Deep-Sea Research I 82 (2013) 60–71

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Major contribution of diatom resting spores to vertical flux in the sub-polar North Atlantic

T.A. Rynearson [a,*], K. Richardson [b], R.S. Lampitt [c], M.E. Sieracki [d], A.J. Poulton [c], M.M. Lyngsgaard [b], M.J. Perry [e]

[a] Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA
[b] Center for Macroecology, Evolution and Climate, University of Copenhagen, Copenhagen, Denmark
[c] Ocean Biogeochemistry and Ecosystems, National Oceanography Centre, Southampton, UK
[d] Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA
[e] Darling Marine Center, School of Marine Sciences, University of Maine, Walpole, ME, USA

ARTICLE INFO

Article history:
Received 14 January 2013
Received in revised form
19 July 2013
Accepted 29 July 2013
Available online 13 August 2013

Keywords:
Aggregates
Carbon flux
Chaetoceros
Diatom resting spores
Floating sediment traps
North Atlantic spring bloom

ABSTRACT

The mass sinking of phytoplankton cells following blooms is an important source of carbon to the ocean's interior, with some species contributing more to the flux of particulate organic carbon (POC) than others. During the 2008 North Atlantic Bloom Experiment in the Iceland Basin, we examined plankton community composition from surface waters and from sediment traps at depths down to 750 m. Samples collected with neutrally buoyant Lagrangian sediment traps captured a major flux event. Diatoms comprised $\geq$99% of cell flux into the sediment traps, with vegetative cells and resting spores of the genus *Chaetoceros* contributing 50–95% of cell flux. Resting spores of one species, identified as *Chaetoceros* aff. *diadema*, were dominant, comprising 35–92% of cell flux. The flux of resting spores ranged from 2 to 63 mg C m^{-2} day^{-1} and was significantly correlated with POC flux ($p=0.003$). Over the course of 10 days, the flux of resting spores increased by 26 fold, suggesting that the cells sank en masse, possibly in aggregates. In contrast, vegetative cells of *C.* aff. *diadema* sampled from surface waters during the period preceding the flux event generally comprised $<$1% of the diatom community and never exceeded 5.2%. Resting spores of *C.* aff. *diadema* were rarely observed in surface waters but their concentrations increased with depth (to 200 m) below the mixed layer. This increase in resting spore abundance, coupled with increased dissolved silicic acid concentrations at depth, suggest that the morphological changes associated with spore formation may have occurred in the mesopelagic zone, while cells were sinking. The values of variable fluorescence (F_v/F_m) measured on sediment trap material dominated by resting spores were among the highest values measured in the study area at any depth. This, in combination with the rapid germination of resting spores in ship-board incubations, suggests that vegetative cells were not physiologically stressed during spore formation. The degradation-resistant, heavily silicified resting spore valves explain the high relative contribution of *C.* aff. *diadema* resting spores to total plankton carbon at depth. These data emphasize the ephemeral nature of organic carbon flux events in the open ocean and highlight how non-dominant species and transient life stages can contribute more to carbon flux than their more abundant counterparts.

1. Introduction

The biological pump, defined as the process by which surface-generated particulate organic carbon (POC) sinks to depth, has a strong influence on global carbon cycling (e.g., Ducklow et al. (2001), Volk and Hoffert (1985)) and atmospheric CO_2 concentrations (e.g., Kohfeld et al. (2005), Takahashi et al. (2009)). Overall, the biological pump transfers 1–3% of oceanic primary production to the deep sea and sediments (De La Rocha and Passow, 2007; DeVries et al., 2012). The magnitude of POC that is successfully transferred from surface layers to the inner reaches of the ocean depends upon several factors, including the aggregation and disaggregation of particles and organisms, microbial remineralization, and grazing and fecal pellet production by zooplankton (reviewed in De La Rocha and Passow (2007), Ragueneau et al. (2006)). The biological pump is controlled, in part, by the taxonomic and mineral composition of sinking organisms (Legendre and Rivkin, 2002). For example, diatoms and their silica frustules are thought to contribute to pulses of phytodetritus that in some

* Corresponding author. Tel.: +1 401 874 6022.
E-mail address: rynearson@mail.uri.edu (T.A. Rynearson).

0967-0637/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dsr.2013.07.013

locations can deliver the equivalent of the annual average carbon flux to the benthos within days to weeks (Beaulieu, 2002).

Diatoms represent one of the most productive groups of photosynthetic organisms on earth, generating 30–40% of global marine primary productivity each year (Mann, 1999; Nelson et al., 1995). The relative dominance of diatoms in many surface phytoplankton communities is thought to play an important role in regulating the magnitude and efficiency of the biological pump (Buesseler, 1998; Goldman, 1993; Pondaven et al., 2000; Sarmiento, 2006). Even in oligotrophic regions where diatoms are not generally abundant, material of diatom origin frequently dominates POC recovered from depth (Karl et al., 2012; Scharek et al., 1999). A large part of diatom production is generated during blooms, i.e., periods of rapid growth and accumulation of cells, with the most conspicuous of these blooms occurring in temperate and sub-polar waters during spring (Longhurst, 1998). At the end of these blooms, diatoms can contribute to the biological pump by rapidly sinking out of the surface layer, at rates of 100–150 m day^{-1} (Billett et al., 1983), and arriving at the seafloor as relatively intact and sometimes viable cells (Billett et al., 1983; Cahoon et al., 1994; Smith et al., 1996).

Several mechanisms are believed to contribute to the initiation of diatom sinking during a bloom's demise, including nutrient limitation and formation of aggregates (reviewed in Smetacek (1985)). At the onset of nutrient limitation, diatoms undergo senescence and lose their ability to maintain neutral buoyancy (e.g., Waite et al. (1997)). Many bloom-forming diatoms then produce transparent exopolymer particles (TEP), which increase the probability that colliding cells or chains will stick together upon contact and form aggregates (Alldredge and Gotschalk, 1989; Kiørboe and Hansen, 1993; Passow and Alldredge, 1995). Although several phytoplankton taxa can produce TEP, it appears that only diatoms are capable of producing the large quantities of TEP that drive aggregation (Alldredge et al., 1993). High TEP production and aggregate formation tends to occur particularly when cells are nutrient limited and under conditions of intermediate turbulence (Alldredge and Gotschalk, 1989; Alldredge et al., 1993; Kiørboe and Hansen, 1993). Sinking POC also is susceptible to substantial bacterial degradation, hence both sinking rate and susceptibility to remineralization can dramatically influence the amount and quality of organic carbon that reaches mesopelagic waters and below (reviewed in De La Rocha and Passow (2007)).

Here, we determined the species composition of plankton cells recovered from both surface waters and neutrally buoyant sediment traps deployed in the sub-polar North Atlantic during a spring diatom bloom to examine how species composition and life history stage influence the magnitude and efficiency of POC flux. The physiological status of both bulk sediment trap material and individual cells was examined to better understand the nature and viability of sinking cells. We found that diatoms dominated the plankton community both in the sediment traps and surface waters, but that the relative abundances of different species varied greatly. Sediment traps were dominated by heavily-silicified resting spores that germinated rapidly when brought into culture indicating that the POC transported to depth was labile. Our results allowed us to identify that a subset of species from the surface phytoplankton community combined with life cycle stage (i.e., resting spores) significantly influenced the magnitude and efficiency of POC flux associated with the North Atlantic spring diatom bloom.

2. Methods

2.1. Sampling overview

All samples were collected during the North Atlantic Bloom Experiment cruise on the R/V Knorr, 2–20 May 2008, yearday (YD) 123–141. Shipboard samples were collected in the Iceland Basin (Fig. 1), in conjunction with a passively drifting, mixed-layer Lagrangian float (Alkire et al., 2012; Briggs et al., 2011; Mahadevan et al., 2012; Martin et al., 2011).

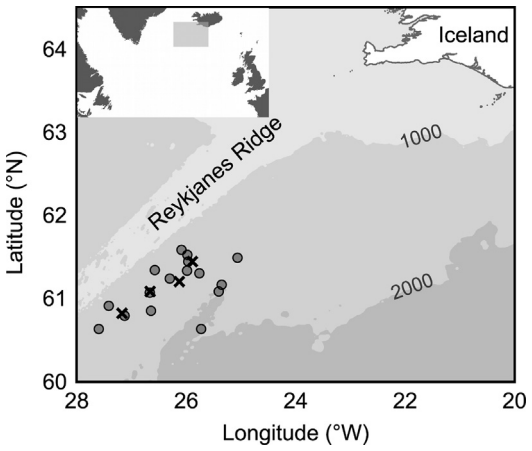

Fig. 1. Location of surface water samples (circles) and four PELAGRA floating sediment trap deployments (crosses). Shading indicates depth intervals in meters. Map of sampling locations is shown as a shaded box in the inset map of the North Atlantic.

Table 1
Date, depth and location of PELAGRA sediment trap deployments. Number of traps deployed on a given date varies; see Table 3.

Date (2008)	Yearday	Deployment	Depths (m)	Latitude (°N)	Longitude (°W)
5 May	126	1	140, 230	60.82	27.17
7 May	128	2	160, 340, 620	61.08	26.65
12 May	133	3	320, 600, 750	61.20	26.12
16 May	137	4	400, 730	61.44	25.88

2.2. PELAGRA sediment traps

Four deployments of neutrally buoyant, Lagrangian sediment traps (PELAGRA; Lampitt et al., 2008) were made between YD 126 and 137 (Fig. 1, Table 1). The traps collected sinking material at depths between 140 and 750 m and time periods of 15–72 h, for a total of 13 independent samples (depth and time). Each PELAGRA trap had four collection funnels (0.115 m^2 each) with attached collection cups. As described in Martin et al. (2011), traps were deployed in isopycnal mode and collection cups were programmed to open 24 h after deployment and to close minutes before the trap ascended to the surface. Collection times were deliberately varied to assess optimal deployment periods (Table 3). For each deployment, both preserved and live trap material was recovered. Live material was recovered from collection cups filled with seawater obtained from below 400 m depth. Preserved material was recovered from collection cups that additionally had a final concentration of 0.5% NaCl and 2% formaldehyde buffered with sodium tetraborate ($Na_2B_4O_7 \cdot 10\,H_20$). After recovery, 1 mL of 40% buffered formaldehyde was added to the contents of each preserved cup.

2.3. Phytoplankton taxa and carbon content in PELAGRA traps

Species composition and abundance for each PELAGRA deployment were determined using 1 mL subsamples from the preserved traps, a Sedgwick Rafter slide and quantitative light microscopy according to Utermöhl (1958). Species were identified following Sunesen et al. (2008) and Tomas (1997). Detailed size measurements

of *Chaetoceros* spp. resting spores for carbon content estimation were determined from 2 mL of preserved trap material from deployment 3. Samples were filtered onto 13 mm, 0.8 μm polycarbonate filters, rinsed with trace ammonia solution (pH ~10) and air dried. A small portion (0.25 cm^2) of the filter was cut from the center, mounted on an aluminum stub and sputter coated with ~2 nm gold. A Leo 1450VP SEM (Zeiss Inc.) with Smart SEM (V5.1) software automatically captured images of consecutive fields of view from a 15 × 15 grid at a magnification of 2500 ×, providing 225 images per sample. The valve diameter, mantle, epivalve and hypovalve heights were determined for >100 *Chaetoceros* spp. resting spores using ImageJ (⟨http://rsbweb.nih.gov.ij/⟩) after scale calibration using a 10 μm scale bar pre-set on each SEM image. Two resting spore shapes were identified (scalene and oblate spheroids) and their volume was determined appropriately. Because no specific carbon to volume (C:Vol) equation exists for the resting spore of the identified species, carbon content was determined two ways; (1) using the C:Vol relationship determined for vegetative diatoms in Menden-Deuer and Lessard (2000), and (2) using the C:Vol relationship calculated for resting spores of the diatom *Chaetoceros curvisetus* (Kuwata et al., 1993). The percentage of POC flux contributed by resting spores was calculated using total POC flux that was measured from the same deployments (Martin et al., 2011).

2.4. Viability of cells in PELAGRA traps

Live PELAGRA material was used to determine variable fluorescence (F_v/F_m), chlorophyll *a* fluorescence and resting spore viability. F_v/F_m, an indicator of maximal quantum efficiency of Photosystem II (Maxwell and Johnson, 2000), was measured on live trap material from the third and fourth PELAGRA deployments. The material was diluted to a chlorophyll *a* concentration of <10 μg L^{-1} with pre-filtered (Whatman GF/F) surface seawater. Three sub-samples were incubated for ≥30 min at approximately 6 °C in dark bottles covered with aluminum foil to prevent light exposure. F_v/F_m was measured within the dark chamber of a FAST^{tracka} II fluorometer (Chelsea Instruments Group Ltd.) mounted and secured in the lab, using FASTpro software (edition 2230-001-HB-A). A single turnover protocol with 30 sequences per acquisition, each including 100 saturation and 50 relaxation flashlets, was utilized. The sequence interval was set to 100 ms; the PMT eht (extra high tension) and LED light source (excitation peak of 470 nm) were optimized for each sample and varied from 380 to 540 and 60 to 90 V, respectively. F_v/F_m was calculated from saturation and relaxation phase fits following Kolber et al. (1998). The first F_v/F_m determination was made on trap material within several hours of retrieval. Samples were incubated at approximately 6 °C in the dark and F_v/F_m determined at regular intervals over the next 7 days (material from the third deployment) or after 48 h (fourth deployment). Differences in F_v/F_m between depths and deployments were tested using two-tailed *t* tests with equal variances, which were determined using *F*-tests (Zar, 1996). Live material from the third deployment was examined for chlorophyll auto-fluorescence with excitation of photosynthetic accessory pigments, 470–490 nm, using an Axioskop microscope (Zeiss Inc.) equipped for epifluorescence. Images were recorded with a SPOT camera (Diagnostic Instruments, Inc.).

To determine if resting spores in live trap material collected from the third deployment were viable, 3–400 μL aliquots of trap material from 300, 600 and 750 m were added to 1 mL volumes of sterile f/20 seawater media (Guillard, 1975) in 48 well plates immediately after trap recovery. Inoculated plates were incubated at ambient surface seawater temperature of 9 °C and 50 μmol photons m^{-2} s^{-1} on a 16:8 h L:D cycle. Cultures were checked microscopically using an Axioskop microscope (Zeiss Inc.) after 14, 41, 65 and 89 h; germination of resting spores was documented at each time point with a SPOT camera (Diagnostic Instruments, Inc.).

Table 2
Date, location and start time for CTD profiles from which water samples were collected for nutrient analyses and cell counts (cast number is the identifier used in the BCO–DMO database to identify CTD profiles).

Date (2008)	Yearday	Time	Cast no.	Latitude (°N)	Longitude (°W)
2 May	123	19:28	1	61.16	25.35
4 May	125	17:14	2	60.85	26.64
5 May	126	13:21	8	60.79	27.12
6 May	127	13:34	12	60.91	27.41
7 May	128	15:57	15	61.07	26.66
8 May	129	13:52	21	60.63	25.72
9 May	130	14:23	29	60.63	27.59
10 May	131	11:38	35	61.34	26.57
11 May	132	11:31	43	61.52	25.97
12 May	133	11:25	46	61.44	25.96
13 May	134	14:12	62	61.33	25.98
14 May	135	13:14	73	61.24	26.29
16 May	137	11:26	89	61.58	26.08
18 May	139	12:47	105	61.08	25.40
19 May	140	11:40	118	61.30	25.75
20 May	141	13:19	121	61.49	25.06

2.5. Surface water sampling

Water samples for phyto- and microzoo-plankton community composition and size as well as nutrient concentrations were determined from water samples collected once per day, typically at midday (Fig. 1, Table 2). Variable fluorescence (F_v/F_m) was also determined on discrete samples taken from 4 depths (5–60 m) at the stations where phyto- and microzoo-plankton samples were collected. Later analysis indicated, however, that despite a minimum 30 min dark treatment prior to measurements, the F_v/F_m determinations made on samples taken in daylight indicated an influence of previous light exposure. To compare surface and sediment trap F_v/F_m, we instead used measurements made on samples taken at 5 m from stations in the study area that were collected at night. F_v/F_m measurements on surface and sediment trap samples were made using the same protocol.

Complete hydrographic data are available from the Biological and Chemical Oceanography Data Management Office for the NAB 2008 project ⟨http://osprey.bcodmo.org/project.cfm?flag=view&id=102&sortby=project⟩. Water samples were collected with Niskin bottles mounted on a CTD-Rosette from 2 depths (usually 10 and 30 m) that were both within the surface mixed layer (50–100 m) (Briggs et al., 2011). Taxonomic composition of the dominant species was similar between depths, hence only data from 10 m is presented. On YD 131, samples for microplankton analysis were taken from 6 depths (5, 30, 80, 120, 200, and 300 m), the only deep profile taken for such analysis.

2.6. Phyto- and microzoo-plankton

Samples were preserved in 1 L brown glass bottles with acidified Lugol's solution (approximately 2% final concentration) for taxonomic determination. The composition of the plankton community was determined using quantitative light microscopy according to Utermöhl (1958). Analyses were carried out by Orbicon A/S (Århus, Denmark). Identification of *Chaetoceros* vegetative cells and spores was based on Jensen and Moestrup (1998) and Rines and Hargraves (1988). To determine cell biovolumes, the linear dimensions of cells were measured and biovolume calculated using the appropriate geometric volume formula. Carbon to volume relationships for either diatoms or for all other plankton

taxa (Menden-Deuer and Lessard, 2000) were used to determine microplankton carbon content.

2.7. Nutrient analyses

Water samples were collected for nutrient analyses between YD 123 and 141 (Table 2). Water from 10 m depth was collected directly from Niskin bottles into acid-cleaned 60 mL polyethylene bottles, pre-rinsed with three aliquots of sample, and frozen unfiltered immediately; samples were stored up to 6 months at −20 °C prior to analysis (Kallin et al., 2011). Samples were analyzed for silicic acid and nitrate plus nitrite (hereafter referred to as nitrate) using a Lachat QuickChem® 8000 Flow Injection Analysis System (Smith and Bogren, 2001; Wolters, 2002). Samples were slowly thawed in the dark at room temperature for 24 h and vigorously vortexed prior to analysis to avoid silica polymerization (Gordon et al., 1994). A depth profile (10–200 m) was collected on YD 131 and analyzed as described above.

2.8. Statistical analyses

Linear regression analysis was used to describe the relationship between resting spore carbon flux and POC flux in SPSS V. 20 (IBM, Inc). Statistical analyses of diatom community composition were conducted using Primer 6 (Clarke and Gorley, 2006) and an alpha of 0.05. Changes in sediment trap community composition were analyzed by examining the four dominant diatom groups in sediment traps: *C.* aff. *diadema* (vegetative cells and resting spores combined), all other *Chaetoceros* spp., *Thalassiosira* spp., and all other diatom species. These groups were compared to three levels of cell flux (<100, 100–400 and >400 cells 10^6 m^{-2} day^{-1}) into the sediment traps. Samples were standardized, transformed using a Bray–Curtis similarity matrix and compared using Analysis of Similarity (ANOSIM). Community composition of surface samples was compared in the same way except surface community compositions were split into two levels of biomass (>30 mg C m^{-3} and <30 mg C m^{-3}) and comparisons were made using class-level taxonomic divisions (e.g., Bacillariophyceae, Prasinophyceae). Comparisons of surface community composition with environmental variables (nitrate, silicic acid, surface water temperature and salinity) were conducted using BEST (Biology-Environment and Stepwise) analysis and class-level taxonomic divisions.

3. Results

3.1. Downward flux of phytoplankton taxa and POC

In contrast to most deep ocean and PELAGRA samples collected in the past, the majority of the material collected during this study was present as identifiable cells. Counts from preserved trap material revealed that diatoms numerically dominated the 13 PELAGRA samples. Other microplankton were present (e.g., foraminifera, dinoflagellates, ciliates) but comprised $<0.25\%$ of cells in the traps. The number of sinking diatoms collected by the PELAGRA cups peaked during the third deployment (retrieved on YD 136), and decreased thereafter (Fig. 2, Table 3). The most numerically dominant cells in the sediment trap material for all deployments were vegetative and resting spores of *Chaetoceros* spp., which together comprised an average of 81% of cells. Resting spores comprised between 35% and 92% of all phytoplankton cells in the traps, and $>49\%$ in 10 of the 13 samples (Table 3). The largest flux of *Chaetoceros* resting spores occurred on YD 136, when cell flux exceeded 400×10^6 cells m^{-2} day^{-1} at all depths. The number of vegetative cells of *Chaetoceros* was highly variable

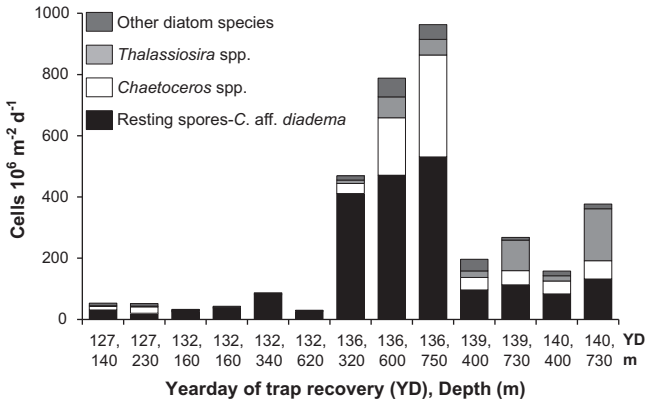

Fig. 2. Flux of diatom cells into each sediment trap. All species listed are vegetative cells except where noted. The category "other diatom species" includes all remaining diatoms including the genera *Thalassionema* and *Pseudo-nitzschia*. Recorded yearday (YD) is for closure of the trap cup; see Table 3.

in trap material, ranging from 2% to 40% of cells. Diatoms in the genus *Thalassiosira* comprised between 2% and 45% of cells in the trap, with a higher numerical dominance during the fourth deployment. All remaining diatom species contributed between 3% and 19% of trapped cells, and included the diatoms *Thalassionema* spp. and *Pseudo-nitzschia* spp.

The majority ($>95\%$) of *Chaetoceros* resting spores were identified as *Chaetoceros* aff. *diadema*. Although these resting spores were morphologically similar to *C. diadema*, there were important differences, including a lack of highly branched spines (Fig. 3). In some cases, resting spores were embedded inside vegetative cell frustules, which were also similar to *C. diadema* except for the foramen, which did not match the taxonomic descriptions of this species. Resting spores of other *Chaetoceros* species whose vegetative cells were abundant in surface waters (see below) were also observed in the traps, but were uncommon ($<5\%$ of total spores); the spores of these species (*C. compressus* and *C. laciniosus*) have very distinct resting spore morphologies (Tomas, 1997). Other dominant *Chaetoceros* species identified from the surface are not known to form spores (e.g., *C. decipiens;* (Tomas, 1997)).

Cell size measurements were used to compute cell carbon content for *C.* aff. *diadema* spores; however, two morphologies were observed in sediment trap material. One was circular in cross-section (Fig. 3A) with an average cell diameter of 8.4 ± 1.5 μm ($n=50$), and the other was elliptical in cross-section (Fig. 3B) with average dimensions of 14.7 ± 2.4 μm and 3.1 ± 0.3 μm ($n=50$). Aside from these differences, they appeared identical. The carbon contents of the two morphologies were 145.6 ± 0.5 and 90.7 ± 0.4 pg C cell^{-1}, respectively, when the C:Vol relationship of vegetative diatoms was used (Menden-Deuer and Lessard, 2000). Carbon content of the resting spores was substantively larger (1020 ± 4.4 and 568.6 ± 3.3 pg C cell^{-1}, respectively) when the C:Vol relationship determined for *C. curvisetus* resting spores was used (Kuwata et al., 1993). Because the two morphologies were only visible in SEM images and were not recorded independently in light microscope cell counts, we calculated carbon flux using a size-averaged estimate for the carbon content per resting spore. Using the equation for vegetative diatoms, the size-averaged C content per cell was 118 ± 0.3 pg C and the average flux contributed by resting spores was 2.4–62.7 mg C m^{-2} day^{-1} or 9–64% of the POC flux (Table 3). Using the C:Vol relationship for *C. curvisetus* resting spores, the size-averaged C content per cell was 794.3 ± 3.8 pg C. Using this relationship, POC flux contributed by resting spores was 16.4–424 mg C m^{-2} day^{-1} or 59–431% of the total measured POC flux.

Table 3
Summary of cell composition from PELAGRA floating sediment traps. Deployment number corresponds to that used in Martin et al. (2011). Cell fluxes of major diatom taxa are for vegetative cells, unless otherwise noted. Carbon fluxes of all *Chaetoceros* resting spores were generated using the C:Vol conversion of Menden-Deuer and Lessard (2000) and compared to total POC flux from Martin et al. (2011).

Deployment	Trap recovery (yearday)	Duration of trap collection (h)	Depth (m)	Total diatoms	*Chaetoceros* resting spores	*Chaetoceros* spp.	*Thalassiosira* spp.	*Thalassionema* spp.	*Pseudo-nitzschia* spp.	Other diatom spp.	Total POC flux[d]	*Chaetoceros* resting spore carbon flux	Resting spore carbon flux (%)
				Flux as cells $\times 10^6$ m^{-2} day^{-1}							mg C m^{-2} day^{-1}		
1	127	16	140	53.2	30.8	12.0	2.2	2.1	3.4	2.7	15.4 ± 0.8	3.6 ± 0.01	23.6
1	127	15	230	52.0	20.5	20.8	2.1	2.5	2.5	3.6	14.4 ± 0.7	2.4 ± 0.01	16.8
2[a]	132	72	160	32.6	25.2	2.7	1.6	1.8	1.1	0.2	10.2 ± 0.3	3.0 ± 0.01	29.2
2[a]	132	72	160	43.0	37.2	0.8	1.3	2.9	0.5	0.3	13.3 ± 0.7	4.4 ± 0.01	33
2	132	48	340	87.2	80.0	2.2	2.0	1.6	1.2	0.2	30.6 ± 1.7	9.5 ± 0.02	30.9
2	132	60	620	29.8	26.2	1.5	0.7	0.9	0.3	0.2	27.2 ± 1.8	3.1 ± 0.01	11.4
3	136	24	320	469.1	411.0	33.1	11.0	11.0	1.2	1.8	76.2 ± 6.1	48.6 ± 0.12	63.7
3	136	24	600	788.0	471.0	188.0	68.0	23.0	20.0	18.0	164 ± 4.1	55.6 ± 0.14	33.9
3	136	24	750	963.0	531.0	333.0	51.0	13.0	11.0	24.0	154 ± 4.8	62.7 ± 0.16	40.7
4[b]	139	24	400	196.3	96.3	41.0	21.0	10.0	14.0	14.0	120 ± 5.8	11.4 ± 0.03	9.5
4[b]	139	24	730	268.1	113.0	46.2	100.0	5.3	1.8	1.8	95.2 ± 4.6	13.3 ± 0.03	14.1
4[c]	140	24	400	157.9	83.3	42.2	17.0	8.0	4.0	3.4	112 ± 5.4	9.8 ± 0.02	8.8
4[c]	140	24	730	377.1	132.0	59.7	170.0	5.7	3.2	6.5	75.1 ± 3.7	15.6 ± 0.04	20.8

[a] Two traps deployed at same depth.
[b] First 24 h collection.
[c] Second 24 h collection.
[d] From Martin et al. (2011).

The largest percentage of POC flux generated by resting spores was observed during the third deployment. Across all deployments, POC flux was tightly correlated with resting spore flux ($p=0.003$, $r^2=0.58$) and less so with community composition in the sediment traps ($p=0.047$, $R=0.275$).

3.2. Viability of cells from PELAGRA traps

Live trap material from the final two deployments was examined shortly after trap recovery for photosynthetic capacity. The variance and mean of F_v/F_m measurements of shallow (320 m) vs. deep (600 and 750 m) samples were not significantly different (F-test, $p=0.7$; t-test, $p=0.9$) from the third deployment (Fig. 4A). The magnitude of F_v/F_m from live trap material was similar to the highest value recorded for surface waters during the cruise, i.e., 0.430 ± 0.018 ($n=2$) on YD 131 for discrete water samples collected at night from 5 m. F_v/F_m in the incubated trap material decreased similarly for all depths over the following days and dropped significantly after one week of incubation to 0.371 ± 0.006 for the 300 m sample (t-test, $p<0.001$) and to 0.373 ± 0.003 for the 750 m sample (t-test, $p<0.001$). From the fourth deployment, F_v/F_m measurements were 0.338 ± 0.074 (300 m) and 0.431 ± 0.012 (700 m). There was no significant difference in the mean or variance of F_v/F_m between depths for the fourth deployment (F-test, $p=0.21$; t-test, $p=0.22$). Under phase contrast microscopy, resting spores were clearly pigmented (Fig. 3D). Resting spores from the largest flux event (third deployment) had bright chlorophyll fluorescence under epifluorescence microscopy (Fig. 4B).

After 14 h of incubation under low irradiance at *in situ* surface water temperature, resting spores from all depths began to germinate by casting off the spiny resting spore valve (Fig. 5A–C). By 41 h, cells from all depths had cast off the smooth resting spore valve and undergone one cell division (Fig. 5D). Cell division continued on days 3 and 4, with formation of 4 and 8 cell chains, respectively (Fig. 5E and F). Frequently, resting spore valves were observed lying next to 4 and 8 cell chains, indicating that the vegetative cell chains originated from a single resting spore. After the initial 14 h incubation, growth rates of germinated resting spores were rapid, approximately 1 doubling day^{-1}.

3.3. Diatoms in surface waters

Surface community composition varied significantly as a function of carbon biomass ($p=0.002$, $R=0.59$). High levels of carbon (>30 mg C m^{-3}) were associated with diatoms and low levels of carbon (<30 mg C m^{-3}) with other phytoplankton, including prymnesiophytes, dinoflagellates and cryptophytes (Fig. 6). At stations sampled on YD 123–128 and 133, diatoms were particularly abundant and contributed the majority of organic carbon (56–88%) associated with the phyto- and microzoo-plankton communities in the upper 10 m of the water column. Total carbon concentrations of microplankton ranged from 16 to 78 mg C m^{-3}.

The same diatom genera identified as the most abundant in sediment traps were also observed in surface waters. *Pseudo-nitzschia* spp. comprised, on average, 28% of cell abundance in surface waters, in stark contrast to its average abundance of 2.6% in sediment traps (Tables 3 and 4). Average abundances of *Thalassionema* spp. and *Thalassiosira* spp. in surface waters (6%, 3% respectively) were more similar to their average abundance in sediment traps (4%, 11% respectively). *Chaetoceros* spp. represented >57% of diatom cells (Table 4) and 36–65% of diatom cell carbon in surface waters on YD 123–128 and 133. On average, *Chaetoceros* spp. represented 31% of cell abundance in surface waters, in contrast to its abundance in the sediment traps (81%). *Chaetoceros diadema* comprised an average of only 1% of diatom

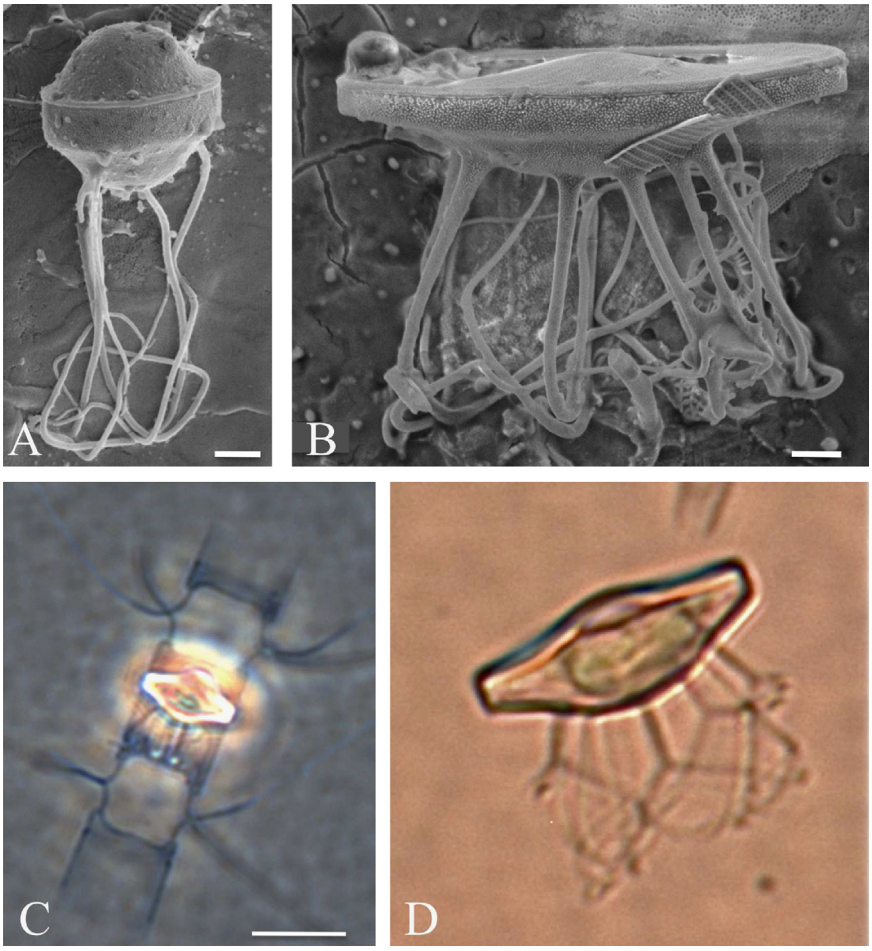

Fig. 3. Scanning electron micrograph images of the most abundant *Chaetoceros* aff. *diadema* resting spores representing two morphologies: (A) the circular cross-section and (B) the elliptical cross-section (both from sediment trap recovery on May 15 (YD 136), 600 m). Light micrographs of a *C.* aff. *diadema* resting spore: (C) inside a vegetative valve and (D) free of the vegetative valve (both from sediment trap recovery May 15 (YD 136), 750 m). Scale bars indicate 2 μm (A, B) and 15 μm (C).

cell abundance in surface waters and its maximum contribution at 10 m during the cruise was 5.2% of cell abundance. This is in stark contrast to the average contribution of resting spores of this species to the sediment traps (63%).

During the time period when *Chaetoceros* was most abundant in surface waters (YD 123–128, 133; Table 4), the most numerous *Chaetoceros* species identified were *C. laciniosus*, *C. compressus*, and *C. decipiens*. They represented, on average, 64% of all *Chaetoceros* cells. Many *Chaetoceros* cells in surface waters were small and lightly silicified and thus it was not possible to identify them from fixed samples; these unidentified cells comprised, on average, 32% of *Chaetoceros* in surface waters.

3.4. Depth distribution of *Chaetoceros aff. diadema*

Few resting spores of *C.* aff. *diadema* were recorded in the upper 30 m of the water column (maximal number of 4×10^3 L^{-1} was observed at 10 m on YD 127). On YD 131, samples were collected between 5 and 300 m on the only deep taxonomic profile. No resting spores were recorded at 5 m (Fig. 7A) and at 30 m, they constituted just 0.1 mg C m^{-3}. Between 80 and 300 m, resting spore carbon concentrations were an order of magnitude higher (between 1.1 and 2.2 mg C m^{-3}). The absolute amount of diatom cell carbon decreased with depth from 30 to 300 m (Fig. 7B); however, the relative contribution of diatom carbon from *C.* aff. *diadema* increased with depth. This species comprised on average $<2\%$ of the diatom carbon biomass at the surface, but comprised 59% and 82%, respectively, at 200 and 300 m.

3.5. Nutrient concentrations

Inorganic nitrogen concentrations were relatively high, remaining over 8 μM in surface waters throughout the cruise. In contrast, dissolved silicic acid concentrations were already low at the beginning of the sampling period (YD 123, <4 μM) and decreased to <0.3 μM by YD 133 (Fig. 8A). For surface waters, there were no significant correlations between changes in community composition and environmental conditions (nitrate, silicic acid, surface water temperature and salinity; $p=0.49$). Nutrients measured from the deep taxonomic profile on YD 131 increased with depth (Fig. 8b). The exception was at 30 m where there was a decrease relative to surface waters in both nitrate and silicic acid.

4. Discussion

In May of 2008, a major particle flux event was observed in the North Atlantic from optical 'spikes' in chlorophyll fluorescence, backscatter, and beam attenuation from the ship and four Seagliders (Briggs et al., 2011). During the flux event, the amount of POC recovered from floating sediment traps increased by over an order of magnitude (Martin et al., 2011). We determined that phytoplankton cells were responsible for generating the flux event and that their abundance in the sediment traps varied by over 30 fold during the two-week deployment period, highlighting the ephemeral nature of downward carbon flux during the North Atlantic spring bloom. Here, we show that a subset of species from the

surface phytoplankton community and a transient life cycle stage that may be highly resistant to degradation (i.e., resting spores) can dominate vertical flux, influencing the magnitude and efficiency of POC flux associated with the North Atlantic spring bloom.

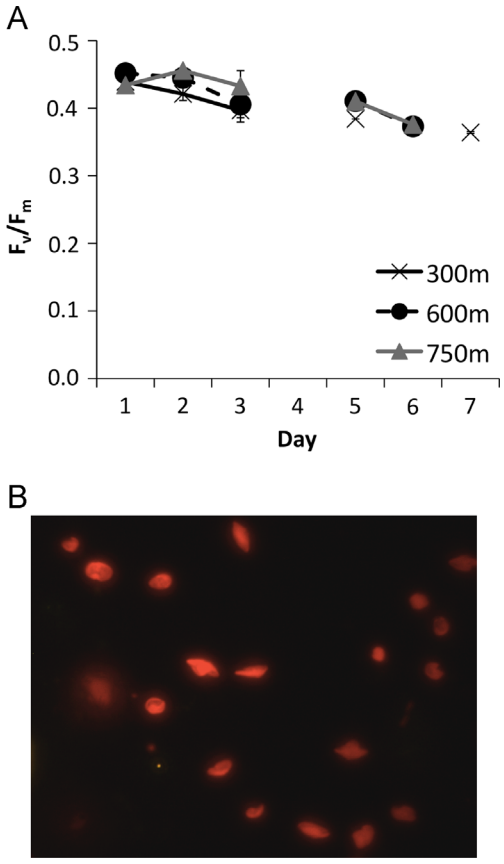

Fig. 4. Photosynthetic capacity of sediment trap material and resting spores from the third sediment trap recovery, May 15 (YD 136). (A) Variable fluorescence (F_v/F_m) of trap material collected from 300, 600 and 750 m and measured between 1 and 7 days after recovery. (B) Chlorophyll *a* autofluorescence of resting spores collected from 750 m; excitation at 470–490 nm.

4.1. *Chaetoceros resting spores dominate plankton flux and POC in the traps*

During the peak POC flux, collected by the third sediment trap deployment (recovered YD 136), *Chaetoceros* resting spores represented 55–88% of all diatom cells (Table 3). Depending on the C:Vol conversion used, resting spores represented up to 64% or 431% of the total POC flux. The C:Vol relationship based on vegetative

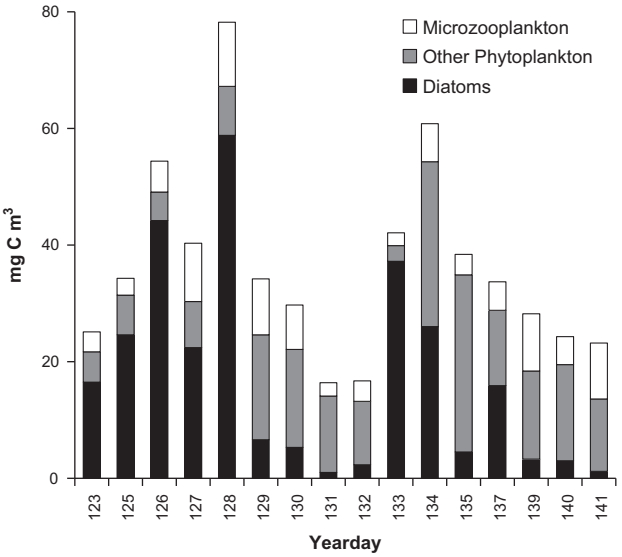

Fig. 6. Microplankton carbon from 10 m, including all diatoms, all other phytoplankton larger than 2 μm (including prymnesiophytes, dinoflagellates and cryptophytes) and all microzooplankton (including ciliates, heterotrophic dinoflagellates and choanoflagellates).

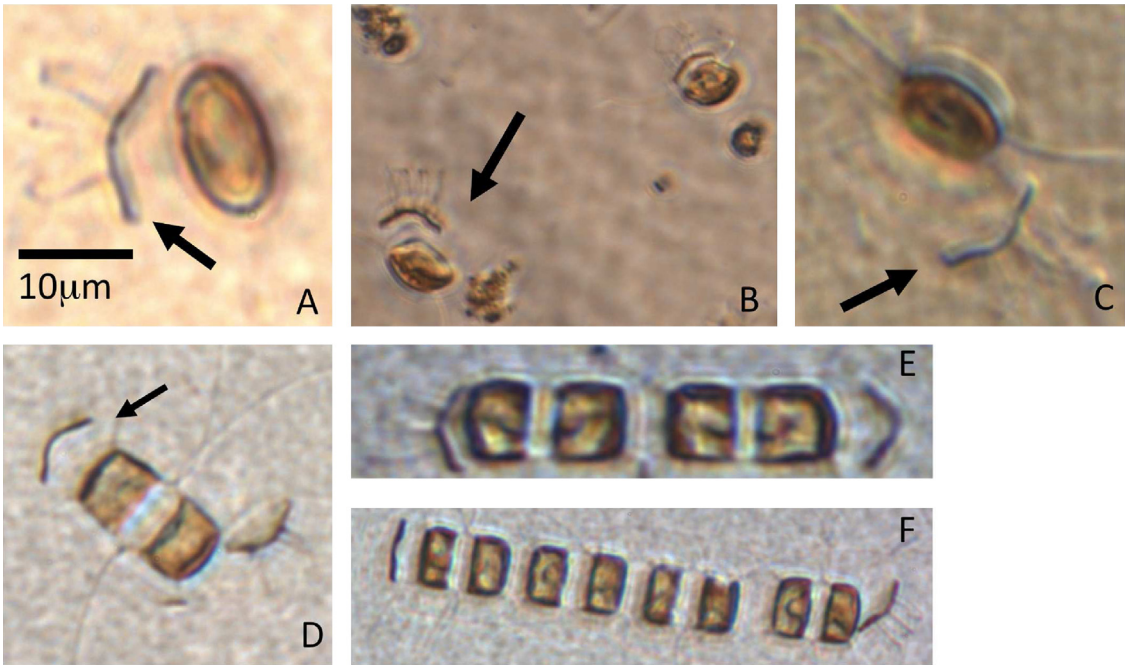

Fig. 5. Germination of resting spores from the third sediment trap recovery on May 15 (YD136). After 14 h incubation, spiny resting spore valves (indicated by arrows) were cast off in trap material from 300 m (A), 600 m (B) and 750 m (C). By 41 h (D), the smooth resting spore valve (denoted by arrow) had been cast off and one cell division had occurred; example cell from 600 m. Cell division after 65 h (E, 4-cell chain) and 89 h (F, 8-cell chain) of incubation for trap material from 750 m.

Table 4
Abundance of diatom cells at 10 m and percentage of cells belonging to each genus included in Table 3. Percent *Chaetoceros* includes *C.* aff. *diadema*.

Year-day	Diatom abundance (10^3 cells L^{-1})	*Chaetoceros* (%)	*C.* aff. *diadema* (%)	*Thalassiosira* (%)	*Thalassionema* (%)	*Pseudo-nitzschia* (%)
123	310	75	0.3	0.6	2.9	16
125	460	69	0.8	0.0	8.5	17
126	540	75	2.6	3.4	2.9	17
127	425	57	3.7	1.3	3.4	24
128	1126	71	1.0	0.1	5.3	17
129	276	17	4.7	0	4.2	17
130	164	2	0.6	4.0	0.8	58
131	82	11	1.6	0	6.3	20
132	19	6	5.2	0	10.5	18
133	598	63	0.2	0	13.9	20
134	441	35	0.2	0.9	14.4	42
135	171	2	0	0	4.6	70
137	40	0.5	0	6.4	0	0
139	49	22	0.8	2.7	8.0	53
140	22	0	0	23.6	0	36
141	31	0.3	0	0.0	6.2	27

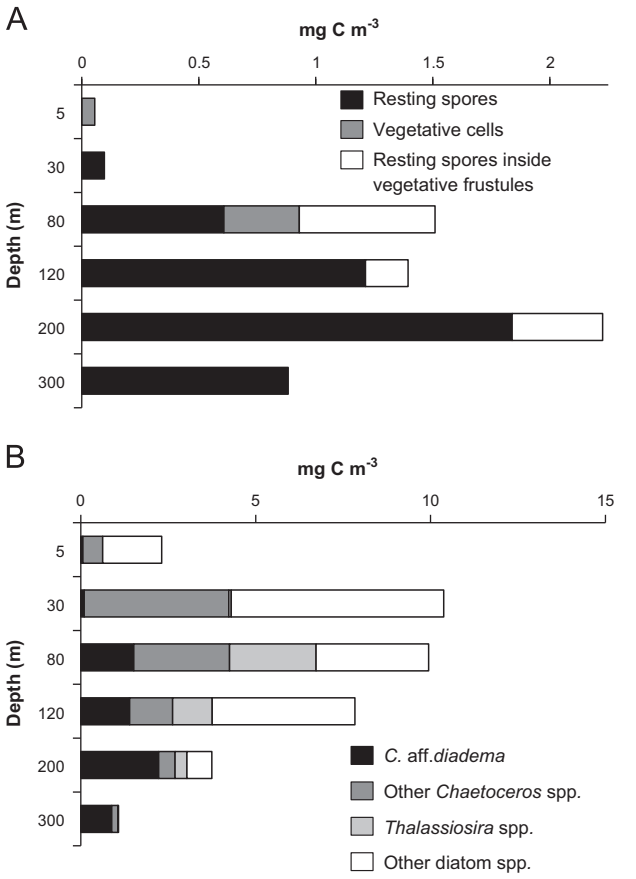

Fig. 7. Depth profile (5–300 m) on May 10 (YD 131). (A) carbon concentration of *C.* aff. *diadema* resting spores, vegetative cells, and resting spores found inside of vegetative frustules, and (B) carbon concentration of all diatoms.

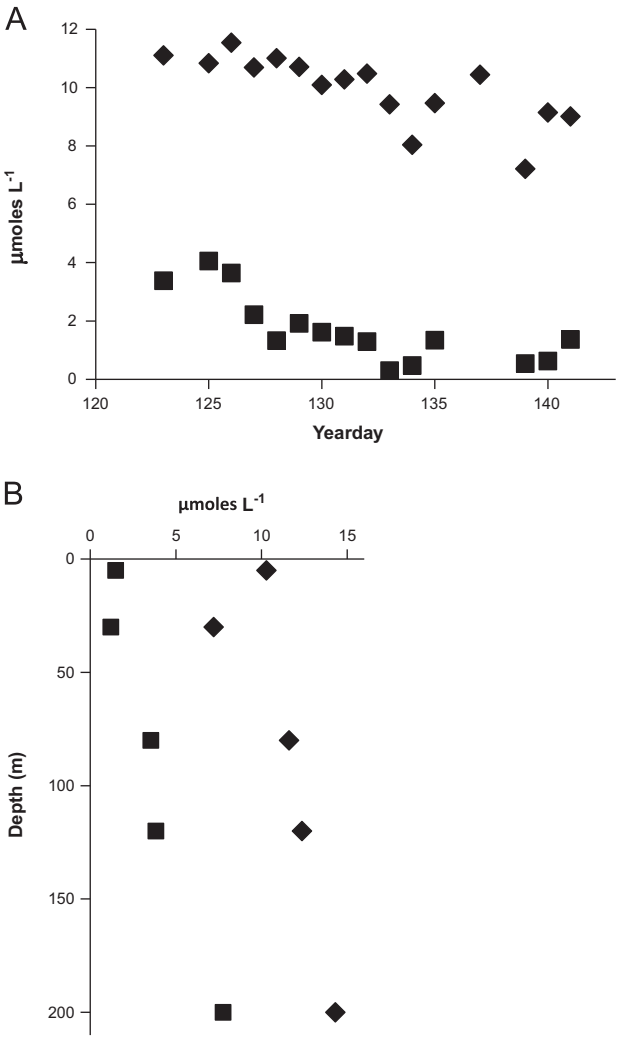

Fig. 8. Concentrations of nitrate plus nitrite (diamonds) and silicic acid (squares) from (A) 10 m collected from stations listed in Table 2 (no silicic acid measurement available for YD 137), and (B) the depth profile on May 10 (YD 131).

diatoms (Menden-Deuer and Lessard, 2000) yielded the 64% estimate and was likely conservative, given that *Chaetoceros* resting spores have been found to contain four to ten times more carbon than vegetative cells (French and Hargraves, 1980; Kuwata et al., 1993). Resting spores are thought to be more carbon dense due to the lack of a vacuole and the presence of storage compounds, including lipids and carbohydrates (Anderson, 1975; Doucette and Fryxell, 1983; Kuwata et al., 1993). Higher carbon density is reflected in the POC flux estimates (up to 431%) based on the C:Vol relationship for *C. pseudocurvisetus* resting spores (Kuwata et al., 1993). The carbon per cell generated using this approach clearly led to an unrealistic overestimation of resting spore contribution to total POC flux, perhaps due to the fact that it was based on resting spores of a different species (although the same genus). For example, *C. pseudocurvisetus* resting spores had a cell volume that was nearly seven times smaller than the

C. aff. *diadema* spores (253 μm^3 vs. 1680 μm^3), and they may have thus been more carbon dense. It has been noted that the C:Vol relationship of individual species can deviate significantly from each other (Menden-Deuer and Lessard, 2000). Using either estimate, the sediment trap results clearly showed that resting spores contributed a significant percentage of the POC flux during the North Atlantic spring bloom.

Prior to and during the flux of resting spores into sediment traps, high backscattering signals were observed in the water column and interpreted as sinking aggregates (Briggs et al., 2011). Material in the PELAGRA traps was examined microscopically immediately after recovery and a surprising feature was that no aggregates were visible, unlike previous PELAGRA deployments where aggregates were observed (Lampitt et al., 2008; Salter et al., 2007). We conclude that the flux event was mediated by fragile aggregates that lost their identity in the sediment trap cup before recovery. Given that resting spores dominated the carbon biomass, it is likely that they were present within the sinking aggregates, as has been observed previously (Alldredge et al., 1995). Aggregate formation may have been enhanced by transparent exopolymer particles (TEP), produced by phytoplankton (Passow and Alldredge, 1995) and identified in large quantities at the peak of POC flux during this study (Martin et al., 2011). Thus, the peak flux of resting spores observed here ($>500\times10^6$ cells m^{-2} day^{-1}, Fig. 2) may have been accelerated by aggregation and TEP production.

The physiological characteristics of resting spores and sediment trap material were suggestive of rapid POC flux with a high transfer efficiency. The F_v/F_m values recorded in the sediment trap material were, for example, similar to those recorded in surface waters at the beginning of our study and among the highest recorded at any station or depth in the study as a whole. The F_v/F_m decreased over the week following retrieval from the sediment traps (Fig. 4A) indicating a loss of photosynthetic capacity over time. Thus, the fact that F_v/F_m values recorded in the sediment trap material were similar from all depths where sediment traps were deployed suggests that sedimentation was occurring rapidly at this time collection. Strong *in vivo* chlorophyll fluorescence of the resting spores from the traps also indicates that the material was relatively fresh, and not degraded (Fig. 4B). This physiological evidence is supported by estimates that the sinking rate of aggregates was $\sim$75 m day^{-1} (Briggs et al., 2011). Furthermore, resting spores collected from down to 750 m depth germinated rapidly after incubation with nutrients and light, showing that these live spores contained high quality, labile POC (Fig. 5). Indeed, the measured transfer efficiency of POC to the sediment traps for this flux event was 30–126% higher than the predicted 19% (Martin et al., 2011). Transfer efficiencies reported for diatom blooms in this region of the North Atlantic are amongst the highest measured in the global ocean (Buesseler and Boyd, 2009), suggesting that the flux of resting spores described here may not represent an isolated event.

Importantly, resting spore formation and flux is not restricted to the North Atlantic. For example, resting spores of the diatom *Eucampia antarctica* var. *antarctica* captured by sediment traps represented up to 71% of all sinking diatom cells following a natural iron fertilization event in the Southern Ocean (Salter et al., 2012). Furthermore, it appeared that the flux of sedimenting material was also comprised of relatively un-degraded organic material, suggesting similarly high transfer efficiencies associated with resting spore flux in the Southern Ocean. This indicates that important flux events of diatom resting spores may be generated by multiple genera and in multiple ocean basins. The significant flux of C generated by resting stages has also been observed in other taxa, such as dinoflagellates. For example, large fluxes of dinoflagellate cysts have been observed in coastal regions of the Pacific (Fujii and Matsuoka, 2006; Pospelova et al., 2010), the North Sea (Godhe et al., 2001), and the Baltic Sea, where one flux event contributed about 45% of the maximum POC flux following a spring bloom (Heiskanen, 1993). Together, these observations have important implications for the disproportionate contribution of particular taxa and importantly, different life stages, to the biological pump.

4.2. Comparison between the surface plankton community and cells collected in sediment traps

Sampling of surface waters took place during the peak of the diatom spring bloom (Alkire et al., 2012). The species composition at the surface was typical of phytoplankton communities during the spring bloom in this region (Moore et al., 2005; Sieracki et al., 1993); phytoplankton chlorophyll and carbon concentrations in surface waters were average for the peak of the North Atlantic spring bloom (Henson et al., 2009). Phytoplankton concentration was patchy during the cruise, as observed in ocean color satellite imagery (cf. Martin et al. (2011)). Chlorophyll a concentrations measured from the ship and by gliders also exhibited patchiness, with concentrations varying by more than five-fold on any given day (e.g., 0.35–2 mg m^3) (Mahadevan et al., 2012). Movement of the ship in and out of patches with elevated chlorophyll was responsible for the variability in diatom abundance among our samples. The sampling strategy allowed for random sampling both inside and outside of patches, and was geographically well distributed in the study area.

At sinking rates of 75 m day^{-1} calculated for the flux event (Briggs et al., 2011), the high number of *C.* aff. *diadema* resting spores recovered on YD 136 at depths of 320–750 m were most likely present in surface waters as vegetative cells until YD 132. Although *Chaetoceros* spp. dominated surface phytoplankton communities, particularly at the beginning of the cruise (YD 123–128), *C.* aff. *diadema* never accounted for more than 5.2% of the diatom cells in surface waters before YD 132 (Table 4). This species was observed in all samples throughout the study area until YD 135, but only at low concentrations both in and out of the patches. We hypothesize that a rarely-occurring species can disproportionally contribute to total organic carbon flux, in this case most likely as a function of its life history strategy of resting spore formation.

The observation that resting spores were in great abundance in sediment traps on YD 136 at all depths, including the shallowest trap (320 m), supports the hypothesis that *C.* aff. *diadema* was a rare species at the surface but a disproportionate contributor to POC flux (Fig. 2). This conclusion gains further support from the fact that the importance of *C.* aff. *diadema* as a percentage of total diatom carbon increased dramatically with depth (Fig. 7). While *C. diadema* occurred together with a number of other *Chaetoceros* spp. in surface waters, it was essentially the only *Chaetoceros* species found at 300 m. This increasing contribution of *C. diadema* carbon at greater depths is likely a consequence of the highly silicified valves of resting spores which appear to be more resistant to the degradation experienced by their vegetative counterparts (Hargraves and French, 1983; Kuwata and Takahashi, 1990).

An alternative hypothesis is that the spores were derived from a major bloom of *C.* aff. *diadema* that occurred prior to our arrival on site. This hypothesis would require very slow sinking rates on the order of <30 m day^{-1}, which is unlikely given sinking rates of $\sim$75 m day^{-1} estimated between YD 123 and 136 (Briggs et al., 2011). Few resting spores were found in shallow traps during the first and second deployment, an observation that is also counter to slow sinking of an early *C.* aff. *diadema* bloom. Furthermore, chlorophyll a and particle concentrations measured from the floats and gliders were very low prior to the start of sampling on YD 123,

suggesting low phytoplankton abundance (Alkire et al., 2012; Mahadevan et al., 2012).

4.3. Initiation of spore formation

The appearance of resting spores in the sediment traps over a very short time period suggests that they formed quickly, consistent with reports that *Chaetoceros* can form resting spores within 6–48 h (reviewed in McQuoid and Hobson (1996)). Resting spore formation is a process that involves wholesale morphological (Hargraves, 1979; Ishii et al., 2011) and metabolic changes from the vegetative life stage (Doucette and Fryxell, 1983; French and Hargraves, 1980; Kuwata et al., 1993). While our data do not allow identification of the trigger(s) for spore formation, they do provide insights into the environmental conditions present before and during the time when resting spores were identified in the water column. This is important because diatom resting spores have not consistently been observed in sinking material (e.g., Billett et al. (1983), Smith et al. (1996)) but when present, can have a large impact on C flux (this study; Salter et al., 2012).

In laboratory experiments, diatom resting spore formation has often been associated with nutrient depletion (French and Hargraves, 1980; Garrison, 1981; Kuwata et al., 1993; Oku and Kamatani, 1999; Pitcher, 1986; Sanders and Cibik, 1985). During the period preceding the appearance of diatom spores in the sediment traps, surface waters remained nitrate replete but dissolved silicic acid concentrations declined from $\sim$4 to $<1\,\mu$M (Fig. 8A). While silicic acid deficiency may have provided a trigger for spore formation, resting spores captured in the deepest sediment traps appeared to be in excellent physiological condition (Fig. 4). When spores were placed in a nutrient enriched medium and provided with light, they began to germinate within 14 h (Fig. 5). Such observations do not reconcile well with the assumption that spores were formed from nutrient stressed, vegetative cells.

An argument that challenges silicic acid depletion as a potential trigger for spore formation is the fact that spores themselves are heavily silicified. Resting spores of some *Chaetoceros* species contain 3–4 times more silicon than vegetative cells and it appears that they can form resting spores only when sufficient silicic acid is present (Kuwata et al., 1993; Kuwata and Takahashi, 1990). Thus, it is difficult to explain the formation of heavily silicified resting spores under dissolved silicic acid concentrations of $<1\,\mu$M in surface waters. This is consistent with our observations of there being no or only very few spores in surface waters at any time during the cruise. Instead, concentrations of spores between 80 and 300 m were found to be an order of magnitude higher than those ever recorded in surface waters (Fig. 7A). Vegetative cells containing spores were only observed between 80 and 200 m, and free spores were observed at 300 m. This observation suggests that the morphological changes associated with spore formation may have occurred primarily below the euphotic zone at depths where silicic acid, needed to form resting spore valves, was found at higher concentrations (Fig. 8B). Alternatively, silicic acid required for production of heavily-silicified resting spores could potentially have come from re-mobilization of silica in the frustules; however, we have no data on frustule density of biogenic silica, either pre or post spore formation, to disprove this hypothesis. The data gathered here suggest a generalized scenario whereby the morphological changes associated with spore formation occurred as cells sank from the surface waters.

4.4. Ecological implications of resting spore flux

The morphological and metabolic changes associated with resting spore formation may ultimately influence their ability to survive and form subsequent blooms. Unlike vegetative cells, whose organic frustule covering can be degraded by bacteria, making the silica readily soluble (Bidle and Azam, 1999), resting spores are both inherently more resistant to bacterial degradation and heavily silicified, thereby enhancing their survival capability. For example, resting spores of *Chaetoceros pseudocurvisetus* appear more resistant to bacterial degradation than vegetative cells (Kuwata and Takahashi, 1990). Resting spores observed here were heavily silicified, potentially enhancing their survival capability. For example, the resting spores of some *Chaetoceros* species were able to germinate after passing through the guts of their copepod predators (Hargraves and French, 1983; Kuwata and Tsuda, 2005). Furthermore, when resting spores are present, copepods lower their filtering rates or actively avoid ingesting the spores suggesting that copepods have evolved strategies to actively avoid resting spore ingestion (Kuwata and Tsuda, 2005). Resting spores sampled here were able to rapidly resume vegetative growth, dividing at rates of $\sim$1 doubling day^{-1}. Rapid germination and growth is consistent with laboratory observations of *C. diadema* resting spores (Hollibaugh et al., 1981). The high growth rates achieved immediately following germination may help to explain the dominance of this spore-forming genus in mid to high latitude blooms. For example, *Chaetoceros* spp. have been shown to dominate spring blooms over multiple years in regions of the Barents and Norwegian Seas (Degerlund and Eilertsen, 2010) as well as the waters just east of the Iceland Basin (Bresnan et al., 2009). Importantly, the germination of *Chaetoceros* spp. resting spores has been observed in the field previously, although primarily in upwelling and coastal regions (Garrison, 1981; Pitcher, 1990).

In open ocean environments, the ability of resting spores to reseed surface waters following germination would depend on a combination of survival time, water depth, winter mixing and circulation. Resting spores of *C. diadema* can survive for long time periods, although the proportion of cells germinating decreases over time. For example, after approximately 50 days in darkness, 85% of *C. diadema* resting spores germinated in the laboratory (Hollibaugh et al., 1981). Maximal survival time has not been measured, but 10% of *C. diadema* cells were still able to germinate after nearly two years in darkness (Hollibaugh et al., 1981). In addition to extended survival ability, resting spores must also be resuspended. While that is unlikely in deeper areas of the North Atlantic, it may be possible in shallower regions, such as coastal waters where *Chaetoceros* is a common diatom genus. For example, the neutrally buoyant sediment traps used here were recovered within 40 km of the Reykjanes Ridge (Fig. 1), which has depths of 500–1000 m in this region. Seagliders operating during this experiment occasionally observed high backscatter measurements below 600 m, indicative of re-suspended sediments and sediment plumes coming from the ridge (Briggs et al., 2011). Deep winter mixing in the Iceland Basin to depths of 600 m could bring re-suspended spores back to the surface (Backhaus et al., 2003). Re-suspended spores could then be transported by prevailing currents to the Iceland Basin and beyond (Pollard et al., 2004). Similar mixing processes in shallow coastal waters off Greenland and North America could allow resting spores to reseed broad areas of the North Atlantic.

5. Conclusions

The data presented here show that biological properties beyond community composition, such as species identity and life cycle stage, can play important roles in the flux of POC to depth. On average, 1–3% of POC leaving the surface ends up below the depth of sequestration, at 1000 m (reviewed in De La Rocha and

Passow (2007)). The amount of initial POC reaching great depth may increase significantly due to the formation and flux of highly-silicified, degradation-resistant diatom resting spores. This is seen in the increased efficiency of POC flux observed in this field study by Martin et al. (2011), with up to 43% of the POC at 100 m transferred to depths of 750 m or greater. Importantly, the species that formed resting spores and contributed most to POC flux during this event was relatively rare in surface waters. Based on sediment cores from the deep ocean, it is clear that resting spores have regularly reached the sediments in large quantities over geologic time (Abelmann et al., 2006; Grimm et al., 1997; Suto, 2006). Resting spores have also been captured in large numbers from deep sediment traps following natural iron enrichment (Salter et al., 2012). This suggests that the formation and sinking of resting spores may essentially "magnify" the effect of surface diatom blooms by transporting proportionally more POC to depth than from blooms where diatoms are not present or do not form resting spores.

Challenges remain in terms of understanding the biogeochemical and ecological impacts of resting spore formation. For example, the massive open ocean flux event of resting spores that we captured in this study was ephemeral and the trigger(s) for spore formation unknown. These characteristics make it a challenge to effectively observe, quantify and predict flux events generated by resting spores. A more complete understanding of the impacts of flux events will benefit from future work to better characterize the chemical and physiological composition of resting spores and how they differ from vegetative cells (e.g., Doucette and Fryxell (1983)). For example, without accurate carbon to volume estimates for resting spores, we were unable to put narrow bounds on the carbon contribution of resting spores to the total POC flux: depending on the carbon-to-biovolume conversion used, our estimates ranged by over six-fold. This highlights that additional basic information about diatom life stage could be used to better understand large-scale events such as the fate of the North Atlantic spring bloom.

Acknowledgments

This work would not have been possible without the vision and hard work of C. Lee and E. D'Asaro who designed the North Atlantic Bloom study and the assistance of our Icelandic colleague K. Guðmundsson. We thank E. Kallin for nutrient analyses, I. Cetinić for assistance with figures, the Captain and crew of the R/V Knorr, and numerous students and colleagues who helped on the cruises. This work was supported by US NSF OCE0727227 (to TAR); US NSF OCE0628379, OCE0628107 and US NASA NNX08AL92G (to MJP with subcontracts to MES); Danish Research Council for Nature and Universe and Danish National Research Foundation (to KR); UK Natural Environment Research Council (to AJP and RSL).

References

Abelmann, A., Gersonde, R., Cortese, G., Kuhn, G., Smetacek, V., 2006. Extensive phytoplankton blooms in the Atlantic sector of the glacial Southern Ocean. Paleoceanography 21 (1), PA1013.

Alkire, M.B., D'Asaro, E., Lee, C., Perry, M.J., Gray, A., Cetinić, I., Briggs, N., Rehm, E., Kallin, E., Kaiser, J., González-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 Research Part I 64, 157–174.

Alldredge, A.L., Gotschalk, C., Passow, U., Riebesell, U., 1995. Mass aggregation of diatom blooms: insights from a mesocosm study. Deep Sea Research Part II 42 (1), 9–27.

Alldredge, A.L., Gotschalk, C.C., 1989. Direct observations of the mass flocculation of diatom blooms: characteristics, settling velocities and formation of diatom aggregates. Deep Sea Research Part A 36 (2), 159–171.

Alldredge, A.L., Passow, U., Logan, B.E., 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep Sea Research Part I 40 (6), 1131–1140.

Anderson, O.R., 1975. The ultrastructure and cytochemistry of resting cell formation in *Amphora coffaeformis* (Bacillariophyceae). Journal of Phycology 11, 272–281.

Backhaus, J.O., Hegseth, E.N., Wehde, H., Irigoien, X., Hatten, K., Logemann, K., 2003. Convection and primary production in winter. Marine Ecology Progress Series 251, 1–14.

Beaulieu, S.E., 2002. Accumulation and fate of phytodetritus on the sea floor. Oceanography and Marine Biology 40, 171–232.

Bidle, K.D., Azam, F., 1999. Accelerated dissolution of diatom silica by marine bacterial assemblages. Nature 397, 508–512.

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.

Bresnan, E., Hay, S., Hughes, S.L., Fraser, S., Rasmussen, J., Webster, L., Slesser, G., Dunn, J., Heath, M.R., 2009. Seasonal and interannual variation in the phytoplankton community in the north east of Scotland. Journal of Sea Research 61 (1), 17–25.

Briggs, N., Perry, M.J., Cetinić, I., Lee, C., D'Asaro, E., Gray, A., Rehm, E., 2011. High-resolution observations of aggregate flux during a sub-polar North Atlantic spring bloom. Deep Sea Research Part I 58, 1031–1039.

Buesseler, K.O., 1998. The decoupling of production and particulate export in the surface ocean. Global Biogeochemical Cycles 12 (2), 297–310.

Buesseler, K.O., Boyd, P.W., 2009. Shedding light on processes that control particle export and flux attenuation in the twilight zone of the open ocean. Limnology and Oceanography 54 (4), 1210–1232.

Cahoon, L.B., Laws, R.A., Thomas, C.J., 1994. Viable diatoms and chlorophylla in continental slope sediments off Cape Hatteras, North Carolina. Deep Sea Research Part II: Topical Studies in Oceanography 41 (4-6), 767–782.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

De La Rocha, C.L., Passow, U., 2007. Factors influencing the sinking of POC and the efficiency of the biological carbon pump. Deep Sea Research Part II 54 (5), 639–658.

Degerlund, M., Eilertsen, H.C., 2010. Main species characteristics of phytoplankton spring blooms in NE Atlantic and Arctic waters (68°–80°N). Estuaries and Coasts 33 (2), 242–269.

DeVries, T., Primeau, F., Deutsch, C., 2012. The sequestration efficiency of the biological pump. Geophysical Research Letters 39 (L13601).

Doucette, G.J., Fryxell, G.A., 1983. *Thalassiosira antarctica*: vegetative and resting stage chemical composition of an ice-related marine diatom. Marine Biology 78 (1), 1–6.

Ducklow, H.W., Steinberg, D.K., Buesseler, K.O., 2001. Upper ocean carbon export and the biological pump. Oceanography 14 (4), 50–58.

French, F.W., Hargraves, P.E., 1980. Physiological characteristics of plankton diatom resting spores. Marine Biology Letters 1, 185–195.

Fujii, R., Matsuoka, K., 2006. Seasonal change of dinoflagellates cyst flux collected in a sediment trap in Omura Bay, West Japan. Journal of Plankton Research 28 (2), 131–147.

Garrison, D.L., 1981. Monterey Bay phytoplankton. II. Resting spore cycles in coastal diatom populations. Journal of Plankton Research 3 (1), 137–156.

Godhe, A., Noren, F., Kuylenstierna, M., Ekberg, C., Karlson, B., 2001. Relationship between planktonic dinoflagellate abundance, cysts recovered in sediment traps and environmental factors in the Gullmar Fjord, Sweden. Journal of Plankton Research 23 (9), 923–938.

Goldman, J.C., 1993. Potential role of large oceanic diatoms in new primary production. Deep Sea Research Part I 40 (1), 159–168.

Gordon, L.I., Jennings, J.C.J., Ross, A.A., Krest, J.M., 1994. A suggested protocol for continuous flow automated analysis of seawater nutrients (phosphate, nitrate, nitrite and silicic acid). The WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study, WOCE Operations Manual vol. 3: The Observational Program, Section 3.1: WOCE Hydrographic Program, Part 3.1.3: WHP Operations and Methods, Woods Hole, Massachusetts, pp. 52.

Grimm, K.A., Lange, C.B., Gill, A.S., 1997. Self-sedimentation of phytoplankton blooms in the geologic record. Sedimentary Geology 110 (3), 151–161.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, USA, pp. 29–60.

Hargraves, P.E., 1979. Studies on marine plankton diatoms IV. Morphology of *Chaetoceros* resting spores. Beiheft zur Nova Hedwigia 64, 99–120.

Hargraves, P.E., French, F.W., 1983. Diatom resting spores: significance and strategies. In: Fryxell, G. (Ed.), Survival Strategies of the Algae. Cambridge University Press, New York, pp. 49–68.

Heiskanen, A.S., 1993. Mass encystment and sinking of dinoflagellates during a spring bloom. Marine Biology 116 (1), 161–167.

Henson, S.A., Dunne, J.P., Sarmiento, J.L., 2009. Decadal variability in North Atlantic phytoplankton blooms. Journal of Geophysical Research: Oceans 114, C04013 (doi: 04010.01029/02008JC005139).

Hollibaugh, J.T., Seibert, D.L.R., Thomas, W.H., 1981. Observations on the survival and germination of resting spores of three *Chaetoceros* (Bacillariophyceae) species. Journal of Phycology 17 (1), 1–9.

Ishii, K.-I., Iwataki, M., Matsuoka, K., Imai, I., 2011. Proposal of identification criteria for resting spores of *Chaetoceros* species (Bacillariophyceae) from a temperate coastal sea. Phycologia 50 (4), 351–362.

Jensen, K.G., Moestrup, Ø., 1998. The genus *Chaetoceros* (Bacillariophyceae) in inner Danish coastal waters. Nordic Journal of Botany 18 (1), 88.

Kallin, E., Cetinić, I., Sauer, M., Perry, M.J., 2011. The 2008 North Atlantic Bloom Experiment Calibration Report #6, Laboratory Analysis Report, http://data.bco-dmo.org/NAB08/Laboratory_analysis_report-NAB08.pdf.
Karl, D.M., Church, M.J., Dore, J.E., Letelier, R.M., Mahaffey, C., 2012. Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation. Proceedings of the National Academy of Sciences 109 (6), 1842–1849.
Kiørboe, T., Hansen, J.L.S., 1993. Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. Journal of Plankton Research 15, 993–1018.
Kohfeld, K.E., Quéré, C.L., Harrison, S.P., Anderson, R.F., 2005. Role of marine biology in glacial–interglacial CO_2 cycles. Science 308 (5718), 74–78.
Kolber, Z., Zehr, J., Falkowski, P.G., 1998. Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. Plant Physiology 88, 923–929.
Kuwata, A., Hama, T., Takahashi, M., 1993. Ecophysiological characterization of two life forms, resting spores and resting cells, of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*, formed under nutrient depletion. Marine Ecology Progress Series 102, 245–255.
Kuwata, A., Takahashi, M., 1990. Life-form population responses of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*, to oligotrophication in regionally upwelled water. Marine Biology 107 (3), 503–512.
Kuwata, A., Tsuda, A., 2005. Selection and viability after ingestion of vegetative cells, resting spores and resting cells of the marine diatom, *Chaetoceros pseudocurvisetus*, by two copepods. Journal of Experimental Marine Biology and Ecology 322 (2), 143–151.
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 Research Part 1 55 (11), 1484–1502.
Legendre, L., Rivkin, R.B., 2002. Fluxes of carbon in the upper ocean: regulation by food-web control nodes. Marine Ecology Progress Series 242, 95–109.
Longhurst, A., 1998. Ecological Geography of the Sea. Academic Press, San Diego.
Mahadevan, A., D'Asaro, E., Lee, C., Perry, M.J., 2012. Eddy-driven stratification initiates North Atlantic spring phytoplankton blooms. Science 337 (6090), 54–58.
Mann, D.G., 1999. The species concept in diatoms. Phycologia 38 (6), 437–495.
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 Research Part I 58 (4), 338–349.
Maxwell, K., Johnson, G., 2000. Chorophyll fluorescence—a practical guide. Journal of Experimental Botany 51, 659–668.
McQuoid, M.R., Hobson, L.A., 1996. Diatom resting stages. Journal of Phycology 32 (6), 889–902.
Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography 45 (3), 569–579.
Moore, C.M., Lucas, M.I., Sanders, R., Davidson, R., 2005. Basin-scale variability of phytoplankton bio-optical characteristics in relation to bloom state and community structure in the Northeast Atlantic. Deep Sea Research Part I 52 (3), 401–419.
Nelson, D.M., Tréguer, P., Brzezinski, M.A., Leynaert, A., Quéguiner, B., 1995. Production and dissolution of biogenic silica in the ocean: revised global estimates, comparison with regional data and relationship to biogenic sedimentation. Global Biogeochemical Cycles 9 (3), 359–372.
Oku, O., Kamatani, A., 1999. Resting spore formation and biochemical composition of the marine planktonic diatom *Chaetoceros pseudocurvisetus* in culture: ecological significance of decreased nucleotide content and activation of the xanthophyll cycle by resting spore formation. Marine Biology 135 (3), 425–436.
Passow, U., Alldredge, A.L., 1995. Aggregation of a diatom bloom in a mesocosm: the role of transparent exopolymer particles (TEP). Deep Sea Research Part II 42 (1), 99–109.
Pitcher, G.C., 1986. Sedimentary flux and the formation of resting spores of selected *Chaetoceros* species at two sites in the southern Benguela System. South African Journal of Marine Science 4, 231–244.
Pitcher, G.C., 1990. Phytoplankton seed populations of the Cape Peninsula upwelling plume with particular reference to resting spores of *Chaetoceros* (Bacillariophyceae) and their role in seeding upwelling waters. Estuarine, Coastal and Shelf Science 31 (3), 283–301.
Pollard, R.T., Read, J.F., Holliday, N.P., Leach, H., 2004. Water masses and circulation pathways through the Iceland Basin during Vivaldi 1996. Journal of Geophysical Research: Oceans 109 (C4), C04004.
Pondaven, P., Ragueneau, O., Treguer, P., Hauvespre, A., Dezileau, L., Reyss, J.L., 2000. Resolving the 'opal paradox' in the Southern Ocean. Nature 405, 168–172.
Pospelova, V., Esenkulova, S., Johannessen, S.C., O'Brien, M.C., Macdonald, R.W., 2010. Organic-walled dinoflagellate cyst production, composition and flux from 1996 to 1998 in the central Strait of Georgia (BC, Canada): a sediment trap study. Marine Micropaleontology 75 (1-4), 17–37.
Ragueneau, O., Schultes, S., Bidle, K., Claquin, P., Moriceau, B., 2006. Si and C interactions in the world ocean: importance of ecological processes and implications for the role of diatoms in the biological pump. Global Biogeochemical Cycles 20 (4), GB4S02.
Rines, J.E.B., Hargraves, P.E., 1988. The *Chaetoceros* Ehrenberg (Bacillariophyceae) Flora of Narragansett Bay, Rhode Island, USA. Cramer, Berlin/Stuttgart.
Salter, I., Kemp, A.E.S., Moore, C.M., Lampitt, R.S., Wolff, G.A., Holtvoeth, J., 2012. Diatom resting spore ecology drives enhanced carbon export from a naturally iron-fertilized bloom in the Southern Ocean. Global Biogeochemical Cycles 26 (1), GB1014.
Salter, I., Lampitt, R.S., Sanders, R., Poulton, A., Kemp, A.E.S., Boorman, B., Saw, K., Pearce, R., 2007. Estimating carbon, silica and diatom export from a naturally fertilised phytoplankton bloom in the Southern Ocean using PELAGRA: a novel drifting sediment trap. Deep Sea Research Part II 54 (18–20), 2233–2259.
Sanders, J.G., Cibik, S.J., 1985. Reduction of growth rate and resting spore formation in a marine diatom exposed to low levels of cadmium. Marine Environmental Research 16, 165–180.
Sarmiento, J.L., Gruber, N. Ocean Biogeochemical Dynamics. Princeton University Press, Princeton, NJ.
Scharek, R., Tupas, L.M., Karl, D.M., 1999. Diatom fluxes to the deep sea in the oligotrophic North Pacific gyre at Station ALOHA. Marine Ecology Progress Series 182, 55–67.
Sieracki, M.E., Verity, P.G., Stoecker, D.K., 1993. Plankton community response to sequential silicate and nitrate depletion during the 1989 North Atlantic spring bloom. Deep Sea Research Part II 40 (1-2), 213–225.
Smetacek, V., 1985. Role of sinking in diatom life-history cycles: ecological, evolutionary, and geological significance. Marine Biology 84 (3), 239–251.
Smith, C.R., Hoover, D.J., Doan, S.E., Pope, R.H., Demaster, D.J., Dobbs, F.C., Altabet, M.A., 1996. Phytodetritus at the abyssal seafloor across 10° of latitude in the central equatorial Pacific. Deep Sea Research Part II 43 (4-6), 1309–1338.
Smith, P., Bogren, K., 2001. Determination of nitrate and/or nitrite in brackish or seawater by flow injection analysis colorimeter: QuickChem Method 31-107-04-1-E, Saline Methods of Analysis. Lachat Instruments, Loveland, CO p. 12.
Sunesen, I., Hernandez-Becerril, D.U., Sar, E.A., 2008. Marine diatoms from Buenos Aires coastal waters (Argentina). V. Species of the genus *Chaetoceros*. Revista de Biología Marina y Oceanografía 43 (2), 303–326.
Suto, I., 2006. The explosive diversification of the diatom genus *Chaetoceros* across the Eocene/Oligocene and Oligocene/Miocene boundaries in the Norwegian Sea. Marine Micropaleontology 58 (4), 259–269.
Takahashi, T., Sutherland, S.C., Wanninkhof, R., Sweeney, C., Feely, R.A., Chipman, D.W., Hales, B., Friederich, G., Chavez, F., Sabine, C., Watson, A., Bakker, D.C.E., Schuster, U., Metzl, N., Yoshikawa-Inoue, H., Ishii, M., Midorikawa, T., Nojiri, Y., Körtzinger, A., Steinhoff, T., Hoppema, M., Olafsson, J., Arnarson, T.S., Tilbrook, B., Johannessen, T., Olsen, A., Bellerby, R., Wong, C.S., Delille, B., Bates, N.R., de Baar, H.J.W., 2009. Climatological mean and decadal change in surface ocean pCO_2, and net sea–air CO_2 flux over the global oceans. Deep Sea Research Part II 56 (8–10), 554–577.
Tomas, C.R., 1997. Identifying Marine Phytoplankton. Academic Press, New York.
Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen—Internationale Vereinigung Für Theoretische und Angewandte Limnologie 9, 1–38.
Volk, T., Hoffert, M.I., 1985. Ocean carbon pumps: analysis of relative strengths and efficiencies in ocean-driven atmospheric CO_2 changes. In: Sundquist, E.T., Broecker, W.S. (Eds.), The Carbon Cycle and Atmospheric CO_2: Natural Variations Archean to Present. AGU, Washington, DC, pp. 99–110.
Waite, A., Fisher, A., Thompson, P.A., Harrison, P.J., 1997. Sinking rate versus cell volume relationships illuminate sinking rate control mechanisms in marine diatoms. Marine Ecology Progress Series 157, 97–108.
Wolters, M., 2002. Determination of silicate in brackish or seawater by flow injection analysis: QuickChem Method 31-114-27-1-D, Methods Manual. Lachat Instruments, Loveland, CO p. 12.
Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Inc., Englewood Cliffs, New Jersey.