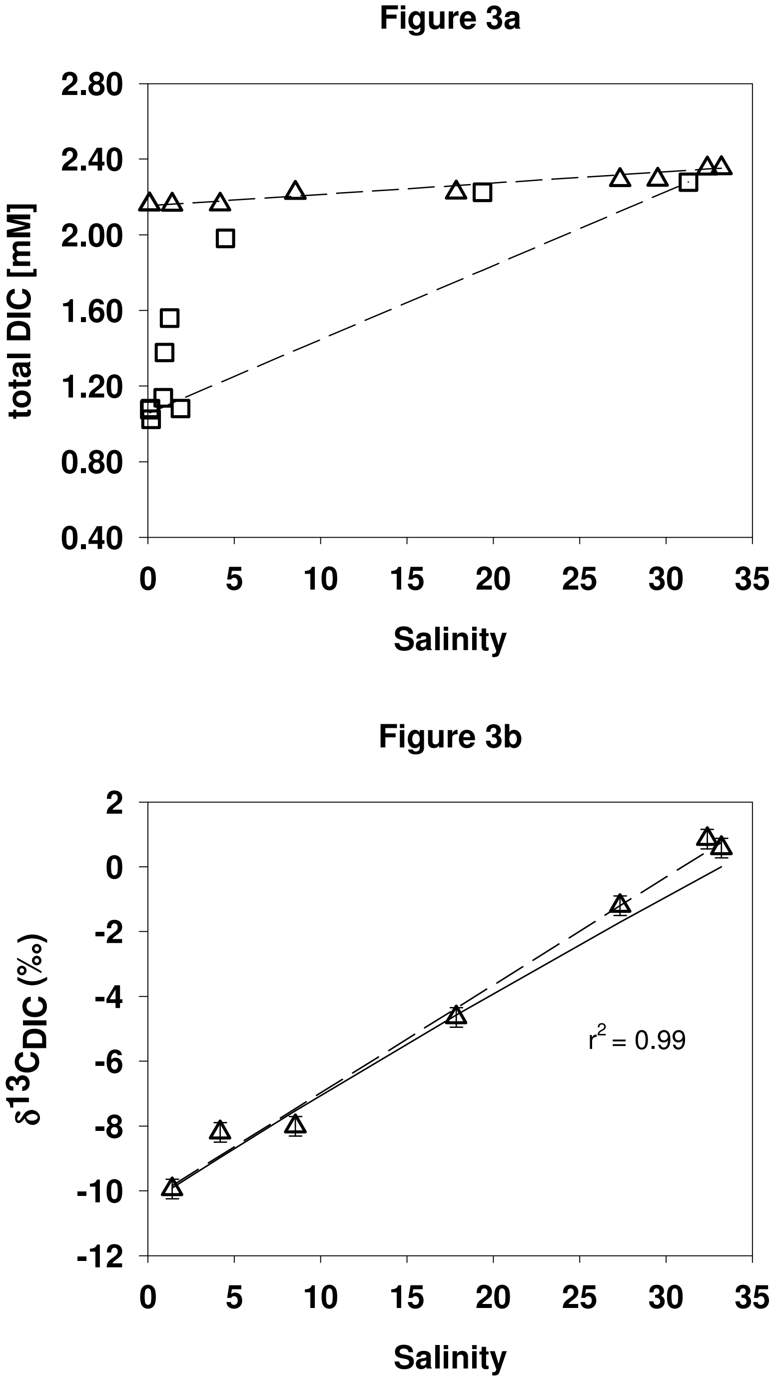

Figure 3. (a) Concentrations of total DIC [mM] and (b) $\delta^{13}C$ values (‰) for DIC ($\delta^{13}C_{DIC}$) in the Tweed Estuary during Jul-03 (open circles) and December 2003 (open squares) plotted versus salinity. The straight dashed lines in Figure 3a represent the concentration of DIC expected due to conservative mixing between riverine and marine sources DIC. The dashed line in Figure 3b represents the linear relationship between $\delta^{13}C$ and salinity ($r^2 = 0.99$, n = 7). The solid line in Figure 3b represents the conservative isotopic mixing curve (Eq. 1). Accuracy and reproducibility was ±0.3‰ on $\delta^{13}C$ values.

during Jul-03 therefore also suggested conservative mixing. Note that $\delta^{13}C_{DIC}$ signatures from the December 2003 survey were not measured.

As opposed to the Tyne, pH in the least saline part of the Tweed estuary during the July 2003 transect (Figure 3c) was relatively high (8.77), and decreased in

Figure 3c

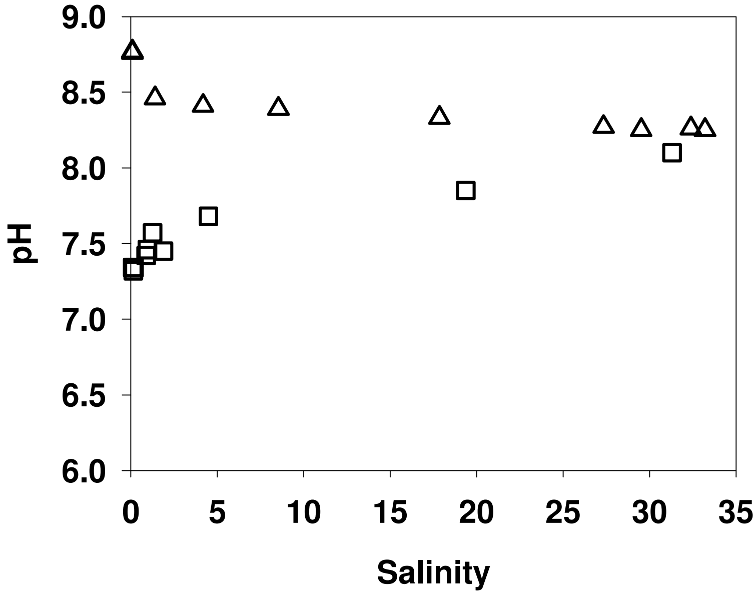

Figure 3d

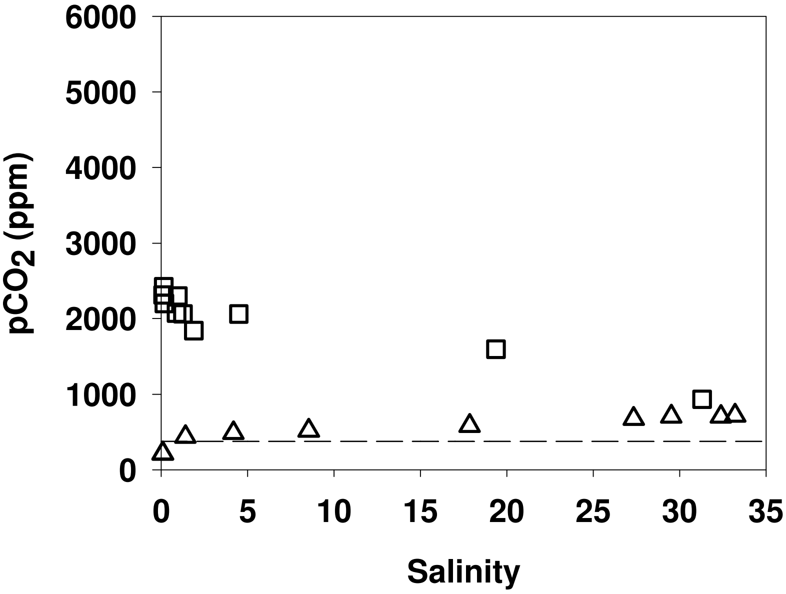

Figure 3. (c) pH and (d) partial pressures of CO_2 (pCO_2) in the Tweed Estuary during July 2003 (open circles) and December 2003 (open squares) plotted versus salinity. The vertical dashed line in Figure 3d represents the atmospheric CO_2 concentration (375 ppm).

a near linear manner to a value of 8.25 at the North Sea end-member. The high pHs and relatively high total DIC found in the Tweed were also reported by others (Howland et al., 2000; Neal, 2002), and are indicative of carbonate-rich groundwater input dominating the riverine DIC during periods of low flow. Conversely, during periods of high flow, concentrations of DIC and pH values in rivers are expected to be lower, mainly because the larger freshwater inputs result to a larger part from overland flow that is less affected by calcium carbonate buffering. These seasonal trends have been previously reported for the Tweed (Howland et al., 2000) and were observed during the December 2003 survey, with total DIC and pH ranging from 1.02 to 2.28 mM (Figure 3a) and 7.32 to 8.10 (Figure 3c), respectively. However, in contrast to the summer, total DIC concentrations in the Tweed during Dec-03 demonstrated non-conservative inputs across the estuary. In July 2003, excess pCO_2 in the Tweed was generally quite low and increased across the salinity gradient from an undersaturated value of 0.6 at the freshwater end-member to a maximum of 1.9 at the North Sea sampling location (Figure 3d). Conversely, during December 2003 excess pCO_2 was much higher, ranging from 2.5 to 6.5 (Figure 3d).

$\delta^{18}O$ signatures of dissolved oxygen ($\delta^{18}O_{DO}$; Figure 4) in the Tyne ranged from +26.1‰ at 13.9 psu to +25.4‰ at 32.5 psu and were all significantly more ^{18}O-enriched than the air-water equilibrium value of +24.5‰. $\delta^{18}O_{DO}$ signatures in the Tweed during July 2003 ranged from +22.5‰ (1.4 psu) to +25.2‰ (33.2 psu), with values < 30 psu being slightly more ^{18}O-depleted than the air-water equilibrium value. During December 2003 $\delta^{18}O_{DO}$ values in the Tweed were relatively invariant across the estuary (+24.9 ±0.2‰), although most were slightly more ^{18}O-enriched than the air-water equilibration value.

In order to verify whether $\delta^{13}C_{DIC}$ signatures obey conservative mixing between marine and riverine sources, a two end-member mass balance equation using both isotopic and concentration data was utilised (Mook and Tan, 1991):

$$\delta^{13}C_{\text{DIC-pred}} = \frac{S_S(DIC_F\delta^{13}C_F - DIC_M\delta^{13}C_M) + S_F DIC_M\delta^{13}C_M - S_M DIC_F\delta^{13}C_F}{S_S(DIC_F - DIC_M) + S_F DIC_M - S_M DIC_F} \quad (1)$$

Where S_S, S_F and S_M and DIC_S, DIC_F and DIC_M refer to the salinities and total DIC of the sample, the freshwater and the marine components, respectively, and $\delta^{13}C_{DIC-}$

$_{\text{pred}}$ refers to the predicted or calculated $\delta^{13}C$ signature for DIC. Unfortunately, precise freshwater end-members were not obtained for either the Tyne or Tweed Estuaries during the July 2003 surveys. Thus, total DIC and $\delta^{13}C_{DIC}$ values measured at the lowest salinities of 1.4 and 13.9 were substituted for the freshwater end-members (S_F) in the Tweed (DIC_F = 2.19 mM, $\delta^{13}C_F$ = -9.9‰) and Tyne (DIC_F = 3.16 mM, $\delta^{13}C_F$ = -5.9‰), respectively. While we cannot test for estuarine mixing behaviour near the freshwater – saline interface, our data together with Eq. 1 can still be used to analyse the mixing behaviour in the remaining salinity range. In any case, plotting salinity vs. $\delta^{13}C_{DIC}$ for the July 2003 data set (Figures 2b and 3b) yields freshwater end-members (at the y-intercept) of approximately -10.3 and -11.3 ‰ for the Tweed and Tyne, respectively. These values are within the narrow range of $\delta^{13}C_{DIC}$ values (-10 to -12‰) reported by (Mook, 1970) for DIC in a variety of rivers in northwestern Europe. S_M, DIC_M and $\delta^{13}C_M$ were measured in the coastal North Sea at the mouth of each estuary and are 33.2, 2.34 mM and +0.6‰ for the Tweed and 32.5, 2.40 mM and +0.1‰ for the Tyne.

The conservative isotopic mixing curves are plotted with the measured $\delta^{13}C_{DIC}$ signatures in Figures 2b and 3b as solid lines. In the Tweed, the measured $\delta^{13}C_{DIC}$ mostly seemed to indicate conservative mixing (Figure 3b), although several data points were slightly higher than the predicted $\delta^{13}C_{DIC}$ values ($\delta^{13}C_{DIC\text{-}pred}$). In the Tyne, however, many of the measured $\delta^{13}C_{DIC}$ values were slightly lower than those predicted by conservative mixing (Figure 2b). The differences between measured $\delta^{13}C_{DIC}$ values and $\delta^{13}C_{DIC\text{-}pred}$ ($\delta^{13}C_{DIC}$ - $\delta^{13}C_{DIC\text{-}pred}$) ranged from -0.9 to +0.2‰ (mean = -0.4 ‰, n = 9) and -0.5 to +1.1‰ (mean = +0.3‰, n = 7) in the Tyne and Tweed Estuaries, respectively (Table 2). Therefore, these systematic differences suggested that in addition to estuarine mixing other processes in these two estuaries were influencing the $\delta^{13}C_{DIC}$ signatures.

As mentioned previously, a proper riverine end-member was not obtained during the Jul-03 survey to the Tyne Estuary. Therefore, it was not possible to ascertain whether DIC distributions reflected a significant estuarine input or whether the higher levels of DIC across the Tyne were a function of a relatively high freshwater concentration, reflecting upriver or watershed soil processes. However, considering the high pCO_2 (up to 14.4 times atm. pres.) measured during this survey, it is likely that the non-conservative behaviour of $\delta^{13}C_{DIC}$ signatures in the Tyne were a result of net *in situ* water column oxidation of ^{13}C-depleted organic matter. Since dissolution of carbonates or atmospheric CO_2 results in $\delta^{13}C_{DIC}$ values of ~ -1 or 0‰, these processes would cause a positive shift in $\delta^{13}C$ and can therefore be excluded. Furthermore, due to the relatively low SPM concentrations reported in the Tyne during the Jul-03 transect (Chapters 4 and 5) is not likely that DIC was being released via fluid mud resuspension (Abril et al., 2004).

The relatively high pCO_2 values in the Tyne are within range of those reported in other estuaries in Western Europe (Abril et al., 2002; Barth et al., 2003; Frankignoulle et al., 1998). In these studies, the large CO_2 fluxes emitted from these estuaries were mostly attributed to the mineralization of labile, pollution-derived organic matter. In the Tyne, however, many of the sampling sites that exhibited high pCO_2 were located upstream of Newcastle, where the pollutant load is generally considered low. In the lower part of the estuary just downstream of the main urban area (Figure 1; Chapter 1) is the Howdon sewage works, one the UK's largest estuarial secondary treatment facilities. Thus, while mineralization of organic carbon originating from contaminated urban sources located in the lower part of the estuary may account for some of the generated CO_2, it is likely that a significant amount of labile terrestrial material originating from within the watershed is also undergoing bacterial oxidation within the estuary. The fact that the highest pCO_2 levels found in the Tyne were not associated with Howdon, which was located nearest the 27.1 psu sampling site during the July 2003 survey, supports this claim.

While $\delta^{13}C_{DIC}$ mostly obeyed conservative mixing, the slightly more ^{13}C-enriched behaviour observed in some $\delta^{13}C_{DIC}$ samples in the Tweed may have been the result of photosynthesis, dissolution of carbonates or atmospheric CO_2, or atmospheric efflux. However, considering the short residence time of the Tweed (~ 1 day), prolonged atmospheric efflux is not considered to play a major role in influencing DIC distributions across this estuary. This leaves either photosynthesis or dissolution of carbonates or atmospheric CO_2 as the two main processes responsible for the slightly ^{13}C-enriched $\delta^{13}C_{DIC}$ values found in this estuary. Considering the lack of a discernible estuarine input of DIC (Figure 3a), it is unlikely that the latter two processes had a major influence on the behaviour of $\delta^{13}C_{DIC}$ signatures across the salinity gradient. However, when DIC is not limiting, fractionation caused during photosynthetic uptake should be more pronounced, which might not be evident in DIC versus salinity profiles. Therefore, while $\delta^{13}C_{DIC}$ values in the Tweed appeared to be mainly controlled by conservative mixing, a secondary influence was the preferential removal of the lighter isotope caused by photosynthetic uptake. However, the most intensive summertime autotrophic activity is probably confined to the non-tidal river, as evidenced by the documented DO supersaturation (Scottish Environmental Protection Agency) and low freshwater pCO_2 levels reported here and in other studies (Howland et al., 2000; Neal et al., 1998). Taking into consideration the low chl-a values (<1.7 μg/L) reported in this study, benthic macrophytes are likely to be the dominant cause of net autotrophy in the Tweed, as has been suggested by others (Howland et al., 2000; Neal et al., 1998).

Other evidence which points to net autotrophy in the Tweed and net heterotrophy in the Tyne in the summer can be found upon examination of $\delta^{18}O$ values for DO (Figure 4). The dashed line in this figure represents the expected $\delta^{18}O_{DO}$ values caused by atmospheric equilibration (+24.5‰). During July 2003 $\delta^{18}O_{DO}$ values from the Tweed mostly plot below +24.5‰, suggesting net autotrophy within the estuary. However, $\delta^{18}O_{DO}$ signatures produced by photosynthesis will also reflect the $\delta^{18}O$ of the source water from which dissolved oxygen is formed during photosynthesis. In an estuarine environment the $\delta^{18}O$ of the water ($\delta^{18}O_{H2O}$) should change linearly with salinity, reflecting conservative mixing between freshwater and seawater. Consequently, while $\delta^{18}O_{DO}$ signatures across the Tweed during July 2003 reflect net autotrophy and mixing with a more air-water equilibrated ^{18}O-enriched marine DO end-member, the positive linear increase in $\delta^{18}O_{DO}$ values observed across the salinity gradient during this period is also a function of the increasing $\delta^{18}O_{H2O}$ signatures. In any case, the most ^{18}O-enriched $\delta^{18}O_{DO}$ signatures were found in the freshwater regions of the estuary, confirming the suggestion made in the previous section that the most intensive autotrophic activity was confined to the non-tidal river.

In contrast to the Tweed summer survey, $\delta^{18}O_{DO}$ isotopes from the Tyne fall above the atmospheric equilibration value of +24.5‰. As shown in Figure 4, there was a consistent DO ^{18}O-enrichment across the entire salinity gradient, with values approaching atmospheric equilibration at higher salinities. Therefore, in conjunction with high pCO_2 values (excess $pCO_2 = 2.4-14.4$) and slightly ^{13}C-depleted $\delta^{13}C_{DIC}$ signatures, $\delta^{18}O_{DO}$ isotopes provide further evidence for the dominance of heterotrophic activity in the Tyne Estuary during July 2003.

$\delta^{18}O_{DO}$ signatures in the Tweed during December 2003 plot slightly above +24.5‰ (Figure 4), suggesting that the large freshwater input into this estuary during the winter survey was approaching equilibration with atmospheric oxygen. The slightly elevated $\delta^{18}O_{DO}$ signatures are associated with higher pCO_2 values (excess $pCO_2 = 2.5-6.5$), confirming net respiration within the Tweed during the winter. Howland et al. (2000) have also reported this reversal in photosynthesis-respiration dynamics in the Tweed between seasons. Since pollution-derived anthropogenic carbon inputs into the relatively pristine Tweed are thought to be minor, these results suggest that a significant amount of terrestrial organic carbon was undergoing mineralization in the Tweed Estuary during the December 2003 survey. Further evidence for this can be seen in DIC distributions, which showed non-conservative inputs across the salinity gradient (Figure 3a).

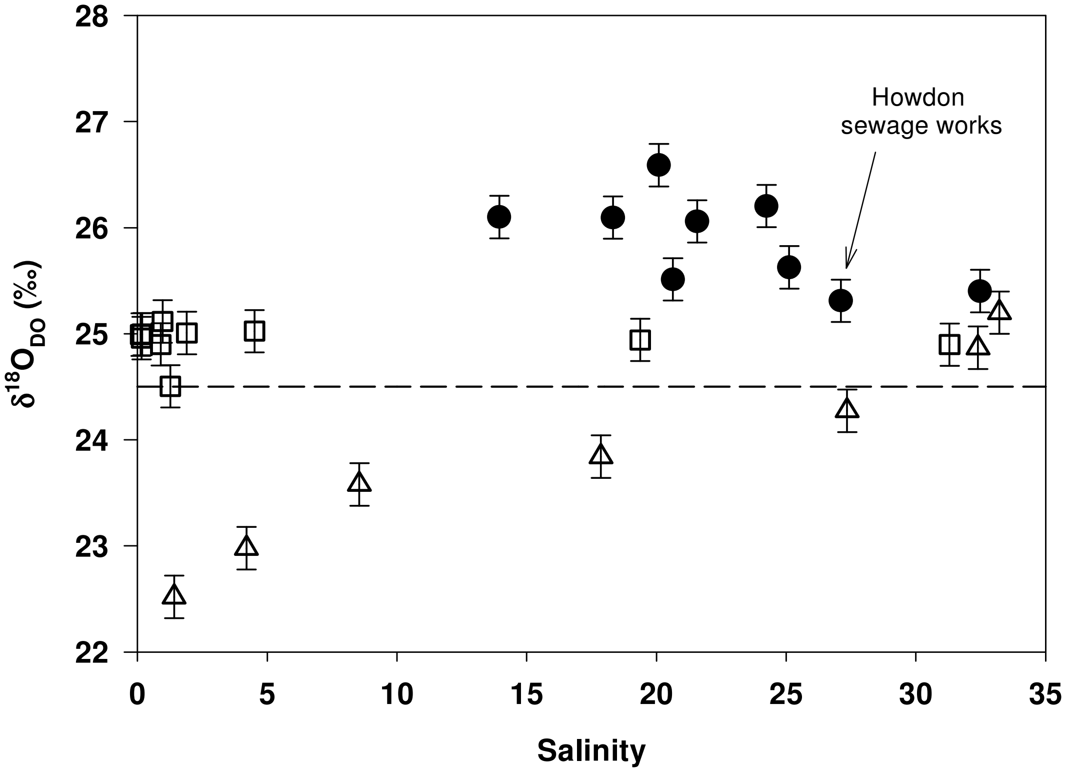

Figure 4. $\delta^{18}O$ values (‰) for dissolved oxygen ($\delta^{18}O_{DO}$) in the Tyne Estuary during July 2003 (closed circles) and in the Tweed Estuary during July 2003 (open triangles) and December 2003 (open squares) plotted versus salinity. The dashed line represents the $\delta^{18}O_{DO}$ air-water equilibrium value of +24.5‰. Vertical error bars represent an accuracy and reproducibility of ±0.2‰ on $\delta^{18}O$ values.

CONCLUSIONS

At first examination it appeared that $\delta^{13}C_{DIC}$ trends across the Tyne and Tweed estuaries in the summer were almost entirely governed by estuarine mixing between riverine and marine sources. However, a concentration dependent isotopic mixing line demonstrated small, yet significant deviations away from conservative behaviour ($\delta^{13}C_{DIC}$ - $\delta^{13}C_{DIC\text{-}pred}$). The deviations were more pronounced in the Tyne, which showed slightly ^{13}C-depleted $\delta^{13}C_{DIC}$ values across the salinity gradient. In the Tweed, where $\delta^{13}C_{DIC}$ signature more closely followed conservative mixing, these deviations were more ^{13}C-enriched. These differences between measured and predicted $\delta^{13}C_{DIC}$ values provided valuable insight into respiration-photosynthesis dynamics, showing that during the summer the Tyne and Tweed Estuaries were net heterotrophic and autotrophic systems, respectively. The relatively low (<2 times atm. pres.) and high (up to 14.4 times atm. pres.) pCO_2 levels in the Tweed and Tyne Estuaries, respectively, helped to substantiate these conclusions. The combined use of $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ isotopes enabled further delineation between DIC changes that were associated with oxidation of organic matter and those associated with net autotrophy. In the Tyne during July 2003, $\delta^{18}O_{DO}$ values were generally heavier than the atmospheric equilibration value (+24.5‰), suggesting net heterotrophy within the estuary. $\delta^{18}O_{DO}$ values in the Tweed during the same period were lighter than +24.5‰, implying net autotrophy. However, during December 2003 $\delta^{18}O_{DO}$ values in the Tweed were slightly ^{18}O-enriched, demonstrating a seasonal reversal in respiration-photosynthesis dynamics. The switch to net heterotrophy in the winter was supported by both non-conservative inputs of DIC and elevated pCO_2 (up to 6.5 times atm. pres.). Since the amount of terrestrial organic matter delivered to the world's oceans can undergo significant modification during estuarine transport, understanding such seasonal and site-specific variability in net heterotrophy is therefore vital.

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CHAPTER 3: Behaviour of dissolved organic carbon in the Tyne and Tweed Estuaries, UK

ABSTRACT

In order to examine the behaviour of terrestrially-derived dissolved organic carbon (DOC) in estuarine environments, concentrations of chlorophyll *a* (chl-a), total, high molecular weight (HMW) and low molecular weight (LMW) dissolved organic carbon (DOC), and stable and radiocarbon isotopes of HMW DOC ($\delta^{13}C$ and $\Delta^{14}C$, respectively) were measured in the Tyne and Tweed Estuaries (NE UK) during both winter and summer periods. Modern $\Delta^{14}C$ values (76-121 ‰) indicated an export of young terrestrial HMW DOC to the North Sea from both rivers. In both estuaries, HMW DOC concentrations showed a large concave removal across the salinity gradient indicating a significant loss (31-70%) of terrigenouos HMW DOC material during estuarine mixing. $\delta^{13}C$ signatures of HMWDOC across the Tyne and Tweed Estuaries exhibited progressive ^{13}C-enrichment with increasing salinity, demonstrating mixing between marine and terrestrial dissolved organic matter. A concentration dependent isotope mixing line was used to evaluate conservative mixing between marine and terrestrial components. This evaluation suggested that conservative mixing alone could not explain the progressive ^{13}C-enrichment across these estuaries. In addition to minor autochthonous inputs, it was hypothesised that the non-conservative behaviour of $\delta^{13}C$ signatures may have been caused by the preferential removal of ^{13}C-depleted HMW DOC during flocculation, biodegradation, and or photochemical oxidation processes. The behaviour of $\delta^{13}C$ signatures across the salinity gradient was closely correlated with $\Delta^{14}C$ values, which demonstrated a ^{14}C-enrichment above modern values with increasing salinity. These elevated $\Delta^{14}C$ values (up to 811‰) were attributed to anthropogenic discharges originating from within the coastal North Sea environment possibly from the nuclear industry.

INTRODUCTION

Rivers carry roughly 1 Gt of carbon to the oceans every year, with around half of this load in dissolved inorganic form (DIC) and the other half in both dissolved (DOC) and particulate (POC) organic forms (Meybeck, 1993; Probst et al., 1994). A significant proportion of organic carbon in rivers is derived from soils and peat, which are thought to be sources of aged, highly-degraded and recalcitrant organic biomolecules such as humic material (Hedges and Keil, 1995). In comparison, contributions by riverine plankton are generally considered negligible globally. If all of the riverine organic carbon reaches the ocean this should make a large contribution to carbon stored in the ocean, particularly with respect to the DOC pool (Raymond and Bauer, 2001b). However, geochemical studies have failed to identify significant proportions of terrestrially-derived organic compounds in the marine DOC pool (Hedges et al., 1997; Meyer-Schulte and Hedges, 1986). This discrepancy may suggest that a large fraction of terrestrial carbon is either quantitatively removed or its diagnostic geochemical properties are altered during transit through estuarine and coastal zones and therefore become similar to those of marine DOC (Bianchi et al., 2004; Moran et al., 2000; Opsahl and Zepp, 2001; Repeta et al., 2002).

Recent studies have demonstrated large net removal of POC by bacterial mineralization in European estuaries (Abril et al., 2002; Frankignoulle et al., 1998) and in the Amazon delta (Keil et al., 1997); however, it is not clear whether the large DOC pool is also subject to estuarine removal and alteration. Previous studies have shown that DOC is mostly refractory across the salinity gradient and hence often exhibits near-conservative mixing (Álvarez-Salgado and Miller, 1999; Mantoura and Woodward, 1983). That is, DOC trends in estuaries generally follow a conservative pattern of decreasing concentrations across the salinity gradient from relatively high riverine towards lower marine concentrations. However, recent work has demonstrated that a linear distribution across an estuary does not necessarily represent conservative mixing, since concurrent sources and sinks may balance each other out (Cifuentes and Eldridge, 1998; Raymond and Bauer, 2000). While most studies documenting non-conservative behaviour of DOC within estuaries have reported elevated concentrations attributed to inputs from such sources as phytoplankton, marshes, and anthropogenic activity (Abril et al., 2002; Benner and Opsahl, 2001; Kattner et al., 1999; Peterson et al., 1994; Raymond and Bauer, 2001a), others have found negative deviations, thus indicating removal of a significant fraction of DOC. At low salinities, this removal of terrestrial DOC has often been attributed to flocculation, whereby the humic-rich, high molecular weight

(HMW) fraction of DOC undergoes aggregation and precipitation leading to burial in sediments (Fox, 1983; Sholkovitz et al., 1978; Uher et al., 2001). Although Sholkovitz et al. (1978) and Fox (1983) found that flocculation was in some cases responsible for a substantial removal of humic material (up to 100%), total DOC distributions still exhibited conservative mixing behaviour across the salinity gradient. In addition to flocculation and adsorption onto suspended sediment (Uher et al., 2001), terrestrial DOC removal within estuaries can also be caused by bacterial respiration (Coffin et al., 1993; Findlay et al., 1992; Moran et al., 1999) Photochemical oxidation is another possibility (Moran and Zepp, 1997), during which large humic molecules are broken down by UV light into more labile components which can subsequently be utilised by aquatic bacteria (Amon and Benner, 1998; Kieber et al., 1989; Miller and Moran, 1997). A recent study by Bianchi et al (2004) has also suggested that a significant amount of terrestrial carbon can be processed via co-metabolism with fresh labile organic carbon (e.g., algal sources). The factors that contribute to such contrast in the behaviour and reactivity of DOC in different estuaries remain to be fully elucidated.

Stable carbon isotopes ($\delta^{13}C$) can provide a valuable means to interpret the origin and behaviour of terrestrial-derived organic carbon within estuarine systems. Traditionally, $\delta^{13}C$ signatures of organic carbon have been used as source indicators within estuarine systems in order to evaluate the proportions of terrestrial and marine material (Canuel et al., 1995; Cifuentes and Eldridge, 1998; Coffin and Cifuentes, 1999; Peterson et al., 1994). Their usefulness in this approach relies on distinct differences in $\delta^{13}C$ values that exist between terrestrial material (i.e., soil, plants) and marine algae. Terrestrial plants use atmospheric CO_2 as their main source of carbon, whereas the main source of carbon for aquatic macrophytes and phytoplankton is DIC (Hillaire-Marcel, 1986). This results in terrestrial plants and soil organic matter having $\delta^{13}C$ values which are generally more ^{13}C-depleted than aquatic plants and marine-produced organic matter (Fry and Sherr, 1984). However, $\delta^{13}C$ signatures may also be of value for discerning biogeochemical processes that lead to systematic changes in stable carbon isotope ratios. This alteration would result from isotope fractionation caused by kinetic effects, which occur as the result of differences in activation energies caused by mass differences (Galimov, 1985). This typically results in the residual substrate becoming more enriched in the heavier isotope, ^{13}C

(i.e., a less negative $\delta^{13}C$ value). For instance, Opsahl and Zepp (2001) found small but significant positive shifts of up to 1.5 ‰ ^{13}C-enrichment in bulk riverine DOC after prolonged exposure to UV light.

The age of terrestrial DOC exported to the world's oceans by rivers will also influence its reactivity during estuarine mixing. It has been suggested that terrestrial DOC exported by rivers is generally younger ^{14}C-enriched material (Hedges et al., 1986). For instance, in a recent study carried out in peat-dominated Arctic Rivers (Benner et al., 2004) the $\Delta^{14}C$ values for DOC ranged between -6 to 307‰, indicating the presence of bomb-produced ^{14}C and hence modern radiocarbon ages. Other studies have shown similar $\Delta^{14}C$ values for DOC in both temperate (Raymond and Bauer, 2001a; Raymond and Bauer, 2001b) and tropical (Hedges et al., 1986) rivers, although in the case of the former older DOC corresponding to radiocarbon ages of between 1000-1400 yrs BP has also been found (Raymond and Bauer, 2001b; van Heemst et al., 2000). Raymond and Bauer (2001a) have demonstrated that during estuarine mixing bacteria preferentially utilise younger, more labile DOC. This results in the residual DOC becoming more ^{14}C-depleted as degradation progresses. From these findings they concluded that rivers can therefore supply organic matter to the world's oceans in a more degraded, pre-aged form (Raymond and Bauer, 2001b). Since geochemical studies have failed to identify significant proportions of terrestrially-derived organic compounds in the marine DOC pool (Hedges et al., 1997; Meyer-Schulte and Hedges, 1986), the export of large amounts pre-aged and altered terrestrial organic carbon could be used to explain the presence of old marine carbon, which is generally a few thousand years old (Williams and Druffel, 1987). Factors that will influence the age of riverine DOC include watershed soil and vegetation characteristics (i.e., the parent age of soil organic matter), weathering patterns, and stream and river residence times (Raymond and Bauer, 2001b).

In this study, we have examined the sources, ages and transformations of dissolved organic carbon in two northern, temperate estuaries (Tyne and Tweed) utilising concentrations of the different size fractions (total, low molecular weight-LMW and HMW) and stable and radiocarbon isotopic signatures of HMW DOC ($\delta^{13}C$ and $\Delta^{14}C$, respectively). C/N ratios (C/N_{HDOM}) of HMW dissolved organic matter (DOM), chlorophyll *a* (chl-a) concentrations and stable carbon isotopes of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) were measured in order to assess the contribution from autochthonous production. Surveys were carried out in summer low flow conditions (July 2003) in the Tyne and Tweed Estuaries and winter high flow conditions (December 2003) in the Tweed Estuary in order to further evaluate the influence of seasonal/hydrologic variability. During each of the five surveys reported here (3 in the Tyne, 2 in the Tweed) there were large net removals in HMW DOC across the salinity gradient. Furthermore, $\delta^{13}C$ signatures often exhibited ^{13}C-enriched, non-conservative behaviour. Therefore, one of the main goals of this study was to ascertain whether these changes in $\delta^{13}C$ values were associated with removal of HMW DOC.

MATERIAL AND METHODS

Description of Study Sites

The Tyne Estuary

The River Tyne, which flows through the densely populated city of Newcastle, has a total drainage area of approximately 2900 km^2 and an average freshwater flow of ~48 m^3/s. Its two main tributaries are the North Tyne, which receives humic-rich waters draining areas of blanket peat afforestation, and the South Tyne, which drains relatively pristine moorland (Baker and Spencer, 2004). The North and South tributaries converge downstream to form the River Tyne, which supplies more than 90% of the total river discharge into the Tyne Estuary (Chapter 1, Figure 1). Most of the remaining freshwater input is derived from the River Derwent. The maximum extent of the tidal estuary is approximately 33 km inland from the North Sea, and the residence time is approximately 5-20 days (A. P. Stubbins, private communication). The Tyne is a partially mixed mesotidal estuary (Baker and Spencer, 2004). Although industrial fluxes to the lower part of the estuary are in decline, it continues to receive significant amounts of urban waste, particularly from sewage treatment facilities located at Howdon in the lower estuary (Baker and Spencer, 2004; Upstill-Goddard et al., 2000). However, the water quality in the Tyne upstream of Newcastle has improved significantly over the past few decades. The Tyne Estuary is fairly deep and gently sloping, with only a very minor extent of salt marshland across the tidal interface.

The Tweed Estuary

The Tweed River and its tributaries drain a rural, relatively sparsely populated region located in the border region between England and Scotland. Most of the Tweed's discharge originates from the main river, although approximately 10% of the freshwater input comes from the Whiteadder, which joins the Tweed approximately 6.5 km upriver from the North Sea. The combined catchment area of the Tweed and Whiteadder is approximately 4900 km^2 and the average freshwater input into the estuary is ~84 m^3/s (Fox and Johnson, 1997). The maximum length of the Tweed estuary (Chapter 1, Figure 1) is around 13 km and the residence time is approximately 1 day (Uncles and Stephens, 1996). The Tweed is a partially mixed to stratified microtidal estuary (Uncles and Stephens, 1996). The catchment is dominated by agricultural activity, ranging from upland areas of moorland used for sheep grazing to more arable regions in the lowlands (Neal, 2002). The Tweed Estuary receives significantly less anthropogenic inputs than the Tyne Estuary, as reflected in its favourable water quality ratings (Robson and Neal, 1997). The Tweed Estuary is relatively shallow, with large areas of exposed mudflats visible during low tide, and is fairly steeply rising (Uncles and Stephens, 1996). Salt marshes in the Tweed Estuary make up <10% of the tidal surface area (Joint Nature Conservation Committee, UK).

Sample Collection

Data reported here (Tables 1-5) are from near-surface water samples collected on single day excursions to the Tyne (Jul-02, Mar-03, Jul-03) and Tweed (Jul-03, Dec-03) Estuaries. Site selection within the estuary was based on *in situ* salinity measurements (using a portable probe) in order to ensure adequate, high-resolution coverage of the full salinity gradient. Once back in the laboratory, salinity was analysed on a pre-calibrated Hanna (model 8633) conductivity meter. Water samples for chlorophyll *a* and DOC analysis were collected in acid-cleaned 4 L and 25 L high density polyethylene (HDPE) containers, respectively, at a depth of 1-2 m from a small boat using a submersed pump. Samples for isotopic analysis of DIC were collected in 30 ml airtight glass vials that were pre-poisoned with $HgCl_2$. River discharge data are reported here as the two-day averages incorporating the days prior

Site/Date	salinity	total DOC [µM]	HMW DOC [µM]	LMW DOC [µM]	C/N$_{HDOM}$	chl-a (µg/L)
Tyne						
11/7/02	33.2	142.9	9.1	136.4	-	0.8
11/7/02	30.9	213.3	15.4	202.5	-	1.4
11/7/02	25.9	283.4	22.2	261.5	-	3.0
11/7/02	19.3	407.6	36.4	388.7	-	2.4
11/7/02	15.0	540.5	45.7	490.7	-	2.4
11/7/02	9.8	576.5	65.1	516.7	-	4.3
11/7/02	4.7	621.1	83.0	554.1	-	2.5
11/7/02	0.2	735.3	130.5	598.0	21.0	1.3
11/7/02	0.2	731.6	131.0	593.5	21.2	1.4
4/3/03	34.1	204.5	6.9	177.5	12.2	0.3
4/3/03	28.7	219.2	14.9	206.4	5.9	0.2
4/3/03	19.0	319.4	17.3	305.4	18.0	0.2
4/3/03	14.8	417.4	34.3	400.5	22.3	0.2
4/3/03	14.1	511.5	36.6	494.4	21.4	0.4
4/3/03	10.5	476.9	45.9	428.6	27.7	0.6
4/3/03	5.3	570.0	71.0	454.4	28.3	1.2
4/3/03	1.3	714.0	157.1	546.3	30.0	0.6
4/3/03	0.1	736.5	261.1	503.5	33.2	0.6
4/3/03	0.1	738.0	260.8	511.1	33.6	0.7
23/7/03	32.5	152.5	5.2	141.2	14.9	1.0
23/7/03	27.1	316.2	13.8	294.2	13.5	2.2
23/7/03	25.1	324.2	21.7	313.6	17.0	0.9
23/7/03	24.2	336.1	22.2	322.0	16.8	1.4
23/7/03	21.6	363.9	29.2	356.9	18.9	2.7
23/7/03	20.6	382.9	30.4	360.1	17.8	0.7
23/7/03	20.1	406.6	33.9	367.0	19.5	1.4
23/7/03	18.3	433.8	37.3	411.0	20.3	2.8
23/7/03	16.4	444.5	43.6	423.9	22.5	1.4
23/7/03	13.9	468.5	51.7	440.1	19.7	1.4

Table 1. Geochemical parameters of water samples from the Tyne Estuary, including salinity, total, low molecular weight (LMW) and high molecular weight (HMW) dissolved organic carbon (DOC) and chlorophyll a (chl-a) concentrations, and C/N ratios of HMW dissolved organic matter.

to and day of sampling. Discharges for the Tyne (Q = 8.6 m^3/s, 10-11 July 2002; Q = 30.7 m^3/s, 03-04 March 2003; Q = 7.3 m^3/s, 22-23 July 2003) and Tweed (Q = 14.5 m^3/s, 07-08 July 2003; Q = 151.2 m^3/s, 02-03 December 2003) were obtained from the UK Environment Agency and from the Scottish Environment Protection Agency, respectively. Care was taken to sample the entire estuarine salinity range. However, flow conditions and tidal status restricted the accessible salinity range on some occasions. During the July 2003 transect in the Tyne Estuary, the combined effects of a spring tidal event with extremely low flow resulted in a low salinity end-member sampling point of 13.9 psu due to inaccessibility of the inner estuary. Conversely, during the December 2003 sampling trip to the Tweed Estuary high river flow resulted in a survey comprised predominantly of low salinity water samples. This strong dependence on surface salinity with freshwater runoff has been previously reported in the Tweed Estuary (Uncles et al., 2000).

Site/Date	salinity	total DOC [μM]	HMW DOC [μM]	LMW DOC [μM]	C/N_{HDOM}	chl-a (μg/L)
Tweed						
8/7/03	33.2	67.2	1.3	64.5	9.7	0.2
8/7/03	32.4	70.9	2.5	70.6	9.5	0.3
8/7/03	29.5	88.0	3.8	80.7	10.4	0.6
8/7/03	27.4	94.3	4.7	84.5	10.5	1.2
8/7/03	17.9	135.1	8.5	124.8	10.7	1.4
8/7/03	8.6	176.7	17.4	145.2	11.0	1.4
8/7/03	4.2	193.8	27.6	151.7	11.0	1.4
8/7/03	1.4	217.6	39.5	161.1	11.1	1.5
8/7/03	0.1	223.5	61.9	163.7	11.8	1.6
8/7/03	0.1	226.1	62.5	165.1	11.9	1.7
3/12/03	31.30	74.9	4.2	70.9	9.0	1.8
3/12/03	19.38	298.2	56.3	264.2	20.1	-
3/12/03	4.51	559.0	181.9	359.2	23.8	-
3/12/03	1.90	590.2	199.4	362.9	24.9	3.9
3/12/03	1.27	607.4	198.4	366.1	24.5	3.1
3/12/03	0.97	644.3	253.7	357.9	27.0	3.4
3/12/03	0.90	651.0	266.3	345.0	25.5	2.1
3/12/03	0.19	658.2	354.0	279.2	24.0	4.6
3/12/03	0.16	664.0	331.8	281.2	22.6	4.0
3/12/03	0.12	668.2	337.9	284.0	21.8	3.8

Table 2. Geochemical parameters of water samples from the Tweed Estuary, including salinity, total, low molecular weight (LMW) and high molecular weight (HMW) dissolved organic carbon (DOC) and chlorophyll a (chl-a) concentrations, and C/N ratios of HMW dissolved organic matter.

Analysis of high molecular weight dissolved organic matter (HMW DOM)

Following sample collection, the 25 L water samples were consecutively pressure-filtered (~1.4 bar) through both a 0.7 μm glass fibre filter and a 0.2 μm capsule filter to remove particulates, algae and most of the bacteria. In order to obtain sufficient material for isotope analysis, dissolved organic matter (DOM) was concentrated via tangential-flow ultrafiltration (UF), a technique that separates a filtrate of known molecular weight. A 1 kDa UF membrane (Millipore) was purchased for this study but after testing was found to have a nominal pore size of 15 kDa, which was capable of separating the high molecular weight (HMW, >15kDa) from the low molecular weight (LMW, <15 kDa) fraction of DOM. The nominal pore size of the UF membrane used in these experiments was slightly higher than those commonly employed by others (Benner et al., 1997; Benner and Opsahl, 2001; Bianchi et al., 2004; Repeta et al., 2002) and was expected to underestimate HMW DOC concentrations relative to these studies. However, the HMW DOC determination reported here are comparable to other studies which have subdivided the HMW fraction into a larger, 10 kDa to 0.2 μM (colloidal organic carbon – COC_{10}) component (Guo and Santschi, 1997; Santschi et al., 1995). In these studies COC_{10} in coastal water samples comprised around 3-7% of total DOC, which is similar to percentages of HMW DOC obtained at our more saline sites. Based on our concentration factor of 30, the percent recovery of total DOC ranged from 90.2 to 107.5%, indicating minimal loss or contamination for bulk carbon.

After initial UF, the HMW DOM samples were diafiltered with 10 L of Milli-Q water (using a 20:1 dilution ratio) in order to remove salts. After diafiltration, HMW DOC samples were freeze-dried, homogenised, re-wetted with Milli-Q water and placed for several days inside a glass desiccator containing concentrated HCl to remove inorganic carbon. They were then vacuum desiccated to remove HCl prior to $\delta^{13}C$ analysis. Total, HMW and LMW DOC values reported here were measured on aliquots of sample which were stored in 20 ml glass scintillation vials using a Shimadzu TOC-5000 analyzer, and the accuracy and precision for these measurements was better than ±5 % RSD.

Analysis of chlorophyll a

Suspended material for chlorophyll *a* (chl-a) analysis was collected on pre-combusted (440°C for 4 h) GF/F filters (Whatman). It has been observed that the retention capability of these filters, which have a nominal pore size of ~0.7 μm, is approximately equal to that of 0.2 μm Nuclepore filters (Chavez et al., 1995). Therefore, the GF/F filters used in this study should have retained the bulk of phytoplankton biomass, including the smaller nanoplankton (2 to 20 μm) and picoplankton (0.2 to 2 μm) size classes. The samples were filtered and then stored at -20°C prior to chl-a extraction. After soaking the filters for several hours in 90% acetone, chl-a in the leachate was determined by measuring fluorescence with a Turner Model 450 fluorometer (Parsons et al., 1984). Based on multiple analyses of standards and samples, the precision for the chl-a measurements was found to be ±10 % RSD.

C/N ratios of HMW DOM and δ^{13}C analysis of HMW DOC and DIC

δ^{13}C signatures of HMW DOC and C/N ratios (reported here as molar ratios) of HMW DOM were determined using a Carlo Erba Instrument NA2500 elemental analyser coupled with a Micromass PRISM III Isotope Ratio Mass Spectrometer (EA-IRMS). δ^{13}C values for DIC ($\delta^{13}C_{DIC}$) were run on an Optima mass spectrometer that was equipped with a Gilson® autosampler. Ratios of ^{13}C to ^{12}C are expressed in delta notation as per mil (‰) differences relative to the VPDB standard, where:

$$\delta^{13}C = [\{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}\} / (^{13}C/^{12}C)_{standard}] \times 1000$$
(1)

Repeat isotope measurements of HMW DOC were made when sufficient material was available, which included all samples collected at salinities <25. At several sites across both estuaries triplicate samples were collected for isotopic analysis of DIC. Based on replicate samples and multiple measurements of standards the 1σ precision for δ^{13}C values presented in this study was ±0.3‰. Based on multiple analyses of standards and samples, the acuracy and reproducibility for %C and %N determined via EA analysis was generally better than ±5% and ±7% RSD, respectively. During the July 2002 survey to the Tyne Estuary HMW DOM samples were not properly diafiltered, resulting in unquantifiable EA-IRMS peak sizes for saline samples. Hence, only the riverine sample from this survey is reported here.

^{14}C dating

HMW DOC samples were prepared to graphite at the Natural Environmental Research Council (NERC) Radiocarbon Laboratory, East Kilbride, UK, and analysed at the Scottish Universities Environmental Research Centre (SUERC), East Kilbride, UK on the 5MV NEC AMS. Graphite was prepared by quantitative recovery of carbon in sealed quartz tubes followed by cryogenic separation of CO_2 (Boutton et al., 1983). Aliquots of CO_2 were converted to an iron/graphite mix by iron/zinc reduction (Slota et al., 1987). A sub-sample of CO_2 was used to measure $\delta^{13}C$ using a dual-inlet mass spectrometer with a multiple ion beam collection facility (VG OPTIMA) in order to normalise ^{14}C data to -25‰ $\delta^{13}C$-PDB. The mass spectrometer was calibrated with international reference materials to a precision of ±0.1‰. Radiocarbon data have been normalised to -25 ‰ $\delta^{13}C$-PDB using values obtained either from on-line measurements made on graphite analysed by AMS or using the $\delta^{13}C$ measurement made on the sub-sample of CO_2 using the dual-inlet mass spectrometer described above. In order to assess the viability of analysing HMW DOM using UF, blanks were prepared by adding internal standard material of known ages to 400 ml of ultrafiltered MilliQ water. The standards were then prepared in the same manner as described above for the samples, including freeze-drying, acidification, re-wetting and vacuum desiccation steps. These UF blanks demonstrated acceptable levels of background contamination. AMS results (Table 3) are reported here as conventional radiocarbon years BP (before AD 1950), absolute percent modern carbon (pMC) and $\Delta^{14}C$ ratios with an overall analytical precision of 1σ. The use of pMC takes into account the ongoing radioactive decay of the international reference standard (oxalic acid) since AD 1950 (Stuiver and Polach, 1977). ^{14}C enrichment > 100 pMC indicates the presence of ^{14}C from atomic weapons testing (post AD 1955). $\Delta^{14}C$ values are expressed according to the convention defined by Stuiver and Polach (1977), where:

$$\Delta^{14}C = (pMC/100 - 1) \times 1000 \qquad (2)$$

Using the error associated with pMC values, and based on results from two replicate analysis (riverine end-members, Tyne Mar-03 and Tweed Dec-03) the 1σ precision for $\Delta^{14}C$ values of HMW DOC presented in this study was better than ±7‰.

RESULTS AND DISCUSSION

Chlorophyll a concentrations

Chl-a concentrations during all surveys in both estuaries were relatively small, ranging from 0.8 to 4.3 μg/L (Jul-02), 0.2 to 1.2 μg/L (Mar-03) and 0.7 to 2.8 μg/L (Jul-03) in the Tyne Estuary and from 0.2 to 1.7 μg/L (Jul-03) and 2.1 to 4.6

µg/L (Dec-03) in the Tweed Estuary. Although comparisons in the literature are not available for the Tyne, chl-a values similar to those reported here have been observed previously in the Tweed (<2 µg/L; Uncles et al., 2000), who suggested these low values were due to rapid flushing which prevents the occurrence of repeated algal cell division within the tidal estuary. The slightly higher chl-a concentrations in the Tweed during December were probably related to higher levels of plant debris in the river during this period of high discharge. Although not measured during the Feb-02 survey to the Tyne Estuary, it is likely that chl-a concentrations during this transect were similar to those measured during the following winter. Based on these low chl-a concentrations we therefore assume that autochthonous production in both the Tweed and Tyne estuaries should have only a minor impact on the carbon cycling of dissolved organic carbon.

Site/Date	salinity	$\delta^{13}C$ ±0.3‰	$\Delta^{14}C$ ±7‰	^{14}C age (yrs BP) ±1σ	^{14}C pMC ±1σ
Tyne					
4/3/03	34.1	-25.7	-		-
4/3/03	28.7	-25.7	-		-
4/3/03	19.0	-27.0	-		-
4/3/03	14.8	-27.1	-		-
4/3/03	14.1	-26.9	-		-
4/3/03	10.5	-27.3	143	modern	114.31 ±0.31
4/3/03	5.3	-27.7	143	modern	114.27 ±0.31
4/3/03	1.3	-27.9	96	modern	109.57 ±0.27
4/3/03	0.1	-28.4	119	modern	111.93 ±0.27
4/3/03	0.1	-28.3	121	modern	112.14 ±0.36
23/7/03	32.5	-24.0	-		-
23/7/03	27.1	-24.8	471	modern	147.05 ±0.47
23/7/03	25.1	-25.1	469	modern	146.86 ±0.38
23/7/03	24.2	-25.3	310	modern	131.00 ±0.41
23/7/03	21.6	-26.1	178	modern	117.79 ±0.36
23/7/03	20.6	-25.6	299	modern	129.91 ±0.36
23/7/03	20.1	-26.6	136	modern	113.64 ±0.28
23/7/03	18.3	-26.2	317	modern	131.66 ±0.41
23/7/03	16.4	-26.9	117	modern	111.66 ±0.34
23/7/03	13.9	-26.8	129	modern	112.92 ±0.35
11/7/02	0.2	-27.6	-		-
11/7/02	0.2	-27.8	-		-

Table 3. $\delta^{13}C$ and $\Delta^{14}C$ signatures, radiocarbon ages, and pMC (% modern absolute carbon) values of high molecular weight dissolved organic carbon (HMW DOC) from the Tyne Estuary.

Behaviour of total, LMW and HMW DOC concentrations

Figures 2, 3 and 4 show the HMW, LMW, and total DOC concentrations in the Tyne and Tweed Estuaries during the July and December 2003 surveys. The straight lines on these figures represent the apparent conservative mixing lines determined from the end-member concentrations. As shown in Figures 2c and 2d, HMW DOC concentrations in the Tweed Estuary exhibited a concave removal across the salinity gradient. HMW DOC in the Tweed decreased from maxima of 62.2 µM (n = 2) and 341.2 µM (n = 3) at the freshwater end-members (≤ 0.2 psu) to values of 1.3 and 4.2 µM at the coastal North Sea sites during July and December 2003, respectively. This concave removal was also observed during the March 2003 survey to the Tyne (Figure 2b), when HMW DOC concentrations decreased from 260.9 µM (n = 2) at the freshwater end-member to 6.9 µM at the mouth of the estuary. Owing to the particularly low flow conditions experienced in the Tyne during the July 2003 survey, a freshwater end-member (≤ 0.2 psu) DOC sample could not be obtained. However, total, HMW and LMW DOC concentrations from the July 2002 survey,

Site/Date	salinity	$\delta^{13}C$ ±0.3‰	$\Delta^{14}C$ ±7‰	^{14}C age (yrs BP) ±1σ	^{14}C pMC ±1σ
Tweed					
8/7/03	33.2	-23.9	-		-
8/7/03	32.4	-23.2	-		-
8/7/03	29.5	-24.0	-		-
8/7/03	27.4	-23.8	-		-
8/7/03	17.9	-24.7	259	modern	125.93 ±0.39
8/7/03	8.6	-25.4	149	modern	114.94 ±0.31
8/7/03	4.2	-25.5	83	modern	108.25 ±0.29
8/7/03	1.4	-26.2	44	modern	104.40 ±0.32
8/7/03	0.1	-26.7	76	modern	107.60 ±0.33
8/7/03	0.1	-26.9	-		-
3/12/03	31.30	-23.3	-		-
3/12/03	19.38	-27.2	811	modern	181.06 ±0.64
3/12/03	4.51	-28.2	123	modern	112.31 ±0.34
3/12/03	1.90	-28.5	123	modern	112.26 ±0.27
3/12/03	1.27	-28.6	153	modern	115.28 ±0.31
3/12/03	0.97	-28.5	129	modern	112.89 ±0.34
3/12/03	0.90	-28.6	-		-
3/12/03	0.19	-28.4	104	modern	110.37 ±0.30
3/12/03	0.16	-28.4	-		-
3/12/03	0.12	-28.6	111	modern	111.05 ±0.34

Table 4. $\delta^{13}C$ and $\Delta^{14}C$ signatures, radiocarbon ages, and pMC (% modern absolute carbon) values of high molecular weight dissolved organic carbon (HMW DOC) from the Tweed Estuary.

during which time river discharge was only slightly higher (Q = 8.6 versus 7.3 m^3/s), showed remarkable similarity with those collected during July 2003. These two surveys are plotted together in Figure 2a. Therefore it was assumed that the

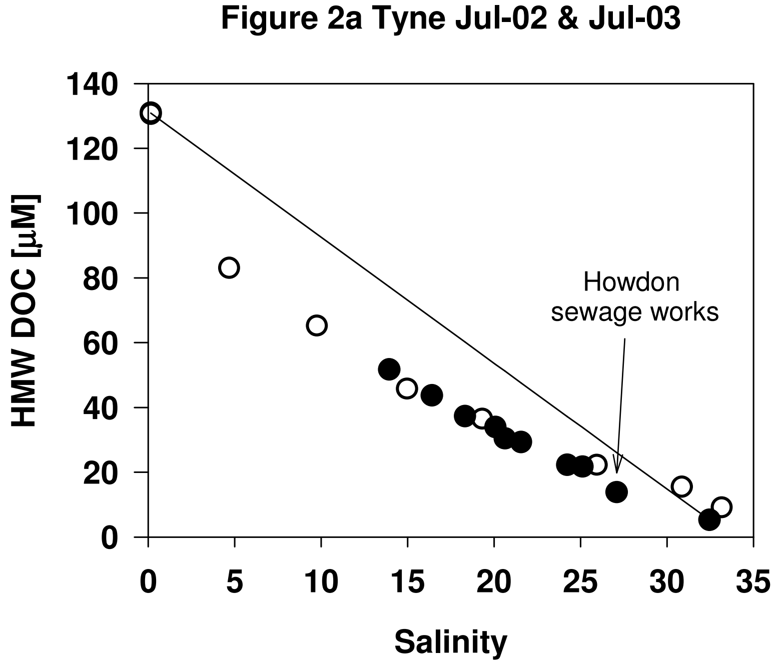


Figure 2a Tyne Jul-02 & Jul-03

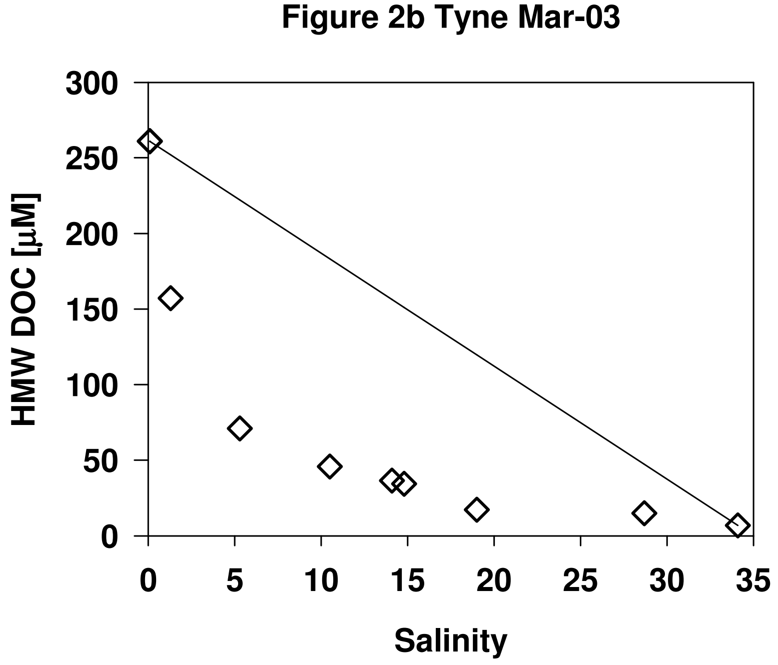
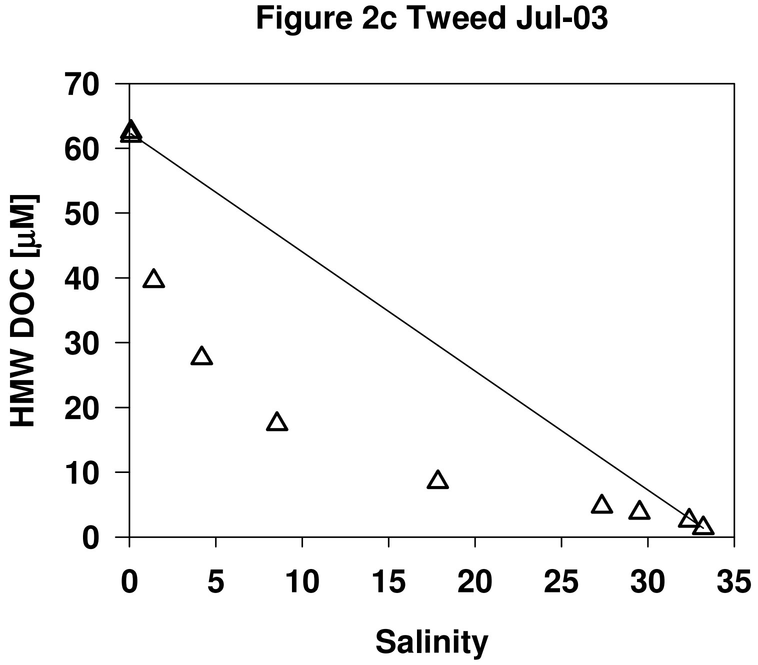


Figure 2b Tyne Mar-03

Figure 2. Concentrations (µM) of high molecular weight dissolved organic carbon (HMW DOC) in the Tyne Estuary during (a) July 2002 (open circles) and July 2003 (closed circles) and (b) March 2003 (open diamonds). The straight line represents the expected concentration due to conservative mixing between marine and riverine sources.

freshwater (0.2 psu) end-member from the July 2002 transect accurately reflects the freshwater DOC input during July 2003. When plotting the July 2002 freshwater HMW DOC end-member (130.8 μM, n = 2) with the July 2003 data, HMW DOC in



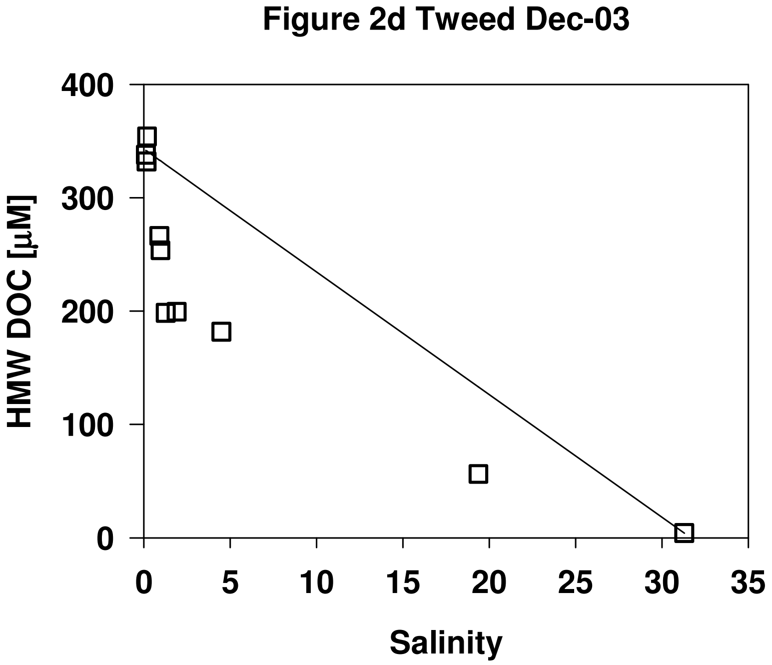


Figure 2. Concentrations (μM) of high molecular weight dissolved organic carbon (HMW DOC) in the Tweed Estuary during (c) July 2003 (open triangles) and (d) December 2003 (open squares) potted versus salinity. The straight line represents the expected concentration due to conservative mixing between marine and riverine sources.

the Tyne during the summer also exhibited a concave removal across the salinity gradient (Fig. 5a), decreasing to 9.1 µM (July 2002) and 5.2 µM (July 2003) at the coastal North Sea sampling sites.

When comparing measured HMW DOC concentrations to those expected from conservative mixing, we found significant differences indicating net removal. The percentage of HMW DOC removal ([1 – (measured HMW DOC / predicted HMW DOC)] × 100) during the three surveys within the mid-salinity range (0.5 to 30 psu) averaged 55 ±13% and 36 ±13% in the Tweed during July and December 2003, respectively, and 31 ±11%, 70 ±15% and 38 ±4% in the Tyne during July 2002, March 2003 and July 2003, respectively. These removals were associated with corresponding decreases in the percentage contribution of HMW to total DOC, which dropped from 28 to 2% and from 54 to 6% in the Tweed during July and December 2003, and from 18 to 6%, 35 to 3% and 11% (at 13.9 psu) to 3% (32.5 psu) in the Tyne during July 2002, March 2003 and July 2003, respectively. The higher concentrations and percentage of HMW DOC found in the freshwater end-members in the winter surveys suggests that more humic-rich material was flushed out of these watersheds and into the Tyne and Tweed estuaries during periods of high river discharge. Therefore, the absolute amount of labile HMW DOC removed in estuaries also increases with flow rate. This suggests that the HWM DOC fraction is inherently reactive during estuarine mixing during all seasons.

In contrast to HMW profiles across the estuaries, the LMW DOC fraction (Figure 3) generally became more dominant with increasing salinity, changing from 73 to 99% (Tweed, July 2003), 42 to 95% (Tweed, July 2003), 81 to 95% (Tyne, July 2002), 68 to 97% (Tyne, March 2003) and 81 to 98% (Tyne, July 2003). Furthermore, in contrast to the significant removal of HMW DOC observed in both estuaries during all five surveys, LMW DOC concentrations showed non-conservative inputs. These LMW DOC additions appeared to be more pronounced during the winter months corresponding with large removals in HMW-DOC. This was particularly evident in the winter Tweed survey (Figure 3d. One explanation for this non-conservative behaviour of LMW DOC is that the HMW DOC in these two estuaries was undergoing degradation to DIC and enzymatic hydrolysis to LMW DOC compounds (Christian and Karl, 1995; Smith et al., 1992). A study by Amon and Benner (1994) suggested that approximately 50% of marine HMW DOC can be removed via microbial degradation, and several studies have shown that DOM reactivity increases with increasing size class (Amon and Benner, 1996; Guo and Santschi, 1997; Santschi et al., 1995).Therefore, if LMW DOC was produced via

Figure 3a Tyne Jul-02 & Jul-03

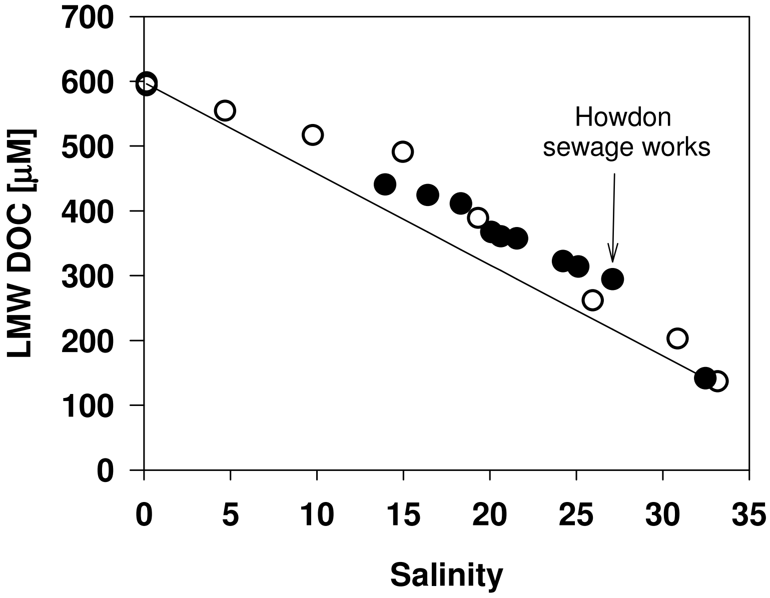


Figure 3b Tyne Mar-03

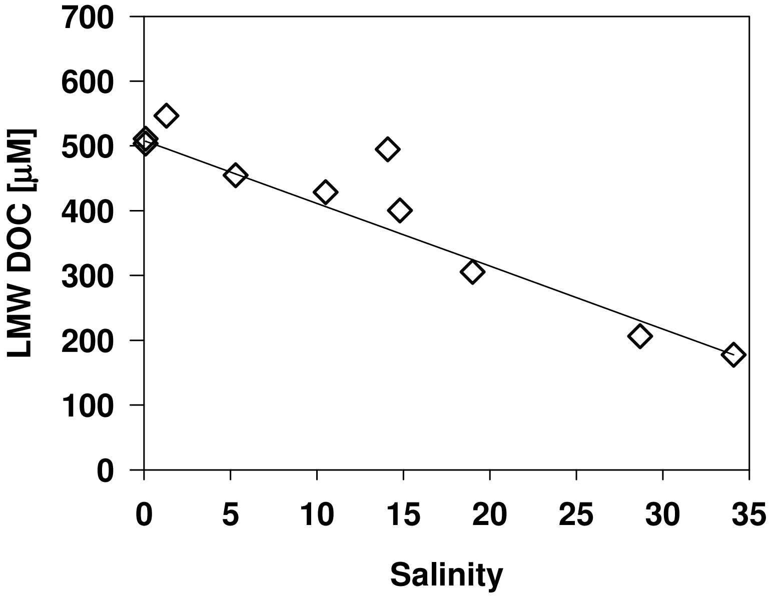
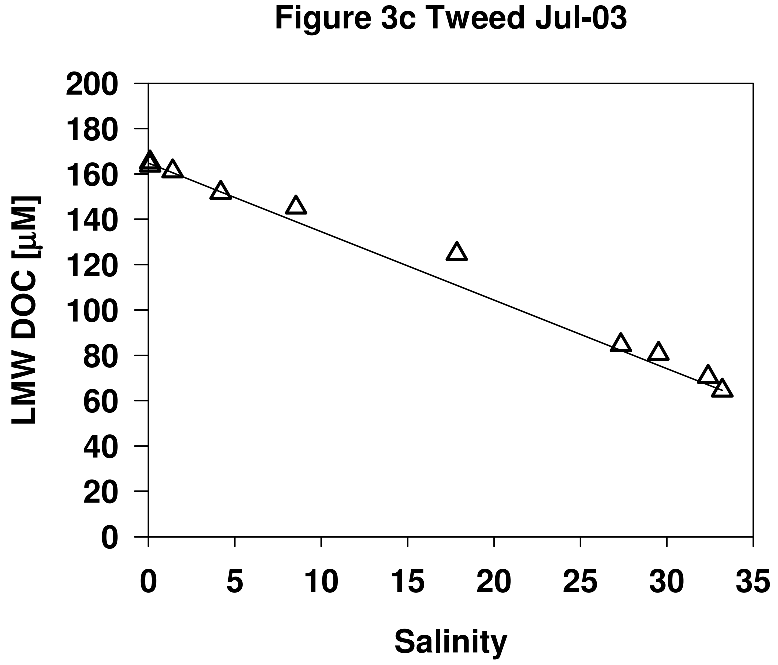


Figure 3. Concentrations (µM) of low molecular weight dissolved organic carbon (LMW DOC) in the Tyne Estuary during (a) July 2002 (open circles) and July 2003 (closed circles) and (b) March 2003 (open diamonds) potted versus salinity. The straight line represents the expected concentration due to conservative mixing between marine and riverine sources.

microbial breakdown of HMW DOC, and there were no significant inputs of additional sources of LMW DOC into the estuary, one might expect to see a removal in the total DOC concomitant with the HMW fraction. This was observed in both



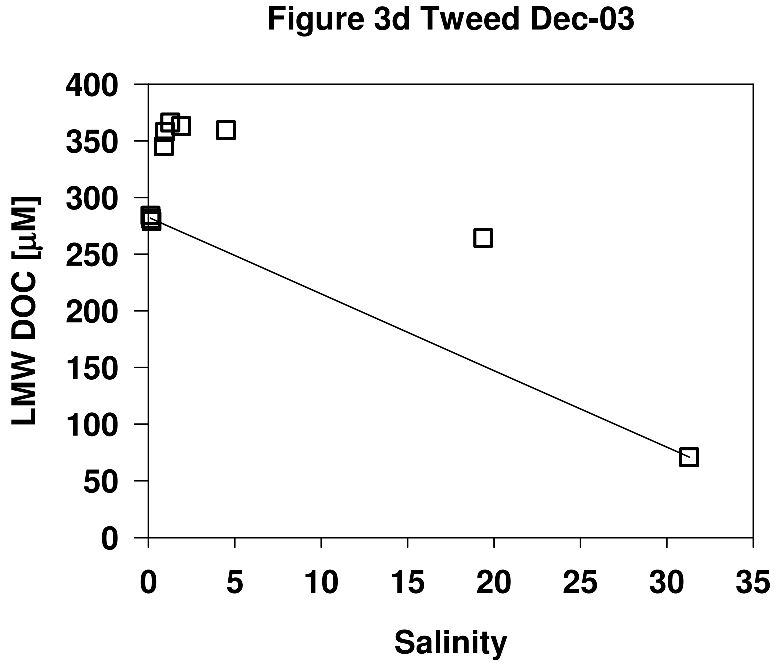


Figure 3. Concentrations (µM) of low molecular weight dissolved organic carbon (LMW DOC) in the Tweed Estuary during (c) July 2003 (open triangles) and (d) December 2003 (open squares) potted versus salinity. The straight line represents the expected concentration due to conservative mixing between marine and riverine sources.

winter transects (Figures 4b and 4d), when the HMW fraction comprised a greater percentage of the total DOC (3-35%, Tyne Mar-03; 5-54%, Tweed Dec-03) compared to the other three summer surveys (6-18%, Tyne Jul-02; 3-11%, Tyne Jul-

Figure 4a Tyne Jul-02 and Jul-03

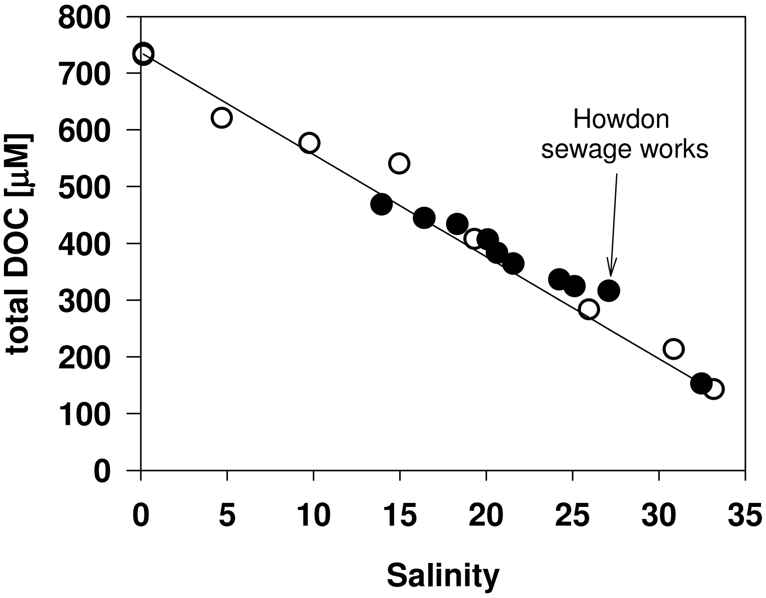


Figure 4b Tyne Mar-03

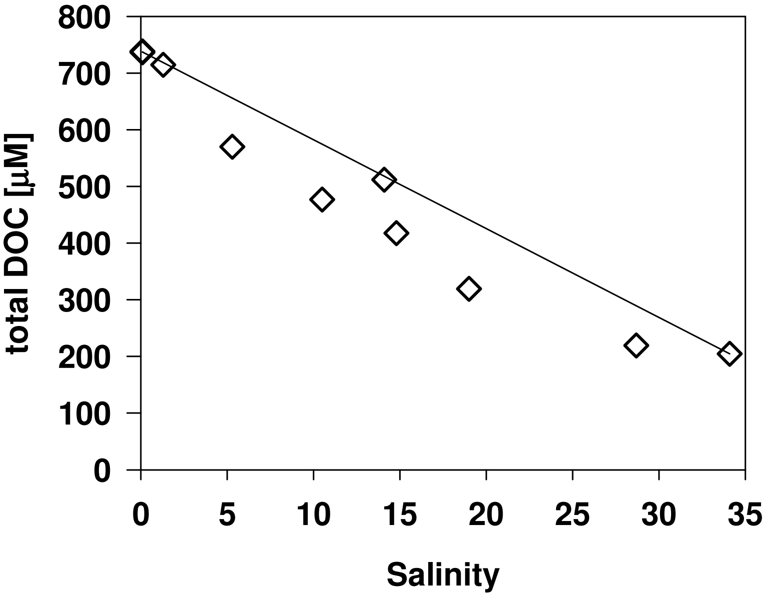


Figure 4. Concentrations (µM) of total dissolved organic carbon (DOC) in the Tyne Estuary during (a) July 2002 (open circles) and July 2003 (closed circles) and (b) March 2003 (open diamonds). The straight line represents the expected concentration due to conservative mixing between marine and riverine sources.

03; 2-28%, Tweed Jul-03). In addition, in the Tweed during Dec-03, a mass balance of LMW DOC + HMW DOC removed indicated that HMW DOC removal could indeed account for the observed non-conservative inputs of LMW DOC within the

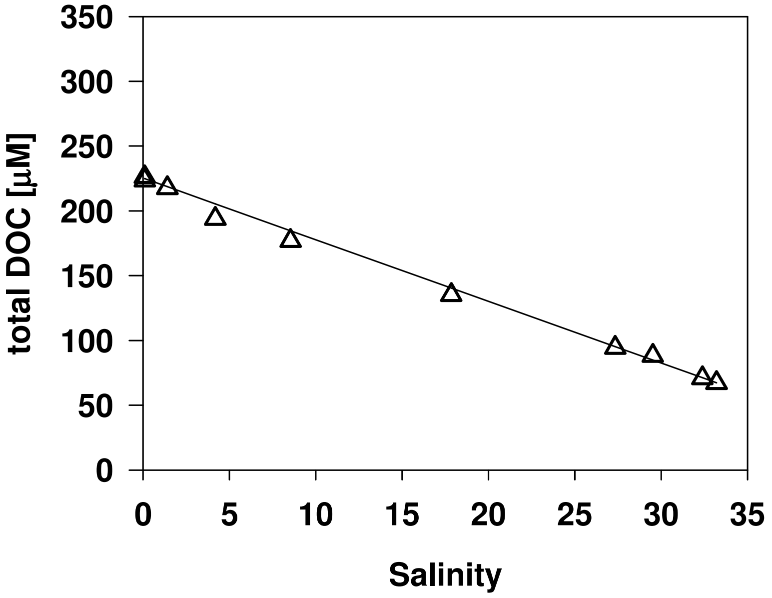


Figure 4c Tweed Jul-03

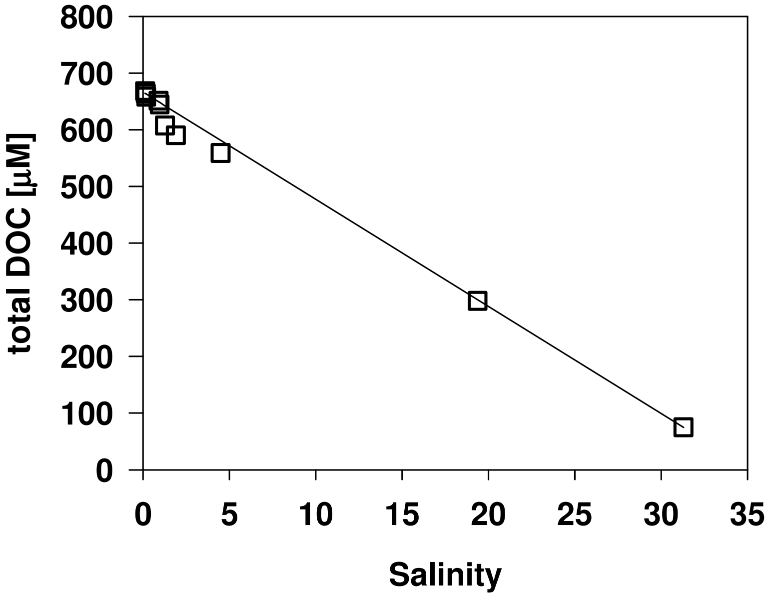


Figure 4d Tweed Dec-03

Figure 4. Concentrations (µM) of total dissolved organic carbon (DOC) in the (c) Tweed Estuary during July 2003 (open triangles) and (d) December 2003 (open squares) potted versus salinity. The straight line represents the expected concentration due to conservative mixing between marine and riverine sources.

estuary, providing evidence for an oxidative transfer of HMW to LMW DOC. The non-conservative behaviour of total DOC in the winter was more pronounced in the Tyne (Figure 4b), with the exception of a few samples collected near 14.1 psu. However, the samples that deviated from the non-conservative removal trend were associated with pronounced spikes in LMW DOC concentrations attributable to point sources from Ouseburn, a heavily contaminated urban river which flows through the city of Newcastle.

During the summer, when HMW DOC comprised a smaller percentage of the total DOC (2-28%), total DOC concentrations across the Tweed Estuary more closely exhibited conservative mixing (Figure 4c). The failure to discern a change in total DOC concentrations across an estuary despite substantial removal in the HMW fraction has been observed by others (Fox, 1983; Sholkovitz et al., 1978). In the Tyne, despite the smaller contribution of HMW DOC to the total DOC pool (6-18%, Jul-02; 3-11%, Jul-03), LMW DOC addition was observed across the estuary during both summer transects, which resulted in a slight convex non-conservative behaviour for the total DOC (Figure 4a). The relatively low percentages of HMW DOC observed during this sampling period suggests a supplementary source for LMW DOC in the Tyne in addition to that derived from the breakdown of HMW DOC. The low chl-a concentrations found in the Tyne (<4.3 μg/L, Jul-02; <2.8 μg/L, Jul-03) imply that contributions from algal material in this estuary during the summer were relatively minor. It is therefore likely that the non-conservative LMW DOC inputs may have originated from mineralization of POC or from anthropogenic contributions (e.g., the Howdon sewage works, 27.1 psu).

The results from this study indicate that a substantial portion of the HMW DOC pool is relatively reactive and is removed during estuarine mixing. In addition, it appears that the absolute amount of HMW DOC removed is higher when concentrations and contributions to total DOC are also higher. This implies that HMW DOC removal is a substrate limited process. The results found here indicating an enhanced reactivity of HMWDOC during estuarine are consistent with other studies. This increasing proportion of LMW relative to HMW DOC downriver across a salinity gradient has also been observed by others (Powell et al., 1996). This study attributed the substantial non-conservative removal in HMW DOC (>10 kD) in the Ochlockonee Estuary (SE US) to flocculation. In contrast, the estuarine inputs of

LMW DOC during periods of removal of large concentrations of HMW-DOC (e.g., Tweed Dec-03) suggests oxidative processes is at least partially responsible for substantial removal HMW DOC in the Tweed. This inference is supported by other evidences such as the seasonal reversal of respiration-photosynthesis dynamics observed in this estuary (Chapter 2, Howland et al., 2000). The lack of sunlight in winter is not the only factor in this seasonal reversal. When the supply of labile HMW DOC is high, heterotrophic activity may therefore dominate over autotrophy in winter months. Thus HMW DOC can make substantial contributions along with POC (Chapter 4) in contributing to net heterotrophy in these estuaries. Furthermore, this study suggests that when HMW DOC comprises of large fraction of riverine DOC inputs, its estuarine removal is more likely to be reflected in total DOC distributions across the estuary.

Behaviour of C/N ratios of HMW DOM (C/N_{HDOM})

As Figure 5 shows, C/N ratios of HMW DOM (C/N_{HDOM}) in both estuaries during summer and winter showed a general decrease with increasing salinities. These changes across the salinity gradient were most pronounced in winter, with a much less pronounced variation in C/N_{HDOM} ratios observed during the July 2003 survey to the Tweed. For instance, C/N_{HDOM} ratios in the Tweed during (Figure 5b) Dec-03 decreased from a high of 27.0 (0.97 psu) to a low of 9.0 at the coastal North Sea (31.30 psu).

The average C/N_{HDOM} ratios in the freshwater (≤ 0.2 psu) end-members in the Tweed were 11.9 ±0.1 (n = 2) and 22.8 ±1.1 (n = 3) during the Jul-03 and Dec-03 surveys, respectively, which were very similar to the total estuarine averages (after combining freshwater samples) of 10.6 ±0.7 (Jul-03; n = 9) and 22.2±5.7 (Dec-03; n = 8). The C/N ratios of terrestrial OM (15-400) are generally higher than those of bacterioplankton and phytoplankton OM, which are typically characterised by C/N ratios less than 8 (Baird and Middleton, 2004; Cowie and Hedges, 1994; Goni and Hedges, 1995). The normal range for C/N ratios of riverine HMW DOM measured in estuaries reported in the literature is around 20-25 (Goni et al., 2003; Guo and Santschi, 1997; Wang et al., 2004). Therefore, exceptionally low C/N ratio of riverine HMW DOM during the summer survey of the Tweed requires an explanation. The low riverine C/N_{HDOM} ratio measured during the Jul-03 survey may suggest the mixing between high C/N terrestrial material and with N-enriched phytoplankton and/or bacterioplankton sources (Goni et al., 2003). While with the data presented here it was not possible to evaluate the contribution of bacterial-derived OM, the low chl-a levels found during the Jul-03 survey (Table 1) implied a

Figure 5a Tyne Estuary

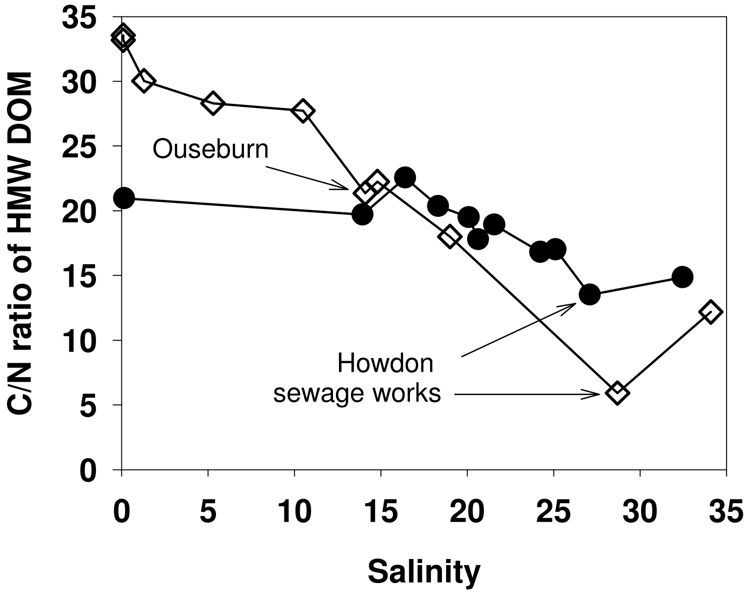


Figure 5b Tweed Estuary

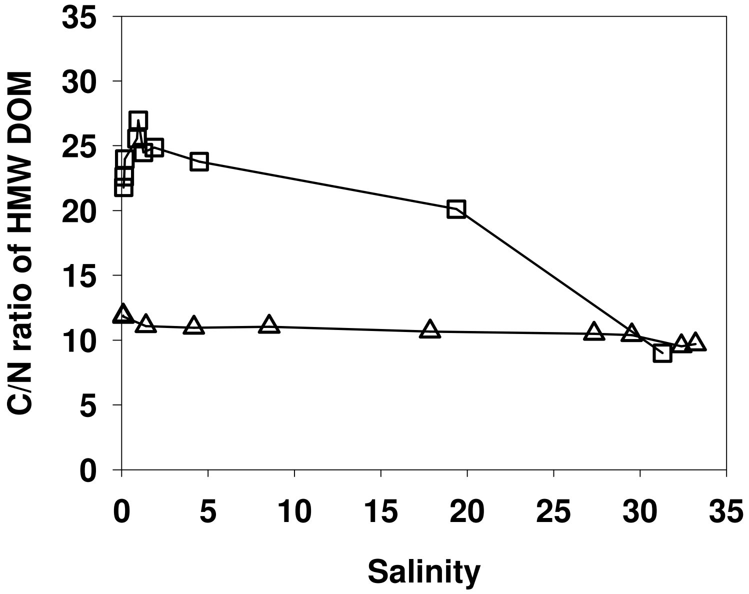


Figure 5. C/N ratios (molar) of high molecular weight dissolved organic nitrogen (C/N) in the (a) Tyne Estuary during Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity.

limited contribution from algal-derived OM. Thus, we argue that the low C/N_{HDOM} ratios found here could be entirely of terrestrial origin. The low riverine C/N_{HDOM} ratio found here was similar to the average value of 9.4 ±1.1 reported for DOC/DON in groundwater draining degraded peatlands (Kalbitz and Geyer, 2002). This is particularly relevant in the Tweed catchment, which is dominated by agriculture activity. These low ratios in degraded agricultural soils were most likely the result of significant alteration by microorganisms, which can lead to the accumulation of nitrogen-rich compounds and hence a higher N content (Goni et al., 2003; Rice and Hanson, 1984). Therefore, this might suggest that soil-derived OM comprised the bulk of the HMW DOM pool. Furthermore, since terrestrial plant litter generally has higher C/N ratios than soil organic matter (Kendall et al., 2001; Weiguo et al., 2003), the seasonal differences in C/N_{HDOM} ratios (and $\delta^{13}C_{HDOC}$ values; next section) in the Tweed can therefore be attributed to changes in the relative concentrations of dissolved C_3 plant material. During the winter, higher river discharge (151.2 m^3/s) would have resulted in a higher contribution from plant litter, and hence higher C/N_{HDOM} ratios. Conversely, during the less turbid, lower flow conditions (14.5 m^3/s) encountered during Jul-03 it is likely that that the relative percentage of soil-derived OM was substantially higher. The C/N_{HDOM} ratios found in the lower part of the estuary during both winter (9.0) and summer (9.7) are typical for marine OM. The similarity between soil-derived and marine C/N_{HDOM} ratios in the Tweed during July 2003 explains the invariance of C/N_{HDOM} ratios across the estuary during this period. Conversely, the large almost linear decline in C/N_{HDOM} ratios in the Dec-03 survey of the Tweed suggests mixing between riverine HMW DOM of high C/N ratios with marine HMW DOM of low ratios.

The average C/N_{HDOM} ratios in the freshwater (≤ 0.2 psu) end-members in the Tyne were 21.1 ±0.2 (n = 2) and 33.4 ±0.3 (n = 2) during the Jul-02 and Mar-03 surveys, respectively. As for the Tweed Estuary, the low chl-a levels found in the Tyne (Table 2) implies that autochthonous production did not make a significant contribution to HMW DOM to explain this seasonal change. C/N_{HDOM} ratios of riverine DOM entering the Tyne Estuary during the summer falls within the normal range (20-25) but during the winter the value (33.4 ±0.3, n = 2) is much higher than those found in other estuaries. It is possible the higher C/N_{HDOM} ratios entering the Tyne estuary during the winter suggest a greater contribution from plant-derived OM relative to soil-derived OM. However, during the Mar-03 survey the percentage of HMW comprising the total DOC in the freshwater end-member (35%) was almost twice as high as that during the Jul-02 survey (18%). Since humic material generally has higher C/N ratios than bulk soil (Seeber and Seeber, 2005), the higher C/N_{HDOM} values found during Mar-03 may therefore reflect a higher component of humic and fulvic acids in Tyne HMW DOM. For instance, in studies carried out in Russian rivers draining vast areas of peat and arctic taiga, C/N ratios for DOM ranging between 30-60 have been reported (Lara et al., 1998; Lobbes et al., 2000).

C/N_{HDOM} ratios in the Tyne also showed a general decrease across the salinity gradient, although in contrast to the Tweed Estuary the coastal North Sea ratios (12.2, Jul-02, 14.9, Mar-03) were slightly higher. Nonetheless, the C/N_{HDOM} ratios measured at the mouth of the Tyne Estuary were within the wide range of values reported for marine HMW DOM (~9-20) in the literature (Benner et al., 1997; Goni et al., 2003; Guo and Santschi, 1997; Wang et al., 2004). During the Mar-03 survey an anomalously low C/N_{HDOM} value (5.9) was measured at the sampling location nearest the Howdon sewage works (28.7 psu), suggesting an estuarial input of sewage-derived OM. In contrast to the winter, the change in C/N_{HDOM} ratio at the site nearest the Howdon sewage works during the summer (27.1 psu) was not as pronounced as during the winter, implying a limited contribution from sewage-derived HMW DOM during the July 2003 survey. In any case, it is likely that during both seasons the C/N_{HDOM} ratios near the mouth of the Tyne Estuary were influenced by HSW-related inputs with low C/N ratios, thus complicating the assignment of a marine C/N_{HDOM} end-member for this system. Nevertheless during both seasons the large estuarial decline in C/N_{HDOM} ratios implies mixing between high C/N peat-derived HMW DOM and low C/N marine- and HSW-derived HMW DOM.

Behaviour of $\delta^{13}C$ signatures of HMW DOC

In Figure 6, $\delta^{13}C$ signatures of HMW DOC ($\delta^{13}C_{HDOC}$) from the Tyne and Tweed Estuaries are plotted versus salinity. In general, $\delta^{13}C$ values across both estuaries became more ^{13}C-enriched with increasing salinity. The riverine (≤ 0.2 psu) $\delta^{13}C$ signature in the Tweed (Figure 6c) was -26.8‰ (n = 2) in July 2003 and -28.5‰ (n =3) during December 2003 (Figure 6d). In the Tyne, riverine $\delta^{13}C$ values of -28.3‰ (n = 2) and -27.7‰ (n = 2) were observed during the March 2003 (Figure

6b) and July 2002 surveys (Figure 6a), respectively. These values are indicative of C_3 plant material, which is most likely dominated by soil and plant litter originating from the upland areas of peat and moorland. In any case, the $\delta^{13}C$ signatures reported here for the riverine end-members in both the Tweed and in the Tyne are still indicative of a terrestrial origin. The $\delta^{13}C$ signatures for the marine end-members (-25.7‰, Tyne Mar-03; -24.0‰, Tyne Jul-03; -23.9‰, Tweed Jul-03; -23.3 ‰, Tweed Dec-03) are generally within the lower range of -18 to -24‰ reported for North Sea DOC (Le Clercq et al., 1997), indicating that these samples are probably influenced by terrestrial material and are more representative of a Coastal North Sea signal. The slightly more ^{13}C-depleted value for the marine end-member from the March 2003 survey to the Tyne suggests an even greater contribution of terrestrially-derived HMW DOC to the Coastal North Sea during this period.

In order to test whether $\delta^{13}C$ signatures obeyed conservative mixing between marine and riverine (i.e., freshwater) sources, a two end-member mass balance equation using both isotopic and concentration data was utilised:

$$\delta^{13}C_{pred} = [(f_M \times \delta^{13}C_M) + (f_F \times \delta^{13}C_F] \tag{3}$$

Here f_F, f_M, and $\delta^{13}C_F$ and $\delta^{13}C_M$ refer to the fraction and $\delta^{13}C$ signatures of the freshwater and the marine components, respectively. The fraction of the marine component was calculated by dividing the assumed concentration of marine HMW DOC expected at a particular salinity due to conservative mixing by the sum of the assumed marine and freshwater (measured HMW DOC – marine HMW DOC) HMW DOC concentrations. The fraction of the freshwater component is $1 - f_M$. For the Jul-03 survey to the Tyne Estuary, where a riverine $\delta^{13}C$ signature was not available, the $\delta^{13}C$ signature from the previous summer (-27.7‰, ± 0.2, n = 2) was used. Using the above equation, we were able to predict the $\delta^{13}C$ signature from conservative two end-member mixing ($\delta^{13}C$) for a sample at a known salinity. The conservative isotopic mixing curves are plotted with the measured $\delta^{13}C$ values in Figure 6 as solid lines. In winter the measured $\delta^{13}C_{HDOC}$ signatures across the Tweed Estuary mostly seemed to indicate conservative mixing (Figure 6d). Note that the majority of these samples during this period were collected at salinities < 2 psu and a proper assessment of conservative behaviour across the full salinity range could not be established. In contrast, $\delta^{13}C_{HDOC}$ values in the summer in both estuaries and in the Tyne during the winter exhibited ^{13}C-enriched, non-conservative behaviour across the full salinity gradient (Figures 6a-c).

Figure 6a Tyne Jul-02 and Jul-03

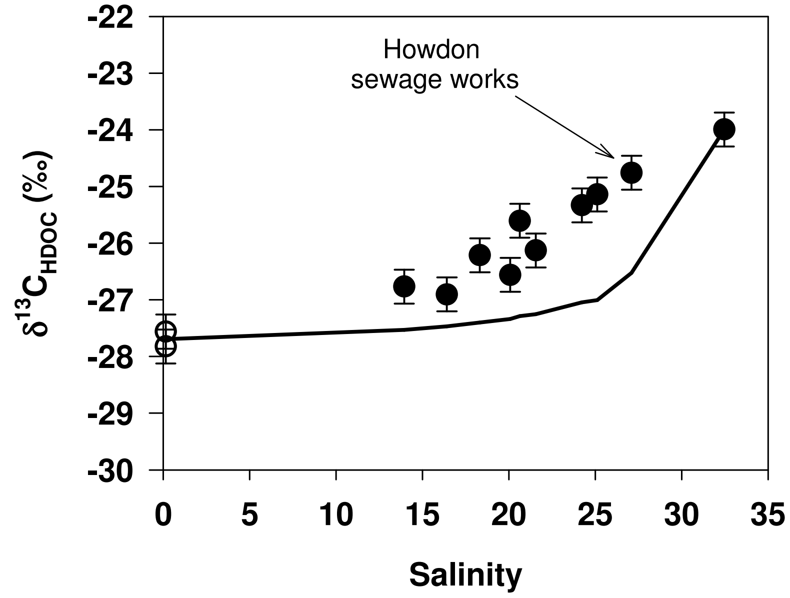


Figure 6b Tyne Mar-03

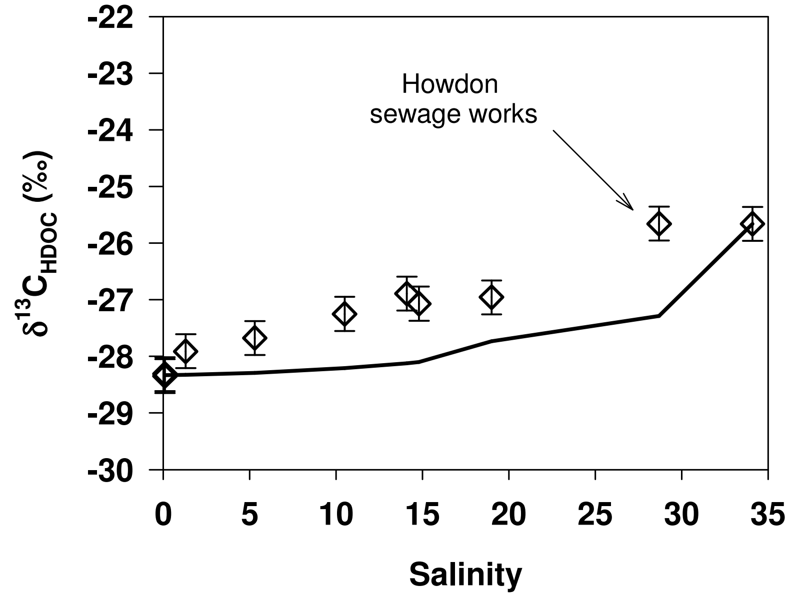


Figure 6. δ^{13}C values ($\delta^{13}C_{HDOC}$) for high molecular weight dissolved organic carbon (HMW DOC) in the (a) Tyne Estuary during July 2002 (open circles) and July 2003 (closed circles) and (b) March 2003 (open diamonds). The solid lines represent the conservative isotopic mixing curve as calculated using Eq. 3. Vertical error bars represent an accuracy and reproducibility of ±0.3‰ on δ^{13}C values.

This non-conservative behaviour in δ^{13}C-DOC values within an estuary has been observed by others (Peterson et al., 1994; Raymond and Bauer, 2001a); however, these studies also documented net inputs of DOC within the estuaries. The elevated δ^{13}C- and values found in these studies were attributed to inputs derived

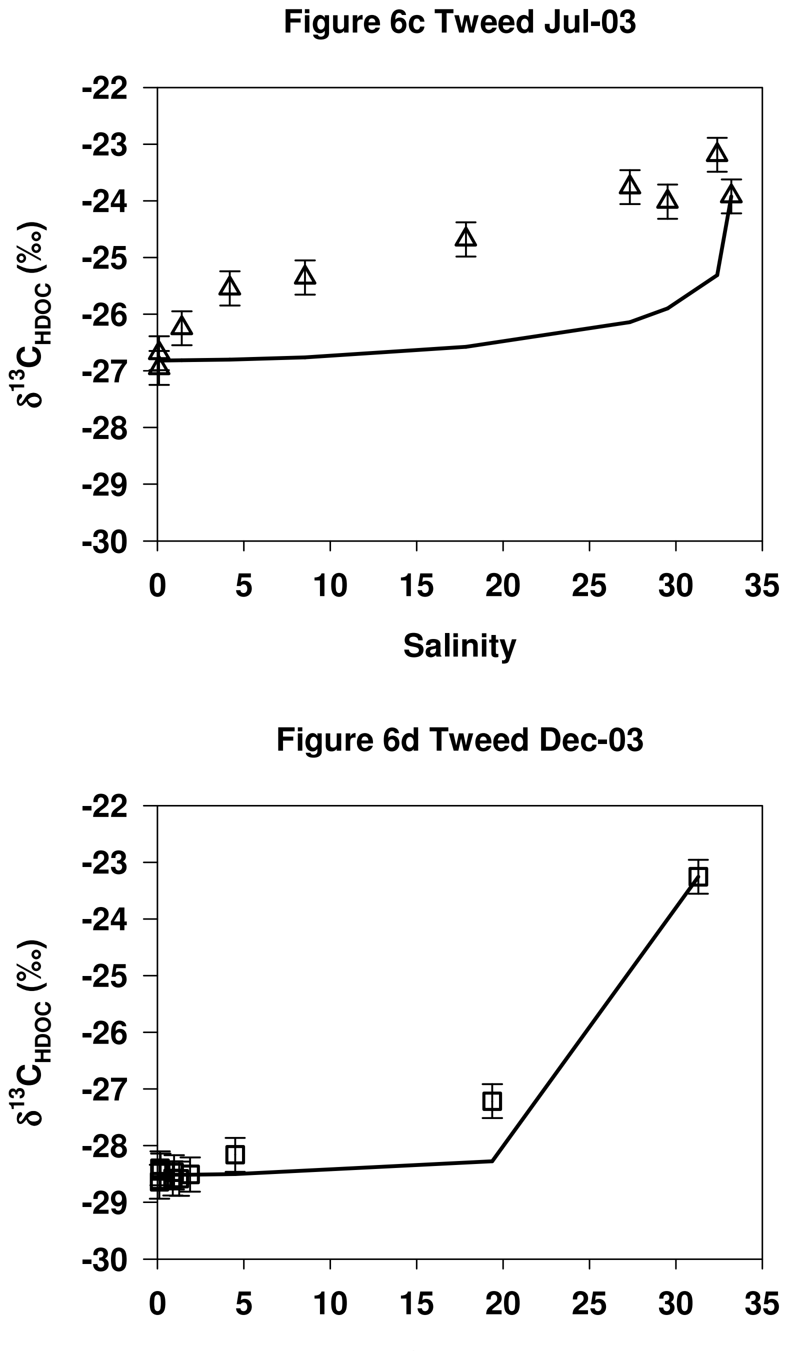


Figure 6. δ^{13}C values ($\delta^{13}C_{HDOC}$) for high molecular weight dissolved organic carbon (HMW DOC) in the Tweed Estuary during (c) July 2003 (open triangles) and (d) December 2003 (open squares) plotted versus salinity. The solid lines represent the conservative isotopic mixing curve as calculated using Eq. 3. Vertical error bars represent an accuracy and reproducibility of ±0.3‰ on δ^{13}C values.

from ^{13}C-enriched sources, such as C$_4$-plant material (e.g., marsh organic matter) and high-salinity algal-derived DOC. In our case, however, there was substantial HMW DOC removal across both estuaries coinciding with ^{13}C-enrichment in HMW DOC and no discernible estuarial inputs. Nonetheless, it was still possible that small estuarial additions may have been masked by the large net concave HMW DOC removals observed in Figure 2. Potential sources of ^{13}C-enriched material in the Tyne and Tweed Estuaries must therefore be addressed.

Due to the low chl-a concentrations measured throughout the Tyne and Tweed Estuaries during the July 2003 transects, it was concluded that phytoplankton-derived OM did not comprise a significant percentage of the HMW DOC pool. Nonetheless, if phytoplankton OM were an isotopically enriched source of OM, then small inputs of this ^{13}C-enriched material could potentially influence δ^{13}C distributions. To investigate this possibility, a theoretical δ^{13}C signature for phytoplankton-derived OM can be estimated using δ^{13}C signatures of DIC ($\delta^{13}C_{DIC}$), assuming a carbon isotopic fractionation between DIC and plankton of -20‰ (Chanton and Lewis, 1999; Raymond and Bauer, 2001a). Using this constant fractionation factor, the estimated δ^{13}C values for phytoplankton-derived organic carbon (Table 5) ranged from -19.9‰ (marine) to -25.9‰ (13.9 psu) in the Tyne and from -19.1‰ (marine) to -29.9‰ (≤2 psu) in the Tweed during the Jul-03 surveys. As shown in Table 5, the differences between δ^{13}C values and the δ^{13}C value of algal organic matter in the low- to mid-salinity region (<30 psu) ranged between 0.9 to 2.6‰ in the Tyne and from -3.7 to 2.6‰ (at 27.4 psu) in the Tweed. In the Tweed, the ^{13}C-depleted differences at salinities < 20 during the summer therefore preclude phytoplankton as a potential source of isotopically heavy δ^{13}C within the upper half of the half of this estuary. Conversely, in the Tyne ^{13}C-enriched differences suggest that in the summer phytoplankton may have indeed been the source for the heavier δ^{13}C signatures. In winter, however, negligible phytoplankton activity excludes algal material as a potential source for ^{13}C-enriched OM.

Considering the low chl-a values (<1.7 µg/L) reported here and in other studies (Howland et al., 2000; Neal et al., 1998), it has been suggested that benthic macroalgae and/or seagrasses are the dominant cause of net autotrophy in the Tweed during the summer (Chapter 2). In a study carried out in the Apalachicola Bay

(Florida, USA), Chanton and Lewis (Chanton and Lewis, 2002) found that the $\delta^{13}C$ signatures of submerged aquatic vegetation tended to increase with salinity, ranging from approximately -27‰ (freshwater) to ~ -15‰ (marine). Therefore, it is possible that small amounts of benthic macroalgae or seagrass OM originating from higher salinities could indeed have contributed to slightly enriched $\delta^{13}C$ signatures in the Tweed Estuary.

Site/Date	salinity	$\delta^{13}C_{DIC}$ ±0.3‰	$\delta^{13}C_{ALG}$ ‰	$\delta^{13}C_{HDOC}$ - $\delta^{13}C_{ALG}$ ‰
Tweed				
8/7/03	33.2	0.6	-19.4	4.5
8/7/03	32.4	0.9	-19.1	4.0
8/7/03	27.4	-1.2	-21.2	2.6
8/7/03	17.9	-4.6	-24.6	0.0
8/7/03	8.6	-8.0	-28.0	-2.6
8/7/03	4.2	-8.2	-28.2	-2.6
8/7/03	1.4	-9.9	-29.9	-3.7
Tyne				
23/7/03	32.5	0.1	-19.9	4.1
23/7/03	27.1	-2.7	-22.7	2.0
23/7/03	25.1	-2.6	-22.6	2.6
23/7/03	24.2	-3.8	-23.8	1.5
23/7/03	21.6	-4.5	-24.5	1.6
23/7/03	20.6	-4.1	-24.1	1.5
23/7/03	20.1	-5.1	-25.1	1.4
23/7/03	18.3	-5.6	-25.6	0.6
23/7/03	13.9	-5.9	-25.9	0.9

Table 5. $\delta^{13}C$ signatures of dissolved inorganic carbon ($\delta^{13}C_{DIC}$), estimated $\delta^{13}C$ signatures of algal-derived OM ($\delta^{13}C_{ALG}$), and differences between the $\delta^{13}C$ signatures of high molecular weight dissolved organic carbon ($\delta^{13}C_{HDOC}$) and $\delta^{13}C_{ALG}$ ($\delta^{13}C_{HDOC} - \delta^{13}C_{ALG}$).

As Figure 5 demonstrates, a trend of decreasing C/N ratios with increasing salinity was observed across the Tyne Estuary during March 2003. The lowest C/N ratio (5.9) was found in close proximity to the Howdon sewage works, and was also associated with a relatively ^{15}N-depleted signature (-5.0‰) of HMW DON (Chapter 5). Therefore, it is likely that sewage-derived HMW DOM may have contributed to the ^{13}C-enriched HMW DOC values found in the Tyne. Because of its terrestrial origin sewage-derived HMW DOC has been shown to have relatively ^{13}C-depleted $\delta^{13}C_{HDOC}$ values (e.g., -30‰; (Wang et al., 2004). However, the subsequent microbial utilisation of this highly labile OM may result in the production of HMW

DOC with a corresponding bacterial fingerprint that could influence the bulk $\delta^{13}C$ signature. Based on lipid compositions and compound-specific $\delta^{13}C$ ratios, Zou et al (2004) (Zou et al., 2004) concluded that a significant amount of HMW DOM is derived from bacteria. In samples collected from four different U.S. estuaries, including Boston Harbour/Massachusetts Bay, Delaware/Chesapeake Bay, San Diego Bay and San Francisco Bay, the average $\delta^{13}C$ signature of bacteria-specific fatty acids present in the HMW DOC fraction was -24.9 ±1.5‰ (Zou et al., 2004). Since the low C/N ratios measured near the Howdon sewage works point to a bacterial origin (Goni and Hedges, 1995; Savin et al., 2001), if bacterial modification of sewage-derived HMW DOM in the Tyne generated similar $\delta^{13}C$ signatures then this would help to explain the ^{13}C-enrichment observed in this estuary.

Preferential removal of ^{13}C-depleted HMW DOC

While anecdotal evidence points to the possible input of ^{13}C-enriched HMW DOM in both the Tyne and Tweed Estuaries, these additions were likely very minor when compared to the relatively large terrestrial HMW DOC concentrations, thereby limiting their influence on overall $\delta^{13}C_{HDOC}$ distributions. Therefore, another explanation for the progressive isotopic enrichment in $\delta^{13}C_{HDOC}$ signatures found in our study was the preferential removal of isotopically lighter terrigenous HMW DOC during estuarine mixing. The bulk $\delta^{13}C$ values reported here are combinations of multiple sources that may contain both relatively ^{13}C-depleted and ^{13}C-enriched fractions. Therefore, it is possible that some of the more ^{13}C-depleted fractions within the terrigenous HMW DOC pool were relatively more reactive compared to the isotopically heavier components, resulting in the ‘residual’ HMW DOC becoming progressively more ^{13}C-enriched with increasing salinity. The potential removal mechanisms of isotopically lighter HMW DOC include flocculation, sorption, biodegradation and/or photochemical oxidation.

Generally, flocculation is most pronounced at lower salinities, where freshwater first comes into contact with more saline water. In addition, this process has been shown to preferentially remove HMW DOC (Fox, 1983; Sholkovitz et al., 1978), which is rich in aliphatic, aromatic and carboxyl carbon (Hedges et al., 1992). Several studies have shown that lignin-derived aromatics are generally depleted in ^{13}C relative to carbohydrates (Benner et al., 1987; Bianchi et al., 2004; Leavitt and

Long, 1986), and recent work has demonstrated that the latter can comprise a significant percentage of freshwater HMW DOC (Repeta et al., 2002). Therefore, while flocculation of bulk OM may not produce a kinetic isotope fractionation effect, it is possible that the preferential removal of isotopically lighter humic material may leave a residual HMW DOC signature that is relatively ^{13}C-enriched.

In the absence of a significant isotope fractionation effect caused by flocculation, this leaves biodegradation and/or photochemical oxidation as the other two main processes capable of producing isotope fractionation in HMW DOC during its removal. Since daylight is substantially reduced in the northeastern UK during winter compared to summer, the effects of photochemical oxidation should be more important during the summer and less pronounced during the winter. Results in Figure 6 support this supposition where the potential ^{13}C-enrichment of HMWDOC during removal in the Tweed is more pronounced in July (Figure 6c) than during December (Figure 6d). This implies that the ^{13}C-enrichment in HMW DOC found in this study during the summer may have been largely influenced by UV-induced decomposition. This is further supported by seasonal variations in SPM, which are generally higher in the Tweed during the winter due to enhanced river discharge (Chapters 4-5, Uncles et al., 2000). Therefore, in conjunction with reduced levels of sunlight the more turbid conditions observed during December 2003 may have also played a role in suppressing UV-induced degradation. However, given the inadequate sampling of the Tweed winter profile this inference remains tentative. In contrast to the Tweed, the extent of ^{13}C-enrichment in HMW DOC signatures in the Tyne during both winter (Figure 6b) and summer (Figure 6a) seemed quite similar. SPM levels across the estuary during March 2003 were much higher than during July 2003 (Chapter 2). Since higher SPM concentrations can lead to both enhanced microbial activity (Abril et al., 2002) and reduced UV penetration, this would suggest that microbial rather than photochemical oxidation of HMW DOC was the dominant process producing ^{13}C-enriched HMW DOC during the winter.

Two of the reasons given for the non-conservative behaviour in $\delta^{13}C_{HDOC}$ values reported here are in agreement with laboratory studies carried out by Opsahl and Zepp (2001), who found a discernible ^{13}C-enrichment (up to 1.5‰) in bulk riverine DOC after exposure to prolonged periods of UV light. As suggested in this study, they concluded that the alteration of δ^{13}C signatures was probably caused by a combination of biodegradation and photochemical oxidation. These laboratory experiments were carried out over a time scale of days to weeks, and provide a good comparison with the isotope fractionation effects observed in the Tyne, taking into account its residence time of 5-20 days. However, our study also suggests that significant isotopic fractionation may happen much more quickly, considering the shorter flushing time (1-2 days) of the Tweed Estuary. Differences in source material and size fraction (total versus HMW DOC) may help to explain the slightly more pronounced ^{13}C-enrichment (~3‰) observed in the Tyne and Tweed Estuaries.

Behaviour of Δ^{14}C signatures of HMW DOC

Δ^{14}C signatures of HMW DOC in both the Tyne and Tweed Estuaries were all greater than 0‰ (i.e., absolute pMC > 100), indicating a contemporary origin post AD 1955 (Table 3). In fact, the Δ^{14}C values reported here, which ranged from 96-143‰ (Tyne, Mar-03), 117-471‰ (Tyne, Jul-03), 44-259‰ (Tweed, Jul-03) and 104-811‰ (Tweed, Dec-03), were much higher than most values for estuarine HMW DOC or total DOC reported in the literature (Guo and Santschi, 1997; Raymond and Bauer, 2001a; Raymond and Bauer, 2001b; Santschi et al., 1995; van Heemst et al., 2000). To the best of our knowledge, one Δ^{14}C signature in particular (811 ‰, 19.38 psu, Tweed, Dec-03) is the most ^{14}C-enriched estuarine HMW DOC or total DOC sample so far recorded. In addition, while most studies have reported a general pattern of decreasing HMW DOC / total DOC age with increasing salinity, indicating mixing with older marine OM or the preferential utilisation of young, ^{14}C-enriched carbon during mineralization (Guo and Santschi, 1997; Raymond and Bauer, 2001a; Raymond and Bauer, 2001b; Santschi et al., 1995), the opposite trend was observed here, whereby Δ^{14}C values showed a general downriver enrichment (Figure 7). The modern ages for HMW DOC are also in complete contrast to the much older (100s to 1000s of years) ages found for the POC pool in these two estuaries (Chapter 4). This contrast between a modern DOC signal draining a much older underlying soil has been previously reported in other studies (O'Brien, 1986; Trumbore et al., 1989), including a peat-dominated headwater stream in the northeastern UK (Palmer et al., 2001). The modern riverine signal found in both estuaries therefore suggests that rivers in the northeastern UK are a source of young, labile terrestrial HMW DOC to the North Sea, as has been reported in other temperate estuaries (Raymond and

Bauer, 2001a; Raymond and Bauer, 2001b) and in peat-dominated Arctic Rivers (Benner et al., 2004).

Figure 7a Tyne Estuary

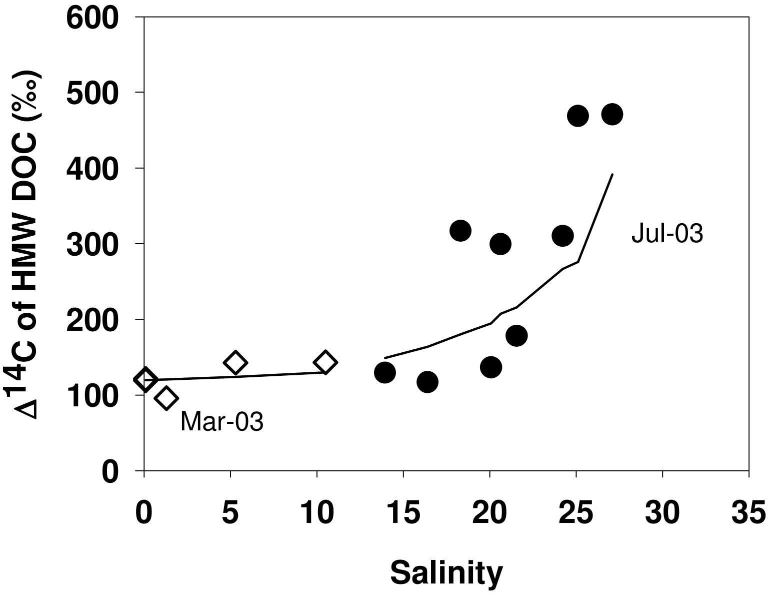


Figure 7b Tweed Estuary

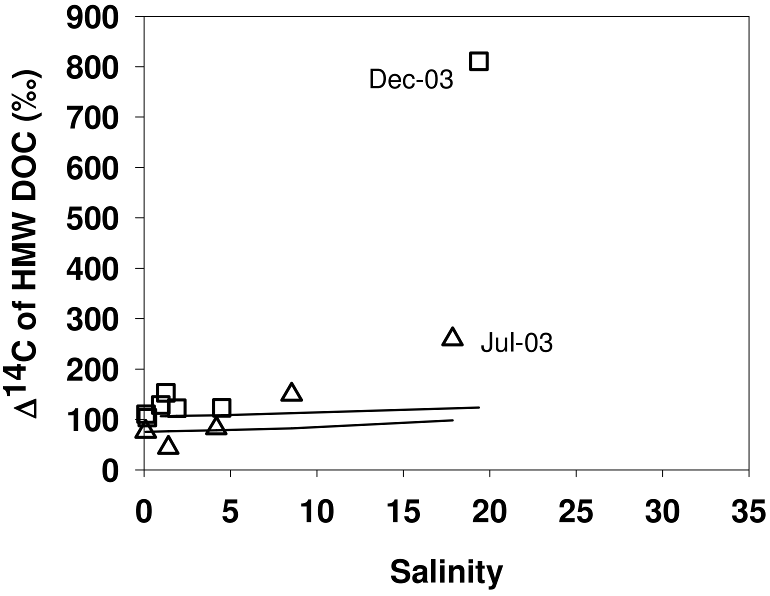


Figure 7. Δ^{14}C values (‰) for high molecular weight dissolved organic carbon (HMW DOC) in the (a) Tyne Estuary during March 2003 (open diamonds) and July 2003 (closed circles) and in the (b) Tweed Estuary during July 2003 (open triangles) and December 2003 (open squares). The solid lines represent the conservative isotopic mixing curve as calculated using Eq. 3. The symbols are larger than the vertical error bars associated with Δ^{14}C measurements s (±7‰).

In order to test whether $\Delta^{14}C$ signatures obeyed conservative mixing between marine and riverine sources, $\Delta^{14}C$ values were substituted for $\delta^{13}C$ values in Eq. 3. Unfortunately, a proper marine $\Delta^{14}C$ end-member was not obtained in any of the surveys. However, given the trend of progressively increasing $\Delta^{14}C$ values with increasing salinity, we assume that the relatively ^{14}C-enriched signature of 1000‰ is a good estimate for coastal North Sea HMW DOC in both estuaries. In addition, while a riverine end-member was not obtained for the Jul-03 survey to the Tyne, considering the similarity in modern $\Delta^{14}C$ signatures for freshwater HMW DOC in both the Rivers Tyne and Tweed, we assume that that a value 120‰ accurately reflects the freshwater signature for this transect. The conservative isotopic mixing curves are plotted with the measured $\Delta^{14}C$ values in Figure 7 as solid lines. As this figure illustrates, measured $\Delta^{14}C$ signatures across the Tyne Estuary mostly seemed to indicate conservative mixing (Figure 7a). However, in the Tweed $\Delta^{14}C$ signatures were much more ^{14}C-enriched than predicted by Eq. 3. One possibility for the inability of this model to explain the Tweed data may be due to an underestimate of the $\Delta^{14}C$ value of the marine-end-member. If this were the case then coastal North Sea HMW DOC near the mouth of the Tweed would have to be even more ^{14}C-enriched than 1000‰. Potential sources for this highly ^{14}C-enriched marine HMW DOC must be addressed.

In the North Sea, $\Delta^{14}C$ signatures of ~200‰ have been found for both DOC (van Heemst, 2000) and DIC (Le Clercq et al., 1997). These elevated $\Delta^{14}C$ values were attributed to nuclear industry related discharges originating from continental Europe. In addition, studies carried out on the west coast of the UK in the Irish Sea have consistently found elevated ^{14}C levels in DIC, DOC, and in various intertidal biota (Cook et al., 1995; Cook et al., 2004; Cook et al., 1998; Gulliver et al., 2001; Gulliver et al., 2004). These relatively high $\Delta^{14}C$ values have been attributed to discharges originating from the Sellafield nuclear fuel reprocessing plant, located in Cumbria, NW England. Consequently, due to the pattern of clockwise water circulation around the UK coastline it is possible that the progressive ^{14}C-enrichment found in HMW DOC samples from the Tyne and Tweed Estuaries reflects a strong Sellafield influence. However, considering the dilution expected due to the large maritime distance between Sellafield and NE England, it is more likely that this

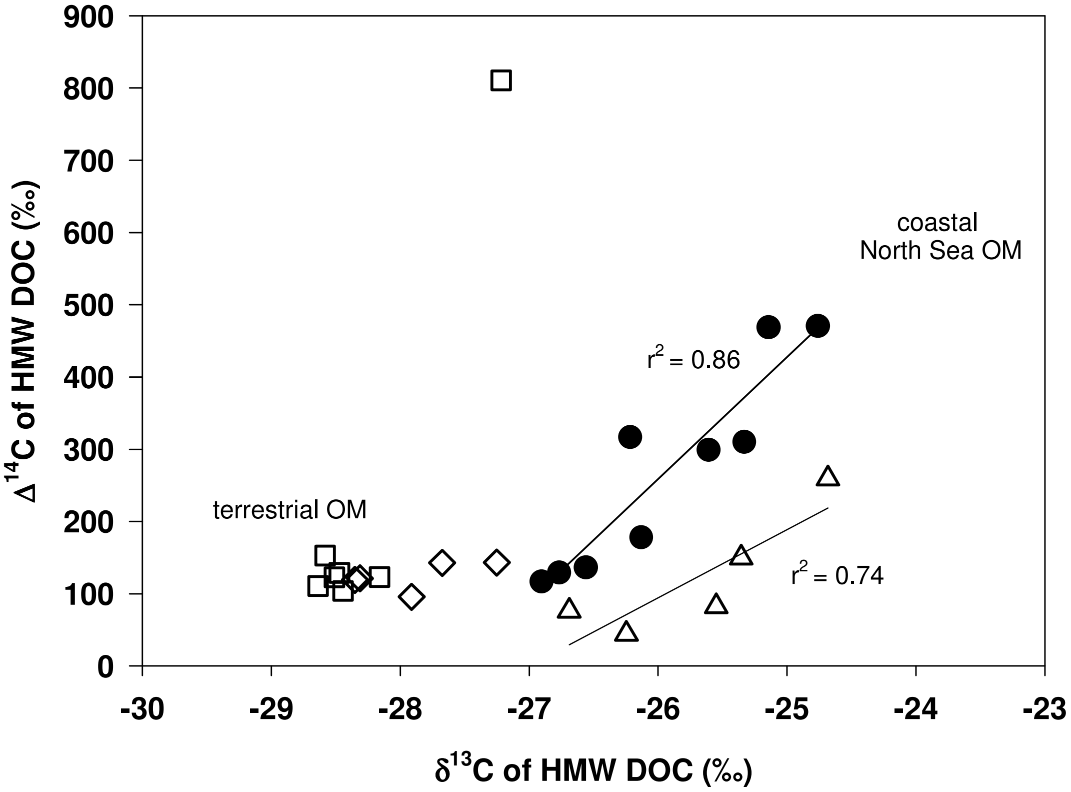


Figure 8. Δ^{14}C values for high molecular weight dissolved organic carbon (HMW DOC) plotted versus δ^{13}C values for HMW DOC in the Tyne Estuary during March 2003 (open diamonds) and July 2003 (closed circles) and in the Tweed Estuary during July 2003 (open triangles) and December 2003 (open squares).

anthropogenic ^{14}C-enriched carbon originated from a nearby source. In a survey carried out in 1999 (Gulliver et al., 2004), significant ^{14}C-enrichments (>400 pMC) were found in mussels collected from the vicinity of the Hartlepool advanced gas-cooled reactor (AGR) nuclear power station, located approximately 40 km down the English coastline from the mouth of the Tyne. But based on the fact that samples collected near Torness AGR (SE Scotland), a reactor with a similar design, did not contain the same level of ^{14}C-enrichment, it was concluded that there was potentially another source of non-nuclear-power related ^{14}C. Whatever the anthropogenic cause, it is clear that the elevated Δ^{14}C values reported here originated from highly ^{14}C-enriched North Sea carbon, either through direct marine contributions from recently fixed North Sea OM, or via uptake of ^{14}C-enriched DIC by primary producers followed by subsequent HMW DOC releases. As discussed previously, these autochthonous releases in the Tyne are potentially derived from phytoplankton, whereas in the Tweed benthic macroalgae are considered as a source for ^{14}C-enriched HMW DOC. These assumptions are supported by the good linear relationships between $\delta^{13}C$ and $\Delta^{14}C$ values during the two summer surveys (Figure 8), which corroborates the suggestion that ^{14}C-enriched North Sea DIC is the main source for autochthonous production in these estuaries.

CONCLUSIONS

Modern $\Delta^{14}C$ values indicated an export of young, labile terrestrial HMW DOC to the North Sea, whether from predominantly peat- (Tyne) or agricultural soil-derived sources (Tweed). Five separate surveys showed significant removal of HMW DOC within both the Tyne and Tweed Estuaries indicating that this size fraction of the carbon pool is reactive during estuarine mixing. Increases in LMW DOC concentrations with increasing salinities in the two estuaries were at least partially caused by the microbial breakdown of HMW DOC. During March 2003 (Tyne) and July 2003 (Tyne and Tweed) $\delta^{13}C$ signatures displayed non-conservative mixing between terrestrial and marine sources, whereas in the Tweed during December 2003 $\delta^{13}C$ values followed conservative mixing more closely.

There was an overall pattern in both the Tyne and Tweed Estuaries of progressive ^{14}C-enrichment in $\Delta^{14}C$ values with increasing salinity. The trends were the opposite of those found in other estuaries, where $\Delta^{14}C$ values of HMW DOC or total DOC often decrease (i.e., gets older) with increasing salinity (Guo and Santschi, 1997; Raymond and Bauer, 2001a; Raymond and Bauer, 2001b; Santschi et al., 1995). The source of the lower-estuarine ^{14}C-enrichment in HMW DOC found here has been attributed to anthropogenic discharges likely associated with nuclear industry related activity within the UK coastal environment. As a result of the parity between $\delta^{13}C$ and $\Delta^{14}C$ signatures, the non-conservative behaviour of $\delta^{13}C$ signatures in both estuaries can therefore be partly attributed to contributions from lower estuary autochthonous material. However, given the large net removals found in HMW DOC concentrations, it was not possible to determine the exact contributions of this autochthonous material relative to the riverine component. Studies have shown that terrestrial DOC can become more ^{14}C-depleted during estuarine mixing as a result of the preferential degradation of younger, more labile carbon (Raymond and Bauer, 2001a). However, the present study has demonstrated that highly ^{14}C-

enriched OM inputs can mask any potential degradation signal caused by this process if these additional inputs cannot be properly quantified.

If the ^{13}C-enriched behaviour of δ^{13}C values found in this study was caused by the preferential removal of ^{13}C-depleted HMW DOC, then rapid estuarine removal of HMW DOC as observed here and by other workers (Guo and Santschi, 1997; Santschi et al., 1995) implies that delineating terrestrial-derived HMW DOC in an estuarine environment may often prove quite difficult when using δ^{13}C signatures. In addition, an enriched carbon isotope signal of residual terrestrial carbon leaving estuaries could lead to the underestimation of terrestrially-derived carbon present in the marine DOC pool when terrestrial end member values are assigned in the partitioning calculations. In order to help verify whether the ^{13}C-enriched non-conservative behaviour observed in bulk HMW DOC was indeed due to an isotope fractionation effect, further laboratory experimentation is required. For instance, quantifying the potential isotope fractionation effects associated with flocculation remains essential.

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CHAPTER 4: Behaviour of suspended particulate matter (SPM) in two contrasting North Sea estuaries: Implications for the fate of terrigenous organic carbon

ABSTRACT

Concentrations, C/N ratios and stable and radiocarbon ($\delta^{13}C$ and $\Delta^{14}C$, respectively) isotope ratios of suspended particulate organic matter (POM) from the Tyne and Tweed Estuaries, NE England, were analysed during winter and summer periods. $\delta^{13}C$ and $\Delta^{14}C$ values showed a net export of old (100-1000s of years), terrigenous POC to the North Sea. Higher C/N ratios in the Tyne indicated a greater proportion of terrestrial plant debris and humic material in riverine POM, whereas in the Tweed the lower C/N ratios were attributed to a predominantly agricultural soil source. Changes in POC% (% of SPM) across the salinity gradient were used to estimate the percentage of riverine POC mineralised, which ranged from 38-76% and 31-39% in the Tyne and Tweed, respectively. These estimations suggested that a significant fraction of terrestrial OM is relatively labile and is oxidised in the estuarine and coastal zone. In the Tyne during summer lower C/N and POC/chl-a ratios indicated a slightly more important POM contribution from estuarine or marine phytoplankton. However, relatively depleted $\delta^{13}C$ and $\Delta^{14}C$ values for POC pointed to a mostly terrestrial and/or anthropogenic origin. Older ages for POC in the Tyne were attributed to a predominantly peat-derived POC source with some sedimentary fossil carbon and/or petroleum product contamination. In the more pristine Tweed, there was a noticeable seasonal trend in the age of POC exported to the North Sea (a difference of ~ 650 years), with more ^{14}C-depleted values found in the summer compared to winter due to an older age for agricultural soil-derived inputs. In the Tyne, decreases in POC% with increasing salinity sometimes coincided with an increase in POC age. This was attributed to mixing with older sediment and to the possible preferential loss of the younger, more labile POC fraction during mineralization.

INTRODUCTION

It is estimated that UK rivers export approximately 0.68 Mt of dissolved organic carbon (DOC) and 0.20 Mt of suspended particulate organic carbon (POC) to the coastal zone each year (Hope et al., 1997). However, the actual value is likely to be about 1.4 Mt a^{-1}, because the data on which these values are based underestimate losses during high flow events (Cannell et al., 1999). This represents about 0.014% of the total UK soil organic C pool of ca. 10,000 Mt (Milne and Brown, 1997). Furthermore, recent studies have measured an increase in concentrations and fluxes of DOC in surface waters derived from upland areas of the UK, the cause of which has been attributed to climate change (Freeman et al., 2001; Freeman et al., 2004; Tranvik and Jansson, 2002). These upland areas are underlain by significant peat deposits, which in Britain comprise over half of all soil C (Milne and Brown, 1997). Since collectively high-latitude blanket peats represent ~20 % of the global soil carbon storage (Post et al., 1982), the fate of peat-derived carbon therefore has important implications to global change. If this exported terrestrial carbon is buried in estuarine and coastal sediments then rivers simply provide a conduit for C transport from one store (soil) to another (estuarine/coastal sediments). On the other hand, if a significant fraction of the terrestrial carbon is oxidised in estuaries and in other coastal environments (Abril et al., 2002; Aller et al., 1996; Frankignoulle et al., 1998; Keil et al., 1997) then it is transferred to a radiatively-active pool in the atmosphere involving positive feedback to global change.

In addition to terrestrial organic matter, OC in estuaries is comprised of autochthonous (i.e., phytoplankton) and marine and coastal material, with anthropogenic wastes making significant contributions in more polluted systems (Abril et al., 2002; Kempe, 1984). In order to determine the relative contributions of these sources and to understand their behaviour during estuarine mixing it is necessary to utilise natural tracers as provenance indicators. Stable carbon isotope ($\delta^{13}C$) analysis of DOC and POC can be an excellent tool for this type of research and has thus been used successfully in many previous studies (Canuel et al., 1995; Cifuentes and Eldridge, 1998; Coffin and Cifuentes, 1999; Peterson et al., 1994). The primary rationale behind utilising $\delta^{13}C$ signatures of organic C as source indicators in estuarine systems is that there exists a distinct difference between the initial $\delta^{13}C$ values between terrestrial material (i.e., soil, plants) and marine algae. Terrestrial plants use atmospheric CO_2 as their main source of carbon, whereas the main sources of carbon for aquatic macrophytes and phytoplankton is DIC (Hillaire-Marcel, 1986), for which the $\delta^{13}C$ signature is the result of mixing of different sources of CO_2. This generally results in most terrestrial plants (C_3 plants) and soil organic matter having more ^{13}C depleted $\delta^{13}C$ signatures than aquatic plants and marine-produced organic matter (Fry and Sherr, 1984). However, interpretation of $\delta^{13}C$ values in estuarine systems is often problematic in that there can be significant overlap in the isotopic signature of the sources of organic matter (i.e., terrestrial, marine, estuarine phytoplankton, etc.). For example, the average $\delta^{13}C$ value of freshwater plankton is approximately -30‰ (Lazerte, 1983; Angradi, 1993; Thorp et al., 1998; McCusker et al., 1999), which can be similar to $\delta^{13}C$ values for many types of terrestrial plants and soil organic matter (Kendall et al., 2001). Thus, if terrestrial material entering the estuary possesses the same $\delta^{13}C$ signature as carbon being produced by estuarine productivity, then delineating the relative contribution of autochthonous estuarine carbon to the overall DOC and POC pools will be extremely difficult. In addition, the carbon isotope composition of old soil carbon can be identical to that of fresh leaf litter and sewage (Kendall et al., 2001), further complicating source differentiation.

The combined use of radiocarbon measurements and stable isotope measurements can substantially reduce signal overlap compared with the use of $\delta^{13}C$ signatures alone. This is primarily due to the wider range in $\Delta^{14}C$ (~ -1000 to +200‰) compared to $\delta^{13}C$ values (~ -32 to -12‰) (Raymond and Bauer, 2001c). It has been suggested that terrestrial DOC exported by rivers is generally younger ^{14}C-enriched material (Hedges et al., 1986b). For instance, in a recent study carried out in peat-dominated Arctic Rivers (Benner et al., 2004) the $\Delta^{14}C$ values for DOC ranged between -6 to 307‰, indicating the presence of bomb-produced ^{14}C and hence modern radiocarbon ages. Other studies have shown similar $\Delta^{14}C$ values for DOC in both temperate (Raymond and Bauer, 2001a; Raymond and Bauer, 2001b) and tropical (Hedges et al., 1986b) rivers, although in the case of the former older DOC corresponding to radiocarbon ages of between 1000-1400 yrs BP has also been found (Raymond and Bauer, 2001b; van Heemst et al., 2000). Conversely, suspended POC is generally considered to be much older than its dissolved counterpart, with $\Delta^{14}C$ values ranging from 24 to –447‰ in rivers draining into the western North Atlantic Ocean (Raymond and Bauer, 2001b), -232 to -544‰ for POC in the Santa Clara River, southern California, USA (Komada et al., 2004), to between 75 to -980‰ for POC in a subtropical mountainous river in Taiwan (Kao and Liu, 1996). Sources of ^{14}C-depleted POC have been attributed to a greater proportion of organic C derived from older soil horizons and sedimentary fossil carbon (Kao and Liu, 1996; Komada et al., 2004; Raymond and Bauer, 2001c).

Despite these preliminary studies, factors controlling the age of terrestrial organic carbon delivered by rivers and the extent to which this carbon is removed or recycled during estuarine mixing remain uncertain. For instance, Raymond and Bauer (Raymond and Bauer, 2001a) have demonstrated that during estuarine mixing bacteria preferentially utilise younger, more labile DOC. This results in the residual DOC becoming more ^{14}C-depleted as degradation progresses. From these findings they concluded that rivers can therefore supply organic matter to the world's oceans in a more degraded, pre-aged form (Raymond and Bauer, 2001b). Since geochemical studies have failed to identify significant proportions of terrestrially-derived organic compounds in the marine DOC pool (Hedges et al., 1997; Meyer-Schulte and Hedges, 1986), the export of large amounts pre-aged and altered terrestrial organic carbon could be used to explain the presence of old marine carbon, which is generally a few thousand years old (Williams and Druffel, 1987).

It has also been suggested that bacteria preferentially utilize younger POC in the same manner as DOC, resulting in a more degraded, older substrate being exported to the world's oceans (Raymond and Bauer, 2001b). Therefore, if a significant amount of terrestrial POC is undergoing mineralization during estuarine mixing, one might find a relationship between age and percent of riverine POC mineralised. Recent studies have demonstrated large net removal of POC by bacterial mineralization in European estuaries (Abril et al., 2002; Frankignoulle et al., 1998), resulting in large emissions of respired CO_2 (Frankignoulle et al., 1998). Much of this large CO_2 flux from European estuaries has been attributed to the respiration of labile, pollution-derived anthropogenic carbon (Abril et al., 2002; Kempe, 1984), with a limited mineralization of terrestrial soil-derived POC. Conversely, a significant mineralization (up to 70% removal) of terrestrial POC has been observed in the Amazon delta (Aller et al., 1996; Keil et al., 1997). One possible explanation for the removal of this supposedly "refractory" material has been attributed co-metabolism with fresh labile organic carbon (e.g., algal sources), as has been suggested by Bianchi et al. (2004). Evidently, the physical and biogeochemical processes in estuaries that lead to efficient remineralization of potentially older terrestrial organic matter (i.e., peat) in temperate estuaries are not fully understood.

In this study, we examined the behaviour of suspended particulate matter (SPM) in two contrasting North Sea estuaries (Tyne and Tweed, NE UK) in order to evaluate the age and reactivity of terrestrially-derived particulate organic carbon during estuarine mixing. This involved 1) determining the relative contributions of marine, autochthonous and terrestrial-derived material to the suspended POC pool, 2) estimating the percentage of riverine POC that was mineralised within the estuaries / coastal zones, and 3) comparing the behaviour of SPM with that of sedimentary OM (in the Tyne). The geochemical parameters used for these purposes and presented in this study include concentrations and C/N ratios of particulate organic matter (suspended and sedimentary) and concentrations of SPM, and chlorophyll a. Chlorophyll a concentrations and C/N ratios (along with isotopic data) were used to assess the proportion of POC which was derived from algal material. Changes in the percentage of POC comprising SPM (POC%) across the salinity gradient were used to estimate riverine POC mineralization. The isotopic data presented here include both stable and radiocarbon isotopes (^{13}C and ^{14}C, respectively) of suspended POC and surface sediments. Surveys were carried out in both summer and winter in order to evaluate the influence of seasonal/hydrologic variability.

MATERIAL AND METHODS

Description of Study Sites

The Tyne Estuary

The River Tyne, which flows through the densely populated city of Newcastle, has a total drainage area of approximately 2900 km^2 and an average freshwater flow of ~48 m^3/s. Its two main tributaries are the North Tyne, which receives humic-rich waters draining areas of blanket peat afforestation, and the South Tyne, which drains relatively pristine moorland (Baker and Spencer, 2004). The North and South tributaries converge downstream to form the River Tyne, which supplies more than 90% of the total river discharge into the Tyne Estuary (Figure 1; Chapter 1). Most of the remaining freshwater input is derived from the River Derwent. The maximum extent of the tidal estuary is approximately 33 km inland from the North Sea, and the residence time is approximately 5-20 days (A. P.

Stubbins, private communication). The Tyne is a partially mixed mesotidal estuary (Baker and Spencer, 2004). Although industrial fluxes to the lower part of the estuary are in decline, it continues to receive significant amounts of urban waste, particularly from sewage treatment facilities located at Howdon (one of the UK's largest estuarial secondary treatment facilities) in the lower estuary (Baker and Spencer, 2004; Upstill-Goddard et al., 2000). However, the water quality in the Tyne upstream of Newcastle has improved significantly over the past few decades.

SPM levels in the Tyne are generally <130 mg/L and show trends of decreasing concentrations with increasing salinities, with a noticeable maximum turbidity zone (MTZ) often located in the low to mid salinity range (Kitidis, 2002). Soils in the catchment are dominated by large areas of peat in the uplands, which provides a substantial store of organic carbon and with stagno-gleys in the majority of the remaining areas (Baker and Spencer, 2004). Generally the soils are slow draining and underlain by shallow or low permeability aquifers, leading to the hydrology of many sub-catchments to be dominated by surface runoff with rapid response to rainfall as a result of saturation excess (Baker and Spencer, 2004). The geology in the upper Tyne basin is comprised of Carboniferous limestones and the Namurian Millstone Grit Series, which is characterised by thick, coarse-grained cross-bedded sandstones, together with fine-grained sandstones, siltstones and mudstones. The Lower and Middle Coal Measures, in which the dominant rock types are shales, mudstones and sandstones, underlie the lower Tyne basin.

The Tweed Estuary

The Tweed River and its tributaries drain a rural, relatively sparsely populated region located in the border region between England and Scotland. Most of the Tweed's discharge originates from the main river, although approximately 10% of the freshwater input comes from the Whiteadder, which joins the Tweed approximately 6.5 km upriver from the North Sea. The combined catchment area of the Tweed and Whiteadder is approximately 4900 km^2 and the average freshwater input into the estuary is ~84 m^3/s (Fox and Johnson, 1997). The maximum length of the Tweed estuary (Figure 1; Chapter 1) is around 13 km and the residence time is approximately 1 day (Uncles and Stephens, 1996). The Tweed is a partially mixed to stratified microtidal estuary (Uncles and Stephens, 1996). The catchment is dominated by agricultural activity, ranging from upland areas of moorland used for sheep grazing to more arable regions in the lowlands (Neal, 2002). The Tweed receives significantly less anthropogenic inputs than the Tyne, as reflected in its favourable water quality ratings (Robson and Neal, 1997).

Due to the rapid flushing time in the Tweed Estuary SPM concentrations are generally quite low (<30 mg/L) and tend to exhibit conservative mixing between river and coastal waters (due to the lack of a MTZ), although higher coastal turbidity resulting from strong wind events and periods of higher discharge can sometimes occur (Uncles et al., 2000). While the Tweed is considered to be a net heterotrophic system during the winter and a net autotrophic system during the summer (Chapter 3, Howland et al., 2000), due to the low chlorophyll a concentrations (<2 μg/L) typically found in this estuary (Chapter 2, Uncles et al., 2000), photosynthetic activity in the Tweed is thought to be dominated by benthic macrophytes. Changing with elevation, soils in the Tweed catchment include well drained brown earths founds in the lowlands, gleys on the southern slopes, podzols on higher land and peats on hill tops and moors (Robson and Neal, 1997). The underlying geology of the Tweed is predominantly comprised of Silurian and Ordovician slates, shales and calcareous mudstones and Old Red Sandstone, with Carboniferous limestones and basaltic intrusions cropping out in the eastern part of the basin. As opposed to the Tyne, the Tweed estuary has not undergone considerable dredging and is fairly steeply rising and shallow.

Sample collection and analysis of surface sediments

SPM, POM and chlorophyll a (chl-a) data reported here (Tables 1-5) are from near-surface water samples collected on single day excursions to the Tyne (Feb-02, Jul-02, Mar-03, Jul-03) and Tweed (Jul-03, Dec-03) Estuaries. Site selection within the estuary (Table 1) was based on *in situ* salinity measurements (using a portable probe) in order to ensure adequate, high-resolution coverage of the full salinity gradient. Once back in the laboratory, salinity was analysed on a pre-calibrated Hanna (model 8633) conductivity meter. Water samples (Appendix 2) for SPM, POM and chlorophyll *a* (chl-a) were collected in 4 L high density polyethylene (HDPE) containers at a depth of 1-2 m from a small boat using a submersed pump. River discharge data is reported here as the two-day average incorporating the days

Site/Date	km from N Sea	salinity	SPM (mg/L)	POC (mg/L)	POC % (of SPM)	% algal POC	C/N at	chl-a (µg/L)
Tyne								
28/2/02	0.0	31.7	31.3	1.42	4.5		18.1	
28/2/02	2.5	17.7	30.1	1.79	5.9		24.8	
28/2/02	5.9	15.0	36.7	1.97	5.4		24.0	
28/2/02	7.4	9.9	35.3	2.41	6.8		21.1	
28/2/02	9.6	6.9	38.6	3.28	8.5		25.2	
28/2/02	15.1	3.0	48.2	4.43	9.2		21.1	
28/2/02	16.9	2.1	52.3	4.58	8.8		21.2	
11/7/02	0.0	33.2	15.7	0.33	2.1	9.4	15.2	0.8
11/7/02	5.9	30.9	17.0	0.83	4.9	6.7	15.5	1.4
11/7/02	15.1	25.9	19.7	0.89	4.5	13.5	20.8	3.0
11/7/02	19.3	19.3	35.1	2.62	7.5	3.7	19.3	2.4
11/7/02	22.8	15.0	34.9	3.04	8.7	3.1	20.4	2.4
11/7/02	27.6	9.8	30.4	2.67	8.8	6.5	18.9	4.3
11/7/02	30.2	4.7	29.1	2.53	8.7	4.0	21.4	2.5
11/7/02	32.0	0.2	15.9	1.99	12.5	2.6	22.5	1.3
11/7/02	32.0	0.2	17.9	2.05	11.4	2.8	22.5	1.4
4/3/03	0.0	34.1	15.7	0.95	6.1	1.1	19.9	0.3
4/3/03	5.9	28.7	20.2	1.56	7.7	0.5	18.0	0.2
4/3/03	9.6	19.0	29.4	1.88	6.4	0.4	20.1	0.2
4/3/03	15.1	14.8	55.3	2.77	5.0	0.4	20.3	0.2
4/3/03	15.9	14.1	147.9	5.75	3.9	0.3	20.3	0.4
4/3/03	17.5	10.5	232.1	6.75	2.9	0.3	21.5	0.6
4/3/03	22.8	5.3	261.9	6.43	2.5	0.7	20.4	1.2
4/3/03	27.6	1.3	92.8	4.92	5.3	0.5	19.6	0.6
4/3/03	32.0	0.1	21.9	2.32	10.6	1.1	20.9	0.6
4/3/03	32.0	0.1	22.0	2.25	10.2	1.3	20.5	0.7
23/7/03	0.0	32.5	1.8	0.19	10.1	21.2	11.4	1.0
23/7/03	5.9	27.1	7.2	0.43	6.0	20.7	10.5	2.2
23/7/03	15.1	25.1	8.3	0.47	5.6	8.1	12.6	0.9
23/7/03	22.8	24.2	13.5	0.55	4.1	9.9	13.1	1.4
23/7/03	26.2	21.6	16.4	0.63	3.8	17.4	12.2	2.7
23/7/03	17.5	20.6	12.8	0.50	3.9	5.4	13.0	0.7
23/7/03	27.6	20.1	17.4	0.53	3.0	10.7	12.9	1.4
23/7/03	23.7	18.3	25.4	0.91	3.6	12.3	13.7	2.8
23/7/03	30.2	16.4	13.8	0.49	3.5	11.3	11.2	1.4
23/7/03	30.5	13.9	13.5	0.47	3.5	12.1	11.3	1.4

Table 1. Geochemical parameters of water samples from the Tyne Estuary, including salinity, total, suspended particulate matter (SPM), particulate organic carbon (POC), POC % of SPM, % of POC that is algal-derived (% algal POC), C/N ratio of POM (C/N_{POM}), suspended particulate Al concentrations, and chlorophyll a concentrations (chl-a).

prior to and day of sampling. Discharges for the Tyne (Q = 144.6 m^3/s, 27-28 February 2002; Q = 8.6 m^3/s, 10-11 July 2002; Q = 30.7 m^3/s, 03-04 March 2003; Q = 7.3 m^3/s, 22-23 July 2003) and Tweed (Q = 14.5 m^3/s, 07-08 July 2003; Q = 151.2

m^3/s, 02-03 December 2003) were obtained from the UK Environment Agency and from the Scottish Environment Protection Agency, respectively. During the Jul-03 transect in the Tyne Estuary, the combined effects of a spring tidal event with extremely low flow resulted in a low salinity end-member sampling point of 13.9 psu due to inaccessibility of the inner estuary. Conversely, during the Dec-03 sampling trip to the Tweed Estuary high river flow resulted in a survey comprised predominantly of low salinity water samples. This strong dependence on surface salinity with freshwater runoff has been previously reported in the Tweed Estuary (Uncles et al., 2000).

Surface sediment samples reported here were collected across the full salinity gradient from the Tyne Estuary in July 2001 and from four different sampling locations during February 2002, July 2002 and March 2003 using a light-weight gravity corer lowered from a small inflatable ribbed boat. All cores were inspected for the intact collection of the sediment water-interface. In July 2001 only the top few centimetres were collected while in Feb-02, Jul-02 and Mar-03 the cores were brought back to the laboratory and sectioned in 1-2 cm intervals across their full length. The sediment was freeze-dried, homogenised with a mortar and pestle, and then acidified with 2N HCl prior to isotopic and chemical analysis to remove inorganic carbon. Concentrations of major elements (Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn, P) in Tyne sediments were also analyzed by X-ray fluorescence spectrometry are presented in Appendix 3.

Analysis of suspended POM, SPM and chlorophyll a

Concentrations and stable and radioactive carbon isotopes (^{13}C and ^{14}C, respectively) of suspended POM and concentrations of SPM and chl-a were determined by direct analysis of the material collected on pre-combusted (440°C for 4 h), pre-weighed glass fibre filters (GF/F, Whatman) by pressure filtration (~1.4 bar) in the laboratory within 10 hours after water samples were collected. After sufficient material had been collected the filters were rinsed with Milli-Q water to remove salts. Filters used for POM analysis were placed inside a glass desiccator containing concentrated HCl for several days to remove inorganic carbon. The filters were then vacuum desiccated to remove HCl, had excess glass fibre removed, were weighed, and then were subsequently homogenised with a mortar and pestle and stored in glass vials prior to concentration and isotopic analysis. SPM concentrations were determined by weighing dry filters prior to acidification. Based on analyses of duplicate GF/F filters from the same sampling locations, the precision for SPM concentrations was ±5% RSD. Chl-a samples were filtered and then stored at -20°C prior to extraction. After soaking the filters for several hours in 90% acetone, chl-a in the leachate was determined by measuring fluorescence with a Turner Model 450 fluorometer (Parsons et al., 1984). Based on multiple analyses of standards and samples, the precision for the chl-a measurements was found to be ±10% RSD.

Site/Date	km from N Sea	salinity	SPM (mg/L)	POC (mg/L)	POC % (of SPM)	% algal POC	C/N_{POM}	chl-a (µg/L)
Tweed								
8/7/03	0.00	33.2	1.0	0.08	8.1	11.5	8.6	0.2
8/7/03	2.02	32.4	1.2	0.12	9.6	11.3	10.7	0.3
8/7/03	3.53	29.5	1.7	0.18	11.0	14.0	10.1	0.6
8/7/03	4.54	27.4	3.4	0.34	10.0	14.5	10.8	1.2
8/7/03	5.67	17.9	3.8	0.44	11.7	12.3	9.1	1.4
8/7/03	5.92	8.6	3.7	0.50	13.7	11.4	8.5	1.4
8/7/03	6.43	4.2	3.3	0.40	12.0	13.9	9.3	1.4
8/7/03	7.18	1.4	3.6	0.51	14.2	11.9	8.5	1.5
8/7/03	8.19	0.1	3.2	0.45	13.9	14.7	9.9	1.6
8/7/03	8.19	0.1	3.3	0.48	14.5	14.2	10.0	1.7
3/12/03	0.00	31.30	17.5	0.67	3.8	11.0	12.6	1.8
3/12/03	0.13	19.38						
3/12/03	0.25	4.51						
3/12/03	3.02	1.90	19.2	1.34	7.0	11.7	12.3	3.9
3/12/03	0.50	1.27	18.0	1.23	6.9	10.2	12.7	3.1
3/12/03	0.76	0.97	16.6	0.95	5.8	14.1	12.4	3.4
3/12/03	1.76	0.90	18.5	1.02	5.5	8.1	11.6	2.1
3/12/03	4.79	0.19	24.0	1.55	6.5	11.7	12.5	4.6
3/12/03	6.80	0.16	23.4	1.45	6.2	11.0	11.5	4.0
3/12/03	6.87	0.12	23.3	1.40	6.0	10.8	11.3	3.8

Table 2. Geochemical parameters of water samples from the Tweed Estuary, including salinity, total, suspended particulate matter (SPM), particulate organic carbon (POC), POC % of SPM, % of POC that is algal-derived (% algal POC), C/N ratio of POM (C/N_{POM}), suspended particulate Al concentrations, and chlorophyll a concentrations (chl-a).

Concentrations, C/N and $\delta^{13}C$ ratios of suspended POM and surface sediments

Concentrations, C/N (reported here as molar ratios) and $\delta^{13}C$ ratios for suspended POM and surface sediments were determined using a Carlo Erba Instruments NA2500 elemental analyser coupled with a Micromass PRISM III

Isotope Ratio Mass Spectrometer (EA-IRMS). Ratios of ^{13}C to ^{12}C and are expressed in delta notation as per mil (‰) differences relative to the VPDB standard, where:

$$\delta^{13}C = [\{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}\} / (^{13}C/^{12}C)_{standard}] \times 1000 \quad (1)$$

With the exception of the most saline sample collected from the Tyne (32.5 psu) and samples taken from the Tweed at salinities >20 during the Jul-03 surveys, $\delta^{13}C$ values reported here are the result of replicate analyses. Based on these replicate samples and multiple measurements of standards the 1σ precision for $\delta^{13}C_{POC}$ presented in this study was ±0.3‰. Based on multiple analyses of standards and samples, the acuracy and reproducibility for %C and %N determined via EA analysis was ±5% and ±8% RSD, respectively.

^{14}C dating

Suspended POM samples and surface sediments were prepared to graphite at the Natural Environmental Research Council (NERC) Radiocarbon Laboratory, East Kilbride, UK, and analysed at the Scottish Universities Environmental Research Centre (SUERC), East Kilbride, UK on the 5MV NEC AMS. Graphite was prepared by quantitative recovery of carbon in sealed quartz tubes followed by cryogenic separation of CO_2 (Boutton et al., 1983). Aliquots of CO_2 were converted to an iron/graphite mix by iron/zinc reduction (Slota et al., 1987). A sub-sample of CO_2 was used to measure $\delta^{13}C$ using a dual-inlet mass spectrometer with a multiple ion beam collection facility (VG OPTIMA) in order to normalise ^{14}C data to -25‰ $\delta^{13}C$-PDB. The mass spectrometer was calibrated with international reference materials to a precision of ±0.1‰. Radiocarbon data have been normalised to -25 ‰ $\delta^{13}C$-PDB using values obtained either from on-line measurements made on graphite analysed by AMS or using the $\delta^{13}C$ measurement made on the sub-sample of CO_2 using the dual-inlet mass spectrometer described above. In order to assess the viability of analysing GF/F filters using this methodology, blanks were prepared by adding internal standard material of known ages to 4 L of MilliQ water. Using the same suspended POM extraction procedures described above, GF/F blanks demonstrated acceptable levels of background contamination. AMS results (Table 3) are reported here as conventional radiocarbon years BP (before AD 1950), absolute percent modern carbon (pMC) and $\Delta^{14}C$ ratios with an overall analytical precision of 1σ. The use of pMC takes into account the ongoing radioactive decay of the international reference standard (oxalic acid) since AD 1950 (Stuiver and Polach, 1977). ^{14}C enrichment >100 pMC indicates the presence of ^{14}C from atomic weapons testing (post AD 1955). $\Delta^{14}C$ values are expressed according to the convention defined by (Stuiver and Polach, 1977), where:

$$\Delta^{14}C = (pMC/100 - 1) \times 1000 \qquad (2)$$

Using the error associated with pMC values, the 1σ precision for $\Delta^{14}C$ values presented in this study was better than ±3‰ for POC and ±10‰ for sediments.

RESULTS AND DISCUSSION

Behaviour of SPM, POC and chlorophyll a concentrations

In Figure 2, concentrations of SPM and suspended POC from all six surveys to the Tyne and Tweed Estuaries are plotted versus salinity. In general, both SPM and POC concentrations showed similar distribution trends. In the Tyne, the highest SPM and POC concentrations were often associated with a well-defined low- to mid-salinity range maximum turbidity zone (MTZ). These MTZs were most pronounced during the Jul-02 (Figure 2b) and Mar-03 (Figure 2c) surveys. During Feb-02 (Figure 2a) very high river discharge following a storm event precluded the build-up of sediments in the mid-salinity region, thereby limiting the role of the MTZ. The lowest SPM and POC concentrations measured in the Tyne were during Jul-03 (Figure 2d), when river discharge was the lowest of all the surveys reported here and followed an unusually dry summer period. The low SPM and POC concentrations measured in the Tyne during Jul-03 are attributed to the combined effects of unusually low river flow ($Q = 7.3\ m^3/s$) reducing particle inputs from the river and a spring tidal event. In the Tweed during both summer (Figure 2e) and winter (Figure 2f) transects there was no observable MTZ, although most samples during the Dec-03 survey (with the exception of one) were limited to the marine end-member and salinities <2. However, the lack of substantial long-term accumulation of muddy sediments and hence the absence of a distinct maximum turbidity zone in the Tweed

Figure 2a Tyne Feb-02

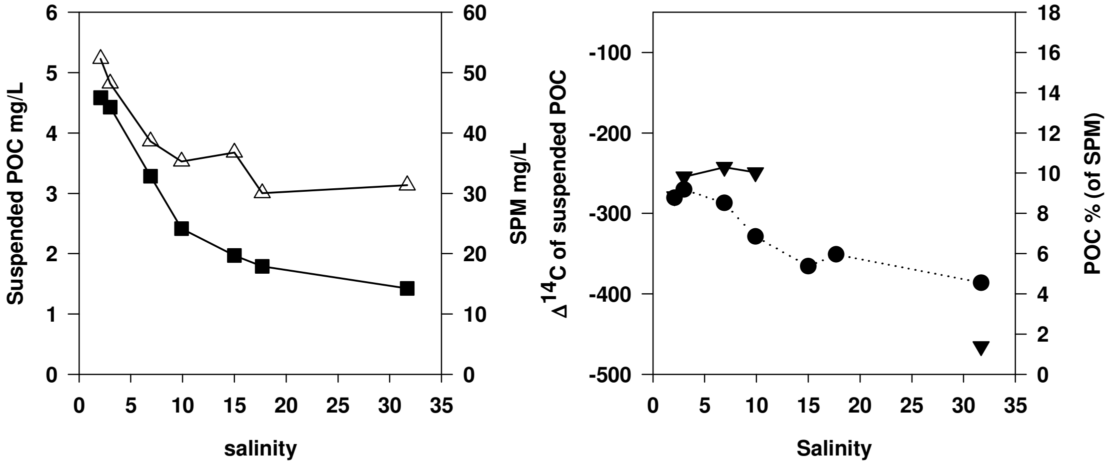

Figure 2b Tyne Jul-02

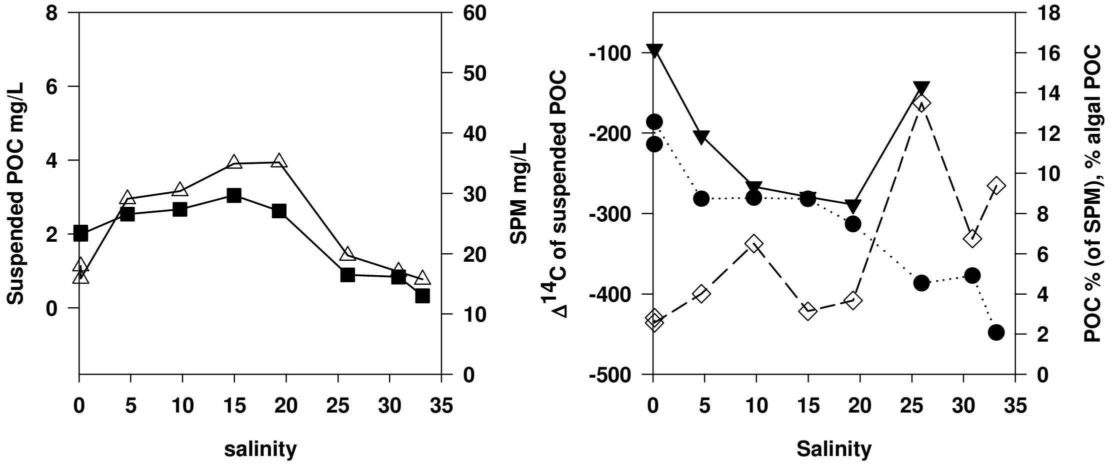

Figure 2c Tyne Mar-03

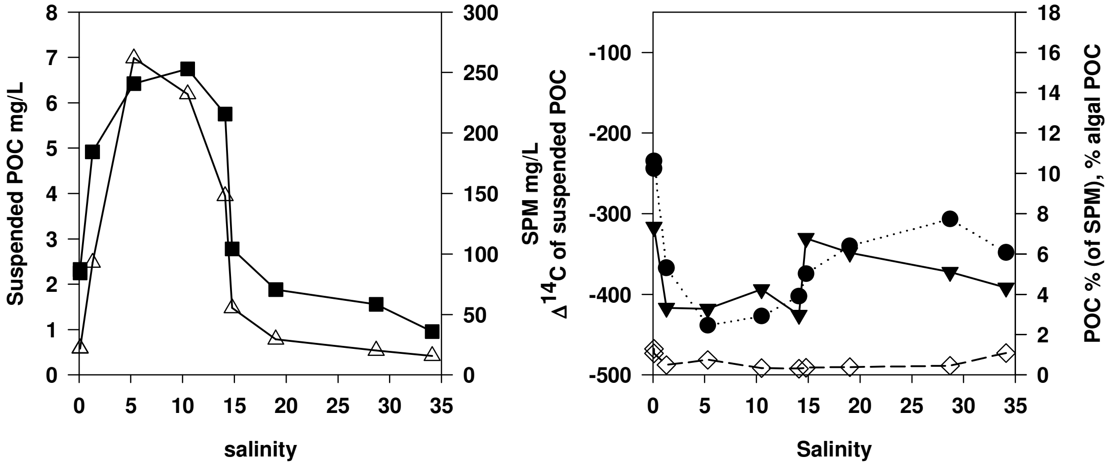

Figure 2. Concentrations of suspended particulate organic carbon (POC; open triangles), suspended particulate matter (SPM; closed squares), particulate organic carbon % (POC %) of suspended particulate matter (SPM) (closed circles), estimated % algal POC (open diamonds), and $\Delta^{14}C$ values of POC (closed inverted triangles) in the Tyne Estuary during (a) Feb-02, (b) Jul-02, (c) Mar-03 and (d) Jul-03, and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. Note that estimated % algal POC values were not measured in the Tyne during Feb-02.

Figure 2d Tyne Jul-03

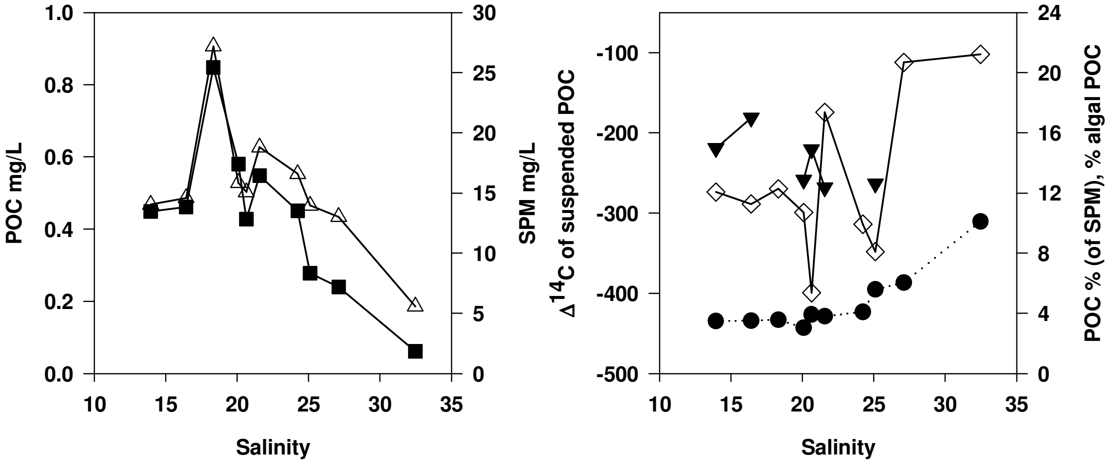

Figure 2e Tweed Jul-03

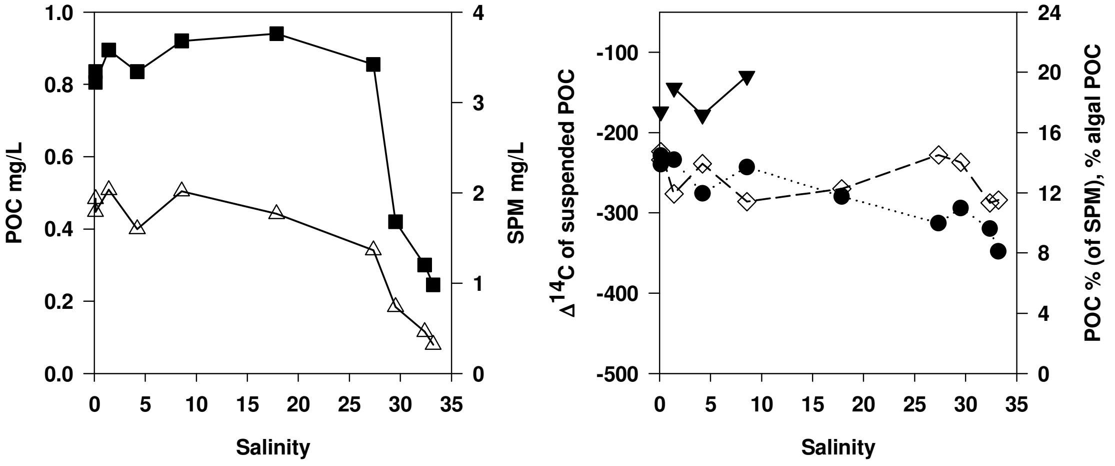

Figure 2f Tweed Dec-03

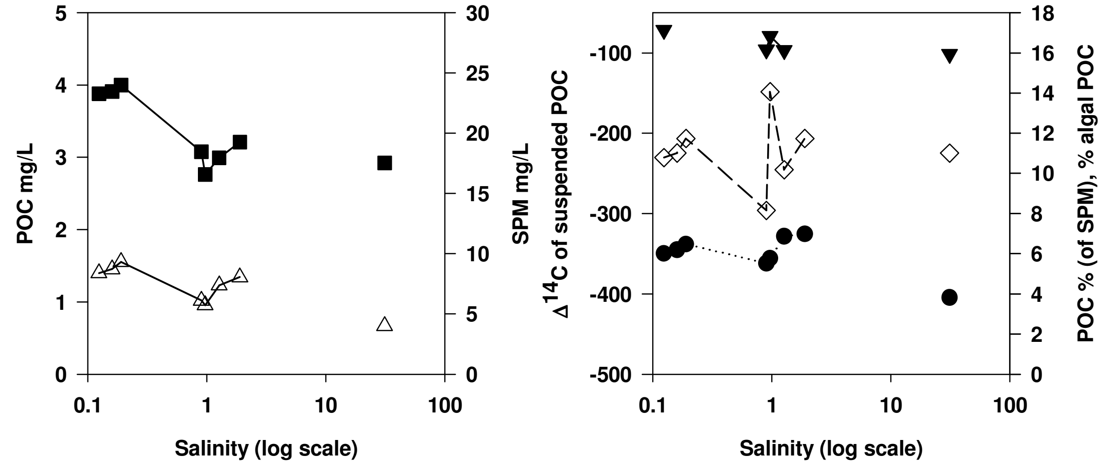

Figure 2. Concentrations of suspended particulate organic carbon (POC; open triangles), suspended particulate matter (SPM; closed squares), particulate organic carbon % (POC %) of suspended particulate matter (SPM) (closed circles), estimated % algal POC (open diamonds), and Δ^{14}C values of POC (closed inverted triangles) in the Tyne Estuary during (a) Feb-02, (b) Jul-02, (c) Mar-03 and (d) Jul-03, and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. Note that estimated % algal POC values were not measured in the Tyne during Feb-02.

Estuary has been documented by others (Uncles et al., 2000), and is thought to be primarily a function of the estuary's short residence time. Suspended POC concentrations in the Tyne were generally higher than those in the Tweed, ranging between 1.42 to 4.58 mg/L (Feb-02), 0.33 to 3.04 mg/L (Jul-02), 0.95 to 6.75 mg/L (Mar-03) and 0.169 to 0.91 mg/L (Jul-03) in the former compared with 0.08 mg/L to 0.50 mg/L (Jul-03) and 0.67 to 1.55 mg/L in the latter. The concentrations of riverine POC entering the Tyne and Tweed Estuaries increased with river discharge, and the riverine POC fluxes (mol C hr^{-1}) during these surveys were 2.0×10^5 (Tyne, Feb-02), 5.2×10^3 (Tyne, Jul-02) 2.1×10^4 (Tyne, Mar-03), 2.0×10^3 (Tweed, Jul-03), and 6.7×10^4 (Tweed, Dec-03).

Chl-a concentrations during all surveys in both estuaries were relatively small, ranging from 0.8 to 4.3 μg/L (Jul-02), 0.2 to 1.2 μg/L (Mar-03) and 0.7 to 2.8 μg/L (Jul-03) in the Tyne Estuary and from 0.2 to 1.7 μg/L (Jul-03) and 2.1 to 4.6 μg/L (Dec-03) in the Tweed Estuary. Although comparisons in the literature are not available for the Tyne, chl-a values similar to those reported here have been observed previously in the Tweed (Uncles et al., 2000). These authors attributed the low chl-a concentrations (<2 μg/L) to a rapid flushing which prevents the occurrence of repeated algal cell division within the tidal estuary. The slightly higher chl-a concentrations in the Tweed during December were probably related to higher levels of plant debris in the river during this period of high discharge. Although not measured during the Feb-02 survey to the Tyne Estuary, it was likely that chl-a concentrations during this transect were similarly low as those measured during the following winter.

A semi-quantitative estimate of contributions from algal-derived suspended POC were made by assuming that algal material has a POC/ chl-a ratio of 40 ((Abril et al., 2002), and references therein). These estimates are presented as percentages of algal-derived suspended POC in the total POC pool (% algal POC). In the Tyne, the % algal POC ranged 2.6-13.5% (Jul-02; Figure 2b), 0.3-1.3% (Mar-03; Figure 2c) and 5.4-21.2% (Jul-03; Figure 2d), with the highest values found in the middle and lower parts of the estuary. In the Tweed, the estimated % algal POC were relatively constant during both summer and winter surveys, ranging from 11.3-14.7% during Jul-03 (Figure 2e) and 8.1-14.1% during Dec-03 (Figure 2f). As noted above, the % algal POC calculated in the winter in the Tweed are most likely inflated due to a elevated chl-a concentrations caused by a higher influx of plant debris. Therefore, with the exception of the two most saline samples collected from the Tyne Estuary during the July 2003 transect (27.1 and 32.5 psu), algal-derived organic carbon comprised a minor fraction (<18%) of suspended POM. However, % algal POC estimated for the Jul-02 and Jul-03 surveys were in general slightly higher (compared to Mar-03). The relative increase in algal contributions during summer months is a response to higher autochthonous production and lower inputs of terrestrial POC. However, some of the high % algal POC values in the lower part of the Tyne Estuary during the summer (e.g., Jul-02 transect, 25.9 psu; Jul-03 transect, 27.1 and 32.5 psu) may represent localized increases in autochthonous production within the estuary and/or marine algae advected into the estuary. This will be evaluated later using $\delta^{13}C$ and C/N ratios.

Since POC concentrations showed the same distributions as SPM, it was concluded that more useful information pertaining to organic matter cycling could be obtained through the interpretation of POC expressed as a dry weight percentage of SPM (POC%). Therefore, following the convention utilised by others (Abril et al., 2002; Zhang et al., 1998), the behaviour of POC in the Tyne and Tweed Estuaries was expressed in terms of relative changes within the total SPM pool (Figure 2). By evaluating the changes in POC% across the salinity gradient, it was possible to estimate the amount of riverine POC mineralised in the estuarine / coastal zone, (Abril et al., 2002):

$$\text{\% of riverine POC mineralised} = SPM_{river} \times (POC\%_{river} - POC\%_{MTZ}) \qquad (3)$$

Here SPM_{river} refers to the average SPM riverine concentration, $POC\%_{river}$ is the POC% (% of SPM) in the river and $POC\%_{MTZ}$ is the POC% (% of SPM) in the maximum turbidity zone. Since the Tweed does not possess a well-defined MTZ, and contributions from algal material were relatively minor in this estuary, the lowest POC% value observed at the mouth of the estuary was substituted for the $POC\%_{MTZ}$ in Eq. 2 (Abril et al., 2002). This was also done for the Tyne Feb-02 survey, due to the lack of a pronounced mid-salinity MTZ during this period caused by higher river flow. Furthermore, owing to the particularly low flow conditions experienced in the Tyne during the Jul-03 survey, a freshwater end-member (≤ 0.2 psu) POC sample could not be obtained. Therefore, the POC% from the Jul-02 transect (12.0 ±0.8%) was used as a riverine end-member for the following summer. All the parameters used in these calculations are listed in Tables 2 and 3. The estimated percentages of riverine POC mineralised were 48, 38, 76 and 70% in the Tyne during Feb-02, Jul-2, Mar-03 and Jul-03, respectively, and were 31% (Jul-03) and 39% (Dec-03) in the Tweed. The higher percentage of riverine POC loss estimated in the Tyne can be partially attributed to this estuary's longer residence time. A positive correlation between mineralization intensity and the percentage of terrestrial POC removed has been demonstrated in other NW European Estuaries (Abril et al., 2002). In conjunction with variations in the estimated % algal POC, a detailed discussion of the trends in POC% across the Tyne and Tweed Estuaries is provided in context with isotopic data in subsequent sections.

Behaviour of C/N ratios of suspended POM

In Figure 3, C/N ratios of suspended POM from all six surveys to the Tyne (Figure 3a) and Tweed (Figure 3b) Estuaries are plotted versus salinity. C/N ratios were generally higher in the winter than in the summer, and also higher in the Tyne than in the Tweed Estuary. Changes in C/N ratios POM entering the estuary could be attributed to variations in terrestrial sources. For example, in a study carried out by Weiguo et al. (2003) in rivers draining the Loess Plateau, NW China, C/N ratios as low as 9.1 were measured in suspended matter that contained approximately equal proportions of soil- and C_3 plant-derived OM. Conversely, when the suspended matter was comprised almost entirely of plant-derived OM, the C/N ratio increased to 14.3.

The C/N ratios in the Tyne during both winter surveys were relatively invariant across the estuary, scattering around means (after combining freshwater end-members, ≤ 0.2 psu, when appropriate) of 22.2 ±2.5 (n = 7) and 20.1 ±1.0 (n = 9) during the Feb-02 and Mar-03 surveys, respectively. During the Jul-02 survey, C/N ratios showed a general decrease with increasing salinity, although the average ratio (19.2 ±2.7, n = 8) was still relatively high. In the Tweed during Dec-03 the average C/N ratio was 12.2 ±0.5 (n = 6) and during Jul-03 was 9.5 ±0.9 (n = 9). The relatively high C/N ratios found in the Tyne during the Feb-02, Mar-03 and Jul-02 surveys imply that much of the suspended POM was derived from terrestrial plant

Figure 3a

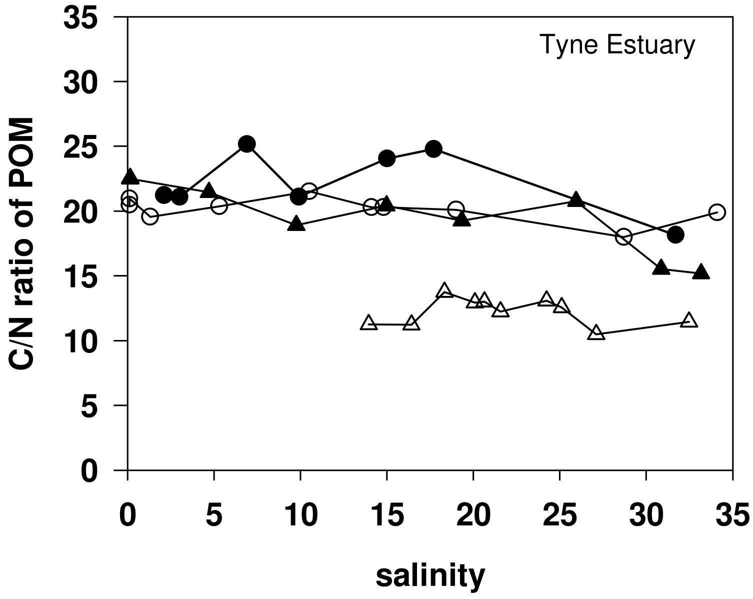

Figure 3b

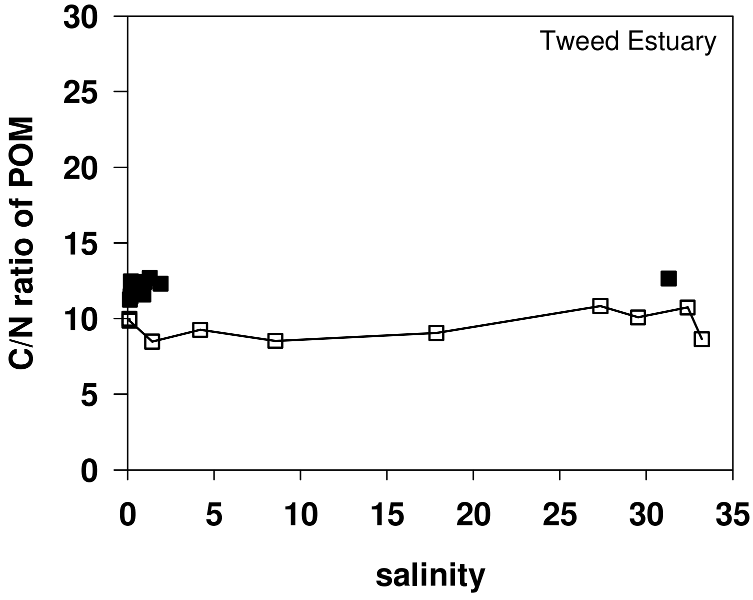

Figure 3. C/N ratios (molar) of particulate organic matter (C/N_{POM}) in the (a) Tyne Estuary during Feb-02 (closed circles), Jul-02 (closed triangles), Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity.

debris (Boutton, 1996; Kendall et al., 2001). However, the C/N ratios of POM encountered in the Tyne were still relatively high when compared with C/N ratios of suspended POM reported in other studies (e.g., Middelburg and Nieuwenhuize, 1998; Rostad et al., 1997; Verity, 2002). These high ratios could be attributed to

POM sources from peat, which could exhibit relatively high C/N ratios relative to other soil types (e.g., >30; Novák et al., 1999) due to its greater humic content (Seeber and Seeber, 2005). The relatively low C/N_{POM} ratios in the Tweed are attributed to inputs of N-enriched agricultural soil. The C/N ratios of soil organic matter (SOM) have been shown to be heavily influenced by land-use patterns, with cultivated soils generally possessing lower C/N ratios (Seeber and Seeber, 2005). This lowering of the C/N ratio in SOM is related to soil decomposition, whereby the more easily decomposed components are removed and nitrogen becomes immobilised in microbial biomass and decay products, leaving behind a residual SOM which contains a higher proportion of recalcitrant material (Post et al., 1985). Low C/N ratios for soil-derived suspended sediment in the River Tweed (~ 10-12) have been observed by others (Neal et al., 1997). Thus, the broad variations in C/N ratios of the two estuaries reflect their catchment characteristics and POM sources.

The lower C/N ratios found in the Tweed during the Jul-03 survey cannot be attributed to peat or agricultural soil-derived sources. This sampling period corresponded with unusually low river discharge (Q = 7.3 m3/s). While the isotopic signatures of estuarine phytoplankton can occupy a wide range of values, in general phytoplankton growth is evidenced by substantially lower (≤ 8) C/N ratios (Baird and Middleton, 2004; Cowie and Hedges, 1994; Goni and Hedges, 1995; Hellings et al., 1999; Kendall et al., 2001; Middelburg and Nieuwenhuize, 1998). Thus the low C/N ratios in the Tyne during July can be attributed to estuarine and marine algal contributions, as confirmed by the higher estimated % algal POC values during this period relative to all other surveys in the Tyne Estuary (Figure 2). Furthermore, the relatively low C/N ratios measured during the Jul-03 Tyne survey might also indicate a sewage contribution, as has been observed in C/N ratios for sewage-derived high molecular weight dissolved organic matter (HMW DOM) in the Tyne Estuary (Chapters 3 and 5).

The average C/N ratio for suspended POM found in the Tweed was 9.5 ±0.9 (n = 9) and 12.2 ±0.5 (n = 6) during the summer and winter surveys, respectively. One could suppose this seasonal difference with slightly lower values in the summer could be attributed to additional algal contributions to soil-derived organic matter. However, unlike the Tyne Jul-03, the chl-a concentrations in the Tweed also reflects plant debris derived by benthic macrophytes and/or seagrasses (Chapters 1-3).

Indeed, chl-a ratios normalised to POC are matched by higher C/N during Jul-03 survey of the Tweed (Chapter 5). Therefore, we attribute the slightly higher C/N ratios in the Dec-03 survey compared to the Jul-03 survey to increased mixing of low C/N soil-derived OM with fresh litter with higher C/N ratios (e.g., Weiguo et al., 2003).

Behaviour of C/N and δ^{13}C ratios of surface sediments

In Figure 4a are plotted the δ^{13}C signatures of surface sediments collected in the Tyne Estuary during Jul-01, Feb-02, Jul-02 and Feb-03 versus the sampling distance from the North Sea. These data are listed in Table 3. As this figure

Date	km from N Sea	%C wt*	C/N at	δ^{13}C ±0.3‰	^{14}C age (yrs BP) ±1σ	^{14}C pMC ±1σ	Δ^{14}C ±10‰
Jul-01	4.2	6.3	21.7	-22.9			
Jul-01	5.9	5.4	23.4	-23.0			
Jul-01	7.9	7.2	21.2	-23.7			
Jul-01	14.5	15.5	29.1	-23.2			
Jul-01	15.1	4.0	23.2	-24.4			
Jul-01	16.0	9.2	27.7	-24.4			
Jul-01	18.8	10.2	26.4	-24.3			
Jul-01	21.6	8.3	22.1	-25.0			
Jul-01	22.8	8.3	21.5	-25.4			
Jul-01	24.3	7.5	20.2	-24.9			
Jul-01	25.7	9.5	18.6	-25.3			
Jul-01	26.0	8.5	19.4	-25.2			
Jul-01	26.1	7.6	21.0	-24.8			
Jul-01	27.2	4.4	21.0	-25.5			
Jul-01	28.8	8.8	17.5	-25.7			
Jul-01	29.1	8.4	21.0	-24.8			
Feb-02	4.2	6.5	18.6	-22.7	9082 ±63	32.07 ±0.25	-538
Feb-02	15.9	8.2	19.0	-25.7	4175 ±28	59.08 ±0.21	-679
Feb-02	24.0	8.7	21.1	-24.9	6145 ±37	46.23 ±0.22	-409
Jul-02	4.2	6.3	20.5	-22.6	11137 ±76	24.84 ±0.24	-752
Jul-02	15.9	8.1	21.2	-25.0	6516 ±36	44.14 ±0.20	-559
Jul-02	24.0	9.1	24.5	-24.5	8006 ±47	36.67 ±0.22	-633
Jul-02	25.8	8.8	20.2	-26.4	3624 ±25	63.27 ±0.20	-367
Mar-03	4.2	6.5	27.5	-22.7	12921 ±100	19.89 ±0.25	-801
Mar-03	15.9	6.4	23.5	-24.5	9721 ±62	29.62 ±0.23	-704
Mar-03	24.0	8.3	19.4	-25.0	6429 ±36	44.63 ±0.20	-554
Mar-03	25.8	7.0	22.4	-24.9	7348 ±42	39.80 ±0.21	-602

Table 3. Geochemical and isotopic parameters (%C wt, C/N at ratios, δ^{13}C, radiocarbon ages, pMC-% modern absolute carbon, Δ^{14}C) of surface sediments collected from the Tyne Estuary during Jul-01, Feb-02, Jul-02 and Mar-03.
*%C wt values have not been salt corrected

illustrates, sedimentary $\delta^{13}C$ values ostensibly indicated a near linear mixing between terrestrial ($\delta^{13}C$ ~ -26‰) and marine ($\delta^{13}C$ ~ -23‰) OM. Most $\delta^{13}C$ values in the mid- and upper estuary pointed to a predominantly terrigenous origin for organic matter deposited in sediments. The relatively ^{13}C-enriched $\delta^{13}C$ values for sediment collected near mouth of the Estuary are typical for marine OM and are similar to the value of -23.2 ‰ reported in the nearby Forth (Graham et al., 2001) and Tay (Thornton and McManus, 1994) Estuaries, both located in SE Scotland. However, while sedimentary $\delta^{13}C$ values apparently reflected mixing between terrestrial and marine sources, as observed in the Tay Estuary (Thornton and McManus, 1994), the C/N ratios of sedimentary OM did not. As Figure 4b demonstrates, C/N ratios across the salinity gradient did not indicate mixing between terrestrial (~ 20, as reported here in the Tyne) and marine (typically <10), as has been observed in the Schelde Estuary, NW Europe (Middelburg and Nieuwenhuize, 1998). Instead, C/N ratios across the entire estuary were relatively high, ranging from 17.5 (28.8 km upriver) to 29.1 (14.7 km upriver), and even showed a slight increase towards the North Sea. Similar elevated C/N ratios in Tyne sediment have been reported by others (Matthiessen et al., 1998). The highest C/N ratios for sedimentary POM were found where the River Tyne flows through the heart of the City of Newcastle (15-20 km from the North Sea). This points to an anthropogenic source being the main source of carbon enriched material such as coal or petroleum-derived contaminants. Furthermore, in this part of the estuary, the seven bridges that span the river should play a significant role in increasing turbidity. This could also concentrate contaminants in sediments such as coarser grained coal debris. In addition, increased winnowing may subsequently enhance the microbial breakdown of OM in sediments, which can lead to an increase in C/N ratio of residual organic matter in sediments due to the preferential degradation of N-bearing compounds (Ganeshram et al., 1999; Hedges et al., 1986a; Rosenfeld, 1981). The inability of C/N ratios to show mixing between marine and terrestrial source has been observed by others (Graham et al., 2001; Thornton and McManus, 1994). In these studies, the relatively high and variable C/N ratios found in sediment across the Forth and Tay Estuaries were also attributed to biochemical alteration of the original POM source signature and anthropogenic inputs.

Figure 4a

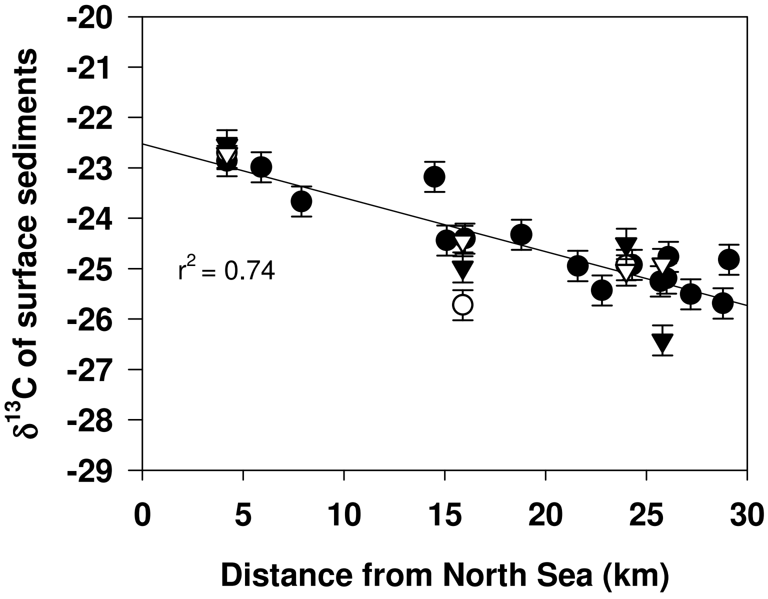

Figure 4b

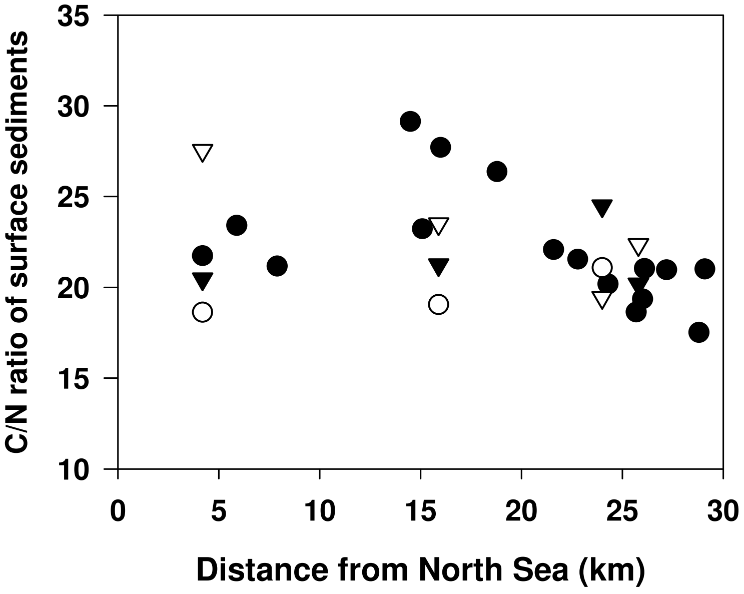

Figure 4. (a) δ^{13}C values and (b) C/N ratios of surface sediments in the Tyne Estuary collected during Jul-01 (closed circles), Feb-02 (open circles), Jul-02 (closed inverted triangles) and Mar-03 (open inverted triangles) plotted versus distance from the North Sea.

Behaviour of δ^{13}C signatures of suspended POC

In Figure 5, variations in δ^{13}C signatures of suspended POC from all six surveys to the Tyne (Table 4) and Tweed Estuaries (Table 5) are plotted versus

Site/Date	salinity	$\delta^{13}C_{POC}$ ±0.3‰	$^{14}C_{POC}$ age (yrs BP) ±1σ	$^{14}C_{POC}$ pMC ±1σ	$\Delta^{14}C_{POC}$ ±3‰
Tyne					
28/2/02	31.7	-24.8	4976 ±31	53.47 ±0.19	-465
28/2/02	17.7	-28.0			
28/2/02	15.0	-26.9			
28/2/02	9.9	-27.9	2247 ±28	75.11 ±0.26	-249
28/2/02	6.9	-28.1	2178 ±36	75.75 ±0.33	-243
28/2/02	3.0	-27.7	2307 ±26	74.55 ±0.24	-255
28/2/02	2.1	-27.7	2574 ±29	72.11 ±0.26	-279
11/7/02	33.2	-25.2			
11/7/02	30.9	-25.6			
11/7/02	25.9	-23.6	1178 ±52	85.80 ±0.56	-142
11/7/02	19.3	-25.8	2687 ±27	71.10 ±0.24	-289
11/7/02	15.0	-26.3	2581 ±27	72.05 ±0.23	-280
11/7/02	9.8	-27.3	2438 ±27	73.34 ±0.24	-267
11/7/02	4.7	-27.4	1771 ±25	79.70 ±0.25	-203
11/7/02	0.2	-28.0	754 ±26	90.45 ±0.29	-95
11/7/02	0.2	-28.0			
4/3/03	34.1	-24.9	3944 ±46	60.81 ±0.34	-392
4/3/03	28.7	-25.0	3690 ±33	62.76 ±0.25	-372
4/3/03	19.0	-25.8	3389 ±58	65.15 ±0.47	-349
4/3/03	14.8	-25.8	3170 ±30	66.95 ±0.25	-331
4/3/03	14.1	-25.2	4401 ±36	57.44 ±0.25	-426
4/3/03	10.5	-25.2	3967 ±27	60.63 ±0.19	-394
4/3/03	5.3	-25.3	4298 ±33	58.19 ±0.22	-418
4/3/03	1.3	-25.4	4282 ±33	58.30 ±0.23	-417
4/3/03	0.1	-26.2	3005 ±28	68.34 ±0.23	-317
4/3/03	0.1	-26.4			
23/7/03	32.5	-21.0			
23/7/03	27.1	-23.6			
23/7/03	25.1	-24.8	2403 ±23	73.66 ±0.21	-263
23/7/03	24.2	-25.5			
23/7/03	21.6	-26.6	2457 ±20	73.17 ±0.18	-268
23/7/03	20.6	-25.1	1950 ±20	77.94 ±0.20	-221
23/7/03	20.1	-26.4	2352 ±21	74.14 ±0.19	-259
23/7/03	18.3	-26.1			
23/7/03	16.4	-26.9	1539 ±21	81.86 ±0.19	-181
23/7/03	13.9	-27.3	1936 ±19	78.07 ±0.19	-219

Table 4. Isotopic parameters of water samples from the Tyne Estuary, including $\delta^{13}C$ signatures, radiocarbon ages, pMC (% modern absolute) and $\Delta^{14}C$ signatures of suspended particulate organic carbon (POC).

salinity. With the exception of the high salinity samples (> 30 psu) analysed during the July 2003 surveys to the Tyne (-21.0‰) and Tweed (-22.6 and -22.9‰), most of the $\delta^{13}C$ values indicated a predominantly terrestrial origin for suspended POC, although evidence for mixing with heavier δ^{13}C values of marine OM could also be seen. In winter, δ^{13}C signatures from both the Tyne and Tweed Estuaries were relatively invariant across the freshwater to mid-salinity range (0-30 psu), ranging from -26.9 to -28.1‰ during Feb-02 (Tyne), -26.3 to -25.2‰ during Mar-03 (Tyne), and from -27.6 to -27.9‰ in the Tweed during the Dec-03 survey. This indicates that both these estuaries are dominated by terrestrial POM in winter when the riverine fluxes are high. In contrast, δ^{13}C signatures of POC in summer showed much more gradual and systematic increases from terrestrial to marine values across the salinity gradient, ranging from -28.0 to -23.6‰ during Jul-02 (Tyne), -27.3 to -23.6‰ during Jul-03 (Tyne) and from -26.5 to -23.3‰ during Jul-03 in the Tweed. These gradients imply that during the summer months marine and algal material are proportionately more important within the estuary when terrestrial POM delivery from the river is diminished and algal levels are higher at the mouth of the estuary. Associated with

Site/Date	salinity	$\delta^{13}C_{POC}$ ±0.3‰	$^{14}C_{POC}$ age (yrs BP) ±1σ	$^{14}C_{POC}$ pMC ±1σ	$\Delta^{14}C_{POC}$ ±3‰
Tweed					
8/7/03	33.2	-22.6			
8/7/03	32.4	-22.9			
8/7/03	29.5	-23.7			
8/7/03	27.4	-23.3			
8/7/03	17.9	-25.1			
8/7/03	8.6	-25.5	1062 ±20	87.05 ±0.21	-130
8/7/03	4.2	-26.6	1523 ±26	82.19 ±0.26	-178
8/7/03	1.4	-26.1	1202 ±27	85.54 ±0.29	-145
8/7/03	0.1	-26.5			
8/7/03	0.1	-26.4	1484 ±22	82.59 ±0.23	-174
3/12/03	31.30	-24.6	809 ±26	89.83 ±0.29	-102
3/12/03	19.38				
3/12/03	4.51				
3/12/03	1.90	-27.7			
3/12/03	1.27	-27.7	762 ±21	90.35 ±0.24	-97
3/12/03	0.97	-27.9	611 ±18	92.08 ±0.21	-79
3/12/03	0.90	-27.7	757 ±24	90.41 ±0.27	-96
3/12/03	0.19	-27.6			
3/12/03	0.16	-27.7			
3/12/03	0.12	-27.7	542 ±18	92.86 ±0.21	-71

Table 5. Isotopic parameters of water samples from the Tweed Estuary, including δ^{13}C signatures, radiocarbon ages, pMC (% modern absolute) and Δ^{14}C signatures of suspended particulate organic carbon (POC).

Figure 5a Tyne Feb-02

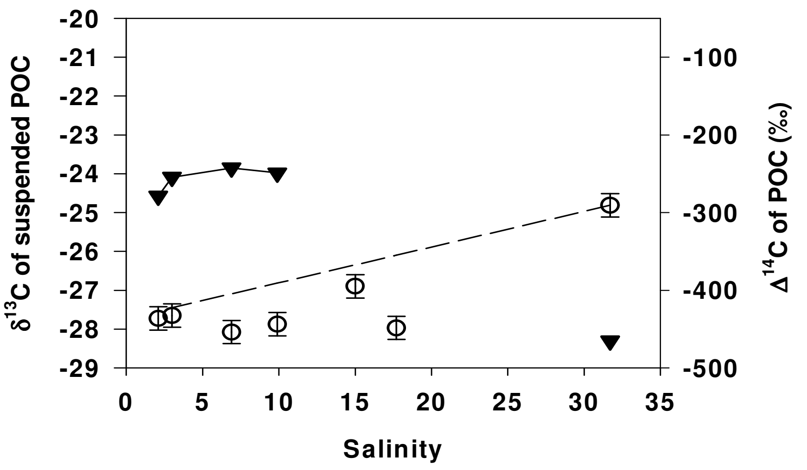

Figure 5b Tyne Jul-02

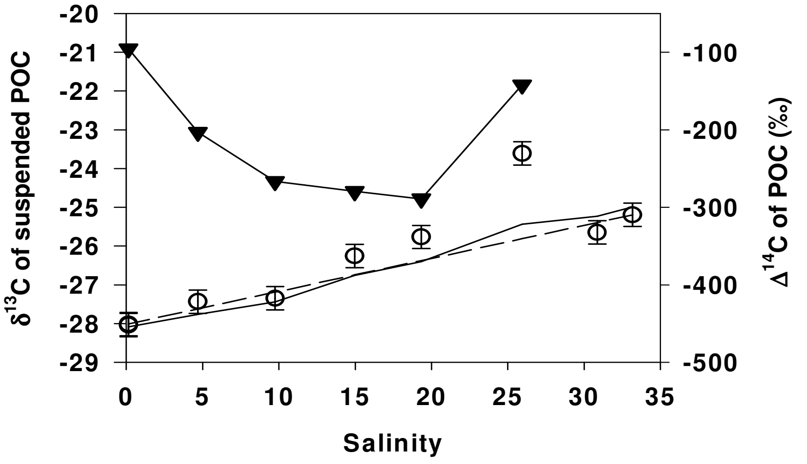

Figure 5c Tyne Mar-03

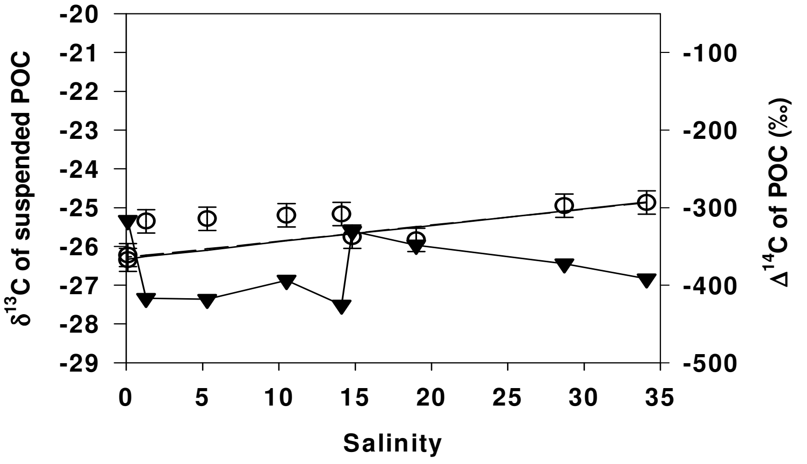

Figure 5. $\delta^{13}C$ (open circles) and $\Delta^{14}C$ (closed diamonds) values of suspended particulate organic carbon (POC) in the Tyne Estuary during (a) Feb-02, (b) Jul-02, (c) Mar-03 and (d) Jul-03, and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. The dashed lines represent a two-component (marine and terrestrial organic matter) $\delta^{13}C$ mixing curve, whereas the solid lines represent a three component (marine, terrestrial and algal organic matter) $\delta^{13}C$ mixing curve (see text).

Figure 5d Tyne Jul-03

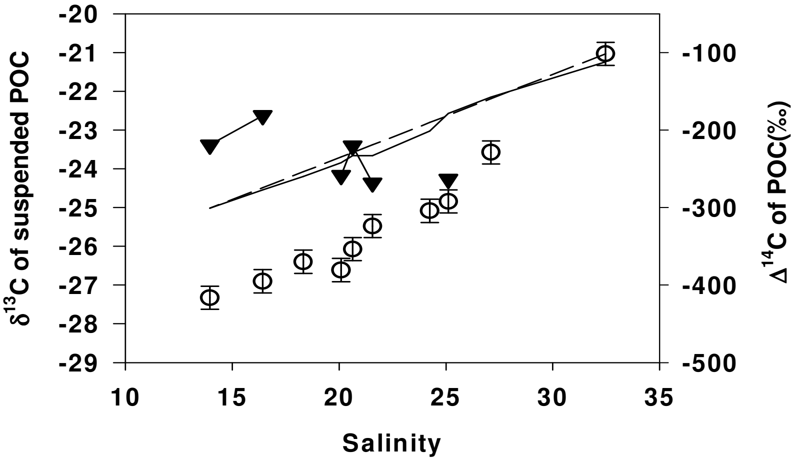

Figure 5e Tweed Jul-03

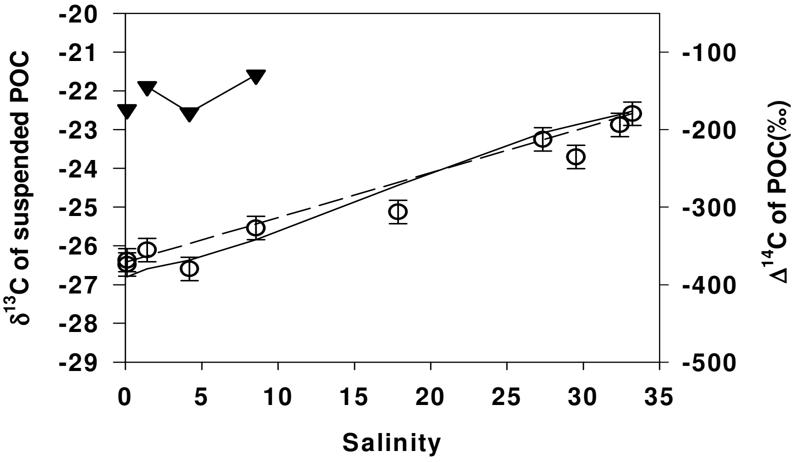

Figure 5f Tweed Dec-03

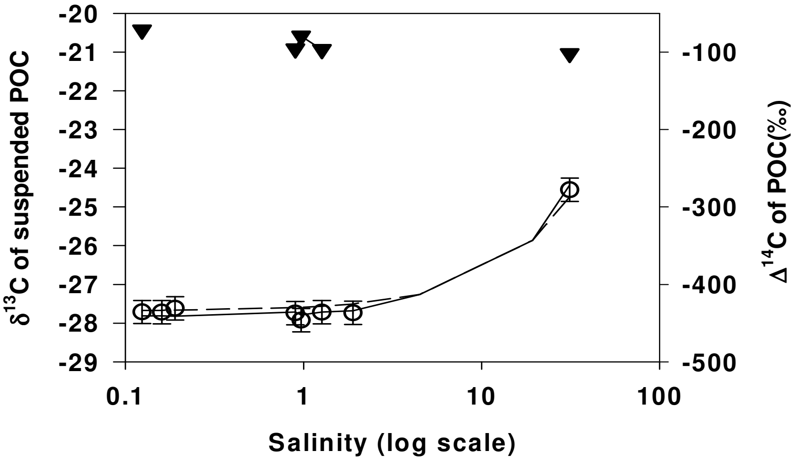

Figure 5. $\delta^{13}C$ (open circles) and $\Delta^{14}C$ (closed diamonds) values of suspended particulate organic carbon (POC) in the Tyne Estuary during (a) Feb-02, (b) Jul-02, (c) Mar-03 and (d) Jul-03, and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. The dashed lines represent a two-component (marine and terrestrial organic matter) $\delta^{13}C$ mixing curve, whereas the solid lines represent a three component (marine, terrestrial and algal organic matter) $\delta^{13}C$ mixing curve (see text).

low river discharges, the highest salinity $\delta^{13}C$ signatures measured in the Tyne (-21.0‰, 32.5 psu) and Tweed (-22.6‰, 33.2 psu) Estuaries during the Jul-03 surveys were the most representative coastal North Sea marine values.

The estuarine trends in $\delta^{13}C$ signatures were evaluated using an isotope mixing model which took into account conservative mixing of marine, terrestrial and estuarine algal POM. The rationale for this model is to assess systematic $\delta^{13}C$ deviations from conservative mixing, which can then be related to estuarine behaviour of various POM components. These three-component isotopic mixing curves were calculated for each survey using salinity, % algal POC and $\delta^{13}C$ values:

$$\delta^{13}C_{Pred} = \delta^{13}C_M f_M + \delta^{13}C_T f_T + \delta^{13}C_A f_A \tag{4}$$

Here $\delta^{13}C_M$, $\delta^{13}C_T$ and $\delta^{13}C_A$ and f_M, f_T and f_A refer to the $\delta^{13}C$ values and fraction of the marine, terrestrial and autochthonous components, respectively, and $\delta^{13}C_{Pred}$ refers to the predicted or calculated $\delta^{13}C$ signature for POC.

Prior to the inclusion of the autochthonous fraction in Eq. 4, the fraction of the marine ($f_{M\text{-prior}}$) and terrestrial ($f_{T\text{-prior}}$) components were determined based on salinity and hence the model assumes conservative mixing between marine and terrestrial POM, where:

$$f_{T\text{-prior}} + f_{M\text{-prior}} = 1 \tag{5}$$

However, because the percentages of algal POC (f_A) were previously determined from chl-a concentrations (Tables 1 and 2; Figure 2) and based on the total POC concentrations, it was necessary to correct the fractions of terrestrial and marine components that were calculated based solely on salinity:

$$f_M = f_{M\text{-prior}} - (f_A \times f_{M\text{-prior}}) \tag{6}$$

$$f_T = f_{T\text{-prior}} - (f_A \times f_{T\text{-prior}}) \tag{7}$$

Owing to the particularly low flow conditions experienced in the Tyne during the July 2003 survey, a freshwater end-member ($\leq$0.2 psu) suspended POC sample could not be obtained. However, it was assumed that the δ^{13}C freshwater end-member from the previous summer, during which time river discharge was only slightly higher (Q = 8.6 versus 7.3 m^3/s), would accurately reflect the riverine δ^{13}C value. In addition, while a precise freshwater end-member was not obtained in the Tyne during Feb-02, because of the relative invariance in δ^{13}C values across the low salinity range it was assumed that a value of -27.7‰ measured at a salinity of 2.1 would be similar to the riverine signature.

By assuming a constant isotopic fractionation between DIC and uptake by riverine and estuarine plankton, $\delta^{13}C_{DIC}$ values (Chapter 2) could be used to estimate the δ^{13}C signature for autochthonous material ($\delta^{13}C_A$) in both the Tyne and Tweed Estuaries. While $\delta^{13}C_{DIC}$ values were only measured in the Tyne and Tweed Estuaries during July 2003, the $\delta^{13}C_{DIC}$ distribution in both estuaries showed similar near linear distributions across the salinity gradient (Chapter 2). Using a value of -20‰ for carbon isotopic fractionation between DIC and plankton (Chanton and Lewis, 1999; Raymond and Bauer, 2001a) and assuming similar $\delta^{13}C_A$ values in both estuaries, we could therefore estimate the average $\delta^{13}C_A$ value expected in three different salinity ranges: 0-10 psu (-29‰), 10-25 psu (-25‰) and 25-35 psu (-22‰).

The mixing curves calculated using Eq. 4 are potted together with δ^{13}C values of suspended POC as straight lines. The dashed lines on Figure 5 represent mixing between marine and terrestrial OM assuming no algal ($\delta^{13}C_Af_A$) component. In general, the differences between these two predicted curves were not very substantial. The similarities between these two lines can be attributed to the fact that autochthonous organic matter comprised a relatively minor fraction of the POC pool in both estuaries (Figure 2). The general close agreement between these two models can also be attributed to some overlap between estimated $\delta^{13}C_A$ values with $\delta^{13}C_{Pred}$ values calculated by simple two-end member mixing between marine and terrestrial components.

In the Tyne during Feb-02 (Figure 5a), the measured δ^{13}C values for suspended POC were generally more ^{13}C-depleted than the mixing curve (assuming 0% algal POC). This survey was conducted following a storm event under high river flow when the estuary was dominated by freshwater input. The sharp salinity gradient near the mouth of the estuary during this period resembled a freshwater plume separated from marine waters offshore. Under such conditions there was very little opportunity for mixing between marine and terrestrial OM pools. This was reflected in $\delta^{13}C$ values where terrigenous OM dominated most of the estuary while at the seaward side of the freshwater front there was a much heavier marine OM $\delta^{13}C$ signal. During Jul-02 (Figure 5b) most of the measured $\delta^{13}C$ signatures obey both the algal and non-algal mixing lines. One notable exception, however, was the sample collected at a salinity of 25.9 ($\delta^{13}C$ = -23.6 ‰). As illustrated in Figure 2b, this sample had a relatively high chl-a concentration, which was reflected in the elevated % algal POC (13.5%) compared to the others. However, the relatively high C/N ratio of this sample (20.8) implied a limited contribution from algal material. Therefore, since this data point could not be explained by the three component isotopic mixing line, we must assume that there was an additional, non-algal anthropogenic input. During Mar-03 (Figure 5c), the measured $\delta^{13}C$ signatures indicated a slight ^{13}C-enrichment in the area corresponding to the MTZ (Figure 2c), which during this period was situated roughly between a distance of 16-25 km from the North Sea. As illustrated in Figure 4a, the $\delta^{13}C$ signatures of sedimentary POC within this part of the estuary varied between a fairly narrow range of values (-25.4 to -24.3 ‰, average = -24.8‰, n = 8). Therefore, it was likely that this non-conservative increase in suspended POC $\delta^{13}C$ in the MTZ was the result of mixing with isotopically heavier sediment. It should be noted that this survey exhibited a well developed MTZ (Figure 2c) with some of the highest SPM levels recorded in the Tyne estuary over the four transects.

During July 2003 (Figure 5d) the predicted $\delta^{13}C$ values are much lighter than those measured in the field. The use of an erroneous terrestrial end-member value ($\delta^{13}C_T$ = -28.0‰) would help to explain the generally more ^{13}C-enriched $\delta^{13}C_{Pred1}$ estimated for the Tyne during July 2003. For instance, if a terrestrial end-member of ~-34‰ is used the measured $\delta^{13}C$ signatures show and excellent correlation with $\delta^{13}C_{Pred1}$values. However, the most ^{13}C-depleted freshwater POC signature observed in the Tyne was one used in Eq. 1 (-28.0‰, July 2002). Since we can rule out mixing with sedimentary POC, which were > -27‰ (Figure 5a), a more feasible explanation for the relatively ^{13}C-depleted signatures may be attributed to the hydrodynamic conditions in the Tyne Estuary during this period caused by the combined effects of low flow and spring tide. Since North Sea POC levels during this period were relatively minor, then a low river discharge and high spring tide event may have lead to the accumulation of terrigenous material at higher than expected salinities, as evidenced by the high salinity (13.9 psu) measured in the normally freshwater region of the river. However, it also possible that these relatively ^{13}C-depleted signatures were caused by an increased contribution from sewage-derived OM across the estuary, which generally possess more negative δ^{13}C signatures (Tucker et al., 1999; Wang et al., 2004). And as previously mentioned, the relatively low C/N ratios of POM measured during this survey (Figure 3a) also point to a potential sewage contribution.

As illustrated in Figures 5e and 5f, Eq. 4 satisfactory describes the relative POC contributions in the Tweed Estuary during both July 2003 and December 2003 surveys. Due to the relatively low and consistent % algal POC values, these mixing curves also adequately describe POC behaviour in the Tweed when the algal contribution is eliminated from Eq. 4. The lack of a pronounced MTZ and no discernible anthropogenic inputs are two other factors which would lead to a better predicative capability for Eq. 4 in the Tweed. Owing to similar high river discharge conditions, one might have expected to find a similar behaviour for δ^{13}C signatures of POC in the Tweed during the winter. However, since POM samples were limited to salinities <2 and the marine-end-member the predominance of terrigenous material in this estuary during the Dec-03 survey could not be assessed.

The isotopic mixing models utilized here provided a useful tool in the delineation of potential POC sources within the Tyne and Tweed Estuaries. The relatively shorter freshwater flushing time in the Tweed resulted in a better predictive capability for this estuary, since additional estuarine inputs (i.e., phytoplankton, resuspended sediment, sewage-derived OM) were minimal compared to the Tyne. Within the context of the information pertaining to the sources and biogeochemical of particulate organic carbon obtained thus far, the behaviour of Δ^{14}C signatures for both surface sediments (Tyne) and suspended POC (Tyne and Tweed) will now be evaluated.

Behaviour of $\Delta^{14}C$ signatures of suspended POC and surface sediments

$\Delta^{14}C$ signatures of suspended POC across the Tyne (Table 4) and Tweed (Table 5) Estuaries are plotted together with variations in POC % and % algal POC in Figure 3 and with $\delta^{13}C$ values in Figure 5, and in Figure 6 are plotted versus C/N

Figure 6a

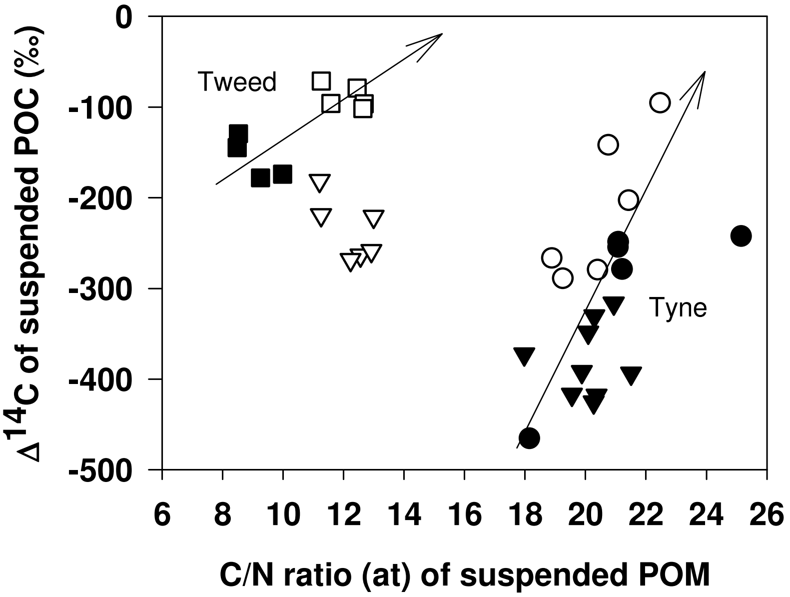

Figure 6b

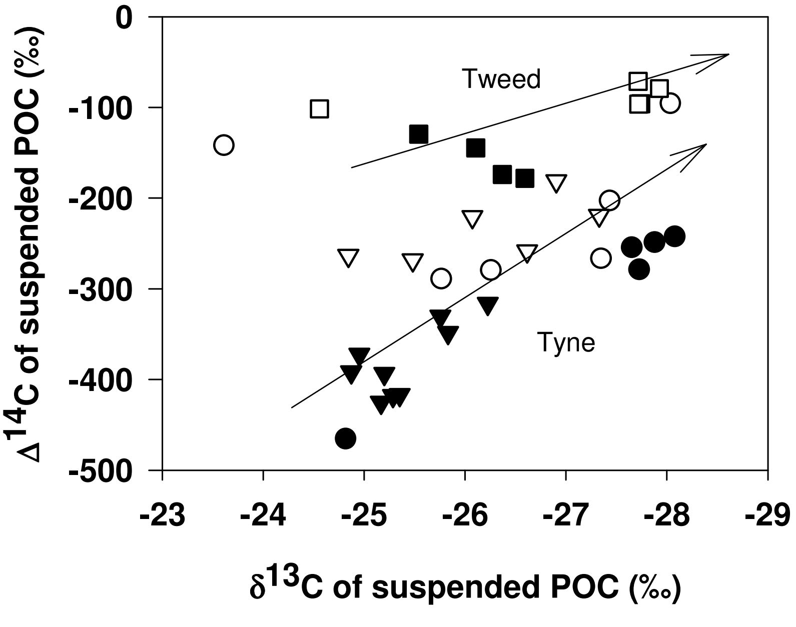

Figure 6. $\Delta^{14}C$ values of suspended POC in the Tyne Estuary during Feb-02 (closed circles), Jul-02 (open circles), Mar-03 (closed inverted triangles) and Jul-03 (open inverted triangles) and in the Tweed Estuary during Jul-03 (closed squares) and Dec-03 (open squares) plotted versus (a) C/N ratio and (b) $\delta^{13}C$ signature.

and δ^{13}C. In general, Δ^{14}C values were more depleted in the Tyne compared to the Tweed, indicating an older age for POC (see Tables 4 and 5 for corresponding ages and pMC values). Δ^{14}C values ranged from -465 to -243‰ (Tyne, Feb-02), -289 to -95.5‰ (Tyne, Jul-02), -317 to -426‰ (Tyne, Mar-03), -181 to -268‰ (Tyne, Jul-03), -130 to -178‰ (Tweed, Jul-03) and -71 to -97‰ (Tweed, Dec-03). The relatively old, ^{14}C-depleted (~750 to 4000 years BP) ages for estuarine POC measured here are not unexpected (Megens et al., 2002; Raymond and Bauer, 2001b), and are similar to those found for peat and riparian soils in NE Scotland (Palmer et al., 2001). As demonstrated by the arrows plotted on Figure 6, in general there was an overall trend of progressively heavier Δ^{14}C values with higher C/N ratios and more depleted δ^{13}C signature in both estuaries.

In the Tweed Estuary (Figures 2e, 2f, 5e, 5f) a limited number of ^{14}C dates that were mostly restricted to salinities < 10 psu precluded a thorough assessment of changes in age during estuarine mixing. However, the concentration weighted average Δ^{14}C values of POC for July 2003 (-155 ±20.3‰) and December 2003 (-87 ±12‰) demonstrated a pronounced difference between seasons. These seasonal changes in Δ^{14}C values coincided with changes in the C/N (Figure 6a) and δ^{13}C (Figures 6b) ratios between the two surveys, which suggested that in the summer suspended POC in the Tweed was comprised predominantly of older, more degraded soil-derived OM, whereas in the winter a higher contribution of C_3 plant debris was being discharged into the North Sea during this period of enhanced river discharge. Furthermore, since the estimated % algal POC contents were similar during both seasons (~10-14%), this confirms that changes in soil characteristics was the main controlling factor in influencing the seasonal differences in POM. These findings therefore show that seasonal and hydrological conditions play an important role in determining the age and lability of riverine POM which is transported to the coastal North Sea.

In the Tyne, Δ^{14}C values for suspended POC did not show the same seasonal trends as observed in the Tweed. The most ^{14}C-enriched (i.e., youngest) riverine end-member was measured during Jul-02 (Δ^{14}C = -95‰, 754 years BP, Figure 2b, 5b), whereas the oldest riverine end-member was found during the Mar-03 survey (Δ^{14}C = -317‰, 3005 years BP, Figure 2c, 5c). These differences may be partly attributed to dissimilarities in the underlying geology of the two watersheds. As opposed to the underlying geology of the Tweed drainage basin, coal-rich sedimentary rocks are found throughout the eastern section of the Tyne watershed. And while its importance has diminished in recent decades, coal-mining activity in this region of northeastern England has been taking place for hundreds of years, likely resulting in relatively high levels of buried coal particles in estuarine sediments. In order to qualitatively check for the presence of coal particles in SPM measured in the Tyne Estuary, backscatter scanning electron microscopy (SEM) was performed on nucleopore filters filtered with water collected during these surveys (Appendix 4). Using this technique, coal particles could be recognised by their distinct, jagged shape and dark colour. The presence of coal particles in Tyne SPM (but not in Tweed SPM) therefore confirmed the possibility that the older ages for suspended POC found in the Tyne reflect a higher component of ^{14}C-depleted sedimentary fossil carbon. Nonetheless, it is not likely that a contribution from sedimentary organic carbon was the only factor in controlling the age of POC in the Tyne Estuary.

During the Feb-02 survey (Figure 2a, 5a), when there was likely no significant mixing with resuspended sedimentary material during this period of high river discharge, $\Delta^{14}C$ signatures showed less variation across the low- to mid-salinity range than during the other surveys. This implies that during high river flow the Tyne Estuary acts as a conduit for the delivery of old, terrigenous OM to the North Sea. In addition to the presence of ^{14}C-depleted sedimentary fossil carbon, the older ages for POC in the Tyne compared to the Tweed during high river flow / winter conditions are likely indicative of different terrigenous source material characteristics for each river system, with a higher component of older, peat-derived OM found in the Tyne.

During the Jul-02 (Figure 2b, 5b) and Mar-03 (Figure 2c, 5c) surveys, when river discharge was lower and there were more pronounced MTZ regions, there was much more variability in $\Delta^{14}C$ values across the estuary. In both these surveys an initial decrease in POC % in the low-salinity region coincided with a decrease in $\Delta^{14}C$ (i.e., an increase in age). For instance, during Jul-02 the $\Delta^{14}C$ signature of POC decreased (i.e., the age increased) from a freshwater end-member value of -95‰ in a near linear manner to a value of -289‰ at a salinity of 19.3, and during Mar-03 the $\Delta^{14}C$ signature showed an initial decrease from -317‰ to -417‰. During both the Jul-02 and Mar-03 surveys to the Tyne Estuary $\Delta^{14}C$ values for suspended POC also showed subsequent increases at higher salinities, increasing to -142 ‰ at 25.9 psu

(Jul-02) and to -331‰ at 14.8 psu (Mar-03). As described earlier, the peak in % algal POC and δ^{13}C observed at a salinity of 25.9 during Jul-02 cannot be attributed solely to an increase in phytoplankton biomass. It is likely that these elevated Δ^{14}C values found at lower salinities were caused by mixing with a relatively more ^{14}C-enriched anthropogenic OM source originating from a number of potential sites within the estuary, such as the Howdon sewage works or the Ouseburn, a heavily urbanised and polluted tributary of the Tyne which converges with the main river approximately 10 km inland from the North Sea. In addition to the most pronounced peak in % algal POC observed during the Jul-02 survey, another smaller increase was found at a salinity of 9.8 psu. In contrast to the sample collected at 25.9 psu, the % algal POC peak at 9.8 psu did not correspond with a slightly more elevated Δ^{14}C signature. However, since this sample was collected in a relatively more turbid part of the estuary, it is likely that dilution with older, more ^{14}C-depleted sediment was masking the Δ^{14}C value associated with the elevated algal contribution.

The highest estimated percentages of algal POC (up to 21.2%) estimated in the Tyne Estuary were found during Jul-03 (Figure 2d), when river discharge was lowest and the entire length of the tidal estuary was dominated by salinities >13 psu. Despite the relatively high % algal POC and relatively low C/N ratios (Figure 3a), however, Δ^{14}C values across the mid-salinity region were not much heavier than those measured during the Feb-02 transect, when algal contributions were considered to be negligible. It is possible that the age of terrigenous POC was even older during Jul-03 and that more ^{14}C-enriched algal contributions acted to “balance” the Δ^{14}C signature. However, as indicated by lower than predicted δ^{13}C signatures and lack of correlation with the % algal POC mixing curve (Figure 5d), the relatively ^{14}C-depleted values for POC corroborate the suggestion that suspended POC during this survey was comprised predominantly of terrigenous and/or sewage-derived material. The small variations in Δ^{14}C values across the estuary can be attributed partly to varying proportions of algal-, terrestrial, and sewage-derived OM. Yet in contrast to the Jul-02 survey, a mid-estuarine peak in % algal POC did not correspond with an elevated Δ^{14}C signature. In fact, the lowest % algal POC measured during this transect (5.4%, 20.6 psu) was associated with a slight increase in Δ^{14}C, suggesting a relatively ^{14}C-enriched anthropogenic source. These types of discrepancies illustrate the difficulties in interpreting variations in POC radiocarbon ages across an estuarine salinity gradient.

One explanation for the trends indicating decreases in POC % with a corresponding increase in age in the upper estuary observed during Jul-02 and Mar-03 can be attributed to the mixing of resuspended surface sediments with lower POC contents and older POC ages, as suggested by a correlation between SPM levels and slightly enriched $\delta^{13}C$ signatures for POC during the Mar-03 survey (Figures 2c, 5c). In Figure 7a the $\Delta^{14}C$ values of surface sediments are plotted versus distance from the North Sea. As this graph illustrates, $\Delta^{14}C$ values of surface sediments become more ^{14}C-depleted (i.e., get older) towards the lower part of the estuary. In addition, the $\Delta^{14}C$ values of surface sediments were in general much more negative than those of suspended POC, ranging between -367 to -801‰ (Table 3). The average age of the sediment in the sampling location closest to the North Sea was ~11,000 years BP, which is older than what one would expect if peat-derived carbon were the sole component. As shown in Figure 7b, this downriver increase in age also corresponded with an increase in $\delta^{13}C$ signature ($r^2 = 0.89$, $n = 8$). Therefore, while $\delta^{13}C$ signatures ostensibly showed a general mixing trend between marine and terrigenous OM (Figure 4a), it is likely that the age of surface sediments was influenced by mixing with ^{14}C-depleted material from the lower part of the estuary, since phytoplankton-derived marine OM should be derived from a relatively more modern carbon source (Bauer et al., 2002; Megens et al., 2001). In addition to a sedimentary fossil carbon source, another possibility for ^{14}C-free material in Tyne sediments may be attributed to contamination for petroleum products. A study by Matthiessen et al. (1998) has shown that sediment throughout the Tyne Estuary is heavily contaminated with weathered and degraded oil, with total hydrocarbon concentrations ranging between 2000 to 6000 μg/g dry weight. This contamination signal is also supported by the relatively high surface sediment C/N ratios found in this part of the estuary (Figure 4b), which showed a trend of increasing C/N ratio corresponding with an increase in age (Figure 7c; $r^2 = 0.40$, $n = 8$).

Contamination from coal particles and hydrocarbons may not be the only explanation for the older ages for both surface sediments and POC observed in the Tyne estuary. In a study by Megens et al (2001), bulk POM samples collected from the Dutch coastal waters showed a distinct seasonal contrast, demonstrating more

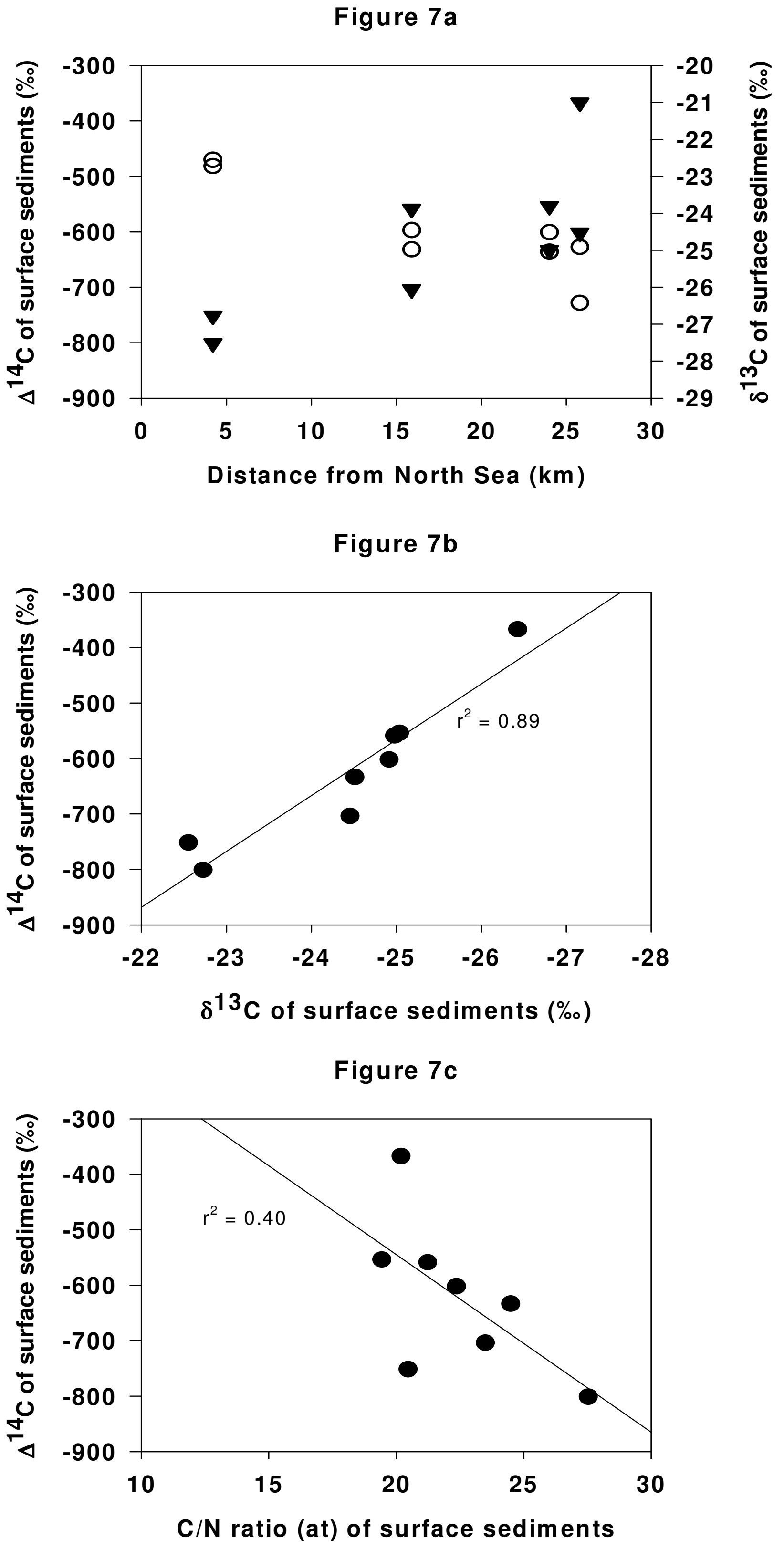

Figure 7. (a) δ^{13}C (open circles) and Δ^{14}C (closed inverted triangles) values of surface sediments in the Tyne Estuary collected during Jul-02 and Feb-03 plotted versus distance from the North Sea, (b) Δ^{14}C values of surface sediments plotted versus δ^{13}C signatures, and (c) Δ^{14}C values of surface sediments plotted C/N ratios.

^{14}C-depleted signatures in winter and autumn compared with spring and summer. These seasonal differences were attributed to lower primary productivity and a greater contribution from resuspended surface sediment during the winter. In the Megens et al. study (2001), the most ^{14}C-depleted sample (February, Δ^{14}C = -120‰) also possessed the most ^{13}C-depleted signatures (-23.2‰). Therefore, it is likely that the older ages for surface sediment and suspended POC found in the lower part of the Tyne Estuary reflect contributions from both coal particle and hydrocarbon contamination and resuspended coastal North Sea sediments, as evidenced by the relatively high C/N ratio (18.1), ^{13}C-enriched δ^{13}C value (-24.8‰) and ^{14}C-depleted Δ^{14}C signature (-465‰) found at the mouth of the Tyne Estuary during the Feb-02 survey (Figure 2a, 5a). Further evidence for this can be seen in the overall relationship between δ^{13}C and Δ^{14}C values for suspended POC (Figure 6b), which was similar to that observed for surface sediments (Figure 7b). In the Tweed, the marine-end-member collected during Dec-03 (31.30 psu) was the most ^{14}C-depleted POC sample from this period (-102‰), suggesting a contribution from older resuspended North Sea sediments. However, in comparison to Tyne POC and sediment samples, its younger age confirms the minor contribution from fossil carbon within this estuary.

While mixing with ^{14}C-depleted material may explain some of the variation in Δ^{14}C signatures found in Tyne Estuary, another possibility for the trends indicating decreases in POC % with a corresponding increase in age in the upper estuary observed during Jul-02 and Mar-03 may be due to the preferential removal of a younger, more labile fraction of POC. This removal would thus result in a more degraded, older substrate being exported to the North Sea. Within the Tyne Estuary the %C measured in surface sediments generally ranged between 6 to 9% (Table 6), with an average of value of 7.9 ±2.1% (n = 27) for the samples reported here. The POC % in SPM during the four surveys reported here were often much lower than this value (Figures 2a-d), and in Jul-02 and Mar-03 showed significant decreases within the MTZ. For instance, during Mar-03, when SPM concentrations were highest (Figure 2c) the POC % within the low salinity region decreased from a freshwater value of 10.4 ±0.3 (n = 2) to lows of 2.5 and 2.9 at salinities of 5.3 and 10.5, respectively. Therefore, these decreases in POC% in the MTZ (and the corresponding increases in POC age) cannot be attributed solely to mixing with older, relatively more carbon-poor surface sediment. The same is also true for the Jul-03 transect, when the POC % between salinities of 10-25 ranged from 3.5 to 4.1%. Furthermore, in contrast to the trends observed in surface sediments, C/N ratios for suspended POC in the Tyne showed an overall corresponding increase with younger, more ^{14}C-enriched Δ^{14}C signatures (Figure 6a). This trend therefore indicates a lower level of fossil carbon contamination for terrigenous POC entering the Tyne Estuary during the sampling periods, and also illustrates the relatively small contribution of riverine algal material to the terrestrial particulate pool

CONCLUSIONS

In both the Tyne and Tweed Estuaries there was a higher influx of terrigenous POC in the winter during periods of elevated river discharge. Higher C/N ratios in the Tyne indicated a greater proportion of terrestrial plant debris and humic material in riverine POM, whereas in the Tweed the lower C/N ratios were attributed to a predominantly agricultural soil source. Changes in POC% (% of SPM) across the salinity gradient were used to estimate the percentage of riverine POC mineralised, which ranged from 38-76% and 31-39% in the Tyne and Tweed, respectively. These estimations suggested that a significant fraction of terrestrial OM is relatively labile and is oxidised in the estuarine and coastal zone.

A three-component isotopic (δ^{13}C) mixing model for suspended POC incorporating terrigenous, autochthonous and marine sources adequately described the behaviour of δ^{13}C values in the Tweed but not in the Tyne. During the Feb-02 and Jul-03 surveys to the Tyne, relatively ^{13}C-depleted signatures for measured versus predicted δ^{13}C values were attributed to hydrodynamic effects caused by high river discharge (Feb-02) and a combined spring tide / low flow event (July 2003). In Feb-02, the relatively ^{13}C-depleted signatures coincided with high C/N ratios and Δ^{14}C values that indicated a riverine flushing of old, peat-derived OM. In the Tyne during summer lower C/N ratios (Jul-03) and relatively higher % algal POC estimates (Jul-02 and Jul-03) indicated a slightly more important POM contribution from estuarine or marine phytoplankton. However, relatively ^{13}C- and ^{14}C-depleted values for POC pointed to a mostly terrestrial and/or anthropogenic origin. During Mar-03, relatively ^{13}C-enriched δ^{13}C values for POC in the MTZ of the Tyne Estuary were attributed to mixing with isotopically heavier sediment.

This study has demonstrated large variations in the ages of suspended POC during estuarine mixing. In the Tweed, there was a noticeable seasonal trend in the age of POC exported to the North Sea, with more ^{14}C-depleted values found in the summer compared to winter. This large difference in the age of low-salinity POC in the Tweed (~650 years) between winter and summer was attributed to seasonal differences in soil characteristics. Considering the large differences in POC flux between the Jul-03 (2.0×10^3 mol/hr) and Dec-03 (6.7×10^4 mol/hr) surveys, the results from this study therefore suggest that high river discharges associated with increased winter runoff can lead to the flushing of younger, relatively more labile POM into the North Sea. In the Tyne during Jul-02 and Mar-03 decreases in POC % in the upper part of the Tyne Estuary corresponded with an increase in POC age. This trend of increasing age was associated with an increase in SPM levels, pointing to mixing with older, more ^{14}C-depleted surface sediment that contained higher levels of sedimentary fossil carbon and/or petroleum product contamination. However, it is also possible that the preferential removal of younger, more labile components of the terrigenous POC played an important role in controlling the age of POC in the Tyne Estuary, as postulated by Raymond and Bauer (2001). Unfortunately, this study was not able to clearly elucidate between these two processes. This research therefore demonstrates that interpreting variations in the age of POC during estuarine mixing becomes rather complicated in heavily populated and urbanised systems, and in geologic settings that can potentially act as a significant source of sedimentary fossil carbon (e.g., coal). However, as illustrated in the Tweed, in estuaries with shorter residence times and a smaller level of urbanisation, Δ^{14}C values of POC can be used much more successfully to evaluate seasonal changes in the source and age of terrigenous POM delivered to the world's oceans.

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CHAPTER 5: Evaluating the sources and biogeochemical cycling of organic nitrogen in two temperate North Sea estuaries using stable isotopes

ABSTRACT

Concentrations, C/N ratios and nitrogen isotope ratios ($\delta^{15}N$) of high molecular weight dissolved organic nitrogen (HMW DON) and suspended particulate organic nitrogen (PON) from the Tyne and Tweed Estuaries, NE England, were analysed during winter and summer periods. In the Tweed, agricultural soil-derived sources in the summer dominated the estuary as evidenced by relatively enriched $\delta^{15}N$ and lower C/N ratios for both particulate organic matter (POM) and HMW dissolved organic matter (DOM) pools, whereas in the winter the admixture of fresher surface derived plant debris produced slightly lower $\delta^{15}N$ and higher C/N ratios. A decent relationship between $\delta^{15}N$ signatures of PON and HMW DON ($r^2 = 0.61$, n = 18) confirmed that soil-derived material was the chief source for organic nitrogen in this estuary. In the Tyne the trends were much more variable. During the winter relatively invariant C/N and $\delta^{15}N$ ratios for POM found across the estuary demonstrated that the bulk of the PON pool was comprised predominantly of peat-derived OM. However, due to either the direct or indirect influence of sewage inputs $\delta^{15}N$ values for PON in the summer were generally more ^{15}N-depleted, as were $\delta^{15}N$ values for HMW DON during both seasons. A very poor relationship between $\delta^{15}N_{PON}$ and $\delta^{15}N_{HDON}$ signatures ($r^2 = 0.02$, n = 22) confirmed that in the Tyne, in addition to terrestrial organic matter sources, estuarial inputs and biogeochemical processing played a more important role in the cycling of HMW DON.

INTRODUCTION

Estuarine environments play an important role in regulating terrestrial material entering marine systems. While estuaries can act as conduits for the delivery of terrestrial organic matter and nutrients to the world's oceans (Hedges and Keil, 1995; Meybeck, 1982), estuarine mixing removes, alters, transforms and contributes to these significant carbon and nitrogen pools. A burgeoning human population has likewise led to a corresponding increase in the amount of terrestrial-derived nitrogen that enters rivers and estuaries, and this trend is expected to continue in the near future (Galloway et al., 1995). Understanding the main controls on the biogeochemical processes active within estuaries is therefore essential in determining the extent to which these systems regulate and modify organic nitrogen inputs to marine systems.

Nitrogen isotope signatures ($\delta^{15}N$) can be a useful tool for examining the sources and biogeochemical cycling of organic nitrogen during estuarine mixing. With regards to the particulate organic nitrogen (PON) pool, which is generally defined as the material retained on a GF/F (~0.7 μm nominal pore size) glass fibre filter, nitrogen isotope analysis has now become a routine procedure carried out during these types of investigations. Traditionally, estuarine PON has been characterised by increasing $\delta^{15}N$ values seaward, due to mixing between terrestrial and marine sources, with the latter generally possessing more enriched $\delta^{15}N$ signatures. For instance, in a study carried out in the Scheldt Estuary, Mariotti et al (1984) reported that in the winter suspended organic matter is a mixture of two components: a continental detrital component characterised by a low $\delta^{15}N$ signature of 1.5‰ and a marine component with a mean $\delta^{15}N$ value of 8‰. In the warmer months, from May to October, intensive primary production led to an additional source of organic material characterised by enriched $\delta^{15}N$ values (maximum 24‰) that reflected the isotopic composition of the ammonia assimilated by phytoplankton. However, in cases where nitrogen is not limiting to primary production, isotopically light dissolved inorganic nitrogen (DIN) should be preferentially utilised during uptake and assimiliation (Altabet et al., 1991; Altabet and McCarthy, 1985; Saino and Hattori, 1980). As a result of this isotope fractionation, $\delta^{15}N_{PON}$ signatures influenced by phytoplankton should be less than or equal to the DIN source (Mariotti et al., 1984; McCusker et al., 1999; Ostrom et al., 1997).

In relatively polluted estuaries and coastal regions anthropogenic inputs can also influence the $\delta^{15}N$ signature of PON. $\delta^{15}N_{PON}$ signatures for sewage-derived organic matter (which is terrestrial in origin) are generally isotopicallly light compared to marine signatures (Tucker et al., 1999). However, increased biological productivity caused by this nutrient rich waste can mask the initial signal (Thornton and McManus, 1994). Depending on the dominant biogeochemical processes and the isotopic signatures of the DIN precursors, this can result in either relatively lighter or heavier $\delta^{15}N_{PON}$ signatures. For example, the comparatively ^{15}N-enriched $\delta^{15}N_{PON}$

signatures (12-14‰) found in the polluted Oder River (Baltic Sea) by Voss and Struck (1997) were attributed to algal uptake and fractionation of isotopically heavy nitrate. Conversely, Tucker et al. (1999) were able to track the relatively light $\delta^{15}N_{PON}$ value measured in sewage-derived effluent away from its source and seaward across Boston Harbour and Massachusetts Bay. Clearly, the effects of anthropogenic inputs can greatly complicate the interpretation of $\delta^{15}N_{PON}$ signatures in estuarine environments.

In contrast to the PON pool, relatively few studies have thus far have examined the isotopic behaviour of dissolved organic nitrogen (DON) within estuarine systems. Studies have shown that DON, which is conventionally defined as the fraction of dissolved matter containing nitrogen that is not retained by a GF/F filter, accounts for most of the organic nitrogen in surface ocean water (Abell et al., 2000; Libby and Wheeler, 1997). In addition, DON is a major component of riverine N, sometimes exceeding DIN by a factor of 2 (Meybeck, 1993). Nitrogen isotope analysis of DON in marine and estuarine environments has been typically hindered by generally low concentrations and a lack of suitable sampling techniques capable of concentrating the DON into an amount necessary to obtain accurate $\delta^{15}N$ values. However, tangential-flow ultrafiltration (UF) has provided a relatively efficient and reliable method for the isolation of high molecular weight dissolved organic nitrogen (HMW DON) for biomolecular and isotopic analysis (Benner et al., 1997; Guo et al., 2003; Wang et al., 2004). Using this technique, Benner et al. (1997) reported a range of +6.6 to +10.2‰ for $\delta^{15}N$ values of HMW DON ($\delta^{15}N_{HDON}$) from the Atlantic and Pacific Oceans and the Gulf of Mexico. Relatively large changes in the isotopic composition of HMW DON within the same geographic region were used to infer a rapidly cycling component of DON, whereby a substantial portion of DIN taken up by phytoplankton is rapidly released as DON (Bronk et al., 1994). This phytoplankton-derived DON is subsequently utilised by heterotrophic bacteria, thus completing the cycle. In a study by Guo et al. (2003), $\delta^{15}N_{HDON}$ signatures from Chesapeake Bay, the Middle Atlantic Bight, Galveston Bay and the Gulf of Mexico ranged between +3.2 to +9.5‰ and displayed a convex pattern across the salinity gradient, first increasing with salinity until approximately 20 psu, then decreasing with increasing salinity in the coastal waters. This distribution of $\delta^{15}N_{HDON}$ values showed that instead of simply being a mixture between terrestrial and marine sources, DON was undergoing significant microbial processing during estuarine mixing. Another study (Wang et al., 2004) has reported a range of +2.8 to +8.9‰ for $\delta^{15}N_{HDON}$ values from four different US estuaries (San Francisco Bay, Boston Harbour/Massachusetts Bay, San Diego Bay, Chesapeake/Delaware Bays). Slightly lower $\delta^{15}N_{HDON}$ values were attributed to a greater contribution from terrestrial sources, which at many sampling locations was associated with sewage inputs. Relatively higher $\delta^{15}N_{HDON}$ values were attributed to a greater contribution of marine material. However, it was also suggested that variations in $\delta^{15}N_{HDON}$ signatures were the consequence of *in situ* production of HMW DON.

The three studies mentioned above highlight the inherent complexity associated with biogeochemical of nitrogen in estuarine environments. As a consequence, the relationships between $\delta^{15}N$ signatures of PON and HMW DON have not been clearly established. In the Benner et al. study (1997), while $\delta^{15}N$ signatures for HMW DON showed the same trends as those for ultrafiltered PON (UPON; 0.1-60 μm), $\delta^{15}N$ values for UPON were consistently heavier than $\delta^{15}N_{HDON}$ values collected at the same station, having an offset of approximately 2.8 to 6.9‰. Since HMW DON showed less isotopic variability than UPON, it was also suggested that the smaller PON reservoir is more dynamic than the larger DON reservoir.

Considering the relatively limited data available in the literature, one of the main goals of this study was to evaluate the isotopic behaviour of HMW DON during estuarine mixing. In addition, in order to ensure that a significant fraction of the total organic nitrogen pool had been examined, the behaviour of PON was also studied. Utilising a combination of concentrations, C/N ratios and $\delta^{15}N$ values, it was hoped that both sources and biogeochemical processes could be delineated. Two geographically close yet fundamentally contrasting temperate estuaries were chosen as study areas (Tyne and Tweed Estuaries, NE UK). This was done to assess potential variability in organic nitrogen sources and cycling dynamics caused by differences in aquatic ecology, water chemistry, anthropogenic perturbations, and watershed land-use characteristics. Within each particular estuary, samples were also collected during different seasonal and hydrological conditions.

MATERIALS AND METHODS

Description of Study Sites

Tyne Estuary

The River Tyne, which flows through the densely populated city of Newcastle, has a total drainage area of approximately 2900 km^2 and an average freshwater flow of ~48 m^3/s. Its two main tributaries are the North Tyne, which receives humic-rich waters draining areas of blanket peat afforestation, and the South Tyne, which drains relatively pristine moorland (Baker and Spencer, 2004). The North and South tributaries converge downstream to form the River Tyne, which supplies more than 90% of the total river discharge into the Tyne Estuary (Figure 1; Chapter 1). Most of the remaining freshwater input is derived from the River Derwent. The maximum extent of the tidal estuary is approximately 33 km inland from the North Sea, and the residence time is approximately 5-20 days (A. P. Stubbins, private communication). The Tyne is a partially mixed mesotidal estuary (Baker and Spencer, 2004). Although industrial fluxes to the lower part of the estuary are in decline, it continues to receive significant amounts of urban waste, particularly from sewage treatment facilities located at Howdon (one of the UK's largest estuarial secondary treatment facilities) in the lower estuary (Baker and Spencer, 2004; Upstill-Goddard et al., 2000). However, the water quality in the Tyne upstream of Newcastle has improved significantly over the past few decades.

SPM (suspended particulate matter) levels in the Tyne are generally <130 mg/L and show trends of decreasing concentrations with increasing salinities, with a noticeable maximum turbidity zone (MTZ) often located in the low to mid salinity range (Kitidis, 2002). The Tyne Estuary is considered to be a net heterotrophic system during late winter (February, early March) and mid-summer (July), with relatively low chlorophyll a concentrations (≤ 4.3 µg/L) in both seasons signifying that authochtonous material comprises a relatively minor component of the organic matter (Chapters 1-4). Soils in the catchment are dominated by large areas of peat in the uplands, which provides a substantial store of organic carbon and with stagno-gleys in the majority of the remaining areas (Baker and Spencer, 2004). Generally the soils are slow draining and underlain by shallow or low permeability aquifers, leading to the hydrology of many sub-catchments to be dominated by surface runoff with rapid response to rainfall as a result of saturation excess (Baker and Spencer, 2004).

Tweed Estuary

The Tweed River and its tributaries drain a rural, relatively sparsely populated region located in the border region between England and Scotland. Most of the Tweed's discharge originates from the main river, although approximately 10% of the freshwater input comes from the Whiteadder, which joins the Tweed approximately 6.5 km upriver from the North Sea. The combined catchment area of the Tweed and Whiteadder is approximately 4900 km^2 and the average freshwater input into the estuary is ~84 m^3/s (Fox and Johnson, 1997). The maximum length of the Tweed estuary (Figure 1; Chapter 1) is around 13 km and the residence time is approximately 1 day (Uncles and Stephens, 1996). The Tweed is a partially mixed to stratified microtidal estuary (Uncles and Stephens, 1996). The catchment is dominated by agricultural activity, ranging from upland areas of moorland used for sheep grazing to more arable regions in the lowlands (Neal, 2002). The Tweed receives significantly less anthropogenic inputs than the Tyne, as reflected in its favourable water quality ratings (Robson and Neal, 1997).

Due to the rapid flushing time in the Tweed Estuary SPM concentrations are generally quite low (<30 mg/L) and tend to exhibit conservative mixing between river and coastal waters (due to the lack of a MTZ), although higher coastal turbidity resulting from strong wind events and periods of higher discharge can sometimes occur (Uncles et al., 2000). While the Tweed is considered to be net heterotrophic system during the winter, photosynthesis becomes the dominant process during the summer (Chapter 2; Howland et al., 2000). However, considering the low chlorophyll a concentrations (<2 μg/L) typically found in the Tweed Estuary (Chapters 1-4; Uncles et al., 2000), photosynthetic activity in the Tweed is thought to be dominated by benthic macrophytes. Changing with elevation, soils in the Tweed catchment include well drained brown earths founds in the lowlands, gleys on the southern slopes, podzols on higher land and peats on hill tops and moors (Robson and Neal, 1997).

Sample collection

Data reported here (Tables 1-4) are from near-surface water samples collected on single day excursions to the Tyne (Feb-02, Jul-02, Mar-03, Jul-03) and Tweed (Jul-03, Dec-03) Estuaries. Site selection within the estuary was based on *in situ* salinity measurements (using a portable probe) in order to ensure adequate, high-resolution coverage of the full salinity gradient. Once back in the laboratory, salinity was analysed on a pre-calibrated Hanna (model 8633) conductivity meter. Samples were collected at a depth of 1-2 m from a small boat using a submersed pump. Water samples for concentration and isotopic analysis of high molecular weight dissolved organic matter (HMW DOM) and POM were collected by large volume pumping into acid-cleaned 25 L and 4 L high density polyethylene (HDPE) containers, respectively (Appendix 1). River discharge data is reported here as the two-day average incorporating the days prior to and day of sampling. Discharges for the Tyne ($Q = 144.6$ m^3/s, 27-28 February 2002; $Q = 8.6$ m^3/s, 10-11 July 2002; $Q = 30.7$ m^3/s, 03-04 March 2003; $Q = 7.3$ m^3/s, 22-23 July 2003) and Tweed ($Q = 14.5$ m^3/s, 07-08 July 2003; $Q = 151.2$ m^3/s, 02-03 December 2003) were obtained from the UK Environment Agency and from the Scottish Environment Protection Agency, respectively. During the Jul-03 transect in the Tyne Estuary, the combined effects of a spring tidal event with extremely low flow resulted in a low salinity end-member sampling point of 13.9 psu due to inaccessibility of the inner estuary. Conversely, during the Dec-03 sampling trip to the Tweed Estuary high river flow resulted in a survey comprised predominantly of low salinity water samples. This strong dependence on surface salinity with freshwater runoff has been previously reported in the Tweed Estuary (Uncles et al., 2000).

Isolation of dissolved organic matter (DOM)

Following sample collection, the 25 L water samples collected for HMW DOM were consecutively pressure-filtered (~1.4 bar) through both a 0.7 μm glass fibre filter and a 0.2 μm capsule filter to remove particulates, algae and most of the bacteria. In order to obtain sufficient material for isotope analysis, dissolved organic matter (DOM) was concentrated via tangential-flow ultrafiltration (UF), a technique that separates a filtrate of known molecular weight. A 1 kDa UF membrane (Millipore) was purchased for this study but after testing was found to have a

Site/Date	salinity	SPM (mg/L)	PON% (of SPM)	PON [μM]	C/N_{POM}	HMW DON [μM]	C/N_{HDOM}
Tyne							
28/2/02	31.7	31.3	0.29	6.5	18.1	NM*	NM*
28/2/02	17.7	30.1	0.28	6.0	24.8	NM*	NM*
28/2/02	15.0	36.7	0.26	6.8	24.0	NM*	NM*
28/2/02	9.9	35.3	0.38	9.5	21.1	NM*	NM*
28/2/02	6.9	38.6	0.39	10.9	25.2	NM*	NM*
28/2/02	3.0	48.2	0.51	17.5	21.1	NM*	NM*
28/2/02	2.1	52.3	0.48	18.0	21.2	NM*	NM*
11/7/02	33.2	15.7	0.16	1.8	15.2	NM*	NM*
11/7/02	30.9	17.0	0.37	4.5	15.5	NM*	NM*
11/7/02	25.9	19.7	0.25	3.6	20.8	NM*	NM*
11/7/02	19.3	35.1	0.45	11.4	19.3	NM*	NM*
11/7/02	15.0	34.9	0.50	12.4	20.4	NM*	NM*
11/7/02	9.8	30.4	0.54	11.8	18.9	NM*	NM*
11/7/02	4.7	29.1	0.47	9.9	21.4	NM*	NM*
11/7/02	0.2	15.9	0.65	7.4	22.5	6.2	21.0
11/7/02	0.2	17.9	0.59	7.6	22.5	6.2	21.2
4/3/03	34.1	15.7	0.36	4.0	19.9	0.6	12.2
4/3/03	28.7	20.2	0.50	7.2	18.0	2.5	5.9
4/3/03	19.0	29.4	0.37	7.8	20.1	1.0	18.0
4/3/03	14.8	55.3	0.29	11.4	20.3	1.5	22.3
4/3/03	14.1	147.9	0.22	23.6	20.3	1.7	21.4
4/3/03	10.5	232.1	0.16	26.1	21.5	1.7	27.7
4/3/03	5.3	261.9	0.14	26.3	20.4	2.5	28.3
4/3/03	1.3	92.8	0.32	21.0	19.6	5.2	30.0
4/3/03	0.1	21.9	0.59	9.2	20.9	7.9	33.2
4/3/03	0.1	22.0	0.58	9.2	20.5	7.8	33.6
23/7/03	32.5	1.8	1.03	1.4	11.4	0.4	14.9
23/7/03	27.1	7.2	0.67	3.5	10.5	1.0	13.5
23/7/03	25.1	8.3	0.52	3.1	12.6	1.3	17.0
23/7/03	24.2	13.5	0.37	3.5	13.1	1.3	16.8
23/7/03	21.6	16.4	0.36	4.3	12.2	1.5	18.9
23/7/03	20.6	12.8	0.35	3.2	13.0	1.7	17.8
23/7/03	20.1	17.4	0.27	3.4	12.9	1.7	19.5
23/7/03	18.3	25.4	0.30	5.5	13.7	1.8	20.3
23/7/03	16.4	13.8	0.37	3.6	11.2	1.9	22.5
23/7/03	13.9	13.5	0.36	3.5	11.3	2.6	19.7

*NM, not measured

Table 1. Geochemical parameters of water samples from the Tyne Estuary, including concentrations of suspended particulate matter (SPM), particulate organic nitrogen (PON), high molecular weight dissolved organic nitrogen (HMW DON), C/N ratios (molar) of particulate organic matter (C/N_{POM}) and C/N ratios (molar) of high molecular weight dissolved organic matter (C/N_{HDOM}). The PON% is the percentage of PON which comprises the SPM.

nominal pore size of 15 kDa, which was capable of separating the high molecular weight (HMW, >15kDa) from the low molecular weight (LMW, <15 kDa) fraction of DOM. For more information on the UF procedure used here the reader is asked to refer to Chapter 3. After initial UF, the HMW DOM samples were diafiltered with 10 L of Milli-Q water (using a 20:1 dilution ratio) in order to remove salts. After diafiltration, HMW DOM samples were freeze-dried, homogenised, re-wetted with Milli-Q water and placed for several days inside a glass dessicator containing concentrated HCl to remove inorganic carbon as required for the δ^{13}C analyses reported elsewhere (Chapter 3). They were then vacuum desiccated to remove HCl prior to isotopic analysis.

Site/Date	salinity	SPM (mg/L)	PON% (of SPM)	PON [μM]	C/N$_{\text{POM}}$	HMW DON [μM]	C/N$_{\text{HDOM}}$
Tweed							
8/7/03	33.2	1.0	1.09	0.8	8.6	0.1	9.7
8/7/03	32.4	1.2	1.05	0.9	10.7	0.3	9.5
8/7/03	29.5	1.7	1.27	1.5	10.1	0.4	10.4
8/7/03	27.4	3.4	1.07	2.6	10.8	0.4	10.5
8/7/03	17.9	3.8	1.51	4.1	9.1	0.8	10.7
8/7/03	8.6	3.7	1.88	4.9	8.5	1.6	11.0
8/7/03	4.2	3.3	1.51	3.6	9.3	2.5	11.0
8/7/03	1.4	3.6	1.95	5.0	8.5	3.6	11.1
8/7/03	0.1	3.2	1.64	3.8	9.9	5.2	11.8
8/7/03	0.1	3.3	1.69	4.0	10.0	5.3	11.9
3/12/03	31.30	17.5	0.35	4.4	12.6	0.5	9.0
3/12/03	19.38	NM[*]	NM[*]	NM[*]	NM[*]	2.8	20.1
3/12/03	4.51	NM[*]	NM[*]	NM[*]	NM[*]	7.6	23.8
3/12/03	1.90	19.2	0.66	9.1	12.3	8.0	24.9
3/12/03	1.27	18.0	0.63	8.1	12.7	8.1	24.5
3/12/03	0.97	16.6	0.54	6.4	12.4	9.4	27.0
3/12/03	0.90	18.5	0.56	7.3	11.6	10.4	25.5
3/12/03	0.19	24.0	0.61	10.4	12.5	14.8	24.0
3/12/03	0.16	23.4	0.63	10.5	11.5	14.7	22.6
3/12/03	0.12	23.3	0.62	10.3	11.3	15.5	21.8

[*] NM, not measured

Table 2. Geochemical parameters of water samples from the Tweed Estuary, including concentrations of suspended particulate matter (SPM), particulate organic nitrogen (PON), high molecular weight dissolved organic nitrogen (HMW DON), C/N ratios (molar) of particulate organic matter (C/N$_{\text{POM}}$) and C/N ratios (molar) of high molecular weight dissolved organic matter (C/N$_{\text{HDOM}}$). The PON% is the percentage of PON which comprises the SPM.

Isolation of suspended particulate organic matter (POM)

Suspended particulate matter (SPM) and suspended particulate organic matter (POM) concentrations were determined by direct analysis of the material collected on pre-combusted (440°C for 4 h), pre-weighed glass fibre filters (GF/F, Whatman) by pressure filtration (~1.4 bar) in the laboratory within 10 hours after water samples were collected. After sufficient material had been collected the filters were rinsed with Milli-Q water to remove salts. As done for the HMW DOM, inorganic carbon on the GF/F filters was removed via vapour-phase exposure with concentrated HCl as required for the $\delta^{13}C$ analyses reported elsewhere (Chapter 4). The filters were then vacuum desiccated to remove HCl, had excess glass fibre removed, were weighed, and then were subsequently homogenised with a mortar and pestle and stored in glass vials prior to concentration and isotopic analysis. SPM concentrations were determined by weighing dry filters prior to acidification. Based on analyses of duplicate GF/F filters from the same sampling locations, the precision for SPM concentrations was ± 5% relative standard deviation (RSD).

Concentration and $\delta^{15}N$ analysis of HMW DON and PON

Concentrations and C/N ratios (reported here as molar ratios) for POM (C/N_{POM}) and HMW DOM (C/N_{HDOM}), and $\delta^{15}N$ ratios for PON ($\delta^{15}N_{PON}$) and HMW DON ($\delta^{15}N_{HDON}$) were determined using a Carlo Erba Instruments NA2500 elemental analyser coupled with a Micromass PRISM III Isotope Ratio Mass Spectrometer (EA-IRMS). HMW DON concentrations were calculated using the C/N_{HDOM} ratio and previously measured HMW DOC concentrations reported elsewhere (Chapter 3). PON concentrations were calculated by dividing the mass of N by the volume of water passed though the GF/F filters.

Ratios of ^{14}N to ^{15}N are expressed in delta notation as per mil (‰) differences relative to an internationally recognised atmospheric nitrogen standard, where:

$$\delta^{15}N = [\{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{standard}\} / (^{15}N/^{14}N)_{standard}] \times 1000 \qquad (1)$$

$\delta^{15}N$ values (and concentrations) reported here for HMW DON are the averages of replicate sample analyses. With the exception of the most saline sample collected from the Tyne (32.5 psu) and samples taken from the Tweed at salinities >20 during the Jul-03 surveys, $\delta^{15}N$ signatures (and concentrations) reported here for PON are also the averages of replicate analyses. The error associated with $\delta^{15}N$ values in this study incorporates both internal reproducibility and accuracy associated with EA-IRMS analysis, and was ≤ 0.4‰ for $\delta^{15}N$ values of PON and HMW DON. Based on multiple analyses of standards and samples, the acuracy and reproducibility for %C and %N determined via EA analysis was ± 5% and ± 8% RSD, respectively. During the July 2002 survey to the Tyne Estuary HMW DOM samples were not properly diafiltered, resulting in unquantifiable EA-IRMS peak sizes for saline samples. Hence, only the riverine sample from this survey is reported here.

RESULTS AND DISCUSSION

Behaviour of particulate organic nitrogen (PON) concentrations

In Figure 2, concentrations of PON and SPM from all six surveys to the Tyne and Tweed Estuaries are plotted versus salinity. In general, concentrations of PON in both the Tyne (Figures 2a-d) and Tweed (Figures 2e-f) Estuaries decreased with increasing salinity, and exhibited similar distributions as reported for POC concentrations (Chapter 4). In the Tyne, the highest PON concentrations were often associated with a well-defined low- to mid-salinity range maximum turbidity zone (MTZ). The highest PON concentrations in the Tyne (26.3 μM) was measured in the MTZ (5.3 psu) during the relatively turbid Mar-03 survey, when the turbidity maximum was well developed. During the Jul-02 survey a more broadened mid-salinity MTZ was observed and PON concentrations tracked the SPM levels. The much higher river discharge in Feb-02 inhibited the formation of a MTZ, which is reflected in the estuarine PON concentrations during these periods. Similarly, a discernible MTZ is also absent during Jul-03 survey under very low river discharge and spring tide conditions. Hence, elevated PON levels within the estuary were not evident during the Jul-03 survey. Slight increases in PON concentrations at the sampling location nearest the Howdon sewage works (HSW) were observed during most of the surveys to the Tyne Estuary. The close association between PON concentrations MTZ locations indicates that the residence time of particulate N in the Tyne Estuary is variable with seasons and river flow conditions.

Unlike the Tyne, the Tweed lacks a distinct MTZ. The lack of substantial long-term accumulation of muddy sediments and hence the absence of a distinct maximum turbidity zone in the Tweed Estuary has been documented by others

Figure 2a. Tyne Feb-02

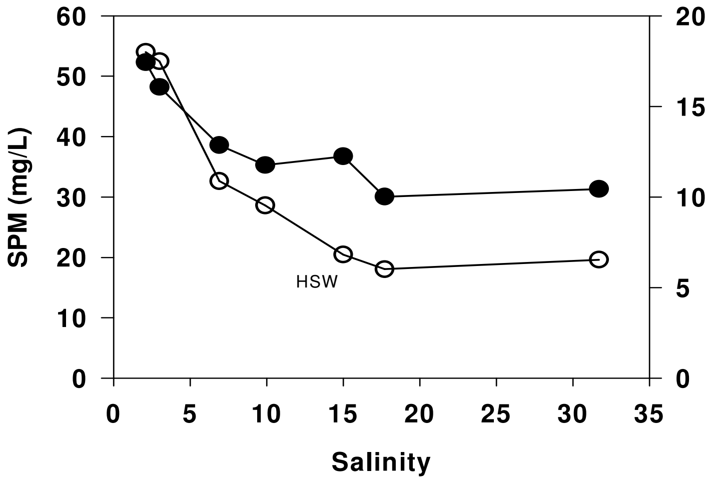

Figure 2b. Tyne Jul-02

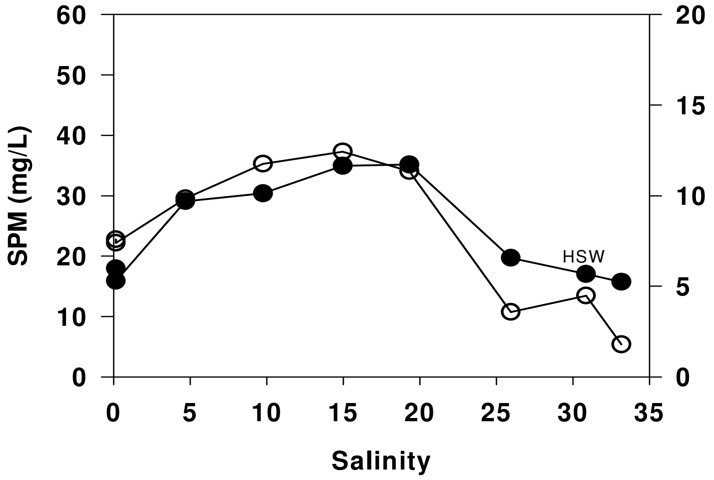

Figure 2c. Tyne Mar-03

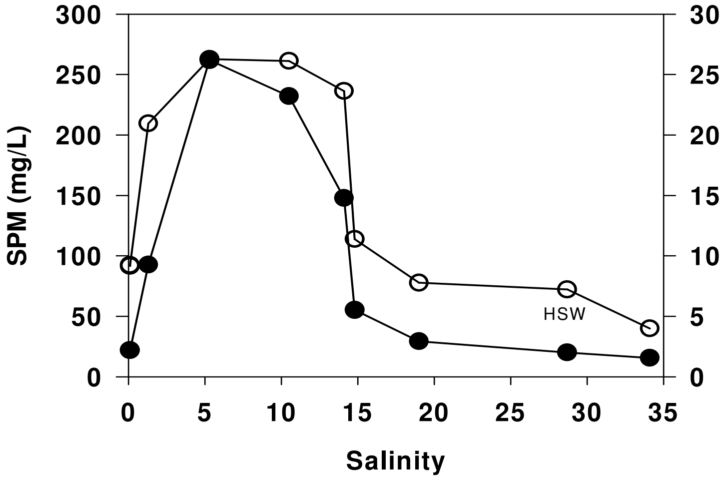

Figure 2. Concentrations of particulate organic nitrogen (PON; open circles) and suspended particulate matter (SPM: closed circles) in the Tyne Estuary during (a) Feb-02, (b) Jul-02 and (c) Mar-03 plotted versus salinity. The location of the Howdon sewage works (HSW) during each of the surveys has been indicated.

Figure 2d. Tyne Jul-03

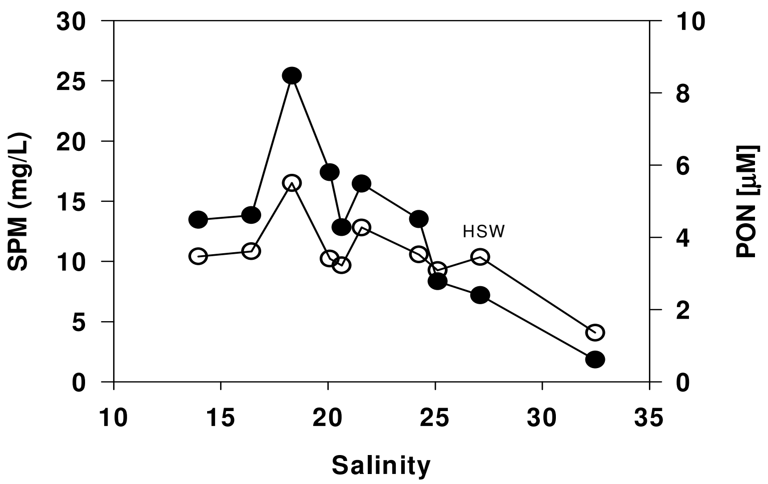

Figure 2e. Tweed Jul-03

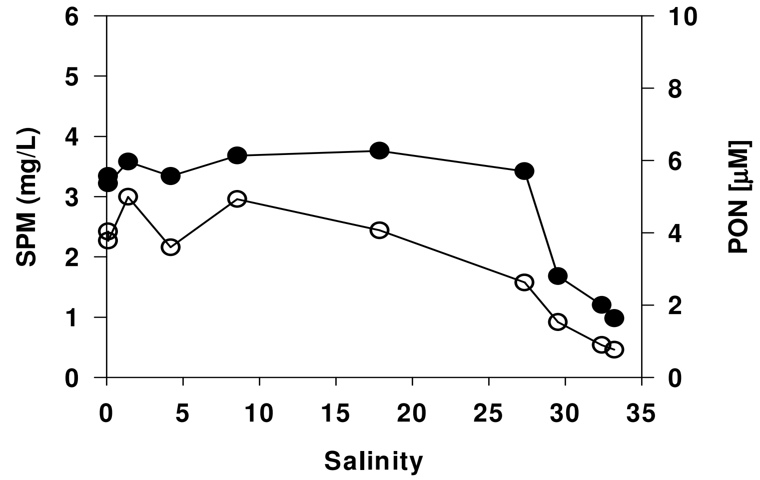

Figure 2f. Tweed Dec-03

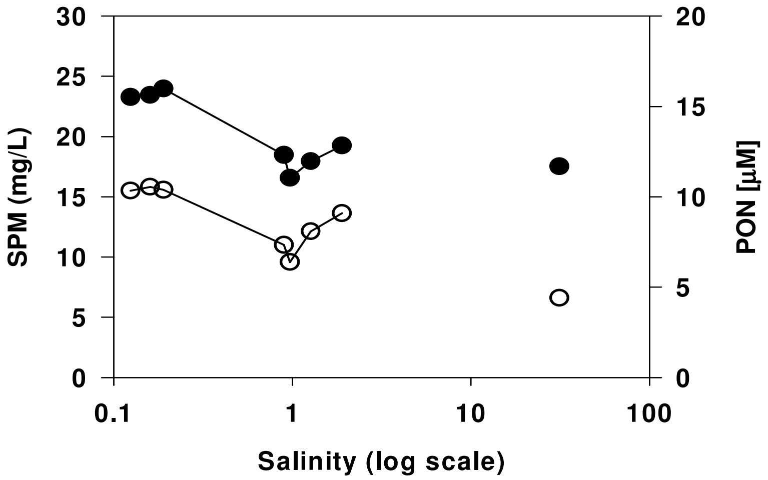

Figure 2. Concentrations of particulate organic nitrogen (PON; open circles) and suspended particulate matter (SPM: closed circles) in the Tyne Estuary during (d) Jul-03 and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. The location of the Howdon sewage works (HSW) during the Jul-03 survey to the Tyne Estuary has been indicated.

(Uncles et al., 2000). Hence the PON concentrations in the Tweed are much lower in all cases (<11 μM) relative to the Tyne. Furthermore, the lowest PON values during the two surveys to the Tweed were encountered near the coast.

The riverine fluxes of PON showed a positive correlation with river discharge both in the Tyne and the Tweed (Figure 3). In the Tyne, the Feb-02 survey records the very high river flow (Q = 144.6 m^3/s) following a winter storm event. This period also recorded the highest PON discharge into the Tyne estuary. Similarly, during the unusually low flow conditions of Jul-03 the lowest PON levels were encountered in the estuary. A positive correlation between concentrations of suspended sediment and river flow has been observed by others in the Tweed (Neal et al., 1997). This also seemed to apply to PON levels in the Tweed, where higher river discharge in the winter was associated with a larger riverine PON load entering the estuary.

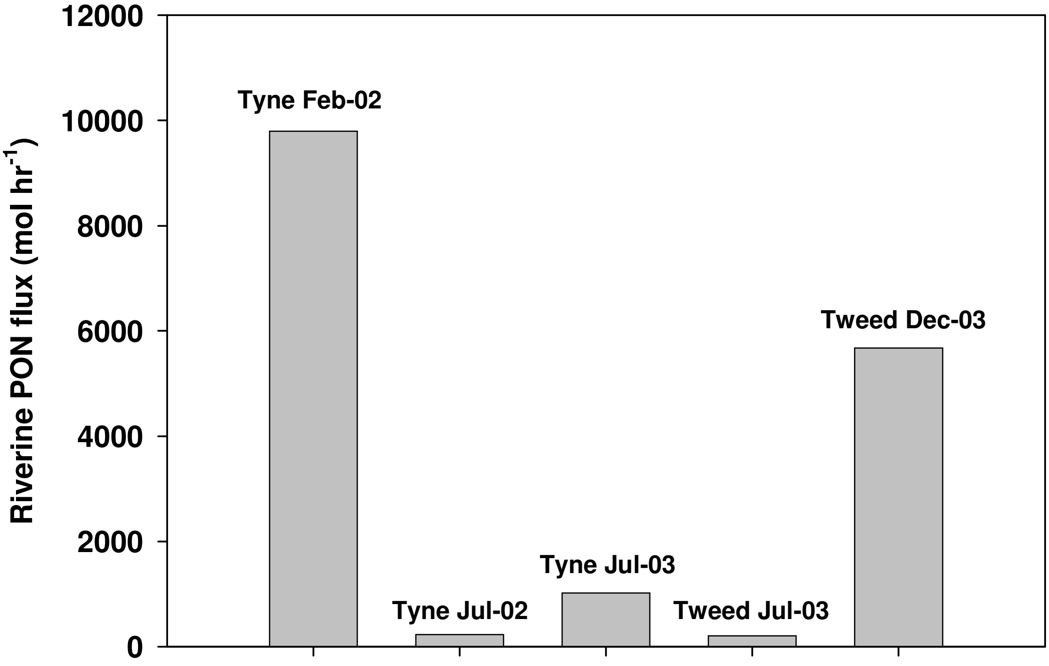

Figure 3. Riverine particulate organic nitrogen (PON) fluxes (mol hr^{-1}) in the Tyne and Tweed.

There appeared to be a general decline in PON concentrations with increasing salinity in both estuaries. This was very clearly seen in the Tweed Estuary, where the lowest PON values during the two sampling periods were encountered near the coast. In the Tyne estuary, this trend was only collectively evident in the seaward side of the turbidity maximum (Figure 2a-d); however in some cases this was obscured by point source from HSW. This is evaluated further by using the percentage of PON comprising the SPM pool (PON%).

In Figure 4, PON% is plotted against salinity for the Tyne and Tweed transects. Plotting PON concentrations as a percent of SPM concentrations removes the effect of the MTZ and allows assessments to be made on the relative enrichment of nitrogen in the SPM pool. In the Tweed, there was a general decline in PON% with an increase in salinity that matched the PON concentration changes. In the Tyne, a similar declining trend was evident during the Feb-02 and Jul-02 surveys. In the Mar-03 survey, there was a large decline in PON% from the river to the low- to mid-salinity MTZ zone followed by an increase seaward of the MTZ. In Jul-03, there was an increasing trend of PON% seaward. Point source PON inputs associated with HSW as seen in the concentration data are evidence for N-enriched sources in the Tyne Estuary. Thus, the trends shown in Figure 4a-d are generally indicative of estuarine loss of terrestrially-derived PON relative to SPM during estuarine mixing. In Mar-03 and Jul-02 additional N-enriched HSW-derived inputs of PON are inferred. Sewage-derived particulate organic matter is generally expected to be enriched in nitrogen relative to carbon in comparison to terrestrially-derived plant remains (Andrews et al., 1998). However, the increasing trend in PON% seaward during Jul-03 survey cannot be explained by HSW inputs alone. This very high enrichment in PON% is related to marine algal sources in addition to HSW input. This large seaward increase in PON% is clearly related to a larger contribution of N-enriched algal PON to the SPM pool.

To provide a quantitative estimate of algal-derived suspended PON, the relative contribution of non-algal PON to the total PON can be determined using PON/chl-a ratios. A ratio of 40 has been used to predict an algal end-member algal for POC distributions from various European estuaries (Abril et al., 2002, and references therein), therefore based on a value of 5 (assuming a C/N ratio of 8 for algal material) estimates of the percentages of algal-derived suspended PON were calculated. In the Tyne (Figure 4a), the % algal PON ranged 6.2-30.0% (Jul-02), 0.7-2.8% (Mar-03) and 7.5-26.0% (Jul-03), with the highest values found in the middle and lower parts of the estuary. In the Tweed (Figure 4b), the estimated % algal PON were relatively constant during both summer and winter surveys, ranging from 10.4-16.8% during Jul-03 and 10.1-18.7% during Dec-03. The % algal PON values

Figure 4a. Tyne Feb-02

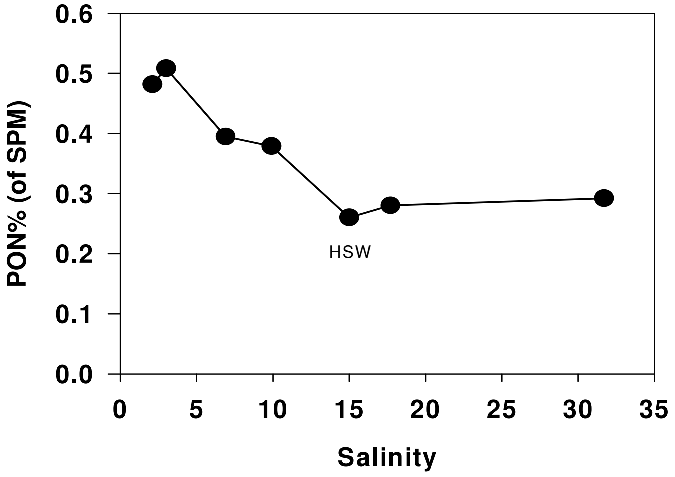

Figure 4b. Tyne Jul-02

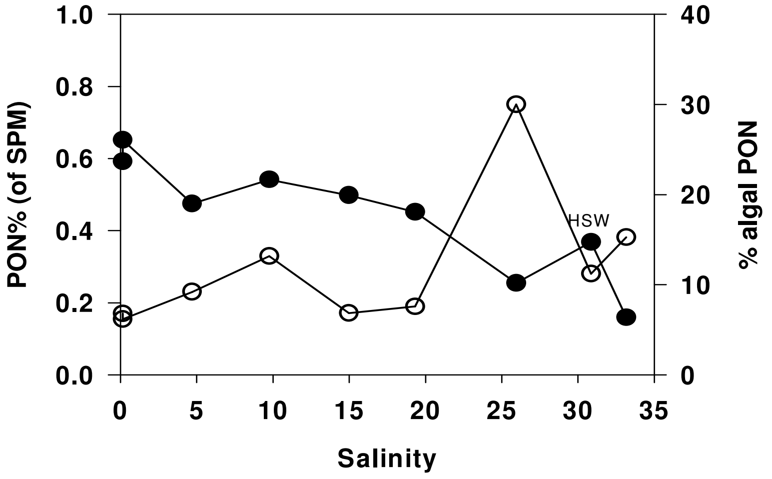

Figure 4c. Tyne Mar-03

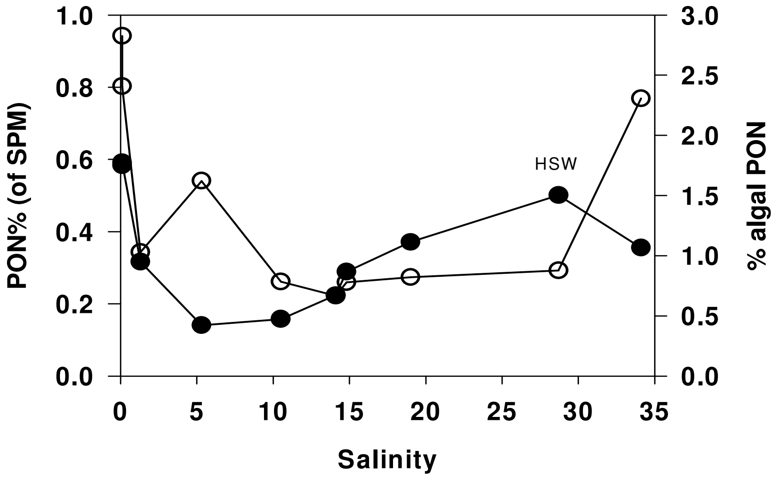

Figure 4. The percentage of PON comprising the SPM (PON%; closed circles) and the estimated % algal PON (open circles) in the Tyne Estuary during (a) Feb-02, (b) Jul-02 and (c) Mar-03 plotted versus salinity. The location of the Howdon sewage works (HSW) during each survey has been indicated.

Figure 4d. Tyne Jul-03

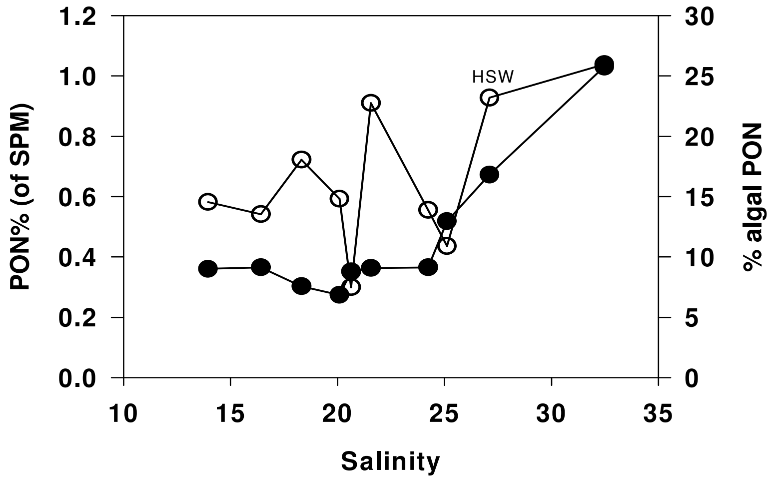

Figure 4e. Tweed Jul-03

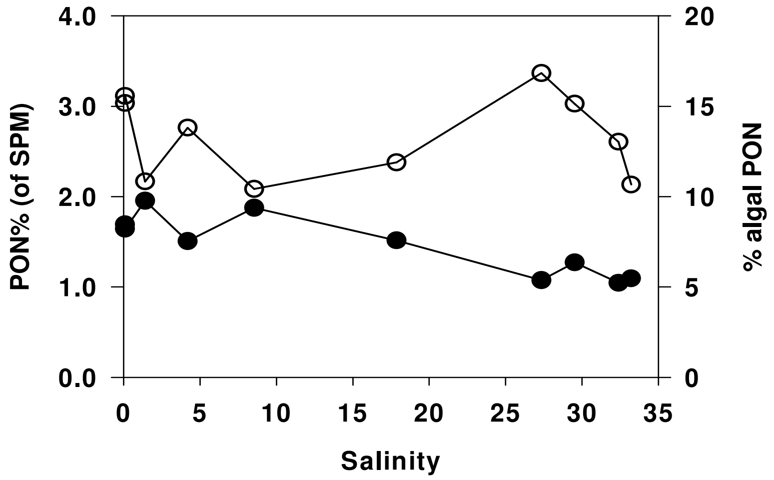

Figure 4f. Tweed Dec-03

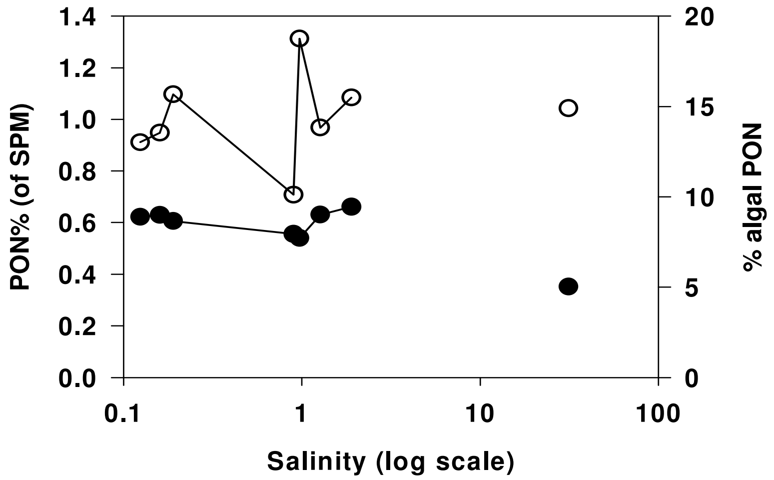

Figure 4. The percentage of PON comprising the SPM (PON%; closed circles) and the estimated % algal PON (open circles) in the Tyne Estuary during (d) Jul-03 and in the Tweed Estuary during (e) Jul-03 and (f) Dec-03 plotted versus salinity. The location of the Howdon sewage works (HSW) during the Jul-03 survey to the Tyne Estuary has been indicated.

calculated in the winter in the Tweed are most likely inflated due to a elevated chl-a concentrations caused by a higher influx of plant debris during this period of elevated river discharge.

As demonstrated in Figure 4, it appeared that algal PON was only important when riverine inputs of terrestrial PON were low in the summer months. In the Tyne, the percentage of nitrogen in SPM entering the estuary from the river appeared to be relatively constant, with a PON% value of roughly 0.6%. Thus, changes in riverine PON concentration with flow rates were not associated with N-enriched particulate sources but with higher amounts of similarly N-enriched particulates. This is in contrast to the Tweed where PON% values were highly elevated in summer months (>1.6% PON). This contrast reflects the fundamental difference in PON sources in the two estuaries and will be discussed in relation to C/N ratios and $\delta^{15}N$ values.

Mineralization of PON

Given that most PON% trends showed a loss of PON in the low- to mid-salinity range, one could estimate the percentage of PON lost using the method described by Abril et al. (2002) for estimating riverine POC removal (Chapter 4):

$$\text{\% of riverine PON mineralised} = SPM_{river} \times (PON\%_{river} - PON\%_{MTZ}) \qquad (2)$$

Here SPM_{river} refers to the average SPM riverine concentration, $PON\%_{river}$ is the PON% (% of SPM) in the river and $PON\%_{MTZ}$ is the PON% (% of SPM) in the maximum turbidity zone. Since the Tweed does not possess a well-defined MTZ, and contributions from algal material were relatively minor in this estuary, the lowest PON% value observed at the mouth of the estuary was substituted for the $PON\%_{MTZ}$ in Eq. 2. This was also done for the Tyne Feb-02 survey, due to the lack of a pronounced MTZ during this period caused by higher river flow. Furthermore, owing to the particularly low flow conditions experienced in the Tyne during the Jul-03 survey, a freshwater end-member (≤0.2 psu) PON sample could not be obtained. Therefore, the PON% from the Jul-02 transect (0.62 ±0.04%) was used as a riverine end-member for the following summer. All the parameters used in these calculations are listed in Tables 1 and 2. The estimated percentages of riverine PON mineralised were 39, 27, 76 and 51% in the Tyne during Feb-02, Jul-02, Mar-03 and Jul-03, respectively, and were 34% (Jul-03) and 43% (Dec-03) in the Tweed. Based on compounded error from SPM and PON measurements, the precision associated with these calculations was approximately ±10%. The trends in these estimations are approximately the same as those calculated for POC (Chapter 4), which substantiates the claim that a significant amount of terrestrially-derived PON is being oxidised in the estuarine and/or coastal zones.

Behaviour of C/N_{POM} ratios

In Figure 5, C/N_{POM} ratios from all six surveys to the Tyne (Figure 5a) and Tweed (Figure 5b) Estuaries are plotted versus salinity. In general, C/N_{POM} ratios were higher in the Tyne than in the Tweed Estuary. The C/N_{POM} ratios in the Tyne during both winter surveys were relatively invariant across the estuary, scattering around means (after combining freshwater end-members, ≤ 0.2 psu, when appropriate) of 22.2 ±2.5 (n = 7) and 20.1 ±1.0 (n = 9) during the Feb-02 and Mar-03 surveys, respectively. During the Jul-02 survey, C/N_{POM} ratios showed a general decrease with increasing salinity, although the average ratio (19.2 ±2.7, n = 8) was still relatively high. In the Tweed during Dec-03 the average C/N_{POM} ratio was 12.2 ±0.5 (n = 6). The relatively high C/N_{POM} ratios found in the Tyne during the Feb-02, Mar-03 and Jul-02 surveys and imply that much of the POM was derived from terrestrial C_3 plant debris or from soil organic matter dominated by C_3 plants (Boutton, 1996; Kendall et al., 2001). Since terrestrial plant litter generally has higher C/N ratios than soil organic matter (Kendall et al., 2001; Weiguo et al., 2003), the lower C/N_{POM} ratios found in the Tweed during the winter suggest a greater relative proportion of the latter. Low C/N ratios for soil-derived suspended sediment in the River Tweed (~ 10-12) have been observed by others (Neal et al., 1997).

C/N_{POM} ratios across the Tyne and Tweed Estuaries during the Jul-03 surveys were lower, scattering around means (after combining freshwater end-members, ≤0.2 psu) of 12.2 ±1.0 (n = 10) and 9.5 ±0.9 (n = 9), respectively. During these two summer surveys Tyne (Q = 7.3 m^3/s) and Tweed (Q = 14.5 m^3/s) River discharges were unusually low. Since POM enters streams through mechanical weathering, which may erode deeper/older soil horizons (Raymond and Bauer, 2001), this may suggest that the soil-derived POM found in these estuaries during these low baseflow conditions was entering the estuaries in a more pre-degraded from than during the

Figure 5a

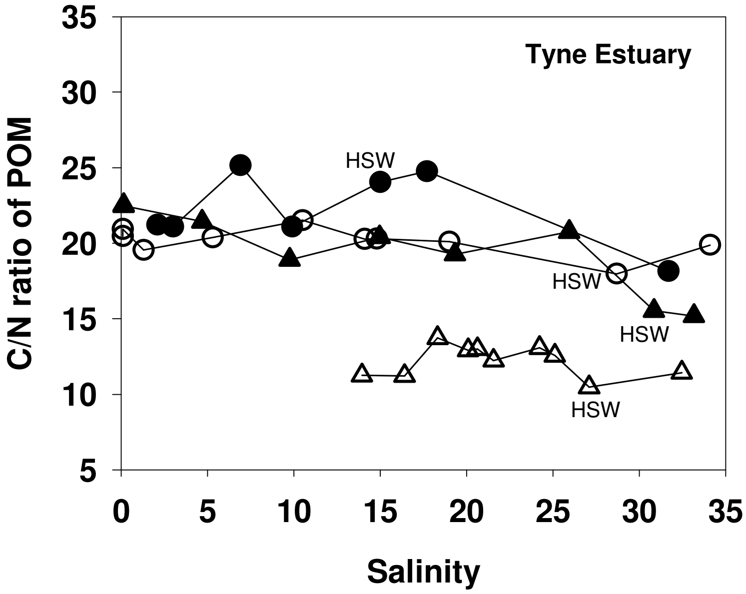

Figure 5b

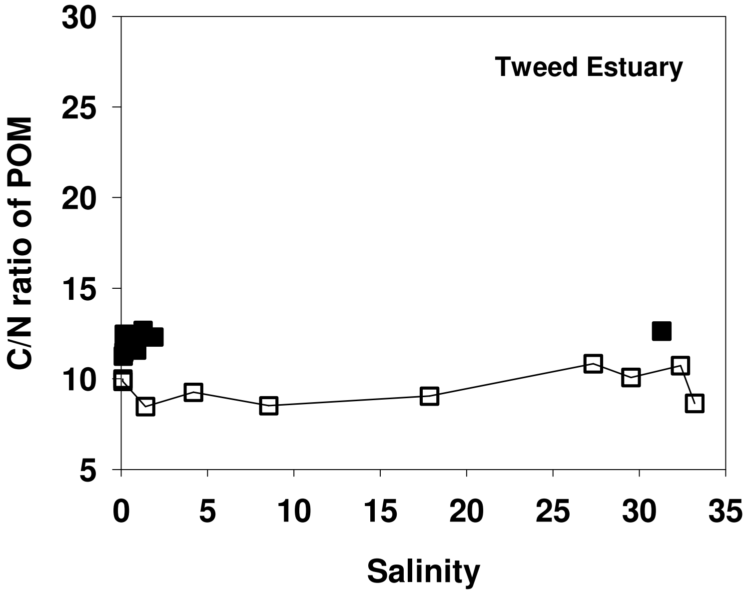

Figure 5. C/N ratios (molar) of particulate organic matter (C/N_{POM}) in the (a) Tyne Estuary during Feb-02 (closed circles), Jul-02 (closed triangles), Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity. The location of the Howdon sewage works (HSW) during each of the four surveys to the Tyne Estuary has been indicated.

winter. In native soils C/N ratios tend to decrease with depth (Killham, 1994). This trend corroborates the suggestion that soil-derived POM originating from deeper horizons comprised a significant part of the PON pool during the summer, with smaller contributions coming from suspended plant debris during the less turbid, lower flow conditions.

Chlorophyll a concentrations in both the Tyne (<3.0 μg/L) and Tweed (<2.0 μg/L) Estuaries during Jul-03 were quite low (Chapters 1-4), implying a minor POM contribution from phytoplankton. However, C/N_{POM} ratios during the summer could still have been influenced by autochthonous production since lower terrestrial inputs can increase the *relative* importance of algal contributions. While the isotopic signatures of estuarine phytoplankton can occupy a wide range of values, in general phytoplankton growth is evidenced by substantially lower (≤8) C/N ratios (Baird and Middleton, 2004; Cowie and Hedges, 1994; Goni and Hedges, 1995; Hellings et al., 1999; Kendall et al., 2001; Middelburg and Nieuwenhuize, 1998). The average C/N_{POM} found in the Tweed during the summer was 9.5 ±0.9 (n = 9); however, values as low as 8.5 were observed in the mid-salinity range (Figure 3b). Therefore, in addition to having lower C/N ratios attributed to seasonal variations in suspended soil-derived OM characteristics, the C/N ratios for suspended POM in the Tweed during the summer might also reflect a relatively increased proportion of autochthonous material. Since the short water residence of the Tweed is thought to inhibit numerous phytoplankton cell divisions (Uncles et al., 2000), macroalgal and/or seagrass debris are considered the main source for suspended algal material within this estuary.

In order to assess whether the suspended algal material in the low to mid-salinity regions (≤30 psu) of the Tweed Estuary during the summer was mainly derived from macroalgal and/or seagrass, C/N_{POM} ratios were plotted against the estimated % algal PON values. As demonstrated in Figure 6, there was a very good positive linear correlation between these two parameters ($r^2 = 0.94$, n = 8), indicating a relatively high C/N ratio for this algal material. Since phytoplankton-derived OM is typically characterised by C/N ratios less than 8 (Baird and Middleton, 2004; Cowie and Hedges, 1994; Goni and Hedges, 1995), the high C/N ratio for algal material in the Tweed Estuary supports our conclusion that chl-a concentrations were largely controlled by macroalgae and benthic plants debris, for which the average C/N ratio is ~18.3 (Atkinson and Smith, 1983; Baird and Middleton, 2004). Furthermore, since the method used to estimate the % algal PON utilized a phytoplankton algal end-member PON/chl-a ratio of 5, the % algal PON values in the Tweed are overestimates for the contribution from non-phytoplankton sources.

The relatively low C/N$_{POM}$ ratios in the Tweed can therefore be attributed to inputs of N-enriched agricultural soil. The C/N ratios of soil organic matter (SOM) have been shown to be heavily influenced by land-use patterns, with cultivated soils generally possessing lower C/N ratios (Seeber and Seeber, 2005). This lowering of the C/N ratio in SOM is related to soil decomposition, whereby the more easily decomposed components are removed and nitrogen becomes immobilised in microbial biomass and decay products, leaving behind a residual SOM which contains a higher proportion of recalcitrant material (Post et al., 1985). In the Tyne watershed, where agricultural activity is less dominant, the generally higher C/N ratios for suspended POM are indicative of the greater component of peaty soil, which has been shown to exhibit relatively high (>30) bulk C/N ratios (Novák et al., 1999). Lower C/N ratios found in Jul-03 were attributed to a greater component of

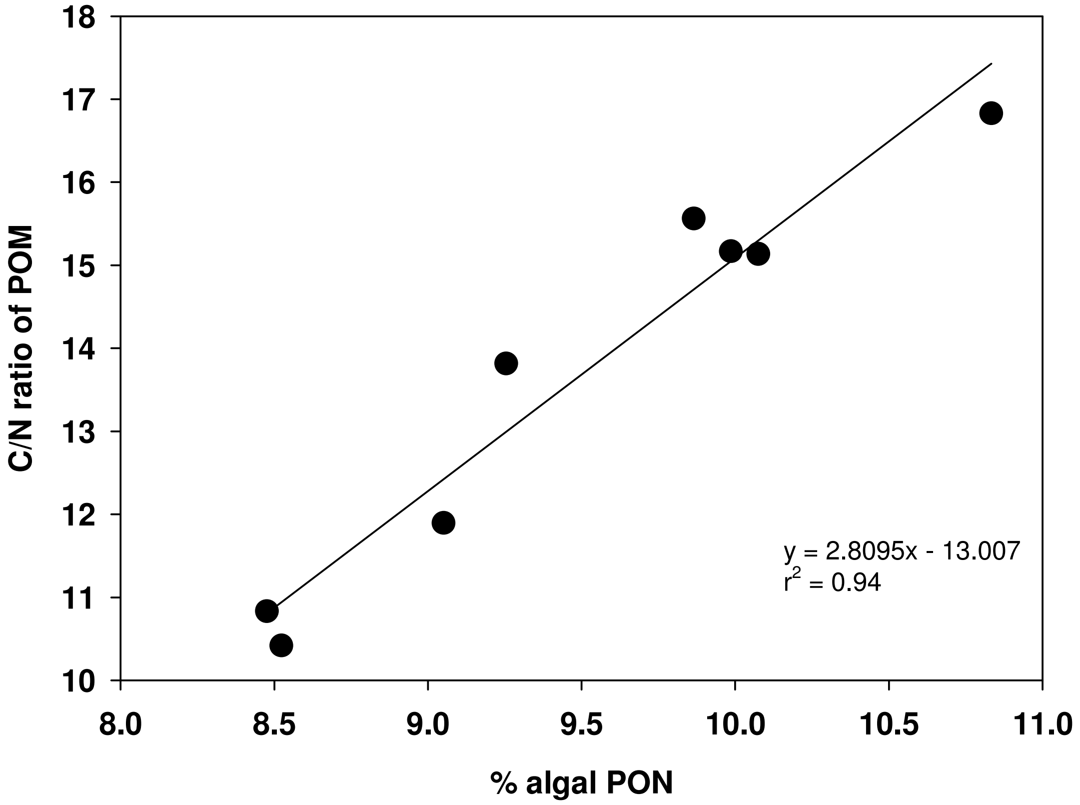

Figure 6. C/N ratios of particulate organic matter (C/N$_{POM}$) in the low- to mid-salinity region (≤ 30 psu) of the Tweed Estuary plotted against the estimated % algal PON values.

sewage- and phytoplankton-derived OM during this period of low riverine PON input. The contributions from these sources will be further evaluated using $\delta^{15}N$.

Figure 7a

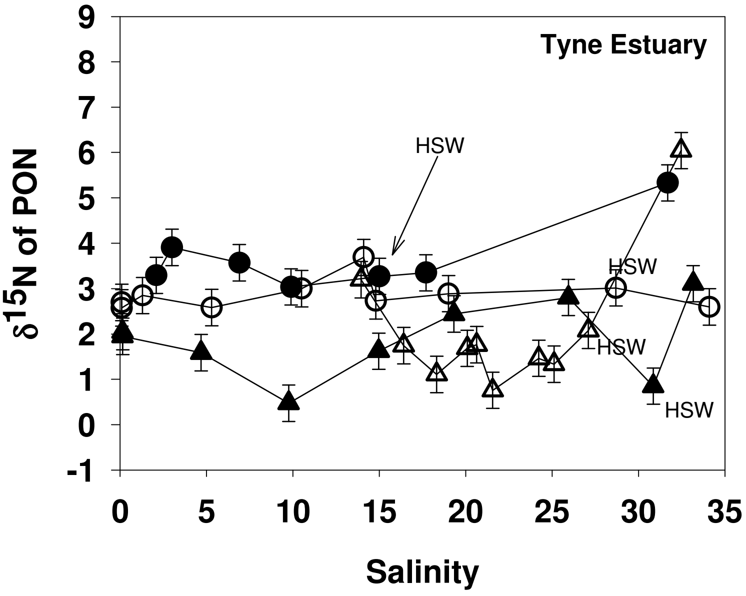

Figure 7b

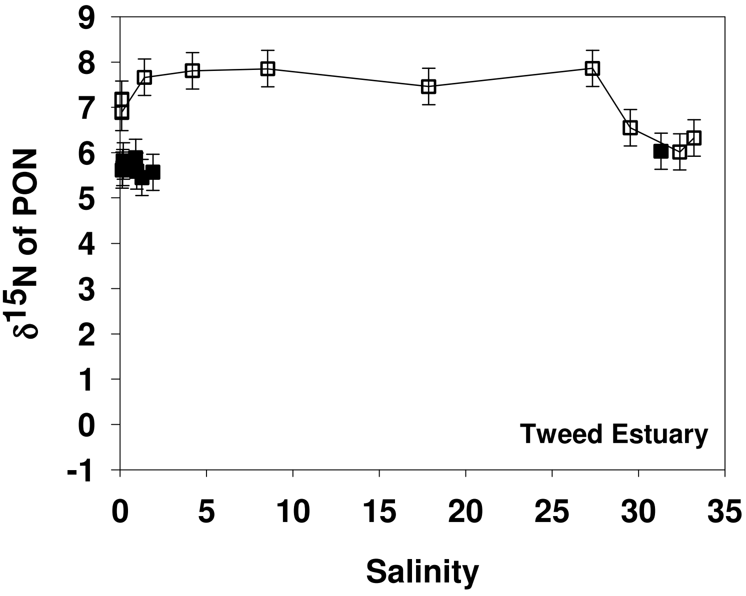

Figure 7. $\delta^{15}N$ values of particulate organic nitrogen ($\delta^{15}N_{PON}$) in the (a) Tyne Estuary during Feb-02 (closed circles), Jul-02 (closed triangles), Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity. The location of the Tyne Estuary's Howdon sewage works (HSW) during the four surveys to the Tyne Estuary has been indicated.

$\delta^{15}N_{PON}$ signatures: General trends

In Figure 7, variations in $\delta^{15}N$ signatures of PON from all six surveys to the Tyne (Figure 7a) and Tweed Estuaries (Figure 7b) are plotted versus salinity. During both winter and summer, with the exception of the coastal North Sea end-members for the Feb-02 and Jul-03 surveys ($\delta^{15}N$ = +5.3‰, +6.0 ‰, respectively), $\delta^{15}N_{PON}$ values in the Tyne were significantly more ^{15}N-depleted than those found in the Tweed. In winter, $\delta^{15}N$ signatures in the Tyne Estuary were relatively invariant across the salinity gradient, ranging from +3.0 to +3.9‰ during Feb-02 (excluding the North Sea end-member), and +2.6 to +3.7‰ during Mar-03. In contrast, $\delta^{15}N$ signatures of PON in the Tyne during the summer were variable and generally depleted, with values ranging from +0.5 to +2.8‰ during Jul-02 and +0.8 to +6.0‰ during Jul-03. In contrast to the Tyne, the Tweed Estuary showed very little in estuarine variability during each survey in $\delta^{15}N$ values of PON, which ranged from +6.0 to +7.9‰ and +5.5 to +5.9‰ during Jul-03 and Dec-03, respectively. The $\delta^{15}N$ signature of the riverine end-member was very similar in the Tyne during all seasons, ranging from +2.0 to +3.3 ‰. This is in contrast to the Tweed, where the riverine inputs were relatively depleted in winter (+5.7‰) and elevated in the summer (+7.0‰).

Contribution from terrestrial $\delta^{15}N_{PON}$ sources

In the Tyne winter transect when low salinity freshwater samples (<0.2 psu) were obtained (Mar-03), the riverine end-member had a $\delta^{15}N_{PON}$ signature of +2.6 ±0.1‰ (n = 2). In the Tweed, the riverine $\delta^{15}N_{PON}$ signature during the winter (Dec-03) was 5.7 ±0.1‰ (n = 3). Since contributions from phytoplankton during the winter were considered insignificant, this approximate 3‰ difference in terrestrial $\delta^{15}N_{PON}$ signatures between the two rivers during similar conditions must be related to variations in soil and plant litter properties. The isotopic signatures of riverine PON in both the Tyne and Tweed Estuaries fall within the wide range of $\delta^{15}N$ values reported for soil organic matter (Broadbent et al., 1980; Kendall et al., 2001). However, it has been suggested that the main process controlling $\delta^{15}N$ differences in soil profiles is related to ^{15}N-enrichment during mineralization (Nadelhoffer and Fry, 1988). In conjunction with decreasing C/N ratios, $\delta^{15}N$ signatures in soils typically exhibit a ^{15}N-enrichment with increasing depth (Novák et al., 1999; Novák et al., 2003), and in general since cultivation greatly accelerates loss of soil N through enhanced decomposition, there can be a pronounced increase in the δ^{15}N value of the remaining soil N (Amundson, 2003). Furthermore, the chronic application of large amounts of nitrate and ammonium can also lead to higher δ^{15}N values in agricultural soils, since isotopic fractionations of these compounds can become more pronounced when the substrate is not limited (Nadelhoffer and Fry, 1994). Thus, the highly enriched δ^{15}N values of PON entering the Tweed relative to the Tyne may reflect the predominant source from agricultural soils as opposed to peat, which dominates the Tyne catchment. This is supported by the high PON% and the low C/N of POM in Tweed relative to the Tyne, with the exception of the Jul-03 Tyne survey. Seasonal differences in riverine δ^{15}N values in the Tweed also reflect the relative predominant agricultural soil source. The enriched δ^{15}N values in the summer survey (Jul-03) was matched by very low C/N and high PON% in the riverine end-member, thus indicating the prevalence of agricultural soil inputs. In the Tweed during the winter the relatively depleted δ^{15}N values were matched by higher C/N and lower PON%. During the Dec-03 survey higher river discharge and overland surface flow conditions may have incorporated higher proportions of plant detritus and fresh litter to the agricultural soil-derived organics. Hence, the differences in freshwater $\delta^{15}N_{PON}$ signatures between the seasons in the Tweed are at least partially due to differences in river discharge and the dilution of ^{15}N-enriched terrestrial agricultural soil OM by ^{15}N-depleted fresh plant litter-derived remains in the winter months. Results from ^{14}C AMS analyses support this conclusion, since POM samples were found to be hundreds of years older in the summer compared to the winter (Chapter 4).

Since both winter transects reported here for the Tyne had higher river discharge than the summer surveys, one would expect to find seasonal differences in PON sources in this estuary, whereby $\delta^{15}N_{PON}$ values show a ^{15}N-enrichment during summer baseflow conditions. However, results from this study indicated a lack of seasonal difference in Tyne in riverine source signatures. Firstly, this may reflect the dominance of peat-derived material during both seasons. The large organic inputs from peat with relatively depleted δ^{15}N values and high C/N ratios may predominate during all seasons. This deduction is backed up by ^{14}C radiocarbon ages which showed no clear seasonal trends (Chapter 4). In fact, the oldest POM from the four surveys to the Tyne reported here were measured during Mar-03. Secondly, peat-derived sources may not have a distinct $\delta^{15}N$ signature from fresh litter captured by winter surface flow, thereby producing $\delta^{15}N$ values that are seasonally invariant.

Influence of marine PON in the Tyne and Tweed Estuaries

The N isotopic composition of freshly produced North Atlantic plankton is expected to be around 5 to 6‰ (Waser et al., 2000). Given the short residence time of settling particles in the upper ocean we would therefore expect marine plankton-derived PON to make a significant contribution to estuarine PON during the summer months. In addition, % algal PON increases in the summer months at higher salinities in the Tyne estuary (Figure 4) suggest the possible influence of marine PON. Because of the low river discharges during the Jul-03 surveys, it is assumed that the most saline $\delta^{15}N_{PON}$ signatures measured in the Tyne (+6.0‰, 32.5 psu), and the Tweed (+6.3 ‰, 33.2 psu) during this period were the most representative coastal North Sea samples taken during this study. During the Jul-03 Tyne survey, the sharp increase in $\delta^{15}N$ values seaward is attributed to the influence of marine PON advected into the mouth of the estuary during spring tide conditions. This inference is supported by increases in the proportions of algal PON matching the increase in $\delta^{15}N$ seaward (Figure 4d). The most saline sample in the Tyne Feb-02 survey also possessed a $\delta^{15}N_{PON}$ signature which was more representative of marine material (+5.3‰). This sample was collected during an incoming tidal front associated with a winter storm event. Therefore, it is likely that this relatively heavy $\delta^{15}N_{PON}$ value, in connection with its elevated PON and SPM (Figure 2a) concentrations was the result of increased re-suspension of coastal sediments with marine $\delta^{15}N_{PON}$ signatures near the mouth of the estuary. In the Tweed Jul-03 survey, the sharp decline in $\delta^{15}N_{PON}$ values in salinities >25 psu can be attributed to the mixing of marine PON with relatively ^{15}N-enriched terrigenous PON during this season.

Potential isotopic effects caused by PON mineralization

Mineralization of organic matter has been shown to cause isotopic enrichment of PON in estuarine systems. For instance, in the marine environment isotopic enrichment of up to 5‰ in $\delta^{15}N_{POM}$ values has been shown during grazing by microzooplankton (Hoch et al., 1996), and many authors have attributed ^{15}N-enrichments in POM to microbial breakdown (Altabet and McCarthy, 1986; Macko et al., 1987; Saino and Hattori, 1980). The large declines in PON% in the low-salinity region (≤10 psu) in the Tyne Estuary may therefore engender fractionation of $\delta^{15}N$ values of PON. However, such an isotopic shift was not apparent in our data. As shown in Figure 8, there was no significant linear relationship between PON% and $\delta^{15}N_{POM}$ values ($r^2 = 0.08$, n = 12), suggesting that $\delta^{15}N_{PON}$ values in the Tyne Estuary were not significantly influenced by estuarial mineralization. Similarly the estuarine mineralization of PON appears to have little effect on the C/N ratios (Figure 8), which are invariant in the low- to mid-salinity range indicating the lack of preferential mineralization of N relative to C.

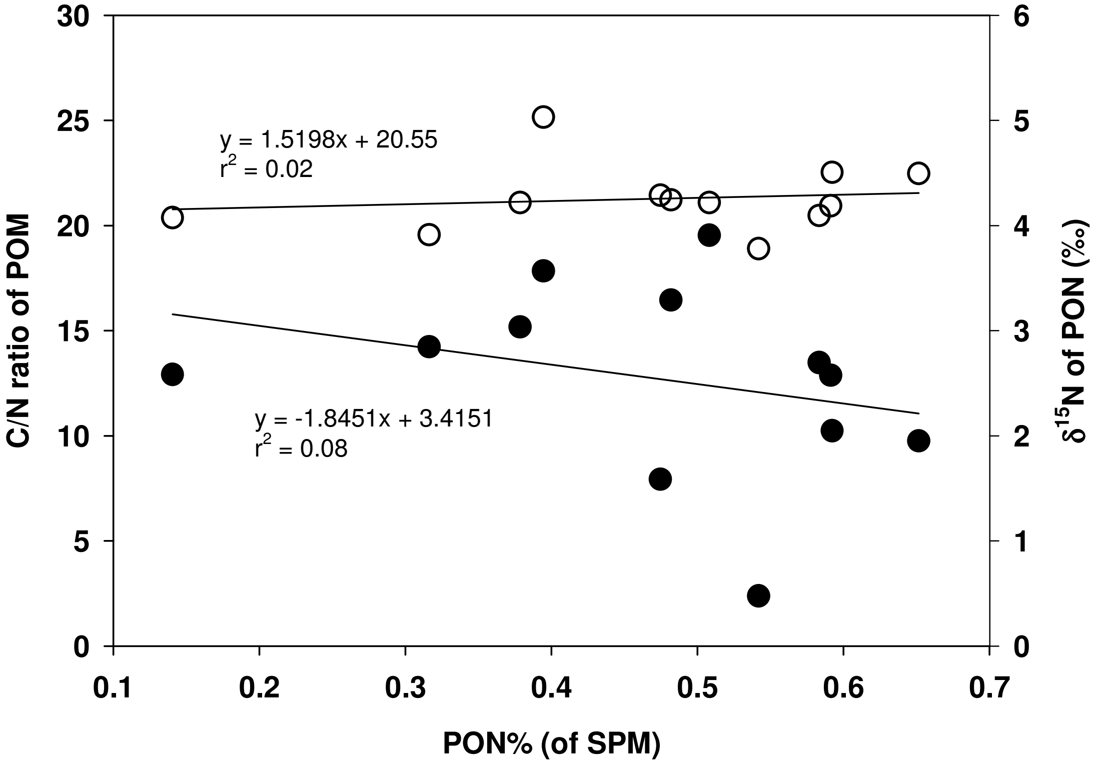

Figure 8. $\delta^{15}N$ values of particulate organic nitrogen ($\delta^{15}N_{PON}$: closed circles) and C/N ratios of particulate organic nitrogen (C/N_{POM}: open circles) in the low-salinity region (≤ 10 psu) of the Tyne Estuary plotted versus the percentage of PON comprising the SPM (PON%).

The influence of HSW discharge on $\delta^{15}N_{PON}$ signatures

In the Tyne, $\delta^{15}N$ values are generally lighter across the estuary in summer (Jul-02 and Jul-03 surveys) than in the winter. The lower river discharge during these periods therefore may have allowed the influence of Howden sewage works (HSW) discharge to be evident in their isotopic composition. Indeed, $\delta^{15}N$ values as low as -1.1‰ have been recorded for sewage-derived sludge (van Dover, 1992), and in general since sewage-derived OM is terrestrial in origin it usually possesses relatively ^{15}N-depleted $\delta^{15}N$ signatures (Tucker, 1999). During Jul-02, the sampling station closest to HSW showed a relatively ^{15}N-depleted signature (+0.8‰). The other $\delta^{15}N_{PON}$ values during this period were variable but generally lighter than the winter values. Similarly, during Jul-03 the stations landward of HSW had very depleted values. We attribute the depleted $\delta^{15}N$ values either to direct influence of HSW-derived PON and indirectly by the uptake of ammonia from Howden in the algal and/or bacterial biomass, since it has been shown that GF/F filters may retain 50-100% of total bacterial numbers (Altabet, 1990; Kirchman et al., 1994; Lee and Fuhrman, 1987).

As mentioned before, in cases where nitrogen is not limiting to primary production, isotopically light DIN should be preferentially utilised during uptake and assimiliation (Altabet et al., 1991; Altabet and McCarthy, 1985; Saino and Hattori, 1980). As a result of this isotope fractionation, $\delta^{15}N_{PON}$ signatures influenced by phytoplankton and/or bacteria should be less than or equal to the DIN source (Mariotti et al., 1984; McCusker et al., 1999; Ostrom et al., 1997). Due to discharges originating from HSW, ammonium is the most abundant species of DIN in the mid- to high-salinity regions of the Tyne Estuary (Chapter 1). Since it has been suggested that phytoplankton prefer NH_4^+ to NO_3^- (McCarthy, 1980), the high concentrations of ammonium would therefore imply that NH_4^+ is the more frequently used DIN source in the Tyne. The $\delta^{15}NH_4^+$ signature of HSW-derived ammonium in the Tyne was remarkably consistent throughout all four surveys (concentration weighted average = +10.6 ±0.5‰, Chapter 1). Therefore, it is possible that this potentially infinite supply of ^{15}N-enriched ammonium resulted in significant isotopic fractionation during algal and/or microbial uptake, thereby contributing relatively depleted $\delta^{15}N$ values to the POM pool.

Behaviour of high molecular weight dissolved organic nitrogen (HMW DON) concentrations

In Figure 9 concentrations of HMW DON across the Tyne (Mar-03 and Jul-03) and Tweed (Jul-03 and Dec-03) Estuaries during four different surveys are plotted versus salinity. In the Tyne Estuary (Figure 9a), HMW DON concentrations ranged from values of 7.9 to 0.6 μM and 2.6 to 0.4 μM during the Mar-03 and Jul-03 surveys, respectively. HMW DON concentrations in the Tweed Estuary (Figures 9b) ranged from values of 5.3 to 0.1 μM and 15.5 to 0.5 μM during the Jul-03 and Dec-03 surveys, respectively. During the four surveys HMW DON concentrations generally decreased with increasing salinity, showing net concave removals across both the Tyne and Tweed Estuaries. This removal was also observed in the HMW DOC fraction indicating the high reactivity of HMW DOM during transit through estuaries (Chapter 3).

Despite the net removal of HMW DON in the Tyne Estuary during the Mar-03 survey, minor non-conservative inputs could be seen at several sampling sites, most noticeably at salinities of 14.1 and 28.7. The small peak at 14.1 psu corresponds to the sampling site located nearest the Ouseburn, a heavily polluted minor tributary of the Tyne which converges with the main river approximately 10 km inland from the North Sea. The more noticeable input at 28.7 psu corresponds to the sampling site nearest the Howdon sewage works (Figure 1; Chapter 1). A significant contribution of sewage-derived HMW DOM in estuarine and coastal regions has been suggested by Wang et al. (2004). However, despite the noticeable inputs of HMW DON, increases in the HMW DOC fraction at these sampling were hardly perceptible, although large increases in LMW DOC were observed (Chapter 3). This suggests that the HMW DOM discharged by HSW in more enriched in N relative to other estuarine DOM sources. In addition, during the Jul-03 survey, HMW DOM contributions from HSW also appeared to be relatively minor (this study, Chapter 3). However, as indicated by the elevated ammonium concentrations across the entire salinity gradient, the influence of the HSW plume during this period could be found in parts of the estuary located far inland from the point of discharge.

Owing to the particularly low flow conditions experienced in the Tyne during the Jul-03 survey, a true freshwater end-member HMW DON sample could not be

Figure 9a

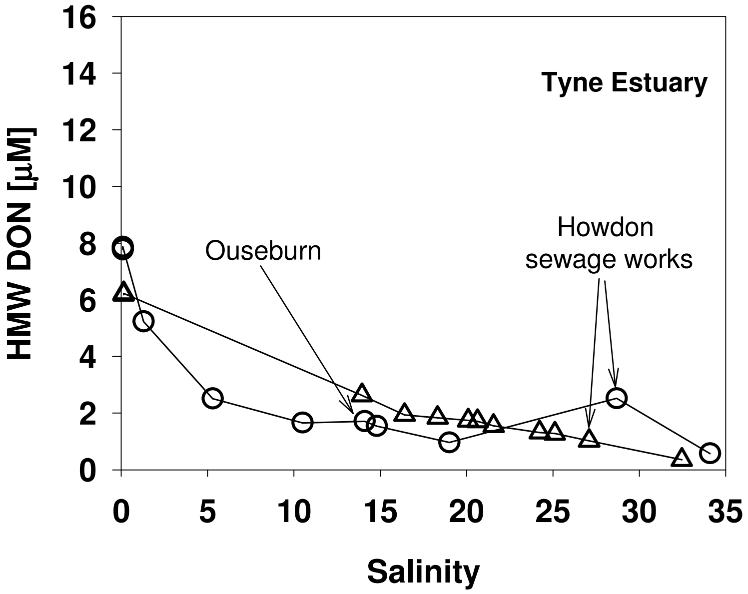

Figure 9b

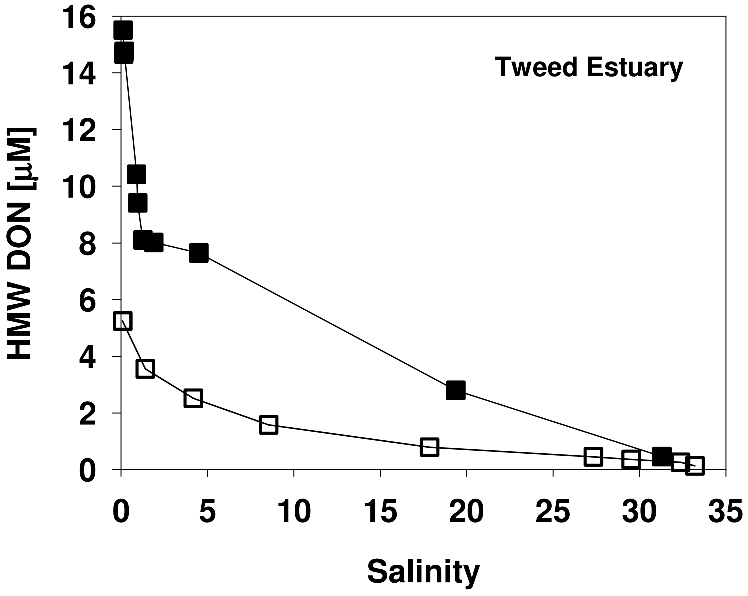

Figure 9. Concentrations of high molecular weight dissolved organic nitrogen (HMW DON) in the (a) Tyne Estuary during Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity. The riverine end-member from Jul-02 is plotted together with the data from the Jul-03 survey.

obtained. Therefore, it was not possible to assess the behaviour of HMW DON in the upper part of the Tyne estuary during the summer. However, samples collected during Jul-02, during which time river discharge was only slightly higher (Q = 8.6 versus 7.3 m^3/s), showed similar behaviour in total, LMW and HMW DOC concentrations across the salinity gradient (Chapter 3). Therefore, it was assumed that the freshwater (0.2 psu) end-member from the Jul-02 transect reflects an approximation of the freshwater HMW DON input during Jul-03. When plotting the Jul-02 freshwater HMW DON end-member (6.2 µM) with the Jul-03 data, HMW DON in the Tyne exhibited a slight removal across the salinity gradient, decreasing toward a value of 0.4 µM in the coastal North Sea.

In both the Tyne and Tweed Estuaries riverine (mean of values ≤ 0.2 psu) HMW DON inputs were higher in winter (7.8 µM, Mar-03, Tyne; 15.0 µM, Dec-03, Tweed) than during the summer (6.2 µM, Jul-02, Tyne; 5.2 µM, Jul-03, Tweed). However, the seasonal difference was less pronounced in the Tyne Estuary. If we assume that DOM fluxes are positively correlated with river discharge, as has been suggested by others (Clair et al., 1994; Tranvik and Jansson, 2002), then this may help to explain the smaller disparity found in riverine HMW DON concentrations between the two Tyne surveys (Q = 30.7 versus 8.6 m^3/s during Mar-03 and Jul-02, respectively) and the larger difference between Tweed surveys (Q = 151.2 m^3/s versus 14.5 m^3/s during Dec-03 and Jul-03, respectively).

Behaviour of C/N ratios of HMW DOM (C/N_{HDOM})

As Figure 10 shows, C/N ratios of HMW DOM (C/N_{HDOM}) in both estuaries during summer and winter showed a general decrease with increasing salinities. These changes across the salinity gradient were most pronounced in winter, with a much less pronounced variation in C/N_{HDOM} ratios observed during the July 2003 survey to the Tweed. For instance, C/N_{HDOM} ratios in the Tweed during (Figure 7b) Dec-03 decreased from a high of 27.0 (0.97 psu) to a low of 9.0 at the coastal North Sea (31.30 psu).

The average C/N_{HDOM} ratios in the freshwater (≤ 0.2 psu) end-members in the Tweed were 11.9 ±0.1 (n = 2) and 22.8 ±1.1 (n = 3) during the Jul-03 and Dec-03 surveys, respectively, which were very similar to the total estuarine averages (after combining freshwater samples) of 10.6 ±0.7 (Jul-03; n = 9) and 22.2±5.7 (Dec-03; n = 8). The C/N ratios of terrestrial OM (15-400) are generally higher than those of bacterioplankton and phytoplankton OM, which are typically characterised by C/N ratios less than 8 (Baird and Middleton, 2004; Cowie and Hedges, 1994; Goni and Hedges, 1995). The normal range for C/N ratios of riverine HMW DOM measured in estuaries reported in the literature is around 20-25 (Goni et al., 2003; Guo and Santschi, 1997; Wang et al., 2004). Therefore, exceptionally low C/N ratio of riverine HMW DOM during the summer survey of the Tweed requires an explanation. The low riverine C/N_{HDOM} ratio measured during the Jul-03 survey may suggest the mixing between high C/N terrestrial material and with N-enriched phytoplankton and/or bacterioplankton sources (Goni et al., 2003). While with the data presented here it was not possible to evaluate the contribution of bacterial-derived OM, the low chl-a levels found during the Jul-03 survey (Chapters 1-4) implied a limited contribution from algal-derived OM. Thus, we argue that the low C/N_{HDOM} ratios found here could be entirely of terrestrial origin. The low riverine C/N_{HDOM} ratio found here was similar to the average value of 9.4 ±1.1 reported for total DOC/DON in groundwater draining degraded peatlands (Kalbitz and Geyer, 2002). This is particularly relevant in the Tweed catchment, which is dominated by agriculture activity. These low ratios in degraded agricultural soils were most likely the result of significant alteration by microorganisms, which can lead to the accumulation of nitrogen-rich compounds and hence a higher N content (Goni et al., 2003; Rice and Hanson, 1984). Therefore, this might suggest that soil-derived OM comprised the bulk of the HMW DOM pool, as concluded for POM. Furthermore, since terrestrial plant litter generally has higher C/N ratios than soil organic matter (Kendall et al., 2001; Weiguo et al., 2003), the seasonal differences in C/N_{HDOM} ratios (and $\delta^{13}C_{HDOC}$ values; Chapter 3) in the Tweed can therefore be attributed to changes in the relative concentrations of dissolved C_3 plant material. During the winter, higher river discharge (151.2 m^3/s) would have resulted in a higher contribution from plant litter, and hence higher C/N_{HDOM} ratios. Conversely, during the less turbid, lower flow conditions (14.5 m^3/s) encountered during July 2003 it is likely that that the relative percentage of soil-derived OM was substantially higher. The C/N_{HDOM} ratios found in the lower part of the estuary during both winter (9.0) and summer (9.7) are typical for marine OM. The similarity between soil-derived and marine C/N_{HDOM} ratios in the Tweed during July 2003 explains the invariance of C/N_{HDOM} ratios across the estuary during this period. Conversely, the large almost linear decline in C/N_{HDOM} ratios in the Dec-03 survey of the Tweed suggests mixing between riverine HMW DOM of high C/N ratios with marine HMW DOM of low ratios.

Figure 10a

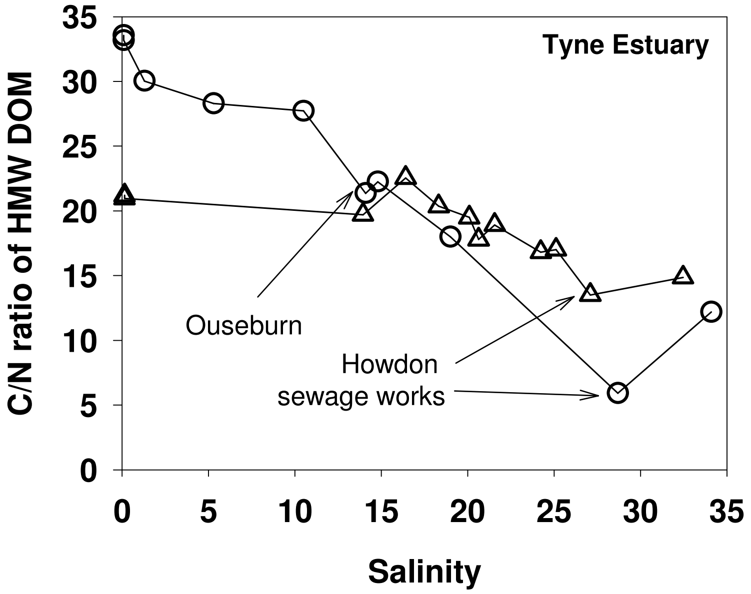

Figure 10b

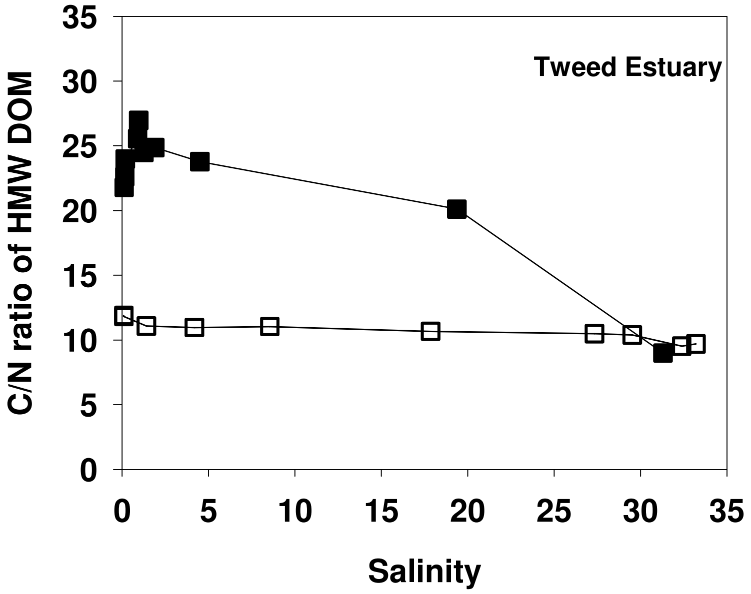

Figure 10. C/N ratios (molar) of high molecular weight dissolved organic nitrogen (C/N_{HDOM}) in the (a) Tyne Estuary during Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity. The riverine end-member from Jul-02 is plotted together with the data from the Jul-03 survey.

The average C/N_{HDOM} ratios in the freshwater (≤ 0.2 psu) end-members in the Tyne were 21.1 ±0.2 (n = 2) and 33.4 ±0.3 (n = 2) during the Jul-02 and Mar-03 surveys, respectively. As for the Tweed Estuary, the low chl-a levels found in the Tyne (Chapters 1-4) implies that autochthonous production did not make a significant contribution to HMW DOM to explain this seasonal change. C/N_{HDOM} ratios of riverine DOM entering the Tyne Estuary during the summer falls within the normal range (20-25) but during the winter the value (33.4 ±0.3, n = 2) is much higher than those found in other estuaries. It is possible the higher C/N_{HDOM} ratios entering the Tyne estuary during the winter suggest a greater contribution from plant-derived OM relative to soil-derived OM. However, during the Mar-03 survey the percentage of HMW comprising the total DOC (Chapter 3) in the freshwater end-member (35%) was almost twice as high as that during the Jul-02 survey (18%). Since humic material generally has higher C/N ratios than bulk soil (Seeber and Seeber, 2005), the higher C/N_{HDOM} values during the Mar-03 survey may therefore reflect a higher component of humic-rich peaty-soil, which as previously mentioned has been shown to exhibit relatively high (>30) C/N values (Novák et al., 1999).

C/N_{HDOM} ratios in the Tyne also showed a general decrease across the salinity gradient, although in contrast to the Tweed Estuary the coastal North Sea ratios (12.2, Jul-02, 14.9, Mar-03) were slightly higher. Nonetheless, the C/N_{HDOM} ratios measured at the mouth of the Tyne Estuary were within the wide range of values reported for marine HMW DOM (~9-20) in the literature (Benner et al., 1997; Goni et al., 2003; Guo and Santschi, 1997; Wang et al., 2004). During the Mar-03 survey an anomalously low C/N_{HDOM} value (5.9) was measured at the sampling location nearest the Howdon sewage works (28.7 psu), suggesting an estuarial input of sewage-derived OM. In contrast to the winter, the change in C/N_{HDOM} ratio at the site nearest the Howdon sewage works during the summer (27.1 psu) was not as pronounced as during the winter, implying a limited contribution from sewage-derived HMW DOM during the July 2003 survey. In any case, it is likely that during both seasons the C/N_{HDOM} ratios near the mouth of the Tyne Estuary were influenced by HSW-related inputs with low C/N ratios, thus complicating the assignment of a marine C/N_{HDOM} end-member for this system. Nevertheless during both seasons the large estuarial decline in C/N_{HDOM} ratios implies mixing between high C/N peat-derived HMW DOM and low C/N marine- and HSW-derived HMW DOM.

$\delta^{15}N_{HDON}$ signatures: General trends

Figure 11 shows the $\delta^{15}N$ values of HMW DON ($\delta^{15}N_{HDON}$) plotted versus salinity in the Tyne (Figure 11a) and Tweed (Figure 11b) Estuaries during four

Figure 11a

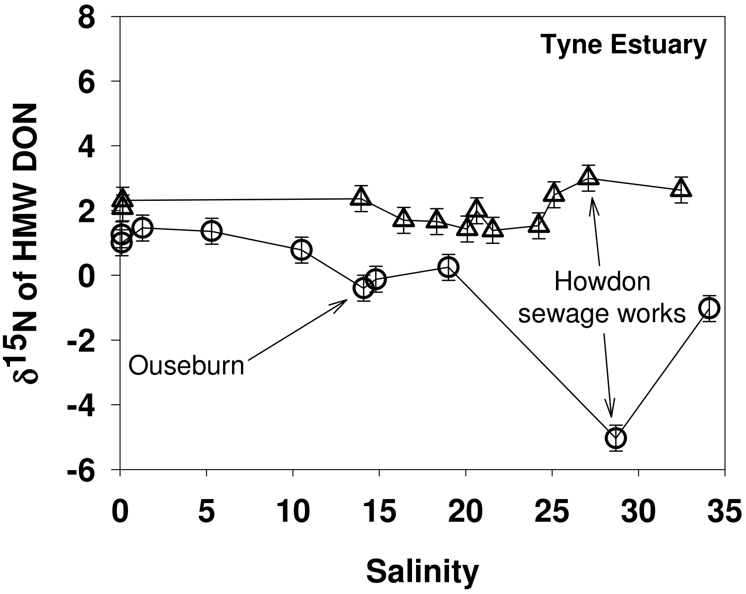

Figure 11b

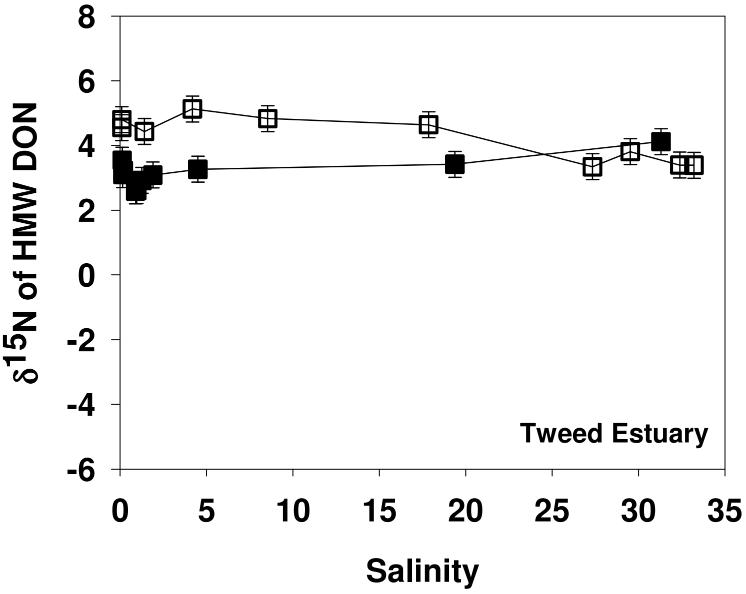

Figure 11. δ^{15}N values of high molecular weight dissolved organic nitrogen ($\delta^{15}N_{HDON}$) in the (a) Tyne Estuary during Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares) plotted versus salinity. The riverine end-member from Jul-02 is plotted together with the data from the Jul-03 survey.

different surveys. In general, more ^{15}N-depleted $\delta^{15}N_{HDON}$ signatures were found in the winter and in the Tyne, with the lowest value (-5.0‰) recorded during Mar-03 at the sampling site nearest HSW. A slight decrease in $\delta^{15}N_{HDON}$ signatures was also found at the sampling locations close to the Ouseburn, the heavily polluted minor tributary. In the Tyne during the Mar-03 and in the Tweed during Jul-03, $\delta^{15}N$ signatures of HMW DON generally decreased across the estuary with increasing salinity. Conversely, in the Tyne during Jul-03 and in the Tweed during Dec-03, the opposite trend was found, in which $\delta^{15}N$ HDON values showed a slight ^{15}N-enrichment with increasing salinity. In the Tyne during Jul-03, the trend of lighter values in the mid-salinity region is attributed to the landward advection of HSW-derived discharge. Heavier $\delta^{15}N_{HDON}$ values at higher salinities are suggestive of the greater influence of marine-derived HMW DON (+2.6‰, 32.5 psu) in the lower part of the estuary. In the Tweed, a similar marine end-member was found for both seasons (>30 psu, +3.6 ± 0.4‰, n = 3), indicating that mixing with marine-derived HMW DON was responsible for the seaward trends of decreasing $\delta^{15}N_{HDON}$ values during summer and increasing $\delta^{15}N_{HDON}$ values during winter.

Contribution from terrestrial $\delta^{15}N_{HDON}$ sources

Since there was a significant difference in $\delta^{15}N_{HDON}$ signatures (~ 2‰) between the combined freshwater end-members in the Tyne (+1.6 ±0.5‰, n = 2) and Tweed (+3.7 ±0.8‰, n = 2) Estuaries, differences in the soil characteristics between the two watersheds presumably play an important role in controlling $\delta^{15}N_{HDON}$ values, as discussed for C/N_{HDOM} ratios. The lower C/N_{HDOM} ratios found in the Tweed were attributed to a greater proportion of agricultural soil-derived OM. In addition, Kalbitz and Geyer (2002) have suggested that DON release in degraded peatlands is promoted when inorganic nitrogen is added to the soil. The main cause of the elevated nitrate concentrations found in the River Tweed (Chapter 1; Balls, 1994, Uncles et al., 2003) is likely a result of agricultural activity in the watershed. Therefore, one would expect that HMW DON levels would be relatively high in the Tweed (compared to HMW DOC concentrations), resulting in lower C/N_{HDOM} ratios. According to Kalbitz and Geyer (2002), DON from these ‘degraded’ soils should also possess relatively more ^{15}N-depleted $\delta^{15}N$ signatures, which is not the case for the Tweed samples. However, after changing the land use from intensive crop production to unimproved grassland, Kalbitz and Geyer (2002) noted a significant positive shift (>1‰) in $\delta^{15}N_{HDON}$ signatures from these degraded peatlands. The more ^{15}N-enriched values for HMW DON were attributed to isotopic fractionation caused by enhanced microbial alteration. Since the Tweed catchment basin is predominantly rural, unimproved grasslands should not be uncommon in this region. Therefore, the more ^{15}N-enriched $\delta^{15}N_{HDON}$ values found in the freshwater regions and throughout the rest of the Tweed Estuary may be the result of isotopic fractionation caused by microbial alteration at the source, as indicated by low C/N_{HDOM} ratios. This may explain why $\delta^{15}N_{HDON}$ signatures in the Tweed exhibited similar isotopic enrichment patterns as previously described for soil-derived PON.

If soil-derived organic matter was the chief source for both HMW DON and PON, as has been inferred in the Tweed Estuary, then one might expect to find a close link between $\delta^{15}N_{PON}$ and $\delta^{15}N_{HDON}$ signatures. In Figure 12, $\delta^{15}N_{PON}$ and $\delta^{15}N_{HDON}$ signatures from both estuaries and from both seasons are plotted against each other. As this figure illustrates, there is a much stronger linear relationship between $\delta^{15}N_{PON}$ and $\delta^{15}N_{HDON}$ signatures in the Tweed Estuary ($r^2 = 0.61$, n = 18) than there is in the Tyne Estuary ($r^2 = 0.02$, n = 22). The strong correlation between $\delta^{15}N$ values of PON and HMW DON in the Tweed strengthens the argument that organic nitrogen in this estuary is predominantly terrestrial in origin, with minor contributions coming from either algal or anthropogenic sources. Conversely, the very poor correlation between $\delta^{15}N$ values of PON and HMW DON in the Tyne suggests that in addition to terrigenous organic matter, estuarial inputs and/or biogeochemical processing have a more important role in the cycling of organic nitrogen within this system.

Microbial influences and contribution from sewage-derived HMW DON sources

The direct input of ^{15}N-depleted HMW DON into the Tyne from HSW or the Ouseburn may provide one explanation for the relatively light $\delta^{15}N_{HDON}$ values found within this estuary. As mentioned previously, sewage-derived organic nitrogen should possess relatively ^{15}N-depleted $\delta^{15}N$ signatures. Therefore, the light $\delta^{15}N_{HDON}$ signature (-5.0‰) found near HSW during the Mar-03 survey indicates a local input. A relative ^{15}N-depletion in HMW DON signatures influenced by anthropogenic contamination has been observed by others in the coastal environment (Wang et al., 2004). However, in addition to DON originating directly from the effluent, it is also possible that HMW DON was produced within the sewage plume, since production

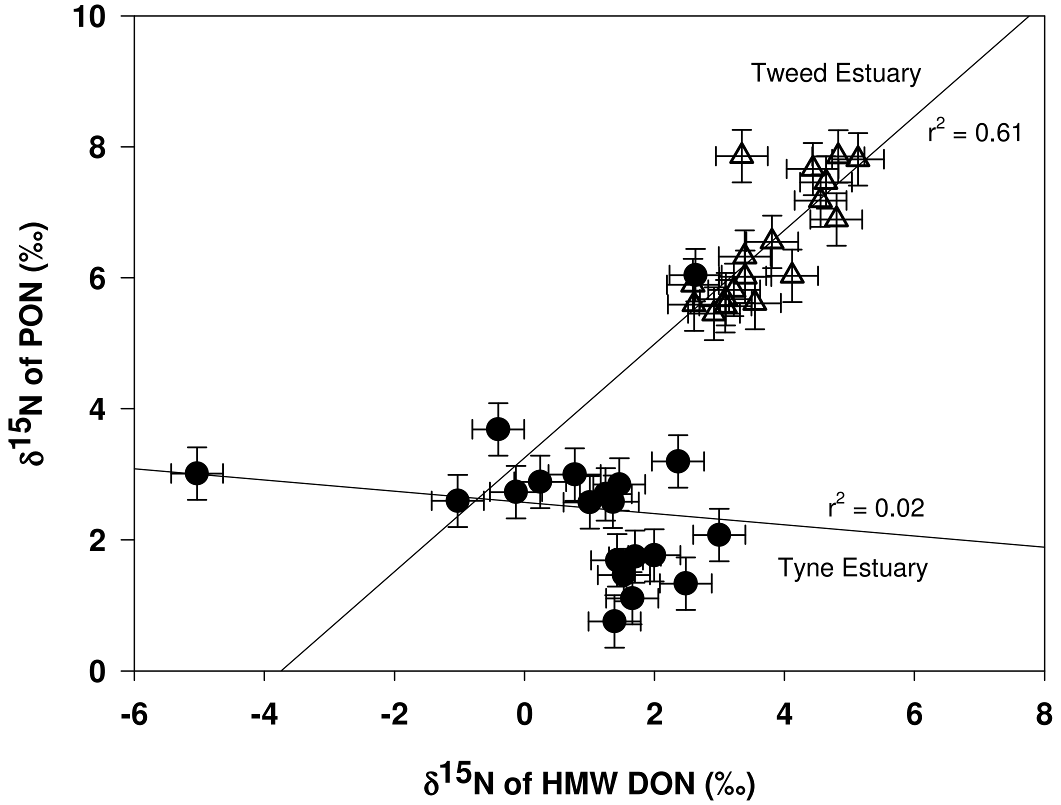

Figure 12. Relationship between $\delta^{15}N$ values of particulate organic nitrogen ($\delta^{15}N_{PON}$) and $\delta^{15}N$ values of high molecular weight dissolved organic nitrogen ($\delta^{15}N_{HDON}$) in the Tyne (Mar-03, Jul-03) and Tweed (Jul-03, Dec-03) Estuaries.

would result in isotopically lighter material. According to Ward and Bronk (2001), a major mechanism for DON release in surface marine waters is through grazing of phytoplankton and bacteria by zooplankton and protozoan. Furthermore, studies have shown that a significant fraction of HMW DOM can be derived from bacteria (Zou et al., 2004). Since we can rule out the influence of phytoplankton during the Mar-03 survey, and large bacterial numbers would be expected within or near a sewage outlet, it is possible that the consumption of heterotrophic bacteria (bactivory) resulted in the generation of ^{15}N-depleted HMW DON. In addition, the relatively low C/N_{HDOM} ratio associated with the Howdon signal also suggests a large contribution from bacterial matter, which generally has a C/N ratio of around 5 (Savin et al., 2001). Isotopically light DON associated with microbial food web interactions has been previously documented in experiments carried out by Hoch et al. (1996), who by mass difference calculations found a ^{15}N-depletion in total DON relative to NH_4^+ and PON in microzooplankton batch cultures.

The large ammonium peaks consistently observed near the Howdon sewage works (Chapter 1) supports the theory that the spike in HWM DON and its associated isotopically light signature found during the Mar-03 survey was related to bacterial growth at this site. Using d-amino acids as a biomarker to trace microbial-derived dissolved and particulate organic matter in the Arctic Ocean, Dittmar et al (2001) found evidence for a bacterial contribution to marine DON. Therefore, as previously described for trends observed in $\delta^{15}N_{PON}$ signatures, if bacteria present near HSW were producing HMW DON via assimilation of NH_4^+ then the $\delta^{15}N_{HDON}$ signature should be more ^{15}N-depleted than the $\delta^{15}NH_4^+$ substrate due to the preferential removal of isotopically light ammonium. In any case, whether through direct sewage inputs or via secondary processing, it is clear that HSW played an important role in generating light $\delta^{15}N_{HDON}$ values in the Tyne Estuary.

CONCLUSIONS

The unique relationships between C/N_{POM} and $\delta^{15}N_{PON}$ ratios in both the Tyne and Tweed Estuaries (Figure 13) led us to conclude that terrigenous material and to a lesser extent algal and/or bacterial uptake (in the Tyne) were the main factors in controlling the rather dynamic $\delta^{15}N_{PON}$ values found in theses systems. As these plots illustrate, relatively lower C/N_{POM} ratios in the Tweed (Figure 13b) generally corresponded with heavier $\delta^{15}N_{PON}$ values, with this tendency becoming more pronounced during the summer. This relationship in the Tweed reflects mainly seasonal variations in sources and their dominance in estuarine PON signatures. Agricultural soil-derived sources in the summer dominated the estuary with enriched $\delta^{15}N$ and lower C/N ratios, whereas during the winter the admixture of fresher surface derived plant debris produced slightly lower $\delta^{15}N$ and higher C/N ratios. In the Tyne the trends were much more variable (Figure 13a). The winter transects closely clustered around $\delta^{15}N$ and C/N_{POM} ratios of around +3.2 ±0.7‰ and 21 ±2.1, respectively, reflecting the predominance of peat-derived inputs of POM into the estuary during this period. However, the summer values were highly variable due to direct and indirect influence of Howden and marine algal material. During Jul-03 both depleted $\delta^{15}N$ values landward and enriched $\delta^{15}N$ values seaward were produced by the influence of Howden and marine algae, respectively, and these were

Figure 13a

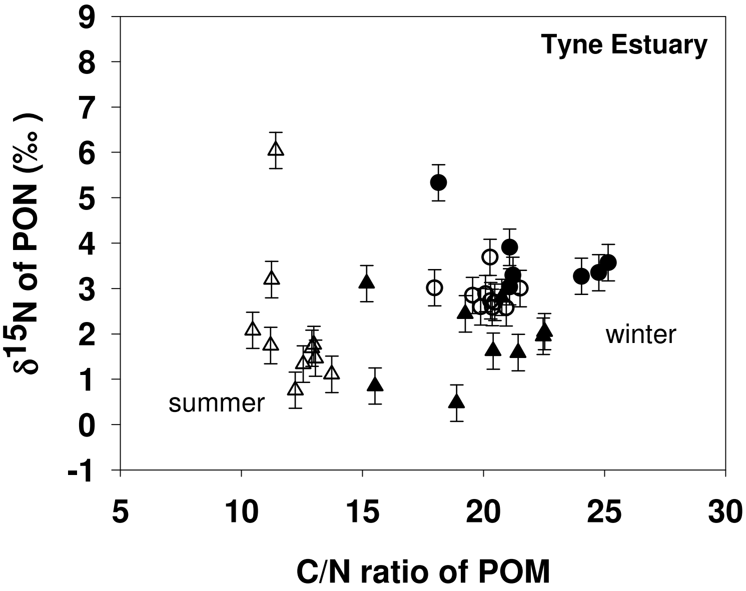

Figure 13b

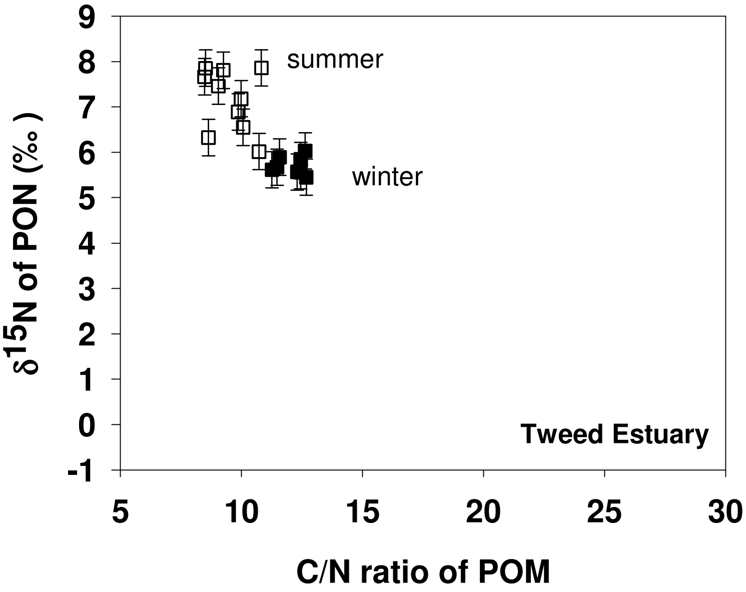

Figure 13. Relationship between δ^{15}N values of particulate organic nitrogen ($\delta^{15}N_{PON}$) and C/N$_{POM}$ ratios in the (a) Tyne Estuary during Feb-02 (closed circles), Jul-02 (closed triangles), Mar-03 (open circles) and Jul-03 (open triangles) and in the (b) Tweed Estuary during Jul-03 (open squares) and Dec-03 (closed squares).

matched by lower C/N$_{POM}$ ratios. In Jul-02, the C/N$_{POM}$ ratios were slightly higher than those measured during the following summer, indicating a larger algal contribution in the POM pool during Jul-03. The higher contribution from algal sources during this transect was confirmed by higher estimates of % algal PON

(Figure 4). In addition to direct inputs of ^{15}N-depleted HSW-derived POM, the large supply of isotopically enriched ammonium originating from HSW (Chapter 1) is thought to generate ^{15}N-depleted PON values due to isotopic fractionation caused by algal and/or bacterial assimilation.

In the Tweed, no additional source inputs of HMW DON were observed across the estuary during either summer or winter surveys. While distinct seasonal differences in C/N_{HDOM} ratios were found, these were attributed mainly to changes in soil-derived HMW DOM dynamics. A decent relationship between $\delta^{15}N_{PON}$ and $\delta^{15}N_{HDON}$ signatures ($r^2 = 0.61$, $n = 18$) indicated that soil-derived organic matter was the chief source for both PON and HMW DON in the Tweed Estuary. In the Tyne, however, a noticeable peak in HMW DON was observed near the HSW during the Mar-03 survey. This non-conservative input was associated with a low C/N_{HDOM} ratio and a very light $\delta^{15}N_{HDON}$ signature. Therefore, the lighter $\delta^{15}N_{HDON}$ values found throughout the Tyne Estuary during both winter and summer were largely influenced by either direct inputs or secondary processing of sewage-derived discharges. A very poor relationship between $\delta^{15}N_{PON}$ and $\delta^{15}N_{HDON}$ signatures ($r^2 = 0.02$, $n = 22$) confirmed that in the Tyne, in addition to terrestrial organic matter sources, estuarial inputs and biogeochemical processing played a more important role in the cycling of HMW DON.

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SUMMARY AND CONCLUSIONS

This study examined the sources and transformations of organic matter and dissolved inorganic nitrogen (DIN) in two contrasting North Sea Estuaries using a wide variety of geochemical and isotopic parameters. In order to properly assess the potential variability within and between these two estuaries, samples were collected from the Tyne (Feb-02, Jul-02, Mar-03, Jul-03) and Tweed (Jul-03, Dec-03) during different seasonal and hydrological conditions. In addition to seasonal and hydrological controls, this study has shown that land use patterns, sewage inputs and freshwater flushing time are the main influences in determining the behaviour and origin of organic matter and DIN entering the coastal North Sea in these two systems.

In both the Tyne and Tweed Estuaries *in situ* processing of dissolved inorganic nitrogen (DIN) was relatively minor (although more significant during the summer surveys), with mixing between different sources being the main factor in controlling the distribution of nitrate and ammonium across the salinity gradient. In the Tyne, ammonium concentrations and $\delta^{15}NH_4^+$ signatures were primarily determined by large discharges emanating from the Howdon sewage works (HSW). These elevated inputs of ammonium lead to enhanced nitrification in adjacent North Sea waters, as evidenced by isotopically light $\delta^{15}NO_3^-$ values found near the mouth of the Tyne indicating a source of nitrification-generated nitrate. Nitrification caused by these high ammonium concentrations leads to high N_2O supersaturations in the Tyne (Barnes, 2003), demonstrating that sewage-derived nitrogen inputs can indirectly result in large emissions of a very effective greenhouse gas which has tremendous implications for global climate change (Galloway et al., 1995; Lashof and Ahuja, 1990). This study has also demonstrated that during periods of elevated river discharge associated with winter storm events atmospherically-derived nitrate comprises a significant percentage of the riverine nitrate entering the North Sea. In the Tweed, enhanced agricultural activity in the watershed leads to elevated nitrate inputs into the estuary relative to the Tyne. These findings have important implications on a global scale. For instance, results from this thesis imply that anthropogenic inputs of agricultural-, sewage- and atmospheric-derived DIN may bypass estuarine processing in some systems and have a greater impact in coastal environments. Furthermore, as illustrated by Figure 6, Chapter 1, the bulk of the anthropogenic nitrate was delivered to the North Sea during rarely sampled storm events. If these trends are applicable to other estuarine systems then the estimated flux of anthropogenic fixed nitrogen delivered to the world's oceans (~ 59 Tg N yr^{-1}; Galloway et al., 1995) is likely too low, leading to a greater perturbation of the global nitrogen cycle than previously envisioned.

Slightly ^{13}C-enriched δ^{13}C values of dissolved inorganic carbon ($\delta^{13}C_{DIC}$), combined with low pCO_2 (<2 times atm. pres.) and ^{18}O signatures of dissolved oxygen ($\delta^{18}O_{DO}$) lower than expected for equilibrium with the atmosphere across the salinity gradient pointed to net autotrophy in the summer in the Tweed Estuary. Conversely, in the Tyne during the summer and in the Tweed during the winter higher pCO_2 (up to 6.5 and 14.4 times atm. pres. in the Tweed and Tyne, respectively), more ^{13}C-depleted $\delta^{13}C_{DIC}$ and ^{18}O-enriched $\delta^{18}O_{DO}$ indicated heterotrophy as the dominant process. However, in both the Tyne and Tweed Estuaries relatively low chlorophyll a (chl-a) levels (<5 μg/L) indicated that autochthonous production had only a minor impact on the biogeochemical cycling of carbon and nitrogen. This was verified by high suspended particulate organic carbon (POC) to chl-a ratios, which demonstrated that algal material generally comprised no greater than 15% of the POC pool. Considering the quick flushing time of the Tweed, which would preclude significant algal cell division in the water column (Uncles et al., 2000), it was concluded that autotrophy during the summer was dominated by bed-anchored algae, as has been suggested by others for this estuary (Howland et al., 2000).

As indicated by relatively depleted $\delta^{13}C_{POC}$ and $\delta^{13}C_{HDOC}$ values and C/N ratios greater than the Redfield ratio (~7), the bulk of the suspended particulate organic matter (POM) and high molecular weight dissolved OM in the Tyne and Tweed estuaries was dominated by terrigenous material. Higher C/N ratios and lower δ^{15}N values for OM in the Tyne were indicative of the greater contribution form peaty soils and C_3 vascular plant debris, whereas lower C/N ratios and higher δ^{15}N values in the Tweed (e.g., POM during the summer) suggested the greater influence of N-enriched agricultural soil inputs. A higher component of marine- (Tyne and Tweed) and sewage-derived OM (Tyne) was observed in the lower part of the estuaries during the Jul-03 surveys, when dry summer conditions resulted in lower river discharges. A HSW-derived point source input was also observed in the Tyne during the Mar-03 survey, as indicated by an elevated HMW DON concentration and substantially ^{15}N-depleted δ^{15}N signature. In addition to a direct input of HSW-derived material, slightly ^{15}N-depleted δ^{15}N values for PON in the summer (Chapter 5) suggested an enhanced biological modification of DIN caused by the preferential removal of ^{14}N during bacterial and/or phytoplankton uptake. This study therefore demonstrates that large inputs of anthropogenic ammonium will have both direct and indirect impacts on the cycling of organic nitrogen within estuaries.

Relatively old radiocarbon ages (100s to 1000s of years) confirmed the predominantly terrestrial origin for POM in both the Tyne and Tweed Estuaries. Indeed, since algal contributions were low, it is not surprising that the POM was comprised mainly of old, recalcitrant soil-derived OM. More ^{14}C-depleted (i.e., older) Δ^{14}C values in the Tyne suggested a source of sedimentary fossil carbon (i.e., coal) in addition to peat-derived OM. During higher winter discharge, the River Tweed exported a larger amount of younger, more labile POC to the coastal zone. In the maximum turbidity zone (MTZ) of the Tyne, decreases in POC % with increasing salinity in some instances coincided with an increase in POC age. In addition to mixing with older sediments, it is possible that this increase in age was caused by the preferential loss of the younger, more labile fraction during mineralisation.

Significant removals of both HMW DOC (Chapter 3) and POC (Chapter 4) were observed across the salinity gradient. These removals were associated with relatively high pCO_2 values that are comparable to those reported in other estuaries in Western Europe (Abril et al., 2002; Barth et al., 2003; Frankignoulle et al., 1998). In these studies, the large CO_2 fluxes emitted from these estuaries were mostly attributed to the mineralisation of labile, pollution-derived organic matter. While HSW-derived OM likely contributed to CO_2 production in the Tyne, pollution-derived anthropogenic carbon inputs into the relatively pristine Tweed are considered minor. Therefore, contrary to the popularly held notion that terrigenous OM is recalcitrant and refractory during estuarine mixing (Abril et al., 2002; Álvarez-Salgado and Miller, 1999; Mantoura and Woodward, 1983), this study has demonstrated that a significant percentage of terrestrially-derived entering North Sea Estuaries is relatively labile and is oxidized to CO_2, providing a positive feedback mechanism for climate change.

Large, concave removals of terrigenous high molecular weight (HMW) DOC were associated with a non-conservative ^{13}C-enrichment in δ^{13}C signatures. Due to the lack of any identifiable estuarine inputs, it was proposed that this isotopic enrichment may have been the result of the selective removal of isotopically lighter terrestrial HMW DOC caused by flocculation, bacterial and/or photochemical oxidation. If the ^{13}C-enriched behaviour of δ^{13}C values found in this study was indeed caused by the preferential removal of ^{13}C-depleted HMW DOC, then rapid estuarine removal of HMW DOC as observed here and by other workers (Guo and Santschi, 1997; Santschi et al., 1995) implies that delineating terrestrial-derived HMW DOC in an estuarine environment may often prove quite difficult when using δ^{13}C signatures. In addition, an enriched carbon isotope signal of residual terrestrial carbon leaving estuaries could lead to the underestimation of terrestrially-derived carbon present in the marine DOC pool when terrestrial end member values are assigned in the partitioning calculations.

While radiocarbon dates showed an export of old (100-1000s of years) terrigenous POC to the North Sea, ^{14}C-enriched Δ^{14}C values indicated a riverine input of young (modern) terrestrially-derived HMW DOC. The large disparity in age between HMW DOC and POC found in this study shows that older, more refractory peat-derived carbon is exported to the world's oceans in particulate rather than dissolved form. Concurrent with significant removals across the salinity gradients, this study has therefore demonstrated that HMW DOC is a very reactive component of the total DOC pool, whether from predominantly peat- (Tyne) or agricultural soil-derived sources (Tweed). Thus, it is highly likely that similar removals in HMW DOC during estuarine mixing are occurring in other systems, although in many cases these removals may be masked by simultaneous estuarine inputs.

This study has found a progressive increase in Δ^{14}C signatures of HMW DOC across the salinity gradient, indicating mixing with a highly ^{14}C-enriched North Sea source attributed to anthropogenic releases (Cook et al., 1998; Gulliver et al., 2004). . While the exact source of the elevated Δ^{14}C values in North Sea OM remains unknown, it is likely that this ^{14}C-enrichment made its way into the marine carbon cycle via the DIC reservoir. However, studies have shown that the Δ^{14}C signature of North Sea DIC is only around 200‰ (Le Clercq et al., 1997), much lower than the value of ~1000‰ predicted for North Sea HMW DOC in this thesis. Considering the large difference in ^{14}C-enrichment between the organic and inorganic fractions, this implies that there must be a significant 'lag' effect between the Δ^{14}C signature of DIC and that of recently fixed carbon. Reasons for this include mixing and dilution of anthropogenic ^{14}C with the much older North Sea DIC pool and loss of DIC due to equilibration with the atmosphere. Therefore, it appears that anthropogenic ^{14}C may become rather persistence in the OM fraction, exhibiting ^{14}C-enrichment long after its initial entry into the marine food chain.

As this thesis has demonstrated, it is difficult to asses the origins of DIN and organic matter in river-dominated estuaries without fully delineating the source end-members. Future studies into similar systems should incorporate more detailed end-member sampling protocols in order to reduce potential ambiguity in signal overlap. For instance, while this study found pronounced differences in $\delta^{15}NO_3^-$ signatures within and between the Tyne and Tweed Estuaries, due to isotopic source signal overlap we were not able to distinguish between several possible origins. For example, during the Feb-02 survey to the Tyne the relatively high inputs of nitrate were substantially ^{15}N-depleted compared to the other three surveys to this estuary. While these light $\delta^{15}NO_3^-$ values suggested a greater proportion of atmospheric-derived nitrate, it was unclear as to what extent ^{15}N-depleted soil-derived nitrate also contributed to the overall $\delta^{15}NO_3^-$ signature. Studies have demonstrated that a more precise isotopic fingerprint for nitrate can be obtained when δ^{15}N is used in conjunction with δ^{18}O (Chang et al., 2002; Kendall, 1998). Therefore, in order to narrow the range of potential sources for terrestrial-derived nitrate inputs into estuarine systems, in the future the dual isotope approach should be considered.

This study has also demonstrated the difficulty involved with fully assessing estuarine sources for ^{14}C- and ^{13}C-enriched bulk HMW DOC (Chapter 3). While it was suggested that anthropogenic discharges resulted in elevated Δ^{14}C values for North Sea OM, bulk HMW DOC measurements precluded the possibility of elucidating the exact molecular fingerprint of this ^{14}C-enriched material. In conjunction with a more detailed sampling strategy that includes a wide variety of different potential organic matter sources across the entire catchment basins (i.e., soil samples, marsh organic matter, macrophytic material, etc.), future studies would benefit from the use of compound-specific isotopic analysis (CSIA) of individual terrestrial, estuarine and marine biomarkers. For instance, CSIA has previously demonstrated its potential to delineate pathways of carbon cycling in microbial communities in peat deposits (Pancost and Sinninghe Damsté, 2003; Pancost et al., 2000) and in providing evidence for an important bacterial role in the formation of HMW DOM is estuarine environments (Zou et al., 2004). CSIA may thus be able to provide valuable insight into the extent of microbial utilisation of terrestrial OM along its entire journey from source to sea.

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APPENDIX 1: Total DIC Determinations

Tyne

Date	**Temp [oC]**	**K CO2**	**K H2CO3**	**K HCO3**	**tot alk (mM)**	**tot alk (M)**	**pH**	**H2CO3 (M)**	**HCO3 (M)**	**CO3 (M)**	**DIC (mM)**	**pCO2**	**pCO2 [ppm]**
23-Jul-03	14.7	4.68E-02	3.74E-07	3.65E-11	2.34	0.002339	8.17	4.22E-05	2.33E-03	1.25E-05	2.39	9.02E-04	902
23-Jul-03	16.2	4.46E-02	3.85E-07	3.79E-11	2.79	0.002789	7.85	1.02E-04	2.79E-03	7.44E-06	2.89	2.29E-03	2292
23-Jul-03	16.6	4.40E-02	3.88E-07	3.82E-11	2.73	0.002729	7.81	1.09E-04	2.73E-03	6.71E-06	2.84	2.47E-03	2471
23-Jul-03	17.9	4.23E-02	3.98E-07	3.95E-11	2.67	0.002669	7.68	1.40E-04	2.67E-03	5.02E-06	2.81	3.31E-03	3314
23-Jul-03	18.8	4.11E-02	4.04E-07	4.03E-11	2.76	0.002759	7.57	1.84E-04	2.76E-03	4.12E-06	2.94	4.46E-03	4464
23-Jul-03	15.5	4.56E-02	3.80E-07	3.72E-11	2.79	0.002789	7.64	1.68E-04	2.79E-03	4.52E-06	2.96	3.69E-03	3687
23-Jul-03	19.2	4.06E-02	4.07E-07	4.07E-11	2.91	0.002909	7.55	2.01E-04	2.91E-03	4.19E-06	3.11	4.95E-03	4954
23-Jul-03	18.4	4.16E-02	4.01E-07	4.00E-11	2.85	0.002849	7.50	2.24E-04	2.85E-03	3.59E-06	3.07	5.39E-03	5389
23-Jul-03	19.3	4.05E-02	4.08E-07	4.08E-11	2.73	0.002729	7.52	2.02E-04	2.73E-03	3.68E-06	2.93	4.99E-03	4986
23-Jul-03	19.3	4.05E-02	4.08E-07	4.08E-11	2.73	0.002729	7.51	2.07E-04	2.73E-03	3.60E-06	2.94	5.10E-03	5103

Tweed

			K1	K2									
Date	**Temp [oC]**	**K CO2**	**K H2CO3**	**K HCO3**	**tot alk (mM)**	**tot alk (M)**	**pH**	**H2CO3 (M)**	**HCO3 (M)**	**CO3 (M)**	**DIC (mM)**	**pCO2**	**pCO2 [ppm]**
08-Jul-03	12.0	5.11E-02	3.53E-07	3.40E-11	2.31	0.002309	8.25	3.66E-05	2.30E-03	1.38E-05	2.35	7.18E-04	718
08-Jul-03	12.2	5.08E-02	3.54E-07	3.41E-11	2.31	0.002309	8.26	3.57E-05	2.30E-03	1.42E-05	2.35	7.02E-04	702
08-Jul-03	12.7	4.99E-02	3.58E-07	3.46E-11	2.25	0.002249	8.25	3.52E-05	2.24E-03	1.37E-05	2.29	7.04E-04	704
08-Jul-03	13.1	4.93E-02	3.61E-07	3.50E-11	2.25	0.002249	8.27	3.33E-05	2.24E-03	1.45E-05	2.29	6.76E-04	676
08-Jul-03	14.8	4.66E-02	3.74E-07	3.65E-11	2.19	0.002189	8.33	2.72E-05	2.18E-03	1.68E-05	2.22	5.84E-04	584
08-Jul-03	16.5	4.42E-02	3.87E-07	3.81E-11	2.19	0.002189	8.39	2.29E-05	2.18E-03	2.01E-05	2.22	5.19E-04	519
08-Jul-03	17.3	4.31E-02	3.93E-07	3.89E-11	2.13	0.002129	8.41	2.10E-05	2.12E-03	2.09E-05	2.16	4.86E-04	486
08-Jul-03	17.8	4.24E-02	3.97E-07	3.94E-11	2.13	0.002129	8.46	1.85E-05	2.12E-03	2.36E-05	2.16	4.36E-04	436
08-Jul-03	18.0	4.21E-02	3.98E-07	3.96E-11	2.13	0.002129	8.77	8.97E-06	2.10E-03	4.74E-05	2.16	2.13E-04	213
08-Jul-03	18.0	4.21E-02	3.98E-07	3.96E-11	2.13	0.002129	8.76	9.18E-06	2.11E-03	4.64E-05	2.16	2.18E-04	218

APPENDIX 1: Total DIC Determinations (continued)

Tweed

Date	**Temp [oC]**	**K CO2**	**K H2CO3**	**K HCO3**	**tot alk (mM)**	**tot alk (M)**
03-Dec-03	9.0	5.65E-02	3.30E-07	3.13E-11	0.94	0.000945
03-Dec-03	9.0	5.65E-02	3.30E-07	3.13E-11	0.94	0.000945
03-Dec-03	8.9	5.66E-02	3.30E-07	3.12E-11	0.90	0.000900
03-Dec-03	8.7	5.70E-02	3.28E-07	3.11E-11	1.02	0.001020
03-Dec-03	8.5	5.74E-02	3.27E-07	3.09E-11	1.24	0.001245
03-Dec-03	8.5	5.74E-02	3.27E-07	3.09E-11	1.44	0.001439
03-Dec-03	8.4	5.76E-02	3.26E-07	3.08E-11	0.97	0.000975
03-Dec-03	8.1	5.82E-02	3.24E-07	3.06E-11	1.86	0.001859
03-Dec-03	8.2	5.80E-02	3.24E-07	3.06E-11	2.13	0.002129
03-Dec-03	8.0	5.84E-02	3.23E-07	3.05E-11	2.22	0.002219

pH	**H2CO3 (M)**	**HCO3 (M)**	**CO3 (M)**	**DIC (mM)**	**pCO2**	**pCO2 [ppm]**
7.34	1.31E-04	9.44E-04	6.47E-07	1.08	2.31E-03	2314
7.32	1.37E-04	9.44E-04	6.18E-07	1.08	2.42E-03	2423
7.34	1.25E-04	8.99E-04	6.14E-07	1.02	2.20E-03	2201
7.42	1.18E-04	1.02E-03	8.32E-07	1.14	2.07E-03	2071
7.46	1.32E-04	1.24E-03	1.11E-06	1.38	2.30E-03	2300
7.57	1.19E-04	1.44E-03	1.65E-06	1.56	2.06E-03	2065
7.45	1.06E-04	9.74E-04	8.45E-07	1.08	1.84E-03	1841
7.68	1.20E-04	1.86E-03	2.71E-06	1.98	2.06E-03	2061
7.85	9.26E-05	2.13E-03	4.60E-06	2.22	1.60E-03	1597
8.10	5.45E-05	2.21E-03	8.45E-06	2.28	9.33E-04	933

APPENDIX 2: Volumes of water collected for SPM/POM analysis

The volumes of water filtered (ml) for the 1st GF/F filter collected at each site (2 to 4 filters were collected at most sampling stations):

Tyne - Feb 2002

salinity	Vol water filtered (ml)
31.7	980
17.7	480
15.0	470
9.9	340
6.9	280
3.0	260
2.1	260

Tyne - July 2002

salinity	Vol water filtered (ml)
33.2	3360
30.9	1140
25.9	1100
19.3	540
15.0	440
9.8	440
4.7	500
0.2	540
0.2	600

Tyne - March 2003

salinity	Vol water filtered (ml)
34.1	1020
28.7	880
19.0	400
14.8	320
14.1	360
10.5	220
5.3	220
1.3	220
0.1	460
0.1	440

Tyne - July 2003

salinity	Vol water filtered (ml)
32.5	3610
27.1	1560
25.1	1370
24.2	940
21.6	800
20.6	960
20.1	870
18.3	750
16.4	910
13.9	960

Tweed - July 2003

salinity	Vol water filtered (ml)
33.2	3820
32.4	3910
29.5	3050
27.4	1700
17.9	1620
8.6	1460
4.2	1360
1.4	1430
0.1	1200
0.1	1210

Tweed - December 2003

salinity	Vol water filtered (ml)
1.27	430
0.97	430
0.90	450
31.30	920
1.90	420
0.19	390
0.16	430
0.12	430
4.51	460
19.38	520

*Note: 25 L of water was collected at each site for high molecular weight dissolved organic matter (HMW DOM) analysis

APPENDIX 3:

Concentrations (wt %) of major elements (Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn and P) in Tyne surface sediments analyzed by X-ray fluorescence spectrometry

Date collected	grab sample, 0-1 or 0-2 cm core top	km from N Sea	%Si	%Al	%Fe	%Mg	%Ca
Jul-01	gs	4.2	23.17	6.74	3.53	1.44	3.61
Jul-01	gs	5.9	28.03	5.24	2.89	1.09	2.53
Jul-01	gs	7.9	20.96	7.72	4.37	1.48	3.05
Jul-01	gs	14.5	20.42	6.99	3.52	1.06	0.78
Jul-01	gs	15.1	33.18	4.26	2.73	0.69	1.02
Jul-01	gs	16.0	23.19	7.73	4.42	0.90	0.68
Jul-01	gs	18.8	23.12	6.99	3.93	0.92	0.99
Jul-01	gs	21.6	24.21	6.80	3.98	1.09	1.64
Jul-01	gs	22.8	21.84	7.54	4.38	1.14	1.63
Jul-01	gs	24.3	33.03	4.57	3.03	0.71	1.00
Jul-01	gs	25.7	20.62	7.99	4.98	1.27	1.52
Jul-01	gs	27.2	35.20	3.83	2.80	0.49	0.59
Jul-01	gs	28.8	27.01	6.47	4.24	0.84	1.02
Jul-01	gs	29.1	23.31	7.69	4.38	1.06	1.24
Jul-01	gs	26.0	24.24	7.63	4.26	0.93	1.52
Jul-01	gs	26.1	25.58	7.39	4.21	0.88	1.17
Feb-02	0-2	4.2	21.70	7.28	4.01	1.39	3.93
Feb-02	0-2	15.9	23.63	7.78	4.71	1.03	1.33
Feb-02	0-2	24.0	22.84	8.07	4.67	1.03	1.49
Jul-02	0-2	4.2	24.76	6.55	3.68	1.30	3.90
Jul-02	0-1	15.9	23.90	6.54	3.95	1.12	1.89
Jul-02	0-2	24.0	23.59	8.24	3.92	1.07	0.81
Jul-02	0-1	25.8	25.24	6.38	4.43	0.87	0.86
Mar-03	0-1	4.2	22.15	6.95	3.89	1.65	4.46
Mar-03	0-1	15.9	26.15	6.95	3.89	1.03	1.52
Mar-03	0-1	24.0	22.52	7.65	4.61	1.21	2.16
Mar-03	0-1	25.8	24.45	6.99	4.32	1.10	1.91

Note: These wt % values have not been salt corrected

APPENDIX 3 (continued):

Concentrations (wt %) of major elements (Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn and P) in Tyne surface sediments analyzed by X-ray fluorescence spectrometry

Date collected	grab sample, 0-1 or 0-2 cm core top	km from N Sea	%Na	%K	%Ti	%Mn	%P
Jul-01	gs	4.2	2.03	1.59	0.39	0.04	0.10
Jul-01	gs	5.9	1.48	1.30	0.31	0.03	0.07
Jul-01	gs	7.9	1.91	1.72	0.44	0.04	0.18
Jul-01	gs	14.5	2.02	1.47	0.38	0.03	0.12
Jul-01	gs	15.1	0.96	1.06	0.30	0.04	0.10
Jul-01	gs	16.0	1.36	1.62	0.45	0.03	0.12
Jul-01	gs	18.8	1.36	1.48	0.43	0.04	0.18
Jul-01	gs	21.6	1.26	1.53	0.43	0.05	0.17
Jul-01	gs	22.8	0.93	1.56	0.44	0.05	0.18
Jul-01	gs	24.3	0.78	1.05	0.27	0.04	0.10
Jul-01	gs	25.7	1.60	1.71	0.46	0.09	0.24
Jul-01	gs	27.2	0.42	0.94	0.25	0.07	0.08
Jul-01	gs	28.8	0.56	1.41	0.38	0.10	0.15
Jul-01	gs	29.1	1.12	1.64	0.44	0.10	0.21
Jul-01	gs	26.0	0.40	1.64	0.45	0.06	0.22
Jul-01	gs	26.1	0.85	1.58	0.43	0.06	0.17
Feb-02	0-2	4.2	1.47	1.76	0.43	0.07	0.11
Feb-02	0-2	15.9	0.70	1.73	0.47	0.10	0.16
Feb-02	0-2	24.0	0.37	1.74	0.47	0.07	0.18
Jul-02	0-2	4.2	1.37	1.66	0.39	0.04	0.10
Jul-02	0-1	15.9	1.29	1.50	0.41	0.05	0.16
Jul-02	0-2	24.0	0.99	1.78	0.48	0.03	0.16
Jul-02	0-1	25.8	1.15	1.46	0.40	0.08	0.15
Mar-03	0-1	4.2	1.28	1.68	0.41	0.05	0.11
Mar-03	0-1	15.9	1.08	1.57	0.42	0.04	0.12
Mar-03	0-1	24.0	0.79	1.74	0.46	0.06	0.16
Mar-03	0-1	25.8	0.76	1.65	0.44	0.05	0.14

Note: These wt % values have not been salt corrected

APPENDIX 3 (continued): XRF Analysis Methodology

Concentrations of major elements (Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn and P) in sediments collected from the Tyne Estuary were analyzed by X-ray fluorescence spectrometry of fused glass discs (45 mm diameter) using a method similar to that of Norrish and Hutton (1969). Approximately 1 g of powdered sediment was accurately weighed into a Pt-Au crucible and diluted with a 4:1 ratio (flux:sample) of lithium tetraborate flux (Johnson Matthey SPECTROFLUX 105). The crucible was then heated to 1100°C in the muffle furnace for 20 minutes. After cooling any further weight loss was made up with additional flux and the crucible reheated over a Bunsen burner. When completely fused the material was swirled and then moulded into a disc on a graphite mould (kept at 220°C on a hot plate) with a clean aluminium plunger (also at 220°C). The resulting glass disc was self supporting and could be placed directly in the X-ray beam. Standardization was achieved using USGS and other international rock standards which had been prepared in exactly the same manner as the samples. Based on multiple analyses of standards and samples, the precision for Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn and P measurements was found to be better than ±3% relative standard deviation (RSD).

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APPENDIX 4:

Scanning Electron Microscopy (SEM) Identification of Coal Particles in Suspended Particulate Matter (SPM) from the Tyne Estuary

(SEM analysis of a Nucleopore polycarbonate membrane (37 mm diameter)

Sample collected during the July 2002 survey (at a salinity of 19.3)