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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

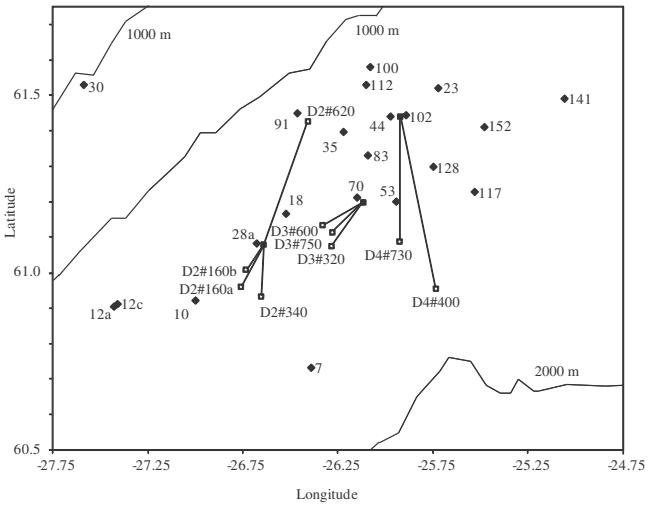

Fig. 1. Map of the study site, showing location of CTD casts (7–152, solid diamonds) and PELAGRA sediment traps (deployments: solid squares; recovery: open squares). For CTD and trap numbering system see Tables 2 and 3.
Adapted from figure in Martin et al., 2011

(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