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Chlorophyll-*a* transformations associated with sinking diatoms during termination of a North Atlantic spring bloom

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ABSTRACT

A research cruise in the North Atlantic during the annual diatom bloom provided an ideal platform to study chlorophyll-*a* (chl-*a*) transformations associated with a large scale diatom bloom and export below the photic zone. On one deployment, Lagrangian sediment traps captured a significant flux of aggregated diatom cells produced during the termination of the main bloom. We examined the distribution of chl-*a* transformation products in sinking particles from the sediment traps and in suspended particles from the water column using high-resolution HPLC with multistage mass spectrometry (LC–MS^n). There was a dramatic change in the distribution of chl-*a* and its transformation products between the pre-sinking period, when the average chl-*a* concentration integrated over the upper 50 m was 68 ± 36 mg m^{-2}, and the post-sinking period, when it was 30 ± 11 mg m^{-2}. Before the diatom bloom left the euphotic zone (pre-sinking), suspended particles contained a considerably higher percentage of pheophorbide-*a* and other chl-*a* transformation products (27%) than during the post-sinking period (10%). Despite high levels of spatial variability in the chl-*a* concentration, and despite sampling from both within and outside a main bloom patch, the chl-*a* transformation products in suspended particles did not exhibit spatial variability. Sinking particles associated with the diatom bloom export had low POC:chl-*a* ratios (52–97), suggesting undegraded phytoplankton cells. However, the samples with especially low POC:chl-*a* ratios exhibited similar distributions of chl-*a* transformation products to those with a higher ratio. The proportions of demetalated and de-esterified transformation products increased with depth of suspended particles, although significant levels of these products were also found in the uppermost 20 m during the bloom. This suggests processes in both surface waters and through the water column led to the formation of these products.

1. Introduction

Spring phytoplankton blooms are a recurrent feature in the North Atlantic (Henson et al., 2009), and can lead to substantial downward export of phytoplankton biomass (Lampitt, 1985; Turner, 2002). The decline of phytoplankton blooms may be linked to zooplankton herbivory, bacterial infection, viral lysis, or nutrient limitation (Agusti et al., 1998; Bidle and Falkowski, 2004; Brussaard et al., 1995; Kirchman, 1999), which represent different possible fate processes for the phytoplankton biomass. Overall, however, our knowledge of phytoplankton bloom decline is limited, which hampers our understanding of the marine carbon cycle.

Different fate processes cause the break-down of chlorophyll-*a* (chl-*a*) into a range of different transformation products, the profile of which might yield insights into which fate processes dominate in a particular instance. Chl-*a* transformation products have hence been widely studied in laboratory culture experiments examining the effects of zooplankton grazing (Goericke et al., 2000; Harris et al., 1995a; Head and Harris, 1992; Kashiyama et al., 2012; Shuman and Lorenzen, 1975; Talbot et al., 1999), phytoplankton senescence (Bale et al., 2011; Franklin et al., 2012; Louda et al., 1998, 2002; Owens and Falkowski, 1982; Spooner et al., 1994), viral lysis (Bale et al., 2013; Llewellyn et al., 2007) and bacterial infection (Satoh and Hama, 2013; Spooner et al., 1994; Szymczak-Zyla et al., 2008). Chl-*a* transformation products have also been studied in the natural environment (Goericke et al., 1999; Hallegraeff, 1981; Head and Horne, 1993; King and Wakeham, 1996; Llewellyn et al., 2008; Walker and Keely, 2004).

These studies have shown that degraded biomass can be readily detected by the appearance of common degradation products such as pheophorbides. More specifically, sterol chlorin esters (SCEs) and

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carotenol chlorin esters (CCEs) have been associated only with zooplankton grazing in the natural environment (Chen et al., 2003a, 2003b; Goericke et al., 1999; King and Repeta, 1991), although a culture study showed that microbial degradation could yield minor amounts of SCEs (Szymczak-Zyla et al., 2008). Further, cyclic pheophorbide enols have been associated with protist herbivory (Kashiyama et al., 2012, 2013), as well as sulfide-mediated reactions in carbonate marls (Louda et al., 2000).

So far, however, other chl-*a* transformation products could not be linked specifically to individual fate processes. Rather, the particular transformation products produced appears to depend on the species of phytoplankton (Bale et al., 2011; Hallegraeff, 1981; Jeffrey and Hallegraeff, 1987; Louda et al., 1998, 2002; Owens and Falkowski, 1982), the physiochemical environment (Louda et al., 1998, 2002; Yacobi et al., 2001), and, in the case of grazing, on the species of grazer and on whether the grazing is primary or secondary (coprophagy) (Burkill et al., 1987; Gieskes et al., 1991; Goericke et al., 2000; Hallegraeff, 1981). Furthermore, North Atlantic spring diatom blooms are often thought to be terminated by silicic acid limitation (Henson et al., 2006; Leblanc et al., 2009; Savidge et al., 1995), with major export of phytodetrital aggregates taking place upon bloom termination (Billett et al., 1983). However, while many studies have attributed certain chl-*a* transformation products to terms such as 'cell stress', 'senescence' and 'death', transformation products associated with potentially nutrient-depleted phytodetrital aggregates have not been studied.

Here, we applied high-resolution HPLC with multistage mass spectrometry (LC–MS^n) to examine the distribution of chl-*a* transformation products in suspended and sinking particles during a cruise to study a North Atlantic spring diatom bloom. A major particle export event was observed upon bloom collapse and thoroughly characterized by independent methods, providing an ideal opportunity to examine the chl-*a* transformation which occur during such an event (Briggs et al., 2011; Martin et al., 2011; Rynearson et al., 2013). The objectives of this work were to gain a better understanding of chl-*a* transformation products in the marine environment during a field study and to examine whether specific chl-*a* transformation processes were associated with nutrient-limited diatom demise. We hypothesized that the distribution of chl-*a* and its transformation products would indicate spatial and temporal differences in phytoplankton fate processes and provide insights into the different processes that act on phytoplankton derived particles through the water column.

2. Methods

2.1. Cruise and study site

The 2008 North Atlantic Bloom (NAB08) project was designed to investigate a spring bloom from onset to collapse, and to track the fate of the phytoplankton biomass (Perry et al., 2012). A Lagrangian bio-optical float and four Seagliders were deployed before the main cruise, which was conducted aboard R/V Knorr in the region of 61° N, 26° W ca. 400 km southwest of Reykjavik, Iceland. The cruise track and the gliders followed the Lagrangian float, while also surveying adjacent waters.

2.2. Sample collection, extraction and analysis

Particles were collected from 5th to 21st May from Niskin bottles and from PELAGRA neutrally buoyant sediment traps (Fig. 1). We consider these samples to represent "suspended" and "sinking" particles, respectively. However, we note that "suspended" particles in bottle samples would include healthy phytoplankton cells and detrital particles, some of which would be sinking. Suspended particles were collected in 10-liter Niskin bottles mounted on a rosette equipped with a SeaBird CTD. Samples were kept cool and in the dark and filtration (2–6 L through 25 mm Whatman GF/F glass fiber filters) was started immediately following collection. Neutrally buoyant PELAGRA

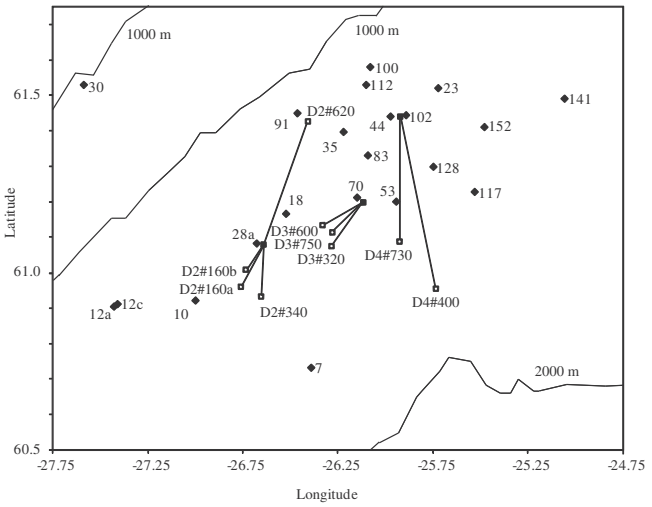

Fig. 1. Map of the study site, showing location of CTD casts (7–152, solid diamonds) and PELAGRA sediment traps (deployments: solid squares; recovery: open squares). For CTD and trap numbering system see Tables 2 and 3.
Adapted from figure in Martin et al., 2011

(Particle Export using a Lagrangian trap) sediment traps were used to collect sinking particles (Lampitt et al., 2008). Each trap can simultaneously collect four independent samples, and while the collection jars are usually filled with a formaldehyde preservative, one jar on each deployment was filled with unpoisoned seawater. Multiple PELAGRA traps were deployed on four occasions, with each trap collecting at a specific depth between 160 m and 750 m (Martin et al., 2011). Samples for pigment analysis were taken from three of the four deployments. For HPLC analysis, subsamples from unpoisoned jars were filtered onto GF/F filters (Whatman, 25 mm).

Filters of suspended and sinking particles were flash-frozen in liquid nitrogen and stored at a maximum temperature of −70 °C for a maximum period of 2 months until HPLC analysis either on board or in the laboratory. Samples were extracted in acetone (3 mL, 100%) by sonication (35–70 s, 4 W). After centrifugation (10 min, 3220 g, 4 °C), the supernatant was removed and re-centrifuged to remove fine particulates (4 min, 16,000 g, room temperature). In the case of the sinking particle samples the extraction procedure was repeated until the samples were colorless. Samples and extracts were kept on ice at all times during the extraction process. The extracts were concentrated using SPE cartridges (Phenomenex, 3 mL, 500 mg, C18) (Mantoura and Llewellyn, 1984). The SPE cartridges were first primed with methanol (2 mL), then Milli-Q water (2 mL) then acetone:water (50:50, 2 mL). The acetone extracts were combined with Milli-Q water (50:50) and passed through the SPE cartridges by syringe. The colored bands were eluted in the minimum volume of 100% acetone. For suspended particles this was between 0.2 mL and 1.1 mL and for sinking particles this was between 1.5 mL and 4 mL.

All pigment extracts were analyzed by high resolution pigment HPLC (Airs et al., 2001) on a 1100 series HPLC system (Agilent Technologies UK Ltd, Stockport, Cheshire, UK), comprising a quaternary pump (G1311A), an autosampler (G13291A) and a diode array detector (G1315B) at wavelengths 440 nm and 660 nm. Extracts were prepared for injection in the autosampler using an automated mixing program (90:10 sample:Milli-Q water) and then injected onto two reversed-phase mode Spherisorb ODS2 columns in series (3 μm, 150 mm × 4.6 mm i.d; total column length 300 mm) (Waters, Milford, MA, USA) in-line with a pre-column containing the same phase (10 mm × 5 mm i.d.). To prevent rapid deterioration of the pre-column, a Phenomenex (Macclesfield, UK) pre-column filter (Security Guard, ODS C18, 4 mm × 3 mm i.d) was used. For analysis of the sinking particle samples from the sediment traps, the resolution of early-eluting components was

Table 1
Assignment of components detected in pigment extracts, with UV/Vis absorbance bands and principle MS ions.

Component	Main UV/Vis bands (nm)	Assignment	$[M + H]^+$ (m/z)	Prominent fragment ions (m/z)	Esterifying alcohol
1	432, 666	Chlide-*a*	(615)		Methanol
2	456, 486	Chl-c_3	(653)		
3[1]	448, 584, 632	Chl-c_2 and chl-c_1	(609)		
4	412, 666	Phide-*a* methyl ester	607	547	Methanol
5	416, 668	Phide-*a* methyl ester′	(607)		Methanol
6	410, 666	Pyrophide-*a* methyl ester	549	521, 435	Methanol
7	414, 666	$C_{414,666}$			
8	414, 672	$C_{414,672}$			
9	426, 666	$C_{426,666}$			
10	464, 650	Chl-*b*	907	629, 597	
11	420, 658	$C_{420,658}$			
12	432, 664	13^2-hydroxy-chl-*a*	909	631, 553	Phytol
13	432, 664	Chl-*a*	893	615, 583, 555	Phytol
14	434, 668	Chl-*a*′	893	615, 583, 555	Phytol
15	408, 666	Phytin-*a*	871	593, 533	Phytol
16	412, 668	Pyrophytin-*a*	813	535	Phytol
17	410, 668	Pyrophide-*a* ester	915	535, 507	$C_{28:2}$ sterol

Values in brackets not seen in this study.

improved by adjusting the starting composition of the eluent from 5% to 15% ammonium acetate and from 80% to 70% methanol (Table S1). This resulted in a minor shift in retention.

Selected samples were further analyzed by APCI (atmospheric pressure chemical ionization) LC–MS^n on an Agilent Technologies 1200 Series system comprising a G1367B autosampler, a G1312A binary pump, a G1315B diode array detector and a 6330 ion trap equipped with an APCI source operated in positive ionization mode. Interface settings were: drying gas temperature 350 °C, vaporizer temperature 450 °C, nebulizer pressure 60 psi, and drying gas flow rate 5 L min^{-1}. The instrument was tuned by direct infusion of chl-*a* in acetone (0.01 mM). Selected extracts were methyl esterified by adding diazomethane solution in diethyl ether (approximately 1 mL) to the acetone extracts before they were reduced to dryness under a flow of nitrogen. The extracts were then re-dissolved in 100% acetone. The diazomethane was produced in a diazomethane generator (Sigma Aldrich Company Ltd) while cooled in an ice bath using diazogen (0.3 g) (Molekula Ltd, Shaftesbury, Dorset, UK) combined with diethyl ether (1 mL) and ethanol (1 mL). Potassium hydroxide solution (37% w/v) (Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK) was

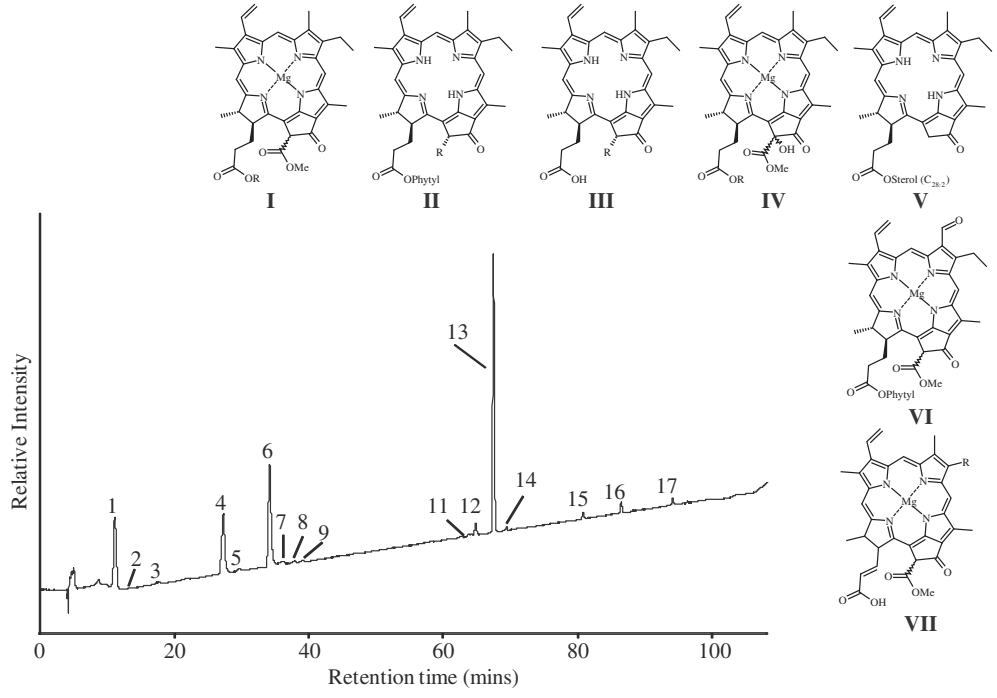

Fig. 2. Representative chromatogram (660 nm) of a sediment trap sample (D2#160a). For peak assignments see Table 1. Insert: chemical structures cited in text. **Ia** chlorophyll-*a* (R = phytyl, 13^2 (*R*) $COOCH_3$); **Ib** chlorophyll-*a* (R = phytyl, 13^2 (*S*) $COOCH_3$); **Ic** chlorophyllide-*a* (R = H, 13^2 (*R*) $COOCH_3$); **IIa** pheophytin-*a* (R = phytyl); **IIb** pyropheophytin-*a* (R = H); **IIIa** pheophorbide-*a* (R = COOCH3); **IIIb** pyropheophorbide-*a* (R = H); **IV** 13^2-hydroxy-chlorophyll-*a*; **V** pyropheophorbide-*a* $C_{28:2}$ sterol ester; **VI** chlorophyll-*b*; **VIIa** chlorophyll-*c1* (R = CH_2CH_3); **VIIb** chlorophyll-*c2* (R = $CHCH_2$).

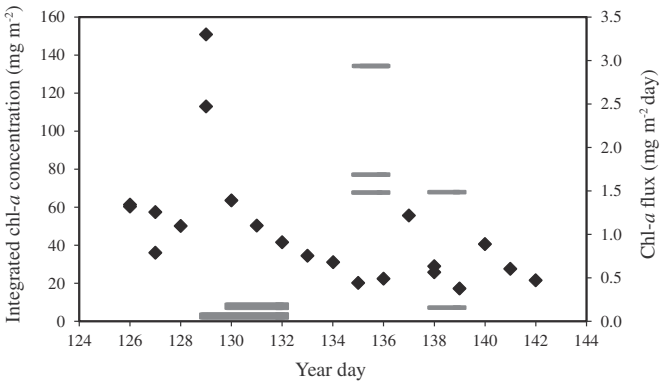

Fig. 3. Time series of chl-*a* in water column integrated over uppermost 50 m (mg m^{-2}; diamond symbols) and the flux of chl-*a* to the sediment traps (mg m^{-2} day^{-1}, gray lines). The thicker lines above days 129–132 and 130–132 denote the deployment of two sediment traps rather than one.

added drop-wise and a yellow solution of diazomethane was produced over a period of 2 h. The diazomethane was used immediately and any remaining product was destroyed by adding 0.1 M HCl.

A chl-*a* standard was obtained from DHI (Hørsholm, Denmark). pheophytin-*a* (phytin-*a*) was prepared by shaking a solution of chl-*a* (0.01 mM, 20 mL) (DHI, Denmark) in diethyl ether (20 mL) with 2 N HCl (5 mL, Sigma Aldrich Company Ltd). The ether layer was separated, washed five times with Milli-Q water and reduced to dryness under a flow of nitrogen. The dry phytin-*a* was re-dissolved in 90% acetone. The absorbance maxima of chl-*a* and phytin-*a* were measured at 660 nm using a spectrophotometer (CE9200 Super Aquarius, Cecil Instruments, Cambridge, UK). Components in the extracts were quantified by HPLC spectrophotometry using the following extinction coefficients (at 660 nm): chl-*a*: 67.55 L g^{-1} cm^{-1}; pheophytin-*a* (phytin-*a*): 40.79 L g^{-1} cm^{-1}; pheophorbide-*a* (phide-*a*): 59.15 L g^{-1} cm^{-1}; chl-*b*: 40.06 L g^{-1} cm^{-1}; chl-*c*: 38.08 L g^{-1} cm^{-1}. Compounds not listed were quantified using the extinction coefficient from the most similar structure (Table S2).

2.3. Detection and identification of chlorophylls and chl-a transformation products

The pigments detected in the extracts are given in Table 1 and structures are shown in Fig. 2. Alongside chl-*a*, a range of its Type I transformation products were detected. These include pheophytin-*a* (phytin-*a*), pheophorbide-*a* (phide-*a*), pyropheophorbide-*a* (pyrophide-*a*), the 13^2-hydroxy chl-*a* allomer (OH-chl-*a*), the C-13^2 diastereomer of chl-*a* (chl-*a*′), chlorophyllide-*a* (chlide-*a*), pyropheophytin-*a* (pyrophytin-*a*) and a sterol chlorin ester (SCE; pyrophide-*a* esterified to a C_{28} sterol with two double bonds). Four peaks 7, 8, 9, 11; (Fig. 2; Table 1) gave chlorin-type UV/Vis spectra, but LC/MS^n data could not be assigned. Given their UV/Vis properties and relative retention times, we assume that they are unknown chlorophyll derivatives and they were hence assigned as $C_{414,666}$, $C_{414,672}$, $C_{426,666}$ and $C_{420,658}$ respectively. For data handling, the percentages of OH-chl-*a*, $C_{414,666}$, $C_{414,672}$, $C_{426,666}$ and $C_{420,658}$ were combined as a single group with the addition of chl-*a*′ (which consistently contributed only ≤ 1.4%). This group was called "chl-*a* allomers and similarly eluting chlorins" (chl-*a* allo). Two diastereomers of phide-*a* were detected (components 5 and 6). Peak 6 was a very minor relative to 5, so the area of the two peaks were combined to give one value for 'phide-*a*'. The HPLC method we used to analyze the suspended particle extracts did not provide sufficient separation of the early-eluting chlide-*a* for it to be confidently quantified. For the sinking particle extracts, which were analyzed with the modified solvent gradient, we were able separate and quantitate chlide-*a*.

3. Results and discussion

3.1. Bloom dynamics, sampling periods and particle flux

The surface physical and biogeochemical setting is described in detail by Alkire et al. (2012), Briggs et al. (2011), Martin et al. (2011) and Mahadevan et al. (2012). Briefly, the onset of the bloom appears to have been triggered by eddy-induced stratification, with chl-*a* concentrations starting to rise around ten days prior to arrival of the R/V Knorr. Sampling for this chl-*a* transformation study occurred across an area which contained several bloom patches, with high levels of spatial variability in chl-*a* concentration (see satellite images in Martin et al. (2011)). The Lagrangian float located inside a discrete patch of elevated chl-*a*, referred to hereafter as the bloom patch. Chl-*a* and diatom cell abundance in the bloom patch peaked around year-day (YD) 129 (8 May), with integrated chl-*a* over the upper 50 m reaching 150 mg m^{-2} (Alkire et al., 2012; Rynearson et al., 2013). The bloom was heavily dominated by diatoms, which contributed 56–88% of the combined phyto- and microzooplankton carbons (Rynearson et al., 2013), but after YD 129 a sharp decline in chlorophyll-*a* (Fig. 3; (Alkire et al., 2012)) and a shift towards picoeukaryotes were observed (M. Sieracki, pers. comm.). The concentration of nitrate in the surface mixed layer decreased roughly from 11 to 8.5 μM (Alkire et al., 2012), while silicic acid decreased from 4 to <1 μM during the cruise (Martin et al., 2011).

Particle flux was low on the first two deployments, but all PELAGRA traps on the third deployment (YD 136) caught a large pulse of particles, with diatom resting spores contributing the majority of the carbon flux even in the deepest trap at 750 m. Fluxes during the final trap deployment were somewhat lower, with much lower fluxes of intact diatom cells, especially in the shallower trap (Martin et al., 2011; Rynearson et al., 2013). That a major export event started between YD 128 and YD133 was also indicated by depletion of ^{234}Th and the appearance of

Table 2
Deployment of PELAGRA sediment traps.
POC data from Martin et al. (2011).

	Deployment latitude	Longitude	Cup opening	Year day	Recovery latitude	Longitude	Cup opening	Year day	Depth	Flux of chl-*a* (mg m^{-2} day^{-1})	Flux of POC	Ratio POC/chl-*a*
D2#160a	61.08	−26.64	08/05/08 19:25	129	60.97	−26.75	11/05/08 19:25	132	160	0.04	10.3	234
D2#160b	61.08	−26.64	08/05/08 19:10	129	61.01	−26.73	11/05/08 19:10	132	160	0.08	13.3	170
D2#340	61.07	−26.65	09/05/08 18:55	130	60.94	−26.65	11/05/08 18:55	132	340	0.19	30.6	161
D2#620	61.07	−26.66	09/05/08 06:40	130	61.42	−26.41	11/05/08 18:40	132	620	0.15	27.2	177
D3#320	61.20	−26.12	14/05/08 17:40	135	61.14	−26.31	15/05/08 17:40	136	320	1.48	76.2	52
D3#600	61.20	−26.11	14/05/08 16:10	135	61.12	−26.27	15/05/08 16:10	136	600	1.69	164	97
D3#750	61.20	−26.11	14/05/08 15:55	135	61.08	−26.27	15/05/08 15:55	136	750	2.94	154	52
D4#400	61.44	−25.92	17/05/08 17:50	138	60.97	−25.74	18/05/08 17:50	139	400	0.16	120	769
D4#730	61.45	−25.93	17/05/08 17:05	138	61.10	−25.92	18/05/08 17:05	139	730	1.49	95.2	64

spikes in the gliders' optical sensors (Briggs et al., 2011; Martin et al., 2011). Resting cysts of *Chaetoceros* aff. *diadema* contributed the majority of particulate carbon flux during the first three trap deployments. The biomass caught in the traps was physiologically healthy (Rynearson et al., 2013), and consisted of a large volume of very fine particles, rather than discrete aggregates (Martin et al., 2011)

Fluxes of organic carbon, biominerals and estimated diatom carbon into the traps have already been published (Martin et al., 2011; Rynearson et al., 2013). The chl-*a* flux into the sediment traps was 0.04–0.2 mg m^{-2} day^{-1} during Deployment 2, 1.5–2.9 mg m^{-2} d^{-1} during Deployment 3, and 0.2 and 1.5 mg m^{-2} d^{-1} during Deployment 4 (Fig. 3, Table 2).

3.2. Spatial variability in chl-a transformation products

It is important to note that water column samples (suspended particles) analyzed in this study were collected from both inside and outside the bloom patch. The stations were geographically scattered (cf. Fig. 1) and the bloom exhibited 'patchiness' (Martin et al., 2011). An assessment, based on temperature and salinity of the water mass ((Briggs,

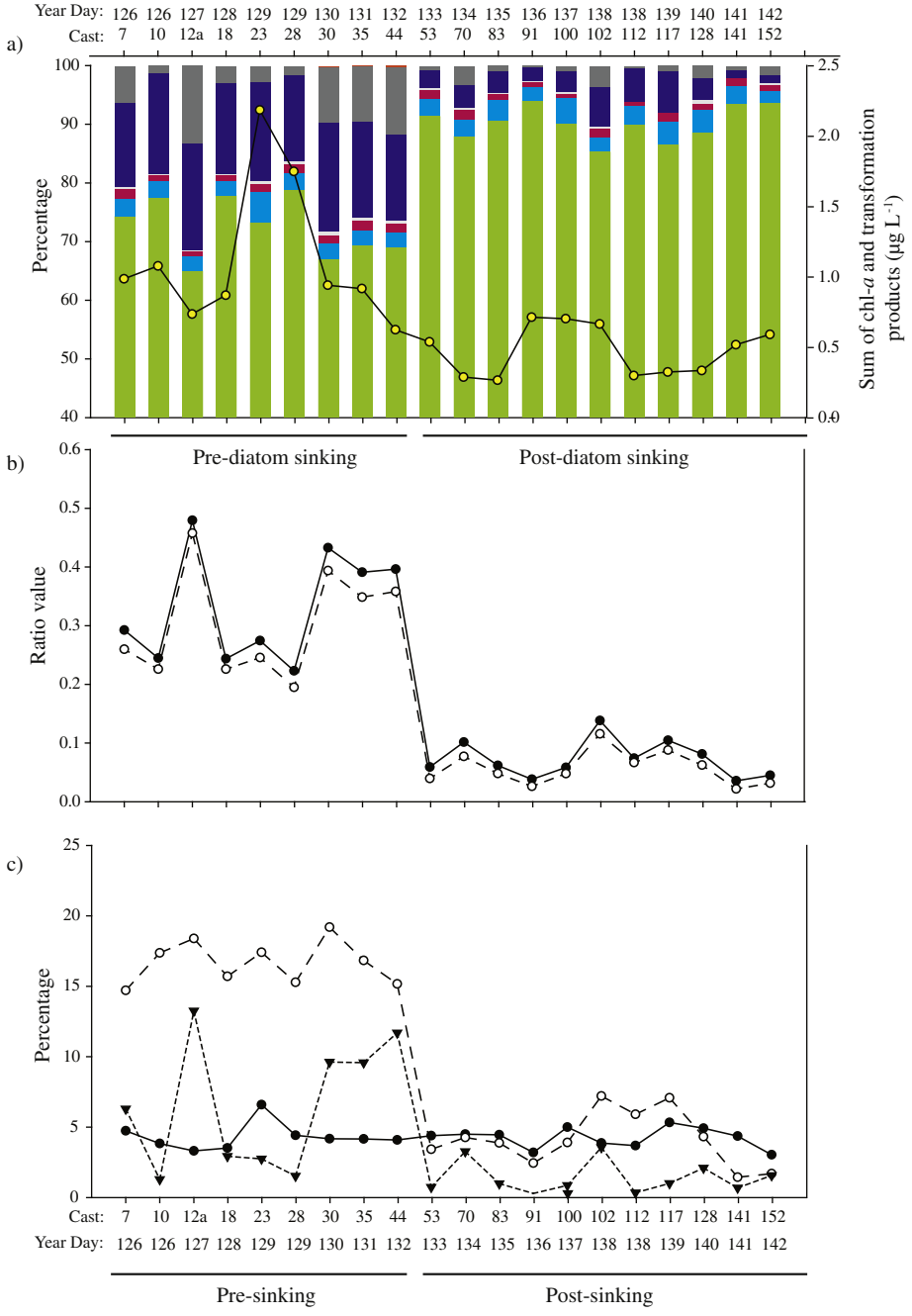

Fig. 4. Time series of water column samples from 20 m showing a) the percentage of chl-*a* and its transformation products (from bottom to top: chl-*a* in green, chl-*a* allo in light blue, phytin-*a* in pink, pyrophytin-*a* in light gray, phide-*a* in dark blue, pyrophide-*a* in dark gray and SCE in orange). Overlaid with their sum (µg L^{-1}; black line with yellow circle symbols). b) The ratio values of demetalated:metalated chlorins (filled symbols, solid line) and of de-esterified:esterified chlorins (open symbols, dashed line) and c) the percentage of products which have undergone one degree of transformation (1°trans, filled circles, solid line), two degrees of transformation (2°trans, open circles, dashed line) and three of more degrees of transformation (3+°trans, filled triangles, dotted line). Unaltered chl-*a* makes up the remainder.

2014); Table S3), determined that eight casts were from inside the patch. No relationship was found between samples being in or out of patch and their chl-*a* transformation products. Indeed, the only significant effect on the concentration and distribution of chl-*a* and its transformation products was the loss of diatoms from the euphotic zone.

3.3. Temporal changes in chl-a transformation products

During the onset of mass sinking, estimated to have started between YD 128 and YD 133 (Martin et al., 2011), there was a distinct change in the pigment distribution in the suspended particles, which at 20 m was most distinct between YD 132 and YD 133 (Fig. 4a; for the distribution at 20 m, for other depths see Table S4). For the purpose of this study we classed the casts from YD 126–132 as 'pre-sinking', as they exhibited higher average chl-*a* concentrations (0.82 ± 0.40 μg L^{-1}; Table 3) and a relatively consistent distribution of chl-*a* transformations products (Fig. 4a). The casts from YD 133–142 also gave a consistent chl-*a* transformation product distribution, distinct from the pre-sinking period (Fig. 4a), and a lower average chl-*a* concentration (0.41 ± 0.18 μg L^{-1}; Table 3), and are thus classed as 'post-sinking'. The difference in chl-*a* concentration at 20 m between the two periods was significant (*t*-test, P = 0.008). The difference between the two periods was particularly evident in terms of the average percentage of chl-*a* (Fig. 4a), which was 73 ± 5% in the pre-sinking casts and 90 ± 3% post-sinking (significantly different, *t*-test, P = <0.001). With this relative increase in chl-*a* there was a concomitant decrease in the percentage of phide-*a*, pyrophide-*a* and to a lesser degree pyrophytin-*a*. However it was phide-*a*, the dominant transformation product pre-sinking (av. 16 ± 1.6%), that decreased to the greatest extent post-sinking (av. 3.9 ± 2%) (significantly different, *t*-test, P = <0.001).

Phide-*a* is generally considered to be a biomarker for grazing (Cailliau et al., 1997; Hallegraeff, 1981; Llewellyn et al., 2008; Shuman and Lorenzen, 1975; Walker and Keely, 2004; Wright et al., 2010), hence our data indicate that a much higher proportion of the suspended particles collected from 20 m were associated with zooplankton grazing on phytoplankton pre-sinking than post-sinking. Unfortunately, grazing rates were not estimated during the cruise. However, certain zooplankton have been shown to feed selectively on diatoms, e.g. *Calanus helgolandicus* (Irigoien et al., 2000) and *Calanus finmarchicus* (Meyer-Harms et al., 1999). During the 1989 North Atlantic Bloom Experiment, zooplankton ingestion rates were highest when the phytoplankton community was dominated by diatoms, and decreased very rapidly with a shift to a more mixed phytoplankton community (Dam et al., 1993). It should, however, be noted that phide-*a* and even pyrophide-*a* can also derive from long-term cellular senescence (Louda et al., 1998, 2002). Furthermore, pyrophide-*a* has also been proposed as a more reliable marker than phide-*a* for grazing (Bianchi et al., 1998; Head and Harris, 1992; King and Repeta, 1991).

Common transformation products of chlorophyll can be categorized by the presence/absence of the central chelating magnesium and/or the presence or absence of phytol esterified at the $C13^2$ position. The ratio of demetalated (phide-*a*, pyrophide-*a*, phytin-*a*, pyrophytin-*a*, SCE) to metalated (chl-*a*, chl-*a*′, chl-*a* allo) chlorins, and the ratio of de-esterified (phide-*a*, pyrophide-*a*, SCE) to esterified (chl-*a*, chl-*a*′, chl-*a* allo, phytin-*a*, pyrophytin-*a*) chlorins were calculated and examined for the suspended particle samples from 20 m (Fig. 4b; see Table S3 for other depths). Both ratios were similar for all casts, both were on average higher (0.3 ± 0.09 and 0.3 ± 0.1 respectively) in the pre-sinking period than during the post-sinking period (0.06 ± 0.03 and 0.07 ± 0.03 respectively). The change in the demetalated:metalated chlorins ratio between the two periods was significant (*t*-test, P = <0.001) as was the change in the de-esterified:esterified chlorins ratio (*t*-test, P = <0.001). This indicates that the lower amount of chl-*a* transformation in the post-sinking period was not due specifically to less demetalation reactions or to less de-esterification reactions. In order to further examine whether the change in transformation product distribution could be assigned to specific reactions, the percentages of the transformation products were grouped by 'degree of transformation' according to the number of chemical modifications that had taken place (cf. (Satoh and Hama, 2013)); as one degree of transformation (1°trans) from chl-*a* (chl-*a*′, chl-*a* allo and phytin-*a*), two degrees of transformation (2°trans) from chl-*a* (phide-*a*, pyrophytin-; *a*) and three or more degrees of transformation (3 + °trans) from chl-*a* (pyrophide-*a*, SCE). Overall, in the 20 m samples, the percentage of 1°trans products did not exhibit a distinct change between the pre- and post-sinking periods, it was average 3.9 ± 1.6% pre-sinking and 4.3 ± 0.7% post-sinking (Fig. 4c; see Table S3 for other depths). The percentage of 2°trans products did exhibit a significant change between the pre- and post-sinking casts (*t*-test, P = <0.001). Pre-sinking it was the major group, on average 15 ± 5.5%, whereas after the sinking event it declined significantly (*t*-test, P = <0.005) to a level similar to the 1°trans products, on average 4.1 ± 1.9%. Similarly, the sum of the 3 + °trans products was higher in the pre-sinking casts, on average 5.9 ± 4.8% than post-sinking, when it was on average 1.4 ± 1.1%.

Table 3
Positions and sampling times of CTD casts.

Station	Latitude longitude	Date/time	Year day (YD)	Chl-*a* concentration at 20 m (ug L^{-1})	Integrated chl-*a* concentration over upper 50 m (mg m^{-2})	Pre- or post-sinking
7	60.73–26.39	05/05/08 05:15	126	0.73	61.3	Pre
10	60.92–27.00	05/05/08 16:45	126	0.83	60.3	Pre
12a	60.91–27.42	06/05/08 09:33	127	0.48	36.0	Pre
12c	60.91–27.41	06/05/08 13:10	127	–	57.4	Pre
18	61.17–26.52	07/05/08 08:45	128	0.68	50.1	Pre
23	61.52–25.72	08/05/08 05:02	129	1.60	150.9	Pre
28	61.07–26.66	08/05/08 22:02	129	1.38	112.9	Pre
30	61.53–27.59	09/05/08 05:34	130	0.63	63.50	Pre
35	61.40–26.22	10/05/08 16:41	131	0.64	50.5	Pre
44	61.44–25.98	11/05/08 16:35	132	0.43	41.5	Pre
53	61.20–25.94	12/05/08 19:33	133	0.49	34.5	Post
70	61.21–26.14	13/05/08 19:34	134	0.25	31.0	Post
83	61.33–26.09	14/05/08 20:52	135	0.24	20.2	Post
91	61.15–26.46	15/05/08 08:57	136	0.67	22.4	Post
100	61.58–26.08	16/05/08 11:10	137	0.63	55.6	Post
102	61.44–25.91	17/05/08 00:48	138	0.57	25.8	Post
112	61.53–26.10	17/05/08 17:46	138	0.27	28.9	Post
117	61.23–25.53	18/05/08 16:09	139	0.28	17.1	Post
128	61.30–25.75	19/05/08 11:40	140	0.30	40.6	Post
141	61.49–25.06	20/05/08 13:19	141	0.48	27.5	Post
152	61.41–25.48	21/05/08 11:41	142	0.55	21.5	Post

3.4. Chl-a transformation products with depth in the water column

Commonly, an increase in the proportion of demetalated and de-esterified transformation products is observed with increasing depth in the water column (Cuny et al., 2002; Walker and Keely, 2004). The ratio of demetalated to metalated chlorins and the ratio of de-esterified to esterified chlorins were calculated and examined through the water column. In two casts which represent the pre-sinking period, from YD 126 and 129, both ratios exhibited a local maximum at the surface, decreased with depth to 100 m, and then increased below 100 m (Fig. 5a, b). On YD 129, when the diatom bloom was at its peak, as judged by chl-*a* concentration (Table 3), both ratios were around 0.75 at 10 m, as compared to ratios maxima of around 0.4 at 3 m on YD 126. Post-sinking, both ratios had minimum values near the surface, and increased with depth (YD 135, Fig. 5c), or reached a peak at 200 m with lower values at 300 m (YD 140, Fig. 5d).

Transformation products were also classed by 'degree of transformation' as described above. On YD 126 and YD 129, products which had undergone 2°trans represented the highest percent (12–17%), especially near the surface (Fig. 5a, b). For both YD 126 and YD 129, the percent of 1°trans and 3+°trans were quite similar, although 3+°trans generally saw the greatest increase in percent with depth (Fig. 5a, b). By YD 135 and 140, post-sinking, 1°trans products represented the highest proportion in the uppermost 20 m (4–7%), followed by 2°trans products (3–5%), with 3+°trans products contributing only a minor proportion (1–2%; Fig. 5c, d). Below 20 m, 3+°trans products contributed the highest proportion and 1°trans products the lowest proportion. The proportion of 2°trans products did not change markedly with depth on either cast.

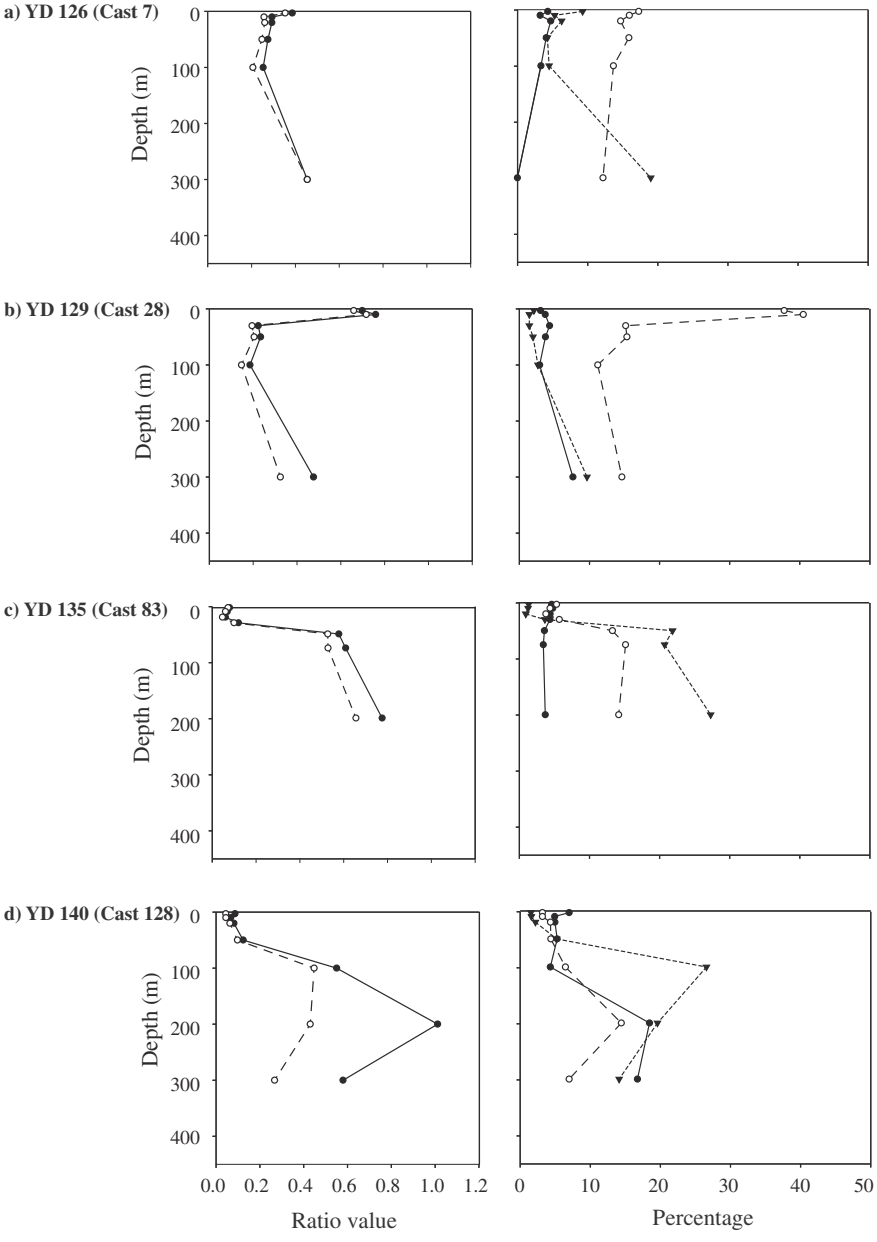

Fig. 5. Depth profiles of water column samples from a) YD 126 (Cast 7), b) YD 129 (Cast 28), c) YD 135 (Cast 83) and d) YD 140 (Cast 128) showing in the left hand column the ratio values of demetalated:metalated chlorin (filled symbols, solid line) and of de-esterified:esterified chlorins (open symbols, dashed line) and in the right hand column the percentage of products which have undergone one degree of transformation (1°trans, filled circles, solid line), two degrees of transformation (2°trans, open circles, dashed line) and three of more degrees of transformation (3+°trans, filled triangles, dotted line).

3.5. Sediment traps

The relative proportions of chl-*a* and its transformation products in the sinking particles was quite uniform in the D2 and D3 traps (Fig. 6a), despite the great difference in total chl-*a* and its transformation product flux. In samples from these two deployments chl-*a* contributed 31–39% to the sum of chl-*a* and its transformation products. The most abundant transformation products were pyrophide-*a* (19%–34%), phide-*a* (15%–24%) and chlide-*a* (6%–25%). Many diatoms have highly active chlorophyllase systems and, as such, will preferentially form chlide-*a* and then pyrophide-*a* (loss of Mg^{2+}) upon cellular disruption (grazing) and/or breakdown of intracellular compartmentalization during senescence (Jeffrey and Hallegraeff, 1987; Louda et al., 2011).

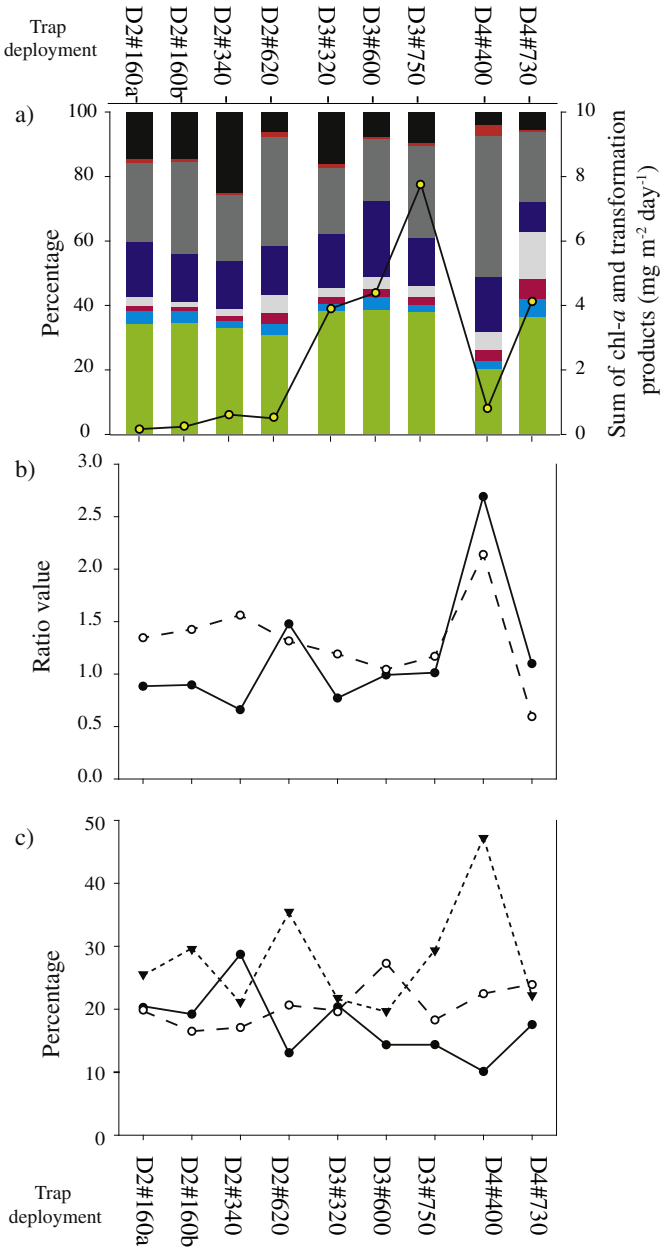

Fig. 6. Time series of samples from sediment traps showing a) the percentage of chl-*a* and its transformation products (from bottom to top: chl-*a* in green, chl-*a* allo in light blue, phytin-*a* in pink, pyrophytin-*a* in light gray, phide-*a* in dark blue, pyrophide-*a* in dark gray, SCE in orange and chlide-*a* in black). Overlaid with their sum (mg m^{-2} day^{-1}; black line with yellow circle symbols). b) The ratio values of demetalated:metalated chlorin (filled symbols, solid line) and of de-esterified:esterified chlorins (open symbols, dashed line) and c) the percentage of products which have undergone one degree of transformation (1°trans, filled circles, solid line), two degrees of transformation (2°trans, open circles, dashed line) and three of more degrees of transformation (3+°trans, filled triangles, dotted line). Unaltered chl-*a* makes up the remainder.

The POC:chl-*a* ratio for the D2 traps ranged between 161 and 234 (average 186 ± 33); while for the D3 traps it was much lower, between 52 and 97 (average 67 ± 26) (Table 2). In contrast, during D4, the 400 m trap contained a higher proportion of pyrophide-*a* (44%) than those before (at the expense of chl-*a*), while the 730 m trap contained a higher proportion of both phytin-*a* (6%) and pyrophytin-*a* (14%) than the earlier traps (Fig. 6a). The POC:chl-*a* ratio values for the D4 trap samples were highly disparate, 769 at 400 m yet only 64 at 730 m (Table 2). However, the percentage of chl-*a* did exhibit a significant negative correlation with the POC:chl-*a* ratio ($\rho = -0.82$, $P \leq 0.01$, $n = 9$).

The ratios of demetalated:metalated chlorins and of de-esterified:esterified chlorins were calculated as described earlier. However, for the sinking particles, chlide-*a* was included in the sum of metalated chlorins and in the sum of de-esterified chlorins (due to insufficient separation which prevented it being confidently quantified in suspended particle samples) and thus the ratios are not directly comparable between sinking and suspended particles. Both ratios were relatively constant in the sinking particles from the D2 and D3 traps, the ratio of demetalated:metalated chlorins ranged from 0.7 to 1.5, while the ratio of de-esterified:esterified chlorins ranged from 1.0 to 1.6 (Fig. 6b). Again, there were clear differences between the two D4 traps, sinking particles from D4#400 had very high ratios, both >2, while the ratios in D4#730 were low (that of de-esterified:esterified chlorins was less than half that in the other traps). Of the two ratios, the ratio of de-esterified:esterified chlorins exhibited a significant correlation with the POC:chl-*a* ratio ($\rho = 0.7$, $P \leq 0.05$, $n = 9$). This is interesting as the ratio of de-esterified:esterified chlorins did not exhibit a greater increase with depth in the water column suspended samples than the ratios of demetalated:metalated chlorins (e.g. Fig. 5).

To examine the transformation products in the sediment traps in terms of degree of transformation, chlide-*a* was added to the sum of 1°trans products, so the results of sinking and suspended particles are not directly comparable. The percentages of the 1°trans products, 2°trans products and 3+°trans were quite variable, but generally the 3+°trans products contributed a high percentage (20–47%), while the contribution of 2°trans products (16–27%) and the 1°trans products (10–29%) were similar (Fig. 6c).

Although the total particle flux differed greatly between different traps, the proportions of chl-*a* and its transformation products were quite uniform over time and depth, suggesting that the type of material and degree of degradation did not change strongly. However, the POC:chl-*a* ratios contradict this view, with the low ratios in the D3 traps suggesting less degraded material than in the D2 traps. Indeed, no live diatom cells aside from *Chaetoceros* cysts were found in the D2 traps, and those were estimated to contribute only 10–30% of total POC flux (Rynearson et al., 2013). In contrast, *Chaetoceros* cysts contributed 30–60% of total POC flux during D3, and were accompanied by substantial fluxes of vegetative diatom cells (Rynearson et al., 2013). In fact, chl-*a* did contribute a slightly higher percentage in the D3 than the D2 traps, while trap D4#400, which had a very high POC:chl-*a* ratio of 770, also contained the highest proportion of chl-*a* transformation products.

Overall, the trap material contained a much higher proportion of transformation products than the water column samples from 20 m, as would be expected for material that has experienced further degradation during sedimentation. The relatively high ratios of demetalated:metalated chlorins and of de-esterified:esterified chlorins in the trap material are also consistent with a greater degree of degradation relative to the suspended particles from 20 m, also given the particularly high values in trap D4#400.

3.6. Sterol chlorin esters

A sterol chlorin ester (SCE) was detected in three samples of suspended particles from 20 m (from YD 130–132), where it represented 0.2–0.3% of chl-*a* and its transformation products (Fig. 4a). The SCE was also present in six casts at 50 m at a percentage of 0.2–0.3% and in only two casts at 200 m at 0.3–0.5% (Table S4). A higher percentage of the SCE was detected in the sediment traps: 0.6–1.4% in D2, 0.6–1.1% in D3, and 0.7–3.1% in D4 (Fig. 6a). The SCE we detected (pyrophide-*a* esterified to a C_{28} sterol with two double bonds) was previously found experimentally be to be the most abundant SCE produced when diatoms were grazed by copepods (Harradine et al., 1996; Talbot et al., 1999). While the presence of SCE has been associated with microbial activity in culture, most significantly under anoxic conditions (Szymczak-Zyla et al., 2008, 2011; Szymczak-Zyla and Kowalewska, 2009), SCE in the water column can be assigned with more certainty as evidence of grazing than phide-*a* (Harris et al., 1995a; King and Repeta, 1991; King and Wakeham, 1996; Prowse and Maxwell, 1991; Riffe-Chalard et al., 2000; Squier et al., 2002; Talbot et al., 1999, 2000; Tani et al., 2009). In the suspended particles, the SCE was only ever present at very low abundances, even in the high phide-*a* samples. The sediment traps contained a higher percentage of SCE, although very little was detected at 200 m in the water column (Table S4). There has only been one previous report of SCEs in seawater samples, in suspended particles from the Gulf of Gdańsk (Baltic Sea) (Szymczak-Żyła and Kowalewska, 2007). It should be noted that we did not detect a number of other products associated with grazing, including carotenol chlorin esters (CCEs) (Goericke et al., 1999), chlone-*a* (Chen et al., 2003a, 2003b; Chillier et al., 1993; Goericke et al., 1999; Harris et al., 1995b; Louda et al., 2000; Ocampo, 1999; Sakata et al., 1990; Walker and Keely, 2004; Watanabe et al., 1993; Yamamoto et al., 1992) and 13^2,17^3-cyclo phide-*a* enol, which has been particularly associated with protist grazing (Kashiyama et al., 2012). The absence of 13^2,17^3-cyclo phide-*a* enol might be an artifact of our separation and detection method (Goericke et al., 2000), as extreme measures are required to avoid its oxidation (Kashiyama et al., 2012). However, the method we used can detect chlorophyllones (Airs et al., 2001), but none were found in our samples.

4. Conclusions

One of the central aims of this study was to examine whether the distribution of chl-*a* and its transformation products would reflect spatial and temporal differences in phytoplankton fate processes during the pre- and post-sinking of the diatom bloom. Indeed, in terms of temporal changes, the profile of chl-*a* transformation products in suspended particles changed sharply during the decline of the diatom bloom, with distinct signatures observed before and after a mass diatom sinking event. While it was not possible to associate specific chl-*a* transformation processes with diatom bloom demise due to nutrient limitation, degree of transformation of the pigments provided a clear signature of the two different periods studied. In terms of spatial variability, the chl-*a* transformation products in suspended particles did not reflect the high levels of heterogeneity in the chl-*a* concentration observed. This suggests that community composition is a more important factor in determining the distribution of chl-*a* and its transformation products than bloom intensity.

Changes in the transformation product distribution with depth in the water column was also expected to provide insights into the different processes that act on phytoplankton-derived particles as they sink through the water column. However, it was found that sinking particles caught in sediment traps were dominated by relatively fresh diatom-derived material that, despite having a higher ratio of transformation products to chl-*a*, exhibited a similar profile of transformation products to that found in suspended particles from 20 m. Furthermore, despite a wide range in the downward flux and a range in the material's freshness, as indicated by their POC:chl-*a* ratios, the distribution of chl-*a* transformation products was relatively consistent between traps. These findings point to the complexity of processes affecting the transformation of chlorophylls throughout the water column.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marchem.2015.03.005.

References

Agusti, S., Satta, M.P., Mura, M.P., Benavent, E., 1998. Dissolved esterase activity as a tracer of phytoplankton lysis: evidence of high phytoplankton lysis rates in the northwestern Mediterranean. Limnol. Oceanogr. 43, 1836–1849.

Airs, R.L., Atkinson, J.E., Keely, B.J., 2001. Development and application of a high resolution liquid chromatographic method for the analysis of complex pigment distributions. J. Chromatogr. A 917, 167–177. http://dx.doi.org/10.1016/S0021-9673(01)00663-X.

Alkire, M.B., D'Asaro, E., Lee, C., Perry, M.J., Gray, A., Cetinic, I., Briggs, N., Rehm, E., Kallin, E., Kaiser, J., Gonzalez-Posada, A., 2012. Estimates of net community production and export using high-resolution, Lagrangian measurements of O_2, NO_3^-, and POC through the evolution of a spring diatom bloom in the North Atlantic. Deep Sea Res. Part I 64, 157–174. http://dx.doi.org/10.1016/j.dsr.2012.01.012.

Bale, N.J., Airs, R.L., Llewellyn, C.A., 2011. Type I and Type II chlorophyll-a transformation products associated with algal senescence. Org. Geochem. 42, 451–464. http://dx.doi.org/10.1016/j.orggeochem.2011.03.016.

Bale, N.J., Airs, R.L., Kimmance, S.A., Llewellyn, C.A., 2013. NOTE Transformation of chlorophyll-a during viral infection of *Emiliania huxleyi*. Aquat. Microb. Ecol. 69, 205–210. http://dx.doi.org/10.3354/ame01640.

Bianchi, T.S., Bauer, J.E., Druffel, E.R.M., Lambert, C.D., 1998. Pyrophaeophorbide-a as a tracer of suspended particulate organic matter from the NE Pacific continental margin. Deep Sea Res. Part II 45, 715–731. http://dx.doi.org/10.1016/S0967-0645(97)00099-4.

Bidle, K.D., Falkowski, P.G., 2004. Cell death in planktonic, photosynthetic microorganisms. Nat. Rev. Microbiol. 2, 643–655. http://dx.doi.org/10.1038/nrmicro956.

Billett, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C., 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature 302, 520–522. http://dx.doi.org/10.1038/302520a0.

Briggs, N., 2014. Using temporal variability in optical measurements to quantify phytoplankton production, particle size, and aggregation during the North Atlantic spring bloom. (Ph.D.). University of Maine, School of Marine Sciences, Orono, Maine.

Briggs, N., Perry, M.J., Cetinic, I., Lee, C., D'Asaro, E., Gray, A.M., Rehm, E., 2011. High-resolution observations of aggregate flux during a sub-polar North Atlantic spring bloom. Deep Sea Res. Part I 58, 1031–1039. http://dx.doi.org/10.1016/j.dsr.2011.07.007.

Brussaard, C.P.D., Riegman, R., Noordeloos, A., Cadee, G., Witte, H., Kop, A., Nieuwland, G., Vanduyl, F., Bak, R., 1995. Effects of grazing, sedimentation and phytoplankton cell-lysis on the structure of a coastal pelagic food-web. Mar. Ecol. Prog. Ser. 123, 259–271. http://dx.doi.org/10.3354/meps123259.

Burkill, P.H., Mantoura, R.F.C., Llewellyn, C.A., Owens, N.J.P., 1987. Microzooplankton grazing and selectivity of phytoplankton in coastal waters. Mar. Biol. 93, 581–590. http://dx.doi.org/10.1007/BF00392796.

Cailliau, C., Claustre, H., Giannino, S., 1997. Chemotaxonomic analysis of phytoplankton distribution in the Indian sector of the Southern Ocean during late austral summer. Oceanol. Acta 20, 721–732.

Chen, N., Bianchi, T.S., Bland, J.M., 2003a. Novel decomposition products of chlorophyll-a in continental shelf (Louisiana shelf) sediments: formation and transformation of

carotenol chlorin esters. Geochim. Cosmochim. Acta 67, 2027–2042. http://dx.doi.org/10.1016/S0016-7037(02)01297-8.
Chen, N., Bianchi, T.S., Bland, J.M., 2003b. Implications for the role of pre- versus post-depositional transformation of chlorophyll-a in the Lower Mississippi River and Louisiana shelf. Mar. Chem. 81, 37–55. http://dx.doi.org/10.1016/S0304-4203(02)0138-X.
Chillier, X., Gulacar, F., Buchs, A., 1993. A novel sedimentary lacustrine chlorin — characterization and geochemical significance. Chemosphere 27, 2103–2110. http://dx.doi.org/10.1016/0045-6535(93)90121-K.
Cuny, P., Marty, J.-C., Chiavérini, J., Vescovali, I., Raphel, D., Rontani, J.-F., 2002. One-year seasonal survey of the chlorophyll photodegradation process in the northwestern Mediterranean Sea. Deep Sea Res. Part II 49, 1987–2005. http://dx.doi.org/10.1016/S0967-0645(02)00023-1.
Dam, H., Miller, C., Jonasdottir, S., 1993. The trophic role of mesozooplankton at 47°N, 20°W during the North-Atlantic bloom experiment. Deep Sea Res. Part II 40, 197–212. http://dx.doi.org/10.1016/0967-0645(93)90013-D.
Franklin, D.J., Airs, R.L., Fernandes, M., Bell, T.G., Bongaerts, R.J., Berges, J.A., Malin, G., 2012. Identification of senescence and death in *Emiliania huxleyi* and *Thalassiosira pseudonana*: cell staining, chlorophyll alterations, and dimethylsulfoniopropionate (DMSP) metabolism. Limnol. Oceanogr. 57, 305–317. http://dx.doi.org/10.4319/lo.2012.57.1.0305.
Gieskes, W.W.C., Engelkes, M.M., Grakaay, G.W., 1991. Degradation of diatom chlorophyll to colourless, non-fluorescing compounds during copepod grazing. Hydrobiol. Bull. 25, 65–72. http://dx.doi.org/10.1007/BF02259591.
Goericke, R., Shankle, A., Repeta, D.J., 1999. Novel carotenol chlorin esters in marine sediments and water column particulate matter. Geochim. Cosmochim. Acta 63, 2825–2834. http://dx.doi.org/10.1016/S0016-7037(99)00155-6.
Goericke, R., Strom, S.L., Bell, R.A., 2000. Distribution and sources of cyclic pheophorbides in the marine environment. Limnol. Oceanogr. 45, 200–211.
Hallegraeff, G., 1981. Seasonal study of phytoplankton pigments and species at a coastal station off Sydney — importance of diatoms and the nanoplankton. Mar. Biol. 61, 107–118. http://dx.doi.org/10.1007/BF00386650.
Harradine, P.J., Harris, P.G., Head, R.N., Harris, R.P., Maxwell, J.R., 1996. Steryl chlorin esters are formed by zooplankton herbivory. Geochim. Cosmochim. Acta 60, 2265–2270. http://dx.doi.org/10.1016/0016-7037(96)00132-9.
Harris, P.G., Eglinton, G., Carter, J.F., Maxwell, J.R., Head, R.N., Harris, R.P., 1995a. Identification of chlorophyll transformation products in zooplankton faecal pellets and marine sediment extracts by liquid chromatography/mass spectrometry atmospheric pressure chemical ionization. Rapid Commun. Mass Spectrom. 9, 1177–1183. http://dx.doi.org/10.1002/rcm.1290091218.
Harris, P.G., Pearce, G.E.S., Peakman, T.M., Maxwell, J.R., 1995b. A widespread and abundant chlorophyll transformation product in aquatic environments. Org. Geochem. 23, 183–187. http://dx.doi.org/10.1016/0146-6380(95)00006-Z.
Head, E., Harris, L., 1992. Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Mar. Ecol. Prog. Ser. 86, 229–238. http://dx.doi.org/10.3354/meps086229.
Head, E., Horne, E., 1993. Pigment transformation and vertical flux in an area of convergence in the North-Atlantic. Deep Sea Res. Part II 40, 329–346. http://dx.doi.org/10.1016/0967-0645(93)90020-N.
Henson, S.A., Sanders, R., Holeton, C., Allen, J.T., 2006. Timing of nutrient depletion, diatom dominance and a lower-boundary estimate of export production for Irminger Basin, North Atlantic. Mar. Ecol. Prog. Ser. 313, 73–84. http://dx.doi.org/10.3354/meps313073.
Henson, S.A., Dunne, J.P., Sarmiento, J.L., 2009. Decadal variability in North Atlantic phytoplankton blooms. J. Geophys. Res. 114, C04013. http://dx.doi.org/10.1029/2008JC005139.
Irigoien, X., Head, R.N., Harris, R.P., Cummings, D., Harbour, D., Meyer-Harms, B., 2000. Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel. Limnol. Oceanogr. 45, 44–54.
Jeffrey, S.W., Hallegraeff, G.M., 1987. Chlorophyllase distribution in 10 classes of phytoplankton — a problem for chlorophyll analysis. Mar. Ecol. Prog. Ser. 35, 293–304. http://dx.doi.org/10.3354/meps035293.
Kashiyama, Y., Yokoyama, A., Kinoshita, Y., Shoji, S., Miyashiya, H., Shiratori, T., Suga, H., Ishikawa, K., Ishikawa, A., Inouye, I., Ishida, K., Fujinuma, D., Aoki, K., Kobayashi, M., Nomoto, S., Mizoguchi, T., Tamiaki, H., 2012. Ubiquity and quantitative significance of detoxification catabolism of chlorophyll associated with protistan herbivory. Proc. Natl. Acad. Sci. U. S. A. 109, 17328–17335. http://dx.doi.org/10.1073/pnas.1207347109.
Kashiyama, Y., Yokoyama, A., Shiratori, T., Inouye, I., Kinoshita, Y., Mizoguchi, T., Tamiaki, H., 2013. 13^2,17^3-Cyclopheophorbide b enol as a catabolite of chlorophyll b in phycophagy by protists. FEBS Lett. 587, 2578–2583. http://dx.doi.org/10.1016/j.febslet.2013.06.036.
King, L.L., Repeta, D.J., 1991. Novel pyropheophorbide steryl esters in Black Sea sediments. Geochim. Cosmochim. Acta 55, 2067–2074. http://dx.doi.org/10.1016/0016-7037(91)90044-6.
King, L.L., Wakeham, S.G., 1996. Phorbin steryl ester formation by macrozooplankton in the Sargasso Sea. Org. Geochem. 24, 581–585. http://dx.doi.org/10.1016/0146-6380(96)00073-3.
Kirchman, D.L., 1999. Oceanography — phytoplankton death in the sea. Nature 398, 293–294. http://dx.doi.org/10.1038/18570.
Lampitt, R.S., 1985. Evidence for the seasonal deposition of detritus to the deep-sea floor and its subsequent resuspension. Deep Sea Res. Part I 32, 885–897. http://dx.doi.org/10.1016/0198-0149(85)90034-2.
Lampitt, R.S., Boorman, B., Brown, L., Lucas, M., Salter, I., Sanders, R., Saw, K., Seeyave, S., Thomalla, S.J., Turnewitsch, R., 2008. Particle export from the euphotic zone: estimates using a novel drifting sediment trap, ^{234}Th and new production. Deep Sea Res. Part I 55, 1484–1502. http://dx.doi.org/10.1016/j.dsr.2008.07.002.
Leblanc, K., Hare, C.E., Feng, Y., Berg, G.M., DiTullio, G.R., Neeley, A., Benner, I., Sprengel, C., Beck, A., Sanudo-Wilhemy, S.A., Passow, U., Klinck, K., Rowe, J.M., Wilhelm, S., Brown, C.W., Hutchins, D.A., 2009. Distribution of calcifying and silicifying phytoplankton in relation to environmental and biogeochemical parameters during the late stages of the 2005 North East Atlantic Spring Bloom. Biogeosciences 6, 2155–2179.
Llewellyn, C.A., Evans, C., Airs, R.L., Cook, I., Bale, N., Wilson, W.H., 2007. The response of carotenoids and chlorophylls during virus infection of *Emiliania huxleyi* (Prymnesiophyceae). J. Exp. Mar. Biol. Ecol. 344, 101–112. http://dx.doi.org/10.1016/j.jembe.2006.12.013.
Llewellyn, C.A., Tarran, G.A., Galliene, C.P., Cummings, D.G., De Menezes, A., Rees, A.P., Dixon, J.L., Widdicombe, C.E., Fileman, E.S., Wilson, W.H., 2008. Microbial dynamics during the decline of a spring diatom bloom in the Northeast Atlantic. J. Plankton Res. 30, 261–273. http://dx.doi.org/10.1093/plankt/fbm104.
Louda, J.W., Li, J., Liu, L., Winfree, M.N., Baker, E.W., 1998. Chlorophyll-a degradation during cellular senescence and death. Org. Geochem. 29, 1233–1251. http://dx.doi.org/10.1016/S0146-6380(98)00186-7.
Louda, J.W., Loitz, J.W., Rudnick, D.T., Baker, E.W., 2000. Early diagenetic alteration of chlorophyll-a and bacteriochlorophyll-a in a contemporaneous marl ecosystem; Florida Bay. Org. Geochem. 31, 1561–1580.
Louda, J.W., Liu, L., Baker, E.W., 2002. Senescence- and death-related alteration of chlorophylls and carotenoids in marine phytoplankton. Org. Geochem. 33, 1635–1653.
Louda, J.W., Mongkhonsri, P., Baker, E.W., 2011. Chlorophyll degradation during senescence and death-III: 3–10 yr experiments, implications for ETIO series generation. Org. Geochem. 42, 688–699.
Mahadevan, A., D'Asaro, E., Lee, C., Perry, M.J., 2012. Eddy-driven stratification initiates North Atlantic spring phytoplankton blooms. Science 337, 54–58. http://dx.doi.org/10.1126/science.1218740.
Mantoura, R.F.C., Llewellyn, C.A., 1984. Trace enrichment of marine algal pigments for use with HPLC-diode array spectroscopy. J. High Resolut. Chromatogr. 7, 632–635. http://dx.doi.org/10.1002/jhrc.1240071107.
Martin, P., Lampitt, R.S., Perry, M.J., Sanders, R., Lee, C., D'Asaro, E., 2011. Export and mesopelagic particle flux during a North Atlantic spring diatom bloom. Deep Sea Res. Part I 58, 338–349. http://dx.doi.org/10.1016/j.dsr.2011.01.006.
Meyer-Harms, B., Irigoien, X., Head, R., Harris, R., 1999. Selective feeding on natural phytoplankton by *Calanus finmarchicus* before, during, and after the 1997 spring bloom in the Norwegian Sea. Limnol. Oceanogr. 44, 154–165.
Ocampo, R., 1999. Structural determination of purpurin-18 (as methyl ester) from sedimentary organic matter. Org. Geochem. 30, 189–193.
Owens, T.G., Falkowski, P.G., 1982. Enzymatic degradation of chlorophyll a by marine phytoplankton in vitro. Phytochemistry 21, 979–984. http://dx.doi.org/10.1016/S0031-9422(00)82401-2.
Perry, M.J., D'Asaro, E., Lee, C., 2012. An autonomous study of the subpolar North Atlantic spring bloom — NAB08. Ocean Carbon Biogeochem. News 5, 1–5.
Prowse, W.G., Maxwell, J.R., 1991. High molecular weight chlorins in a lacustrine shale. Org. Geochem. 17, 877–886. http://dx.doi.org/10.1016/0146-6380(91)90029-J.
Riffe-Chalard, C., Verzegnassi, L., Gulacar, F.O., 2000. A new series of steryl chlorin esters: pheophorbide a steryl esters in an oxic surface sediment. Org. Geochem. 31, 1703–1712. http://dx.doi.org/10.1016/S0146-6380(00)00072-3.
Rynearson, T.A., Richardson, K., Lampitt, R.S., Sieracki, M.E., Poulton, A.J., Lyngsgaard, M.M., Perry, M.J., 2013. Major contribution of diatom resting spores to vertical flux in the sub-polar North Atlantic. Deep Sea Res. Part I 82, 60–71. http://dx.doi.org/10.1016/j.dsr.2013.07.013.
Sakata, K., Yamamoto, K., Ishikawa, H., Yagi, A., Etoh, H., Ina, K., 1990. Chlorophyllone-a, a new pheophorbide-a related compound isolated from *Ruditapes philippinarum* as an antioxidative compound. Tetrahedron Lett. 31, 1165–1168. http://dx.doi.org/10.1016/S0040-4039(00)88754-7.
Satoh, Y., Hama, T., 2013. Stepwise alteration from fluorescent to non-fluorescent chlorophyll derivatives during early diagenesis of phytoplankton in aquatic environments. J. Exp. Mar. Biol. Ecol. 449, 36–44. http://dx.doi.org/10.1016/j.jembe.2013.08.011.
Savidge, G., Boyd, P., Pomroy, A., Harbour, D., Joint, I., 1995. Phytoplankton production and biomass estimates in the northeast Atlantic Ocean, May–June 1990. Deep Sea Res. Part I 42, 599–617. http://dx.doi.org/10.1016/0967-0637(95)00016-Y.
Shuman, F., Lorenzen, C., 1975. Quantitative degradation of chlorophyll by a marine herbivore. Limnol. Oceanogr. 20, 580–586.
Spooner, N., Harvey, H., Pearce, G., Eckardt, C., Maxwell, J., 1994. Biological defunctionalisation of chlorophyll in the aquatic environment. 2. Action of endogenous algal enzymes and aerobic-bacteria. Org. Geochem. 22, 773–780. http://dx.doi.org/10.1016/0146-6380(94)90138-4.
Squier, A.H., Hodgson, D.A., Keely, B.J., 2002. Sedimentary pigments as markers for environmental change in an Antarctic lake. Org. Geochem. 33, 1655–1665. http://dx.doi.org/10.1016/S0146-6380(02)00177-8.
Szymczak-Zyla, M., Kowalewska, G., 2009. Chloropigments a in sediments of the Gulf of Gdansk deposited during the last 4000 years as indicators of eutrophication and climate change. Palaeogeogr. Palaeoclimatol. Palaeoecol. 284, 283–294. http://dx.doi.org/10.1016/j.palaeo.2009.10.007.
Szymczak-Żyła, M., Kowalewska, G., 2007. Chloropigments a in the Gulf of Gdańsk (Baltic Sea) as markers of the state of this environment. Mar. Pollut. Bull. 55, 512–528. http://dx.doi.org/10.1016/j.marpolbul.2007.09.013.
Szymczak-Zyla, M., Kowalewska, G., Louda, J.W., 2008. The influence of microorganisms on chlorophyll a degradation in the marine environment. Limnol. Oceanogr. 53, 851–862. http://dx.doi.org/10.4319/lo.2008.53.2.0851.
Szymczak-Zyla, M., Kowalewska, G., Louda, J.W., 2011. Chlorophyll-a and derivatives in recent sediments as indicators of productivity and depositional conditions. Mar. Chem. 125, 39–48. http://dx.doi.org/10.1016/j.marchem.2011.02.002.
Talbot, H.M., Head, R.N., Harris, R.P., Maxwell, J.R., 1999. Distribution and stability of steryl chlorin esters in copepod faecal pellets from diatom grazing. Org. Geochem. 30, 1163–1174. http://dx.doi.org/10.1016/S0146-6380(99)00095-9.

Talbot, H.M., Head, R.N., Harris, R.P., Maxwell, J.R., 2000. Discrimination against 4-methyl sterol uptake during steryl chlorin ester production by copepods. Org. Geochem. 31, 871–880. http://dx.doi.org/10.1016/S0146-6380(00)00057-7.

Tani, Y., Nara, F., Soma, Y., Soma, M., Itoh, N., Matsumoto, G.I., Tanaka, A., Kawai, T., 2009. Phytoplankton assemblage in the Plio-Pleistocene record of Lake Baikal as indicated by sedimentary steryl chlorin esters. Quat. Int. 205, 126–136. http://dx.doi.org/10.1016/j.quaint.2009.02.004.

Turner, J.T., 2002. Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquat. Microb. Ecol. 27, 57–102. http://dx.doi.org/10.3354/ame027057.

Walker, J.S., Keely, B.J., 2004. Distribution and significance of chlorophyll derivatives and oxidation products during the spring phytoplankton bloom in the Celtic Sea April 2002. Org. Geochem. 35, 1289–1298. http://dx.doi.org/10.1016/j.orggeochem.2004.06.017.

Watanabe, N., Yamamoto, K., Ihshikawa, H., Yagi, A., Sakata, K., Brinen, L.S., Clardy, J., 1993. New chlorophyll-a-related compounds isolated as antioxidants from marine bivalves. J. Nat. Prod. 56, 305–317. http://dx.doi.org/10.1021/np50093a001.

Wright, S.W., van den Enden, R.L., Pearce, I., Davidson, A.T., Scott, F.J., Westwood, K.J., 2010. Phytoplankton community structure and stocks in the Southern Ocean (30-80°E) determined by CHEMTAX analysis of HPLC pigment signatures. Deep Sea Res. Part II 57, 758–778. http://dx.doi.org/10.1016/j.dsr2.2009.06.015.

Yacobi, Y.Z., Ostrovsky, I., Talbot, H.M., 2001. Occurrence of high concentrations of a unique degradation product of chlorophyll-a in particles residing below the thermocline throughout a period of oxygen depletion in Lake Kinneret. J. Limnol. 60, 201–207. http://dx.doi.org/10.4081/jlimnol.2001.1.201.

Yamamoto, K., Sakata, K., Watanabe, N., Yagi, A., Brinen, L.S., Clardy, J., 1992. Chlorophyllonic acid and methyl ester, a new chlorophyll a related compound isolated as an antioxidant from short-necked clam, *Ruditapes philippinarum*. Tetrahedron Lett. 33, 2587–2588. http://dx.doi.org/10.1016/S0040-4039(00)92250-0.