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Temporal dynamics in the diet of two marine polychaetes as inferred from fatty acid biomarkers

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ABSTRACT

We investigated the temporal variation of pelagic and benthic food sources in the diet of two marine polychaetes: a macrobenthic omnivore (*Nephtys hombergii*) and a suspension-deposit feeder (*Lanice conchilega*) by means of fatty acid (FA) biomarkers and compound-specific stable isotope analysis (CSIA). FA biomarkers in the suspended particulate matter roughly mirrored phytoplankton dynamics in the water column, consisting of a small diatom dominance early spring, succeeded by a mass *Phaeocystis* peak followed by a mixed diatom-dinoflagellate bloom. Deposition and subsequent bacterial degradation of the phytoplankton bloom were also reflected in sediment FA biomarkers. The main distinction in FA biomarker concentration within macrobenthic tissue was observed at the species level (48% of variation), the diet of *L. conchilega* consisting of bacteria and diatoms and that of *N. hombergii* also of diatoms, but including more dinoflagellates and invertebrates. Temporal variation explained 17%: the two species retained more bacterial and *Phaeocystis* markers before the bloom, while they accumulated more poly-unsaturated FA after the bloom. CSIA revealed increased accumulation or biosynthesis of poly-unsaturated FA from the suspended matter in *L. conchilega* upon bloom deposition, which is probably related to energy storage for gametogenesis. In contrast, bloom-dependent accumulation or biosynthesis of FA was not detected in *N. hombergii*, probably because of its reliance on invertebrate prey.

1. Introduction

Phytoplankton blooms are the major source of organic matter [OM] in shallow seas like the North Sea. In the Southern Bight of the North Sea, phytoplankton dynamics display strong seasonal patterns. The phytoplankton succession is initiated in late winter–early spring by a diatom bloom, in its late phase succeeded by a large *Phaeocystis* peak which is again followed by a diatom bloom (Rousseau et al., 2002, 2008). Although the bulk of phytoplankton production is mineralised in the water column by microzooplankton and bacteria (Rousseau et al., 2000), still about a quarter is deposited on the sediment of the Belgian part of the North Sea (BPNS), 65% of it being *Phaeocystis* colonies (Lancelot et al., 2005). Shallow benthic communities depend on the seasonal input of fresh OM (Graf, 1992). Seasonal variability in the diet of benthic consumers can therefore be expected, especially in suspension-feeders which rely strongly on the input from the phytoplankton blooms during spring and summer and exhibit opportunistic behaviour in winter (Herman et al., 2000; Lefebvre et al., 2009; Page and Lastra, 2003). *Phaeocystis* is a significant component of the phytoplankton communities during spring (Rousseau et al., 2000, 2008). However, its trophic significance is controversial, because of the relatively low nutritional value and the adverse effects (e.g. mechanical clogging of gills) of the gelatinous matrix of the colonies (Weisse et al., 1994). Nevertheless, *Phaeocystis* is known to be assimilated by zooplankton organisms (e.g. Cotonnec et al., 2001), while its possible contribution as a food source for benthic organisms has only been reported for gastropods in tidal flats (Cadée, 1996), for the mussel *Mytilus edulis* (Smaal and Twisk, 1997) and for subtidal nematodes (Franco et al., 2008).

To unravel the links between the pelagic and benthic realm, biomarkers can be helpful as food web tracers. Apart from gut content analysis and stable isotope ratios (^{13}C, ^{15}N), lipid biomarkers are frequently used (see reviews of Budge et al., 2006; Dalsgaard et al., 2003; Parrish et al., 2000; Sargent and Whittle, 1981). Neutral lipids ([NL]; mainly triacylglycerols [TAG]) are used as energy stores within an organism, whereas polar lipids ([PL], phospholipid derived fatty acids and glycolipids) are important constituents of cell membranes (Dalsgaard et al., 2003). Some PL are specific for bacteria and

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phytoplankton (e.g. Dunstan et al., 1993; Viso and Marty, 1993) and are turned over within days after cell death, which makes them good biomarkers for living biomass (Boschker and Middelburg, 2002). Well-known phytoplankton and bacterial PL biomarkers are presented in Table 1. Because it is energetically more efficient to incorporate FA and to catabolise dietary carbohydrates, FA from the diet are partly routed intact or with minor modification into consumer tissues (Ruess et al., 2005). The PL fraction (cell membranes) of a consumer is under metabolic control and only provides long-term dietary information (Koussoroplis et al., 2010). In contrast, the NL found in the reserve lipids of the primary consumers are to a great extent left unmodified during trophic transfer (Caramujo et al., 2008), which makes them useful in identifying food sources and feeding modes on the short-term. The nutritional value of a prey item is often indicated by the amount of ω3 poly-unsaturated fatty acids [PUFA] such as the long-chained eicosapentaenoic (20:5ω3, EPA) and docosahexaenoic (22:6ω3, DHA) acids (Dalsgaard et al., 2003 and references therein). These PUFA are important for organisms (Viso and Marty, 1993), since they are essential for survival and growth (Sargent and Whittle, 1981). Since no single FA can be assigned uniquely to any one species and not even to any single group of phytoplankton or bacteria (Table 1) (Dalsgaard et al., 2003), a combination of FA biomarkers and the stable isotope signature of these FA might help in the differentiation. This compound-specific isotope analysis (CSIA) of FA can provide important insights into the biogeochemical conditions under which carbon fixation occurred (Bieger et al., 1997) and can be used to distinguish possible sources of organic matter in the diet of organisms (Ramos et al., 2003; Van den Meersche et al., 2004).

In this study, we investigated the temporal variation in the importance of benthic and pelagic food sources for two marine polychaetes in the *Abra alba–Kurtiella bidentata* community (Van Hoey et al., 2004) by means of FA biomarkers and CSIA. The investigated model organisms of this macrobenthic community are a predator and broad-spectrum omnivore (*Nephtys hombergii*) preying on juvenile polychaetes, molluscs and crustaceans but also consuming detritus and plankton (Warwick et al., 1979) and a suspension-deposit feeder (*Lanice conchilega*) feeding on sorted or unsorted sediment and plankton (Hartmann-Schröder, 1996). The two species have been shown to exert contrasting effects on the benthic ecosystem in terms of bioturbation and biogeochemical fluxes (Braeckman et al., 2010), in structuring nematode distribution and community composition (Braeckman et al., 2011a) and in influencing nematode community feeding dynamics (Braeckman et al., 2011b). In addition, aggregations of *L. conchilega* have been found to enhance macrofaunal densities, species richness and community composition (Rabaut et al., 2007). This field study on the seasonal variation in the diet of these two polychaetes investigates their role in linking the pelagic and the benthic realm of the study area. We tested whether (1) the seasonal succession in phytoplankton composition could be traced with PL biomarkers and their compound specific stable isotopes, (2) the diet composition (NL) of the two model macrobenthic organisms could be distinguished and (3) the temporal variation in the relative abundance of pelagic and benthic food sources in the diet of these species could be established on the short-term (NL) and on the long-term (PL).

2. Material and methods

2.1. Study site and sampling

From February to September 2008, the suspended matter in the water column and sediment from a sandy coastal station in front of Oostende (51°19.27′N, 2°52.09′E, 18 m depth, 9 km offshore) were sampled 7 times (Table 2) with respectively a Niskin bottle and a Reineck Boxcorer from the RV Zeeleeuw. Sediment median grain size at the study site is 215 ± 0.2 µm with a mud fraction (<63 µm) of 5.8 ± 0.2% (Braeckman et al., 2011a). We sampled the pelagic food sources by means of a 10 l Niskin bottle, 3 m below the surface and filtered these seston samples (without sieving to exclude zooplankton) on pre-combusted GF/F glass microfiber filters for pigments (triplicate) and lipid analysis (single sample).

Data on phytoplankton composition and abundance were retrieved from the closest monitoring station, 10 km south west (51°13.30′N, 2°51.30′E, 13 m depth, 1.5 km offshore). It is known from Rousseau et al. (2008) that the Belgian coastal area is spatially quite homogeneous in terms of phytoplankton composition. There might be a small temporal lag between of about 1 month in phytoplankton bloom arrival between the south western area and the north eastern area of the Belgian part of the North Sea (Muylaert et al., 2006). However, since both the sampling and the monitoring station are situated in the south western area, we are confident that these stations were at each sampling time similar in terms of phytoplankton composition. For the monitoring of phytoplankton composition, 1 l samples for phytoplankton counts were collected monthly or fortnightly (during the spring bloom) with a Niskin bottle; samples were fixed with a gluteraldehyde-lugol solution (final concentration 1%) and stored in the dark. Counts were performed with a Zeiss Axiovert inverted microscope using the Utermöhl method (Hasle, 1978).

Benthic food sources were sampled by means of 8 ml (4 cm deep into the surface layer) sediment subsamples of a Reineck Box corer that was deployed several times. These subsamples were used for

Table 1
Most important PL biomarkers in phytoplankton.

Polar lipid fatty acid biomarker	*Group*	*Reference*
10-Me16:0, cy-17:0, cy-19:0	Bacterial, might be *Desulfobacter*	Dowling et al., 1986
Short branched PL: i-14:0, i-15:0, ai-15:0, i-16:0, -i17:0, ai-17:0	Bacterial: Cytophaga–Flavobacteria and Gram-positive bacteria	Dalsgaard et al., 2003
18:1ω7c	Bacterial, mainly Gram-negative Proteobacteria	
16:1ω7c	Diatoms, bacteria	Dalsgaard et al., 2003
16:4ω1	Diatoms	Dunstan et al., 1993; Viso and Marty, 1993
16:2, 16:3	Diatoms	Ahlgren et al., 1992; Dunstan et al., 1993; Volkman et al., 1989
20:5ω3 (EPA), 22:5ω3	Diatoms (Bacillariophyceae)	Ahlgren et al., 1992; Dunstan et al., 1993Volkman et al., 1989
18:1ω9c	*Phaeocystis*	Al-Hasan et al., 1990; Claustre et al., 1990
Low content of 18-PUFA, high content of SFA 14:0, 16:0, 18:0	*Phaeocystis*	Claustre et al., 1990; Cotonnec et al., 2001; Hamm and Rousseau, 2003; Volkman et al., 1998
High amounts of 18:5ω3 and 18-PUFA in general	*Phaeocystis*, dinoflagellates	Dalsgaard et al., 2003; Hamm and Rousseau, 2003
22:6ω3 (DHA)	Dinoflagellates, *Phaeocystis*	Dalsgaard et al., 2003
20:1ω9, 22:1ω11	Carnivorous feeding (directly or indirectly on copepods)	Budge et al., 2006; Dalsgaard et al., 2003; Mansour et al., 2005
High 18:1ω9/18:1ω7 and DHA/EPA ratio	Carnivorous feeding	Dalsgaard et al., 2003; Falk-Petersen et al., 2000; Graeve et al., 1997

the determination of pigments (in duplicate) and lipid analysis (single sample) of the sediment organic matter. Pigment samples were stored at −80 °C until analysis. Sediment samples (approx. 3 g of sediment) and water filters for lipid analysis were immediately transferred to extraction liquid (methanol/chloroform). Pigments were determined by HPLC (Gilson) analysis according to Wright and Jeffrey (1997).

Macrobenthos was sampled at 4 occasions (Table 2) using a Van Veen grab, sieved on board and adult specimens of *L. conchilega* and *N. hombergii* were brought back alive to the lab. Upon return to the lab, triplicate samples of macrobenthic organisms were immediately dissected to remove the gut and its content. Remaining tissue was blended and this slurry was stored frozen (−80 °C) until lipid extraction. The gut was not removed from the April samples. To test for the gut presence effect, we sampled extra *L. conchilega* (intertidal area of Bay of Heist) and *Nephtys* spp. (subtidal) individuals in June 2009 from which we compared the relative PL and NL FA composition of entire samples, gutless samples and guts.

2.2. Fatty acid extraction and analysis

The lipids were extracted from the suspended matter, sediment and macrobenthic slurries using a modified Bligh and Dyer method (Boschker, 2004; Boschker et al., 1999). In short, total lipid samples were extracted in chloroform–methanol–water (1:2:0.8/v:v:v) and fractionated on a heat-activated silicic acid column (Silica 60 Merck, Darmstadt, Germany) by sequential elution with chloroform, acetone and methanol to obtain two polarity fractions: neutral lipids containing the TAG reserve lipids (NL) in the chloroform fraction and polar lipids containing mainly membrane lipids (PL) in the methanol fractions. The acetone fraction containing mostly pigments was not retained. Fatty acid methyl esters (FAME) were obtained from both polar and neutral lipid extracts using derivatisation by mild alkaline transmethylation. FAME concentrations were determined by gas chromatograph-combustion-interface-isotope ratio mass spectrometer (GC-c-IRMS, Hewlett Packard G1530 GC; polar BPX-70 column 50 m, 0.32 mm film 0.25 μm, SGE054607; Type-III combustion interface; Thermo Finnigan Delta-plus IRMS). Samples were injected in splitless mode and concentrations were calculated based on the added amount of the internal standard (19:0). The use of GC-c-IRMS for concentration measurement and CSIA implies a higher detection limit for individual compounds than when using GC-MS or GC-FID. Identification of FAME was based on the comparison of relative retention times and compared to those of internal standards (12:0, 16:0, 19:0) and FAME reference sediment mixtures, which were also used to check the accuracy of the isotopic ratios determined by the GC-c-IRMS. The ratio's of the heavy to the light stable carbon isotope ($\delta^{13}C$) of individual FA (PL or NL) were calculated by correcting for methyl C atoms added during derivatisation following: $\delta^{13}C_{FA} = ((n+1) \times \delta^{13}C_{FAME} - 1 \times \delta^{13}C_{Methanol})/n$, where n is the number of C atoms in the FA, $\delta^{13}C_{FAME}$ is the isotope ratio of measured methylated FA and $\delta^{13}C_{Methanol}$ is the isotopic ratio of the used methanol. Stable carbon isotope data are expressed in the delta notation relative to Vienna Pee Dee Belemnite (V-PDB). Blanks and a standard mixture were measured regularly in between the samples to check for system stability and possible contamination. We use a FA shorthand notation of the form *A:BωX*, where A represents the number of carbon atoms, B gives the number of double bounds and X gives the position of the double bond closest to the terminal methyl group (Guckert et al., 1985).

2.3. Data analysis

Results are expressed in terms of relative FA biomarker composition within the PL and NL fraction. The effect of the presence of the gut on the PL and NL composition of the macrobenthic species was explored with a 2-factor permutational ANOVA (Permanova) with Species and Gut Presence as factors, based on a Bray–Curtis resemblance matrix on untransformed data. The temporal variations in the macrobenthic diet (NL) and structural PL were explored with both multi- and univariate techniques. Two-factor permutational ANOVAs (Permanova) were carried out to test the effect of Species [Sp] and Months [M] on the -relative abundance of all (multivariate, Bray–Curtis resemblance matrix) NL and PL biomarkers and on specific (univariate, Euclidean distance resemblance matrix) NL biomarkers. In case a significant month effect was found, pair-wise tests within this factor were carried out, with p-values drawn from Monte Carlo [MC] permutations, because of the restricted number of possible permutations (Anderson and Robinson, 2003). The two-factor design was used for both multivariate and univariate analyses since Permanova allows to perform univariate ANOVAs with p-values obtained by permutation (Anderson and Millar, 2004), thus avoiding the assumption of normality. A Principal Coordinates analysis (PCO) produces an equivalent ordination to a PCA when a Euclidean distance matrix is used as the basis of the analysis. However, for this analysis, we used the same Bray–Curtis resemblance matrix used for Permanova. PCO visualised those FA from NL and PL that correlated >70% with one of the first 2 PCO axes. Difference in $\delta^{13}C$ of the macrobenthic NL (all and specific biomarkers) among species and months was also tested with a two-factor permutational ANOVA using Euclidean distance based resemblance matrices. Within the NL samples, 1 replicate from *L. conchilega* in early June and 1 from *N. hombergii* at the end of June had poorly resolved chromatograms and were therefore omitted from the analysis. All analyses were performed within PRIMER v6 with PERMANOVA add-on software (Anderson et al., 2008; Clarke and Gorley, 2006).

Results of phytoplankton counts and PL of suspended particulate matter (SPM) and sediment are unique samples, pigments from the sediment are averages of duplicates ± SD and pigments from the suspended matter and PL and NL in macrobenthos are represented as averaged triplicates ± SE. SPM and sediment samples are background data for this study of which the main focus is the temporal variation in the feeding ecology of the polychaetes. As such, our sampling strategy for SPM and sediment aims at gathering data that give us the opportunity to evaluate the temporal variation at one point rather than the spatial variation at one point in time. Therefore, the lack of spatial replication should not be an issue.

3. Results

3.1. Microscopic observations of phytoplankton in the water column

Diatom abundances in the water column started to increase slightly as of February, followed by small peaks of dinoflagellates and *Phaeocystis* (Fig. 1a, b). The main *Phaeocystis* bloom occurred mid April, 14 days later followed by a prominent diatom and dinoflagellate peak. By half May, *Phaeocystis* and diatom blooms disappeared, while dinoflagellates bloomed again from June to September.

3.2. Pigments and PL biomarkers in suspended matter and sediment column

The peak in suspended matter chl-*a* occurred in early May and coincided with high values of chl-*a* observed in the sediment (Fig. 1c); note however that the chl-*a* peak was time-delayed with regard to the Phaeocystis, diatom and dinoflagellate blooms. This is because the phytoplankton composition and chl-*a* were not measured at the same timepoint. The chl-*a* concentrations in the suspended matter and sediment were strongly reduced by the end of May (Fig. 1c).

3.2.1. PL composition

Suspended matter PL consisted mainly of saturated fatty acids (SFA, 14:0 + 16:0 + 18:0; 23.5–35.9%), EPA (20:5ω3; 12.3–23.3%),

Table 2

Relative fatty acid concentrations in polar lipids of suspended matter in the water column and of the sediment and average fatty acid concentrations in neutral lipid fraction of *Lanice conchilega* and *Nephtys hombergii* from March to September (in %). Important biomarkers are highlighted in bold.

	Water							*Sediment*								*Nephtys hombergii*				*Lanice conchilega*			
	07/03	**03/04**	**09/05**	**30/05**	**27/06**	**08/08**	**03/09**	**07/03**	**03/04**	**09/05**	**30/05**	**27/06**	**08/08**	**03/09**	**03/04**	**03/06**	**27/06**	**03/09**	**03/04**	**03/06**	**27/06**	**03/09**
10Me-16:0	–	–	–	–	–	–	–	**5.18**	**5.76**	**1.53**	–	**1.94**	**4.56**	**5.69**	**4.48**	**0.75**	**0.46**	**0.46**	**5.99**	**2.49**	**2.30**	**1.31**
10Me-17:0	–	–	–	–	–	–	–	1.20	–	–	–	–	–	–	0.31	–	0.01	–	–	–	–	–
10Me-18:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.88	0.06	0.57	0.12	0.75	0.19	0.62	0.07
10Me-18:0	–	–	–	–	–	–	–	1.83	–	0.52	–	0.85	0.84	0.60	–	–	–	–	–	–	–	–
2-OH_10:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24	0.04	–	–	0.27	–	–
2-OH_12:0	6.04	7.29	4.51	2.30	2.94	2.85	4.24	–	0.36	–	–	–	–	–	–	–	–	0.14	–	–	–	0.54
2-OH_14:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3-OH_12:0	–	–	–	–	–	–	–	–	0.36	–	0.15	–	–	–	–	–	0.03	0.05	–	–	0.54	0.28
ai-15:0	–	–	–	**2.30**	–	–	–	**5.02**	**2.23**	**4.44**	**1.33**	**7.19**	**7.36**	**6.98**	–	–	**0.05**	–	–	**0.38**	**0.14**	**0.06**
i-17:0	–	–	–	–	–	–	–	**0.84**	**0.89**	**0.67**	–	**0.88**	**1.09**	**0.99**	**0.24**	**0.16**	–	**0.25**	**0.85**	**0.49**	**0.48**	**0.80**
i-19:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.75	–	–	–	–	–	–
14:0	9.67	17.97	13.67	7.74	9.71	6.05	8.90	2.88	0.84	3.95	4.61	3.49	2.95	2.54	3.59	1.99	1.68	1.58	3.48	4.17	6.96	5.54
14:1ω5	–	–	–	–	–	–	–	–	–	–	0.94	–	–	–	–	–	–	–	–	–	–	–
15:0	–	–	–	–	–	–	–	1.26	0.64	1.03	0.90	1.46	1.38	1.21	2.59	0.88	0.99	0.68	1.80	0.49	1.07	0.54
15:1ω5	–	–	–	–	–	–	–	–	4.44	–	–	–	–	–	–	0.12	0.07	–	–	–	–	–
16:0	14.50	17.97	14.36	12.34	12.65	11.03	15.25	11.67	4.33	10.25	11.98	13.14	13.59	13.87	14.81	9.23	16.63	10.59	13.53	15.20	18.57	16.86
16:1ω7c	**17.52**	**13.02**	**10.85**	**14.85**	**12.06**	**6.41**	**13.56**	**14.65**	**6.38**	**12.87**	**5.38**	**15.47**	**15.20**	**18.22**	**3.45**	**2.66**	**7.68**	**1.75**	**9.13**	**7.24**	**14.16**	**11.75**
16:1ω7t	–	–	–	1.88	–	–	–	–	–	1.76	0.35	1.49	–	–	–	0.17	0.21	–	–	–	–	0.28
16:2ω4	–	–	2.67	–	1.47	–	–	0.89	–	0.63	0.14	0.70	0.44	0.26	2.15	1.31	1.18	1.83	0.47	0.63	0.54	0.57
16:2ω7	–	–	–	–	–	–	–	1.15	1.30	1.01	0.62	0.82	0.91	0.90	–	0.17	0.42	0.78	0.64	0.60	0.49	0.18
16:3ω3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16:3ω4	–	–	–	–	–	–	–	1.26	0.39	0.89	0.18	1.21	0.66	0.73	–	–	0.06	–	–	–	–	–
16:4ω3	–	–	–	–	–	–	–	–	0.89	–	–	–	–	–	–	0.06	–	–	–	–	–	–
17:0	–	–	–	–	–	–	–	1.05	0.64	0.89	0.69	1.40	1.38	1.16	–	–	–	–	–	–	–	–
17:1ω7c/cy_17:0	–	–	–	–	–	–	–	**1.57**	**8.49**	**0.97**	**0.30**	**1.33**	**1.49**	**1.51**	–	**0.23**	**0.43**	**0.13**	**0.16**	–	–	**0.06**
18:0	–	–	3.13	3.77	4.71	6.41	4.24	2.83	1.37	3.15	5.02	3.46	3.28	2.37	7.14	4.85	6.65	9.51	4.67	8.18	2.87	3.72
18:1ω7c	5.44	5.21	5.65	7.32	6.47	3.91	6.78	11.51	4.85	11.82	5.25	11.10	13.08	15.68	3.62	3.33	2.40	2.29	7.47	7.02	6.69	4.14
18:1ω9c	–	**12.24**	**11.00**	**2.93**	**5.29**	**4.63**	**3.81**	**4.40**	**10.02**	**4.20**	**3.69**	**4.73**	**6.12**	**6.38**	**4.04**	**3.15**	**1.71**	**1.55**	**4.06**	**4.15**	**2.29**	**1.65**
18:1ω9t/16:4ω1	5.44	–	–	–	1.76	–	–	0.37	0.25	0.69	2.22	0.52	0.36	0.34	0.84	0.57	0.87	1.40	1.66	1.08	1.78	1.70
18:2ω6c	–	–	–	–	–	–	–	0.89	0.39	0.67	0.50	0.88	1.13	1.21	–	0.18	0.33	0.23	–	–	–	0.12
18:2ω6t	–	–	–	–	–	–	–	0.37	–	0.83	0.13	0.27	–	–		–	–	–	–	–	–	–
18:3ω3	–	–	2.22	–	–	–	–	–	–	0.34	0.35	0.18	–	–	–	–	0.09	0.06	–	–	–	0.05
18:3ω4	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–	–	–	–	–	–	–	–	–

18:3ω6	–	–	–	–	–	–	–	0.58	8.75	–	0.23	–	–	–	–	–	–	–	–	–	–	–
18:4ω3	–	–	–	–	–	–	–	1.67	–	1.21	1.06	1.58	1.17	0.69	–	–	0.06	–	0.19	0.65	0.19	0.16
18:5ω3 (12–15)	–	–	–	–	**2.94**	–	–	**0.99**	**8.18**	**0.71**	**3.42**	**0.46**	**0.69**	–	**7.09**	**6.17**	**6.79**	**13.27**	**2.59**	**1.03**	**0.66**	**4.57**
18:5ω3 (12–16)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**0.43**	–	–	–	–	–
20:0	4.23	–	5.88	3.35	4.41	13.17	5.51	–	0.18	–	–	–	–	–	–	–	–	0.09	–	–	–	–
20:1ω9c	–	–	–	–	–	–	–	**0.47**	–	**0.48**	**1.03**	**0.52**	**0.66**	**0.60**	**1.69**	**0.78**	**0.81**	**0.83**	–	**0.47**	–	**0.06**
20:2ω9	–	–	–	–	–	–	–	–	–	0.28	0.86	–	–	–	3.69	2.42	4.62	2.30	–	–	–	0.35
20:3ω3	–	–	–	–	–	–	–	–	–	–	2.59	–	–	–	–	–	–	–	–	–	–	–
20:3ω6	–	–	–	1.46	–	–	–	–	–	–	0.23	–	–	–	–	–	–	–	–	–	0.73	–
20:4ω3	–	–	–	–	–	–	–	–	4.42	–	0.91	–	–	–	–	0.04	0.07	0.08	–	0.16	–	0.13
20:4ω6	–	–	–	–	–	–	–	4.08	0.57	2.50	–	2.85	2.81	1.64	1.47	1.53	0.88	0.75	0.72	0.31	2.18	0.49
20:5ω3	**23.27**	**12.76**	**12.30**	**12.55**	**14.12**	**13.88**	**20.76**	**5.08**	**1.12**	**9.56**	**16.53**	**4.73**	**2.81**	**2.28**	**8.14**	**11.74**	**12.60**	**13.29**	**12.47**	**7.55**	**13.93**	**16.66**
21:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.60	0.34	0.89	1.39	–	1.55	0.19	0.09
22:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22:1ω11c	–	–	–	–	–	–	–	–	–	**0.97**	–	–	–	–	**0.33**	**0.25**	**0.80**	**1.52**	–	**0.88**	–	**0.40**
22:1ω9c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22:4ω6	–	–	–	–	–	–	–	–	–	0.48	–	–	–	–	3.54	11.87	2.19	6.00	3.07	2.82	1.56	2.82
22:5ω3	–	–	–	–	–	–	–	0.52	0.61	1.09	3.19	0.33	–	–	4.57	6.51	3.59	7.15	7.03	6.14	6.16	7.79
22:6ω3	**13.90**	**13.54**	**13.75**	**19.25**	**17.35**	**25.27**	**16.95**	**1.78**	**0.82**	**5.20**	**15.97**	**1.85**	**1.20**	**0.99**	**8.78**	**16.51**	**10.12**	**12.77**	**7.71**	**9.16**	**8.53**	**9.79**
23:0/22:2ω6	–	–	–	–	2.65	6.41	–	–	3.01	–	–	–	–	–	5.98	5.32	1.36	1.99	5.35	1.33	1.12	0.97
24:0	–	–	–	–	–	–	–	–	1.69	–	0.95	–	–	–	1.41	1.43	1.73	1.40	0.78	0.54	0.22	1.23
24:1ω9c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.19	–	–	–	–	–
cy_19:0	–	–	–	–	–	–	–	**0.73**	–	–	–	–	–	–	–	–	–	**0.08**	–	–	–	–
i-14:0	–	–	–	**6.28**	**1.47**	–	–	**1.05**	**0.43**	**1.65**	**0.69**	**2.03**	**1.42**	**1.12**	–	–	–	–	–	**0.20**	–	–
i-15:0	–	–	–	**1.67**	–	–	–	**3.77**	**1.53**	**3.67**	–	**3.88**	**4.45**	**4.52**	–	**0.04**	**0.06**	–	**0.21**	**1.34**	**0.52**	**0.13**
i-16:0/15:1ω5c	–	–	–	–	–	–	–	1.20	0.84	0.99	0.76	1.58	1.60	1.46	–	0.13	0.16	0.13	0.46	0.75	0.65	0.18
i-17:0	–	–	–	–	–	–	–	**2.67**	**1.34**	**2.78**	**2.47**	**3.28**	**2.99**	**2.71**	**0.84**	**0.70**	**0.20**	**0.57**	**3.66**	**4.23**	**3.05**	**2.82**
i-18:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	0.05	0.07	–	0.19	–	0.07
i-20:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Σ SFA	**28.40**	**35.94**	**37.05**	**27.20**	**31.47**	**36.65**	**33.90**	**19.68**	**9.68**	**19.27**	**24.14**	**22.94**	**22.59**	**21.15**	**31.14**	**20.47**	**28.57**	**25.24**	**24.27**	**30.13**	**29.87**	**27.97**
Σ SFA — 14-16-18	**24.17**	**35.94**	**31.17**	**23.85**	**27.06**	**23.49**	**28.39**	**17.37**	**6.54**	**17.35**	**21.61**	**20.08**	**19.83**	**18.79**	**25.54**	**16.07**	**24.96**	**21.68**	**21.68**	**27.54**	**28.40**	**26.12**
Σ *Desulfobacter* markers	–	–	–	–	–	–	–	**7.48**	**14.26**	**2.50**	**0.30**	**3.28**	**6.05**	**7.20**	**4.48**	**0.98**	**0.89**	**0.67**	**6.15**	**2.49**	**2.30**	**1.37**
Σ bacterial markers	**5.44**	**5.21**	**5.65**	**17.57**	**7.94**	**3.91**	**6.78**	**32.34**	**25.53**	**27.52**	**10.05**	**31.64**	**36.44**	**39.21**	**9.18**	**5.20**	**3.59**	**3.78**	**18.35**	**16.17**	**13.19**	**9.32**
DHA/EPA	0.61	1.06	1.81	1.54	1.21	0.82	1.54	0.82	0.35	0.75	0.55	0.97	0.39	0.43	0.44	0.62	1.22	0.61	0.59	1.08	1.41	0.90
18:1ω9/**18:1**ω7	1.03	2.27	1.26	0.41	1.14	0.60	0.41	0.60	0.42	2.11	0.42	1.13	0.48	0.50	0.43	0.77	0.62	0.61	0.81	1.35	1.11	1.33
Σ 16-PUFA	–	–	**2.67**	–	**1.47**	–	–	**3.30**	**2.57**	**2.54**	**0.94**	**2.73**	**2.00**	**1.90**	**2.15**	**1.54**	**1.66**	**2.61**	**1.11**	**1.23**	**1.03**	**0.75**
Σ 18-PUFA	–	–	**2.22**	–	**2.94**	–	–	**4.50**	**17.31**	**3.77**	**5.76**	**3.37**	**2.99**	**1.90**	**7.09**	**6.35**	**7.70**	**13.56**	**2.78**	**1.69**	**0.85**	**4.89**
Σ invertebrate markers	–	–	–	–	–	–	–	**0.47**	–	**1.45**	**1.03**	**0.52**	**0.66**	**0.60**	**2.02**	**1.03**	**1.61**	**2.35**	–	**1.35**	–	**0.46**
Σ PUFA	**37.17**	**26.30**	**30.94**	**33.26**	**38.53**	**45.55**	**37.71**	**19.26**	**30.43**	**25.40**	**46.97**	**15.87**	**11.81**	**8.70**	**45.40**	**63.83**	**44.80**	**60.50**	**40.25**	**30.39**	**36.09**	**44.63**

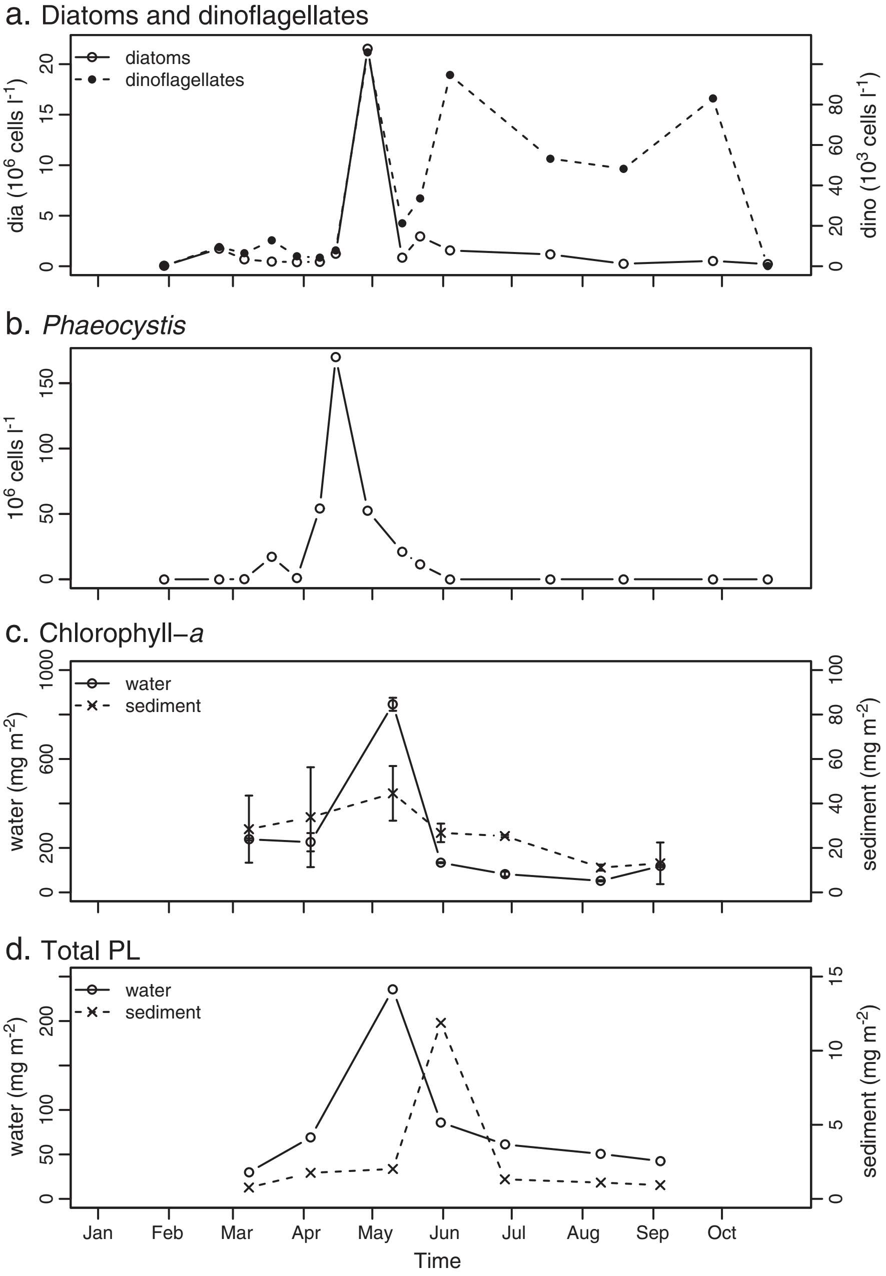

Fig. 1. Microscopic counts of the major phytoplankton groups (panels a, b) in the water column, average integrated average chlorophyll-*a* content of suspended matter ± SE (n = 3) (18 m), sampled on 29/1, 22/2, 5/3,17/3, 28/3, 7/4,14/4, 13/5, 21/5, 3/6, 17/7, 18/8, 26/9, 20/10 and sediment ± SD (n = 2) (4 cm) (panel c) and polar lipids in suspended matter and sediment during the time course (panel d). Mind different scales on y-axes.

DHA (22:6ω3; 13.5–25.3%), 16:1ω7c (6.4–17.5%) and the bacterial short branched PL i-14:0, i-15:0, ai-15:0, i17:0, ai-17:0 and 18:1ω7 (3.9–17.6%) (Table 2).

The *Phaeocystis* bloom that started in April (as observed in Fig. 1b) coincided with elevated concentrations of 18:1ω9c (Fig. 2g) and the saturated fatty acids 14:0, 16:0 and 18:0 (comprising about 35% of water PL at that time) (Fig. 2i). The main PL peak in the suspended matter did not appear until early May, as did the chl-*a* peak (Fig. 1c), and was made up by relatively high amounts of EPA, DHA, 16:1ω7c and specific peaks in 18-PUFA, while 18:1ω9c and SFA concentrations also remained high. This corresponds with the mixed composition of the bloom with *Phaeocystis*, diatoms and dinoflagellates (Fig. 1a, b). By the end of May, the *Phaeocystis* markers had declined and bacterial markers peaked in the suspended matter (Fig. 2a). Dinoflagellate cell count peaks in late summer were mirrored in high relative DHA concentrations at the end of September. If detectable in suspended matter, 16- and 18-PUFA attained only low relative abundances (Table 2; Fig. 2f).

Although some peaks in 18:1ω9c (*Phaeocystis*) and bacterial markers were observed in the sediment in early April (Fig. 2a,g), the main peak in total PL in the sediment occurred 3 weeks later than the bloom in the water column (Fig. 1d). The spring bloom settlement was characterised by high relative contributions of EPA and DHA (together 32.5%) and SFA (24%) (Table 2). In addition, Table 2 shows that the share of EPA + DHA is the same for suspended matter and sediment at the end of May, with maximal levels in the sediment sample at that sampling day. This indicates that sediment material is very fresh at that date, with minor compositional differences to the suspended matter. The relative contribution of the bacterial and general bacterial/diatom markers in the

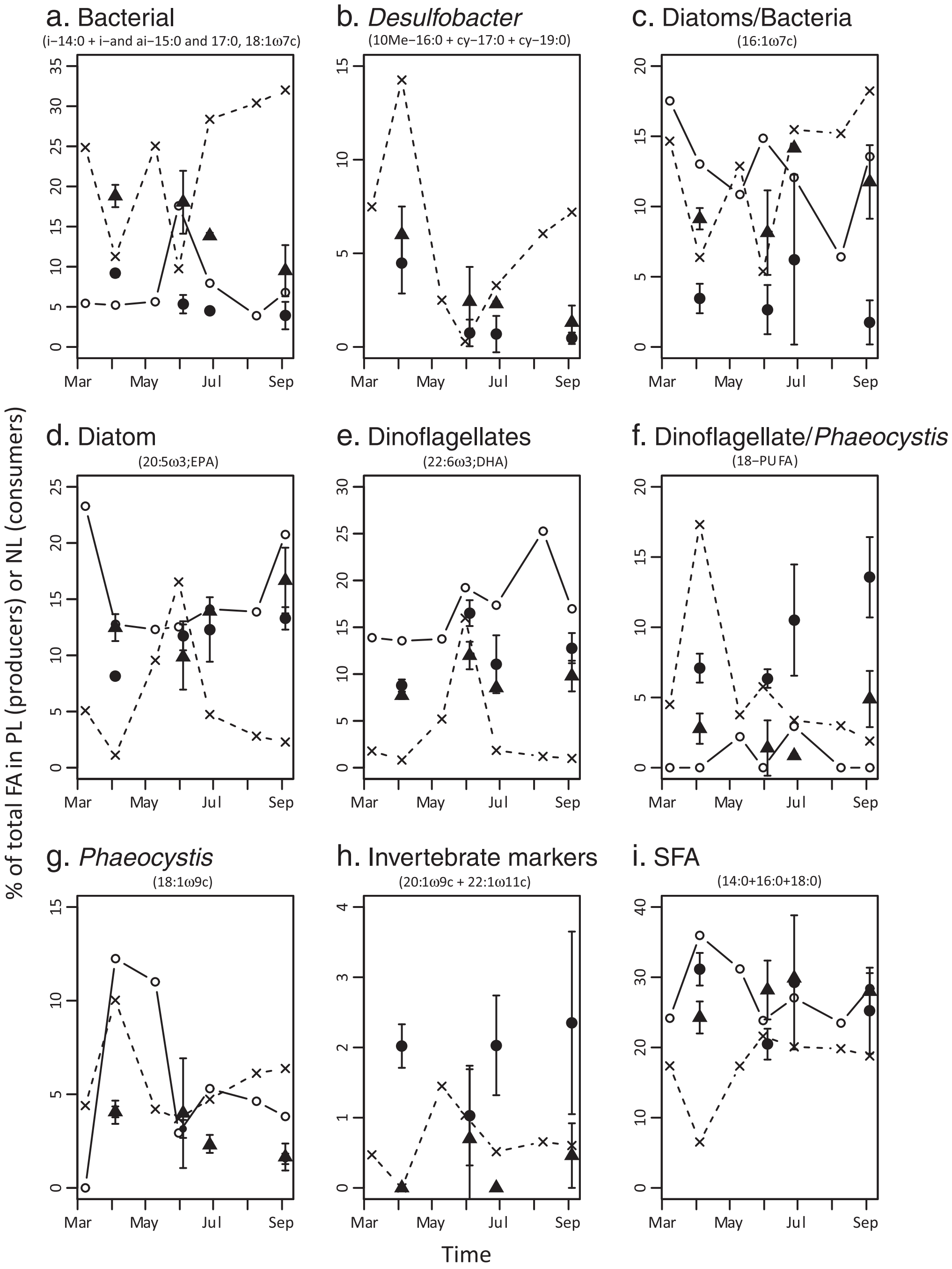

Fig. 2. Relative abundance of specific FA biomarkers within polar lipids of suspended matter (○, solid line), sediment (x, dashed line) and average of these specific FA biomarkers ± SE within neutral lipids of *Lanice conchilega* (▲) and *Nephtys hombergii* (●) during the time course. Mind different scales on y-axes.

sediment was at that time lowest. 10Me-16:0, a possible marker for *Desulfobacter* (references in Table 1) contributed about 14% to the total PL composition of the sediment in early spring, but dropped in relative abundance to virtually zero during the bloom and increased steadily to a relative abundance of 7% again in the post-bloom period (Fig. 2b).

3.2.2. $\delta^{13}C$ signature of PL in suspended matter and sediment

The $\delta^{13}C$-values of the bacterial markers, EPA, DHA, *Phaeocystis* marker 18:1ω9c and SFA in the PL fraction of the suspended matter varied between −23 and −35‰ (Fig. 3a,d,e,g,i). The PUFA had a more depleted $\delta^{13}C$-value than the short-chain FA. All FA showed a relatively enriched value during the bloom period (April–June, depending on the blooming group). At the end of June, a sudden decrease in the $\delta^{13}C$-value of the bacterial markers, EPA, DHA, *Phaeocystis* marker 18:1ω9c and SFA in this fraction of the suspended matter was noticed (Fig. 3a,d,e,g,i). The general diatom/bacterial marker 16:1ω7c fluctuated with time, but a depleted $\delta^{13}C$-value was also observed in this PL at the end of June (Fig. 3c).

The $\delta^{13}C$-values of the sediment PL varied over a smaller range than in the suspended matter (between −26 and −30‰), except for DHA of which the $\delta^{13}C$-value was depleted (−34‰) in April, but increased from early May to −25‰ (Fig. 3e). All sedimentary bacterial markers and SFA were more depleted than EPA and DHA (Fig. 3a,d, e,i). The bacterial markers and the 10Me16:0 *Desulfobacter* marker showed a steady increase in $\delta^{13}C$-values over time (Fig. 3b), while

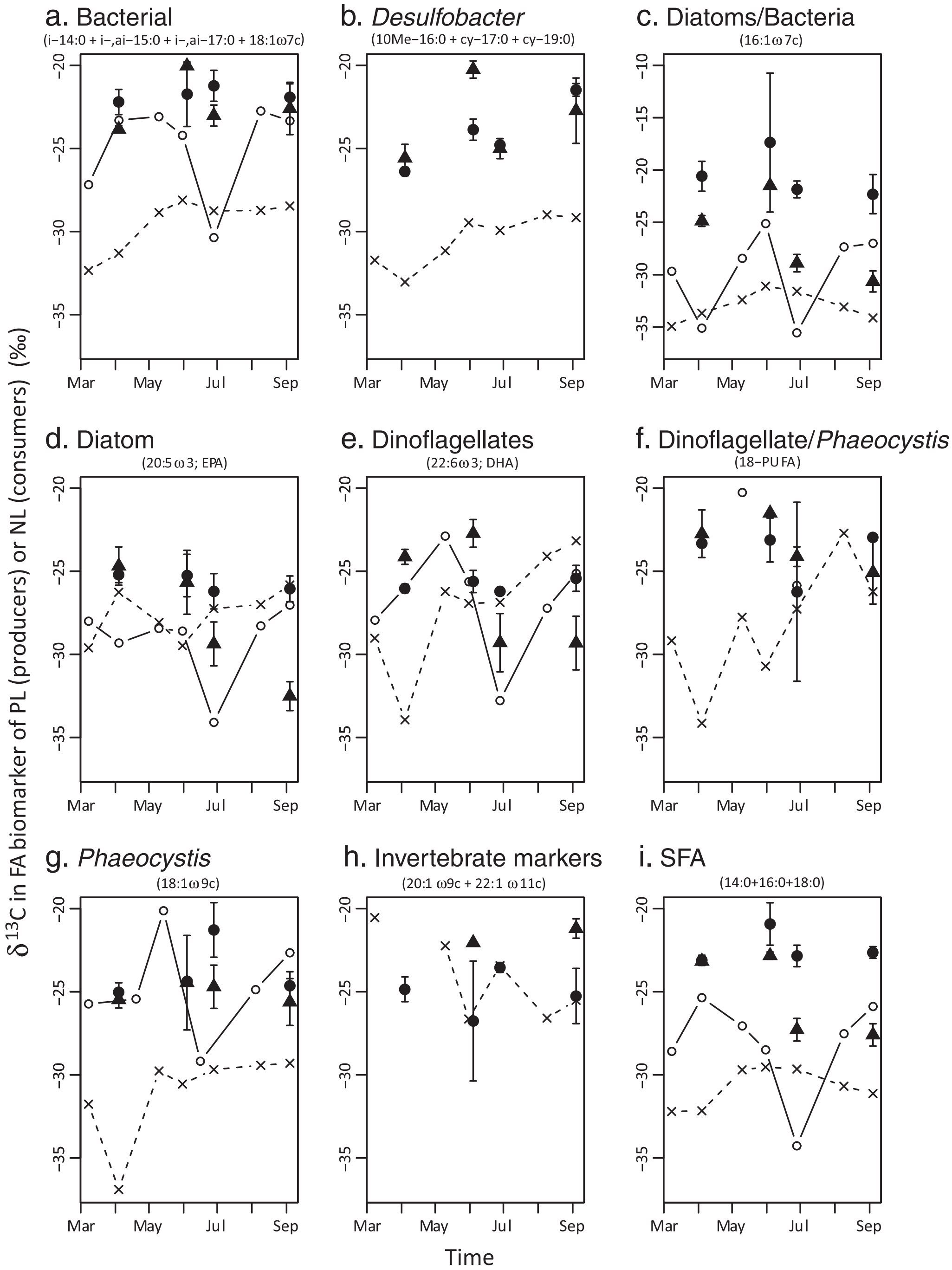

Fig. 3. δ^{13}C of specific FA biomarkers within polar lipids of suspended matter (○), sediment (x) and average of these specific FA biomarkers ± SE within neutral lipids of *Lanice conchilega* (▲) and *Nephtys hombergii* (●) during the time course. Mind different scales on y-axes in panel c. Invertebrate markers (panel h) were not found in the sediment in April.

the general diatom/bacterial marker 16:1ω7c peaked at the end of May and decreased afterwards (Fig. 3c).

3.3. Lipid composition of macrobenthos

3.3.1. Potential effects of gut removal

Two-factor permutational ANOVA (Permanova) on NL and PL only indicated a difference in FA composition on species level (NL: Pseudo-$F_{1,16} = 17.46$, $p < 0.001$; PL: Pseudo-$F_{1,16} = 8.11$; $p = 0.015$). No difference in composition between entire organism samples, gut samples and gutless samples was found. Even in the specific biomarker FA such as EPA, DHA and the bacterial markers, no differences were noted. Therefore, we included the samples obtained in April in the analyses discussed below.

3.3.2. FA in macrobenthos

Within the lipid fraction, PL were generally much more abundant than NL (Fig. 4). A 2-factor Permanova indicated that the proportions of PL and NL were constant through time in both species (Species × Month [SpxM] interaction, single terms Species and Months: $p > 0.05$).

3.3.3. Fatty Acids in reserve lipid fraction (NL)

3.3.3.1. Multivariate. The composition of the macrobenthic NL fraction (short-term diet) differed among species (2-factor Permanova

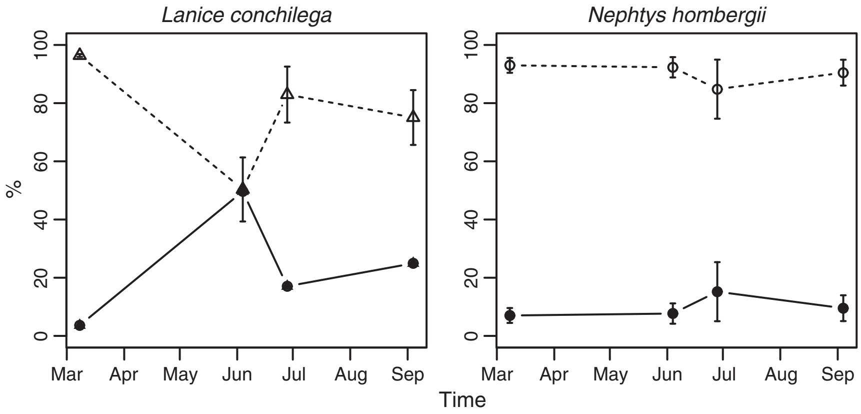

Fig. 4. Fluctuations in the average relative proportions ± SE of polar lipids (open symbols) and neutral lipids (closed symbols) in *Lanice conchilega* and *Nephtys hombergii*.

pseudo-$F_{1,15} = 12.69$, $p < 0.001$) and months (pseudo-$F_{3,15} = 2.82$, $p = 0.001$), but an interaction between the two factors was not detected ($p > 0.05$). In April, the diet was different from the other months (pair wise tests, $p < 0.024$). The PCO analysis in Fig. 5 shows that the FA biomarkers allow distinguishing the species groups and time succession (Pearson correlation coefficient with one of the first 2 PCO axes >0.7). The distinction between *L. conchilega* and *N. hombergii* explained almost 50% of the total variation and results from the higher relative abundance of 16:1ω7c (general biomarker for diatoms/bacteria) and the bacterial markers i-17:0 and 18:1ω7c in the reserve lipids of *L. conchilega*. Relatively more short-branched SFA were present in *L. conchilega* (14:0 and 16:0). In *N. hombergii* relatively more 16:2ω4 (diatoms), 22:6ω3 (dinoflagellates), 18:5ω3 (*Phaeocystis* or dinoflagellate marker) and 22:1ω11c (invertebrate marker) were found. 18:0 was the characteristic SFA for the diet of *N. hombergii*. The temporal distinction explained <20% of the total variation and was characterised by higher relative concentrations of 10-Me16:0 (*Desulfobacter*), 18:1ω9c (*Phaeocystis*), a peak of 23:0/22:2ω6 in pre-bloom and 20:5ω3 (diatoms) in post-bloom periods.

3.3.3.2. Univariate. Within the NL fraction, 16:0 was the most dominant FA biomarker (Table 2), representing on average 14.4 ± 1.1% of the total NL in the two macrobenthic species, followed by EPA (12.1 ± 0.7%) and DHA (10.3 ± 0.7%). In the NL fraction (short-term diet) of *N. hombergii* significantly more PUFA (Sp effect: pseudo-$F_{1,15} = 17.35$, $p < 0.001$), DHA (pseudo-$F_{1,15} = 14.56$, $p = 0.002$), 18-PUFA (Sp effect: pseudo-$F_{1,15} = 45.46$, $p < 0.001$) and invertebrate markers (Sp effect: pseudo-$F_{1,15} = 17.53$, $p = 0.002$) were present. Also, significantly higher ratio's of DHA/EPA (Sp effect: pseudo-$F_{1,15} = 11.17$, $p = 0.001$) and 18:1ω9/18:1ω7 (pseudo-$F_{1,15} = 9.40$, $p = 0.007$) were found (Table 2). In contrast, the NL fraction (short-term diet) of *L. conchilega* was characterised by higher levels of 16:1ω7c (Sp effect: pseudo-$F_{1,15} = 41.24$, $p < 0.001$) and bacterial markers (Sp effect: pseudo-$F_{1,15} = 71.57$, $p < 0.001$). Several PUFA, such as 20:4ω6 and 22:4ω6, were present in the macrobenthic species, but absent from the suspended matter and/or sediment sources (Table 2). The temporal effect on the NL fraction (short-term diet) of both species was demonstrated in a gradual decrease in dietary contribution of general bacterial (M effect: pseudo-$F_{3,15} = 3.83$, $p = 0.033$; pair wise test: early June > September: $p(MC) = 0.032$) (Fig. 2a), *Desulfobacter* (M effect: pseudo-$F_{3,15} = 7.65$, $p = 0.004$; pair wise tests: April > other months $p(MC) < 0.022$) (Fig. 2b) and *Phaeocystis*-specific markers – 18:1ω9c (M effect: pseudo-$F_{3,15} = 7.69$, $p = 0.003$; pair wise tests: April > end of June, Sept, $p(MC) < 0.002$ and end of June > Sept, $p(MC) = 0.046$) (Fig. 2g). In contrast, EPA proportions increased from April to September (M effect: pseudo-$F_{3,15} = 5.38$, $p = 0.01$; pair wise tests: April < end of June $p(MC) = 0.025$, April and early June < September, $p(MC) < 0.038$) (Fig. 2d), as did DHA concentrations (M effect: pseudo-$F_{3,15} = 12.35$, $p < 0.001$; pair wise tests: April < early June, September and early June > end of June, $p(MC) < 0.015$) (Fig. 2e). The DHA/EPA ratio was significantly higher in both species' diets in early June compared to the other months (M effect: pseudo-$F_{3,15} = 4.95$, $p = 0.008$) (Table 2). Finally, 18-PUFA were highest in September compared to April and early June (M effect: pseudo-$F_{3,15} = 45.46$, $p(MC) < 0.001$; pair wise tests: $p < 0.026$) (Fig. 2f).

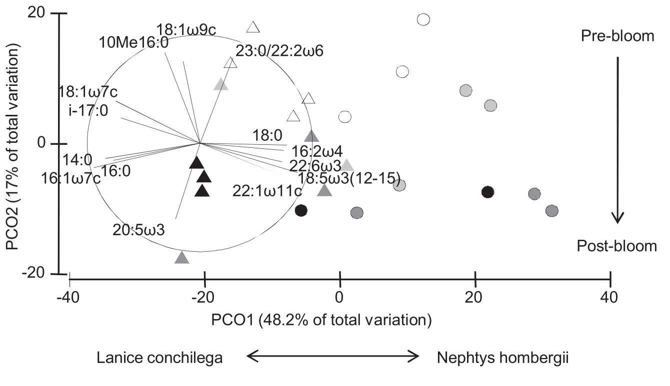

Fig. 5. PCO analysis of relative abundance of neutral lipids in *Lanice conchilega* (Δ) and *Nephtys hombergii* (○) over the time course (April: □ – end of May: ▨ – end of the end of June: ▨ – September: ■). Vectors represent specific FA biomarkers correlating > 70% with one of the first 2 PCO axes.

3.3.4. δ^{13}C signature of NL in macrobenthic consumers

The δ^{13}C-values of the bacterial, invertebrate and *Phaeocystis*/dinoflagellate (18-PUFA) and unique *Phaeocystis* (18:1ω9c) markers did not change through time and among species (Permanova on SpxM: $p > 0.05$ for all factors) (Fig. 3a,f,g,h). The diatom/bacterial marker 16:1ω7c was significantly more depleted in *L. conchilega* (Sp effect: pseudo-$F_{1,15} = 11.09$ $p = 0.005$) (Fig. 3c). The δ^{13}C-values of EPA, DHA and SFA differed among species and time (SpxM $p < 0.05$): the δ^{13}C-values stayed constant through time in *N. hombergii*, while these biomarkers became significantly more depleted later in the year in *L. conchilega* (Fig. 3d,e,i).

3.3.5. Structural fatty acids (PL)

The composition of the PL differed among species and time (SpxM interaction: pseudo-$F_{3,17} = 3.42$, $p < 0.001$) in that April clustered apart from early June and September in *L. conchilega* (pair-wise tests $p(MC) < 0.037$) and apart from the end of June in *N. hombergii* (pair-wise test $p = 0.021$). The structural fatty acids of *L. conchilega* were characterised by the saturated fatty acids 14:0 and 16:0 and the rather short-branched FA and mono-unsaturated FA (MUFA) i16:0/15:1ω5c, 16:1ω7c, 18:1ω7c and 18:1ω9t/16:4ω1 and only one PUFA (22:5ω3) (Fig. 6). *N. hombergii* structural FA mainly consisted of 20 and 22 PUFA and 2 MUFA (17:1ω7c and 18:1ω9c). 20:5ω3 was the only PUFA explaining some of the temporal variation in the PL composition.

4. Discussion

Well-established biomarkers in food web studies are the short-branched bacterial FA (Boschker and Middelburg, 2002) and EPA and DHA as tracers of respectively diatoms and dinoflagellates (Dalsgaard et al., 2003). However, attributing PL to unique food sources is not straightforward and depends on the governing phytoplankton groups, hence on the ecosystem studied (Parrish et al., 2000). FA biomarkers such as 16:1ω7c are more difficult to interpret, since in our study area, they are present in both bacteria and diatoms (Hamm and Rousseau, 2003; Stoeck et al., 2002). In addition, 10Me16:0 is not an unambiguous marker for *Desulfobacter* either (Boschker et al., 2001), but its application in the investigated area is sustained by similar trends in other *Desulfobacter* markers cy-17:0 and cy-19:0 (Dowling et al., 1986). Similarly, 18-PUFA can either be attributed to *Phaeocystis* or dinoflagellates (Dalsgaard et al., 2003; Hamm and Rousseau, 2003). However, this marker was absent from the SPM samples at the time of the *Phaeocystis* bloom and contributed only 2% to the mixed bloom early May. In addition, all herbivores convert the 18-PUFA 18:3ω3 to EPA and DHA, which might occur immediately upon digestion (Budge et al., 2006). Therefore, in this study, we do not consider 18-PUFA to be a good marker for *Phaeocystis*, neither for algal identification nor for tracing food sources in animal diets. Instead, the *Phaeocystis* cell counts converged with the peak in 18:1ω9c and SFA 14:0, 16:0 and 18:0 (Claustre et al., 1990). The combination of these markers is thus a better tracer for *Phaeocystis* in the investigated area. The use of 18:1ω9c as a marker for *Phaeocystis* in the diet of consumers, however, is ambiguous, since it is the precursor of EPA and DHA in all animals (Dalsgaard et al., 2003) and the 18:1ω9/18:1ω7 ratio is indicative of carnivory (Falk-Petersen et al., 2000; Graeve et al., 1997, and other references in Dalsgaard et al., 2003). Finally, the markers 20:1ω9c and 22:1ω11c are very characteristic for predation of pelagic fish on calanid copepods (Dalsgaard et al., 2003), but also for benthic invertebrate carnivores and omnivores consuming zooplankton remains (Drazen et al., 2008; Kürten, 2010; Mansour et al., 2005; Würzberg et al., 2011). Below, we will discuss the characterisation and temporal variability in food sources and consumers taking these considerations into account.

4.1. Characterisation of pelagic and benthic food sources

Diatoms in the SPM started to increase slightly from February onwards and were reflected in high relative EPA and 16:1ω7c concentrations. A pronounced *Phaeocystis* peak followed between mid-April and mid-May, characterised by elevated relative abundances of 18:1ω9c and the SFA 14:0, 16:0 and 18:0. The *Phaeocystis* bloom was quickly succeeded by a mixed diatom and dinoflagellate bloom in the first half of May (high relative EPA, 16:1ω7c and DHA concentrations) and a long lasting presence of dinoflagellates from the end of May till mid September (high relative DHA concentrations). By the end of May, the *Phaeocystis* markers had declined and bacterial markers peaked in the suspended matter, possibly reflecting bacterial degradation of the algal matter (Becquevort et al., 1998). High numbers of dinoflagellates in late summer were mirrored in high relative DHA concentrations at the end of September. These trends are well in accordance with the general phytoplankton dynamics in the Belgian part of the North Sea (Rousseau et al., 2002).

Sediment PL content in early April was dominated by the *Phaeocystis* marker 18:1ω9c, accounting for about 12% of the total PL inventory at that time. This specific sediment peak coincided with the dominance of *Phaeocystis* in the suspended matter. This suggests that organic matter is constantly deposited on the sea floor during the *Phaeocystis* bloom (Lancelot et al., 2005). The main sediment peak in PL occurred 3 weeks later than the peak in the suspended

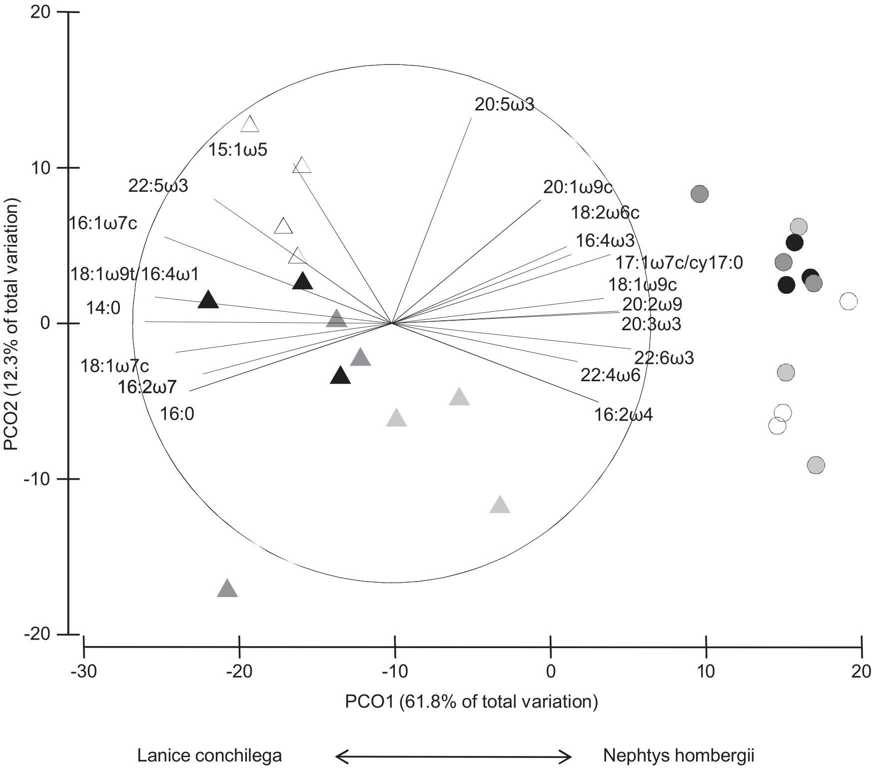

Fig. 6. PCO analysis of relative abundance of polar lipids in *Lanice conchilega* (Δ) and *Nephtys hombergii* (○) over the time course (April: □ – end of May: ■ – end of June: ■ – September: ■). Vectors represent specific biomarkers correlating > 70% with one of the first 2 PCO axes.

matter, which is in accordance with the time lag observed for the phytoplankton in water and sea floor (van Oevelen et al., 2009). This PL peak consisted mainly of EPA, DHA, 18-PUFA and SFA. Similar to the suspended matter pattern, sedimentary bacterial markers only started to increase from the end of June onwards. All biomarker proportions in the sediment were quite similar to those in the suspended matter, except for the bacterial markers, which contributed up to 39.2% of all sedimentary PL, while they comprised only 3.9–17.6% of total PL in the suspended matter of the water column. Heterotrophic marine bacteria are particularly abundant in sediments (Sargent and Whittle, 1981) partly as colonizers of settling particulate matter after a bloom (Becquevort et al., 1998).

The temporal trends observed in the isotopic composition of particulate organic carbon ($\delta^{13}C$) in SPM and sedimentary FA recorded in this study fit very well in the ranges observed for bulk $\delta^{13}C$ of SPM in the Belgian part of the North Sea (between -22.4 ± 0.1 and -21.5 ± 0.1‰ during the pre-bloom period, increasing to peak values of -19.7 ± 0.1‰ during the bloom and subsequently dropping below -24‰ and then finally rising again to -20.5 ± 0.3 in late summer and autumn (van Oevelen et al., 2009)). We refer to their work for interpretation of this temporal trend. The isotopic composition of the individual FA is somewhat more depleted than the bulk POM, which is related to an isotope fractionation of 3–5‰ relative to the total biomass occurring during lipid synthesis (Hayes, 2001). Taking this fractionation into account, we can deduce that the FA in suspended matter in this study originate from locally produced OM and not from another, allochthonous C source. The dip in the $\delta^{13}C$-value of *Phaeocystis* biomarkers and diatoms coincides with that of dinoflagellates and bacteria. However, the peaks show a temporal variation, in that it is earlier for the bacterial and dinoflagellate (22:6ω3) marker than for the *Phaeocystis* and diatom/bacterial (16:1ω7c) marker. For the latter, a variable contribution of diatom and bacterial sources to the stable isotope signature might explain this pattern: before and during the bloom, the signal rather reflects the signature of diatom EPA, while at the end of May, it mirrors that of bacterial FA biomarkers. This corresponds with the timing of the diatom and dinoflagellate bloom and subsequent bacterial degradation of the bloom (Becquevort et al., 1998).

4.2. Characterisation of the macrobenthic diet

Generally, the level of polar lipids in both macrobenthic species was very high as opposed to the neutral lipids, a feature that is somehow counterintuitive since most animals need energy reserves for their metabolism. Nevertheless, low NL levels have been reported for polychaetes before and were attributed to uninterrupted feeding (Drazen et al., 2008; Phleger et al., 2005; Würzberg et al., 2011). As the NL and PL fractions emphasise respectively the short and long-term diet, they are discussed separately.

4.2.1. Short term diet (NL)

4.2.1.1. Species specific diet. The diet of *L. conchilega* was characterised by markers of diatoms and bacteria and by elevated concentrations of the saturated fatty acids 14:0 and 16:0. SFA are ubiquitous in organisms (Volkman et al., 1998) and apart from their elevated concentrations in *Phaeocystis* (Claustre et al., 1990; Cotonnec et al., 2001), they are not very indicative of a specific phytoplankton group. As a suspension-deposit feeder, *L. conchilega* assembles its menu from available OM in the pelagic realm and the settled OM at the sediment surface. *L. conchilega* in the estuary of Baie des Veys (France) mainly feeds on fragmented macroalgae and microphytobenthos as inferred from stable isotopes of bulk samples (Lefebvre et al., 2009). The bacterial markers in the diet of *L. conchilega* in this study are an interesting addition to the characterisation of its diet, pointing to its flexibility to switch between food sources. Moreover, assimilation of bacterial FA markers in invertebrates has not been frequently reported (Kürten, 2010; Parrish et al., 2009; Soler-Membrives et al., 2011). This is remarkable, since deposit-feeders most likely ingest bacteria associated with OM.

In contrast to *L. conchilega*, the diet composition of *N. hombergii* inferred from fatty acid markers is more indicative of carnivory (higher DHA/EPA and 18:1ω9/18:1ω7 ratio's and increased relative abundances of invertebrate markers), and consumption of dinoflagellates (18-PUFA 18:5ω3 and DHA), which corroborates previous reports on its mixed predatory-omnivorous feeding habits (Caron et al., 2004; Schubert and Reise, 1986; van Oevelen et al., 2009). Overall, the reserves of *N. hombergii* contained a higher amount of PUFA, which makes the species less susceptible to environmental stress (Bell and Sargent, 2003; Kanazawa, 1997). The ambiguous diatom/bacterial biomarker 16:1ω7c was the only FA demonstrating different $\delta^{13}C$-values among species. The significantly more depleted $\delta^{13}C$-signature of 16:1ω7c in *L. conchilega* is consistent with the higher relative concentrations of this FA, indicating its enhanced storage in the tube-building polychaete.

Nevertheless, the applicability of FA biomarkers to assess the diet of higher trophic level organisms is constrained by the degree to which they alter their FA signature through *de novo* biosynthesis and breakdown (oxidation) of dietary FA (Caramujo et al., 2008). When a dietary FA is ingested by a consumer, it can be (1) incorporated directly and unmodified in the reserves of the dietary FA or (2) modified at some point between absorption in coelome and incorporation, by elongation or shortening of the C-chain or by (de)saturation (i.e. removal or addition of double bonds). Once incorporated, FA can be used in the organism's metabolism to produce energy catabolised to ATP, or PL can be used as precursors for hormones and secondary messengers (Budge et al., 2006). This is dependent on life history stages, environmental conditions and lipid storage reserves (Dalsgaard et al., 2003). The reserves therefore can also consist of endogenously derived FA resulting from *de novo* synthesis or conversion of FA (biosynthesis). Because of these modifications, a consumer FA profile never exactly mirrors the FA profile of its food source (Budge et al., 2006). Marine animals can convert 18-PUFA to 20-PUFA and subsequently to 22-PUFA only to a strictly limited rate (Pond et al., 2002). Therefore, most marine animal dietary fatty acids are dominated by EPA and DHA. Part of the elevated EPA and DHA concentrations in *L. conchilega* and *N. hombergii* can however be a result of biosynthesis, since herbivorous marine invertebrates can modify dietary 18:3ω3 to EPA and DHA at very slow rates (Dalsgaard et al., 2003). However, in contrast to other invertebrates such as crustaceans (see review of Caramujo et al., 2008; Dalsgaard et al., 2003), bivalves (e.g. Kawashima and Ohnishi, 2004) and echinoderms (e.g. Jeffreys et al., 2009), biosynthesis by polychaetes has not been frequently reported (Drazen et al., 2008; Jeffreys et al., 2009).

4.2.1.2. Seasonal variability. Although NL reflects the diet composition of primary consumers on the short-term, a time lag between pelagic and benthic food sources and appearance in macrobenthic reserves must be expected. Based on laboratory studies with polychaetes (Hentschel, 1998; van Oevelen et al., 2009) adopted a time lag of 2–-2.5 months to account for stable isotope equilibration between the time of food ingestion and food signal appearance in the tissue.

PUFA content fluctuated between 40 and 60% but overall showed a constant contribution to the NL fraction, which is necessary for macrobenthic reserves (Sargent and Whittle, 1981). Similar proportions of PUFA throughout the sampling period suggest that the animals are well able to sequester ω3 FA from their food (Parrish et al., 2009). The temporal shift in the diet of the two macrobenthic consumers was observed in decreasing dietary contributions of 18:1ω9c (*Phaeocystis*), general bacterial and *Desulfobacter*-specific markers, making space for increasing dietary contributions of EPA, DHA and 18-PUFA. This pattern is in accordance with the sequence in available food sources in the SPM and sediment.

Organisms with increased PUFA levels are of higher nutritional value for their consumers and are thus of importance for C transfer in aquatic sediments. As such, the nutritive value of *L. conchilega* and *Nephtys* spp. as prey organisms for juvenile flat fish (Beyst et al., 1999; Rabaut, 2009; Rijnsdorp and Vingerhoed, 2001) and sea birds (De Smet et al., submitted for publication; Godet et al., 2008.) increases from summer on (Fig. 2d,e).

Carbon from a food source is incorporated into the tissue of consumers such that the carbon isotopic ratio (^{13}C/^{12}C) generally reflects that of the diet. Similarly to patterns in FA concentrations, the isotopic composition of individual FA is controlled by the nature and the isotopic fractionation accompanying metabolism and biosynthesis in the animals. A slightly higher ratio in comparison to the food source as noted in both macrobenthic organisms has been attributed to a greater loss of light carbon during respiration, preference for ^{13}C rich compounds when food is assimilated, and/or mechanisms of enzyme-mediated biochemical reactions (Murphy and Abrajano, 1994). A constant signal with time as observed in dietary bacterial, invertebrate and dinoflagellate/*Phaeocystis* markers in both species might indicate independence from this food source or alternatively, non-selective feeding. A progressive depletion in compound-specific stable isotope signature indicates that 1) the according FA accumulated from the diet, *or* if the δ^{13}C-signature is even lower than that of the food source, 2) the FA is being biosynthesised from other macronutrients originating from the food source, such as proteins and carbohydrates (Ruess et al., 2005; Treignier et al., 2009). The sudden depletion of the δ^{13}C-signature in *L. conchilega* PUFA occurred at the same time as the post-bloom depletion of suspended matter PL (Fig. 3d,e). As such, the δ^{13}C-signature in *L. conchilega* at the end of June was close to the δ^{13}C-signature of the suspended matter in the water column at the end of May. While the δ^{13}C-signature of the suspended matter in the water column increased again after the single depleted point at the end of May, the δ^{13}C-signature of *L. conchilega* PUFA stayed depleted until September. Taking into account the equilibration period of about 2 months between macrobenthic assimilation of food sources with a certain δ^{13}C-signature and displaying this signature, the δ^{13}C-signatures of *L. conchilega* PUFA roughly mirror the δ^{13}C-signature of EPA and DHA in the suspended matter in the water column at the end of June. The distinction between increased accumulation and biosynthesis of suspended matter PUFA is therefore not easily made, which is one of the limitations of the CSIA method. The timing of this depletion however coincides with the time of spawning (Van Hoey, 2006) and since PUFA are known to be crucial in reproduction (Demott and Müller-Navarra, 1997; Wacker and Von Elert, 2003), it is probably related to energy storage for gametogenesis. Similar to *L. conchilega*, *N. hombergii* is also spawning in May and September (Olive et al., 1997). However, in *N. hombergii*, although higher PUFA concentrations were also observed in this species during summer, no such phytoplankton-bloom dependent depletion of the δ^{13}C-signature was observed. This suggests that this species is not dependent on the seasonal input of organic matter (Graeve et al., 1994). This is reflected in the more or less constant abundance of *N. hombergii* through time (Buchanan et al., 1974), in contrast with the extreme seasonal fluctuations in *L. conchilega* abundance (Van Hoey, 2006).

4.2.2. Long-term diet reflection (PL)

Polar lipids constitute the basic matrix of cellular membranes in which cholesterol, proteins and other elements are embedded. Most of them are PUFA (ω3 and ω6), ensuring membrane fluidity under different temperature and light regimes (Dalsgaard et al., 2003). PL concentrations remained constant through time, but their composition was subject to turnover because PUFA in animal polar lipids are essentially dietary in origin and can only in plants be formed *de novo* (Sargent and Whittle, 1981). The PL composition in the polychaetes *L. conchilega* and *N. hombergii* was variable with time in that the month of April clustered apart from early June (*L. conchilega*) and from the end of June and September (*N. hombergii*). This suggests that essential lipids derived from organic matter input of the spring phytoplankton bloom are incorporated or biosynthesised during summer. Although the PL of both species are even more distinct than their NL, tracing of the food sources is slightly more difficult because of this biosynthesis and metabolism of FA biomarkers. Several of these FA biomarkers are not present in the pelagic and benthic food sources: In *L. conchilega*, 22:5ω3 might be synthesised from EPA (20:5ω3) by chain elongation (Mansour et al., 2005), while in *N. hombergii*, 22:4ω6 possibly originates from 18-PUFA (18:2ω6 and 18:3ω3) via 20:4ω6 through chain elongation and 20:3ω3 via chain elongation and/or desaturation (Sargent and Whittle, 1981).

4.3. General conclusion

The FA biomarker and compound-specific stable isotope approach proved useful in the characterisation of seasonal variation of primary producer importance within water column and sediment as well as in the diet of the two model macrobenthic organisms. Phytoplankton dynamics in the water column and deposition in the sediment were roughly followed by group-specific FA biomarkers. The diet of *L. conchilega* was characterised by diatom and bacterial markers, while that of *N. hombergii* was dominated by invertebrate and dinoflagellate markers. Early in the year (April), both species apparently relied to some extent on *Desulfobacter* and sedimentary bacteria in general and *Phaeocystis*. After the bloom, an accumulation of ω3 FA was detected in both species, but most pronounced in *L. conchilega*, seemingly relying on suspended matter PUFA for energy storage during the period of gametogenesis. In contrast, bloom-dependent metabolism of FA biomarkers was not detected in *N. hombergii*, which might be explained by its reliance on invertebrates.

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