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Dynamics of particulate organic matter $\delta^{15}N$ and $\delta^{13}C$ during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France)

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ABSTRACT: Two cruises (April and June 1997) were carried out in the Bay of Seine, a nitrate- and ammonium-enriched ecosystem of Western Europe, to identify the major mechanisms that control $\delta^{15}N$ and $\delta^{13}C$ in spring particulate organic matter (POM). Particulate organic nitrogen (PON) $\delta^{15}N$ ranged between 0.8 and 5.2‰ in April and between 2.2 and 6.2‰ in June, while particulate organic carbon (POC) $\delta^{13}C$ ranged between –24.3 and –19.7‰, and between –20.0 and –16.2‰ during the same periods. During spring 1997, POM was highly dominated by autochthonous phytoplankton. It is shown that the variation of PON $\delta^{15}N$ is due to both nitrate mixing between river and marine waters and fractionation of N stable isotopes during nitrate utilization by phytoplankton. Therefore, similarly to what was previously shown for open ocean, $\delta^{15}N$ can be used as a proxy of spring fractional nitrate utilization in coastal ecosystems. It is also shown that POC $\delta^{13}C$ in spring is controlled by POC concentration and C:N ratio (in addition to 'temperature effects'), which are considered here as indicators of primary production and phytoplankton degradation, respectively. The co-variation of $\delta^{13}C$ and $\delta^{15}N$ describes the spring phytoplankton dynamics: at the start of phytoplankton development, nitrate concentration is high (low $\delta^{15}N$) and phytoplankton production is low (low $\delta^{13}C$); then primary production increases ($\delta^{13}C$ becomes higher) and the nitrate pool diminishes ($\delta^{15}N$ becomes higher); at a later stage, the nitrate pool is depleted (high $\delta^{15}N$), part of the phytoplankton becomes degraded and production is still high (high $\delta^{13}C$).

KEY WORDS: C and N stable isotopes · Phytoplankton · Coastal ecosystem · Nitrate utilization · Bay of Seine

INTRODUCTION

In aquatic biogeochemistry and ecology, carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively) of suspended particulate organic matter (POM) are widely used to determine the origin (terrestrial, freshwater or marine reservoir) and fate of POM (Gearing 1988), to characterize the nutrient utilization by autotrophs (Raven & Johnston 1991, Altabet 2001) and the metabolism pathways (Descolas-Gros & Fontugne 1985, Goericke et al. 1994), to study C and N cycles (Altabet et al. 1999, Battle et al. 2000) and to describe

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the food web (Fry & Sherr 1984, Peterson 1999). In marine systems (open ocean and coastal areas), phytoplankton represents the major food source for primary consumers and constitutes the first biological step in the C and N cycles. Phytoplankton is therefore of major interest for biogeochemical studies.

Phytoplankton $\delta^{13}C$ and $\delta^{15}N$ (hereafter $\delta^{13}C_\varphi$ and $\delta^{15}N_\varphi$, respectively) vary widely throughout aquatic ecosystems (Gearing 1988, Wada & Hattori 1991, Hoefs 1997) due to numerous factors. The main steps leading to the phytoplankton isotope signature can be summarized as (Hayes 1993, Fry 1996): (1) the isotope ratios of the nutrient source (Owens 1987, Fry 1996), (2) the isotopic effects associated with nutrient assimilation (O'Leary 1981, Fogel & Cifuentes 1993) and (3) the isotopic effects associated with biosynthesis (Descolas-Gros & Fontugne 1990, Wada & Hattori 1991, Riebesell 2000). Nutrient availability and isotopic fractionation (ε; see 'Materials and methods' for definition) during nutrient uptake are therefore of major importance (Rau et al. 1992, Pennock et al. 1996, Korb et al. 1998). Because the lighter isotope is preferentially incorporated over the heavier one, nutrient uptake results in the enrichment of the heavier isotope in the remaining nutrient pool. When the nutrient stock is large and/or when the uptake is small, this enrichment is negligible. In contrast, when the nutrient stock becomes depleted (during the course of a phytoplankton bloom), its enrichment in ^{15}N becomes large and the isotope ratio of the phytoplankton incorporating residual nutrient rises. Nitrate $\delta^{15}N$, particulate organic nitrogen (PON) $\delta^{15}N$, and isotopic fractionation during the conversion of NO_3^- into PON have been widely described using the Rayleigh fractionation model for open oceans (Wada 1980, Altabet & François 1994, Altabet et al. 1999), coastal ecosystems (Goering et al. 1990, Horrigan et al. 1990) and lakes (Teranes & Bernasconi 2000). However, this model is valid for closed systems, i.e. for systems where there is no input and no output of material. Because this assumption may not be valid for environments such as upwelling or coastal areas (where nutrient input is usually significant), an open system model has also been used (Altabet 2001, Altabet & François 2001).

Isotopic fractionation is not constant and may depend on the environmental conditions such as nutrient concentrations, light, etc. Indeed, nutrient demand (i.e. the difference between the internal and external nutrient concentrations; see Farquhar et al. 1982, Rau et al. 1992, 1996) and growth rate (μ) play an important role in isotopic fractionation. In the late 1990s, several authors have well documented the inverse relationship between ε and the $\mu/[CO_2]_{aq}$ ratio (Laws et al. 1995, 1997, Popp et al. 1998, Burkhardt et al. 1999b). The influence of phytoplankton growth rate on isotopic fractionation is illustrated by the 2-step model of carbon fixation described by Park & Epstein (1960), O'Leary (1981) and Fry (1996):

$$DIC_e \rightleftarrows DIC_i \rightarrow \text{fixed DIC}$$

DIC_e and DIC_i are cell-external and -internal dissolved inorganic carbon (DIC) pools, respectively. The first step (reversible) consists of the DIC incorporation/transport (and the possible leakiness of DIC from the internal pool back to the external pool) associated with low or no fractionation; the second step (irreversible) is the enzyme-catalyzed carboxylation (i.e. photosynthesis mechanism) associated with potentially large fractionation (Fry 1996). If the photosynthesis rate is low (i.e. low growth rate, low nutrient demand), the intracellular DIC pool is large and uptake is limited by the second step (carboxylation). Because of the large fractionation during carboxylation, the expected fractionation for the whole reaction is thus high. In contrast, if the photosynthesis rate is high (i.e. high growth rate, high nutrient demand), the intracellular DIC pool is low and uptake is limited by the first step (incorporation/transport). If all (or almost all) the DIC entering the cell is carboxylated, no (or little) fractionation occurs during the whole reaction.

During the course of a spring phytoplankton bloom in natural ecosystems, growth rate is first low (high nutrients, low light), then high (high nutrients, high light), then low (low nutrients, high light). Because of growth-rate variations and nutrient depletion, $\delta^{13}C_\varphi$ and $\delta^{15}N_\varphi$ are supposed to exhibit large variations. For a controlled ecosystem enclosure experiment, Nakatsuka et al. (1992) described 3 phases of a phytoplankton bloom from an isotopic point of view: (1) the early stage, when nitrate is in excess (low $\delta^{15}N_\varphi$) and the growth rate is high (no light limitation; high $\delta^{13}C_\varphi$); (2) the late stage, when nitrate is recently depleted (high $\delta^{15}N_\varphi$) but growth rate is still high (high $\delta^{13}C_\varphi$); and (3) the steady-state phase, when the balance between NH_4^+ and PON is established (high $\delta^{15}N_\varphi$) and the growth rate becomes lower (low $\delta^{13}C_\varphi$). The occurrence of this third phase actually contradicts Checkley & Entzeroth (1985), who indicated that the mineralization of NH_4^+ from PON is accompanied by an isotopic fractionation leading to a lower $\delta^{15}N$ of the newly produced NH_4^+ pool and a higher $\delta^{15}N$ of the remaining PON stock than the $\delta^{15}N$ of the initial PON (i.e. before degradation). However, only few studies report $\delta^{13}C$ and/or $\delta^{15}N$ dynamics during phytoplankton blooms in natural ecosystems (e.g. Mariotti et al. 1984, Goering et al. 1990, Altabet et al. 1991, Dehairs et al. 1997, Kukert & Riebesell 1998, Rolff 2000).

Thus, high variations of stable isotope ratios occur during spring phytoplankton blooms, and understanding the processes that control $\delta^{13}C$ and $\delta^{15}N$ dynamics is not trivial, especially in complex ecosystems such as coastal areas. This study aims to determine the major biological mechanisms that steer the variations of POM $\delta^{15}N$ and $\delta^{13}C$ during spring phytoplankton blooms in a DIN-enriched coastal area (the Bay of Seine, France). Our approach consists of (1) demonstrating that autochthonous phytoplankton is the main component of sampled POM, and then (2) modelling and discussing N and C stable isotope variations during spring phytoplankton blooms. $\delta^{15}N$ and $\delta^{13}C$ are treated in separate sections. As a conclusion, $\delta^{15}N$ and $\delta^{13}C$ co-variations are used to identify 2 stages in the spring phytoplankton dynamics.

MATERIALS AND METHODS

Study area. The Bay of Seine is located on the northern coast of France, in the eastern English Channel (Fig. 1). It is ca. 45 × 100 km wide and 10 to 30 m deep. The maximum tidal range in the eastern part of the Bay of Seine is 7.5 m. The mean annual continental water supply is about 450 $m^3\ s^{-1}$, of which 400 $m^3\ s^{-1}$ are discharged by the Seine River in the SE zone. The Seine River discharge usually ranges between 140 and 1500 $m^3\ s^{-1}$. Its drainage basin (75 000 km^2) accounts for 20% of agricultural and 30% of urban activity of France (Cabioch 1986a). Nutrient inputs from the Seine into the Bay (for the mean annual flow) are about 85 000 t of nitrogen and 6000 t of phosphorus (Aminot et al. 1998). Nutrient enrichment generates intense phytoplankton blooms, with several chlorophyll maxima that can exceed 40 µg l^{-1} in the outer river plume during spring and summer (Aminot et al. 1998). The Bay of Seine is thus of particular interest when studying phytoplankton processes. General hydrodynamical, hydrological and sedimentological conditions in the Bay are well documented (Cabioch 1986b, Aminot et al. 1997), since numerous French national programs have focused on this area during the last 2 decades (GRECO-Manche, PNEC-Baie de Seine I and II, SEINAVAL).

Sampling strategy. To obtain data at different stages of phytoplankton development and nutrient consumption, 2 spring cruises (Dynamo I: 24 to 27 April 1997, and Dynamo II: 17 to 21 June 1997) were undertaken in the study area. The first cruise was scheduled at the period when the first significant phytoplankton bloom generally takes place (Aminot et al. 1997), and the second one at the period when nitrate was partially depleted in the Bay. The cruises were undertaken at low Seine River discharge (around 250 $m^3\ s^{-1}$) to minimize continental and estuarine POM export toward the study area. Only the eastern Bay of Seine, closest to the Seine estuary mouth, was of interest because of the continental nutrient inputs. The list and location of sampling stations are shown in Table 1 and Fig. 1, respectively. In order to ensure that the upstream POM was not significantly discharged into the Bay, the Seine estuary was sampled during the June cruise. Physical (salinity, temperature, suspended particulate matter [SPM]), chemical (dissolved inorganic nitrogen [DIN]) and biological (chlorophyll [chl] *a*, pheopigments, par-

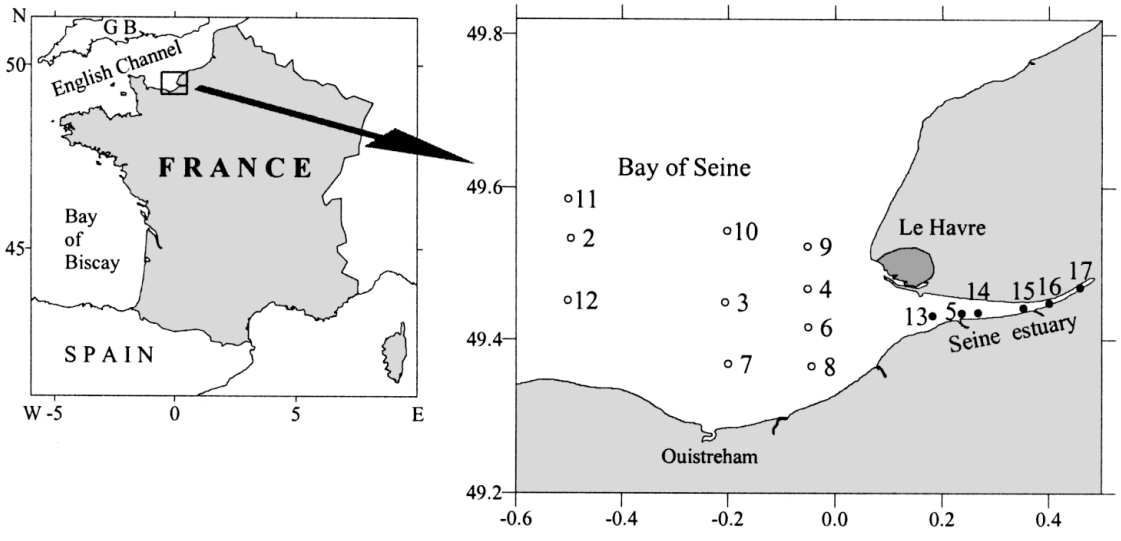

Fig. 1. Location of the sampling stations in the Bay of Seine ecosystem during the DYNAMO I (24–27 April 1997) and II (17–21 June 1997) cruises. ○: Bay of Seine proper stations (salinity > 30); ●: estuary stations (salinity < 30)

Table 1. Measured and calculated (*) data in surface water during the 2 Dynamo cruises (24–27 April and 17–21 June 1997). Data are ranked by decreasing salinity for each cruise. SPM: suspended particulate matter; NO_3^-: nitrate+nitrite; chl: chlorophyll *a*; POC: particulate organic carbon; PON: particulate organic nitrogen; C:N: POC:PON; $\delta^{13}C_{15°C}$: temperature-normalized $\delta^{13}C$; *u'*, *u''*: apparent fractional nitrate utilization (see the text for details). nd: no data; nc: not calculated

Stn	Date	Salinity	Temp. (°C)	[SPM] (mg l^{-1})	[NO_3^-] (µM)	[NH_4^+] (µM)	[Chl] (µg l^{-1})	[POC] (mg l^{-1})	POC:chl* (g g^{-1})	[PON] (µg l^{-1})	C:N* (mol mol^{-1})	$\delta^{13}C$ (‰)	$\delta^{13}C_{15°C}$* (‰)	$\delta^{15}N$ (‰)	*u'**	*u''**
11	27 Apr	34.7	10.3	0.93	10.4	0.14	5.8	0.286	49	47	7.1	–24.3	–22.6	0.8	0.38	0.68
2	24 Apr	34.3	10.2	1.21	7.6	0.23	13.3	0.556	42	92	7.1	–21.7	–20.0	2.7	0.66	0.80
3	24 Apr	33.9	11.4	1.15	10.8	0.11	11.4	0.654	57	114	6.7	–22.0	–20.7	2.5	0.60	0.75
10	27 Apr	33.7	10.1	2.07	14.8	0.56	19.8	0.665	34	125	6.2	–22.4	–20.6	3.7	0.51	0.68
9	27 Apr	33.2	10.3	3.26	16.3	0.48	24.6	0.881	36	192	5.4	–21.9	–20.2	4.3	0.56	0.69
8	26 Apr	33.0	10.4	2.64	8.5	0.39	28.8	1.33	46	247	6.3	–19.7	–18.1	5.2	0.79	0.85
6	26 Apr	32.1	10.6	5.02	21.6	0.46	42.7	1.55	36	309	5.8	–21.1	–19.5	4.2	0.58	0.68
4	25 Apr	31.9	10.1	5.30	44.1	0.21	11.5	0.648	56	125	6.0	–23.1	–21.3	2.5	0.21	0.38
5	25 Apr	25.7	11.1	20	136	0.99	18.8	1.25	66	268	5.4	–24.9	nc	2.5	nc	nc
12	20 Jun	33.9	14.9	1.53	0.57	0.24	8.0	0.460	58	82	6.5	–20.0	–20.0	3.6	0.98	0.99
9	19 Jun	33.7	15.5	1.87	1.08	0.64	9.5	0.777	82	123	7.4	–17.9	–18.1	2.2	0.96	0.98
10	18 Jun	33.4	16.5	1.60	0.37	0.03	9.3	0.940	101	112	9.8	–16.9	–17.4	4.0	0.99	1.00
11[a]	18 Jun	33.1	16.1	1.81	0.16	0.04	9.4	1.18	126	133	10.4	–16.7	–17.1	5.2	1.00	1.00
6	20 Jun	32.7	16.0	5.16	0.41	0.18	17.0	1.39	82	232	7.0	–18.4	–18.8	6.1	0.99	1.00
11[a]	18 Jun	32.7	18.1	2.58	0.34	0.04	12.9	1.82	141	214	9.9	–16.2	–17.3	6.0	0.99	1.00
8	21 Jun	32.6	16.0	5.91	1.82	0.24	17.4	1.20	69	250	5.6	–17.8	–18.2	5.8	0.96	0.98
7	20 Jun	32.5	16.2	3.13	1.91	0.15	20.8	1.85	89	276	7.8	–16.8	–17.2	6.2	0.96	0.98
3	19 Jun	31.8	16.8	3.69	6.2	0.14	22.7	2.13	94	307	8.1	–16.9	–17.5	5.6	0.89	0.94
4	17 Jun	30.4	16.8	5.97	28.6	0.21	15.4	1.20	78	208	6.7	–18.6	–19.2	5.6	0.63	0.76
13	21 Jun	25.7	16.6	43	94.3	3.47	17.2	1.98	115	nd	nc	–23.7	nc	5.3	nc	nc
14	21 Jun	20.2	17.2	62	167	4.41	17.2	2.46	143	420	6.8	–24.0	nc	4.3	nc	nc
15	21 Jun	14.1	18.2	90	250	3.57	13.0	3.37	259	450	8.7	–25.8	nc	5.9	nc	nc
16	21 Jun	10.1	18.6	168	304	2.83	10.0	6.12	612	865	8.3	–26.1	nc	7.2	nc	nc
17	21 Jun	3.6	19.4	92	395	1.55	10.2	3.33	326	513	7.6	–26.9	nc	7.9	nc	nc

[a]Sampled at both high and low tide

ticulate organic carbon [POC] and PON) parameters were studied in order to both characterize the POM and understand their importance on the carbon and nitrogen stable isotope ratios.

Analytical procedures. Surface water was sampled using 8 l PVC samplers (Niskin type). Subsamples were drawn off immediately through on-line 47 mm filter holders fitted to the sampler tap. On-line holders were equipped with a 200 µm nylon net for particulate matter subsampling in order to remove macrodetritus and macrozooplankton from samples, and with a 10 µm polypropylene membrane for nutrient subsampling. For particulate matter determinations, filtration (through Whatman GF/F disks) was performed on board without delay for chlorophyll, total SPM and POM (POC, PON, $\delta^{13}C$ and $\delta^{15}N$). Filters as well as samples for DIN measurement were immediately frozen and stored at –20°C for subsequent analysis at the shore laboratory.

Salinity in discrete samples was measured using a Guildline Portasal salinometer on the Practical Salinity Scale (1978) (PSS78). SPM concentrations were measured by weight. Nitrate + nitrite concentrations were determined according to Mullin & Riley (1955) and Tréguer & Le Corre (1975), and ammonium concentrations according to Kerouel & Aminot (1997). Chl *a* was analyzed spectrophotometrically according to Lorenzen (1967).

Samples for POC, PON, $\delta^{13}C_{POC}$ and $\delta^{15}N_{PON}$ analyses were decarbonated by contact with HCl vapours in a vacuum-enclosed system. After decarbonation, the filters were introduced in an elemental analyzer (Carlo Erba NA 2100 configured for C and N analysis) for the determination of POC and PON contents (precision: ±5% for POC and ± 4% for PON). Resulting gases (CO_2 and N_2) were then injected into the isotope ratio mass spectrometer (Finnigan Delta S) through a Conflo interface in order to measure carbon and nitrogen stable isotope ratios. The isotope ratios are expressed in the usual delta notation:

$$\delta^{13}C_{sample},\ \delta^{15}N_{sample} = 1000 \times \left(\frac{R_{sample}}{R_{reference}} - 1 \right) [‰]$$

$R = {}^{13}C/{}^{12}C, {}^{15}N/{}^{14}N$, respectively.

Reference is VPDB (cretaceous PeeDee Belemnite) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Ten replicates yielded a standard deviation of 0.2‰ for $\delta^{13}C$ and of 0.3‰ for $\delta^{15}N$.

Isotopic fractionation is defined as

$$\varepsilon = 10^3 \ln\left(\frac{R_A}{R_B} \right) \approx \delta_A - \delta_B$$

where δ is $\delta^{13}C$ or $\delta^{15}N$, and A and B are reagent and product of the reaction A $\rightarrow$ B, respectively.

Statistical treatments. Statistical tools were used to test data and support the discussion. Especially, multilinear regressions were performed. According to Scherrer (1984), multilinear regression must be done between a dependent variable and independent variables with no correlation among the independent variables. In order to determine whether data sets are correlated or not, 2 tests are useful: the Pearson correlation test is performed when data is normally distributed (standardized skewness and standardized kurtosis tests) and the Spearman rank correlation test when data is not normally distributed. All statistical tests were performed using the software Statgraphics plus. The significance of each test (p-value) is given in text and/or tables.

RESULTS

The Bay of Seine

In the Bay of Seine proper (Fig. 1), surface water salinity was higher than 30 (Table 1). During the study periods, surface temperature increased from April (ca. 10°C) to June (ca. 16°C), while average DIN concentrations strongly diminished (from 15.9 to 4.15 µM for nitrate and from 0.32 to 0.19 µM for ammonium), until depletion in the western part of the study area ($[NO_3^-] < 0.5$ µM in June; see Table 1). In contrast to the average POC and PON concentrations, which increased during this period (from 0.83 to 1.29 mg l^{-1} for POC and from 157 to 194 µg l^{-1} for PON), average chl *a* concentrations were higher in April (20.4 µg l^{-1}) than in June (14.2 µg l^{-1}). These variations are those generally encountered from the beginning of the phytoplankton production period to late spring in the eastern Bay of Seine (Aminot et al. 1997). C and N isotope ratios also showed large variation from late April to mid-June: $\delta^{13}C$ average increased from –22.0 to –17.6‰ and $\delta^{15}N$ average from 3.2 to 5.0‰.

Large geographical variations were encountered within the study area. Salinity and nutrients were distributed according to the classical westward or northwestward gradient (i.e. from the mouth of the estuary to the open Bay) due to the freshwater input. In the Bay proper (salinity > 30), nitrate concentration ranged from 7.6 to 44 µM (April) and from 0.16 to 29 µM (June), while ammonium concentrations ranged from 0.11 to 0.56 µM (April) and from 0.03 to 0.64 µM (June). Large variations were also encountered for chl *a* (5.8 to 42.7 µg l^{-1} in April; 8.0 to 22.7 µg l^{-1} in June), for POC (0.286 to 1.55 mg l^{-1} in April; 0.460 to 2.13 mg l^{-1} in June) and PON (47 to 309 µg l^{-1} in April; 82 to 307 µg l^{-1} in June) concentrations, for $\delta^{13}C$ (–24.3 to –19.7‰ in April; –20.0 to –16.2‰ in June) and for $\delta^{15}N$ (0.8 to 5.2‰ in April; 2.2 to 6.2‰ in June), but no simple overall spatial pattern was observed. However, the lowest chl *a*, POC and PON concentrations were measured from the open Bay seawater (Fig. 2b,c). POC and PON maxima were observed at Stn 6 in April and at Stn 3 in June, coinciding with chl *a* maxima.

Such a wide range in POM $\delta^{15}N$ (from 0.8 to 6.2‰) values is usual for coastal areas where marine water is influenced by freshwater (Owens 1987, Wada & Hattori 1991). However, when POM $\delta^{13}C$ values of –24 to –18‰ are commonly reported in the literature for a coastal ecosystem (Gearing 1988), the highest values (>–18‰) are infrequent (Fry & Wainright 1991).

The Seine estuary

The Seine estuary (salinity < 30) was studied in June. Nitrate concentrations followed a linear (i.e. conservative) dilution line, whereas ammonium concentrations showed a bell-shaped curve with a maximum of 4.4 µM at salinity 20 (Fig. 2a). POC and PON exhibited maxima (6.12 mg l^{-1} and 865 µg l^{-1}, respectively) at the turbidity maximum ([SPM] = 168 mg l^{-1}; salinity = 10), and a decrease with increasing salinity (Fig. 2b,c).

POM $\delta^{13}C$ values increased almost linearly from upstream ($\delta^{13}C$ = –26.9‰ at salinity 3.6) to downstream ($\delta^{13}C$ = –23.7‰ at salinity 25.7). Along the salinity gradient, POM $\delta^{15}N$ ranged between 4.3 and 7.9‰ and exhibited its minimum at salinity 20, coinciding with the ammonium-concentration maximum. These estuarine POM $\delta^{13}C$ and $\delta^{15}N$ values are consistent with previous data reported in the literature (Owens 1987, Gearing 1988, Wada & Hattori 1991).

DISCUSSION

Particulate organic matter characterization

Because suspended POM is a mixture of living as well as detrital material (phytoplankton, bacteria, zoo-

plankton, fecal pellets, continental detritus, etc.) originating from marine, estuarine, freshwater and/or terrigenous reservoirs, POM samples have to be characterized and the major contributor(s) has (have) to be determined. Since collected water was poured through a 200 µm net before subsampling (see 'Materials and methods: Analytical procedures'), macrozooplankton and macrodetritus were removed from the POM to be sampled.

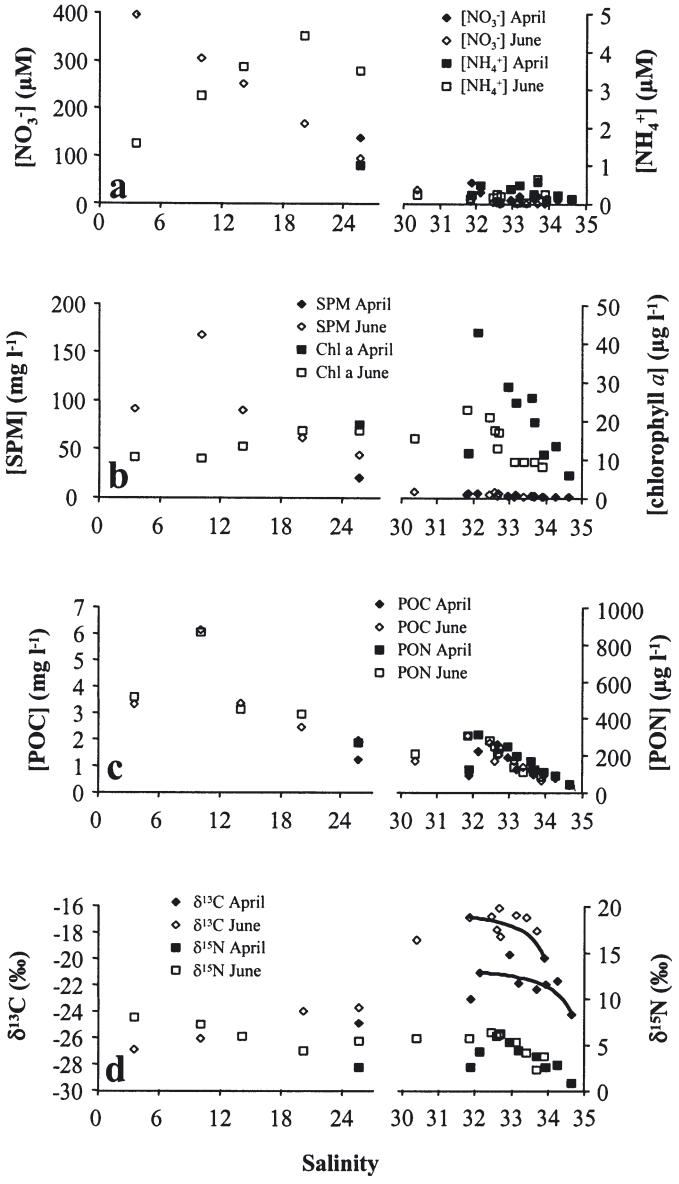

Fig. 2. (a) Nitrate and ammonium concentrations, (b) suspended particulate matter (SPM) and chlorophyll *a* (chl *a*) concentrations, (c) particulate organic carbon (POC) and nitrogen (PON) concentrations, and (d) $\delta^{13}C_{POC}$ and $\delta^{15}N_{PON}$ versus salinity during April and June 1997 in the Seine estuary (salinity < 30) and the Bay of Seine (salinity > 30). Curves in (d) correspond to mixing model (Eq. 1)

POM is commonly characterized by its C:N and POC:chl *a* (POC:chl) ratios. The C:N molar ratio generally ranges between 6 and 10 for phytoplankton (Brzezinski 1985, Montagnes et al. 1994, Créach 1995) and between 3 and 6 for zooplankton and bacteria (Faganeli et al. 1988, Gorsky et al. 1988, Le Fèvre-Lehoerff et al. 1993, Créach 1995, Fagerbakke et al. 1996), and it is typically higher than 12 for terrestrial organic matter (Bordovskiy 1965, Kukal 1971, cited by Faganeli et al. 1988, Thornton & McManus 1994). This ratio is therefore useful when one wishes to distinguish phytoplankton from heterotrophs and from terrigenous material. However, this tool must be used with caution because of its variation in the course of POM degradation: phytoplankton PON is preferentially degraded compared to POC, leading to a C:N increase (Bordovskiy 1965, Smith et al. 1992), whereas terrestrial organic matter (high C:N ratio) colonization by bacteria (low C:N ratio) lowers the initial terrestrial matter C:N ratio (Bordovskiy 1965, Thornton & McManus 1994). Thus degraded phytoplankton and bacteria-colonized terrestrial organic matter could have similar C:N ratios (Lancelot & Billen 1985). In this study, the C:N ratios of the Bay POM ranged between 5.4 and 10.4 (April and June cruises), and generally between 6 and 10 for most of the stations (Table 1), with an average value of 6.3 in April and 7.9 in June. These values seem to indicate that phytoplankton was the major contributor of the POM during the study periods in the Bay of Seine.

Several authors use the POC:chl ratio to characterize the POM in coastal or open ocean waters (Cifuentes et al. 1988, Richard et al. 1997, Bentaleb et al. 1998). For living phytoplankton, POC:chl ratios vary with temperature, growth rate, day length, phytoplankton species, but especially with irradiance (Heath et al. 1990, Montagnes et al. 1994, Cloern et al. 1995, Geider et al. 1998, Thompson 1999). According to previous studies, the POC:chl ratio of 'fresh' living phytoplankton is close to 40 (Montagnes et al. 1994), lower than 70 (Geider 1987), lower than 100 (Head et al. 1996) or lower than 140 (Thompson et al. 1992). Thus, Cifuentes et al. (1988) estimated that a POC:chl ratio value lower than 200 is characteristic of a predominance of newly produced phytoplankton in POM, and that a value higher than 200 is characteristic of detrital or degraded material. Bentaleb et al. (1998) assumed that a POC:chl cut-off of 200 distinguishes autotrophic (<200) from mixo/heterotrophic (>200) predominance. In the Bay of Seine proper, the average POC:chl ratios of 43 in April and of 92 in June (range: 34 to 141; Table 1) confirm that phytoplankton was the major contributor to the Bay POM during the 2 cruises, as suggested by the C:N ratios.

However, the phytoplankton could be autochthonous (produced in the Bay), allochthonous (from a

riverine or an estuarine origin) or mixed. Since the discussion hereafter focuses on the relationship between the POM C and N stable isotopes and the environmental parameters, it is of major importance to determine the autochthonous or allochthonous nature of the POM.

Samples were collected during a period of low river discharge (ca. 255 $m^3 s^{-1}$ for the 2 cruises) and following moderate and low river discharge periods (275 and 215 $m^3 s^{-1}$ on average during the 15 d preceding the April and June cruises, respectively). These values have to be compared with the average (391 $m^3 s^{-1}$), the lowest (166 $m^3 s^{-1}$) and the highest (1255 $m^3 s^{-1}$) discharge values for the year 1997 (see also 'Materials and methods: Study area'). Within the Seine River–Bay of Seine ecosystem, as generally occurs in megatidal and macrotidal ecosystems, most of the river and estuarine SPM is evacuated during winter and spring river floods (Bassoulet 1979, Avoine 1986, Avoine & Crevel 1986). In the Seine River, Avoine (1986) reported that 75% of the yearly SPM flux is exported towards the Bay during the river flood period (ca. 2 to 3 mo yr^{-1}). Thus, during the study periods, there were no favorable conditions for SPM (and especially POM) export out of the estuary.

When the mixing of material between upstream (u) and downstream (d) end-members is the only process occurring along a salinity gradient, concentrations (e.g. nutrient, POC, PON concentration) exhibit a linear relationship with salinity, and $\delta^{13}C$ and $\delta^{15}N$ exhibit the following relationship with salinity (Middelburg & Nieuwenhuize 2001):

$$\delta = \frac{C_d \delta_d f + C_u \delta_u (1-f)}{C_d f + C_u (1-f)} \quad (1)$$

with

$$f = \frac{S - S_u}{S_d - S_u} \quad (2)$$

where f represents the fraction of downstream end-member water, δ is $\delta^{13}C$ or $\delta^{15}N$, C is the concentration of the studied material and S the salinity.

As there is no linear relationship either between POC and salinity or between PON and salinity along the estuary (Fig. 2c), physical mixing between riverine or estuarine and marine end-members is not a dominant process in the distribution of POM within the estuary–bay system. This is in agreement with the unfavourable conditions of POM export from the river and/or the estuary to the Bay, as explained above. In contrast, a linear relationship exists between POC or PON and salinity in the salinity range of 32.1 to 34.7 (April) and 31.8 to 33.9 (June). Is this due to a physical mixing of POM within the bay waters? If yes, isotope ratios should be consistent with Eq. (1). $\delta^{13}C$ is commonly used (and used preferentially over $\delta^{15}N$) to determine the origin of the POM and to trace the upstream/downstream mixing of the POC in the continental–ocean continuum (Fontugne & Jouanneau 1987, Gearing 1988, Thornton & McManus 1994). The mixing model (Eq. 1) was thus tested for $\delta^{13}C$ using Stns 6 and 11 (April) or Stns 3 and 12 (June) as end-members. Fig. 2d points out large discrepancy between the data and this model (curves in Fig. 2d), indicating that the mixing of upstream and downstream POM within the bay waters is unlikely. The observed POC and PON linearity is attributed to the phytoplankton consumption of nitrate, initially (i.e. in winter and early spring) linearly linked to salinity.

Because (1) phytoplankton is the major contributor to the POM, (2) there is no or minor riverine/estuarine POM export and (3) there is no or minor mixing of POM between the bay waters, it is assumed that POM was mainly composed of autochthonous phytoplankton in the Bay of Seine proper.

Toward $\delta^{15}N$ as a recorder of fractional nitrate utilization in coastal ecosystems

Open-system model of fractionation

As reported above, Rayleigh fractionation is widely used to describe PON and nitrate $\delta^{15}N$ variations and fractionation during the conversion of nitrate into PON. One of the main assumptions of this model is that the study system is closed (Owens 1987). In the Bay of Seine, this assumption is not valid because the nitrate supply from the DIN enriched Seine River cannot be neglected over the study period. We therefore prefer to use the open-system equation (Altabet & François 2001, from Sigman et al. 1999)

$$\delta^{15}N_{PON} = \delta^{15}NO_3^-{}_{initial} - \varepsilon(1-u) \quad (3)$$

where $\delta^{15}NO_3^-{}_{initial}$ is the nitrate $\delta^{15}N$ at the start of the production period, ε is the isotopic fractionation during the conversion of nitrate into PON and u is the fractional nitrate utilization as defined by Altabet (2001):

$$u = NO_3^- \text{ utilization}/NO_3^- \text{ availability} \quad (4)$$

However, in river plume systems $\delta^{15}NO_3^-{}_{initial}$ varies with salinity. Indeed, winter nitrate distribution results from the mixing between marine and riverine nitrate, and it has been shown that riverine nitrate usually exhibits higher $\delta^{15}NO_3^-$ than marine nitrate (Scheldt estuary: 5‰ compared to 2‰ [Mariotti et al. 1984]; Chesapeake Bay system: 10‰ compared to 6‰ [Horrigan et al. 1990]; Thames estuary: 9‰ compared to 7‰ [Middelburg & Nieuwenhuize 2001]). The $\delta^{15}NO_3^-$ variation along the salinity (S) gradient, as a result of physical mixing between riverine (r; $S_r = 0$) and marine

(m) end-members, is obtained by rearranging Eq. (1) using Eq. (2):

$$\delta^{15}NO_3^-{}_{initial}\,(S) = \frac{(S/S_m)[NO_3^-]_m\,\delta^{15}NO_3^-{}_m + (1-S/S_m)[NO_3^-]_r\,\delta^{15}NO_3^-{}_r}{(S/S_m)[NO_3^-]_m + (1-S/S_m)[NO_3^-]_r} \quad (5)$$

Rearranging Eq. (3) using Eq. (5) gives

$$\delta^{15}N_{PON} = \frac{(S/S_m)[NO_3^-]_m\,\delta^{15}NO_3^-{}_m + (1-S/S_m)[NO_3^-]_r\,\delta^{15}NO_3^-{}_r}{(S/S_m)[NO_3^-]_m + (1-S/S_m)[NO_3^-]_r} - \delta(1-u) \quad (6)$$

The major assumption of this model (Eq. 6) is that nitrate constitutes the DIN source for the PON formation. Generally, nitrate and ammonium constitute the main source of nitrogen for phytoplankton in coastal waters. During spring, ammonium concentrations are very low compared to nitrate concentrations (see Fig. 2a and Table 1 for this study) and spring phytoplankton production is generally mainly sustained by nitrate over ammonium (Dugdale & Wilkerson 1992). In the Bay of Seine, this was illustrated by Maguer et al. (1998), who reported an *f*-ratio of 0.85 in surface waters during spring 1993. Thus, it is assumed that nitrate was the main DIN source for phytoplankton during the study period.

Calculation of the fractional nitrate utilization

The fractional nitrate utilization is defined in Eq. (4). In the coastal zone, spring nitrate concentration measured at a given time is the result of nitrate input (winter stock + river supply) and nitrate output (autotroph utilization + dilution with open ocean water [exported nitrate]). u can thus be calculated using

$$u = \left([NO_3^-]_{winter\ stock} + [NO_3^-]_{river\ supply} - [NO_3^-]_{exported} - [NO_3^-]_{measured}\right)/\left([NO_3^-]_{winter\ stock} + [NO_3^-]_{river\ supply} - [NO_3^-]_{exported}\right) \quad (7)$$

The winter NO_3^- stock is calculated from the mixing diagram (regression of early spring [25 March 1997] NO_3^- concentration versus salinity [Eq. 6]). Data were provided by the national monitoring network RNO (Réseau National d'Observation de la qualité du milieu marin).

$$[NO_3^-]_{winter\ stock}\ (\mu M) = -13.90S + 498.4 \quad (R^2 = 0.997;\ n = 26) \quad (8)$$

In contrast, the term '$[NO_3^-]_{river\ supply} - [NO_3^-]_{exported}$' (and especially $[NO_3^-]_{exported}$) is not easy to estimate. An underestimated apparent fractional nitrate utilization (u') may be calculated neglecting this term (Eq. 9). However, the river supply can be estimated from freshwater nitrate concentration and river discharge. Taking into account this river supply estimation and neglecting the exported nitrate, an overestimated apparent fractional nitrate utilization (u'') may be calculated (Eq. 10). Therefore, $u' = u = u''$.

$$u' = \left([NO_3^-]_{winter\ stock} - [NO_3^-]_{measured}\right)/\left([NO_3^-]_{winter\ stock}\right) \quad (9)$$

$$u'' = \left([NO_3^-]_{winter\ stock} + [NO_3^-]_{river\ supply} - [NO_3^-]_{measured}\right)/\left([NO_3^-]_{winter\ stock} + [NO_3^-]_{river\ supply}\right) \quad (10)$$

u' ranged between 0.21 and 0.79 in April and between 0.63 and 1.00 in June, while u'' ranged between 0.38 and 0.85 and between 0.76 and 1.00 during the same periods. The use of this under- and overestimation of u in Eq. (6) leads to under- and overestimate ε. Apparent fractional nitrate utilization and apparent fractionation values have to be considered as low and high limits for u and ε, respectively.

$\delta^{15}N_{PON}$ and fractional nitrate utilization

Using u' and subsequently u'', and the end-member nitrate concentrations given by Eq. (8) ($[NO_3^-]$ = 498 and 11.9 µM at salinity 0 and 35, respectively), Eq. (6) was solved to best fit the data acquired from the April and June cruises. The results indicate that Eq. (6) is a relevant model, either using u' or u''. The regression of calculated $\delta^{15}N_{PON}$ versus measured $\delta^{15}N_{PON}$ is strong and significant (R^2 = 0.88, $p < 0.001$, and R^2 = 0.90, $p < 0.001$, using u' and u'', respectively; see Fig. 3). However, Eq. (6) was not solved using the whole data set, since 1 sample exhibited strongly deviatoric behaviour (Fig. 3): in June, Stn 9 had an unexpectedly low $\delta^{15}N$ value (2.2‰) given the u' or u'' value (0.96 or 0.98, respectively) and salinity (33.7). This station had the highest ammonium concentration (0.64 µM) and the highest ammonium fraction within the DIN pool (37%). Since the nitrate stock was depleted ($[NO_3^-]$ = 1.1 µM) and ammonium is generally preferentially incorporated by phytoplankton over nitrate (Collos & Slawyk 1980), the PON might, to a large extent, have been derived from NH_4^+. Checkley & Entzeroth (1985) have shown that mineralized ammonium displays low $\delta^{15}N$ values. Consequently, the $\delta^{15}N$ of phytoplankton incorporating mineralized ammonium is lowered. Thus, the low $\delta^{15}N$ at Stn 9 in June is attributed to the consumption of the ^{15}N-depleted mineralized ammonium by the phytoplankton.

Fitting Eq. (6) with the data set provides apparent fractionation of 3.5‰ and 5.0‰ (using u' and u'', respectively). Because u' and u'' (apparent fractional nitrate utilization) were used instead of u, 'real' apparent fractionation is in fact somewhere between

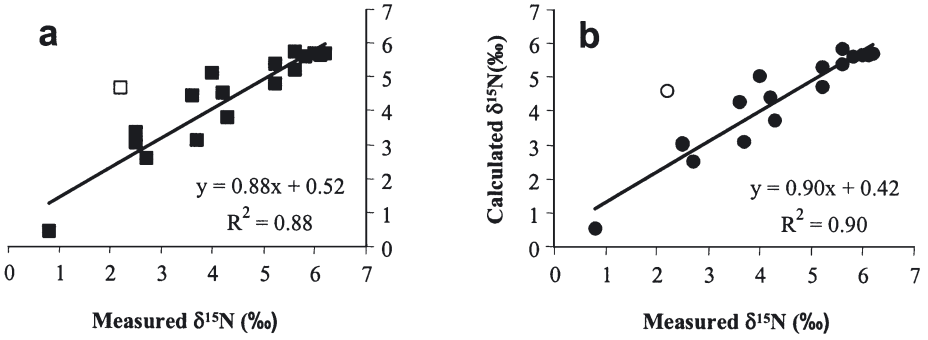

Fig. 3. Calculated (Eq. 6) versus measured $\delta^{15}N$ in the Bay of Seine proper. (a) Using u' (Eq. 9); (b) using u'' (Eq. 10). □ and ○ (Stn 9 in June) are not taken into account for the regressions (see text for details)

3.5 and 5.0‰. Therefore, the average value of 4.2 ± 0.8‰ is more suitable. This value is within the range of fractionation values reported by the literature (Table 2).

From the above results, it appears that using Eq. (6) to model spring $\delta^{15}N_{PON}$ in the Bay of Seine is pertinent. This indicates that PON stable isotope ratios are essentially governed by the fractional nitrate utilization and the salinity (i.e. nitrate $\delta^{15}N$). In turn, $\delta^{15}N_{PON}$ appears to be a good proxy for spring fractional nitrate utilization in 'open' coastal ecosystems, and especially in the river plume, as previously shown for the open ocean (Altabet 2001).

$\delta^{13}C$ variations in the course of the spring phytoplankton blooms

Temperature normalization of $\delta^{13}C_{POC}$

Temperature has a large indirect effect on isotopic fractionation between phytoplankton carbon and CO_2, and therefore on phytoplankton $\delta^{13}C$. An elevation of temperature physically lowers the solubility of CO_2 in water (Weiss 1974), and a decrease in the CO_2 concentration in water leads to a decrease in the isotopic fractionation, i.e. an increase in $\delta^{13}C_\varphi$ (Rau et al. 1992, Bentaleb et al. 1998, Korb et al. 1998, Burkhardt et al. 1999a,b). In addition, the fractionation between HCO_3^- and CO_2 during the equilibrium reaction diminishes (i.e. the $\delta^{13}C_{CO_2}$ increases) as temperature augments (Mook et al. 1974, Zhang et al. 1995, Halas et al. 1997), leading to a potential $\delta^{13}C_\varphi$ increase. Such a relationship between temperature and $\delta^{13}C_{POC}$ (as a proxy for $\delta^{13}C_\varphi$ in open ocean) was described long ago (e.g. Wong & Sackett 1978, Fontugne & Duplessy 1981).

Because this study aims to investigate the influence of biological parameters on $\delta^{13}C_{POC}$, and because there was a high increase in temperature (T) between the 2 study periods (from 10.4°C in April to 16.3°C in June, in average), the data were corrected for the 'temperature effects', i.e. $\delta^{13}C_{POC}$ values were 'normalized' using a $\delta^{13}C_{POC} = f(T)$ equation. This equation can be provided (1) indirectly by combining the equations $[CO_2] = f(T)$ and $\delta^{13}C_{POC} = f([CO_2])$, or (2) directly by the literature-empiric $\delta^{13}C_{POC} = f(T)$ equations. The first approach seems to be more precise, since each step is described. However, although the $[CO_2] = f(T)$ equation is well established (Weiss 1974, Mook 2001),

Table 2. Isotopic fractionation (ε) during nitrate assimilation by phytoplankton from various ecosystems and cultures

ε (‰)	Comment	Source
Culture		
0–19	*Phaeodactylum tricornum*	Wada & Hattori (1978)
0–10	*Dunaniella tertiolecta*	Wada & Hattori (1978)
9.0	*Skeletonema costatum*	Pennock et al. (1996)
0.7–6.3	Cultures	Montoya et al. (1990)
0–2.5	*Chaetoceros* sp.	Wada & Hattori (1978)
Open ocean		
9	Sub-arctic Pacific	Altabet & François (1994)
8–9	North Atlantic	Altabet et al. (1991)
5.2	Equatorial Pacific	Altabet (2001)
4–6	Southern Ocean	Sigman et al. (1999)
7.0 ± 0.95	Southern Ocean	Altabet & François (2001)
Coastal area		
8	Weddell Sea	Biggs et al. (1988)
7.0	Chesapeake Bay	Horrigan et al. (1990)
4	Auke Bay, Alaska	Goering et al. (1990)
4.2 ± 0.8	Bay of Seine	Present study
Lake		
3.0	Switzerland lake	Terranes & Bernasconi (2000)

the $\delta^{13}C_{POC}$ = f([CO_2]) equation is specific for each phytoplankton species (Burkhardt et al. 1999a,b). In addition, this approach does not take into account the *T*-effect on the HCO_3^-–CO_2 fractionation or on the Rubisco activity, which diminishes with increasing temperature, leading to a phytoplankton $\delta^{13}C$ decrease (Descolas-Gros & Fontugne 1990). For these reasons, the second approach is preferred to normalize POC $\delta^{13}C$.

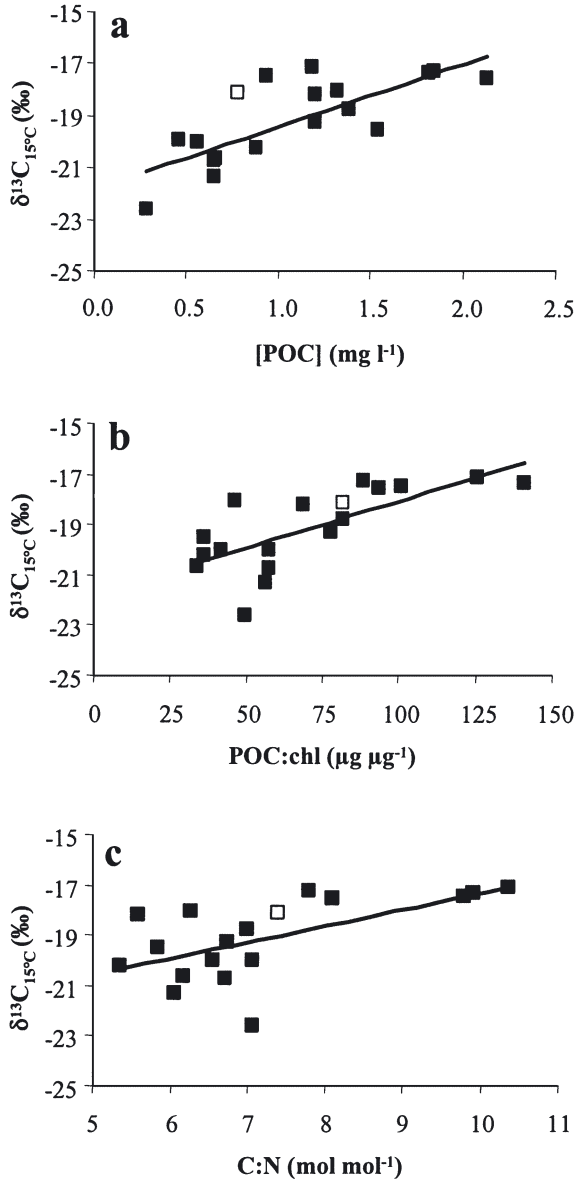

Fig. 4. Temperature-normalized $\delta^{13}C$ ($\delta^{13}C_{15°C}$) versus (a) particulate organic carbon concentration ([POC]), (b) POC:chl *a* ratio (POC:chl), and (c) particulate organic carbon to nitrogen ratio (C:N), in the Bay of Seine proper. □ (Stn 9 in June) are not taken into account for the regressions (see text for details)

The literature provides several linear relationships between phytoplankton isotope ratios or fractionation and temperature. Some of them cannot be used here because they are monospecific (Wong & Sackett 1978, Hinga et al. 1994) or they have been established in particular experimental conditions (Degens et al. 1968). However, 3 slopes are reported from field data for the world ocean: 0.23‰ °C^{-1} (–1.8 = *T* = 25°C; Sackett et al. 1965), 0.35‰ °C^{-1} (–1 = *T* = 35°C; Fontugne & Duplessy 1981) and 0.5‰ °C^{-1} (–2 = *T* = 20°C; Fontugne 1983). These 3 slopes represent the overall variation of $\delta^{13}C$ versus sea-surface temperature, whatever the environmental conditions. An average value of 0.36‰ °C^{-1} is used in the discussion below. POC $\delta^{13}C$ was normalized at the arbitrary temperature of 15°C using

$$\delta^{13}C_{15°C} = \delta^{13}C - s(T - 15) \quad (11)$$

where $\delta^{13}C_{15°C}$ is the normalized-temperature $\delta^{13}C$, *T* the seawater temperature in °C, and *s* the slope of the linear regression $\delta^{13}C = f(T)$ in ‰ °C^{-1}. The impact of variable slopes ranging between 0.23 and 0.5 ‰ °C^{-1} was also tested. The results are the same as those described below, taking into account the uncertainties on each estimate of the model.

The statistical model

$\delta^{13}C_{15°C}$ was studied with respect to the environmental parameters. This study was realized with 17 of the 18 values of the Bay of Seine data set. Indeed, Stn 9 in June was excluded from the data set because of presumably high ammonium consumption (see above). As far as $\delta^{13}C$ is concerned, the ammonium uptake by phytoplankton increases $\delta^{13}C$ through its effect on the β-carboxylation pathway for carbon fixation (Guy et al. 1989, Descolas-Gros & Fontugne 1990, Leboulanger et al. 1995, Dehairs et al. 1997).

Significant regressions (p = 0.05) relate $\delta^{13}C_{15°C}$ to POC concentration (R^2 = 0.61), POC:chl *a* (R^2 = 0.53) and C:N (R^2 = 0.36) ratios (Fig. 4). However, none of these parameters can fully explain the $\delta^{13}C_{15°C}$ variations, indicating that different processes (in addition to the 'temperature effects') determine the phytoplankton isotopic signature. In order to deconvolute these different signals, multilinear regressions were carried out (see 'Materials and methods: Statistical treatments'). A Pearson correlation test reveals that POC:chl ratio is correlated with POC as well as with the C:N ratio (p = 0.05), whereas POC and C:N ratio are not correlated (p > 0.05). Thus, POC and C:N ratio were used as 'independent variables'. The results of the multilinear regression (Table 3, Eq. 12) show that there is a significant correlation (p < 0.001; R^2 = 0.75) between $\delta^{13}C_{15°C}$ and a combination of POC concentra-

tion and the C:N ratio, and that this model (Eq. 12) explains 75% of the $\delta^{13}C_{15°C}$ variations:

$$\delta^{13}C_{15°C} = 2.0[POC] + 0.42C{:}N - 24.4 \quad (12)$$

POC concentration, C:N ratio and POC $\delta^{13}C$

POC is an indicator of the phytoplankton biomass, since POM is dominated by phytoplankton (see 'Particulate organic matter characterization'). In fact, POC is here the result of primary production or growth rate, and it can be considered as a proxy for primary production, temporally integrated on the residence time of POM in surface waters. The relationship between primary production or growth rate and carbon stable isotopes is well documented from *in vitro*, model and *in situ* studies (see 'Introduction'). However, from field studies, such a relationship seems to be significant only in productive areas (Cifuentes et al. 1988, Fogel et al. 1992), since Fontugne (1983) showed that the primary production effect is negligible in the oligotrophic and mesotrophic zones. Eq. (12) indicates that the higher the POC, the higher the $\delta^{13}C_{15°C}$, which is in agreement with the 2-step model of carbon fixation (see 'Introduction'). As a consequence, phytoplankton biomass, and therefore primary production variations, are of great importance in highly productive ecosystems such as the Bay of Seine from a $\delta^{13}C_{POC}$ point of view, confirming the results of previous studies.

In the aquatic reservoir, the variation of the C:N ratio is mainly due to the mixing of different materials and/or the degradation of these materials. As explained above (see 'Particulate organic matter characterization') POM was mainly composed of phytoplankton during the study period. Thus, the C:N variations do not represent the material mixing in this study. In contrast C:N ratio could be considered as an indicator of phytoplankton degradation and/or mineralization (Lancelot & Billen 1985, Martin et al. 1987, Saino 1992, Boyd et al. 1999). Indeed, first there is evidence of a preferential mineralization of N relative to C (in fact, of low C:N components relative to high C:N components) by the bacteria (Smith et al. 1992, Newton et al. 1994), and secondly zooplankton faeces have higher C:N ratio than the ingested phytoplankton (Checkley & Entzeroth 1985). Likewise, it is commonly admitted that chlorophyll is degraded faster than the whole pool of POC, leading to an increase in POC:chl ratio during phytoplankton degradation (Parsons et al. 1977, Cifuentes et al. 1988). Thus, as phytoplankton is being degraded, C:N and POC:chl ratios increase simultaneously. The strong positive correlation ($r = +0.88$; $p < 0.001$) which exists between both ratios brings evidence of their simultaneous increase or decrease. The C:N ratio is thus considered here as a proxy for phytoplankton degradation in this study.

Table 3. Results of the multi-linear regression (n = 17) between temperature-normalized $\delta^{13}C$ ($\delta^{13}C_{15°C}$) (dependent variable), and particulate organic carbon concentration ([POC]) and particulate organic carbon to nitrogen ratio (C:N) (independent variables), from the Bay of Seine data

	Independent parameters			Model
	Constant	[POC]	C:N	
Estimate	–24.41	2.02	0.418	
Standard error	1.07	0.438	0.152	0.882
p-value	0.000	0.000	0.016	0.000
R^2				0.748

The result of the multilinear regression (Eq. 12) points to an alteration of the C stable isotope signal in the course of the phytoplankton degradation. Such an increase in $\delta^{13}C_{POC}$ during POM degradation is poorly documented in the literature. Nonetheless, Boyd et al. (1999) argued that a +4‰ shift in the $\delta^{13}C_{POC}$ values between the sub-surface and depths higher than 250 m in the Pacific Ocean was due to the loss of isotopically lighter labile material by bacterial solubilization. Fischer (1991) measured a 3 to 4‰ increase in $\delta^{13}C$ values between phytoplankton and krill faeces, and attributed this difference to a preferred respiration of ^{12}C by the krill. Likewise, Checkley & Entzeroth (1985), from an *in vitro* experiment, measured copepod faeces enriched in ^{13}C and demonstrated from a mass-balance model that copepod-dissolved excreta was ^{13}C-depleted, both relative to SPM. Laboratory experiments (Macko & Estep 1984, cited by Thornton & McManus 1994) confirm that isotope effects associated with microbial respiration of organic carbon result in the ^{13}C enrichment of residual material. However, some studies have provided evidence that early diagenesis has a minor effect on POM $\delta^{13}C$ in the water column and sedimentary organic matter (see Fontugne & Calvert 1992).

This study of the POC stable isotopes points out that in a highly productive environment such as the Bay of Seine, 2 processes contribute to the C isotopic signature of the phytoplankton in addition to the 'temperature effects': primary production and phytoplankton degradation. Firstly, this study verifies the major contribution of the primary production to the $\delta^{13}C$ signal. Secondly, it brings in new elements to confirm that this signal is also significantly dependent on phytoplankton degradation, an effect poorly documented in earlier $\delta^{13}C$ studies.

CONCLUSIONS

POM $\delta^{15}N$ and $\delta^{13}C$ were studied in a highly productive DIN-enriched embayment during nutrient depletion due to intense spring phytoplankton blooms. Large variations of the C and N isotope ratios were observed during the period when phytoplankton was the major contributor to POM. Using a theoretical and statistical model for $\delta^{15}N$ and $\delta^{13}C$, respectively, these variations were related to biological processes. Indeed, the increase in $\delta^{15}N_{PON}$ was directly influenced by the phytoplankton nitrate utilization during the entire spring, following an open-system equation. $\delta^{15}N_{PON}$ integrates effects over a long time period and constitutes a 'memory' of the nitrate depletion. This indicates that $\delta^{15}N_{PON}$ represents a pertinent tool for studying nitrate utilization in such complex coastal areas, as was previously shown for open ocean and cultures. POC $\delta^{13}C$ was related to POC concentration and the C:N ratio, in addition to 'temperature effects'. These 2 parameters have been considered as primary production and phytoplankton degradation indicators, respectively. Whereas the $\delta^{13}C$ and growth rate or primary production relationship is now well established, $\delta^{13}C$ alteration due to phytoplankton degradation is poorly documented. Our study points out that this process should be considered for further studies of the POC $\delta^{13}C$ variations in coastal ecosystems.

Therefore, C and N stable isotope ratios appear useful for describing phytoplankton dynamics during spring blooms and nutrient depletion. Fig. 5 synthesizes the isotope ratio variations during spring: (1) in a first stage, at the start of the phytoplankton development, the nitrate concentration is high (low $\delta^{15}N$) and the phytoplankton production is low (generally due to light limitation; low $\delta^{13}C$); (2) an intermediate phase is characterized by the increase in production ($\delta^{13}C$ increases) and the decrease in the nitrate pool ($\delta^{15}N$ decreases); (3) a late stage (as described by Nakatsuka et al. 1992) takes place when the nitrate is depleted (high $\delta^{15}N$) and part of the phytoplankton becomes degraded but the production is still high (high $\delta^{13}C$).

This study of the C and N stable isotope dynamics during spring in such a complex coastal ecosystem represents a first step. It would be interesting to extend this $\delta^{13}C$ and $\delta^{15}N$ approach to the whole of the production period (roughly from April to October), and especially to focus on the mineralization processes and the regenerated production. This includes understanding the consequences of the mineralized nutrient (NH_4^+ and CO_2) utilization by the phytoplankton on the newly produced POM $\delta^{13}C$ and $\delta^{15}N$, as well as the consequences of the phytoplankton degradation on the C and N isotope signal of the remaining POM. This is of great importance since nutrient regeneration sustains the main part of the phytoplankton production after the 'new' nutrient depletion (Dugdale & Goering 1967, Ragueneau et al. 1994, Maguer et al. 1998). Finally, coupling enriched isotope techniques (^{15}N, ^{13}C, ^{14}C) and natural abundance isotope determination ($\delta^{13}C$ and $\delta^{15}N$) would provide complementary information. On the one hand, enriched isotope techniques are direct ways to estimate phytoplankton production and nutrient utilization; they are representative of a short time period (several hours to few days) and require incubations (i.e. simulated, but not real, *in situ* conditions). On the other hand, natural abundance isotope techniques are indirect ways to describe phytoplankton production and nutrient utilization; they are representative of a longer time period (several days to several weeks) and real *in situ* conditions.

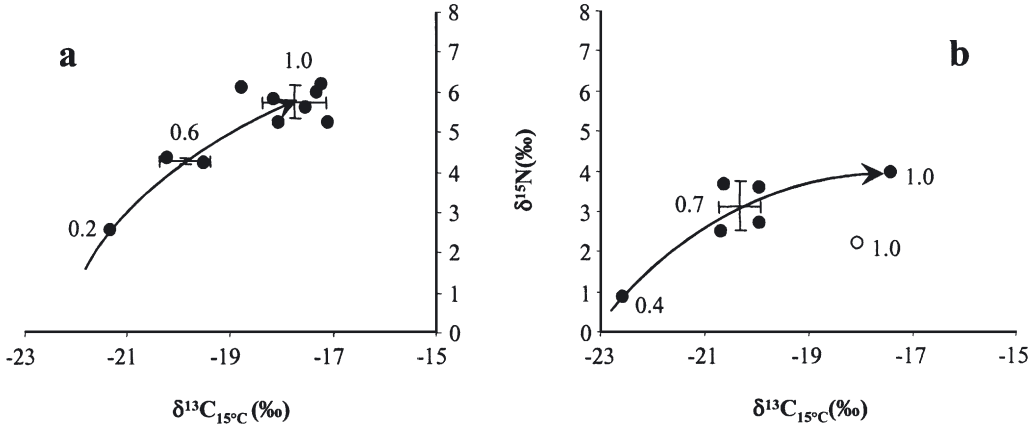

Fig. 5. $\delta^{15}N$ versus temperature-normalized $\delta^{13}C$ ($\delta^{13}C_{15°C}$) in the Bay of Seine proper (April and June 1997). (a) 31.8 ≤ salinity ≤ 33.2; (b) 33.4 ≤ salinity ≤ 34.7. Averages and standard deviations are shown for each point group; numbers correspond to fractional nitrate utilization (u') average for each point group; open circle corresponds to Stn 9 in June (see text for details)

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