Continental Shelf Research
August 2008, Volume 28 (15), Pages 1911-1919
http://dx.doi.org/10.1016/j.csr.2008.03.002

Comparison of C and N stable isotope ratios between surface particulate organic matter and microphytoplankton in the Gulf of Lions (NW Mediterranean)

Mireille Harmelin-Vivien[a, *], Véronique Loizeau[b], Capucine Mellon[c], Beatriz Beker[d], Denise Arlhac[a], Xavier Bodiguel[b], Franck Ferraton[a], c, Rachel Hermand[a], Xavier Philippon[b] and Chantal Salen-Picard[a]

[a] Centre d'Océanologie de Marseille, Université de la Méditerranée, UMR CNRS 6540, Station Marine d'Endoume, 13007 Marseille, France
[b] IFREMER Centre de Brest, Département Biogéochimie et Ecotoxicologie, BP 70, 29280 Plouzané, France
[c] Centre de Recherches Halieutiques Méditerranéenne et Tropicale, IFREMER, Laboratoire Ressources Halieutiques, Avenue Jean Monnet, BP 171, 34203 Sète Cedex, France
[d] Centre d'Océanologie de Marseille, Université de la Méditerranée, UMS CNRS 2196, Station Marine d'Endoume, 13007 Marseille, France

*: Corresponding author : Mireille Harmelin-Vivien, email address : mireille.harmelin@univmed.fr

Abstract:

Carbon and nitrogen stable isotope ratios of particulate organic matter (POM) in surface water and 63–200 µm-sized microphytoplankton collected at the fluorescence maximum were studied in four sites in the Gulf of Lions (NW Mediterranean), a marine area influenced by the Rhone River inputs, in May and November 2004. Some environmental (temperature, salinity) and biological (POM, Chlorophyll *a* and phaeopigments contents, phytoplankton biomass and composition) parameters were also analysed. Significantly different C and N isotopic signatures between surface water POM and microphytoplankton were recorded in all sites and seasons. Surface water POM presented systematically lower $\delta^{13}C$ (~4.2‰) and higher $\delta^{15}N$ (~2.8‰) values than those of microphytoplankton, due to a higher content of continental and detrital material. Seasonal variations were observed for all environmental and biological parameters, except salinity. Water temperature was lower in May than in November, the fluorescence maximum was located deeper and the Chlorophyll *a* content and the phytoplankton biomass were higher, along with low PON/Chl *a* ratio, corresponding to spring bloom conditions. At all sites and seasons, diatoms dominated the phytoplankton community in abundance, whereas dinoflagellate importance increased in autumn particularly in coastal sites. C and N isotopic signatures of phytoplankton did not vary with season. However, the $\delta^{15}N$ of surface water POM was significantly higher in November than in May in all sites likely in relation to an increase in $^{15}N/^{14}N$ ratio of the Rhone River POM which influenced surface water in the Gulf of Lions. As it is important to determine true baseline values of primary producers for analysing marine food webs, this study demonstrated that C and N isotopic values of surface water POM cannot be used as phytoplankton proxy in coastal areas submitted to high river inputs.

Keywords: Particulate organic matter; Phytoplankton composition; Fluorescence maximum; Stable isotopes; C/N ratio; PON/Chl *a*; Seasonal variation

1. Introduction

Stable isotopes of carbon and nitrogen are widely used to analyse food webs in freshwater (Vander Zanden and Rasmussen, 2001; Perga and Gerdeaux 2004), estuarine (Kikuchi and Wada, 1996; Riera et al., 2000) and marine (Thomas and Cahoon, 1993; Pinnegar and Polunin, 2000; Grall et al., 2006) ecosystems. Due to difference in fractionation with trophic levels, these two elements give complementary informations (Peterson et al., 1985). As the $^{13}C/^{12}C$ ratio increases generally from 1-2‰ from prey to predator, $\delta^{13}C$ traces the origin of carbon sources into food webs (De Niro and Epstein, 1978; McCutchan and Lewis, 2002). The large increasein $^{15}N/^{14}N$ ratio in the food web (mean 3.2 to 3.4‰) (Owens, 1987; Sweeting et al., 2007) explains the use of $\delta^{15}N$ to determine the trophic level of organisms (Minagawa and Wada, 1984; Cabana and Rasmussen, 1996; Schmidt et al., 2004). The comprehension of the transfer of organic matter into food webs as well as the determination of trophic level of organisms, imply a precise determination of the isotopic signatures of organic matter sources (Fry and Scherr, 1984; Vander Zanden and Rasmussen, 2001; Philipps and Gregg, 2003). Isotopic signatures of phytoplankton are generally difficult to obtain and imply specific sampling procedures (Descolas-Gros and Fontugne, 1985; Fry and Wainwright, 1991). Therefore most of food web studies rely on the isotopic values of particulate organic matter (POM) in surface water as a proxy of phytoplankton isotopic values (Riera et al., 1999; Michener et al., 2007). However, POM is a complex heterogeneous material composed of a variety of elements, as bacteria, phytoplankton, microzooplankton, fecal pellets and detritus which influence its isotopic values, particularly in coastal areas submitted to river inputs. To determine the accurate values of organic matter end-members in a multisource environment as coastal zones is thus of prime importance in food webs studies.

The Gulf of Lions provided a suitable area to address the fundamental question of the use of surface water POM isotopic ratios as a marine end-member in a coastal area influenced by river runoff, as it is subjected to the high Rhone River inputs. The Gulf of Lions is located in the northwestern Mediterranean Sea and presents complex hydrological dynamics. The circulation is driven by a combination of factors including winds, freshwater dynamics associated with the large Rhone River discharge, coastal upwellings and the Northern Current that flows cyclonically along the continental slope (Millot, 1990). The Rhone River, with a mean annual discharge of 1700 m^3s^{-1} is the most important river in the Mediterranean and is responsible for 50 % of the primary production of the Gulf of Lions (Lochet and Leveau, 1990). The river plume, diverted mostly towards the south-west, is a site of high but variable primary production (Naudin et al., 2001). The influence of the Rhone River particles on surface water and sediment is observed westward all over the gulf area (Durrieu de Madron et al., 1990, 2000; Tesi et al., 2007).

The present work is part of a broad, multidisciplinary programme aimed at studying contaminant transfer and food web functioning of coastal demersal fishes in this region (Ferraton et al., 2007). Analyses of size-fractioned plankton in a pelagic Mediterranean area (Rau et al., 1990), the Atlantic Ocean (Sholto-Douglas et al., 1991) and the Baltic Sea (Rolff, 2000) demonstrated significant differences in $\delta^{13}C$ and $\delta^{15}N$ of suspended particles related to their size and composition. However, no study has been done previously in a river-dominated Mediterranean coastal zone. Thus, to obtain more realistic isotopic values of the phytoplanktonic source in the Gulf of Lions where surface water POM is a mixing of marine and terrestrial organic matter, we analysed differences in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios between surface water POM and 63-200µm-sized microphytoplankton pumped at the fluorescence maximum. Our objectives were (1) to look for differences in $\delta^{13}C$ and $\delta^{15}N$ in POM sampled at sea surface and in the microphytoplankton, (2) to evidence or not seasonal variations in these parameters, and (3) to link C and N isotopic values to the specific composition of the phytoplankton collected.

2. Material and methods

2.1. Study area and sampling

Sampling was performed in four sites located on the continental shelf of the Gulf of Lions (NW Mediterranean) (Fig. 1A). Two sites were located in front of the Rhone River delta (sites 1 and 2) in the Rhone River water dilution zone, and two in front of Agde in the middle of the Gulf (sites 3 and 4) generally outside of the dilution zone to look for difference related to river input level. Sites 1 and 3 were located far from the coast in deep water (90-100 m) and sites 2 and 4 in more coastal shallow

water (30-50 m). Sampling was performed in May 2004 during the spring phytoplankton bloom and in November 2004 at the end of the warm low productive season (Fig. 1B). At each site, the characteristics of the water masses were recorded using a Seabird CTD fitted with a fluorimeter to detect the fluorescence maximum. Surface water was prefiltered on 250 µm-mesh sieves to remove zooplankton and large detritus, and filtered on preweighed Whatman GF/F filters precombusted for 4 h at 500°C. Plankton samples were collected by pumping and filtering large volumes of seawater at the fluorescence maximum with a submersible high flow water pump (320 L min^{-1}). Seawater was filtered through three different vertical nets of decreasing mesh size (200, 63 and 6 µm) set in a large tank. According to previous studies (Rau et al., 1990; Rolff and Elmgren 2000), the 63-200 µm-sized fraction was considered to be the best proxy for analysing the main phytoplankton components of the community. The term "phytoplankton" used in this paper referred to the 63-200 µm fractions which mainly represented microphytoplankton. The present study was not designed to analyse the various fraction of phytoplankton, but to obtain a realistic value of the marine end-member for reconstructing demersal fish food webs (Ferraton et al., 2007). As the herbivorous pathway dominated in coastal zones (Legendre and Rassouzadegan, 1995) and diatoms are grazed by numerous small pelagic crustaceans as well as benthic filter feeders (Riera et al., 1999), we did not take into consideration the small nano- and picoplankton.

In each site a sub-sample of the 63-200 µm-sized fraction was fixed in a lugol solution for species determination. Samples for Chlorophyll a and phaeopigment content determination were obtained by filtration performed on Whatman GF/F filters, rinsed with filtered seawater and stored frozen in the dark. The remaining material retained on net sieve was immediately frozen for further contaminant and isotopic analyses. Quantification of suspended particulate matter (SPM) concentration in surface water (0.7-250 µm-sized particules) and at the fluorescence maximum (63-200 µm-sized particules) was determined by filtration on precombusted and preweighed Whatman GF/F filters. Filters were rinsed with demineralised water to remove salt and dried at 60°C for 48 h before being weighed again (Aminot and Kerouel, 2004) and then stored at -20°C till isotopic analyses. Particulate organic carbon (POC) and particulate organic nitrogen (PON) contents were determined with an elemental analyser (Vario El III) after decarbonatation of filters with HCl vapours in a vacuum-enclosed system, and used to calculate molar C/N ratios. Chlorophyll a and phaeopigments were analysed spectrophotometrically according to Lorenzen (1967) and Aminot and Rey (2002). Biomass of the 63-200 µm microphytoplankton was determined from chlorophyll a concentration according to the following equation:

$Biomass_{Phytoplankton} = (Chl\ a \times R)/\%C$

with Chl a = chlorophyll a concentration in the 63-200 µm fraction, R = C/Chl a mass ratio of phytoplankton, and %C = percentage of carbon in living phytoplankton. In the present study, mean values of R = 60 and %C = 0.28 were used following Aminot and Kerouel (2004).

2.2. Stable isotope analysis

C and N stable isotope analyses were conducted on two sub-samples of surface POM and 63-200 µm-sized fraction. One sub-sample was analysed without any prior treatment for $\delta^{15}N$ determination. The other, used for carbon isotope analysis, was acidified with 10% HCl solution to remove carbonates, rinsed with distilled water and oven-dried at 40°C for 24 h, as carbonates present a higher $\delta^{13}C$ than organic carbon (De Niro and Epstein, 1978). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the samples were determined using continuous-flow isotope-ratio mass spectrometry. The spectrometer (Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser with ANCA Solid/Liquid preparation module) was operated in dual mode allowing $^{13}C/^{12}C$ and $^{15}N/^{14}N$ to be measured simultaneously. The analytical precision was 0.2‰ for both N and C, estimated from internal standards analysed along with the samples (1 mg leucine prepared by freeze drying 50 µl of a 20 mg ml-1 stock solution and calibrated against 'Europa flour' and IAEA standards N1 and N2). Isotope ratios were expressed as parts per thousands (‰) differences from a standard reference material:

$$\delta X = [(R_{sample}/R_{reference})\text{-}1] \times 10^3$$

where X is ^{13}C or ^{15}N, and R the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard reference materials were Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen.

Relative proportions of terrestrial (Ft) and marine (Fm) organic matter in the surface water POM at both seasons were calculated using the equation:

$$\delta^{13}C_{Surface\ POM} = Ft.\delta^{13}C_{MOP\ Rhone} + Fm.\delta^{13}C_{Phytoplankton}$$

with Ft + Fm = 1. The $\delta^{13}C_{MOP\ Rhone}$ was –27.76 ‰ in May and –24.95 ‰ in November 2004 (MHV personal data).

2.3. Phytoplankton composition

Phytoplankton samples were concentrated using Utermöhl settling chambers (Hasle, 1988) and counted with an inverted microscope (Wild M40) equipped with phase contrast. Sedimented volumes were adapted to the particle concentration of each sample. Organisms were identified to the lowest possible taxonomic level with optic microscopy analysis. Numerations were carried out on diametrical tapes with enlargements of 200 and 400 times, or on the totality of the chamber surface, depending on the size and the abundance of the species (Lund et al., 1958). When possible, 400 cells were counted to assure that the error in estimation of cellular abundance remains within the limits of ± 10% (Uehlinger, 1964). Estimates of cell volume for each species are obtained by routine measurements of 30-50 cells of an individual species and application of the geometric formula best fitted to the shape of the cell (Hillebrand et al., 1999). Unfortunately, heterotrophic and autotrophic dinoflagellates could not be distinguished due to lugol fixation of samples.

2.4. Data analysis

Difference in temperature, salinity and quantity of suspended matter in surface water and at the fluorescence maximum according to season (May versus November) were analysed by two-way analyses of variance (ANOVA) after the assumption of normality and homogeneity of data were met using Levene test. Appropriate means were compared using Student-Newman-Keuls test. Difference in mean $\delta^{13}C$, $\delta^{15}N$ and C/N ratios according to the type of material analysed (surface POM versus 63-200 µm-sized plankton) and season (May versus November) were tested by two-way ANOVAs. Relationships between the isotopic signature of the 63-200 µm-sized plankton and the abundance percentage of diatoms and dinoflagellates were tested by Pearson linear regressions combining data from all sites and seasons.

3. Results

3.1. Physical and biological parameters

The mean water temperature at the sampling stations was lower in May (13.9 ± 0.7 °C) than in November (17.7 ± 0.8 °C), and did not differ between the surface and the fluorescence maximum depths (Tables 1 and 2). Salinity did not change with season or depth and averaged 37.2 ± 0.9 for the period studied. A two-way ANOVA showed that SPM concentration varied significantly with season with higher values in May than in November, and depth with higher values in surface water than at the fluorescence maximum. However, interaction between the two factors indicated that SPM concentration in surface water in May was significantly higher than the others. Seasonal difference in SPM quantity occurred thus only in surface water and difference between surface and fluorescence maximum was only observed in May. The fluorescence maximum (F. max) was located significantly deeper in spring than in autumn (17.1 ± 7.7 vs 5.5 ± 3.0 m, P = 0.005), except in site 1 (Table 1). Chlorophyll *a* concentration and biomass of phytoplankton at the fluorescence maximum were both significantly higher in May than in November (1.96 ± 0.14 vs 0.06 ± 0.03 $\mu g.l^{-1}$, P <0.001 and 421.07 ± 30.93 vs 13.39 ± 7.50 $mg.l^{-1}$, P <0.001 respectively). Chlorophyll *a* concentration (1.96 ± 0.14 $\mu g.l^{-1}$) was significantly higher than phaeopigments concentration (0.48 ± 0.31 $\mu g.l^{-1}$) in May (P <0.001), whereas the reverse was observed in November with a higher concentration of phaeopigments (0.18 ± 0.07 $\mu g.l^{-1}$) than Chlorophyll *a* (0.06 ± 0.03 $\mu g.l^{-1}$) (P = 0.019). All the other phytoplankton parameters tested did not differ with season.

3.2. Stable isotope signatures

C and N isotopic values of surface POM significantly differed from those of 63-200 µm microphytoplankton collected at the fluorescence maximum (Tables 3 and 4). Significantly lower $\delta^{13}C$ and higher $\delta^{15}N$ values were recorded for surface POM than for the 63-200 µm microphytoplankton in both seasons (Fig. 2). However, if no seasonal difference in C and N isotopic ratios was observed for the 63-200 µm-sized plankton, $\delta^{15}N$ of surface POM was significantly higher in November than in May (Table 4). No difference in surface POM values was observed with site and season, except for a significantly higher $\delta^{13}C$ value at site 4 in November ($P = 0.005$) and higher $\delta^{15}N$ values in sites 1 and 3 in November also ($P < 0.001$). In contrast, significant difference in isotopic signatures between sites at both seasons was observed for the 63-200 µm microphytoplankton due to the low variance of data ($P < 0.001$ for carbon and $P < 0.001$ for nitrogen). The lowest $\delta^{13}C$ values were generally observed in site 1 and the lowest $\delta^{15}N$ values in sites 3 and 4 (Table 3). Mean differences in $\delta^{13}C$ and $\delta^{15}N$ values between surface POM and the 63-200 µm microphytoplankton (Δ) did not differ with season ($P = 0.867$ and $P = 0.320$ respectively) (Fig. 3), averaging 4.17 ± 1.17 ‰ for $\Delta^{13}C$ and 2.81 ± 1.9 ‰ for $\Delta^{15}N$ in the Gulf of Lions in 2004. Determination of the organic matter origin indicated that the mean proportion of terrestrial matter in the surface water POM was significantly lower in May (56.0 ± 9.5 %) than in November (81.1 ± 19.8 %) (Table 3). This was likely due to the higher phytoplankton biomass recorded in spring (Table 1). Terrestrial matter represented a lower percentage of surface POM near the Rhone mouth than in the middle of the gulf in May, whereas it formed the bulk (>90 %) of the surface POM near the river mouth in November.

3.3. Level of heterotrophy

C/N ratio of surface POM was significantly higher (9.52 ± 2.11) that C/N of the 63-200 µm microphytoplankton (7.56 ± 1.35), and the values recorded in May were generally higher than those recorded in November (Table 4). Lower C/N ratio were systematically observed for the 63-200 µm microphytoplankton collected at the fluorescence maximum at each site and season (Table 3). C/N ratios did not differ with site for surface POM ($F = 1.45$, $P = 0.255$) or 63-200 µm microphytoplankton ($F = 0.83$, $P = 0.495$) at each season. The 63-200 µm microphytoplankton presented higher values of the PON/Chl *a* ratio in November (13.66 ± 8.48 %) than in May (0.99 ± 0.14 %) indicating a higher level of heterotrophy in autumn (Table 1). This ratio was higher near the Rhone River than off Agde, particularly in November.

3.4. Composition of phytoplankton community

Despite a much higher phytoplankton biomass in spring than in autumn 2004, the number of phytoplanktonic species recorded in the 63-200 µm-sized fraction was lower in May than in November in each site (Table 1). The spring phytoplanktonic bloom was thus generated by large populations of a few species, whereas the high diversity of phytoplankton observed in autumn was related to low specific population level. Diatoms dominated largely the community during both seasons averaging 83 % of the phytoplankton abundance in May and 82 % in November. An unidentified chlorophyte was important in site 3 in May, and dinoflagellates were an important component of the community in coastal sites (2 and 4) in November. However, as dinoflagellates presented larger cellular volumes than diatoms (Table 5), their volumetric percentage in the phytoplankton ranged between 9 % and 63 % in May (mean 37 ± 26 %), and between 4 % and 90 % in November (mean (48 ± 41 %). Few species constituted the bulk of the phytoplankton abundance (Table 5) and most of them differed between seasons. In May, some freshwater diatoms, like *Asterionella formosa,* were found in abundance within the river plume (site 1), likely due to the river phytoplankton bloom which also occurred in spring.

No correlation was found between the isotopic signatures of the 63-200 µm microphytoplankton and the abundance percentage of diatoms ($r = 0.62$, $P = 0.096$ for $\delta^{13}C$, $r = -0.20$, $P = 0.629$ for $\delta^{15}N$) or dinoflagellates ($r = -0.42$, $P = 0.302$ for $\delta^{13}C$, $r = 0.20$, $P = 0.643$ for $\delta^{15}N$). Correlations were also not significant between the isotopic ratios and the volumetric percentage of diatoms ($r = 0.57$, $P = 0.138$ for $\delta^{13}C$, $r = -0.40$, $P = 0.324$ for $\delta^{15}N$) or dinoflagellates ($r = -0.57$, $P = 0.141$ for $\delta^{13}C$, $r = 0.40$, $P = 0.323$ for $\delta^{15}N$).

4. Discussion

Marine particulate organic matter (POM) is composed of a mixture of living and detrital material including bacteria, phytoplankton, zooplankton, fecal pellets and continental detritus of terrestrial and freshwater origin, particularly in coastal zones submitted to river outflow. The characteristics of the surface water POM, including their isotopic signatures, could be modified by the quality and quantity of the continental material brought to the sea by rivers. In the Gulf of Lions, both surface and bottom water layers are largely influenced by the Rhone River inputs and continental material was transported all over the area due to the westward water circulation (Durrieu de Madron, et al., 1990, 2000; Sempere et al., 2000; Tesi et al., 2007). The influence of the Rhone river inputs on surface water can be observed very far from the coast and exhibits high seasonal variations in relation to the river flow (Cauwet et al., 1997).

4.1. Difference between surface POM and microphytoplankton

The quantity of suspended particulate matter (SPM) was higher in surface water than at the fluorescence maximum depths. SPM corresponded to 0.7 –250 µm-sized particles in surface water and to 63-200 µm-sized particles at the fluorescence maximum, this implied that both large (200-250 µm) and small (0.7-63 µm) particles constituted an important component of the surface POM, particularly in May. The 63-200 µm sized-fraction collected at the fluorescence maximum was mainly composed of microphytoplankton (mostly diatoms and dinoflagellates). Following the results of Rau et al. (1990) and Rolff and Elmgren (2000), this fraction was considered to be the most representative of large autotrophic phytoplankton organisms. It was used in this study as the best proxy for analysing phytoplankton isotopic signatures in the Gulf of Lions and the term 'phytoplankton' will be used hereafter for '63-200 µm-sized fraction of plankton'. Small-sized phytoplankton organisms (nano- and picoplankton) were not taken into account in our study, whereas picoplankton is the main contributor to chlorophyll *a* content not only in oligotrophic regions, but also in coastal areas (Arin et al., 2005), including the Gulf of Lions (Lantoine and Neveux, 1993). Our study was not designed to analyse the various size classes of phytoplankton, but to provide the best approximation of the isotopic signatures of the marine organic matter source for the study of coastal food webs in the Gulf of Lions. The microphytoplankton was choosen as the most appropriate fraction because the herbivore pathway is dominant in the transfer of carbon into upper trophic levels in high nutrient areas (Legendre and Rassoulzadegan, 1995).

The C/N ratio of the surface POM was higher than that of phytoplankton. As C/N is considered to be an indicator of the mixing of different material and/or their degradation when increasing (Savoye et al., 2003), these results indicated that surface water POM contained a higher proportion of mixed and degraded material than the microphytoplankton fraction collected at the fluorescence maximum. This could be related to the quantity of phytoplanktonic material of decaying state along with a high quantity of detritus linked to both river inputs and planktonic food webs, including fecal pellets, in the surface water. The importance of land-derived detritus was corroborated by the carbon isotopic signature, as continental detritus of terrestrial and freshwater origin exhibit much lower $\delta^{13}C$ (-30 ‰ to –26 ‰) than that of marine phytoplankton (–23 ‰ to -19 ‰) (Fry and Scherr, 1984; Riera et al., 1999; Darnaude et al., 2004 a, b). Thus, systematically lower $^{13}C/^{12}C$ ratios in surface POM in our study indicated a higher proportion of continental POM in this fraction than in phytoplankton, likely due to difference in particle size. Terrestrial organic matter adsorbed onto fine particles is selectively transported away from the Rhone River prodelta (Tesi et al., 2007) and surface POM samples included small sized particles (0.7-63 µm) contrary to phytoplankton samples which was composed of 63-200 µm sized particles. The importance of the smallest sized particles in bearing terrestrial and detrital isotope signatures was confirmed by the analysis of the 6-63 µm fraction at site 4 in May. This fraction exhibited isotopic ratios ($\delta^{13}C$ = -20.10 ± 0.05 ‰, $\delta^{15}N$ = 3.58 ± 0.20 ‰) similar to those of the 63-200 µm fraction ($\delta^{13}C$ = -20.30 ± 0.11 ‰, $\delta^{15}N$ = 3.79 ± 0.02 ‰), but highly different from the 0.7-250 µm surface POM ($\delta^{13}C$ = -24.97 ± 0.75 ‰, $\delta^{15}N$ = 7.71 ± 1.23 ‰) recorded at that site. Thus, we might infer reasonably that difference in isotopic signatures between surface POM and microphytoplankton was likely due to the 0.7-6 µm fraction, constituted by nano- and picoplankton along with detrital material. If we assumed that these small particles represented at least 50 % of the phytoplankton biomass (Lantoine and Neveux, 1993; Arin et al., 2005), their calculated isotopic ratios were –29.74 ‰ for $\delta^{13}C$ and 11.74 ‰ for $\delta^{15}N$. In an area not influenced by river, Rau et al. (1990) recorded $\delta^{13}C$ values of –25.4 to –22.5 ‰

and $\delta^{15}N$ values of –0.5 to 3 ‰ for <0.8 µm particles. The lower $\delta^{13}C$ values and the higher $\delta^{15}N$ values obtained for the 0.7-6 µm particles in our study testified thus from the high importance of terrestrial material in this small sized fraction in the Gulf of Lions. These results corroborated the estimation of the percentage of organic matter of terrestrial origin in the surface POM (68.6 ± 19.6 % as a mean). Significantly higher $\delta^{15}N$ values were observed for POM collected in surface water than at the fluorescence maximum. As $^{15}N/^{14}N$ ratios are higher in bacteria, zooplankton and fecal pellets than in phytoplankton (Minagawa and Wada, 1984; Rolff, 2000; Vander Zanden and Rasmussen, 2001), the higher $\delta^{15}N$ values found in surface POM were related to the higher content of degraded material included in this fraction and revealed by a higher C/N ratio. Thus, particles from continental origin and detrital material of vegetal and animal origin modified the isotopic signatures of surface POM compared to those observed for phytoplankton. In the Gulf of Lions, the difference in isotopic ratios between surface POM and phytoplankton was around 4.2 ‰ for carbon and 2.8 ‰ for nitrogen. Similar differences were observed by Rolff (2000) in the Baltic Sea with higher $\delta^{13}C$ values and lower $\delta^{15}N$ values in phytoplankton (50-100 µm) than in the smallest sized-POM fraction (<5 µm). Fry and Wainright (1991) indicated that phytoplankton can be enriched in ^{13}C by 3 ‰ to 7 ‰ relative to particulate organic matter, and Loick et al. (2007) by 2.4 ‰.

4.2. Seasonal variations

The situation observed in May in the Gulf of Lions for physical and biological parameters was characteristic of the end of the spring phytoplankton bloom as described for the north-western Mediterranean by several authors (Lefevre et al., 1997; Conan et al., 1998; Bosc et al., 2004). The water temperature was low, and the Chlorophyll *a* concentration and the biomass of phytoplankton were high. The depth of the fluorescence maximum was around 20-30 m, except in front of the Rhone River (site 1) where the maximum of phytoplankton production was located in more shallow water at the dilution plume/marine water interface (Naudin et al. 2001). In November, the water temperature was high testifying of the fall water stratification. Chlorophyll *a* concentration and phytoplankton biomass were low and the fluorescence maximum, hardly noticeable, was rather shallow as already observed in this region in autumn (Lefevre et al., 1997; Conan et al., 1998). High particulate organic carbon content in surface water is mainly correlated with primary production and exhibits high seasonal variability in the Gulf of Lions (Cauwet et al., 1997). Maximum of primary production occurs during spring bloom (March to May) whereas low production occurs in autumn (Lefevre et al., 1997). In our study, the phytoplankton communities were numerically dominated at all seasons by diatoms, whereas dinoflagellates might be predominant by volume due to their larger size. Similar observations are related for this region (Lefevre et al., 1997; Naudin et al., 1997) and other temperate waters (Videau et al., 1998; Arin et al., 2005). The increased importance of dinoflagellates in autumn when the abundance of diatoms decreased was also observed in the Bay of Marseille (Travers and Travers, 1962) and the Bay of Seine (L'Helguen et al., 2003).

The lower C/N ratio observed in November in both surface POM and phytoplankton might be the result of a higher bacterial activity as bacterial C/N is lower than that of phytoplankton (Savoye et al., 2003) and a higher importance of heterotrophic organisms in the plankton (Waite et al., 2007). The dominance of phaeopigments along with higher PON/Chl *a* ratios at that season corroborated this hypothesis, as PON/Chl *a* ratio is used as an index of heterotrophy (Waser et al., 2000; Waite et al., 2007). Low PON/Chl *a* ratio is an indication of the importance of phytoplankton as a component of organic matter (Maguer et al., 2000). In the Gulf of Lions, low PON/Chl *a* ratios along with high chlorophyll *a* content in May suggested a high contribution of phytoplankton to the total biomass, whereas high PON/Chl *a* ratios in November indicated highly degraded material.

Carbon isotopic signature of suspended POM and microphytoplankton did not vary with season. However, the $\delta^{15}N$ of surface POM was significantly higher in November than in May, whereas no difference occurred for phytoplankton. Surface water organic matter is largely influence by the Rhone River inputs (Cauwet et al., 1997), and the $^{15}N/^{14}N$ ratio of the Rhone water varied with season, with higher values in November 2004 (8.9 ‰) than in May (6.7 ‰) (MHV personal data). Higher $\delta^{15}N$ of the Rhone River POM in autumn than in spring has been already recorded in 2001 (Darnaude et al. 2004a). Thus, the variations of isotopic ratios recorded in surface POM with higher $\delta^{15}N$ values in November 2004 reflected mainly the stable isotopic variations of the continental organic matter brought by the Rhone River into the Gulf of Lions. The significantly lower $\delta^{13}C$ of surface POM observed in site 1 and the higher $\delta^{15}N$ values recorded in sites 1 and 2 revealed a higher influence of continental material in the sites located in front of the Rhone River delta. This influence was

particularly evident in autumn when >90 % of the surface POM in front of the Rhone River was of terrestrial origin. In May, this proportion was lower due to dilution by the high phytoplankton biomass, especially off the river mouth where the inputs of nutrients favoured the primary production (Sempéré et al., 2000; Naudin et al., 2001). The higher participation of degraded material and bacterial activity in front of the Rhone River was also indicated by the higher PON/Chl *a* ratios in this area whatever the season.

No significant seasonal variations of phytoplankton isotopic signatures were recorded in our study, nor in the Bay of Marseille (Darnaude et al., 2004a), whereas large seasonal variations of stable isotopic ratios in Mediterranean phytoplankton were recorded by Dauby et al. (1990) and Vizzini and Mazzola (2003). Mean isotopic values for microphytoplankton in the Gulf of Lions ($\delta^{13}C$ = –20.1 ± 0.8 ‰; $\delta^{15}N$ = 4.4 ± 0.7 ‰) were close to those observed eastward in the Bay of Marseille ($\delta^{13}C$ = –22.4 ± 0.2 ‰; $\delta^{15}N$ = 2.3 ± 0.1 ‰) (Darnaude et al., 2004 a) and in a coastal Italian lagoon ($\delta^{13}C$ = –20.0 ± 1.9 ‰; $\delta^{15}N$ = 5.9 ± 2.1 ‰) (Vizzini and Mazzola, 2003). All these values are in the range found for typical isotopic composition of marine phytoplankton in temperate seas that varies from –23 % to –19 ‰ for $\delta^{13}C$ (Gearing et al., 1984) and from 3 ‰ to 12 ‰ for $\delta^{15}N$ (Owens, 1987). The lower $\delta^{13}C$ value (–24.4 ± 1.6 ‰) recorded in surface POM by Carlier et al. (2007) westward in the Bay of Banyuls indicates a participation of particles of terrestrial origin issued probably from both the nearby Baillaury River and the distant Rhone River, as it was demonstrated that Rhone small-sized particles are transported westward all over the Gulf of Lions to the Banyuls region (Durrieu de Madron et al., 2000; Tesi et al., 2007).

4.3. Importance of phytoplankton composition

Variability of stable isotope ratios in phytoplankton mainly results from nutrient forms and concentrations, and isotopic fractionation. This last metabolic process depends on species, but also growth rate and environmental conditions such as light, temperature, pH, and vary during the course of a phytoplankton bloom (Decolas and Fontugne, 1985; Savoye et al., 2003). Thus taxonomic data are important for interpreting POM isotopic signatures as the different phytoplanktonic organisms present different isotopic fractionation (Falkowsky, 1991). Typically diatoms present higher $\delta^{13}C$ than dinoflagellates with values ranging from –19 ‰ to –15 ‰ for diatoms compared to –22 ‰ to –20 ‰ for dinoflagellates in marine waters (Fry and Wainwright, 1991). In our study, no relationship was found between C and N isotopic ratios and the numerical or volumetric percentages of diatoms or dinoflagellates, and no seasonal or spatial variations in spite of varying phytoplantonic composition. The mean $\delta^{13}C$ value (-20.1 ‰) recorded for the microphytoplankton of the Gulf of Lions testified from its diatoms and dinoflagellates mixed composition. If we assumed a mean $\delta^{13}C$ of –19 % for diatoms as usually found for temperate species (Waite et al., 2005), the mean $\delta^{13}C$ of dinoflagellates in the Gulf of Lions was –23%, which was in accordance with literature data (Fry and Wainwright, 1991). The 6-63 µm fraction analysed at site 4 contained only half of the chlorophyll *a* content recorded in the 63-200 µm fraction. It was also mainly composed of diatoms (99 % in abundance) and dinoflagellates, more than 50 % of the cells counted in this fraction being represented by the small diatom *Cylindrotheca closterium.* However, the proportion of heterotrophic dinoflagellates in the 63-200 µm fraction could not be quantified. It was likely that seasonal variations of heterotrophy observed with C/N and PON/Chl *a* ratios were not only due to bacterial activity but also to changes in autotrophic/heterotrophic composition of plankton. A more accurate analysis of the different size-classes of phytoplankton, including pico- and nanoplankton remains to be conducted in Mediterranean waters and can be envisaged using the new techniques of flow cytometry with fluorescence activated cell sorting combined with highly sensitive isotopic mass spectrometry.

5. Conclusion

To properly reconstruct marine food webs and determine precise trophic levels of consumers, it is important to use appropriate food web baselines (Post, 2002). As collecting pure samples of phytoplankton is difficult, most scientists use isotope ratios of surface POM as a proxy of phytoplankton isotopic signatures used as marine end-member. In the present paper we demonstrated that a huge discrepancy existed between stable isotope ratios of surface water POM and phytoplankton in an area submitted to high river inputs like the Gulf of Lions. Surface water POM

influenced by organic matter of detrital and continental origin presented lower $\delta^{13}C$ and higher $\delta^{15}N$ values than those of phytoplankton. It is thus of prime importance to collect true phytoplankton material at the fluorescence maximum or outside the area influenced by the river outflow for analysing food webs, particularly in estuarine and deltaic ecosystems.

Acknowledgements

Thanks are expressed to the crews of the R.V. “Europe” and “Téthys” for their collaboration at sea and to Alicia Bautista and Françoise Cubizolles for assistance in sampling. We would like to thank especially Charlie Scrimgeour (Scottish Crop Research Institute) for stable isotope analyses. This study was financially supported by the French research programmes PNEC-MEDOC, MEDICIS and ECCO-PNBC and the regional Languedoc-Roussillon programme SYSCOLAG. We are grateful to the Guest Editor and three anonymous reviewers for most valuable comments and improvement of the manuscript.

References

Aminot, A., Kerouel, R., 2004. Hydrologie des écosystèmes marins. Paramètres et analyses. Edition Ifremer, 336 pp.

Aminot, A., Rey, F., 2002. Chlorophyll *a*: determination by spectrometric methods. ICES Technical and Marine Environmental Sciences 30, 1-18.

Arin, L., Estrada, M., Salat, J., Cruzado, A., 2005. Spatio-temporal variability of size fractionated phytoplankton on the shelf adjacent to the Elbro river (NW Mediterranean). Continental Shelf Research 25, 1081-1095.

Bosc, E., Bricaud, A., Antoine, D., 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from four years of SeaWiFS observations. Global Biogeochemical Cycles 18, GB 1005, doi:10.1029/2003GB002034.

Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences 93, 10844-10847.

Carlier, A., Riera, P., Amouroux, J.M., Bodiou, J.Y., Grémare, A., 2007. Benthic trophic network in the Bay of Banyuls-sur-Mer (northwest Mediterranean, France): An assigment based on stable carbon and nitrogen isotopes analysis. Estuarine, Coastal and Shelf Science 72, 1-15.

Cauwet,G., Miller, A., Brasse, S., Fengler, G., Mantoura, R.F.C., Spitzy, A., 1997. Dissolved and particulate organic carbon in the western Mediterranean Sea. Deep-Sea Research II 44,769-779.

Conan, P., Pujot-Pay, M., Raimbault, P., Leveau, M., 1998. Variabilité hydrologique et biologique du golfe du Lion: II. Productivité sur le bord interne du courant. Oceanologica Acta 21,767-782.

Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004a. trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138, 325-332.

Darnaude, A.M., Salen-Picard, C., Harmelin-Vivien, M.L., 2004b. Depth variation in terrestrial particulate organic matter exploitation by marine coastal benthic communities off the Rhone River delta (NW Mediterranean). Marine Ecology Progress Series 275, 47-57.

Dauby, P., Mosora, F., Vertez, M., 1990. A yearly study of $^{13}C/^{12}C$ isotopic ratio variations in the Calvi’s Bay plankton. Rapport de la Commission Internationale pour la Mer Méditerranée 32, 202.

De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42, 495-506.

Descolas-Gros, C., Fontugne, M.R., 1985. Carbon fixation in marine phytoplankton: carboxylase activities and stable carbon-isotope ratios; physiological and paleoclimatological aspects. Marine Biology 87, 1-6.

Durrieu de Madron, X., Nyfeller, F., Godet, C., 1990. Hydrographic structures and nepheloid spatial distribution in the Gulf of Lions continental margin. Continental Shelf Research 10, 915-929.

Durrieu de Madron, X., Abassi, A., Heussner, S., Monaco, A., Aloisi, J.C., Radakovitch, O., Giresse, P., Buscail, R., Kerhervé, P., 2000. Particulate matter and organic carbon budgets of the Gulf of Lions (NW Mediterranean). Oceanologica Acta 23, 717-730.

Falkowski, P.G., 1991. Species variability in the fractionation of ^{13}C and ^{12}C by marine phytoplankton. Journal of Plankton Research 13 Supp., 21-28.

Ferraton, F., Harmelin-Vivien, M., Mellon-Duval, C., Souplet, A., 2007. Spatio-temporal variation in diet may affect condition and abundance of juvenile European hake in the Gulf of Lions (NW Mediterranean). Marine Ecology Progress Series 337, 197-208.
Fry, B, Scherr, E.B., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27, 13-47.
Fry, B., Wainwright, S.C., 1991. Diatom sources of ^{13}C-rich carbon in marine food webs. Marine Ecology Progress Series 76, 149-157.
Gearing, J.N., Gearing, P.J., Rudnick, D.T., Requejo, A.G., Hutchins, M.J., 1984. Isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. Geochimica et Cosmochimica Acta 48, 1089-1098.
Grall, J., Le Loc'h, F., Guyonnet, B., Riera, P., 2006. Community structure and food web based on stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) analysis of a North Eastern Atlantic maerl bed. Journal of Experimental Marine Biology and Ecology 338, 1-15.
Hasle, G.R., 1988. The inverted microscope method. In: Sournia, A. (Ed.), Phytoplankton manual.UNESCO, Paris.
Hillebrand, H., Durselen, C.D., Kirchttel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35, 403-424.
Kikuchi, E., Wada, E., 1996. Carbon and nitrogen stable isotope ratios of deposit-feeding polychaetes from the Nanakita River estuary, Japan. Hydrobiology 321, 69-75.
Lantoine, F., Neveux, J., 1993. Spectrofluorometric analysis of photosynthetic pigments in the Gulf of Lions (Mediterranean Sea) with an emphasis on the autotrophic picoplankton. Annales de l'Institut Océanographique 69, 173-175.
Lefevre, D., Minas, H.J., Minas, M., Robinson, C., Le B. Williams, P.J., Woodward, E.M.S., 1997. Review of gross community production, primary production, net community production and dark community respiration in the Gulf of Lions. Deep-Sea Research II 44, 801-832.
Legendre, L., Rassoulzadegan, F., 1995. Plankton and nutrient dynamics in marine waters. Ophelia 41, 153-172.
L'Helguen, S., Maguer, J.F., Madec, C., 2003. Seasonal changes of phytoplankton production in response to high nitrogen load in the Bay of Seine. Geophysical Research Abstracts 5, 13898.
Lochet, F., Leveau, M., 1990. Transfers between a eutrophic ecosystem, the river Rhône, and an oligotrophic ecosystem, the north-western Mediterranean Sea. Hydrobiologia 207, 95-103.
Loick, N., Dippner, J., Doan, H.N., Liskow, I., Voss, M. 2007. Pelagic nitrogen dynamics in the Vietnamese upwelling area according to stable nitrogen and carbon isotope data. Deep-Sea Research I 54, 596-607.
Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnology and Oceanography 12, 343-346.
Lund, J.W.G., Kipling, C., Le Cren, E.D., 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11, 143-170.
Maguer, J.F., L'Helguen, S., Le Corre, P., 2000. Nitrogen uptake by phytoplankton in a shallow water tidal front. Estuarine, Coastal and Shelf Science 51, 349-357.
McCutchan, J.H., Lewis, W.M. Jr., 2002. Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. Limnology and Oceanography 47, 742-752.
Michener,R.H., Faufman, L. 2007. Stable isotope ratios as tracers in marine food webs: An update. In: Michener, R., Lajtha, K. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Publ., Oxford, UK, pp. 238-282.
Millot, C., 1990. The Gulf of Lion's hydrodynamics. Continental Shelf Research 10, 885-894.
Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48, 1135-1140.
Naudin, J.J., Cauwet, G., Chretiennot-Dinet, M.J., Deniaux, B., Devenon, J.L., Pauc, H., 1997. River discharge and wind influence upon particulate transfer at the land-ocean interaction. Case study of the Rhone River plume. Estuarine, Coastal and Shelf Science 45, 303-316.
Naudin, J.J., Cauwet, G., Fajon, C., Oriol, L., Terzic, S., Devenon, J.L., Broche, P., 2001. Effect of mixing on microbial communities in the Rhone River plume. Journal of Marine Systems 28, 203-227.
Owens, N.J.P., 1987. Natural variations in ^{15}N in marine environment. Advances in Marine Biology 24, 389-451.
Perga, M.E., Gerdeaux, D., 2004. Changes in the $\delta^{13}C$ of pelagic food webs: the influence of lake area and trophic status on the isotopic signature of whitefish (*Coregonus lavaretus*). Canadian Journal of Fisheries and Aquatic Sciences 61, 1485-1492.
Peterson, B.J., Howarth, R.W., Garritt, R.H., 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science 227, 1361-1363.

Pinnegar, J.K., Polunin, N.V.C., 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122, 399-409.
Philipps, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261-269.
Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83, 703-718.
Rau, G.H., Teyssie, J.L., Rassoulzadegan, F., Fowler, S.W., 1990. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size-fractionated marine particles: implications for their origin and trophic relationships. Marine Ecology Progress Series 59, 33-38.
Riera, P., Montagna, P.A., Kalke, R.D., Richard, P., 2000. Utilization of estuarine organic matter during growth and migration by juvenile brown shrimp *Peneus aztecus* in a South Texas estuary. Marine Ecology Progress Series 199, 205-216.
Riera, P., Stal, L.J., Nieuwenhuize, J., Richard, P., Blanchard, G., Gentil, F., 1999. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. Marine Ecology Progress Series 187, 301-307.
Rolff, C., 2000. Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of size-fractionated plankton at a coastal station in the northern Baltic proper. Marine Ecology Progress Series 203, 47-65.
Rolff, C., Elmgren, R., 2000. Use of riverine organic matter in plankton food webs of the Baltic Sea. Marine Ecology Progress Series 197, 81-101.
Savoye, N., Aminot, A., Tréguer, P., Fontugne, M., Naulet, N., Kérouel, R., 2003. Dynamics of particulate organic matter $\delta^{15}N$ and $\delta^{13}C$ during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France). Marine Ecology Progress Series 255, 27-41.
Schmidt, K., McClelland, J.W., Mente, E., Montoya, J.P., Atkinson, A., Voss, M., 2004. Trophic-level interpretation based on $\delta^{15}N$ values: implications of tissue-specific fractionation and amino-acid composition. Marine Ecology Progress Series 266, 43-58.
Sempéré, R., Charrière, B., Van Vambeke, F., Cauwet, G., 2000. Carbon inputs of the Rhône River to the Mediterranean Sea: Biogeochemical implications. Global Biogeochemical Cycles 14, 669-681.
Sholto-Douglas, A.D., Field, J.G., James, A.G., van der Merwe, N.J., 1991. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios in the Southern Benguela Ecosystem: indicators of food web relationships among different size-classes of plankton and pelagic fish; differences between fish muscle and bone collagene tissues. Marine Ecology Progress Series 78, 23-31.
Sweeting, C.J., Barry, J., Barnes, C., Polunin, N.V.C., Jennings, S., 2007. Effects of body size and environment on diet-tissue $\delta^{15}N$ fractionation in fishes. Journal of Experimental Marine Biology and Ecology 340, 1-10.
Tesi, T., Miserocchi, S., Goñi M.A., Langone, L., 2007. Source, transport and fate of terrestrial organic carbon on the western Mediterranean Sea, Gulf of Lions, France. Marine Chemistry 105, 101-117.
Thomas, C.J., Cahoon, L.B., 1993. Stable isotope analyses differenciate between different trophic pathways supporting rocky-reef fishes. Marine Ecology Progress Series 95, 19-24.
Travers, A., Travers, M., 1962. Recherches sur le phytoplancton du golfe de Marseille. Recueil des Travaux de la Station Marine d'Endoume 41, 7-139.
Uehlinger, V. 1964. Etude statistique des méthodes de dénombrement planctonique. Archives des Sciences, 17, fasc.2, 11-223.
Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 48, 2061-2066.
Videau, C., Ryckaert, M., L'Helguen, S., 1998. Phytoplankton in the Bay of Seine (France). Influence of the river plume on primary productivity. Oceanologica Acta 21, 907-921.
Vizzini, S., Mazzola, A., 2003. Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. Marine Biology 142, 1009-1018.
Waser, N.A.D., Harrison, W.G., Head, E.J.H., Nielsen, B., Lutz, V.A., Calvert, S.E., 2000. Geographic variations in the nitrogen isotope composition of surface particulate nitrogen and new production across the North Atlantic Ocean. Deep-Sea Research I 47, 1207-1226.
Waite, A.M., Gustafsson, Ö., Lindahl, O., Tiselius, P., 2005. Linking ecosystem dynamics and biogeochemistry: sinking fractionation of organic carbon in a Swedish fjord. Limnology and Oceanography 50, 658-671.
Waite, A.M., Muhling, B.A., Holl, C.M., Beckley, L.E., Montoya, J.P., Strzelecki, J., Thompson, P.A., Pesant, S., 2007. Food web structure in two counter-rotating eddies based on $\delta^{15}N$ and $\delta^{13}C$ isotopic analyses. Deep-Sea Research II 54, 1055-1075.

Figures

Fig. 1 A. Location of the four sites studied in the Gulf of Lions in May and November 2004. The average extension of the Rhone River water dilution zone (37.5 surface isohaline), indicated by the dashed line, was derived from a compilation of about 1,500 CTD profiles performed between 1993 and 2006 (courtesy X. Durrieu de Madron).

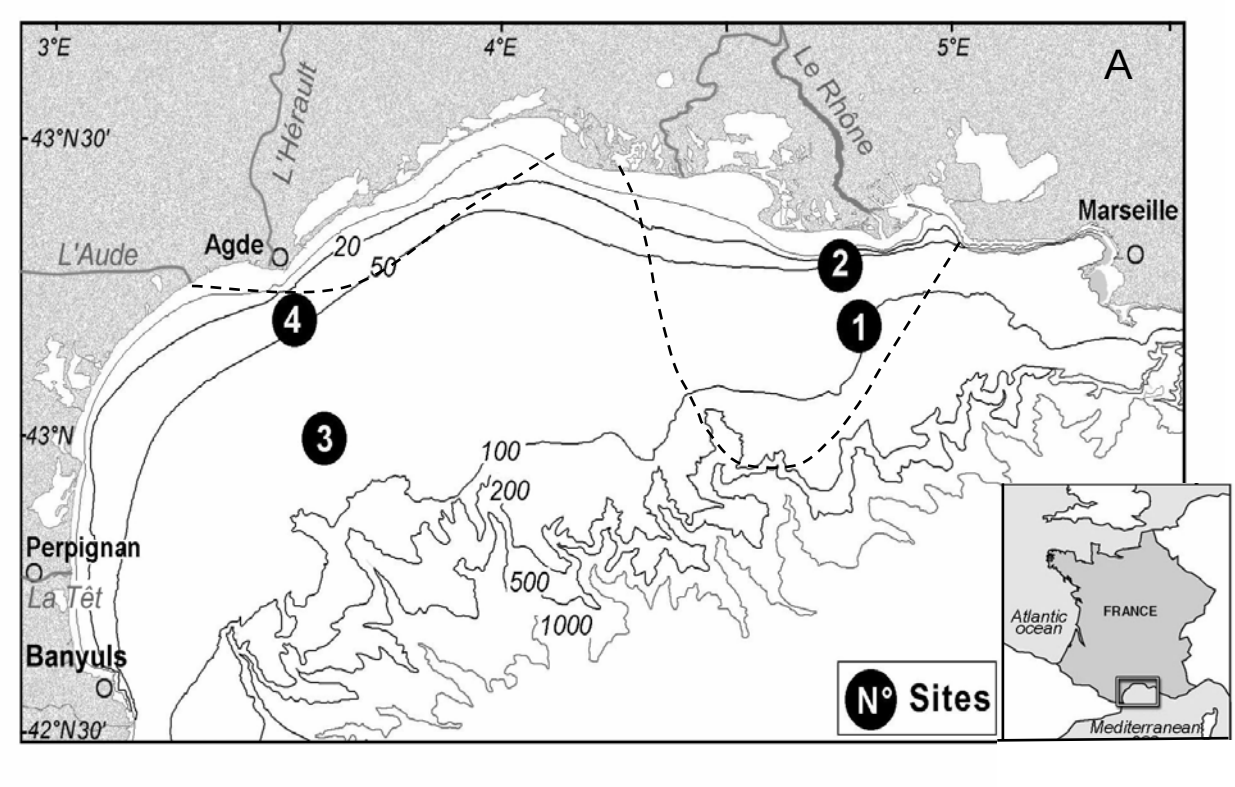


Fig. 1 B. Seasonal variations of the primary production (gC m^{-2} d^{-1}) in the Gulf of Lions averaged for the period 1997 to 2001 (from Bosc et al., 2004) and monthly discharge (m^3 s^{-1}) of the sum of the Rhone, Hérault and Aude Rivers' flows in 2004 (Compagnie Nationale du Rhône, and Banque HYDRO). The Rhone River flow represented 92% of the monthly discharge indicated. Spring (May) and autumn (November) sampling periods are indicated by the vertical dashed lines.

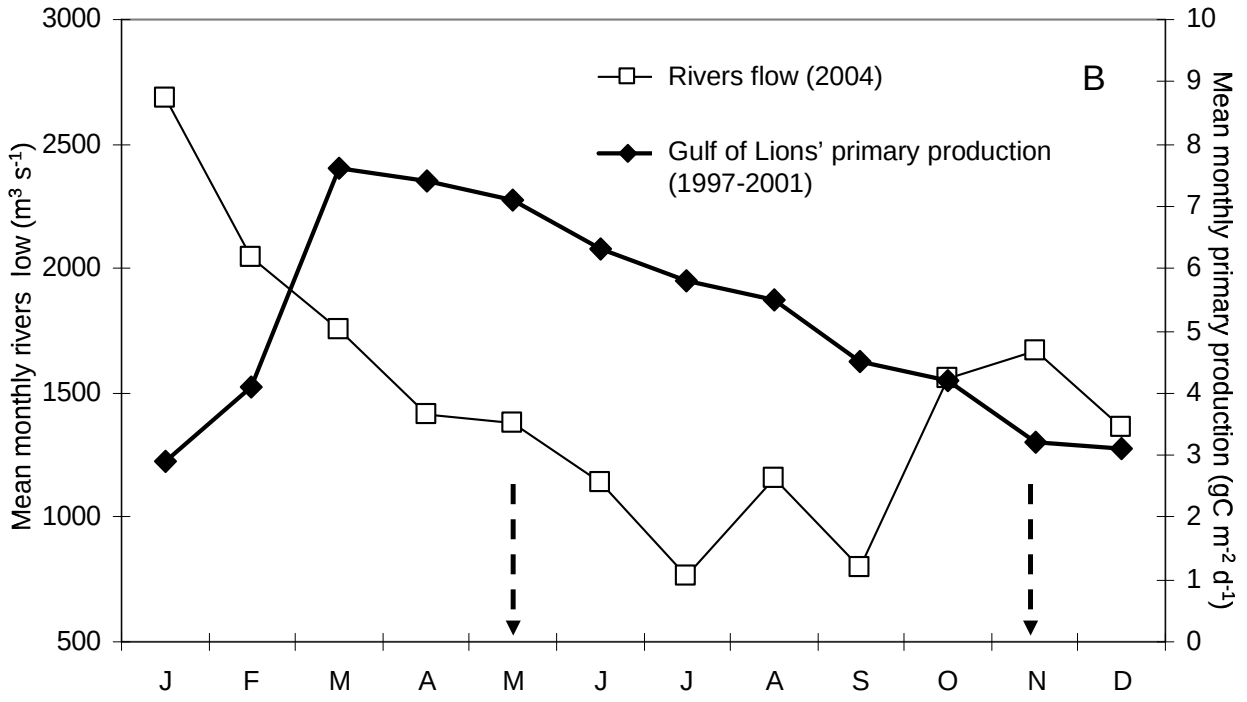


Fig. 2. Mean C and N stable isotope signatures of the surface water POM (Surface POM) and the 63-200 µm microphytoplankton (Phytoplankton) in the Gulf of Lions in May (open symbols) and in November 2004 (dark symbols). Vertical and horizontal bars indicate standard deviations.

Fig. 2

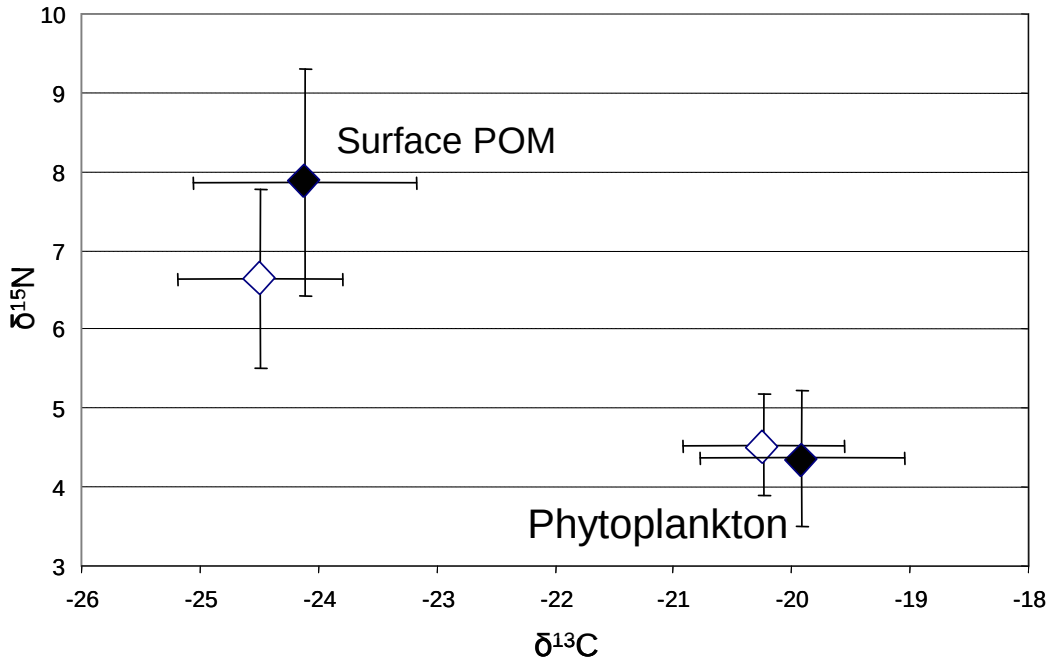
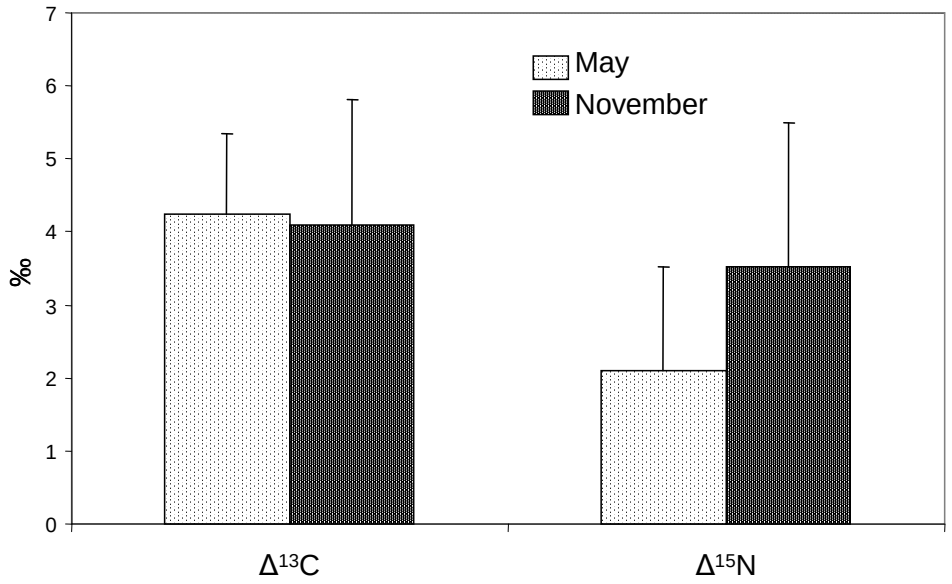


Fig. 3. Mean difference (Δ) between surface water POM and 63-200 µm microphytoplankton isotopic ratios in the Gulf of Lions in May and November 2004. Vertical bars indicate standard deviations.

Figure 3.



Tables

Table 1
Characteristics of the main physical and biological parameters recorded in surface water (Surface) and at the fluorescence maximum (F.max) in the Gulf of Lions in 2004. SPM = suspended particulate matter, Biomass Phyto = biomass of phytoplankton (63-200 µm size-class), PON = particulate organic nitrogen (µmol.l^{-1})

Season	***May***				***November***			
Sites	1	2	3	4	1	2	3	4
Depth	(90-100 m)	(30-50 m)	(90-100 m)	(30-50 m)	(90-100 m)	(30-50 m)	(90-100 m)	(30-50 m)
Date	15/05/04	14/05/04	12/05/04	13/05/04	11/11/04	09/11/04	04/11/04	08/11/04
Depth-F.max. (m)	6.9	17.8	25.7	17.8	9.1	4.6	6.3	0.3
Temperature-Surface (°C)	15.4	13.5	13.8	13.4	16.9	17.2	18.7	18.1
Temperature-F.max. (°C)	14.6	13.4	13.4	13.4	16.9	17.2	18.7	18.1
Salinity-Surface	34.5	37.7	37.1	37.3	37.3	38.0	38.0	36.2
Salinity-F.max.	36.4	37.7	37.4	37.6	37.3	38.0	38.0	36.2
SPM-Surface (mg.l^{-1})	10.0	6.8	4.6	6.1	1.8	3.0	0.6	1.1
SPM-F.max. (mg.l^{-1})	1.8	3.4	2.3	2.8	1.4	2.5	0.6	1.9
Chlorophyll *a*-F.max. (µg.$l^{-1)}$	1.99	2.14	1.94	1.79	0.05	0.02	0.10	0.08
Phaeopigments-F.max. (µg.l^{-1})	0.93	0.27	0.29	0.44	0.13	0.24	0.12	0.25
Biomass Phyto-F.max (µg.l^{-1})	426.43	458.57	415.71	383.57	10.71	4.28	21.43	17.14
PON:Chl *a*-F.max	1.08	1.11	0.81	0.96	15.20	25.00	6.30	8.13
Number of species-F.max	36	32	16	31	66	34	41	39
% Diatoms-F.max	97.54	85.61	50.05	98.94	99.58	76.56	97.34	53.07
% Dinoflagellates-F.max	2.45	14.39	9.88	1.06	0.41	23.29	2.64	46.61
% Others-F.max	0.01	0.00	40.07	0.00	0.01	0.15	0.02	0.32

Table 2
Summary of 2-way ANOVA results performed on temperature (°C), salinity and suspended matter concentration (mg.l^{-1})

Source of variation	Temperature		Salinity		Suspended matter	
	F	P	F	P	F	P
Season	92.02	<0.001	0.69	0.421	22.80	<0.001
Depth	0.16	0.694	0.40	0.539	11.59	0.005
Season x Depth	0.16	0.694	0.40	0.539	7.80	0.016

Table 3
Mean (± sd) $\delta^{13}C$, $\delta^{15}N$ and C/N values of particulate organic matter in surface water (Surface POM) and 63-200 µm-sized phytoplankton at the fluorescence maximum (F.max Phyto.) and mean proportions of organic matter of terrestrial (Ft) and marine (Fm) origin in the Surface POM in the Gulf of Lions in 2004. For each sample n = 3

	May				November			
Sites	1	2	3	4	1	2	3	4
Depth	(90-100 m)	(30-50 m)	(90-100 m)	(30-50 m)	(90-100 m)	(30-50 m)	(90-100 m)	(30-50 m)
$\delta^{13}C$ (‰)								
Surface POM	-23.94 ± 0.25	-24.23 ± 0.07	-24.84 ± 0.97	-24.97 ± 0.75	-24.73 ± 0.32	-24.76 ± 0.41	-23.97 ± 0.28	-22.57 ± 0.26
F.max Phyto.	-20.36 ± 0.22	-21.05 ± 0.07	-19.25 ± 0.04	-20.30 ± 0.11	-20.85 ± 0.16	-18.66 ± 0.42	-20.34 ± 0.12	-19.78 ± 0.06
$\delta^{15}N$ (‰)								
Surface POM	5.26 ± 0.22	6.84 ± 0.58	6.74 ± 0.68	7.71 ± 1.23	8.63 ± 1.93	6.67 ± 0.15	9.01 ± 1.02	7.29 ± 0.55
F.max Phyto.	5.45 ± 0.05	4.24 ± 0.06	4.63 ± 0.14	3.79 ± 0.02	3.82 ± 0.13	5.77 ± 0.13	3.73 ± 0.04	4.18 ± 0.18
C/N								
Surface POM	7.55 ± 0.40	10.01 ± 1.55	13.64 ± 3.13	10.13 ± 0.24	9.61 ± 0.45	8.12 ± 1.53	8.37 ± 0.06	8.86 ± 0.06
F.max Phyto.	6.68 ± 0.05	8.94 ± 0.06	9.44 ± 0.01	9.02 ± 0.05	7.29 ± 0.01	5.66 ± 0.03	6.73 ± 0.02	6.72 ± 0.91
Origin POM (%)								
Ft	48.4	47.4	65.7	62.6	94.6	97.0	78.7	54.0
Fm	51.6	52.6	34.3	37.4	5.4	3.0	21.3	46.0

Table 4
Summary of 2-way ANOVA results on $\delta^{13}C$, $\delta^{15}N$ and C/N of surface POM and 63-200 µm-sized POM at the fluorescence maximum (POM Type) in May and November (Season.)

Source of variation	$\delta^{13}C$		$\delta^{15}N$		C/N	
	F	P	F	P	F	P
POM Type	337.43	<0.001	83.36	<0.001	19,17	<0.001
Season	2.41	0.128	3.05	0.087	15,59	<0.001
POM Type x Season	0.01	0.912	5.10	0.029	0,10	0.7448

Table 5
Name and abundance percentage (%) of the dominant phytoplankton species collected in the Gulf of Lions in May and November 2004. Bv = cell biovolume (μm^3) calculated from simple geometric shapes

Group	Species	Bv (μm^3)	May 2004				November 2004			
			Site 1 (90-100m)	Site 2 (30-50m)	Site 3 (90-100m)	Site 4 (30-50m)	Site 1 (90-100m)	Site 2 (30-50m)	Site 3 (90-100m)	Site 4 (30-50m)
Chlorophytes	Chlorophyte sp.	463	-	-	40.1	-	-	-	-	-
Diatoms	*Asterionella formosa*	420	25.4	-	-	-	-	-	-	-
Diatoms	*Asterionellopsis glacialis*	384	-	4.4	-	3.6	-	-	-	-
Diatoms	*Chaetoceros costatus*	10073	-	-	-	-	21.1	-	34.8	-
Diatoms	*Chaetoceros curvisetus*	6597	-	-	-	-	52.9	-	6.2	-
Diatoms	*Chaetoceros decipiens*	3770	4.1	-	-	3.3	13.4	-	46.3	-
Diatoms	*Chaetoceros densus*	15607	54.3	-	-	-	-	-	-	-
Diatoms	*Chaetoceros laciniosus*	3393	-	-	-	-	5.1	-	-	-
Diatoms	*Chaetoceros rostratus*	2827	-	-	-	-	4.0	-	7.1	-
Diatoms	*Chaetoceros* spp. (broken cells)	5089	-	-	-	23.5	-	-	-	-
Diatoms	*Cyclotella* sp.	170	2.4	-	-	-	-	-	-	-
Diatoms	*Cylindrotheca closterium*	80	5.6	23.5	-	62.3	-	-	-	-
Diatoms	*Paralia sulcata*	5888	-	7.5	22.2	-	-	8.3	-	22.4
Diatoms	*Pinnularia* sp.	40560	-	-	-	-	-	4.3	-	-
Diatoms	*Pleurosigma* sp.1	3864	-	-	27.3	3.2	-	-	-	-
Diatoms	*Pleurosigma* sp2.	58904	-	-	-	-	-	38.9	-	9.2
Diatoms	*Thalassionema nitzschioides*	2040	-	48.5	-	-	-	-	1.8	-
Diatoms	*Thalassiosira cf. excentrica*	15080	-	-	-	-	-	10.1	-	11.0
Dinoflagellates	*"Diplopsalis"* sp 1.	120335	-	4.3	-	-	-	23.0	-	-
Dinoflagellates	*Ceratium fusus*	115925	-	-	-	-	-	-	-	10.9
Dinoflagellates	*Preperidinium meunieri*	60670	-	-	3.4	-	-	-	-	-
Dinoflagellates	*Protoperidinium oblongum*	63334	-	-	-	-	-	-	-	6.7
Dinoflagellates	*Pyrophacus* sp.	139801	-	-	2.7	-	-	-	-	-
% of total phytoplankton abundance per site			91.7	88.1	95.6	95.8	96.4	84.7	96.3	60.2