

# Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment

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**ABSTRACT:** Lipid concentration and composition were measured in net-tow and sediment trap samples during the spring diatom bloom in Conception Bay, Newfoundland, to determine the quantity and quality of material settling from the upper mixed layer into the benthic food web. An experiment was also conducted to estimate trap accuracy and precision, in which trap poisoning was found to reduce bacterially mediated dissolution, but to increase variability in particulate lipid data. Total lipid concentrations in plankton reached a maximum of  $6.3 \pm 2.5\%$  dry weight (mean  $\pm$  SD) at the height of the bloom in April. Bacterial fatty acid markers were at their lowest during the bloom period (2.0 to 2.8% of total fatty acids), while polyunsaturated fatty acids (PUFA) were at their highest from the beginning of the bloom onwards (44 to 53%). The maximum fluxes of total lipids ( $75 \pm 0.7 \text{ mg m}^{-2} \text{ d}^{-1}$ ) in non-poisoned traps at 80 m depth occurred at the bloom maximum while those at 220 m ( $35 \pm 1.7 \text{ mg m}^{-2} \text{ d}^{-1}$ ) occurred 16 d later. The lipid and PUFA supply to the benthos was over 10 times that accumulated by important amphipod and mysid populations in Conception Bay. In the deep traps, PUFA were maintained at high levels (33 to 35%) throughout the bloom period while bacterial markers were at their lowest (2.3 to 2.9%). A comparison of fluxes showed that over half the PUFA survived transit from 80 to 220 m during the bloom period. These data indicate a rapid transfer of highly nutritious material to the benthos during the spring bloom in Conception Bay.

**KEY WORDS:** Lipids · Pigments · CHN · Net-tows · Sediment traps · Integrated fluxes

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## INTRODUCTION

Continental margins are particularly important for carbon cycling. Globally, ~45% of buried organic matter occurs in shelf areas (Hedges & Keil 1995). They are generally highly productive and the shallow water column reduces the opportunity for remineralization. More surface primary production reaches the seabed in shallower water (Tyler 1995) because particle fluxes decrease with depth as would be expected if particles are remineralized or disaggregated during settling (Asper et al. 1992). One question that remains is the variability in the nature of the material arriving in coastal sediments (Goñi et al. 2000).

Lipids are an important component of the productivity of shelf areas. They are carbon-rich with a very high

energy value and are thus important metabolic fuels. Certain constituents of lipids such as polyunsaturated fatty acids (PUFA) are also essential nutrients for animals. Marine invertebrates require PUFA with between 2 and 6 double bonds for survival and growth (Xu et al. 1994, Pond et al. 1996, Milke et al. 2004).

In this lipid biogeochemistry study, cruises were undertaken in Conception Bay, Newfoundland, to sample a sinking spring bloom. Conception Bay is one of several large fjord-like bays on the Atlantic coast of Newfoundland. In order to measure the transfer of bloom material to the benthos we used sediment traps. While traps are the usual means of determining water column fluxes, problems with microbial activity and the use of poisons in traps (Lee et al. 1992) or with dissolution of trapped particles (Kortzinger et al. 1994) are

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well known. In a 3 d sediment trap deployment in Trinity Bay, Newfoundland, we had previously found that the amount of dissolved lipids could often be as high as one third the amount of particulate lipids in the bottom of sediment traps (Parrish 1998). However, by applying the principles of equilibrium chemistry we hypothesized that losses of lipid material due to dissolution would become proportionately less of a concern as the period of trap deployment increased (Budge & Parrish 1998).

Our research site, Conception Bay, provides a unique opportunity to study cold ocean processes at mid-latitudes. The inshore branch of the Labrador Current maintains sub-zero temperatures below 150 m year-round and surface waters are seasonally as cold (Stead & Thompson 2003). However, modelling of over 10 yr of biological and physical data has revealed that the timing of the initiation of the spring phytoplankton bloom is independent of temperature (Tian et al. 2003), while bacteria and especially zooplankton growth may be suppressed in the spring (Pomeroy et al. 1991). Consequently hyperbenthic and benthic animals are the recipients of significant portions of the productivity (Choe et al. 2003, Stead & Thompson 2003). The quality of the settling material should be high, as polar microalgae produce high levels of PUFA (Skerratt et al. 1995, Henderson et al. 1998). Thus in this study we sought to measure the nature and quantity of spring bloom lipids produced at low temperatures and to determine their flux through the water column. We also evaluated the sampling process used to measure this lipid transfer to the benthos.

## MATERIALS AND METHODS

Water and sediment trap samples were taken near the deepest part of Conception Bay in 248 m of water at ~18 d intervals from 6 March to 9 July 1996 (Fig. 1). Conception Bay is ~100 km long and 30 km wide, with a maximum depth of ~300 m and a sill depth of 170 m.

**Water sampling.** Conductivity-temperature-depth casts were conducted throughout the sampling period using a Seabird SBE 25 CTD fitted with a SeaTech fluorometer. Nitrate, silicate and phosphate concentrations were determined in an EnviroFlow 3500 nutrient analyser (Perstorp Analytical) from Niskin bottle samples that were filtered through 0.45  $\mu\text{m}$  cellulose acetate filters. Nitrate and nitrite were measured together since we have previously found that nitrite concentrations are approximately 1% of the nitrate + nitrite concentrations in Newfoundland waters (Parrish 1998). Plankton was sampled by towing a 20  $\mu\text{m}$  mesh net vertically from 80 m to the surface. In these waters, this sampling procedure collects diatoms, dinoflagellates

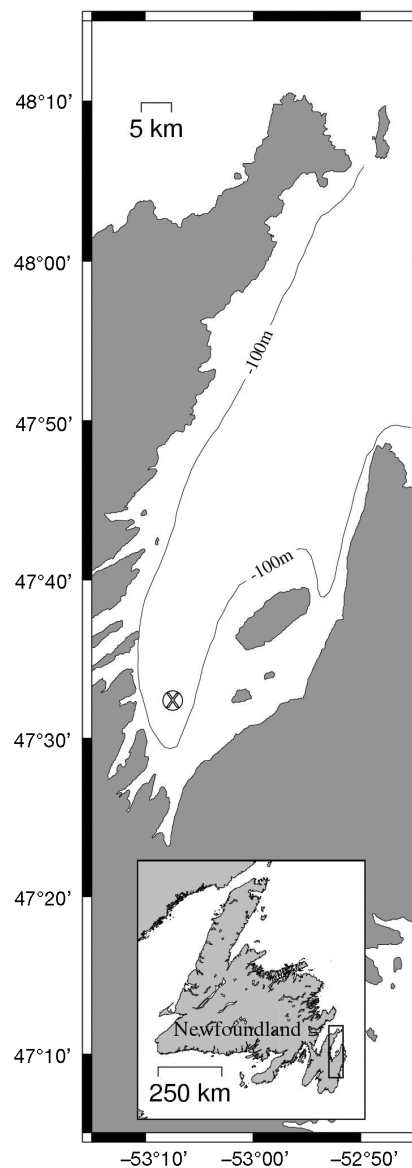


Fig. 1. Station location (X) in Conception Bay on the southeast coast of the island of Newfoundland

and copepod nauplii (Budge & Parrish 1998). Plankton in the cod end were poured into plastic containers and kept in darkness on ice until subsampling for lipid and weight determination in the laboratory within 5 h.

**Sampling settling matter.** Samples of settling matter were collected in a manner very similar to that used in a previous study in Trinity Bay (Parrish 1998). On 6 March 1996 a sediment trap mooring was deployed in 248 m of water in Conception Bay at Stn BRLP5 (47° 32.6' N 53° 07.9' W). Each trap consisted of a 60 cm PVC pipe, sealed at one end and fitted with a spigot placed at about the bottom 1 l mark. Four traps were set at each of 80 and 220 m with 2 at each depth being

poisoned with  $5 \text{ g l}^{-1} \text{ HgCl}_2$  in 40 ppt  $\text{NaCl}_{(\text{aq})}$ , the other 2 having only 40 ppt  $\text{NaCl}$  solution placed in the bottom at the time of deployment. On 27 March 1996, the traps were recovered, samples were taken and the traps were redeployed. Subsamples for lipid, chloropigment, CHN and weight determination were filtered onto pre-combusted Whatman GF/C glass-fibre filters. This procedure was then repeated at  $\sim 18 \text{ d}$  intervals until 9 July 1996.

**Trap performance.** In April 1998 an experiment was conducted to evaluate trap accuracy. A mooring was deployed at Stn BRLP5 in 248 m of water in Conception Bay for 15 d. Four traps were set at each of 50, 75 and 100 m with 2 at each depth being poisoned with  $5 \text{ g l}^{-1} \text{ HgCl}_2$  in 40 ppt  $\text{NaCl}_{(\text{aq})}$ , the other 2 having only 40 ppt  $\text{NaCl}$  solution placed in the bottom at the time of deployment. On the day of deployment and recovery 5 l Go-Flo bottles were used to collect seawater at the same depths as used for trap deployment. The traps were sampled in the usual manner on recovery except that the entire contents of one 50 m non-poisoned and one 50 m poisoned trap were also sampled in 1 l aliquots. In the laboratory all samples were filtered through 47 mm Whatman GF/C glass-fibre filters to obtain both dissolved and particulate fractions from both within and outside the traps. Observations were made on the size and number of zooplankton 'swimmers' visible on the filters before their removal.

**Lipid analyses.** Samples were extracted and analysed based on Parrish (1999). Briefly, samples were extracted in chloroform-methanol (2:1) using a modified Folch procedure. Extracts were analysed with the Chromarod-Iatroscan TLC/FID system for lipid classes (Parrish 1987a). Lipid classes were scanned on the rods in 3 steps with different scan lengths for each step: Iatroscan settings of PPS (partial pyrolysis selection) 22 and 11 for the first 2 chromatograms, and a full scan for the last one. To determine individual fatty acids by gas chromatography, samples were derivatized at  $85^\circ\text{C}$  with 10%  $\text{BF}_3/\text{MeOH}$ . Water was then added and the upper, organic layer was withdrawn and placed in a 2 ml vial. The sample was concentrated to 0.5–1.0 ml under nitrogen and 1  $\mu\text{l}$  was injected into a Varian 3400 gas chromatograph using a Varian 8100 Auto-Sampler. Fatty acids were identified mainly with the use of a comprehensive standard (Supelco 37 component FAME mix) and by careful reference to Ackman (1986).

**Pigment analyses.** Pigments were extracted from sediment trap samples in 90% acetone at  $-20^\circ\text{C}$ . Chloropigments were separated on a 5  $\mu\text{m}$  reverse phase column in a Beckman HPLC using 2 methanol-based solvent systems (Redden 1994).

**Carbon, nitrogen, dry and ash weight determinations.** For organic carbon and nitrogen determination,

samples were dried at  $60^\circ\text{C}$  and combusted in a Perkin-Elmer 240A CHN analyser. Weights were measured on samples that had been washed with 5 to 10 ml of 3% ammonium formate before drying for 16 to 18 h at  $80^\circ\text{C}$ . These samples were then combusted at  $450^\circ\text{C}$  to determine weight loss.

## RESULTS AND DISCUSSION

### Hydrographic conditions

In March 1996, the temperature was below zero throughout the water column at the mooring station in Conception Bay (Fig. 2), and it remained sub-zero below 75 m until mid-August. Below 180 m, salinity and density were greater than 32.8 and 26.4 respectively throughout the sampling period. Salinity and density data suggest a mixing event down to  $\sim 100 \text{ m}$  at the beginning of April. A strong pycnocline developed near the end of June when salinities and densities dropped below 32.0 and 25.0 respectively in the top 15 m of the water column.

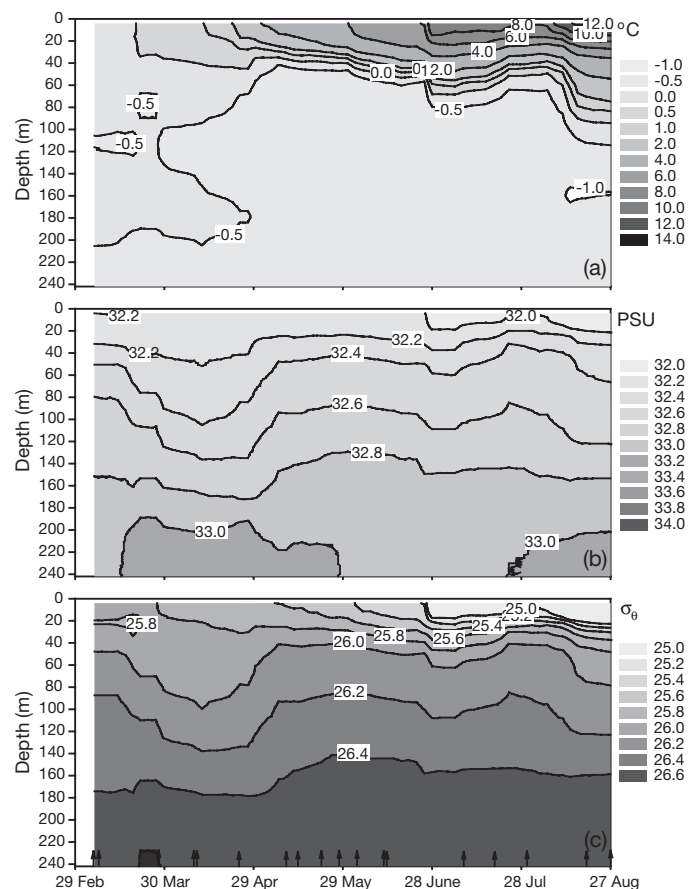


Fig. 2. Temperature ( $^\circ\text{C}$ ), salinity (psu) and density ( $\sigma_t$ ) during the 1996 spring diatom increase in Conception Bay, Newfoundland. Arrows indicate dates of casts

*In situ* fluorescence data were used to track the progress of the 1996 spring diatom increase (Fig. 3a). The timing of the start, the maximum and the arrival to the benthos was determined using unsmoothed data and 2 different contouring programs (Surfer® and SigmaPlot®). The bloom started on 15 March or Julian Day 75 (2.0 contour), the first arrival of bloom material to the benthos was 25 d later (0.5 contour) on 9 April, and the bloom maximum (centre of 6.0 contour) occurred on 24 April, 40 d after the start of the bloom. The depth of the chlorophyll maximum ranged from 15 to 50 m. There was also a small bloom in mid-June on Julian Day 165 (centre of the 2.0 contour).

It is widely accepted that seasonal spring blooms in temperate coastal oceans are terminated by exhaustion of nutrients in the photic zone, which leads to increased sedimentation of phytoplankton cells (Smetacek 1985). Reduced nutrient concentrations in a well-developed diatom bloom can cause diatoms to aggregate, sink and leave the photic zone (Andreassen & Wassmann 1998). In shallow waters, diatoms can reach the sediment surface as intact cells following the spring bloom (Graf 1992).

Silicate and phosphate concentrations decreased by ~50% between 6 March (10–18 m) and 24 April (10 to 50 m) while nitrate decreased to ~10% of the 6 March values (Fig. 3). The highest nitrate concentrations were

Table 1. Composition of particulate matter during the spring bloom in Conception Bay. Overall average proportions (%). Data are mean  $\pm$  SD, n = 6 to 8. Sediment trap data are from unpoisoned traps. POM: particulate organic matter (ash free dry weight); TPM: total particulate matter (dry weight); TL: total lipid (sum of Iatroscan determined lipid classes); Neutral lipids: hydrocarbons, wax and steryl esters, ketones, triacylglycerols, free fatty acids; (FFA), alcohols (ALC), sterols, diacylglycerols; Polar lipids: monoacylglycerols, acetone-mobile polar lipids, phospholipids; LI: lipolysis index [(FFA + ALC) (acyl lipids + ALC)<sup>-1</sup>]; HI: hydrolysis index [(FFA + ALC) (neutral acyl lipids + ALC)<sup>-1</sup>]

	Tows from 80 m	80 m traps	220 m traps
POM/TPM	58.6 $\pm$ 9.49	49.9 $\pm$ 12.77	32.7 $\pm$ 8.12
TL/TPM	3.41 $\pm$ 1.67	1.83 $\pm$ 0.93	0.57 $\pm$ 0.25
TL/POM	5.75 $\pm$ 2.36	0.39 $\pm$ 0.21	0.20 $\pm$ 0.10
Neutral/TL	65.5 $\pm$ 10.32	70.3 $\pm$ 7.97	62.8 $\pm$ 6.91
Polar/TL	34.5 $\pm$ 10.32	29.8 $\pm$ 7.97	37.2 $\pm$ 6.91
LI	19.4 $\pm$ 6.73	17.6 $\pm$ 6.04	18.1 $\pm$ 5.20
HI	31.9 $\pm$ 13.44	26.9 $\pm$ 9.21	30.7 $\pm$ 8.57

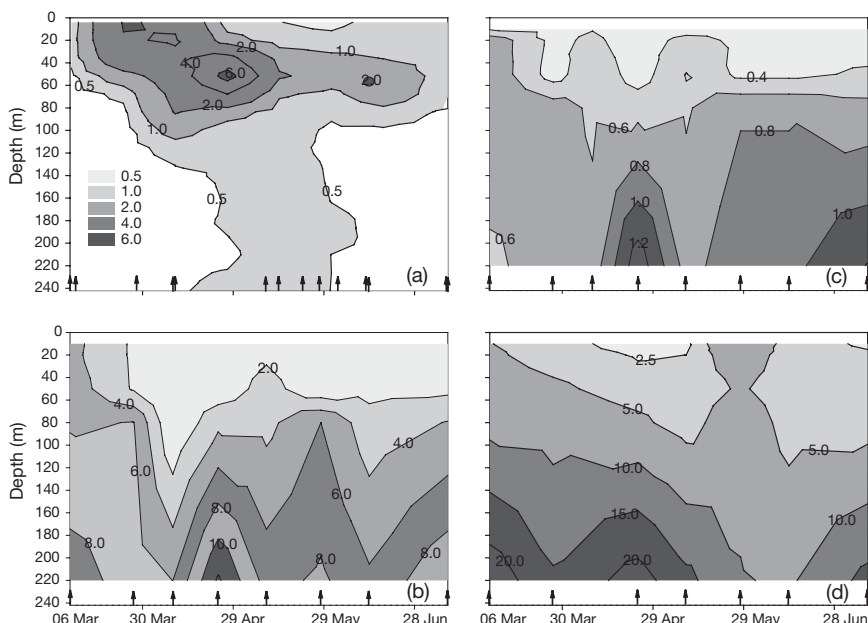


Fig. 3. Fluorescence and nutrient contours. (a) Data from the SeaTech fluorometer are contoured in relative fluorescence units. (b) Nitrate + nitrite ( $\mu\text{M}$ ). (c) Phosphate ( $\mu\text{M}$ ). (d) Silicate ( $\mu\text{M}$ ). Arrows indicate dates of casts

observed that same day at 220 m, together with some of the highest silicate and phosphate concentrations. Such values occurred near the middle of the period of bloom arrival to the benthos, strongly suggesting that remineralization was responsible for the high concentrations. It is noteworthy that this maximum occurred at the same time as the bloom maximum in surface waters (Fig. 3a) and that the N:P molar ratio was 9.3, suggesting regeneration from nitrogen depleted particles.

During the period of trap deployment in April 1998, hydrographic conditions were very similar to those observed in April 1996; however, the 1998 bloom started earlier in March and reached its maximum earlier in April (authors' unpubl. data). Furthermore, the fluorescence maximum was about twice as large in 1998.

## Plankton

Total lipid concentrations in plankton were on average,  $3.4 \pm 1.7\%$  of dry weight (Table 1) and were greater ( $p = 0.040$ ) at the height of the bloom in April, reaching a maximum of  $6.3 \pm 2.5\%$  dry weight (Fig. 4a). There is evidence of another increase in lipids during the small bloom in mid-June on Julian Day 165. In both cases, increases in neutral lipids accounted for most of the increase. Neutral lipids were also slightly elevated at the beginning of the sampling period. The major neutral lipid class in plankton was

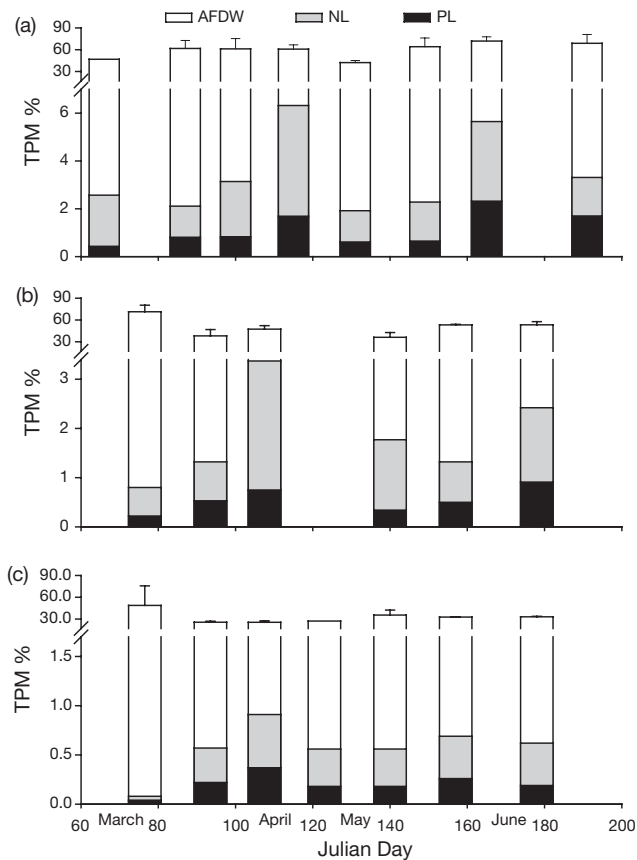


Fig. 4. Ash free dry weight (AFDW) and total lipid content of particulate matter during the spring bloom in Conception Bay, Newfoundland. (a) Vertical plankton tows from 80 m to the surface. (b) 80 m sediment trap samples. (c) 220 m sediment trap samples. The shaded lipid portion is divided into neutral lipid (NL) and polar lipid (PL). Data are mean  $\pm$  SD,  $n = 3$  for net-tows and mean  $\pm \frac{1}{2}$  range,  $n = 2$  for sediment traps. Note factor of 2 in pre-break scalings

Table 2. Lipid composition (% total lipid) of particulate matter during the spring bloom in Conception Bay. Data are mean  $\pm$  SD,  $n = 8$  for plankton and 7 for sediment traps

	Tows from 80 m	80 m traps	220 m traps
Hydrocarbons	1.18 $\pm$ 1.26	1.77 $\pm$ 1.54	1.91 $\pm$ 1.27
Steryl/wax esters	17.2 $\pm$ 13.7	23.4 $\pm$ 11.6	10.2 $\pm$ 8.28
Methyl esters	1.00 $\pm$ 1.22	1.33 $\pm$ 1.64	1.75 $\pm$ 1.45
Triacylglycerols	18.0 $\pm$ 7.42	20.2 $\pm$ 6.97	19.7 $\pm$ 5.35
Free fatty acids	14.7 $\pm$ 6.83	13.8 $\pm$ 6.36	12.2 $\pm$ 5.82
Alcohols	3.31 $\pm$ 1.59	2.94 $\pm$ 0.75	4.16 $\pm$ 1.00
Pink pigment	0.10 $\pm$ 0.29	0.12 $\pm$ 0.20	0.50 $\pm$ 0.66
Sterols	4.99 $\pm$ 1.38	5.64 $\pm$ 2.17	6.61 $\pm$ 1.18
Diacylglycerols	5.06 $\pm$ 1.38	3.88 $\pm$ 1.01	5.77 $\pm$ 1.12
Acetone mobile polar lipids	18.1 $\pm$ 7.88	16.0 $\pm$ 5.16	21.0 $\pm$ 4.46
Phospholipids	16.4 $\pm$ 9.56	15.2 $\pm$ 6.40	16.2 $\pm$ 3.51

triacylglycerol (Table 2). In diatoms and dinoflagellates high triacylglycerol production is usually associated with lower growth rates (Sicko-Goad & Andresen 1991, Parrish et al. 1994), as is often found at the beginning and especially the end of batch culture experiments. Seawater concentrations of particulate triacylglycerol were also slightly elevated near the beginning of a bloom in Bedford Basin, Nova Scotia, and then at their maximum from the time of the chlorophyll maximum onwards (Parrish 1987b).

Proportions of polyunsaturated fatty acids (PUFA) were high from the beginning of the bloom until the end of May (44 to 53%: Fig. 5a). These values are similar to the 31 to 54% observed in net-tows from adjacent Trinity Bay in March-June 1996 (Budge & Parrish 1998), and are about as high as the highest (55%) observed in coastal and fjord plankton in Antarctica

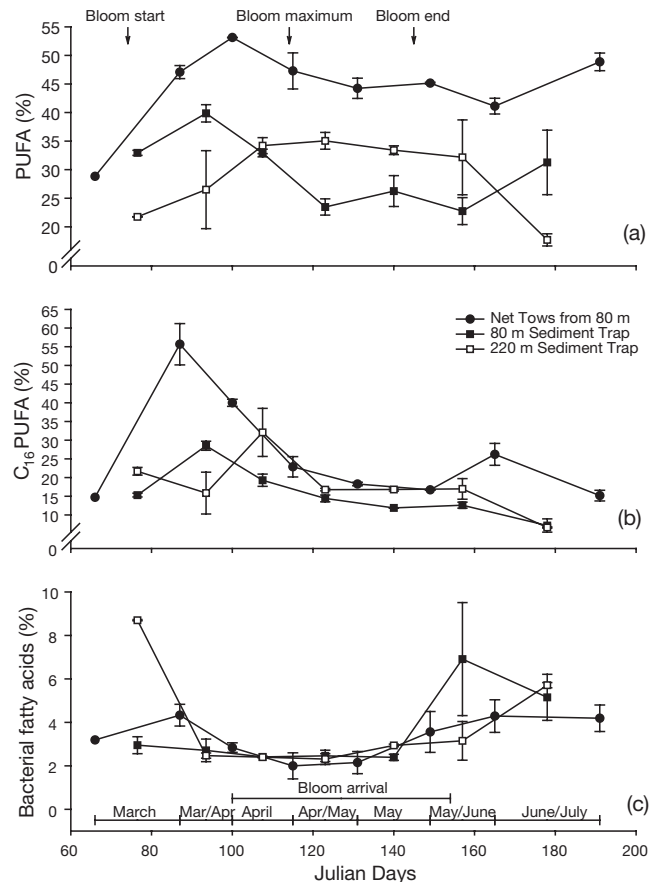


Fig. 5. Fatty acid proportions (%) in plankton and settling particulate matter during the spring bloom in Conception Bay, Newfoundland. (a) Polyunsaturated fatty acids as a proportion of total fatty acids. (b)  $C_{16}$  PUFA ratio (see text for definition). (c) Bacterial fatty acids ( $i15:0 + ai15:0 + 15:0 + i16:0 + ai16:0 + i17:0 + ai17:0 + 17:0$ ) as a proportion of total fatty acids. The periods of trap deployments and bloom arrival to the benthos are indicated at the bottom. Data are mean  $\pm$  SD,  $n = 3$  for net-tows and mean  $\pm \frac{1}{2}$  range,  $n = 2$  for sediment traps

(Skerratt et al. 1995). There was, however, a decrease in PUFA proportions between April and June in Conception Bay during the period of low nutrient concen-

Table 3. Fatty acid composition (% total fatty acids) during the spring bloom in Conception Bay. Data are overall means,  $n = 7$  to 8; trap data are for unpoisoned traps only

	Plankton		80 m trap		220 m trap	
	Mean	SD	Mean	SD	Mean	SD
14:0	9.74	1.69	8.04	2.74	7.28	2.06
<i>i</i> 15:0	0.38	0.29	9.00	4.51	9.43	4.27
<i>ai</i> 15:0	0.44	0.24	6.12	2.43	6.43	2.85
15:0	0.55	0.17	0.68	0.20	0.78	0.44
<i>i</i> 16:0	0.21	0.11	0.28	0.24	0.32	0.23
<i>ai</i> 16:0	0.11	0.09	0.24	0.29	0.16	0.12
16:0	12.19	2.79	15.00	2.68	12.75	2.01
16:1 $\omega$ 7	12.86	4.05	18.43	5.50	22.12	4.12
16:1 $\omega$ 5	0.53	0.41	0.26	0.26	0.44	0.40
<i>i</i> 17:0	1.18	0.76	0.64	0.23	0.77	0.47
<i>ai</i> 17:0	0.27	0.26	0.25	0.13	0.20	0.10
16:2 $\omega$ 4	1.79	0.66	1.44	0.46	1.56	0.43
17:0	0.17	0.14	0.21	0.12	0.25	0.23
16:3 $\omega$ 4	1.86	1.36	1.58	1.23	1.66	0.77
16:4 $\omega$ 3	0.76	0.56	0.37	0.14	0.64	0.47
16:4 $\omega$ 1	5.97	6.65	3.26	2.41	4.38	2.71
18:0	1.05	0.45	1.17	0.54	1.29	0.62
18:1 $\omega$ 9	4.22	3.85	4.94	3.13	4.56	1.61
18:1 $\omega$ 7	1.47	0.82	2.65	1.10	1.91	0.92
18:1 $\omega$ 6?	0.02	0.05	0.00	0.00	0.00	0.00
18:1 $\omega$ 5	0.62	0.41	0.72	0.26	0.41	0.37
18:2 $\omega$ 6	1.02	0.40	1.00	0.42	0.90	0.35
18:2 $\omega$ 4	0.14	0.12	0.30	0.15	0.12	0.07
18:3 $\omega$ 6	0.16	0.10	0.08	0.04	0.06	0.06
18:3 $\omega$ 4	0.07	0.17	0.01	0.04	0.01	0.02
18:3 $\omega$ 3	0.39	0.26	0.25	0.15	0.32	0.15
18:4 $\omega$ 3	2.92	1.39	2.10	1.34	2.06	1.30
18:4 $\omega$ 1	0.11	0.14	0.03	0.04	0.01	0.02
20:0	0.01	0.03	0.02	0.03	0.04	0.06
18:5 $\omega$ 3	0.00	0.00	0.09	0.17	0.01	0.03
20:1 $\omega$ 9(11)	1.36	1.44	1.79	1.10	1.29	0.92
20:1 $\omega$ 7	0.24	0.12	0.44	0.31	0.28	0.17
20:2 $\omega$ 6	0.12	0.10	0.12	0.12	0.29	0.21
20:3 $\omega$ 6	0.00	0.00	0.00	0.02	0.18	0.31
20:4 $\omega$ 6	0.32	0.28	2.51	2.24	0.70	0.80
20:3 $\omega$ 3	0.09	0.23	0.06	0.05	0.03	0.04
20:4 $\omega$ 3	0.68	0.44	0.40	0.21	0.21	0.09
20:5 $\omega$ 3	17.86	6.71	10.90	2.50	12.66	6.47
22:0	0.01	0.04	0.01	0.02	0.01	0.02
22:1 $\omega$ 11(13)	0.82	1.35	1.00	0.65	0.78	0.72
22:1 $\omega$ 9	0.14	0.20	0.53	0.21	0.32	0.23
22:1 $\omega$ 7	0.00	0.00	0.07	0.13	0.01	0.02
21:5 $\omega$ 3	0.22	0.13	0.17	0.06	0.14	0.13
23:0	0.02	0.04	0.03	0.05	0.02	0.06
22:4 $\omega$ 6	0.04	0.09	0.04	0.07	0.03	0.06
22:4 $\omega$ 3	0.16	0.30	0.04	0.06	0.03	0.04
22:5 $\omega$ 6	0.14	0.17	0.10	0.10	0.04	0.04
22:5 $\omega$ 3	0.56	0.47	0.36	0.16	0.16	0.10
24:0 <sup>a</sup>	0.00	0.00	0.00	0.00	0.00	0.00
22:6 $\omega$ 3	9.09	5.26	4.72	3.04	2.48	0.96
24:1	0.05	0.08	0.11	0.14	0.19	0.19

<sup>a</sup>Detected in only one poisoned trap at 0.18%

trations in surface waters. Nitrate (Sukenic & Wahnou 1991), phosphate (Harrison et al. 1990, Reitan et al. 1994) and silica limitation (Harrison et al. 1990) decrease  $\omega$ 3 PUFA proportions in microalgae. However, the ratio of 22:6 $\omega$ 3 to 20:5 $\omega$ 3, the 2 largest contributors (Table 3), increased from May onwards indicating a greater relative contribution of dinoflagellate lipids (Ramos et al. 2003).

Shin et al. (2000) proposed a 'Polyunsaturation Index of C<sub>16</sub> fatty acids' as an indicator of the physiological status of diatoms. They used the ratio (16:2 $\omega$ 7 + 16:3 $\omega$ 4 + 16:4 $\omega$ 1 to 16:0 + 16:1 $\omega$ 9 + 16:1 $\omega$ 7 + 16:1 $\omega$ 5 + 16:2 $\omega$ 7 + 16:3 $\omega$ 4 + 16:4 $\omega$ 1) to show the effects of nitrate limitation. We used a slight modification of both the calculation and the name (Fig. 5b). Our C<sub>16</sub> PUFA ratio is defined as the ratio (16:2 $\omega$ 4 + 16:3 $\omega$ 4 + 16:4 $\omega$ 3 + 16:4 $\omega$ 1 to 16:0 + 16:1 $\omega$ 7 + 16:1 $\omega$ 5 + 16:2 $\omega$ 4 + 16:3 $\omega$ 4 + 16:4 $\omega$ 3 + 16:4 $\omega$ 1). The polyunsaturated 16:2 $\omega$ 4 and 16:4 $\omega$ 3 have been added because they comprise a significant portion (>2%) of the total fatty acids in some species of diatom (Viso & Marty 1993, Dunstan et al. 1994) and were present at about this level in our samples (Table 3). The C<sub>16</sub> PUFA ratio was highest in the net tow sample taken immediately after the start of the bloom. This value of 56% was much higher than the highest Polyunsaturation Index of C<sub>16</sub> fatty acids (33%) measured by Shin et al. (2000) in Japanese waters. The C<sub>16</sub> PUFA ratio maximum preceded the overall PUFA maximum by 13 d and declined rapidly as nitrate concentrations fell (Figs. 3b & 5b).

Bacterial fatty acid markers in the plankton were at their lowest throughout April and into the middle of May (2.0 to 2.8% of total fatty acids; Fig. 5c). Antarctic phytoplankton have shown antibacterial activity (Sieburth 1959), so low contributions by bacteria to the fatty acid pool could indicate allelopathy between plants and bacteria during the bloom period. In a mesocosm experiment in warmer water, bacterial *i*15:0 concentrations were low during a diatom bloom (Hayakawa et al. 1996); however, in Conception Bay comparatively low contributions more likely result from the use of proportions. The lowest proportion (2.00 ± 0.60%) occurred on 24 April when total lipid concentrations were at their highest (Fig. 4a). The highest proportion (4.33 ± 0.50%) occurred on 27 March when total lipid concentrations were at their second lowest, one third of the highest value.

#### Changes in the nature of particles during transfer to the benthos

Overall, organic matter and total lipid concentrations decreased continuously in the transition from plankton to near bottom particles (Table 1), so that deep trap

values were significantly lower ( $p < 0.05$ ). This was not the case for the components of the total lipids, although there were differences in neutral and polar lipid proportions between the 2 trap depths ( $p = 0.049$ ). Differences in neutral lipid proportions suggest catabolism of storage lipids or increases in membrane lipids in deep sediment traps. On the other hand, the average lipolysis index remained the same at 18 to 19% in all samples (Table 1). There was no increase with depth probably because the bloom maximum occurred around 50 m at sub-zero temperatures and the traps were deployed in sub-zero waters throughout. The closely related hydrolysis index (HI; Weeks et al. 1993) was also not different among the samples and not different to the previous study in Trinity Bay (Parrish 1998) where net tow and trap samples were taken from the top 100 m of the water column. The median HI value of 31 for Conception Bay, with a range of 12 to 49, is indicative of low bloom utilization in surface waters (Weeks et al. 1993)

There were no trends with depth in the distributions of any of the major lipid classes nor in some of the minor classes as the organic material sank from the photic zone through the water column (Table 2). However, hydrocarbons, methyl esters, a pink pigment and sterols did increase continuously between net-tow and 220 m trap samples, and free fatty acids surprisingly decreased continuously. The pink pigment has been tentatively identified as a peridinin-like pigment (Parrish 1998). Lipid classes showing no continuous trends in relative proportions with depth were the energy storage triacylglycerols, the breakdown indicator alcohols (Weeks et al. 1993) and diacylglycerols, and the membrane phospholipids. Wax esters in the combined wax ester/steryl ester latroscan peak are zooplankton energy stores, and the acetone-mobile polar lipid peak contains the monoacylglycerol breakdown indicator and the membrane glycolipids.

As a proportion of dry weight, all but one of the lipid classes decreased continuously in the transition from plankton to the 220 m trap, consistent with the large decrease in total lipid as a proportion of dry weight (Table 1). The only exception was, again, a minor component of the extract, the peridinin-like pigment. This characteristic pigment of the dinoflagellates (Goodwin 1974) was present in its highest proportion in the 220 m trap.

Although most lipid classes decreased as a proportion of dry weight during transfer to the benthos, the range in values for each lipid class between the tow data and the 220 m trap data was actually lower than was found among the 8 tow means. This, combined with the almost total lack of significant differences in lipid class proportions (Table 2), suggests that changes in lipids as a proportion of dry weight (Table 1) are due, in part, to admixture of lipid-poor material.

Eicosapentaenoic acid (20:5 $\omega$ 3) was, on average, the major fatty acid in the net-tow samples while 16:1 $\omega$ 7 was the major fatty acid in sediment trap samples (Table 3). Nine PUFA (18:2 $\omega$ 6, 18:4 $\omega$ 3, 18:4 $\omega$ 1, 20:4 $\omega$ 3, 21:5 $\omega$ 3, 22:4 $\omega$ 3, 22:5 $\omega$ 6, 22:5 $\omega$ 3, 22:6 $\omega$ 3) showed a continuous relative decrease with depth, while 7 saturates (*i*15:0, *ai*15:0, 15:0, *i*16:0, 17:0, 18:0, 20:0) and 2 monoenes (16:1 $\omega$ 7, 24:1) showed an increase. Such opposing trends suggest replacement of diatom fatty acids by bacterial ones. The PUFA 18:2 $\omega$ 6, 18:4 $\omega$ 3, 18:4 $\omega$ 1, 20:4 $\omega$ 3, 22:5 $\omega$ 3 and 22:6 $\omega$ 3 have all been identified in a pennate diatom isolated from Canadian waters (Parrish et al. 1991), while the *iso* (*i*) and *anteiso* (*ai*) branched chain saturated fatty acids are major constituents in Gram-positive bacteria (Fulco 1983). However, the replacement is apparently selective since the pennate diatom had 16:1 $\omega$ 7 as its major fatty acid, as is commonly found in diatoms (Volkman et al. 1989, Viso & Marty 1993), and this monoene showed the opposite trend (Table 3). This indicates PUFA may be selectively removed, catabolized or chemically oxidised during passage through the water column, although degradation rates are likely to be slow in cold water (Budge & Parrish 1998). Three of these PUFA, 18:2 $\omega$ 6, 22:5 $\omega$ 6, and 22:6 $\omega$ 3 are essential nutrients for marine invertebrates (Xu et al. 1994, Pond et al. 1996, Milke et al. 2004).

Notwithstanding apparent trends in the nature of the fatty acid composition of particles during transfer to the benthos, it is important to note that the ranges between the plankton and 200 m trap means were, in fact, almost always smaller than found in plankton and trap material over the season. The ranges for PUFA showing trends in Table 3 were always smaller than those found in the 8 net tows, while the ranges in the saturates and monoenes showing upward trends were always smaller than in the 80 m and 220 m trap data. Thus, for these fatty acids, temporal variability was greater than depth variability on average, suggesting that changes in the nature of the lipids during transfer to the benthos were comparatively small. Indeed, cluster analysis has revealed that the fatty acid composition of yellowtail flounder *Limanda ferruginea* in Conception Bay is quite similar to that of plankton and settling particulate matter (Dwyer et al. 2003).

The major fatty acids (means >1% in all samples) showing no trends with depth were 16:0, 16:2 $\omega$ 4, 16:3 $\omega$ 4, 16:4 $\omega$ 1, 18:1 $\omega$ 9, 18:1 $\omega$ 7, 20:1 $\omega$ 9(11) and 20:5 $\omega$ 3. This last long-chain PUFA is an essential fatty acid which has been shown to be important in maintaining fluidity in the membrane of a cold water bivalve (Hall et al. 2002). The saturated fatty acid 16:0 is common to all life forms, while the C<sub>16</sub> polyenes were major fatty acids in the pennate diatom from Atlantic Canada (Parrish et al. 1991). The C<sub>18</sub> monoenes have been used to

indicate feeding behaviour in cold water benthos (Graeve et al. 1997), and the monoene 20:1 $\omega$ 9 (in the combined 20:1 $\omega$ 9 + 20:1 $\omega$ 11 GC peak) is prominent in cold water herbivorous copepods (Albers et al. 1996). Sometimes 20:1 $\omega$ 9 and 20:1 $\omega$ 11 were separately identifiable with an average  $\omega$ 9/ $\omega$ 11 ratio of  $\sim$ 2 in net-tow lipids and 10 in sediment trap lipids. Likewise, on 2 occasions the other long-chain monoenes 22:1 $\omega$ 11 and 22:1 $\omega$ 13 were separately identifiable in sediment trap lipids with  $\omega$ 11/ $\omega$ 13 ratios of 3 and 5. Among the minor fatty acids 3 multiple branched fatty acids were tentatively identified in some samples at proportions between 0.1 and 0.8% each in net-tows and between 0.1 and 1.1% each in sediment traps. These were 4,8,12-trimethyltetradecanoic acid and pristanic and phytanic acids, all believed to be derived from the phytol side-chain of chlorophyll (Ackman et al. 1966, Ackman 1986).

### Fluxes of particulate matter

The maximum fluxes of chlorophyll *a*, phaeopigments, carbon and nitrogen occurred at 80 m during the April deployment/recovery (Fig. 6) and were similar to maxima determined in the Laurentian Trough (Colombo et al. 1996), but higher than in coastal Antarctica (Hayakawa et al. 1997). Comparisons of areas under the flux curves are given in Table 4. The data

Table 4. Areas under flux curves and their ratios (220 m/80 m)

	80 m traps (g m <sup>-2</sup> )	220 m traps (g m <sup>-2</sup> )	Ratio (%)
TPM	90.4	309	342
POM	42.8	91.9	215
Chlorophyll <i>a</i>	0.130	0.087	66.8
Phaeopigments	0.184	0.194	106
Carbon	22.8	20.6	90.1
Nitrogen	5.92	4.66	78.8
Total lipids	3.32	1.75	52.8
Neutral lipids	2.45	1.11	45.5
Polar lipids	0.867	0.660	76.1
PUFA	0.629	0.345	54.8

suggest that most of these 4 organic classes pass through the water column, although the data for dry and ash free dry weight indicate that the fluxes should be compared cautiously. In the case of these TPM and POM data, most of the fluxes at 220 m were higher than those at 80 m, suggesting additional sources for the 220 m trap, most likely from resuspension.

The major peak in the HPLC chloropigment chromatograms of trap samples was almost always chlorophyll *a*, although there were always considerable amounts of several phaeopigments, so that their total flux was usually higher than that of chlorophyll *a*. Nonetheless, at the height of the bloom there was no significant difference between the chlorophyll *a* flux

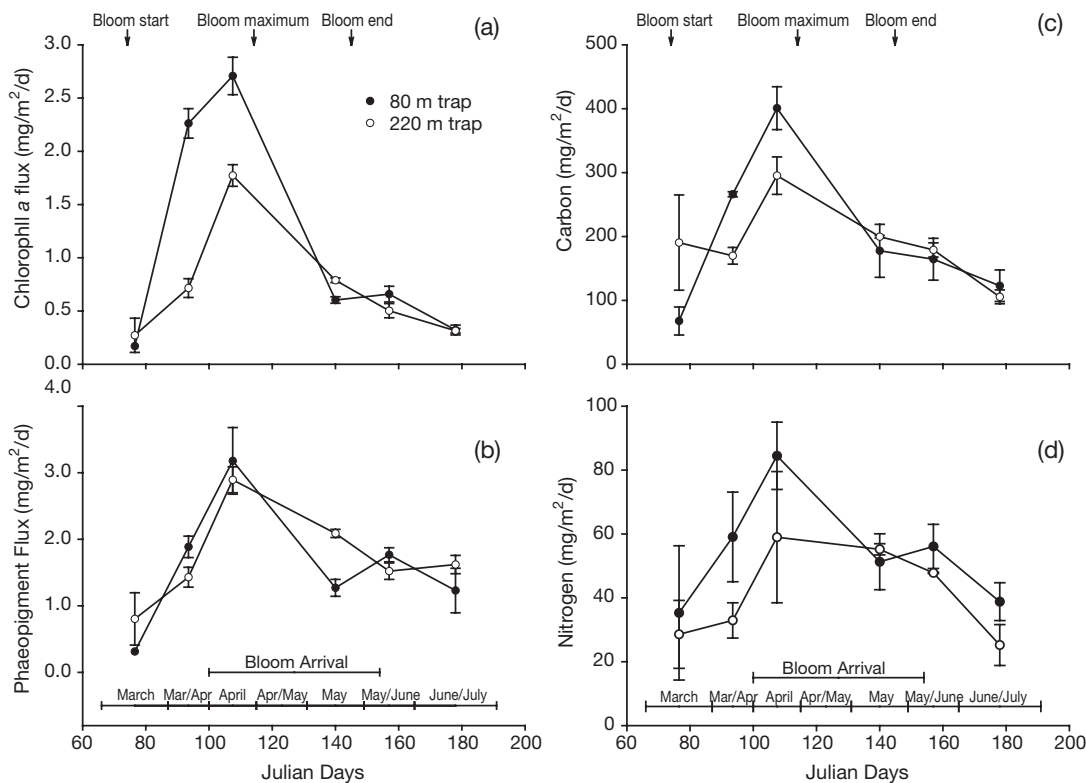


Fig. 6. Fluxes of organic matter during the spring bloom in Conception Bay, Newfoundland. The periods of trap deployments and bloom arrival to the benthos are indicated at the bottom. Data are mean  $\pm$   $\frac{1}{2}$  range,  $n = 2$



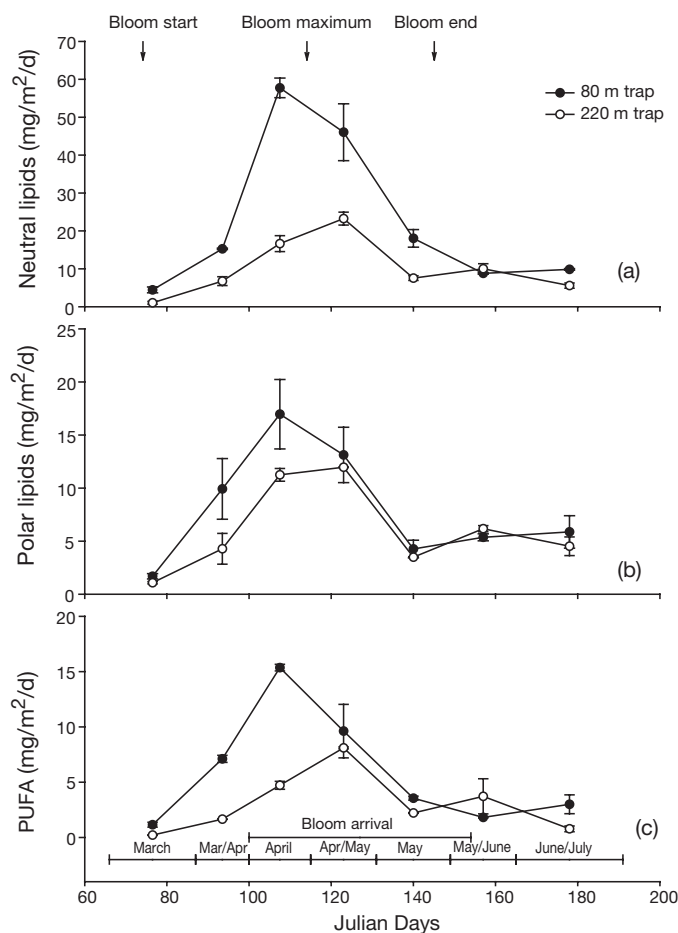


Fig. 7. Neutral lipid, polar lipid and polyunsaturated fatty acid fluxes during the spring bloom in Conception Bay, Newfoundland. The periods of trap deployments and bloom arrival to the benthos are indicated. Data are mean  $\pm$   $\frac{1}{2}$  range,  $n = 2$

and the phaeopigment flux at 80 m (Fig. 6a,b). Over the bloom period, 67% of the undegraded chlorophyll *a* passing through 80 m reached 220 m (Table 4) presumably in intact phytoplankton cells and chains. The proportion was higher for phaeopigments, suggesting transport in fast sinking faecal pellets or additional chlorophyll breakdown in transit.

The maximum fluxes of neutral and polar lipids ( $58 \pm 2.6$  and  $17 \pm 3.3$   $\text{mg m}^{-2} \text{d}^{-1}$ ) in non-poisoned 80 m traps occurred at the bloom maximum, while those at 220 m ( $23 \pm 1.7$  and  $12.0 \pm 0.02$   $\text{mg m}^{-2} \text{d}^{-1}$ ) occurred 16 d later (Fig. 7). This gives an apparent settling velocity of  $8.8$   $\text{m d}^{-1}$  for lipid-containing particles. This is considerably slower than the values of 20 to 23  $\text{m d}^{-1}$  given by Redden (1994) for Conception Bay, calculated on the basis of carbon measurements. This difference may relate to slower settling rates of lipid rich particles, but it is also related to deployment of the traps. Redden deployed traps for shorter periods and at more depths than was possible here.

Integration of the lipid flux data provides the quantities of lipids in settling particulate matter passing through each depth during the spring bloom (Table 4), which in turn can be compared with lipid accumulation in important benthic species in Conception Bay (Richoux et al. 2004a,b). The maximum net accumulation for populations of the amphipod *Acanthostepheia malmgrenii* or the mysid *Mysis mixta* accounts for  $\sim 4\%$  each of the  $1.75$   $\text{g m}^{-2}$  total lipid in the 220 m traps during the 1996 spring bloom. *A. malmgrenii* and *M. mixta* are among the 5 most abundant large species in the suprabenthos in Conception Bay (authors' unpubl. data).

PUFA levels in traps ranged from 18 to 40% of total fatty acids (Fig. 5a), bracketing the year round mean of 20% in Trinity Bay (Budge & Parrish 1998), but remaining higher than the maximum (16.7%) in coastal Antarctica (Hayakawa et al. 1997). In the deep traps, PUFA were maintained at high levels (33 to 35%) throughout the period of bloom arrival while bacterial markers were at their lowest (2.3 to 2.9%). The mean chain length and number of double bonds in fatty acids in the settling particles was the same at the 2 trap depths ( $p > 0.25$ ). Again, the values bracketed those from Trinity Bay (Parrish et al. 2002) and averaged 17 carbon atoms and 2 double bonds. These data, combined with low C/N mass ratios (mean of 5.1) at 220 m, indicate a rapid transfer of highly nutritious material to the benthos during the spring bloom in Conception Bay. In order to quantify the transfer, PUFA proportions (Fig. 5a) and lipid class flux data (Fig. 7a,b) can be combined (Budge & Parrish 2003) to calculate PUFA fluxes (Fig. 7c) and proportions transferred (Table 4). In this way it can be determined that over half the PUFA settling from surface waters reached the benthos. The maximum net PUFA accumulation for populations of *Acanthostepheia malmgrenii* and *Mysis mixta* (Richoux 2004) accounts for  $\sim 2$  and 4% respectively of the  $0.345$   $\text{g m}^{-2}$  in the 220 m traps. The higher value for *M. mixta* suggests that this mysid population was better able to sequester settling PUFA.

### Trap performance

The temperature at the depths of the traps was  $-1^\circ\text{C}$  on deployment and recovery in 1998. A salinity determination on the bottom litre of the non-poisoned 50 m trap sample showed it remained within 93% of its original salinity. This value matched the 95% value we determined for the efficiency of our on-board particle collection procedure for sediment traps. By sampling the particles in the entire collection tube of both poisoned and non-poisoned traps we could compare the particulate lipid concentrations in the bottom with the rest of each

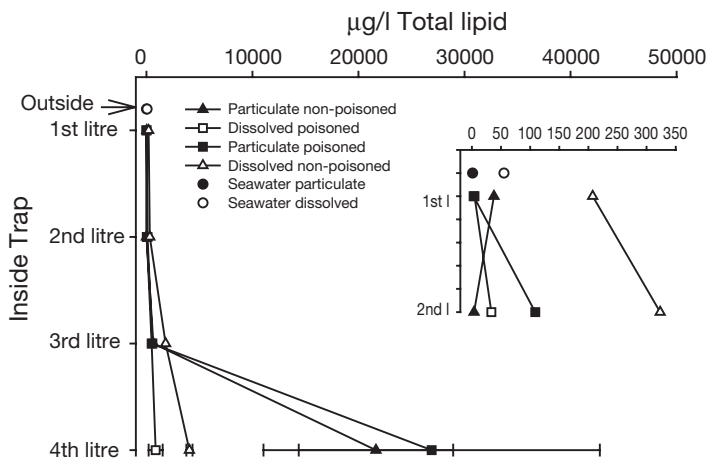


Fig. 8. Dissolved and particulate lipid concentrations within entire sediment traps set at 50 m for 15 d in April 1998. Filled symbols represent particulate samples; open symbols represent dissolved samples. Non-poisoned samples are represented by triangles, poisoned samples by squares. Samples taken outside the trap on the day of recovery are represented by circles. Data for the bottom litre are mean  $\pm$   $\frac{1}{2}$  range

trap (Fig. 8). Almost all the particulate lipids in the collection tube were found in the bottom litre, the part of the tube that we would normally sample.

At the top of the trap, both dissolved and particulate lipid concentrations were similar to those found outside the trap, except for dissolved total lipids in the non-poisoned trap, which appear to be greater (Fig. 8, inset). At the bottom of the trap these concentrations are clearly greater and a statistical analysis of the entire sediment trap array revealed that concentrations of dissolved lipids were indeed significantly higher ( $p = 0.018$ ) in non-poisoned than in poisoned trap bottom layers. By considering the sum of the dis-

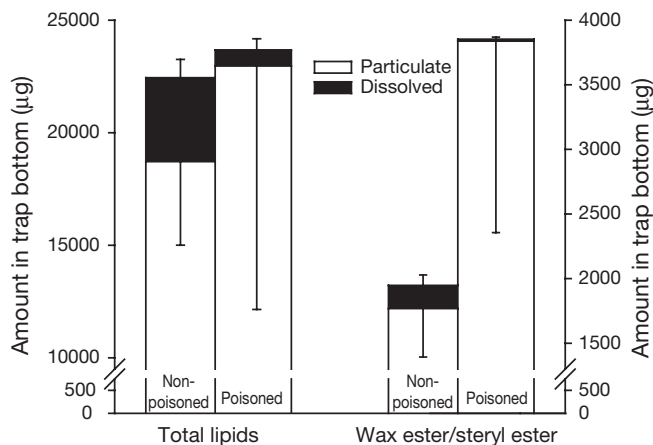


Fig. 9. Dissolved and particulate lipid content in the bottom of 50 m sediment traps following a 15 d deployment in April 1998. Total lipid and wax/steryl ester data are mean  $\pm$   $\frac{1}{2}$  range for dissolved lipids and mean  $\pm$   $\frac{1}{2}$  range for particulate lipids

solved and particulate lipids in the trap bottom (Fig. 9) it can be seen that particulate lipids represent a smaller proportion of the total in the non-poisoned traps. For the entire sediment trap array, particulate lipids accounted for  $86 \pm 7\%$  of the total in non-poisoned traps, which was significantly less ( $p = 0.016$ ) than the  $95 \pm 4\%$  of the total in poisoned traps. The evidence that poisoning is important in the reduction of bacterially mediated dissolution during long-term deployments is supported by the fact that proportions of bacterial fatty acid markers in filtrates were 7 times higher in the bottom layer of non-poisoned traps. However, as expected for low solubility compounds, the ratio of dissolved to particulate lipids was much lower than in short-term deployments (Parrish 1998).

While poisoning reduced lipid dissolution by 9%, this advantage may be outweighed by the considerable increase in variability in particulate lipid values (Figs. 8 & 9). This greater variability, together with the greater lipid content in poisoned traps, also occurred throughout the 1996 sampling season, both at 80 and at 220 m (Ramos et al. 2003). The large and variable wax ester/steryl ester content in poisoned traps (Fig. 9) indicates one reason for this. We observed many more swimmers (mainly copepods) in poisoned samples and are unlikely to have been able to remove all animals, or parts thereof, from all samples.

## CONCLUSIONS

The 1996 Conception Bay spring bloom started in mid-March at  $0^\circ\text{C}$  in the top 40 m of the water column. The  $\text{C}_{16}$  PUFA ratio reached a very high maximum of 56% in plankton 12 d later, thereafter this indicator of nutrient sufficient diatoms declined rapidly as surface nitrate concentrations decreased by 90%. Forty days after the bloom started, deep water nutrient concentrations peaked and N:P ratios indicated regeneration from nitrogen depleted particles.

Total PUFA proportions reached a maximum in the plankton 25 d after the start of the bloom, and total and neutral lipid concentrations peaked 15 d later when the chlorophyll maximum had sunk to 50 m. Samples of settling particulate matter were collected using unpoisoned sediment traps, which may underestimate lipid fluxes by  $\sim 14\%$  due to dissolution in the trap. In addition, there was evidence of admixture of lipid poor material near the bottom of Conception Bay from concentrations in 80 and 220 m traps and from integrated flux comparisons at each depth.

There were small changes in the average lipid class and fatty acid composition among the plankton and settling particle samples, suggesting some selective removal of PUFA between their synthesis in the photic

zone and arrival at the benthos. There was little offset in the timing of maximum lipid concentrations among the net-tow, 80 and 220 m trap samples, indicating a rapid transfer. Overall, half of the total lipids, neutral lipids and PUFA passing through 80 m reached 220 m, and the lipid and PUFA supply were more than 10 times that required by important amphipod and mysid populations in Conception Bay. This efficient flow of energy and essential nutrients remains to be compared with lipid requirements of other large abundant species in the depositional zone.

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