

Seasonal variability of inorganic and organic nitrogen in the North Sea

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Abstract This study considers the cycling of nitrogen in the waters of the North Sea, particularly focussing on organic nitrogen. Dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON) were measured in the North Sea over a one-year period (autumn 2004–summer 2005). The surface water concentrations of nitrate, ammonium, DON and PON during the present study ranged from <0.1 – 7.2 μM , <0.1 – 2.0 μM , 1.9 – 11.2 μM and 0.3 – 5.6 μM , respectively, with DON the dominant fraction of total nitrogen at all times. These nutrient concentrations were significantly lower compared to previous studies in the southern North Sea. The seasonal variations showed high mean surface concentrations of

nitrate (4.7 ± 0.6 μM) and DON (8.9 ± 0.9 μM), low ammonium (<0.1 μM) and PON (0.8 ± 0.1 μM) in winter, shifting to low nitrate (0.3 ± 0.3 μM) and DON (4.2 ± 1.2 μM) in summer, with high ammonium (0.8 ± 0.4 μM) in autumn and PON (2.5 ± 1.2 μM) in spring. Highest mean surface DON concentration was measured in winter and may be due to resuspension of the organic matter from the bottom sediments. For autumn and spring, phytoplankton DON release was likely to be the most significant source of DON as shown by high concentrations of low molecular weight (LMW) DON and its positive correlation to chlorophyll *a*. Low total and LMW DON concentrations during summer were likely to be due to the uptake of the LMW DON fraction by phytoplankton and bacteria and the stratification of the water column. DON is therefore shown to be a potentially important source of nitrogen in shelf seas especially after the spring bloom has depleted nitrate to limiting concentrations.

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Introduction

Nitrogen has been intensively studied due to its role as a limiting nutrient in the marine environment (Ryther & Dunstan, 1971) including the North Sea (Bauerfeind et al., 1990; Brockmann et al., 1990;

Riegman et al., 1990). In seawater, there are three main forms of dissolved inorganic nitrogen (DIN). They are nitrate, nitrite and ammonium. Nitrate is the thermodynamically dominant and most oxidised form of N, and ammonium is the most reduced form of environmental significance, with active exchange between these forms via intermediate nitrite. Other forms of N present in the water column are dissolved and particulate organic N (DON and PON, respectively) with the former representing a major N pool in marine systems (Bronk, 2002). However most studies, especially in the North Sea, have focussed on DIN (Wafar et al., 1983; Baeyens et al., 1984; Radach et al., 1990; Gentilhomme and Lizon, 1998; Ramirez et al., 2005) although Weston et al. (2004) emphasised the importance of PON in the summer cycling and transport of N in the southern North Sea. There have also been few systematic seasonal and spatial surveys of DON in coastal waters (Butler et al., 1979; De Galan et al., 2004; van der Zee & Chou, 2005). Relatively little is therefore known about DON in coastal shelf seas due to the perception that DON is composed mainly of refractory compounds resistant to biological degradation that are

unavailable as a source of N nutrition for phytoplankton (Antia et al., 1991; Bronk, 2002). It has, however, been demonstrated in culture studies that a part of DON can be potentially utilised directly by phytoplankton (Antia et al., 1991; Berman and Chava, 1999; Bronk, 2002; Berman and Bronk, 2003). Hence in a system, such as the North Sea where N is potentially the limiting nutrient, information on DON is essential.

The North Sea is generally defined as the area south of 62° N including the Skaggeak and the Kattegat, and has a total surface area of 575,000 km² (Fig. 1) and volume of 40.3 km³ (Reid et al., 1988). It is a shallow shelf sea with the depth ranging from 30 to 600 m with the shallow region (<50 m) south of and including Dogger Bank. Here, the shallow topography of the basin in conjunction with strong tidal mixing prevents vertical thermal stratification in summer (Howarth et al., 1994). It becomes deeper in the northern region (~200 m) with the deepest area on its eastern margin in the Norwegian Channel. In the central and northern regions, vertical thermal stratification occurs in summer, with a thermocline appearing in late spring, typically at a depth of 50 m

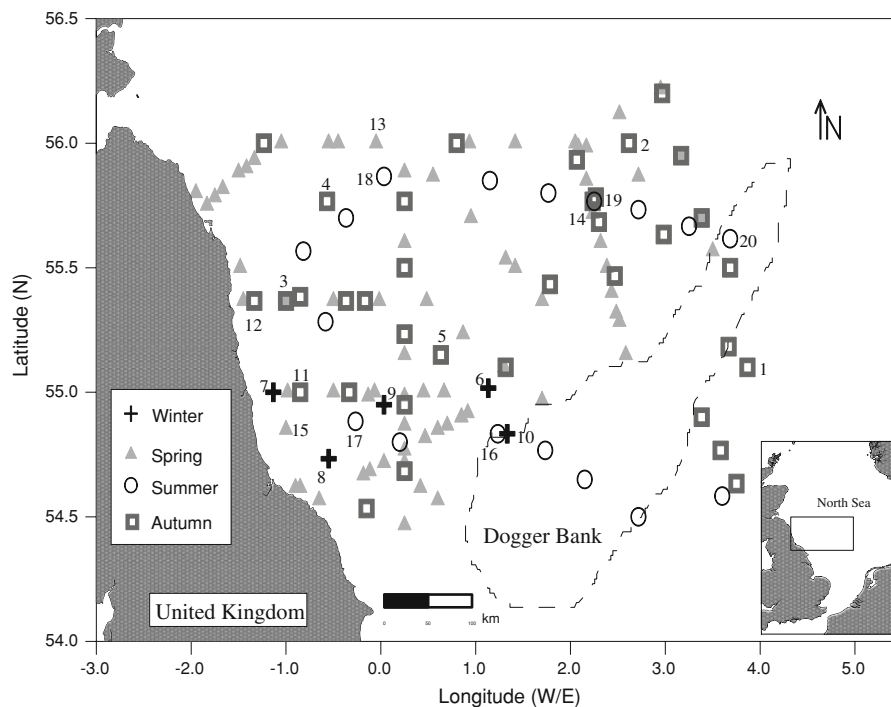


Fig. 1 Map of the study area showing sampling stations for all seasons. Stations where the ultrafiltration experiment was carried out were labelled with number (see Table 2)

(Warrach, 1998). In winter, the whole of the North Sea is well mixed vertically, except in deeper areas in the northern part of the North Sea.

The North Sea and its surrounding area is one of the most studied continental shelf seas due to its location surrounded by some of the industrialised and densely populated areas of northern Europe. It is also economically important for mineral resources (e.g. oil and gas), fishing, sea transportation and tourism (Howarth et al., 1994; Ducrotoy et al., 2000). There is an increasing concern about the ecological effects of anthropogenic nutrient inputs to the North Sea (Charnock et al., 1994; Sundermann, 1994), especially in relation to impacts on primary production in the coastal waters of the southern part of the North Sea (van Bennekom & Wetsteijn, 1990; Laane et al., 1993; Jickells, 1998; Brion et al., 2004). These nutrient inputs to the North Sea are via three major pathways from rivers, the Atlantic Ocean and atmospheric deposition (Jickells, 1998; Brion et al., 2004). Among these inputs, rivers play an important role accounting for 65–80% of the total N input from land (Brion et al., 2004). Many studies have therefore concentrated on the influence of riverine input to the North Sea coastal area (e.g. van Bennekom & Wetsteijn, 1990; Desprez et al., 1992; Nedwell et al., 2002; Skogen et al., 2004). In addition, studies on seasonal and spatial variability of inorganic nutrients in the North Sea (Butler et al., 1979; Wafar et al., 1983; Baeyens et al., 1984; Radach et al., 1990; Laane et al., 1993; Gentilhomme & Lizon, 1998; De Galan et al., 2004; Weston et al., 2004; Ramirez et al., 2005; van der Zee & Chou, 2005) show that the nutrient cycling is broadly driven by biological processes, such as primary production and its subsequent remineralisation (e.g. by grazing), hydrography (stratification and mixing) and on the dynamics of nutrient supply (import–export balance and riverine loads).

In order to determine the seasonal and spatial variations of inorganic and organic nutrients, surveys of the spatial and temporal distribution of inorganic and organic N were undertaken in the North Sea area. Our main aim is to present a quantitative picture of the seasonal changes of N focusing on DON throughout an annual cycle and also to relate this to phytoplankton biomass. In this study, DON was measured as both total and the fraction below a specific molecular weight, with the latter achieved using centrifugal techniques.

Methods

Sampling area and field sampling procedures

A series of cruises were undertaken in the central part of the North Sea in both seasonally stratified and well mixed waters. These cruises were conducted during the autumn (1–8 October 2004), winter (9–12 February 2005), spring (23 April–1 May 2005) and summer (9–23 August 2005). In general, the locations of the sampling stations were not replicated among cruises, but similar regions were covered on all cruises (Fig. 1). The location of sampling areas was determined by weather related accessibility and also the demands of the other research on the cruise. During the winter cruise, only five stations were sampled due to the constraints of poor weather. The sampling strategy was identical for all cruises with surface water sampled from online continuous flow, which sampled at 4 m depth below the surface waters on board the RV CEFAS Endeavour and allowed surface water spatial distribution to be determined. The online continuous flow was fed by a centrifugal pump of impeller diameter 165 mm leading to phosphor bronze pipe work of 65 mm diameter, which fed outlet pipes in the wet laboratory where samples were collected. There were no sedimentation chambers and areas of potential accumulation have been minimised, so that, particulate transport would remain consistent with the intake. Samples were collected at all stations for measurements of DIN, chlorophyll *a* (chl-*a*), DON and PON.

Analytical procedures

Salinity was determined onboard using a Guideline Portasal. The Portasal was calibrated with IAPSO standards at the start and end of each batch to correct for drift with absolute accuracy determined to within ± 0.003 . Samples for nitrate + nitrite (hereafter referred to as nitrate) and ammonium were filtered through a 0.7 μm pore size Whatman GF/F glass fibre filters and preserved with HgCl_2 (100 μl HgCl_2 (16 g/l) in 75 ml samples) and stored in HCl rinsed polypropylene bottles and analysed within 1–2 days. DIN was analysed using a Scalar San Plus Autoanalyser according to Kirkwood (1996). Detection limits for both nitrate and ammonium were

0.1 μM . Quality control was assured by participation in an external quality assurance scheme, Quality Assurance for Marine Environmental Measurements (QUASIMEME). The % error for DIN analyses was <5% (relative to Ocean Scientific International standard). Chl-*a* concentration was measured fluorometrically (Holm-Hansen et al., 1965). Samples for chl-*a* were filtered onto Whatman GF/F glass fibre filters using a vacuum pump (vacuum <50 mm Hg) and stored at -20°C . During analyses, the filters were left for 12 h in 90% acetone at 4°C in the dark. The fluorescence of the extract was measured in a Turner Designs fluorometer. The % error for chl-*a* was <2% (relative to Turner certified reference material).

Samples for DON and PON determinations were filtered immediately after sampling through pre-combusted (400°C ; 4 h) Whatman GF/F glass fibre filters at <50 mm Hg vacuum. The filtrate and filter were used for DON and PON measurements, respectively. The filtrate samples were stored frozen in acid washed polypropylene (PP) tubes at -20°C until analysis at the laboratory. DON was calculated as the difference between total dissolved nitrogen (TDN) and DIN. After thawing, TDN was measured by high temperature catalytic oxidation (HTCO) with a Thermalox TOC/TN analyser (Cambridge, UK), which was coupled to chemiluminescence detector. The detector measured NO_x gases produced by HTCO of all N species (Hopkinson & Cifuentes, 1993). The temperature of the combustion tube was set at $680 \pm 10^{\circ}\text{C}$ and the catalyst was 0.5% Pt/ Al_2O_3 . Calibration of the instrument was performed with KNO_3 (0–25 μM) in ultrapure water (18.2 M Ω). The system blank was estimated for each individual run by injected ultrapure water with typical values of 1.6–3.7 μM ($2.3 \pm 0.6 \mu\text{M}$, $n = 10$) and blank corrections were applied to the TDN data. Certified Reference Material (CRM) of deep seawater from the Sargasso Sea obtained from the Hansell's laboratory, University of Miami was used during the routine analyses of water samples as recommended by Sharp (2002) and Badr et al. (2003). The recovery of the TDN in the CRM was between 91 and 110% ($101 \pm 6\%$, $n = 10$). The oxidation efficiency was also checked on each analytical day by comparing with urea and resulted in values in the range of 96–103% ($100 \pm 2\%$, $n = 10$). The instrument used ≥ 3 injections per

analysis until a precision of $\pm 5\%$ or better was achieved. Possible contribution of DON from the PP tubes during storage has been investigated. This was determined by analysis of DON concentrations of ultrapure water, which was stored in the PP tubes during the duration of study. The results showed minimal contribution (i.e. $0.2 \pm 0.1 \mu\text{M}$ as TDN) and therefore, no correction of TDN from the PP tubes was made. Storage for the duration of the study also did not significantly alter concentrations based on replicate samples stored for varying length of time. The online continuous pump did not affect the integrity of the phytoplankton cells, since comparison of DON concentrations were made with the surface samples and samples collected using the Niskin bottles with no significant difference (one way ANOVA test; $P < 0.05$) between samples and a coefficient of variation (CV) <10%.

For PON measurements, the filter samples were rinsed with 10 ml ultrapure water immediately after filtration, to eliminate NaCl. The filters were then dried at 60°C for 12 h and stored in clean Petri dishes. Filters were then fumed with concentrated HCl in a closed container for 45 min. PON was determined by high temperature oxidation using a Carlo Erba CHNS EA 1108 elemental analyser on triplicate samples with acetanilide used as a standard. The average precision during the analysis was $\pm 15\%$.

Weight fractionation of DON was carried out using an ultrafiltration technique. Surface samples from each cruise were filtered using Centriprep ultrafilter tubes (Amicon #4305; 10 kDa (nominal pore size <10 nm) (Benner et al., 1992)). The tubes were soaked in ultrapure water for 3 days (water was changed daily) and rinsed thoroughly with ultrapure water before use. Samples were placed in the ultrafilters and then centrifuged at 2,800 rpm for 1 h allowing molecules <10 kDa to pass through the membrane. A fraction of the ultrafiltrate in the filtrate collector was removed and the concentration of TDN in this fraction was measured using a Thermalox TOC/TN analyser and we assume all DIN to be <10 kDa. TDN blank from the ultrafilter was determined by using ultrapure water. The ultrapure water was treated as for the sample. The blank from the ultrafilter ranged from 2.0 to 3.1 μM ($2.5 \pm 0.3 \mu\text{M}$, $n = 9$). All samples were, therefore, blank corrected by 2.5 μM .

Results

Surface distribution

For each cruise, in general, all stations showed salinity in the range of 34.2–35.1 and, therefore, show limited freshwater influence in the study area. Despite the limited data for the winter cruise, during this season there is minimal chemical and biological activity that could alter the nutrient concentration and this, together with relatively intense physical mixing, should result in homogeneity throughout the study region (Laane et al., 1993).

During autumn, the highest concentrations recorded for nitrate and ammonium were 4.5 μM and 1.7 μM , respectively (Fig. 2). The lowest concentrations for both nutrients were below detection limit (BDL) ($<0.1 \mu\text{M}$) at stations in the central part of the North Sea. In winter, nitrate was significantly higher compared to autumn with the concentration ranging from 3.9 to 5.5 μM , while ammonium concentrations were uniformly BDL ($<0.1 \mu\text{M}$) for all stations. The range of concentrations during spring for nitrate and ammonium were BDL–7.2 μM and BDL–2.0 μM , respectively. In comparison to autumn, nitrate and ammonium were more frequently BDL during spring, i.e. 53% and 33% were BDL for nitrate and ammonium, respectively. Nitrate concentrations in summer ranged from BDL to 1.4 μM . Lowest and highest ammonium concentrations measured during summer were BDL and 1.3 μM , respectively. Again, for nitrate and ammonium, most of the stations (60%) show BDL concentrations. In general, there were relatively higher nitrate concentrations in coastal waters in comparison to offshore stations in all seasons. With the exception of winter, coastal stations recorded higher concentrations of ammonium compared to offshore stations.

In autumn, the concentrations of DON varied from 4.1 to 11.2 μM (Fig. 3). During winter, the DON concentration ranged from 7.4 to 9.7 μM . The data set in winter is limited as noted earlier due to bad weather, but other samples with similar salinity collected further south in the North Sea did fall in the range of 4.7–15.2 μM . Concentration of DON measured in spring was in a similar range (2.6–11.2 μM) to autumn. Lower concentration of DON was observed in summer (1.9–6.5 μM). Although the winter data set is limited, in general, DON recorded

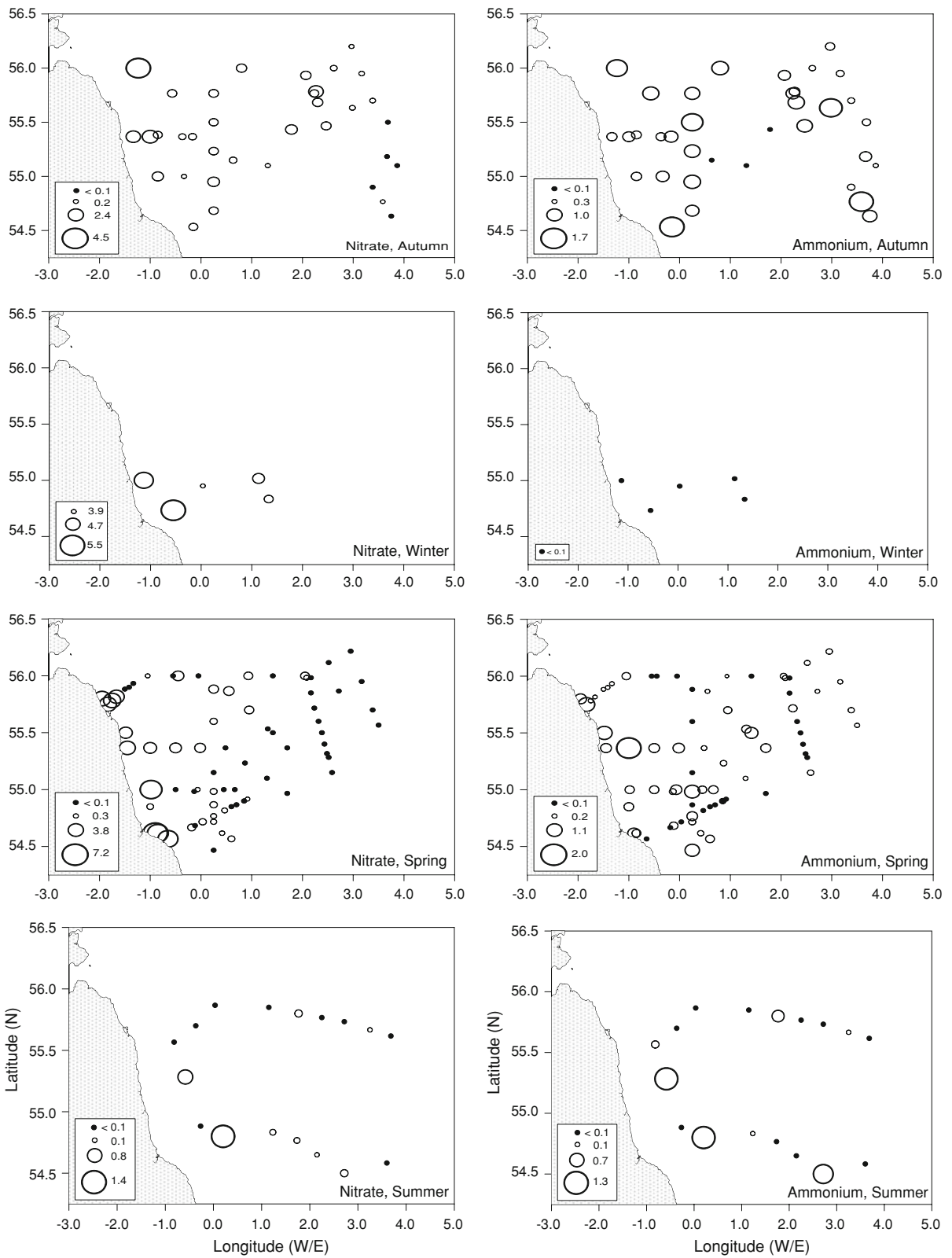
higher concentrations at coastal stations compared to offshore waters in winter with the opposite trend during summer. However, no clear trend was found for the other two seasons. From Fig. 3, the PON concentration was high during the autumn (0.3–4.4 μM) and then decreased to 0.6–0.9 μM during winter. In spring, the concentrations were highest again with the concentrations of 0.7–5.6 μM . Finally, the PON concentrations returned to lower concentrations during summer (0.6–2.2 μM). Comparison between stations showed only in winter, were higher PON concentrations seen for coastal stations compared to offshore stations.

In winter and summer, chl-*a* concentrations were lower with the concentrations ranging from 0.3–0.5 $\mu\text{g/l}$ and 0.1–0.9 $\mu\text{g/l}$, respectively (Fig. 4). Spring sampling showed considerable variability (0.1–6.2 $\mu\text{g/l}$) with the maximum chl-*a* measured, suggesting sampling coincided with the spring bloom at some stations. Higher chl-*a* concentrations were also present in autumn (0.1–4.3 $\mu\text{g/l}$). No clear spatial trend was observed between areas with higher and lower concentrations of chl-*a*.

Seasonal cycle

Table 1 shows mean concentrations for N compounds and chl-*a* in surface waters for all seasons. The data were then tested by one-way ANOVA to a significance level of $P = 0.05$ to show differences between seasons. Both results for nitrate and ammonium mean concentrations clearly exhibited a seasonal variation and showed opposite trends to each other. There was no significant difference in nitrate concentration between autumn and spring ($P > 0.05$), but the concentrations were significantly different for other seasons ($P < 0.05$). Only spring and summer show no significant difference in ammonium concentration ($P > 0.05$) whereas others were significantly different ($P < 0.05$). The relative seasonal abundance of N compounds is shown in Fig. 5 with the combined fraction of nitrate and ammonium (DIN) of total N highest in winter (33%) but lowest in summer (10%).

Statistical tests showed no significant difference in DON concentration between autumn and spring seasons ($P > 0.05$), but other seasons were significantly different ($P < 0.05$). The DON fractions of total N were relatively similar throughout the seasons with the percentage ranging between 61 and 69%.



◀ **Fig. 2** Horizontal distribution of nitrate (μM) and ammonium (μM) for surface waters for all seasons. The filled circles represent the concentration below detection limit ($<0.1 \mu\text{M}$). Note different scales in different seasons

There was no significance difference ($P > 0.05$) in PON concentrations during autumn and summer but differences were significant ($P < 0.05$) for other seasons. This pronounced difference between seasons was also observed for the PON fraction of total N. A high percentage PON was recorded during spring (24%) and summer (21%) and low fraction in autumn (13%) and winter (5%). Lastly, there was no significant difference between seasons for chl-*a* concentrations ($P > 0.05$) in autumn–spring and winter–summer, but autumn–spring seasons was significantly different ($P < 0.05$) to winter–summer seasons.

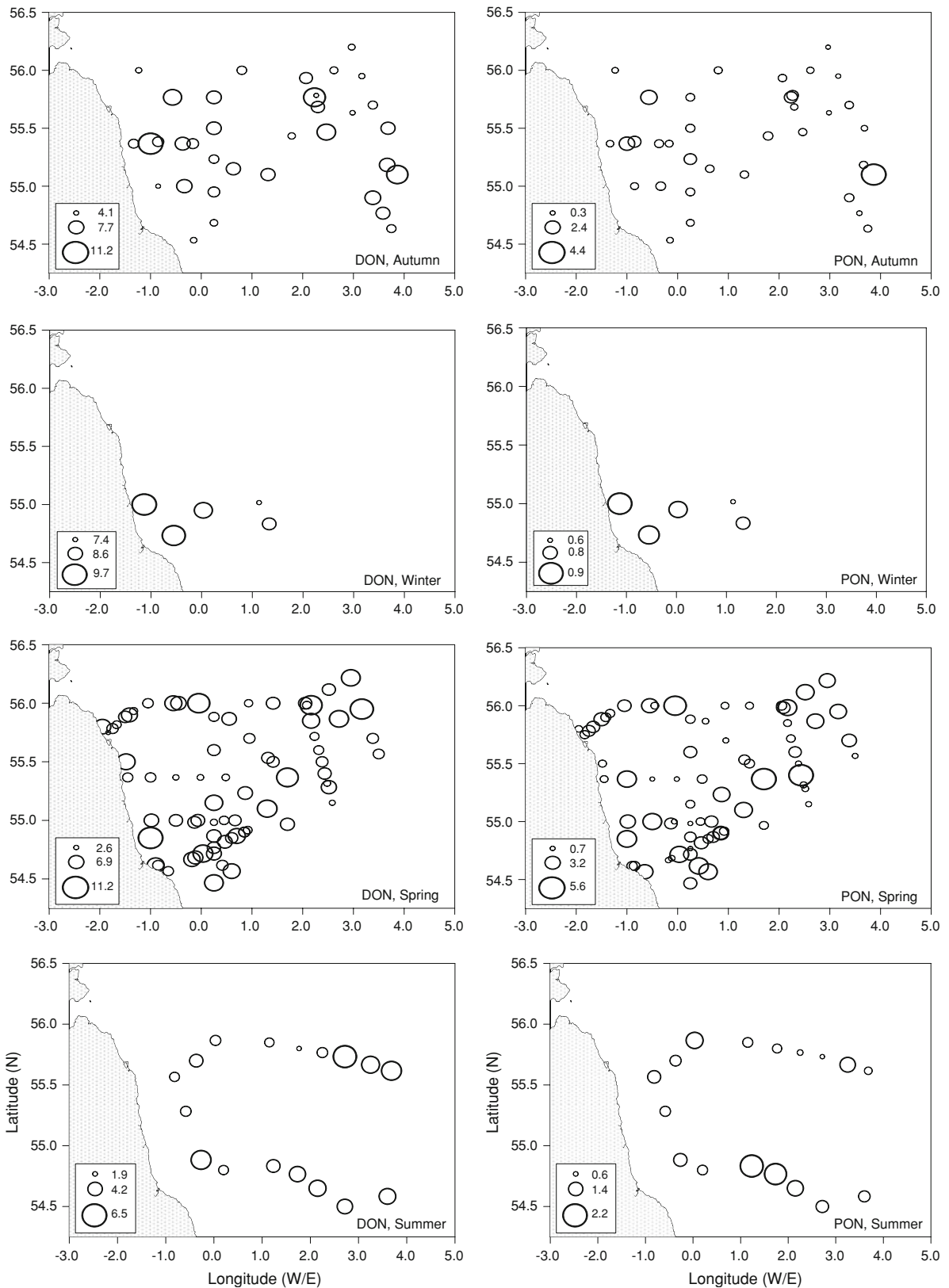
Weight fractionation of DON

In order to investigate a general description of the molecular weight distribution of DON, weight fractionation was determined for 5 samples from each season (Table 2). Locations of the stations at which the samples were taken are presented in Fig. 1. Two types of DON fractions were determined, i.e. low molecular weight (LMW) and high molecular weight (HMW). The term LMW in this study refers to the fraction that passed through a 10-kDa membrane filter and the fraction which does not is therefore the HMW fraction. Table 2 shows a large range of LMW DON concentration for autumn and spring ranging from 4.9–9.8 μM and 3.6–10.6 μM , respectively. A reduced range was measured for winter (1.8–5.4 μM) and summer (1.5–3.2 μM). A higher percentage of LMW DON was present in autumn and spring comprising between 77 and 92% ($83 \pm 7\%$) and 64–94% ($78 \pm 12\%$) of the total DON but lower percentage for winter (16–42%) ($27 \pm 12\%$) and summer (36–56%) ($14 \pm 8\%$). Comparison was made between the percentages of LMW DON with respect to their chl-*a* concentrations (Fig. 6). In general, stations with higher concentrations of chl-*a* also showed higher percentage of LMW DON. Strong positive correlation between percentage of LMW DON and chl-*a* was observed. The relationship was not linear, and a logarithmic regression gave the best fit ($R^2 = 0.53$, $n = 20$).

Discussion

Nutrient distribution in surface waters

DON concentrations ranged from 1.9 to 11.2 μM and were consistently the dominant component compared to nitrate (BDL-7.2 μM), ammonium (BDL-2.0 μM) and PON (0.3–5.6 μM) and were within the range for marine environments summarised in Sharp (1983). Although the results showed considerable spatial variability in all seasons, no obvious trend differentiated the areas which recorded maximum and minimum values. However, in general nitrate values were higher in coastal areas for all seasons, as was also shown by DON and PON during winter. Although these results suggest that the high coastal nutrient concentrations were due to riverine input, this cannot be quantified since property–salinity plots are not possible due to the limited range of salinities. This restricted salinity variation between sampling stations in the coastal and an offshore area was consistent with the salinity distribution in the region reported by Howarth et al. (1994). In comparison with previous studies in the North Sea and the English Channel, this region showed relatively low concentration of all N compounds. In the eastern English Channel, Gentilhomme & Lizon (1998) showed annual concentrations varied between 0–20 μM , 1–8 μM and 36–1400 μM for nitrate, ammonium and PON, respectively. In Belgian coastal waters of the North Sea, the concentrations of nitrate, ammonium and DON ranging from 0.1–140 μM , 0.1–17.5 μM and 3.0–63.5 μM , respectively throughout the whole seasons (De Galan et al., 2004). In agreement with De Galan et al. (2004), van der Zee & Chou (2005) reported nutrient concentrations in the Belgian coastal zone of 5–90 μM (nitrate), 0.5–12 μM (ammonium) and 2–40 μM (DON). Higher nutrient concentrations in Belgian coastal waters were the result of discharges from the highly polluted Rhine, Meuse and Scheldt Rivers. A previous study has shown that the Rhine and Meuse estuaries export 29,000 mmol N y^{-1} (de Vries et al., cited in Nienhuis, 1996) and the Scheldt 5,190 mmol N y^{-1} (Billen et al., 1985). In comparison, the annual nutrient loads to the central and northern North Sea are small, i.e. Thames (2,788 mmol N y^{-1}) and Humber (3,223 mmol N y^{-1}) (Nedwell et al., 2002). Thus, it is suggested that the lower



◀ **Fig. 3** Horizontal distribution of DON (μM) and PON (μM) for surface waters for all seasons. Note different scales in different seasons

concentrations in our study were expected due to lower riverine nutrient inputs in combination with North Atlantic input from the north. To the best of our knowledge, there are no other published data for DON in the open water of the North Sea.

Phytoplankton seasonal growth cycle and inorganic N linkages

The seasonal nitrate concentrations showed a typical annual cycle for high latitude temperate regions where the water column is summer stratified. In winter, the water column was well mixed due to cooling and wind driven mixing bringing deep nitrate into the surface water. Limited sedimentary remineralisation of organic detritus involving ammonification

and nitrification processes also occurs, releasing nitrate to the water column. Since biological activity was low at this time, with phytoplankton growth light-limited as shown by low mean winter chl-*a* concentrations, nitrate concentrations remain high during this period. The annual spring phytoplankton bloom occurs due to the increase of day length and stratification, as shown by high mean spring chl-*a* concentrations, resulting in phytoplankton new production, e.g. nitrate uptake. Low nitrate levels in spring are therefore in part due to this phytoplankton uptake in agreement with other studies in the North Sea region (Butler et al., 1979; Wafar et al., 1983; Radach et al., 1990; Howarth et al., 1994; Gentilhomme & Lizon, 1998; De Galan et al., 2004; van der Zee & Chou, 2005). In summer, low concentrations of nitrate in surface water result from the restricted supply of nitrate from deep water due to stratification. The organic matter due to phytoplankton growth that sinks beneath the thermocline can be

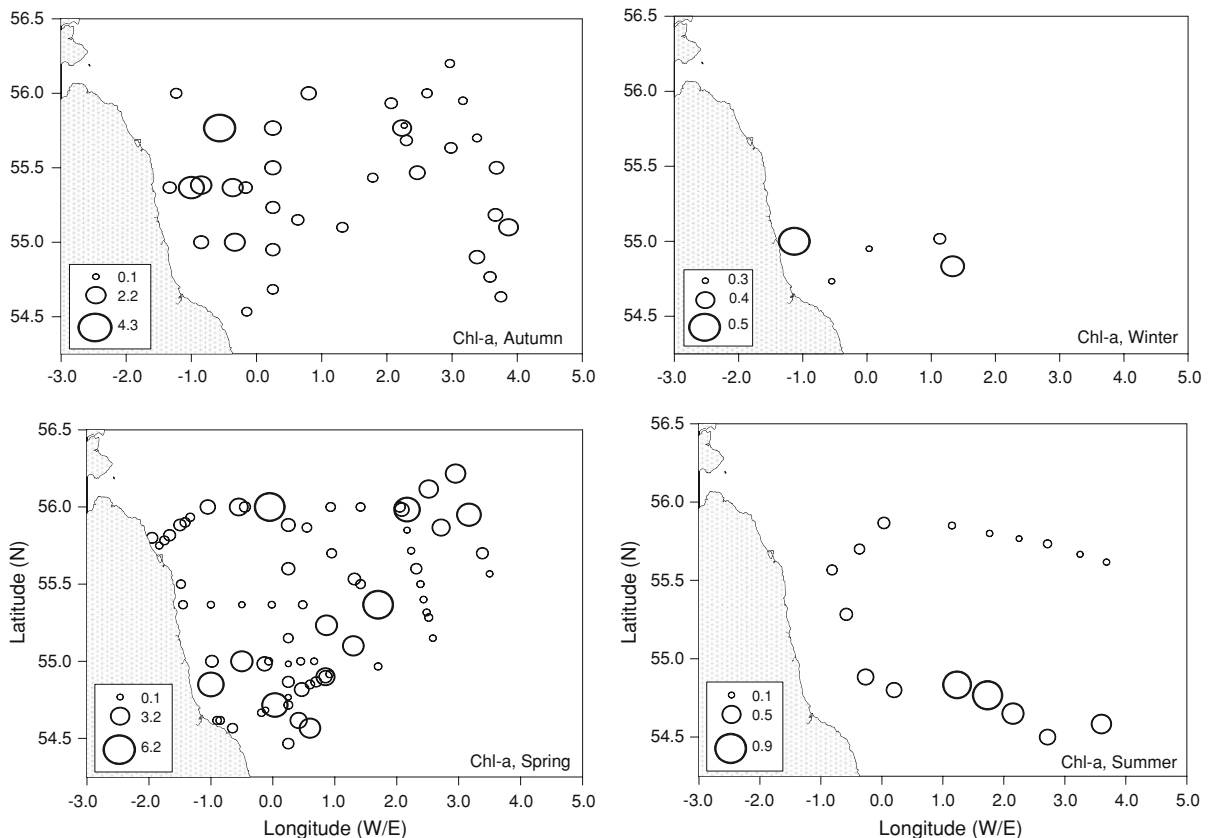


Fig. 4 Horizontal distribution of chl-*a* ($\mu\text{g/l}$) for surface waters for all seasons. Note different scales in different seasons

Table 1 Concentrations for surface waters of N compounds (μM) and chl-*a* ($\mu\text{g/l}$)

	<i>n</i>	Mean	SD
Nitrate			
Autumn	35	1.1	0.9
Winter	5	4.7	0.6
Spring	73	1.2	1.8
Summer	17	0.3	0.3
Ammonium			
Autumn	35	0.8	0.4
Winter	5	<0.1*	-
Spring	73	0.4	0.4
Summer	17	0.3	0.4
DON			
Autumn	35	6.8	1.8
Winter	5	8.9	0.9
Spring	73	6.4	1.9
Summer	17	4.2	1.2
PON			
Autumn	35	1.3	0.8
Winter	5	0.8	0.1
Spring	73	2.5	1.2
Summer	17	1.3	0.4
Chl-<i>a</i>			
Autumn	35	1.5	0.8
Winter	5	0.4	0.1
Spring	73	1.7	1.5
Summer	17	0.4	0.3

In calculating mean, all samples BDL were treated as 0.1 μM

*All stations showed BDL values (<0.1 μM)

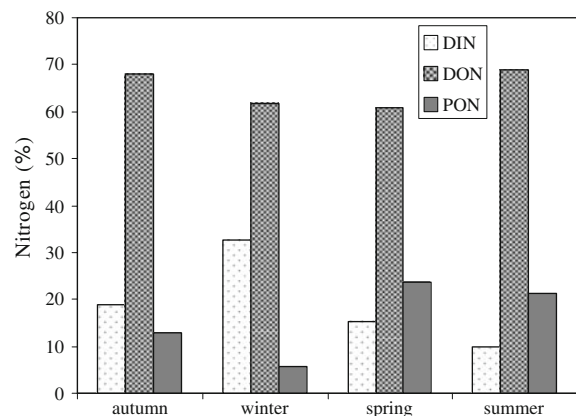


Fig. 5 Relative abundance of N compounds for all seasons

Table 2 Composition of DON for surface waters

Stn No.	DON (μM) ^a	DON (frac. <10 kDa) (μM) ^b	DON (frac. <10 kDa) (%) ^c
Autumn			
1	10.1	7.8	77
2	5.3	4.9	92
3	11.2	9.8	87
4	9.0	7.0	78
5	7.6	6.0	79
Winter			
6	7.4	3.1	42
7	9.7	2.3	24
8	11.9	2.0	17
9	11.1	1.8	16
10	15.2	5.4	36
Spring			
11	7.0	5.3	76
12	5.0	3.6	71
13	10.3	8.7	85
14	4.9	3.2	64
15	11.2	10.6	94
Summer			
16	4.1	1.5	36
17	5.7	3.2	56
18	3.4	1.6	46
19	5.0	1.8	37
20	5.4	2.4	45

^a DON that passes through GF/F filter (as total DON)

^b DON that passes through the membranes in pre-cleaned, ultrafiltration tubes with a nominal molecular weight cutoff of 10 kDa (as LMW DON)

^c Calculated as the percentage of LMW DON in total DON

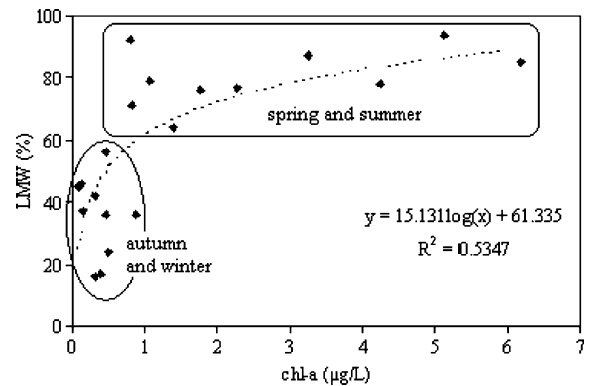


Fig. 6 Relationship between LMW DON and chl-*a* for all seasons

remineralised in the deep waters but the return of this regenerated N to the euphotic zone is inhibited by the thermocline (Weston et al., 2004; Ramirez et al., 2005). Nitrate is therefore ‘trapped’ beneath the thermocline layer with the limited exchange that occurs due to passive diffusion rapidly taken up in the upper mixed layer by phytoplankton during the summer-stratified period (Weston et al., 2005). During autumn, the thermocline breaks down due to mixing resulting from surface cooling and early winter storms, allowing nitrate to be mixed into the surface water. With sufficient light during autumn, this can produce a second smaller phytoplankton bloom (autumn bloom) with higher mean autumn chl-*a* concentrations while the concentrations of nitrate remain low as shown in this study.

The ammonium cycle showed highest mean ammonium concentrations following the autumn phytoplankton blooms. It is likely this ammonium was due to excretion by zooplankton and bacterial remineralisation of organic detritus (Baird et al., 1995). After the autumn phytoplankton blooms, the relatively high water temperatures coupled to the production of phytoplankton biomass will encourage bacterial production and allow the continued remineralisation of organic matter into ammonium (Kemp et al., 1990; Baird et al., 1995). In spring and summer, the mean ammonium concentration was low in part due to its rapid turnover by phytoplankton (Weston et al., 2004) since it is used for phytoplankton growth and nitrate is depleted by the spring bloom (Flynn, 1991), although other factors will also play a significant role in determining the dynamics of the cycling of ammonium (Howarth et al., 1994). Concentrations were BDL during winter when ammonium remineralisation processes were low due to the low temperature in the water column.

DON and PON cycling

The present study shows DON is the dominant form of N throughout the year with seasonal variations in mean concentration although less variation than shown for nitrate and ammonium (Table 1). The maximum mean DON concentration was observed in winter but other seasons showed similar high values. This was in contrast to the seminal study in the English Channel by Butler et al. (1979) which reported a seasonal cycle of N where the two forms

of N alternate in dominance, i.e. nitrate concentration was higher in winter corresponding to lower DON value but during summer the nitrate concentration was lower associated with higher DON. Later studies have observed the same trend as Butler et al. (1979), such as in the Gulf of Mexico (Lopez-Veneroni & Cifuentes, 1994), Chesapeake Bay (Bronk et al., 1998) and coastal waters of Greenland (Skoog et al., 2001). However, studies have also shown no seasonal cycle of DON, e.g. in Santa Monica Bay (Hansell et al., 1993) and at the Bermuda Atlantic Time Series (BATS) in the Sargasso Sea (Hansell & Carlson, 2001). Other studies also record higher DON concentration during winter, e.g. in Belgian coastal waters (De Galan et al., 2004; van der Zee & Chou, 2005). De Galan et al. (2004) proposed that their low sampling frequency missed possible peaks or there is no seasonal cycle at all for DON. Van der Zee & Chou (2005) suggested that their study area was heavily polluted with riverine DON especially in winter. The cycling of DON is therefore highly variable according to local conditions and there is no paradigm suitable for all marine environments.

The high DON concentrations observed during winter are expected to be driven by the resuspension of bottom sediments which have ~5% organic carbon in the study area (Basford et al., 1993). This resuspension was highest in winter due to mixing driven by storms and also winter bottom trawling activities in the North Sea with the latter as important as storm events in sediment resuspension in parts of this region (Jennings et al., 1999). These processes can increase the particulate organic matter concentrations in the water column, and also increase dissolved organic matter concentrations. To the best of our knowledge there have currently been no measurements of DON concentration in bottom water of the North Sea region studied here, however, Saad and Abdel Wahed (2006) have shown that the stirring up of the bottom sediments by strong winds in winter accelerated the release of DON to the water column in the Eastern Harbour of Alexandria, Egypt. In addition, a contribution of DON from bottom sediments has been observed by Lomstein et al. (1998) in summer showing that the efflux of DON from sediments was approximately twice that of DIN in Danish coastal waters due to the organic matter hydrolysis and Middelboe et al. (1998) have also recorded DON as the major form of N releases from

sediments in Limfjord, Denmark. These results suggest that sediments could be an important source of DON for the water column, and we therefore, recommend depth profiling in the future studies to confirm organic sediment release during winter.

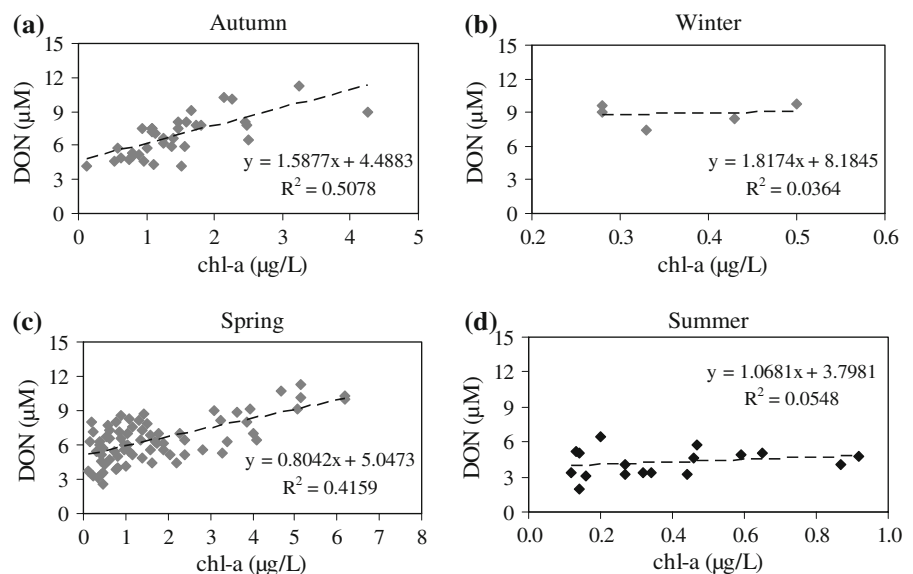
In summer DON decreased due to a portion of the DON, such as urea, being taken up for phytoplankton production. Bacterial DON utilisation and mineralisation will also be high in summer due to the increased water column temperature (Berman & Bronk, 2003; Jorgensen, 2006) with bacteria able to directly transport LMW compounds (500–1,000 Da) through their cell membranes via permeases (Carlson, 2002). HMW compounds may also be utilised by bacteria (Amon & Benner, 1996) after hydrolysis to LMW compounds via hydrolytic enzymes (Christian & Karl, 1995). In addition, the stratification of the water column will inhibit transfer of DON from the sediments into the upper water column during this period. The combination of these processes resulted only in the refractory DON remaining during the summer resulting in low water column total DON concentrations.

The higher range of DON concentrations measured in autumn and spring corresponded with periods of highest chl-*a*. Figure 7 shows the linear regression between DON and chl-*a* for each season with high significant correlation ($P < 0.05$) between DON and phytoplankton in autumn and spring ($R^2 = 0.51$ (autumn, $n = 35$), 0.42 (spring, $n = 73$)) suggesting

that phytoplankton could be an important DON source. This source may be due to release by phytoplankton through excretion as previously shown in culture and field studies (Eberlein et al., 1985; Mykkestad et al., 1989; Collos, 1992; Bronk & Glibert, 1993; Hu & Smith, 1998; Diaz & Raimbault, 2000; Mulholland et al., 2004; Verala et al., 2005) and also due to release of DON by microzooplankton grazing on phytoplankton blooms (Nagata & Kirchner, 1991; Hasegawa et al., 2000). Low correlation ($P > 0.05$) during winter ($R^2 = 0.04$, $n = 5$) and summer ($R^2 = 0.06$, $n = 17$), suggests that at these times DON concentrations were controlled by other factors. In winter, physical factors (e.g. resuspension of bottom sediment) may be the most important. In summer, lower DON was possibly due to DON uptake by phytoplankton and bacteria produced by grazing processes.

The PON also showed a clear seasonal cycle similar to that of chl-*a* as expected due to the formation of biomass during the phytoplankton blooms. In winter and summer, low phytoplankton biomass lead to the decrease of particulates in agreement with Brockmann et al. (1990), Gentilhomme & Lizon (1998) and van der Zee & Chou (2005). The linear regression plot between PON and chl-*a* for surface water in autumn ($R^2 = 0.39$, $n = 35$), spring ($R^2 = 0.59$, $n = 73$) and summer ($R^2 = 0.62$, $n = 17$) shows these two parameters were highly correlated ($P < 0.05$) with similar slopes

Fig. 7 Relationship between DON and chl-*a* for all seasons



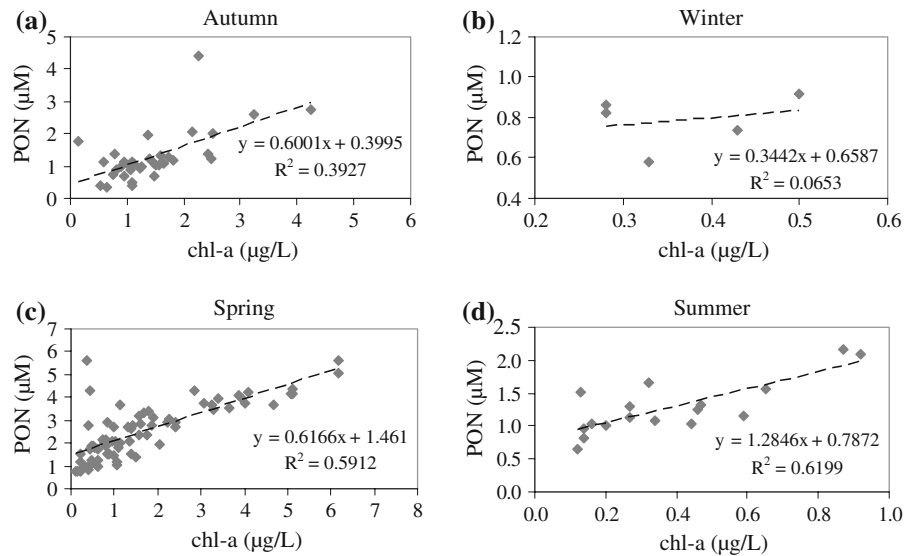


Fig. 8 Relationship between PON and chl-*a* for all seasons. Note different scales in different seasons

(Fig. 8). This shows the distribution patterns of the PON in these three seasons were due to the formation of PON by the phytoplankton as expected. In contrast, the distribution of PON during winter was from another source, e.g. resuspension from bottom waters as shown by low correlation coefficient with chl-*a* ($R^2 = 0.07$, $P > 0.05$, $n = 5$).

DON fractionation

Fractionation of DOM using ultrafiltration is widely used (Ogura, 1974; Tuschall & Brezonik, 1980; Carlson et al., 1985; Bronk & Glibert, 1991; Benner et al., 1992, 1996; Guo et al., 1994; Guo & Santschi, 1996; Pettine et al., 2001). The weight distribution of DOM was used to determine the source of the organic fractions and to understand the fate and functional roles of DOM in aquatic systems. Most DOM fractionation studies have focussed on DOC, with studies of DON fractionation limited (Tuschall & Brezonik, 1980; Bronk & Glibert, 1991; Bauer et al., 1996). There were distinct features of DON fractionation in terms of seasonal variation and its source or fate in the North Sea with DON weight fraction showing seasonal variation, i.e. higher percentage of LMW was recorded during autumn and spring (Table 1). The LMW fraction concentration corresponded to high chl-*a* concentrations (Fig. 6)

suggesting that the source of the LMW DON during autumn and spring is through direct release from the phytoplankton during the bloom as previously discussed. Using ¹⁵N tracer method for measuring the DON release by phytoplankton, Bronk & Glibert (1991; 1993) proposed that the molecular weight of released DON is indicative of the release mechanisms. For example, if LMW DON release represents a large percentage of the total DON release, the dominant mechanism is probably direct release by phytoplankton as exudates from actively growing phytoplankton are often dominated by LMW compounds (Hellebust, 1974; Wiebe & Smith, 1977; Mague et al., 1980). Although no uptake and release experiments were conducted as part of this study the higher percentage of LMW DON observed during autumn and spring was most likely through direct release from phytoplankton. The low percentage of LMW DON in winter was therefore in turn due to low phytoplankton growth, degradation of LMW DON and possibly resuspension of HMW DON from sediments. During summer, the low percentage of LMW DON suggests that part of the labile DON (e.g. urea) released during spring bloom has been utilised by phytoplankton or bacteria in summer. Another reason could be that stratification in summer prevents the upward mixing of LMW DON produced from the sediments to the surface waters.

Conclusion

In the North Sea the seasonal cycle of DON showed DON continuously as the dominant form of N with maximum mean concentrations in winter. High winter DON concentrations were dominated by HMW compounds and may be driven by the resuspension of the organic matter from bottom sediments due to winter storms and trawling. In summer the lowest mean concentrations of DON were measured and also a lower relative proportion of LMW. This was expected to be due to uptake of LMW DON by phytoplankton and bacteria and the stratification of the water column. At other times of the year, there was evidence of DON release by phytoplankton due to the high correlation of DON with chl-*a*. This field study, therefore, supports laboratory-based experiments which have demonstrated that LMW DON can be readily utilised by phytoplankton and bacteria for production, especially when the inorganic nutrients are limiting. This study also shows the importance of measuring DON and in particular its weight fraction, along with the more standard suite of inorganic nutrients, if the controls on phytoplankton growth and nutrient transport in these productive and ecologically important shelf seas are to be understood and effectively modelled.

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