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$\delta^{15}N$ and $\delta^{13}C$ dynamics of suspended organic matter in freshwater and brackish waters of the Scheldt estuary

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Abstract

Suspended particulate organic matter was sampled monthly between June 1999 and April 2000 in the Scheldt river and estuary to investigate the seasonal and spatial patterns of $\delta^{13}C$ and $\delta^{15}N$ signatures. $\delta^{15}N$ of suspended matter showed large seasonal variation. Minimum values ranged from −0.5‰ in the freshwater zone (spring situation) to +2.3‰ in the mesohaline zone (winter situation). Maximum values (summer situation) ranged from +8.8‰ in the freshwater zone to +12.9‰ in the mesohaline zone. $\delta^{13}C$ showed less seasonal variation and ranged overall from −31.1‰ in the freshwater zone to −23.7‰ in the mesohaline zone. During the growth season, decrease of $\delta^{13}C$ and increase of $\delta^{15}N$ of suspended matter were due to local phytoplanktonic and bacterial biomass. There is strong evidence that the ^{15}N enrichment of suspended matter during the growth season reflects the ^{15}N enrichment of the ambient NH_4^+ pool induced by nitrification and NH_4^+ uptake.Zooplankton in the mesohaline section of the river was consistently enriched in ^{15}N relative to suspended matter but followed its seasonal trend. During summer and autumn the isotopic offset between zooplankton and the suspended particulate organic matter was consistent with a pattern of selective feeding on phytoplankton. During summer, $\delta^{15}N$ of zooplankton reached a value as high as +25.5‰, the highest value observed during this study. During spring, present-day $\delta^{15}N$ of suspended matter in the oligohaline and mesohaline section increased compared to the 1970s, probably because today nitrification, which enriches the NH_4^+ pool in ^{15}N, starts earlier in the season. For summer, the discrepancy between present-day suspended matter $\delta^{15}N$ values and those observed in the 1970s was even larger, especially in the oligohaline and freshwater reaches, probably as a result of improved O_2 conditions now favouring nitrification. Likewise, the present decreased input of ^{15}N-depleted sewage will enhance ^{15}N enrichment of suspended matter during the growth season.

Keywords: $\delta^{15}N$; $\delta^{13}C$; NH_4^+; Zooplankton; Suspended organic matter; Seasonal variation

1. Introduction

Numerous studies have illustrated that natural stable isotopes are a useful tool to investigate origin, fate and seasonal processing of suspended particulate organic matter (SPOM) in riverine and estuarine environments (e.g., Gearing et al., 1984; Mariotti et al., 1984; Owens, 1985; Cifuentes et al., 1988, 1989; Montoya et al., 1991; Fichez et al., 1993; Canuel et al., 1995; Qian et al., 1996; Ostrom et al., 1997; Middelburg and Nieuwenhuize, 1998). $\delta^{15}N$ com-

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monly shows larger differences between reservoirs than $\delta^{13}C$ and could be a more sensitive indicator of origins and biogeochemical processing (Ostrom et al., 1997). Stable nitrogen isotope ratios have been studied to track anthropogenic nitrogen in estuarine food webs, to detect causes of eutrophication (McClelland et al., 1997; Riera et al., 2000) and to trace biogeochemical processes that act on the dissolved inorganic nitrogen pool in estuarine systems (McClelland and Valiela, 1998).

The Scheldt estuary is a temperate well-mixed tidal estuary characterised by the occurrence of a maximum turbidity zone (Middelburg and Nieuwenhuize, 1998; Herman and Heip, 1999) and long water residence times of two to three months (Soetaert and Herman, 1995a; Van Damme et al., 1999). Phytoplankton blooms at different timings in different areas of the estuary. In the uppermost freshwater reaches (> km 120), which receive phytoplankton advected from the tributaries, chlorophyll-a concentrations up to 70 µg dm $^{-3}$ are found during spring (Muylaert et al., 1997, 2000). In the lower freshwater reaches (between km 97 and km 120) Chl-a concentrations exceed 100 µg dm $^{-3}$ during the phytoplankton bloom in summer (Muylaert et al., 1997, 2001). The highest Chl-a contents occur in the oligohaline and mesohaline areas (>200 µg dm $^{-3}$) during the bloom period extending from spring to early summer (Soetaert and Herman, 1994; Muylaert and Sabbe, 1999). Lowest Chl-a contents (up to 20 µg dm $^{-3}$) are found in the polyhaline and marine stations (Soetaert and Herman, 1994). In case of long residence times of the water, nutrients and plankton produced in situ, or imported, undergo significant biogeochemical modification (Cifuentes et al., 1988; Middelburg and Nieuwenhuize, 1998) and physical mixing (Cifuentes et al., 1988). Biogeochemical transformations of nutrients and organic matter induce seasonal variability of isotope ratios and affect the isotopic composition to a greater extent than does physical mixing (Cifuentes et al., 1988). The input of organic matter in the Scheldt estuary is high because the river drains one of the most densely populated and industrialised areas of Europe (Frankignoulle et al., 1996; Baeyens et al., 1998) and biogeochemical reprocessing of this material results in a net heterotrophic system sustaining significant CO_2 efflux (Frankignoulle et al., 1998; Hellings et al., 2001). Also, NH_4^+ is efficiently recycled, implying extensive reprocessing of particulate organic matter and a close coupling of production and consumption processes mediated by algae and bacteria (Middelburg and Nieuwenhuize, 2000).

Several earlier studies focused on the C and N isotopic signature of suspended organic matter and phytoplankton in the Scheldt system (e.g., Laane et al., 1990; Middelburg and Nieuwenhuize, 1998; Hellings et al., 1999, 2001), but our knowledge about the different processes in control is still incomplete. The main objective of this study is to further document and understand the seasonal variability of $\delta^{13}C$ and $\delta^{15}N$ of SPOM of the Scheldt estuary and to extend the investigation into the freshwater reaches. Further objectives are to understand the seasonal dependency of zooplankton $\delta^{15}N$ composition on the one of suspended matter and to compare today's seasonal trends of suspended matter isotopic composition with earlier observations. Our hypothesis is that $\delta^{15}N_{SPOM}$ will have increased over the years, following the improved O_2 conditions and related increase in nitrification.

2. Methods

2.1. Study area

The Scheldt river (Fig. 1) is a lowland rain river with a seasonally varying freshwater discharge (average 100 m^3 s^{-1}; Heip, 1988). Freshwater discharge to the estuary is several orders of magnitude smaller than tidal exchange (Soetaert and Herman, 1995a). This results in long water residence times of two to three months (Soetaert and Herman, 1995a; Van Damme et al., 1999) and a salinity gradient intruding to about 100 km upstream from the river mouth (km 0) (Soetaert and Herman, 1995a). The estuary can be divided into three main zones: a marine (km 0 to km 40), brackish (km 40 to km 97) and freshwater zone (km 97 to km 160) that represents one of the largest freshwater tidal areas in Western Europe. The brackish zone itself is divided into a mesohaline (km 40 to km 57) and an oligohaline zone (km 57 to km 97). The latter is characterised by a steep salinity gradient between km 57 and km 80 (Van Damme et al., 1999). The maximum turbidity zone extends roughly from km 90 to km 110 (Van Damme et al., 1999). The maximum tidal amplitude (5.3 m) occurs at Schelle

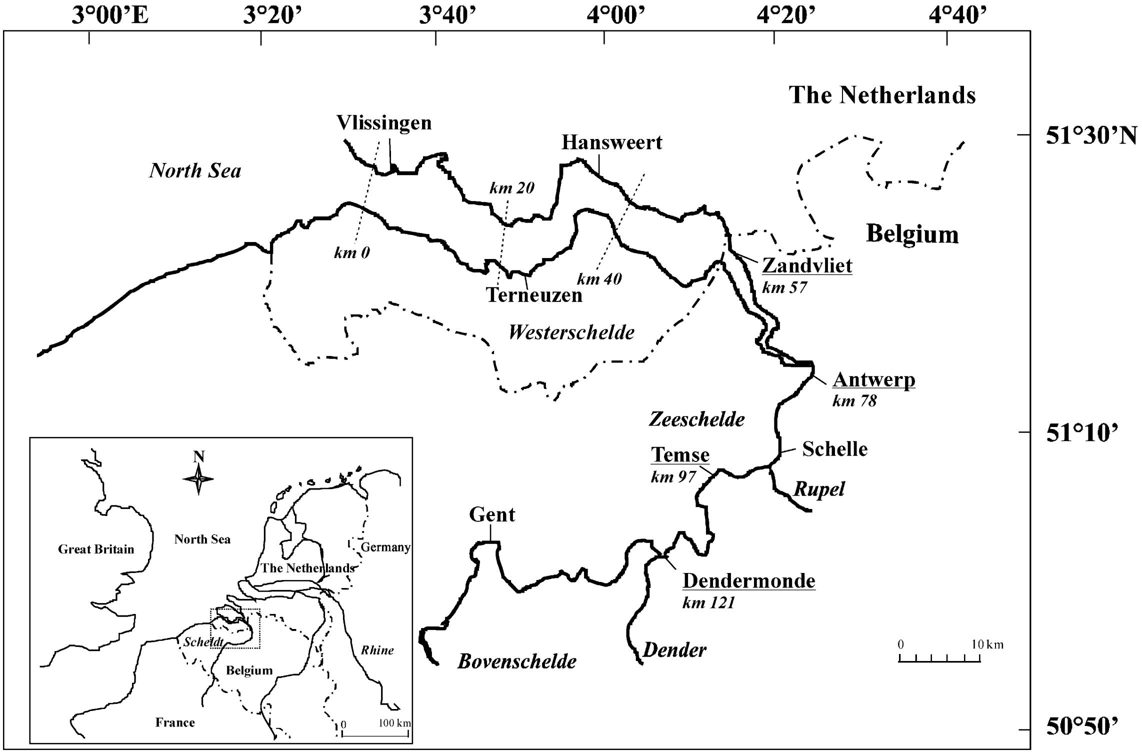

Fig. 1. Map of the Scheldt estuary showing the location of the sampling stations for SPOM and zooplankton. Numbers represent the distance in km from the mouth of the estuary.

(km 90) in the freshwater part of the estuary (Claessens, 1988).

Between June 1999 and April 2000 (December not sampled), sixteen stations along the river and estuary were sampled for physico-chemical parameters (nitrate, ammonium, dissolved oxygen, temperature and salinity). At four of these stations (Dendermonde, Temse, Antwerp and Zandvliet) SPOM and zooplankton were sampled. Dendermonde station (km 121) is located in the freshwater zone. Temse station (km 97) and Antwerp station (km 78) are located in the oligohaline zone, upstream and downstream from the Rupel mouth, respectively. The area Temse – Antwerp is influenced by discharge from the river Rupel receiving untreated sewage from the Brussels sewage collectors. At Temse station, salinity ranges from 0.4 to 1.1 PSU, with a yearly average of 0.7 PSU, while at the more downstream station of Antwerp salinity ranges from 0.4 to 8.7 PSU, with a yearly average of 2.6 PSU. Zandvliet station (km 57) is located in the mesohaline zone where a strong salinity gradient occurs (Van Damme et al., 1999). Here, salinity ranges from 1.8 to 13.8 PSU, while the yearly average is 8.9 PSU.

Fig. 2 shows the freshwater discharge recorded at km 90 (Schelle) for the period between June 1999 and April 2000 (data from Taverniers, 2001). River discharge fluctuated between 50 and 125 m^3 s^{-1} (summer-autumn), while higher values (up to 425 m^3 s^{-1}, late December) were recorded in winter and spring.

2.2. Physico-chemical parameters

Samples for nutrients (NH_4^+ and NO_3^-) were taken just below the water surface with a clean PE bucket. Samples were stored in glass bottles, kept in cool boxes and analysed within 24 h in the home laboratory using a Skalar auto-analyser. Salinity, temperature and

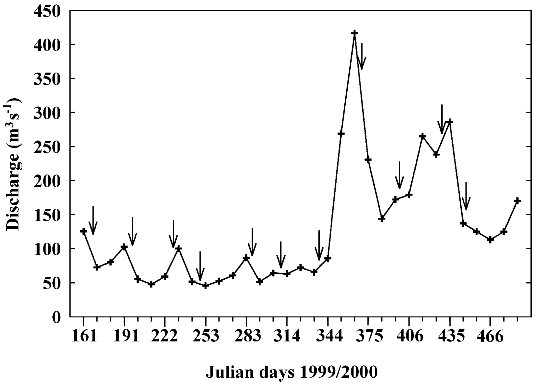

Fig. 2. Discharge ($m^3\ s^{-1}$) of the Scheldt estuary measured at Schelle, km 90 (Data by Taverniers, 2001). Arrows indicate the sampling events.

dissolved O_2 were measured in situ using a HYDROLAB 3® Data Probe.

2.3. SPOM collection

SPOM for isotope analyses was collected by sampling surface water with a clean PE bucket. Depending on suspended matter load, 80 to 300 cm^3 of water were immediately filtered through Whatman GF/C glassfiber filters (∅ = 47 mm). After filtration, samples were quickly frozen using liquid nitrogen. In the laboratory, samples were thawed and dried for several days at 50 °C.

2.4. Zooplankton

Copepods were sampled monthly at Zandvliet station by towing a 300 μm zooplankton net just below the water surface for 5 to 10 min. The copepods were kept in filtered Scheldt water for 2 h for gut content emptying. Then, samples were frozen in liquid nitrogen. In the laboratory samples were thawed by submerging them in distilled water. For each sample, 600 to 800 calanoid copepods were handpicked for N isotope analysis and dried at 50 °C to constant weight (between 0.1 and 2 mg dry weight).

2.5. $\delta^{13}C$ and $\delta^{15}N$ analysis

For $\delta^{13}C$ analysis, a disc (∅ = 10 mm) was cut out of the filter, and pre-treated with HCl acid vapour to remove carbonates. This disc was packed in a tin cup ready for combustion in the elemental analyser (Carlo Erba NA1500). CO_2 gas produced during combustion was led into a boro-silicate vacuum line and cryogenically trapped in glass tubes, which were subsequently sealed with a hand torch (Hellings et al., 1999; Hellings, 2000).

For $\delta^{15}N$, more material was needed. This was obtained by scraping the filtered matter from the filter with a clean scalpel and transferring it into a tin cup. N_2 gas formed during combustion in the elemental analyser was led into in a stainless steel vacuum line and cryogenically trapped in stainless steel tubes fitted with a gas-tight valve and filled with molecular sieve (Marguillier et al., 1997; Bouillon et al., 2002).

Mass spectrometric measurements were performed using a Delta E Finnigan Mat dual inlet isotope ratio mass spectrometer. Reference materials for C were graphite (USGS-24: $\delta^{13}C = -16.1$ ‰), sucrose (IAEA-C-6: -10.4 ‰) and polyethylene foil (IAEA-CH-7: $\delta^{13}C = -31.8$ ‰). Values are expressed relative to the VPDB (Vienna Peedee Belemnite) standard. For nitrogen, high purity tank nitrogen gas was used as a working standard. This working standard was calibrated against ammonium sulphate (IAEA-N1: $\delta^{15}N = +0.4$ ‰, IAEA-N2: $\delta^{15}N = +20.4$ ‰) and potassium nitrate (IAEA-NO-3: $\delta^{15}N = +4.7$ ‰). $\delta^{15}N$ values are expressed relative to atmospheric N_2 reference. The precision for 8 consecutive measurements was ≤ 0.1 ‰ for $\delta^{15}N$ and ≤ 0.04 ‰ for $\delta^{13}C$.

3. Results

3.1. Temporal and spatial variation of physico-chemical parameters

Temporal evolution of temperature and dissolved O_2 at the four stations is shown in Fig. 3. O_2 concentrations during winter were markedly higher than during summer at all stations (Fig. 3). At Antwerp, Temse and Dendermonde, the water was hypoxic (< 2 mg dm^{-3}) during summer and autumn.

Lowest NH_4^+ concentrations (< 100 μM) occurred in summer (July-September); (Fig. 4). Generally, NH_4^+ at Zandvliet was lower than at the more upstream stations. Higher NH_4^+ concentrations occurred in

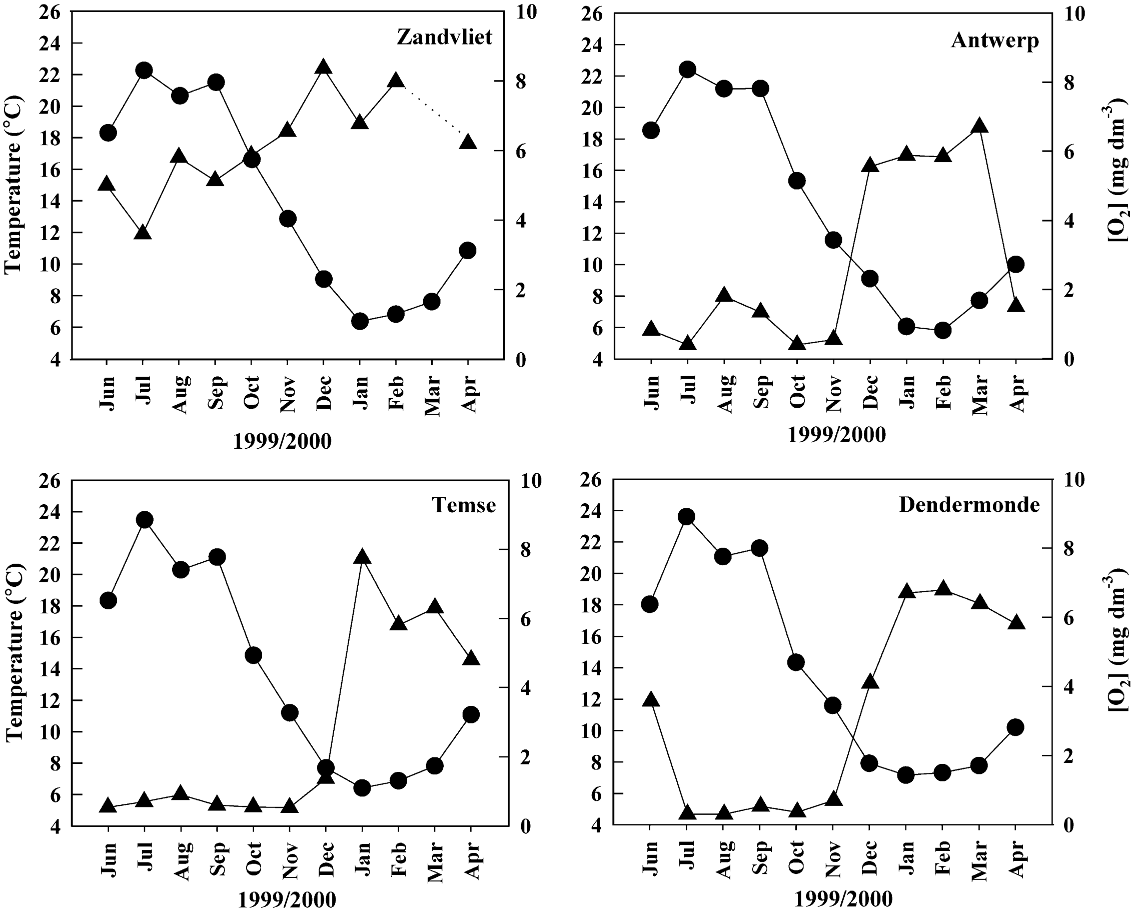

Fig. 3. Temporal variation of temperature (circles) and dissolved oxygen (mg dm^{-3}; triangles) at Zandvliet (km 57), Antwerp (km 78), Temse (km 97) and Dendermonde stations (km 121).

autumn, winter (up to 406 μM, Dendermonde, December). The high discharge in December 1999 (Fig. 2) is probably the cause of the decrease in NH_4^+ concentrations recorded in January 2000, particularly at Temse and Dendermonde. Spatial patterns of NH_4^+ and NO_3^- concentrations for a typical winter, spring, summer and autumn month are shown in Fig. 5. In July, a strong decrease in NH_4^+ between km 155 and km 133 coincided with a sharp increase of NO_3^-. Downstream of km 133, NH_4^+ and NO_3^- were relatively constant, but with NO_3^- largely in excess of NH_4^+. In October, NH_4^+ exceeded NO_3^- for the section upstream of km 85 and a sharp decrease in NH_4^+ with simultaneous increase in NO_3^- occurred between km 88 and km 72. In January, both NO_3^- and NH_4^+ decreased slightly downstream, but NO_3^- largely exceeded NH_4^+. In April also, NO_3^- exceeded NH_4^+ and downstream of km 78, NH_4^+ decreased while NO_3^- increased slightly.

3.2. Temporal and spatial variability of SPOM isotope ratios

3.2.1. Temporal and spatial variation in $\delta^{15}N_{SPOM}$

Considerable temporal variation in $\delta^{15}N_{SPOM}$ was observed at the four sites sampled for SPOM (Fig. 4). Generally, $\delta^{15}N_{SPOM}$ was lower in winter, early spring and increased during spring, reaching a maximum in summer, followed by a decrease in late summer, autumn. However, the timing of extreme $\delta^{15}N_{SPOM}$

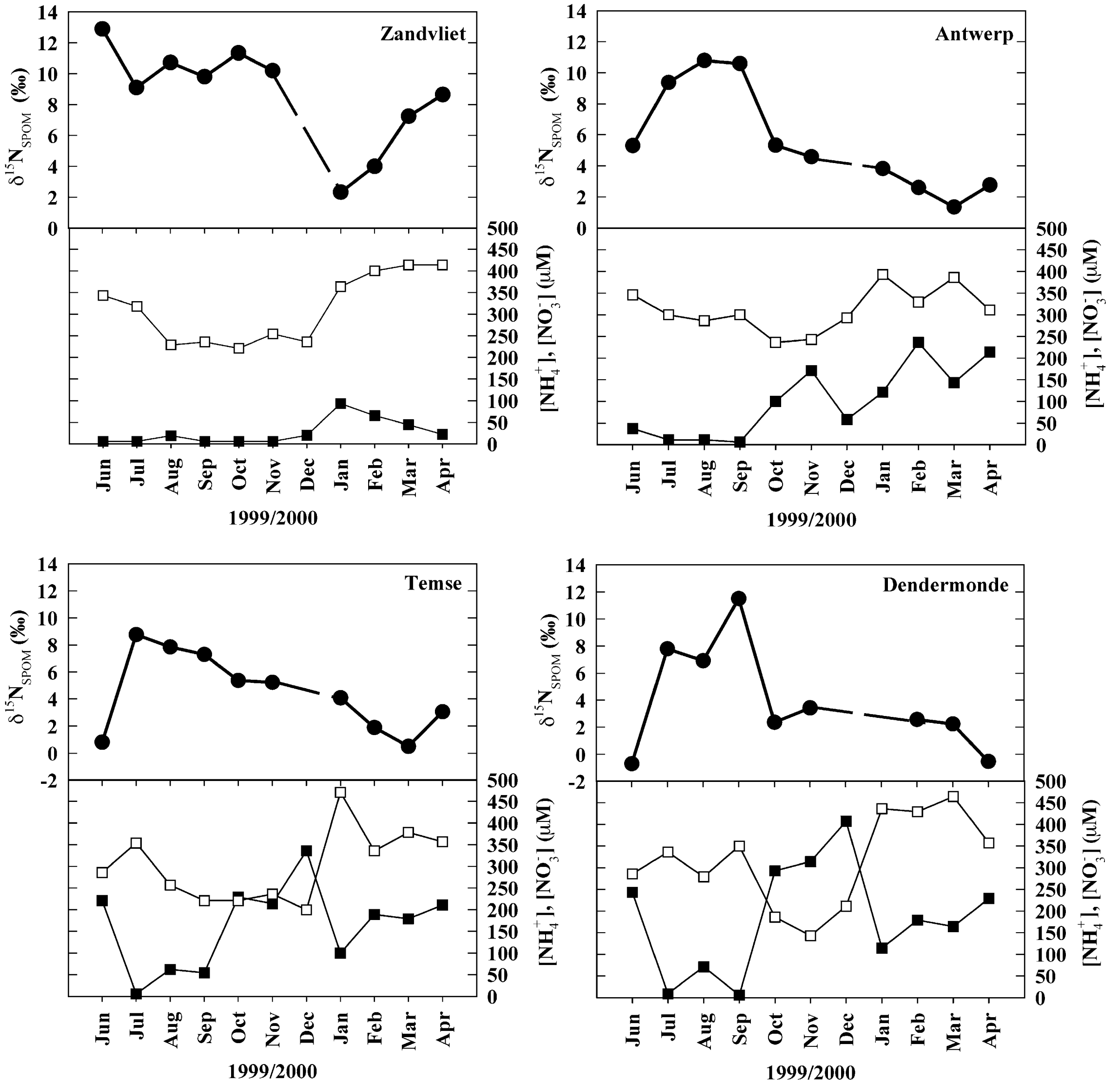

Fig. 4. Temporal variation of ammonium (µM; closed squares), nitrate (µM; open squares) and $\delta^{15}N_{SPOM}$ (circles) at Zandvliet (km 57), Antwerp (km 78), Temse (km 97) and Dendermonde stations (km 121).

values (minimum and maximum) differed for the four stations.

The most salient feature is the large discrepancy between maximum and minimum values at all stations. Extreme values are: Zandvliet: maximum +12.9 ‰ (June), minimum + 2.3 ‰ (January); Antwerp: maximum + 10.8 ‰ (August and September), minimum + 1.3 ‰ (March); Temse: maximum + 8.8 ‰ (July), minimum + 0.5 ‰ (March); Dendermonde maximum + 11.5 ‰ (September), minimum − 0.5 ‰ (April and June).

The yearly averaged $\delta^{15}N_{SPOM}$ value was highest at the most downstream station Zandvliet (+ 8.6 ‰) and decreased upstream, with annual averages for Antwerp, Temse and Dendermonde of + 5.6 ‰, + 4.5 ‰ and + 4.0 ‰, respectively. Zandvliet differed from the other

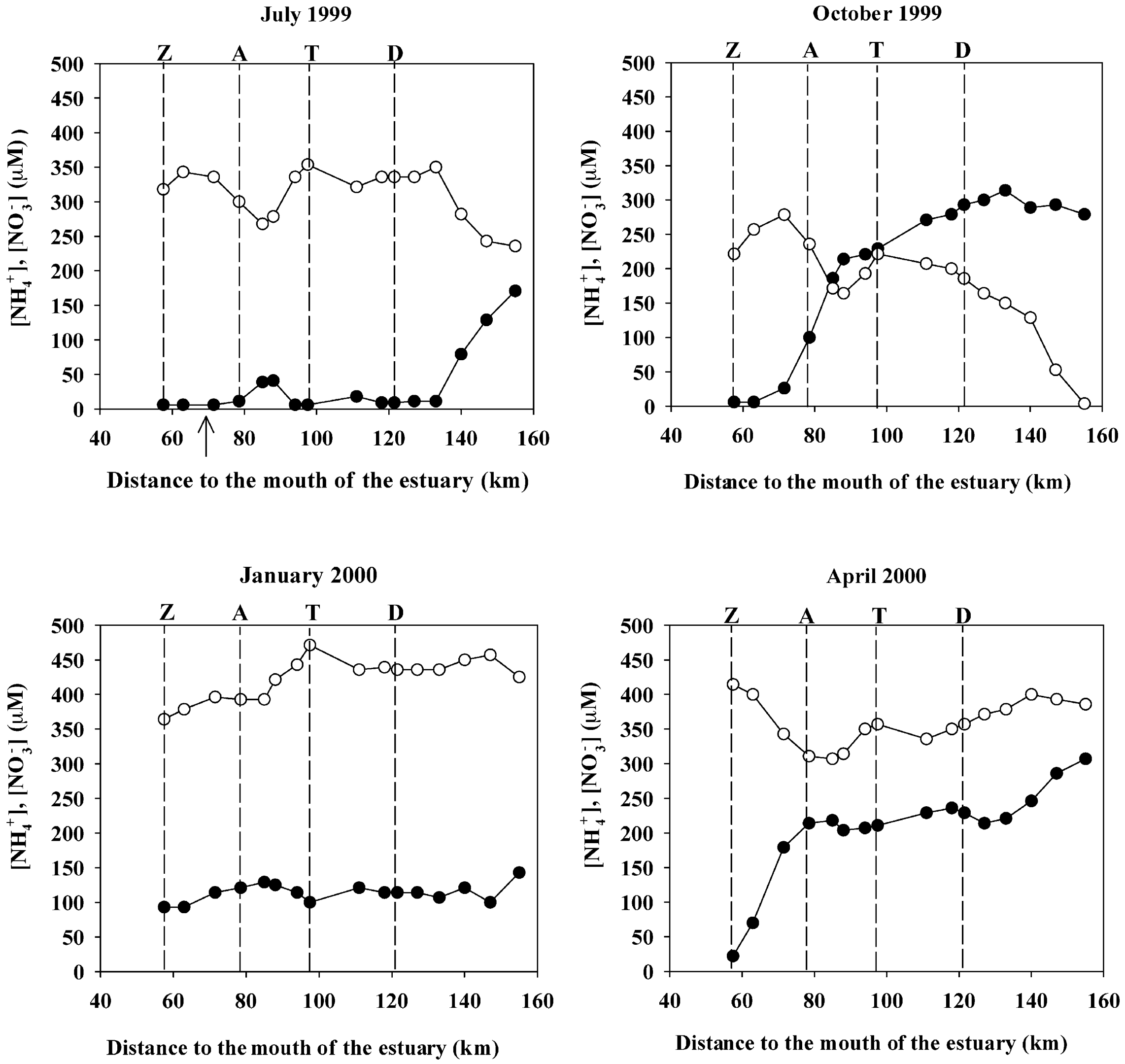

Fig. 5. Spatial variation of ammonium (µM; closed circles) and nitrate (µM; open circles) during a typical summer, autumn, winter and spring season. Z = Zandvliet (km 57), A = Antwerp (km 78), T = Temse (km 97) and D = Dendermonde stations (km 121). The upstream boundary of the area of intense nitrification can be recognised by the sharp decline of NH_4^+ coinciding with a sharp increase of NO_3^-. The arrow indicates the position of the zone of intense nitrification during the seventies according to Mariotti et al. (1984).

stations by showing enriched $\delta^{15}N_{SPOM}$ values throughout spring and summer (March to November). At the other stations $\delta^{15}N_{SPOM}$ started to decrease earlier (from September on).

Our $\delta^{15}N_{SPOM}$ values are generally much higher than those observed some twenty years ago by Mariotti et al. (1984), who reported values ranging from +1.5‰ to +6.0‰ for the oligohaline and mesohaline part of the estuary. However, they are in good agreement with more recent values reported by Middelburg and Nieuwenhuize (1998) and averaging +12.0‰ for the same river section. The very high values for SPOM (up to +24‰) reported by Mariotti et al. (1984) for downstream Scheldt (unspecified area) in early summer were not observed here, nor in Middelburg and Nieuwenhuize (1998).

3.2.2. Temporal and spatial variation in $\delta^{13}C_{SPOM}$

$\delta^{13}C$ values of SPOM ranged from − 31.1 ‰ to − 23.7 ‰ and also showed seasonal patterns (Fig. 6), but these were less pronounced than for $\delta^{15}N$. $\delta^{13}C_{SPOM}$ increased downstream with annual averages of − 29.1 ‰ at Dendermonde and Temse, − 28.1 ‰ at Antwerp and − 26.6 ‰ at Zandvliet.

Generally, $\delta^{13}C_{SPOM}$ values were lowest in spring and summer. For the stations of Zandvliet and Antwerp maximum values were reached in January, while at Temse and Dendermonde maxima were reached only in March. The range of our $\delta^{13}C_{SPOM}$ values in the freshwater, oligohaline and mesohaline sections of the estuary closely overlaps with the ones reported in previous studies (− 25.0 ‰ to − 32.2 ‰; Laane et al., 1990; Middelburg and Nieuwenhuize, 1998; Hellings, 2000; Hellings et al., 1999; 2001).

3.3. Temporal variation of $\delta^{15}N$ of calanoid copepods at Zandvliet

Maximum $\delta^{15}N$ values of calanoid copepods (+ 25.5 ‰) were observed in July and values stayed high until November (Fig. 7). In January $\delta^{15}N$ values had decreased to + 13.5 ‰. A further slight decrease was observed from February to March. Average difference between $\delta^{15}N_{SPOM}$ and $\delta^{15}N_{Calanoids}$ was +

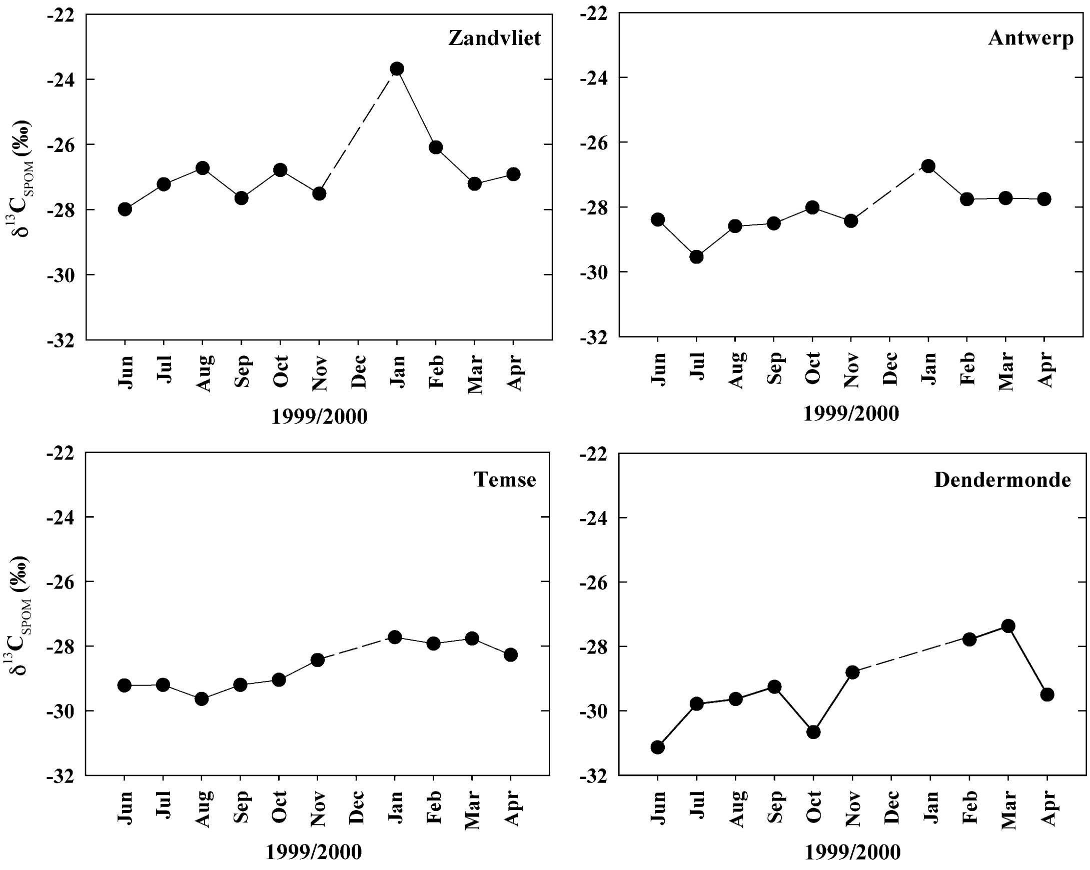

Fig. 6. Temporal pattern of $\delta^{13}C_{SPOM}$ in the Scheldt estuary at Zandvliet (km 57), Antwerp (km 78), Temse (km 97) and Dendermonde stations (km 121).

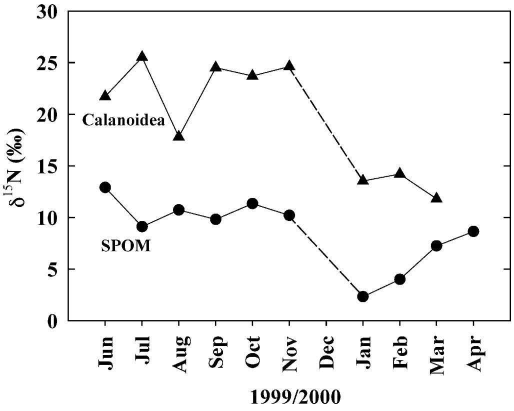

Fig. 7. Temporal variation of $\delta^{15}N_{SPOM}$ (circles) and $\delta^{15}N_{copepods}$ (triangles) at Zandvliet station (km 57).

11.1 ‰, with a maximum difference reached in July (+16.4 ‰) and a minimum in March (+4.6 ‰).

4. Discussion

The $\delta^{15}N$ signature of terrigenous detritus is reported to range between +1.5 ‰ (Mariotti et al., 1984) and +3.5 ‰ (Middelburg and Nieuwenhuize, 1998). SPOM from the sewage collectors of Brussels city, discharging into the Scheldt via the rivers Zenne and Rupel, has an average $\delta^{15}N$ value of +2.0 ‰ (Fisseha, 2000). Admixture of both these sources alone cannot account for the observed ^{15}N enrichment of SPOM in the Scheldt during spring and summer. Likewise, the very low $\delta^{13}C$ values (as low as −31.1 ‰) observed for SPOM during spring and summer cannot be explained by simple admixture of detritus from riparian vegetation (−28.4 ‰; Hellings et al., 1999; Hellings, 2000) and domestic sewage (averaging −25.3 ‰; Fisseha, 2000). Clearly, in situ processes need to be invoked to explain the $\delta^{15}N$ and $\delta^{13}C$ compositions of Scheldt SPOM. SPOM isotopic composition varies seasonally and shows largest ^{15}N enrichments (Mariotti et al., 1984) and largest ^{13}C depletions (Hellings et al., 1999; Hellings, 2000) during the bloom. High $\delta^{15}N$ signals of SPOM are attributed mainly to enhanced contributions of ^{15}N-enriched phytoplankton (Mariotti et al., 1984; Cifuentes et al., 1988, 1989; Ostrom et al., 1997), organic detritus enriched by bacterial processing (Wada, 1980; Owens, 1985; Ostrom et al., 1997) and bacterial biomass (Caraco et al., 1998). Outside the bloom period, isotopic signatures of SPOM are likely to shift towards those for terrigenous detritus and domestic sewage end-members.

4.1. Can microbial biomass account for the ^{15}N enrichment of SPOM during bloom?

Since, at present, there are no $\delta^{15}N$ data for pure phytoplankton and bacteria for the Scheldt system, we estimated the temporal evolution of the microbial (=phytoplankton and bacteria) $\delta^{15}N$ signal from the isotopic composition of the mesozooplankton grazers (the latter data are for Zandvliet only) and from published $\delta^{15}N$ compositions of the Scheldt NH_4^+ pool, taking into account appropriate $^{15}N/^{14}N$ discrimination factors.

The extent of ^{15}N enrichment of copepods relative to their food substrate ranges from 2.2 ‰ to 6 ‰ (Checkley and Entzeroth, 1985; Montoya et al., 1991, 1992; Keough et al., 1996; Wu et al., 1997), averaging 4 ‰. In the brackish part of the Scheldt estuary calanoid copepods consist mainly of *Eurytemora affinis* during winter-spring and *Acartia tonsa* during summer (Soetaert and Van Rijswijk, 1993). Tackx et al. (1995) report selective feeding on phytoplankton during blooms for *A. tonsa*, while *E. affinis* appears to be omnivorous with limited selection for phytoplankton.

Copepod $\delta^{15}N$ composition suggests a strong seasonal variability of the $\delta^{15}N$ signal of their food (Fig. 7). The latter can be estimated considering the trophic level factor of 4 ‰. From May to November, copepod $\delta^{15}N$ (average = +22.9 ‰) indicates that the food substrate $\delta^{15}N$ signal would be +18.9 ‰. This signal is close to 8 ‰ higher than the one observed for SPOM (+10.7 ‰; Fig. 7) and could reflect selective feeding on phytoplankton by *A. tonsa*, the predominant species during summer. In winter (January – February), the discrepancy between $\delta^{15}N$ of the consumed food substrate (calculated as: average copepod signal, +13.9 ‰ − 4 ‰ = +9.9 ‰) and SPOM (+3.2 ‰) is still large. During spring (March), however, there is a good concordance between $\delta^{15}N$ of SPOM (+7.2 ‰) and the food substrate (+7.8 ‰; copepods = +11.8 ‰). The latter situation could reflect both increased contribution of phytoplankton to the SPOM pool and the predominance of *E. affinis* which is known to be less selective for phytoplankton (see above).

We now verify the high microbial $\delta^{15}N$ signal (+18.9‰), calculated above, by computing this isotopic composition starting from the $\delta^{15}N$ signal of the NH_4^+ pool. $\delta^{15}N$ of the NH_4^+ pool increases over the growth season due to the preferential removal of light $^{14}NH_4^+$ by nitrification (Mariotti et al., 1981; Cifuentes et al., 1989; Montoya et al., 1991) and uptake by phytoplankton and bacteria (Cifuentes et al., 1988, 1989). Mariotti et al. (1984) report $\delta^{15}N_{NH_4^+}$ values between +23 and +29‰ for the area in the Scheldt characterised by intense nitrification and a NH_4^+ content lower than 150 μM in summer (mesohaline - polyhaline area). Since for Zandvliet we observe that NH_4^+ concentrations are low (<6 μM) from June to November (except August: 19 μM; Fig. 4), we assume that $\delta^{15}N_{NH_4^+}$ values in the range +23 to +29‰, as reported by Mariotti et al. (1984) apply also at present.

During microbial uptake of NH_4^+ significant discrimination against ^{15}N occurs. For natural marine bacterial assemblages growing in a system with high NH_4^+ regeneration, Hoch et al. (1994) report a discrimination of 10‰ during bacterial NH_4^+ uptake. An average discrimination of 9.1‰ was reported for algae during a bloom period in the Delaware estuary (Cifuentes et al., 1989) while values between 6.5‰ and 8‰ were reported for Chesapeake Bay (Montoya et al., 1991). Both these estuaries have a NH_4^+-based productivity, as is the case for the Scheldt estuary (Mariotti et al., 1984; Middelburg and Nieuwenhuize, 2000). For the purpose of the present discussion we will assume that bacteria and phytoplankton discriminate by 8‰ against the ^{15}N isotope during NH_4^+ uptake (i.e. an average of the values reported by the other authors). Given that $\delta^{15}N_{NH_4^+}$ values vary between +23 and +29‰ during periods of low NH_4^+ and intense nitrification, $\delta^{15}N$ of the microbial community should vary between +15‰ and +21‰. For Zandvliet, this range overlaps with the value we calculated above for the food substrate during June–November (+18.9‰) as based on the isotopic composition of copepods. Since at the other stations, the NH_4^+ concentration is low from July to September (For Antwerp from June to September) the $\delta^{15}N$ signal of the remnant NH_4^+ pool will probably also range between +23 and +29‰, inducing the observed high $\delta^{15}N_{SPOM}$ values for summer. When NH_4^+ concentrations are higher, as occurs outside periods of intense nitrification and uptake, $\delta^{15}N$ values of microbial biomass will be lower than +18.9‰. The dependency of $\delta^{15}N_{SPOM}$ on NH_4^+ content is corroborated by the Rayleigh type relationship we observe between $\delta^{15}N_{SPOM}$ and NH_4^+ (Fig. 8). This relationship indicates that consumption of NH_4^+ can exceed production, despite the occurrence of high NH_4^+ mineralisation rates in the Scheldt as reported by Middelburg and Nieuwenhuize (2000).

Thus, both the magnitude of the NH_4^+ depletion and the ratio of microbial biomass to allochtonous matter will set the $\delta^{15}N_{SPOM}$ signal. The highest ^{15}N enrichment of SPOM occurs at high relative contribution of microbial biomass and low NH_4^+ concentrations. Lower $\delta^{15}N_{SPOM}$ values will be found during periods of abundant NH_4^+ and low productivity. As a result, the discrepancy between $\delta^{15}N_{SPOM}$ and $\delta^{15}N$ of the food substrate effectively consumed by copepods will vary seasonally.

4.2. Spatial and seasonal patterns of $\delta^{13}C_{SPOM}$ and $\delta^{15}N_{SPOM}$

The different temporal patterns of $\delta^{15}N_{SPOM}$ and $\delta^{13}C_{SPOM}$ observed at the four study sites reflect differences in the timing of the phytoplankton bloom (Fig. 9).

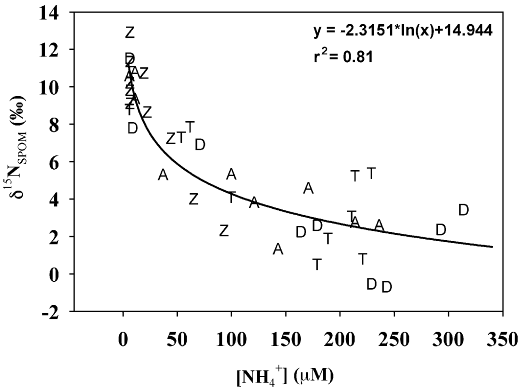

Fig. 8. Relationship between ammonium (μM) and $\delta^{15}N_{SPOM}$ in the Scheldt estuary. Z = Zandvliet (km 57), A = Antwerp (km 78), T = Temse (km 97) and D = Dendermonde stations (km 121).

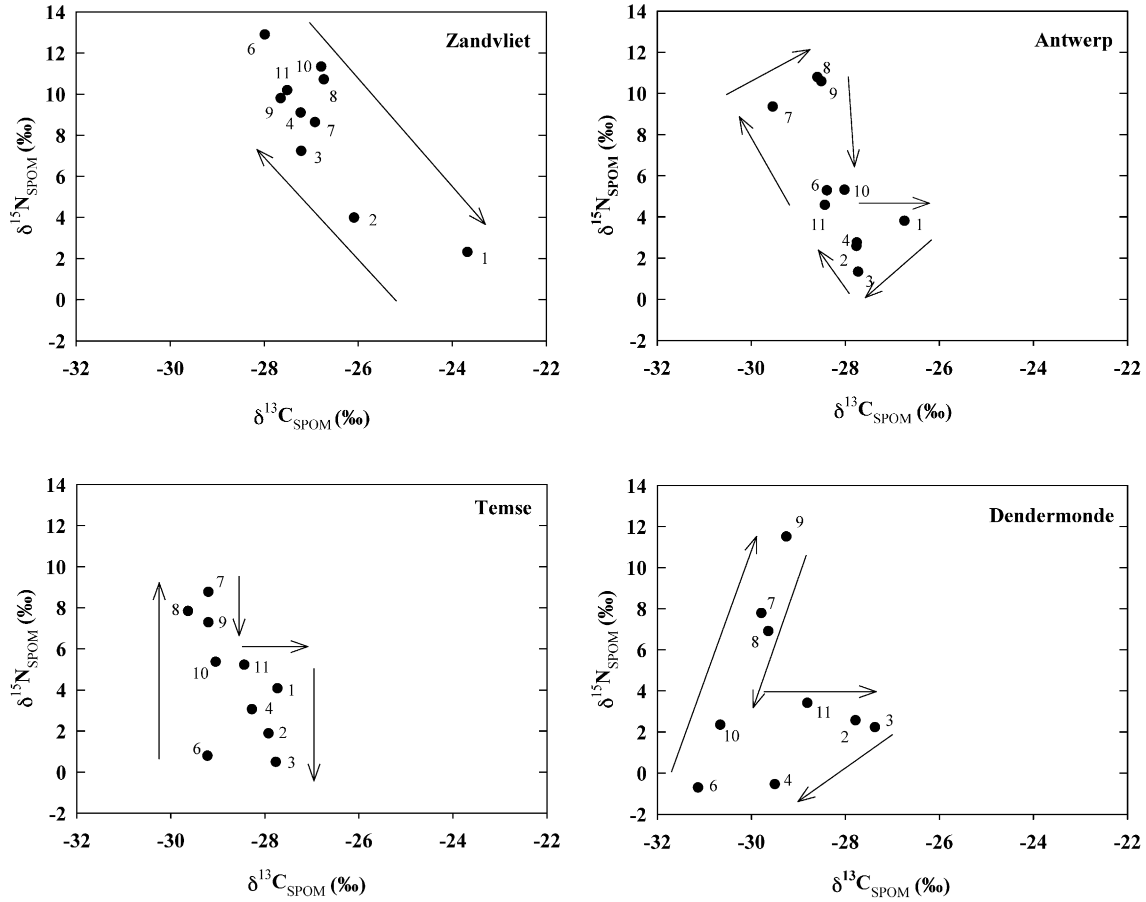

Fig. 9. $\delta^{13}C_{SPOM}$ versus $\delta^{15}N_{SPOM}$ in the Scheldt estuary at Zandvliet (km 57), Antwerp (km 78), Temse (km 97) and Dendermonde stations (km 121). Arrows point to the direction to which $\delta^{13}C_{SPOM}$ and $\delta^{15}N_{SPOM}$ shift along the annual cycle (from June 1999 (month 6) to April 2000 (month 4).

During winter (months 1 to 2) $\delta^{15}N_{SPOM}$ is low and $\delta^{13}C_{SPOM}$ high for all stations. Low microbial biomass and higher discharge are likely reasons for this. Peak discharges such as the one recorded during December (Fig. 2) can advect large amounts of terrestrial organic detritus having lower $\delta^{15}N$ and higher $\delta^{13}C$ signatures (Hellings et al., 1999; Hellings, 2000) and wash out local microbial populations (Brion et al., 2000; Muylaert et al., 2001).

For Antwerp, Temse and Dendermonde the early season (months 1 to 4) shows a slight decrease in $\delta^{15}N$ and $\delta^{13}C$ (the latter not for Temse). This early season decrease in $\delta^{15}N_{SPOM}$ could reflect microbial activity under conditions of high ambient NH_4^+ (Fig. 4), due to the preferential uptake of $^{14}NH_4^+$. Similarly, the decrease in $\delta^{13}C$ of SPOM likely reflects the effect of autotrophic fixation of carbon from a DIC pool enriched in $^{12}CO_2$ during winter (Hellings et al., 1999, 2001; Hellings, 2000).

From late spring to late summer (months 6 to 9), Antwerp, Temse and Dendermonde show a strong increase in $\delta^{15}N_{SPOM}$. This coincides with lowered NH_4^+ due to uptake during bloom events (e.g., Cifuentes et al., 1989) and enhanced nitrification during spring-summer (e.g., Iriarte et al., 1998; Brion, 1997). These processes induce a progressive ^{15}N enrichment of the NH_4^+ pool. During months 6 to 9, $\delta^{13}C_{SPOM}$ remains relatively constant and low at Antwerp and Temse, while at Dendermonde we observe a slight increase in $\delta^{13}C_{SPOM}$. This could reflect an increased demand for CO_2 by blooming phytoplankton depending on dissolved inorganic carbon (DIC) that became progressively enriched in ^{13}C over the growth season as a result of previous phytoplankton activity (Farquhar et al., 1982; Hinga et al., 1994; Rau et al., 1996; Hellings et al., 1999, 2001).

During autumn (months 10 to 11), $\delta^{15}N_{SPOM}$ at Antwerp, Temse and Dendermonde is lower. $\delta^{13}C$ for the former two stations shows little change, while at Dendermonde $\delta^{13}C$ increases after an initial decrease. This situation reflects a decreased microbial and an increased terrestrial detritus contribution to SPOM.

Zandvliet station is peculiar in that $\delta^{15}N_{SPOM}$ is high from month 4 to 11 (+8.6‰; Fig. 9). Because NH_4^+ content is low during this whole period of high $\delta^{15}N_{SPOM}$, it would appear at first sight that local phytoplankton and bacteria thriving on this reduced nutrient pool are responsible for this situation. It is unlikely that these lasting high $\delta^{15}N$ values are sustained by local phytoplankton only, since phytoplankton contribution of SPOM is relatively small in the Zandvliet area compared to upstream stations (Muylaert and Sabbe, 1999) and since the growth season in this mesohaline zone extends only from spring to early summer (Soetaert and Herman, 1994; Muylaert and Sabbe, 1999). For Zandvliet we speculate that the lasting ^{15}N enrichment of SPOM is caused by bacteria processing phytoplankton detritus imported from upstream regions. Indeed, high salinity stress induces phytoplankton mortality (Muylaert and Sabbe, 1999; Van Damme et al., 1999; Goosen et al., 1999) and it is thus possible that phytoplankton washed out from the freshwater reaches dies off in the strong salinity gradient of the mesohaline zone close to Zandvliet. Furthermore, degrading phytoplankton becomes enriched in ^{15}N as a result of bacterial processing (Wada, 1980; Owens, 1985; Ostrom et al., 1997) and bacteria colonising phytoplankton detritus will be enriched in ^{15}N since they experience low ambient NH_4^+ in the Zandvliet area (Fig. 4).

4.3. Long-term variation of $\delta^{15}N_{SPOM}$

Our spring (April) and summer (June to August) $\delta^{15}N_{SPOM}$ values for the mesohaline and oligohaline sections of the estuary are higher than the ones reported for the 1970s by Mariotti et al. (1984). For the Temse to Zandvliet section (km 78 to 57) during April, values ranged from +1.5‰ to +5‰ in the 1970s, while today values range between +2.7 and +8.6‰ (compare their Fig. 8 with our Fig. 4). During summer, the oligohaline section (Temse to Antwerp) also shows an increased $\delta^{15}N_{SPOM}$ signal today (+8.7‰ for Temse in July and +10.8‰ for Antwerp in August; Fig. 4), while for the same section in the 1970s values did not exceed +5‰. However, in the downstream area during summer the situation might be reversed. Indeed values as high as +24‰ were reported for the 1970s by Mariotti et al. (1984), and these were attributed mainly to autochtonous phytoplankton. Our present-day highest summer values at Zandvliet are +12.9‰ (June), but since the downstream summer sampling area is not detailed by Mariotti et al. (1984), it is difficult to compare their values with ours. In any case, Middelburg and Nieuwenhuize (1998), who investigated the Scheldt over its polyhaline to oligohaline sections in 1994 (August), did not observe the very high downstream $\delta^{15}N_{SPOM}$ values of the 1970s, indicating that for that section, too, significant changes have occurred over time.

For the mesohaline – oligohaline section, the increase in $\delta^{15}N_{SPOM}$ probably reflects improved conditions for nitrification, as NH_4^+ concentrations are generally lower at present than in the 1970s (Van Damme et al., 1999). Today ^{15}N enrichment of SPOM occurs earlier in the season and is observed in more upstream areas, probably because nitrification starts earlier in the season (there is even evidence for winter nitrification, N. Brion, unpublished results) and occurs more upstream in the estuary. Our nutrient data indicate that the area of intense nitrification during summer is now situated upstream of km 130 (Fig. 5), a situation already documented in the 1990s (Soetaert and Herman, 1995b), while in the 1970s nitrification was insignificant upstream of km 70 (Mariotti et al., 1984; Billen et al., 1985). This shift occurred despite occasional low O_2 contents (<1 mg dm^{-3}) in these freshwater reaches (Fig. 3), but Van Damme et al. (1999) suggest that nitrification can probably proceed in hypoxic conditions when coupled with oxygen production by phytoplankton. As an alternative explanation for the present generally increased $\delta^{15}N_{SPOM}$

signals for the mesohaline – oligohaline sections in spring and summer, we can invoke a decreased input of domestic sewage. Domestic sewage has a very light $\delta^{15}N$ signal (+2‰; Fisseha, 2000) and a reduction of sewage load relative to other less light N components would increase the present $\delta^{15}N$ signal of SPOM. Indeed, at present the input of untreated sewage comes mainly from the city of Brussels (1.10^6 inhabitants), whereas in the 1970s, mostly untreated sewage was released also by Antwerp and Ghent (6.10^6 inhabitants).

5. Conclusions

During this study, we observed considerable spatio-temporal variability of the $\delta^{15}N$ and $\delta^{13}C$ composition of suspended particulate organic matter in the Scheldt estuary. In general, the $\delta^{13}C_{SPOM}$ signal followed a quite predictable seasonal trend, set mainly by phytoplankton activity, with least negative values in winter and most negative values in spring, summer. The spatio-temporal variation of $\delta^{15}N_{SPOM}$, however, was more complex. At Antwerp, Temse and Dendermonde highest $\delta^{15}N$ values observed during the bloom period (spring, summer) were attributed to the uptake of NH_4^+ enriched in ^{15}N as a result of ongoing uptake and nitrification. The pattern observed for Zandvliet, more downstream in the mesohaline part of the river, was different with high $\delta^{15}N_{SPOM}$ values from spring to autumn. The persistent high $\delta^{15}N$ signal probably resulted from the advection of ^{15}N-enriched phytoplankton detritus from upstream regions and from further ^{15}N enrichment during bacterial processing. Mariotti et al. (1984) observed an increase in $\delta^{15}N_{SPOM}$ during the growth season for the mesohaline section. This trend is confirmed by our results, but there is evidence that the zone of intense nitrification in summer has shifted upstream relative to the situation in the 1970s. Also, the nitrification period now appears to start earlier (in winter-spring) resulting in higher $\delta^{15}N_{SPOM}$ values during spring than in the 1970s. Our $\delta^{15}N_{SPOM}$ data for Dendermonde (range: −0.5‰ to +11.5‰) are the first reported for the freshwater part of the estuary. The largest change in $\delta^{15}N$ composition of SPOM has probably occurred in the freshwater part of the Scheldt that used to be anaerobic in the 1970s and would have experienced low nitrification most of the year.

During most of the year, zooplankton-$\delta^{15}N$ in the mesohaline section followed the one of SPOM but with an offset exceeding by far the normal increment associated with trophic level, probably as a result of selective grazing on phytoplankton. The large ^{15}N enrichment of zooplankton (up to +25.5‰) during summer is among the highest observed in estuarine systems and reflects the intensity of nitrification today. It is likely that the seasonal ^{15}N enrichment will be transferred also to the higher trophic levels. Future studies of trophic relationships in the Scheldt system will have to consider carefully these strong fluctuations of isotopic composition at the lower trophic levels.

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