LIPID CLASS AND CARBOHYDRATE CONCENTRATIONS IN MARINE COLLOIDS

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QINGJUN LIU

LIPID CLASS AND CARBOHYDRATE CONCENTRATIONS IN MARINE COLLOIDS

By

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B.Sc and M.Eng

©

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ABSTRACT

Cross-flow filtration (CFF) was used to concentrate marine colloids from microalgal cultures and surface seawater. Lipid classes in different size fractions were determined using the Chromarod-Iatroscan thin-layer chromatography with the flame ionization detection method. Total carbohydrate concentrations were determined colorimetrically using methyl benzothiazolinone hydrazone reagent. Extensive determinations of CFF blanks and recoveries were conducted for each lipid class to optimize CFF operating procedures. The total lipid blank in the CFF system was approximately 6-7 μg ($\sim$0.5 μM C). Recoveries for the major lipid classes in marine samples varied from 80% to 120%.

Both colloidal lipid and carbohydrate concentrations in microalgal cultures were found to be 120-140 μg/L. Triacylglycerol (TAG) and polar lipids were the major lipid classes in colloids from microalgal cultures. Concentrations of colloidal (>10,000 dalton) lipids and carbohydrates in Newfoundland seawater ranged from 0.9 μg/L to 8.7 μg/L and from 21 μg/L to 30 μg/L, respectively. The major lipid classes present in coastal seawater colloids were TAG, free fatty acids, and phospholipids.

Data from both algal cultures and actual seawater samples showed that marine colloids are characterized by having high proportions of free fatty acids, phospholipids and hydrocarbons by comparison with truly dissolved material. The free fatty acids may be produced by microbial degradation, the phospholipids are probably derived from

biological membranes, and the hydrocarbons are likely preferentially adsorbed from the surrounding water onto the colloids.

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TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

LIST OF TABLES viii

LIST OF FIGURES xiii

ABBREVIATIONS AND SYMBOLS xv

1.0 LITERATURE REVIEW 1
1.1 Marine colloids 1
1.1.1 Introduction 1
1.1.2 Characteristics of marine colloids 2
1.1.3 Origin, formation and fate of marine colloids 5
1.1.4 Importance of marine colloids 7
1.1.5 Chemical composition of marine colloids 10
1.1.6 Sampling of marine colloids 14
1.2 Lipids 18
1.2.1 Introduction 18
1.2.2 GC methods 19
1.2.3 The TLC-FID method 21
1.3 Carbohydrates 22
1.3.1 Chromatographic methods 23
1.3.2 Colorimetric methods 25

2.0 OBJECTIVES 28

3.0 MATERIALS AND METHODS 31
3.1 Materials 31
3.1.1 Chemicals 31
3.1.2 Filters 34
3.2.3 Instruments 35
3.2 Methods 38
3.2.1 Sampling 38
3.2.2 Handling of filters 41
3.2.3 Treatment of samples 42

3.2.4 Measurement of lipid classes 46
3.2.5 Measurement of total carbohydrates 55

4.0 RESULTS AND DISCUSSION 60
4.1 Blank for measurements of lipid classes 60
4.1.1 Chromarod-Iatroscan system blank 60
4.1.2 Extracting solvent blank 63
4.1.3 Cross-flow filtration system blank 63
4.2 Cross flow filtration 67
4.2.1 Characteristics of CFF 67
4.2.2 Optimization of CFF system 69
4.2.3 Mass balance 69
4.3 Lipid class concentrations in seawater samples 73
4.3.1 Total lipids 73
4.3.2 Hydrocarbons (HC) 76
4.3.3 Wax and sterol esters (WE/SE) 81
4.3.4 Triacylglycerols (TAG) 82
4.3.5 Free fatty acids (FFA) 82
4.3.6 Free fatty alcohols (ALC) 84
4.3.7 Sterols (ST) 85
4.3.8 Acetone-mobile polar lipids (AMPL) 87
4.3.9 Phospholipids (PL) 88
4.4 Particulate and dissolved lipids in seawater samples 89
4.5 Lipid classes and carbohydrates in microalgal cultures 98
4.5.1 Cellular lipid class concentration 98
4.5.2 Cellular carbohydrate concentration 102
4.6 Carbohydrate in seawater samples 102
4.6.1 Total dissolved carbohydrates 103
4.6.2 Particulate carbohydrates 106
4.7 Marine colloids 108
4.7.1 Molecular weight of colloidal lipids and carbohydrates 108
4.7.2 Colloidal carbohydrate and lipid class concentrations 110
4.7.3 Composition of colloidal lipids 114
4.7.4 The distribution of lipid classes 119

5.0 CONCLUSIONS 122

REFERENCES 126

APPENDIX 142

LIST OF TABLES

Table		Page
1.1	Percentage of colloidal organic carbon in "dissolved" organic carbon . . .	9
1.2	Cross-flow filtration in marine applications	16
3.1	Marine lipid classes and standards used for their identification and calibration by TLC-FID	32
3.2	Microalgal culture conditions	39
3.3	Station BRLP4 hydrography	39
3.4	Filtration flow diagram for an *Isochrysis galbana* (T-ISO) culture sample.	45
3.5	Operating conditions for the Iatroscan MK-5 TLC-FID analyzer	51
3.6	Flow chart for determining carbohydrates by the MBTH method	57
4.1	Total lipid concentrations in dissolved and particulate fractions of seawater in different locations	61
4.2	Contributions to the blank by each step in the procedure	62
4.3	Comparison of total blank (μg) before filtering culture samples with after cleaning the CFF system with methanol-water (1:1 v/v)	65
4.4	Lipid class concentrations (μg/L, mean $\pm$ S.D., n= 4-8) in unfiltered seawater samples collected in Conception Bay, Newfoundland.	74
4.5	Total hydrocarbon concentrations (μg/L) in seawater. Data from the literature are the sum of dissolved and particulate concentrations.	78
4.6	Total free fatty acid concentrations (μg/L) in seawater. Data from the literature are the sum of dissolved and particulate concentration.	83
4.7	Total sterol concentrations (μg/L) in seawater. Data from the literature are the sum of dissolved and particulate concentration.	86

4.8 Particulate (part.) and dissolved (diss.) lipid concentrations in seawater collected in Conception Bay in 1991 and 1993 92

4.9 Linear regression on lipid class concentration in 75 μm filtration versus ultrafiltrate (10K-F) filtrate 94

4.10 Concentrations (pg/cell, mean ± S.D., n = 4-8) and proportion (%) of solvent-extractable lipid class and total carbohydrate concentrations in microalgal cultures 100

4.11 Dissolved carbohydrate concentrations in filtered seawater 104

4.12 Carbohydrate concentration (μM C/L, mean ± S.D., n = 3-5) in seawater collected from station BRLP4 in Conception Bay, Newfoundland 105

4.13 Total lipid concentrations (μg/L, mean ± S.D., n = 3-6) and total carbohydrate concentrations (μg/L, mean ± S.D., n = 4-8) in different size class particles for microalgal samples 111

4.14 Total lipid concentrations (μg/L, mean ± S.D., n = 3-6) and total carbohydrate concentrations (μg/L, mean ± S.D., n = 3-5) in different size class particles for seawater samples collected from Conception Bay, Newfoundland 112

A1 Flow diagram for the filtration of *Isochrysis galbana* (T-iso) culture (5.8 x 10^6 cell/mL, 0.61 div./day) grown on semicontinuous and N-replete condition 142

A2 Flow diagram for the filtration of *Isochrysis galbana* (T-iso) culture (4.2 x 10^6 cell/mL, 0.90 div./day) grown on semicontinuous and N-replete condition 143

A3 Flow diagram for the filtration of *Chaetoceros muelleri* culture (4.4 x 10^6 cell/mL, 0.24 div./day) grown on semicontinuous and N-replete condition 144

A4 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland on May 19, 1993 145

A5 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland on June 14, 1993 146

A6 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland on August 2, 1993 147

A7 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland on October 5, 1993 148

B1 Analytical data of lipid standard solution by TLC-FID 149

B2 Linear regression between peak area (mV·mm) and lipid class load (μg) 150

B3 The relationship between absorbency of colour complex compound and standard glucose concentration (μM) 151

B4 Regression between absorbency and glucose standard solution (μM) . . . 152

C1 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-12) in different filtration fractions of an *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) culture and recoveries (%) on three filters 153

C2 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-12) in different filtration fractions of an *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) culture and recoveries (%) on three filters 154

C3 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-12) in different filtration fractions of a *Chaetoceros muelleri* (4.4 x 10^6 cells/mL, 0.24 div./day) culture and recoveries (%) on three filters 155

C4 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-12) and distribution (%) in different particle pools and "dissolved" fraction of an *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) culture 156

C5 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-12) and distribution (%) in different particle pools and "dissolved" fraction of an *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) culture 157

C6 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-12) and distribution (%) in different particle pools and "dissolved" fraction of a *Chaetoceros muelleri* (4.4 x 10^6 cells/mL, 0.24 div./day) culture 158

C7 Lipid composition percentage (%, mean $\pm$ S.D., n = 6-12) in different particle pools, the dissolved fraction and the unfiltered fraction for *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) culture 159

C8 Lipid composition percentage (%, mean $\pm$ S.D., n = 6-12) in different particle pools, the dissolved fraction and the unfiltered fraction for *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) culture 160

C9 Lipid composition percentage (%, mean $\pm$ S.D., n = 6-12) in different particle pools, the dissolved fraction and the unfiltered fraction for *Chaetoceros muelleri* (4.4 x 10^6 cells/mL, 0.24 div./day) culture 161

D1 Lipid class concentration (μg/L, mean $\pm$ S.D., n = 6-10) in different filtration fraction of seawater collected in Conception Bay, Newfoundland in May 19, 1993 and recoveries (%) on two filters 162

D2 Lipid class concentration (μg/L, mean $\pm$ S.D., n = 6-10) in different filtration fraction of seawater collected in Conception Bay, Newfoundland in June 24, 1993 and recoveries (%) on two filters 163

D3 Lipid class concentration (μg/L, mean $\pm$ S.D., n = 6-10) in different filtration fraction of seawater collected in Conception Bay, Newfoundland in August 2, 1993 and recoveries (%) on two filters 164

D4 Lipid class concentration (μg/L, mean $\pm$ S.D., n = 6-10) in different filtration fraction of seawater collected in Conception Bay, Newfoundland in October 5, 1993 and recoveries (%) on two filters 165

D5 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-10) and distribution (%) in different particle pools and "dissolved" fraction of seawater collected from Conception Bay on May 19, 1993 166

D6 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-10) and distribution (%) in different particle pools and "dissolved" fraction of seawater collected from Conception Bay on June 24, 1993 167

D7 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-10) and distribution (%) in different particle pools and "dissolved" fraction of seawater collected from Conception Bay on August 2, 1993 168

D8 Lipid class concentrations (μg/L, mean $\pm$ S.D., n = 6-10) and distribution (%) in different particle pools and "dissolved" fraction of seawater collected from Conception Bay on October 5, 1993 169

D9 Lipid composition percentage in different particle pools, dissolved, and total lipid in seawater collected from Conception Bay, Newfoundland, on May 19, 1993 170

D10 Lipid composition percentage in different particle pools, dissolved, and total lipid in seawater collected from Conception Bay, Newfoundland, on June 24, 1993 171

D11 Lipid composition percentage in different particle pools, dissolved, and total lipid in seawater collected from Conception Bay, Newfoundland, on August 2, 1993 172

D12 Lipid composition percentage in different particle pools, dissolved, and total lipid in seawater collected from Conception Bay, Newfoundland, on October 5, 1993 173

D13 Lipid class concentration (μg/L, mean $\pm$ S.E.M, n = 3) in different filtration fractions of seawater collected in Conception Bay on May 19, June 14, August 2, 1993 and mass balance (%) on two filters 174

LIST OF FIGURES

Figure		Page
3.1	Diagram of Millipore cross-flow filtration system	36
3.2	Diagrams for major operating procedures in TLC-FID method	37
3.3	Map showing the location of the BRLP4 sampling station.	40
3.4	Typical TLC-FID chromatogram for lipid analysis in seawater samples. No internal standard was added to this sample.	50
3.5	Calibration curves for each lipid class measured by TLC-FID	52
3.6	Chemical reactions for determination of carbohydrate by the MBTH method .	56
3.7	Calibration curve for carbohydrate measured by the MBTH colorimetric method	59
4.1	Mass balance (%, mean ± S.E.M., n = 3-4) of lipid classes in seawater sample for two filters	72
4.2	Total lipid concentrations in seawater	75
4.3	Concentration distribution of total lipid in particle pools and dissolved fraction	91
4.4	Particulate (3nm-75μm) and dissolved (<3nm) lipid concentrations in surface mixed layer	93
4.5	Linear regression of phospholipid concentration in 75 μm filtrate versus ultrafiltrate (10K-F) fraction.	95
4.6	Colloidal lipid class concentrations (μg/L, mean ± S.D., n = 3-5) for *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) sample	115
4.7	Colloidal lipid class concentration (μg/L, mean ± S.D.n = 3-5) for *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) sample	116

4.8 Colloidal lipid class concentration (μg/L, mean $\pm$ S.D., n = 3-5) for *Chaetoceros muelleri* (4.4 x 10^6 cells/mL, 0.24 div./day) sample 117

4.9 Lipid class concentration (μg/L, mean $\pm$ SEM, n = 3) in different size fraction for surface seawater sample collected from May to August, Conception Bay, NF. 118

4.10 Lipid class distribution (%) in particle pools and "dissolved" fraction for seawater samples collected from May to August, Conception Bay, NF. . 120

ABBREVIATIONS AND SYMBOLS

$\delta^{13}C$	carbon-13 isotope abundance
μm	micrometer
μM C	micromoles of carbon per litre
0.45μm-F	filtrate passed through a filter with 0.45 μm pore size
0.45μm-R	retentate of a filter with 0.45 μm pore size
10K-F	filtrate passed through a filter with 10,000 NMWL cut-off
10K-R	retentate of a filter with 10,000 NMWL cut-off
A/E-F	filtrate passed through a Gelman A/E filter
A/E-R	retentate of a Gelman A/E filter
ALC	free fatty alcohols
AMPL	acetone-mobile polar lipid
AOU	apparent oxygen utilization
AVG	average value
C.V.	coefficient of variation
CFF	cross-flow filtration
COC	colloidal organic carbon
COM	colloidal organic matter
DOC	dissolved organic carbon

DOM	dissolved organic matter
EDS	energy-dispersive x-ray spectroscopy
ESD	equivalent spherical diameter
FFA	free fatty acid
FIA	flow injection analysis
FID	flame ionization detector
GC	gas chromatography
GF/C-R	retentate of a Whatman GF/C filter
GF/C-F	filtrate passed through a Whatman GF/C filter
HC	hydrocarbon
HPLC	high performance liquid chromatography
HTCO	high temperature catalytic oxidation
i.d.	inside diameter
kDa	Dalton x 1000
KET	ketone
MBTH	3-methyl-2-benzothiazolinone hydrazone hydrochloride
MS	mass spectroscopy
MW	molecular weight
nm	nanometer
nM	nanomoles per litre
NMR	nuclear magnetic resonance

NMWL	nominal molecular weight limit
OM	organic matter
PL	phospholipid
POC	particulate organic carbon
rpm	revolutions per minute
RT	room temperature
S.D.	standard deviation
SE	sterol ester
ST	sterol
TAA	total amino acids
TAG	triacylglycerol
TDCHO	total dissolved carbohydrate concentration
TEM	transmission electron microscopy
TLC	thin layer chromatography
UF	unfiltered sample
WCO	wet chemical oxidation

1.0 LITERATURE REVIEW

1.1 Marine colloids

1.1.1 Introduction

Colloids are very small particles with dimensions between 1 nm and 1 μm that exhibit Brownian motion. This definition, involving a wide range of sizes, implies the ubiquity of colloidal systems in the natural world.

In marine science, the definition of the "dissolved" fraction and the "particulate" fraction is usually operational. These definitions are quite simple. For example, the division between dissolved and particulate organic matter depends on whether the particles pass through a 0.45 μm membrane. Obviously, such a definition cannot distinguish the truly dissolved fraction and the colloidal fraction. In fact, the colloidal fraction has been neglected in the traditional operational definition.

In recent years, there has been an increasing awareness that marine colloids play an important role in biogeochemical processes, especially in the oceanic carbon cycling and trace metal scavenging processes. Marine colloids are not only different from larger particles (>1 μm) but also from the truly dissolved fraction (<1 nm). Unlike larger particles, marine colloids do not settle in response to gravitational fields and have a larger liquid/solid interface than larger particles (>1 μm). On the other hand, marine colloids, like dissolved matter, are subjected to the same transportation and mixing processes as the water mass in which they are suspended, but their biological and chemical properties may be vastly different from those of truly dissolved matter of

similar gross chemical composition. Therefore, marine colloids have their own characteristics governing their oceanic biogeochemical processes.

1.1.2 Characteristics of marine colloids

From the operational perspective, marine colloids include clay minerals, organic detritus, picoplankton, bacteria, viruses, and macromolecules. Colloidal particles in seawater have recently been found to be quite abundant. There are about 10^6-10^7 particles (size range 0.38-1 μm) per mL in the upper layer (50 m) of the ocean (Koike *et al.*, 1990). Smaller marine colloids (< 120 nm in size) are at least three orders of magnitude more abundant than larger colloids (size range 0.38-1 μm: Wells and Goldberg, 1991). The size spectra in the open ocean show increases in colloid numbers with decreasing size, often nearly logarithmically in surface waters (Wells and Goldberg, 1993). Particles smaller than 120 nm are extremely abundant in coastal surface water, particularly near the seasonal thermocline. They are 3-6 orders of magnitude more abundant than either bacteria or plankton.

Vertical profiles have shown that colloids < 120 nm in size were highly stratified with a mid-depth maximum (Wells and Goldberg, 1991). This distribution is different from that reported for larger (0.38-1.0 μm) colloids in the northwest Pacific, where the concentration sharply decreased with depth (Koike *et al.*, 1990).

The large concentration of marine colloids in seawater provides a large solid/liquid interface. The surface area is 0.2-0.5 cm^2 mL^{-1} of seawater for particles with

size range 0.36-1.0 μm (Koike *et al.*, 1990). The total surface area of smaller marine colloids (5-120 nm, 10^9 particles mL^{-1}) reaches 0.08 cm^2 mL^{-3} of seawater (Wells and Goldberg, 1992).

Koike *et al.* (1990) found >95% of colloidal particles (0.4-1 μm in size) are non-living but seem to be composed largely of organic material. This finding was confirmed by Wells and Goldberg (1991), when they examined colloidal particles by transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS). The low electron opacity of these colloids suggests that they are composed of largely organic materials though they also may contain some metals such as Fe, Al and Co. TEM examination also indicated that many of smaller colloids are aggregates of granules 2-5 nm in size (Wells and Goldberg, 1992).

The residence time of colloidal matter was studied using naturally occurring ^{234}Th as an *in situ* tracer (Moran and Buesseler, 1992; Santschi *et al.*, 1994). The results show that the ^{234}Th activity associated with colloidal matter (size range 10k Da to 0.2 μm) is similar to that associated with smaller particles (0.2-53 μm). Both macromolecular colloidal matter and smaller particles have a short residence time ($\sim <$ 10 days) and a rapid turnover rate in the upper open ocean. Santschi *et al.* (1994) also confirmed the colloidal pumping model proposed by Honeyman and Santschi (1989). This model involves a more physical rather than a biological driving mechanism for colloid turnover.

Colloidal matter was thought to be chemically refractory and biologically labile. DOC values obtained by the high-temperature catalytic oxidation (HTCO) method are usually higher than those obtained by wet chemical oxidation (WCO) methods. Suzuki (1988) attributed part of the newly enlarged DOM pool to colloidal macromolecules (i.e., nanometre-sized). The extra colloidal organic matter could not be oxidized by UV irradiation or persulfate oxidation, thus, it was thought to be chemically refractory. On the other hand, colloidal organic matter was thought to be biologically labile because of correlations between colloidal DOC and the apparent oxygen utilization (AOU) (Sugimura and Suzuki, 1988; Suzuki *et al.*, 1992; Guo *et al.*, 1994). The statement that colloidal matter is biologically labile could be supported indirectly or directly by the following facts: they have short residence times and rapid turnover rates (Moran & Buesseler, 1992; Santschi *et al.*, 1994); they can be utilized indirectly or directly by bacteria or microzooplankton (Flood *et al.*, 1992); bacterial respiration and cell number both increase when COC and bacteria came into close contact through surface coagulation (Johnson *et al.*, 1986; Kepkay and Johnson, 1988, 1989; and Kepkay, 1990a, b).

Koike *et al.* (1990) found half of the larger colloids (>0.38 μm equivalent spherical diameter (ESD)) can pass through a 0.1 μm Nuclepore filter. This suggests that marine colloids have a highly flexible, amorphous structure.

Thus, from a variety of perspectives, marine colloids do have their own characteristics and behaviour which should greatly affect the chemistry and cycling of OM and trace metals.

1.1.3 Origin, formation and fate of marine colloids

Although the frequency distribution of large particles (1-100 μm) varies geographically and with depth (Sheldon *et al.*, 1972), the similarities in colloid morphology, abundance and size distribution in different oceanic areas suggest that there are some general mechanisms to explain the origin, formation, and removal of marine colloids.

A significant linear correlation between the number of submicrometre particles (0.38-1 μm) and bacterial number in different pelagic stations as well as chlorophyll a indicates a biological origin for these particles. The vertical stratification (nonconservative behaviour) of smaller colloids (<200 nm) and their organic nature suggest they are derived primarily from biological processes.

These colloids were thought to have a biogenic origin, but how were they formed? Marine viruses, bacteria, and picoplankton may contribute to these abundant colloids. They can also originate from release and degradation of microorganisms and the disruption of particulate materials. Monomeric or macromolecular materials of biogenic origin may condense into colloids by flocculation (Sholkowitz, 1976), abiological binding (Carlson *et al.*, 1985), and photochemically initiated cross-linking (Harvey *et al.*, 1983).

They may also be formed by the collapse of bubbles, and by growth *in situ* (Biddanda, 1985; Johnson *et al.*, 1986). At present, the formation mechanism is still not clear.

What is the fate of marine colloids? Since marine colloids do not sink directly to the sediments, only three pathways can be used to explain the fate of marine colloids: dissolution, direct utilization, and aggregation. Dissolution appears to be the least likely explanation for the removal of marine colloids from the water column. Hydrolytic enzymes have been assumed to render aggregates (>0.5 μm) soluble (Smith *et al.*, 1992), but if dissolution was the dominant mechanism for removing marine colloids from the water column, it would be difficult to explain why particle numbers increase with decreasing size.

The coagulation among colloids to form aggregates, and then sinking to sediment or utilization by microzooplankton appears to be the principal mechanism for removing small colloids (McCave, 1984; Wells and Goldberg, 1992a, b). An aggregate-dominated mechanism is consistent with a particle size distribution in which particle number increases nearly logarithmically with decreasing size. McCave (1984) suggested that the aggregation rates of colloids is mainly determined by the collision frequency and coalescence efficiency in Brownian motion. Johnson and Kepkay's (1992) studies also highlight the importance of coagulation and formation of aggregates before larger colloids can be utilized efficiently by bacteria. The evidence shows mobile bacteria in a turbulent flow are the least efficient in their ability to interact with colloids in the 0.1-1 μm size range. However, fine colloidal matter can be directly utilized as food by

microzooplankton. Some studies have suggested COM is chemically refractory but biologically labile. The mean residence time of smaller colloids was reported to be short and similar to larger colloids or larger particles (Morgan *et al.*, 1992; Santschi *et al.*, 1994). All of this suggests colloidal matter can be utilized as large particles by microorganisms. On the other hand, it is interesting that particle-size spectra are reported as log normal distributions with a peak at about 0.5 μm (Lambert *et al.*, 1980; Longhurst *et al.*, 1992). The valley near the peak seems to indicate colloids with 0.35-0.45 μm size range can be utilized directly besides being efficiently utilized after they have formed larger aggregates. This judgement has been supported by new evidence that oikopleurids consume colloidal organic carbon in size ranges down to about 0.2 μm in diameter (Flood *et al.*, 1992).

Although the exact origin, formation, and removal mechanisms of marine colloids are not clear at the moment, the proposed mechanisms of their formation and removal are important to help us recognize the nature and role of marine colloids in the ocean.

1.1.4 Importance of marine colloids

Traditionally, it was believed that larger particles (>1 μm) played the most important role in marine carbon dynamics as they settle out of water column. Because marine colloids do not sink in water column, they were considered not to be as important as larger particles. However, as the nature of marine colloids is being revealed, the

importance of marine colloids in oceanic biogeochemical processes is gradually being recognized.

Firstly, a large quantity of "dissolved" organic carbon is actually present in colloidal form. In 1970, Ogura found that between 8% and 12% of total dissolved organic carbon was in the size range of 0.1 μm and 0.5 μm. A little later, Sharp (1973) also found a large amount of particulate carbon to be present in colloidal size range. Particles with a range of 3 nm to 25 nm contained about 10 to 30% of the total organic carbon present. From the increasing colloidal organic carbon (COC) data available (**Table 1.1**), COC (1 kDa to 1.0 μm) accounts for at least 10% of DOC, although the amount of DOC and COC in the ocean is at present controversial and difficult to compare because of differences in blank corrections, analytical methodology, spatial variability, and use of different filters, and also because of the flexible nature of colloids. However, most of these independent investigations (**Table 1.1**) indicate that a significant fraction of the "dissolved" organic matter in seawater exists in colloidal form and that this colloidal organic carbon is important in the dynamics of the carbon cycle. The latest data (Guo *et al.*, 1994, Bauer *et al.*, 1994) show as much as 45% of DOC is in colloidal form (> 1 kDa MW).

As mentioned above, marine colloids have a large solid/liquid interface (Wells and Goldberg, 1992). Many interactions occur at interfaces. These interfacial reactions include adsorption of trace organics and metals (Means and Wijayaratne, 1982), polymerization of organic materials adsorbed on surfaces (Degens and Matheja, 1967),

Table 1.1 Percentage of colloidal organic carbon (COC) in "dissolved" organic carbon

	Reference	Size Range	Percentage (%)	DOC (μM C)	Sample Location
1973	Sharp	0.025 – 0.8 μm	8	64–145*	West Central North Atlantic Ocean
		3 nm – 0.8 μm	16	64–145*	
1976	Maurer	>1 kDa	10–15	40–75	Gulf of Mexico
		>10 kDa	<10	40–75	
1979	Zsolnay	>20 kDa–0.45μm	11	109±5*	Coast of Shackelford Island, North Carolina
1985	Carlson et al.	1 – 30 kDa	28	67–168*	Coastal and North Atlantic
		30 – 100 kDa	5	67–168*	
		>100 kDa	1	67–168*	
1986	Whitehouse et al.	10 kDa – 1 μm	15.8±5.8	NA	Baltic sea
1988	Sugimura and Suzuki	>1.8 kDa	85		Northwestern Pacific Ocean
1989	Moran and Moore	10 kDa – 0.45 μm	10–15	47–77	Coastal ocean off Nova Scotia
1990	Whitehouse et al.	10 kDa – 1 μm	3.5	28–60*	Shelf break
1991	Brownawell	>5 kDa	18	79–198	Seawater
1991	Hollibaugh et al.	10 kDa – 1.2 μm	28	42–129	Tomales Bay, California
1992	Benner et al.	1 kDa – 0.2 μm	22–33	38–82	Pacific Ocean
1992	Ogawa and Ogura	1 kDa	30–37	70.3–87.5	Northwestern Pacific Ocean
		10 kDa	3.8–4.9	70.3–87.5	
1993	Kepkay et al.	10 kDa – 0.2 μm	4.4 – 16.5	70–158	Bedford Basin, Nova Scotia
1994	Guo et al.	>1 kDa	40–53	50–131	Gulf of Mexico
		>10 kDa	8–14	50–131	
1994	Bauer	>1kDa	45	NA	Pacific Ocean
1994	Sempere et al.	0.01 – 0.7 μm	7–49	82±10	Rhone Delta
		>1kDa	30–50	80	

* Values calculated based on original data.

complexation of trace metals with organic materials (Moore *et al.*, 1979), and coagulation to form aggregates (Johnson and Kepkay, 1992). These reactions can produce macromolecules or larger colloids. Adsorption at particle surfaces not only influences the distribution and concentration of trace metals and biogenic organics but also their bio-availability (Sugai and Henrichs, 1992). The formation of macromolecules and aggregates by interfacial actions is an important step in the bacterial utilization of DOM (Johnson and Kepkay, 1992). Interfacial actions on surfaces of marine colloids are important processes for transferring dissolved substances into particulate (>1 μm) substances (Farley and Morel, 1986; Honeyman and Santschi, 1989, 1991). It is thus apparent that marine colloids can play an important role in enriching particles with OM and trace metals and may be responsible for transporting contaminants into food webs.

1.1.5 Chemical composition of marine colloids

Despite the importance of marine colloids in biogeochemical processes, relatively little progress has been made on determining the chemical structure and composition of colloidal matter, partly because of the apparently complex, macromolecular nature of colloidal matter, the difficulties in isolating sufficient unaltered material for characterization, and the limitation in analytical methodology. Limited studies available mainly focus on elemental components such as nutrient elements (C, N, P) and trace metals (Al, Fe, Mn, Cu, ^{234}Th, ^{7}Be, ^{210}Pb).

From **Table 1.1**, we know about 20-45% of DOC is present in colloidal form. If the average DOC concentration in the ocean is taken to be 80 μM C, there is about 200 μg–400 μg of colloidal organic matter per litre of seawater. Hollibaugh *et al.* (1991) investigated colloidal (size range from 10 kDa to 1.2 μm) and dissolved (< 10 KD) nutrient concentrations in samples from a temperate shore environment (Tomales Bay, California). They found a significant amount of organic carbon (28%), organic nitrogen (27%) and organic phosphorus (71%) in GF/C (1.2 μm) filtrates was present in colloidal form (10 kDa to 1.2 μm). However, only 3% of reactive phosphorus and 1% of ammonium was present in colloidal form. They found no evidence for nitrate plus nitrite or silicate as components of marine colloids. Similarly, Bauer *et al.* (1994) found colloidal organic material (> 1 kDa MW) comprised approximately 45% of the total DOC. The $\Delta^{14}C$ value of this colloidal fraction was identical with those of the total DOC while $\delta^{13}C$ value was significantly lower than those of total DOC. Their results show colloidal organic material was N-poor (C/N ~ 15-19) relative to total dissolved organic matter (C/N ~ 8-10).

Moran and Moore (1989) investigated the distribution of colloidal (10 kDa-0.45 μm) aluminum and organic carbon in coastal and open ocean waters. They found < 5% Al and < 10-15% OC in dissolved (< 10 kDa) fractions were present in colloidal form. Some metals (Fe, Mn, Cu) associated with marine colloids were studied by Whitehouse *et al.* (1990). Baskaran *et al.* (1992) proposed that 80% of Th is associated with colloidal (10 kDa to 0.4 μm) materials in seawater samples from the Gulf of Mexico. 32% of

^{234}Th and 8.3% of ^{7}Be in the "dissolved" (<0.4 μm) fraction of coastal seawater was present in colloidal (10 kDa to 0.4 μm) form (Baskaran and Santschi, 1993).

The biochemical composition (amino acids, carbohydrates, and lipids) of colloidal matter has been investigated mainly in estuarine environments. Colloidal matter in the size range 1.2 nm to 0.4 μm isolated from Chesapeake Bay was studied by pyrolysis GC/MS (Sigleo *et al.*, 1982). The results obtained are consistent with the composition of Chesapeake Bay phytoplankton (approximately 50% protein, 30% carbohydrate, 10% lipid, and 10% nucleotide by dry weight). These results suggest that aquatic microorganisms are the dominant source of colloidal organic material in estuarine surface water. Sigleo *et al.* (1983) also found that the hydrolysable amino acids and associated ammonia account for 80% of the nitrogen that existed in estuarine colloidal (1.2 nm-0.4 μm) samples from surface waters that ranged from fresh to brackish water (12 g/kg salinity). Total colloidal (1.2 nm-0.45 μm) carbohydrates, amino acids, and lipids in estuary water from Chesapeake Bay accounted for 35-60%, 4-13% and less than 1%, respectively, of DOC (<1.2 nm) (Means and Wijayaratne, 1984).

Therefore, estuarine colloids are composed mainly of carbohydrate and proteinaceous materials associated with different amounts of clay minerals and trace metals. What then is the composition of marine colloids? How does the salinity affect the composition of marine colloids? A strong negative correlation between colloidal carbon concentrations and salinity was found, indicating that the bulk of the colloidal matter in rivers precipatated on contact with coastal seawater. Zsolnay (1979) suggested that

coastal and estuarine colloidal material are composed of different compounds. Coastal colloidal material had no significant fluorescence or light absorption in the UV and visible range, indicating a paucity of aromatics and other chromophores (Zsolnay, 1979).

In seawater, colloidal organic matter (COM) with MW between 3,000 and 5,000 Daltons was indicated to be proteinaceous by Degens (1970). It was found that proteinaceous matter can comprise up to 60% of the colloidal nitrogen and over a third of the colloidal carbon (Sharp, 1975). However, recent research work (Bauer *et al.* 1994) showed COM was N-poor OM relative to DOM. Benner *et al.* (1992) reported that colloidal (1 kDa to 0.2 μm) polysaccharides account for ~50% of DOC (<1 kDa) in surface water (10 m) and ~25% of DOC in deep samples. This evidence indicates that carbohydrate-proteinaceous materials are also major components of marine colloids apparently in disagreement with the results of Maurer (1976) and Zsolnay (1979). Maurer (1976) found that only 10-15% of total DOC exists as compounds with a molecular weight > 1,000 and carbohydrate and proteinaceous matter are minor proportion in these compounds.

Biogeochemical processes in oceans can be influenced greatly by the chemical composition of marine colloids. Knowledge of the chemical composition of colloidal matter can explain many biogeochemical processes, as the chemical nature of colloidal matter is an important factor in determining how it will behave in relation to other substances in the water column. With the development of effective sampling techniques

and new analytical methods, it should be possible to find out the relationships among the various processes.

1.1.6 Sampling of marine colloids

Studies that assess the quantity, composition, distribution, and characteristics of colloids in aquatic systems require an efficient collection process for colloidal/macromolecular materials. There is a variety of methods (e.g. ultracentrifugation, size-exclusion chromatography, adsorption and filtration) to isolate macromolecular/colloidal materials.

Wells *et al.* (1991) used ultracentrifugation to collect directly on specimen grids for transmission electron microscopy (TEM) studies of colloidal number, size and distribution in seawater. It is very difficult using this method, to obtain a large collection of macromolecular/colloidal materials from seawater.

The conventional adsorption method for the separation of DOM from seawater is based on the adsorption of acidified DOM onto nonionic XAD resins and depends on an adjustment of pH. The major shortcomings of this method are low recovery and preferences for hydrophobic substances, as well as other constituents of DOM.

Unlike the adsorption method, filtration is based on the size of colloids rather than their chemical properties. This method does not depend on the DOM constituents of colloids, therefore the filtration method is widely used to obtain a colloidal /macromolecular fraction. However, traditional ultrafiltration has a slow filtration rate

(on the order of mL/h) and the filters are easily clogged. With the development of new filtration techniques such as cross-flow filtration, filtration has become a powerful tool in isolating colloids for various marine studies (**Table 1.2**).

Unlike the conventional filtration method, the flow in cross-flow filtration (CFF) is parallel rather than perpendicular to the filter. Tangential flow flushes particles away from the filter thus reducing filter clogging. Application of multiple filters is helpful in increasing the filtration rate. The filtration rate of CFF can reach L/h (Carlson *et al.*, 1985; Whitehouse, 1990). Being able to filter large volumes is very important in accurately determining trace materials in the aquatic colloidal fraction because the use of small samples magnifies problems caused by contamination of trace materials. CFF decreases the conventional problem of larger colloids staying on the surface of membranes, and it maintains an approximately constant nominal filter pore size during filtration. These features make CFF suitable for studies of trace materials in the colloidal fraction.

In the CFF method, colloidal/macromolecular fractions can be obtained by filtering a prescreened sample through ultrafilter membranes. It is obvious that isolation of colloidal/macromolecular fractions depends on the prefiltration filter and ultrafilter membrane. Sheldon *et al.* (1969) reported that all the filters removed many particles from suspension which are much smaller than the minimal pore size of the filters. Therefore, the pore size of a filter is not a good indicator of its effectiveness for separating different size fractions of marine particles.

Table 1.2 Cross-flow filtration (CFF) in marine applications

Reference	CFF apparatus & filter	Particle size range	Volume	Research area
Whitehouse et al. (1986)	Millipore Pellicon CFF Polysulfone PTGC filter	10 kD – 1 μm	200 L	Organic carbon (OC) in seawater
Moran & Moore (1989)	Millipore Pellicon CFF Polysulfone PTGC filter	10 kD – 0.45 μm	70 – 100 L	OC and trace metals in seawater and river water
Whitehouse et al. (1989)	Osmonics Inc. OSMO/AN filter	10 kD – 0.2 μm	–	OC in seawater and river water
Whitehouse (1990)	Millipore Pellicon CFF Polysulfone PTGC filter	10 kD – 1 μm	6–102 L	OC and trace metals in seawater and river water
Baskaran et al. (1992)	Amicon DC10L CFF Amicon H10P10–20 filter	10 kDa–0.4 μm	200 L	Th–234
Benner et al. (1992)	Amicon DC10L CFF spiral-wound polysulfone filter	10 kDa–0.2 μm	200 L	OC and carbohydrates in seawater
Moran & Buesseler (1992)	Millipore CFF 10 k NMW cut-off filter	10 kDa–0.2 μm	80–100 L	Residence time of colloids in seawater
Kepkay et al. (1993)	Millipore Pellicon CFF Polysulfone PTGC filter	10 kDa–0.2 μm	100 L	OC in seawater
Guo et al. (1994)	Amicon DC10L CFF Amicon S10N1 & H10P10–20 filter	1 kDa–10 kDa	200 L	OC in seawater
Santschi et al. (1994)	Amicon DC10L & DC30L CFF 1 k & 10 k NMW cut-off filter	10 kDa–0.2 μm	200–1,000 L	Residence time of colloidal matter in seawater and mechanism for colloid turnover
Benoit et al. (1994)	Amicon H10P10–20 filter (10 k NMW cut-off)	10 kDa–0.5 μm	100–200 L	Trace metals in seawater
This study	Millipore CFF Polysulfone PTGC filter	10 kDa–1 μm	8–18 L	Colloidal lipid classes and carbohydrates in seawater and cultures

After further studying several types of membranes and glass-fibre filters, Sheldon (1972) pointed out that the average minimum size of particles retained by silver membranes (Flotronic®) and polycarbonate membranes (Nuclepore®) was similar to the stated pore size when relatively small seawater samples with moderate concentrations of particles were filtered. However, all cellulose ester membranes retain particles much smaller than the minimal pore size. Borosilicate glass-fibre filters also suffer from this problem.

Although the glass-fibre filters have some drawbacks as mentioned above, they still have been employed widely because of their relatively high flow rates and high loading capacities (Altabet, 1990). Organic contaminants on glass-fibre filters are easily removed by combustion at around 400 °C. Combustion of filters alters the effective pore size toward the larger size. In fact, no filters have a uniform pore size and the specified filter pore size is a mean value. On the other hand, the particle size distribution in the water column is continuous. The definitions of particulate, colloidal, and truly dissolved fraction are arbitrary. Therefore, the isolation of macromolecular/colloidal matter depend greatly on the choice of filters.

Polysulfone ultrafilter membranes are usually employed to set a division between macromolecular/colloidal matter and the dissolved fraction. Carlson *et al.* (1985) re-evaluated the uncertainties of ultrafiltration by measuring amounts of DOM in ultrafiltrates relative to the original solutions. A total of 9 Amicon 43 mm diameter ultrafiltration membranes (nominal MW cut-off range from 500 to 300,000 Da) were

examined and 31 oceanic samples were ultrafiltered through filters with pore sizes ranging from 0.02 to 1.2 μm. The experiment indicated the majority of ultrafilter-derived MW data are consistent with filter patterns shown previously; earlier reports of high-MW DOM in seawater were apparently exaggerated due to procedural and ultrafilter artifacts.

The other problems with filtration involve adsorption of some dissolved organics, especially surface-active materials (Quinn and Meyers, 1971; Gordon and Sutcliffe, 1974; Uno, 1976; Abdel-Moati, 1990) and the release of dissolved biochemicals from cell lysis caused by high filtration pressures or by the filter structure itself (Nagata and Kirchman, 1990). Contamination by filtration membranes (Norrman, 1993) and electrostatic rejection of polyelectrolyte DOM can also be problems. Any researcher interested in colloids should be aware of these problems. Some precautions should be taken in order to isolate representative colloidal/macromolecular materials.

1.2 Lipids

1.2.1 Introduction

Unlike the hydrophilic compounds, amino acids, and carbohydrates, marine lipids constitute a group of hydrophobic organic compounds easily soluble in organic solvents. A characteristic of the chemical composition of lipids is the presence of long alkyl chains in the molecules giving them their hydrophobic character. Because of this, lipids may serve as a means of transport for hydrophobic pollutants in the marine environment.

The composition and quantity of the various lipid classes in the dissolved, colloidal, and particulate fractions can provide clues to the nature and origin of the organic material as well as to its role in food web dynamics and contaminant transfer.

Due to the low concentration and complex composition of lipids in seawater, the methodological difficulties involved in sample processing, and the low sensitivity of analytical instrumentation, reliable quantitative data were unavailable until the late 1960's (Jeffery, 1966; Zsolnay, 1977). Since then, many investigators have measured marine lipids, especially fatty acids and sterols, by GC-FID or GC-MS methods.

1.2.2 GC methods

GC methods necessitate sample handling procedures such as extraction, evaporation, saponification, and derivatization. In order to analyze lipids in seawater, 1-2 L of seawater is usually collected and extracted with $CHCl_3$ at pH 2. Kennicutt and Jeffrey (1981) used a sample-to-solvent ratio of 70:1 for extraction. The extracts were passed through a silica gel column and three fractions (aliphatic, ester/aromatic and a polar fraction) were collected by eluting with different solvents (hexane, benzene and methanol). Each fraction was concentrated in a rotary-evaporator and then analysed by GC with a glass capillary column coated with SP-2100 (OV-101) (Kennicutt and Jeffrey, 1981). Venkatesan *et al.* (1987) fractionated $MeOH/CH_2Cl_2$ extracts into two parts (acidic and non-saponifiable) by saponification with 0.5N KOH in 1:1 $MeOH/H_2O$. The acidic fraction was reacted with BF_3/MeOH and the methyl esters of the fatty acids were

quantified by GC. Hydrocarbons, alcohols, and sterols were isolated from the non-saponifiable fraction by TLC (silica gel with CH_2Cl_2 development). Alcohols and sterols were trimethylsilylated using bis-(trimethylsilyl)-trifluoroacetamide. The silylated derivatives were then analyzed by GC-FID. Hydrocarbons were passed through a silica gel column to separate aliphatic and aromatic fractions, and these were directly quantified by GC. A fused silica column (Durabond DB-5 J & W Scientific Inc; 25 m x 0.25 mm; 0.25 μm film) was programmed from 35 to 290°C at 4°C/min and then held isothermally for about 2 h. The recoveries for sterols ranged from 80% to 92% and for hydrocarbons, alcohols, and fatty acids from 44% to 85%. Obviously, the analytical results were not totally satisfactory because of the low recoveries.

Gomez-Belinchon (1988) determined hydrocarbons and fatty acids in dissolved and particulate matter in a deltaic environment by GC-FID. A column of 25 m x 0.25 mm i.d. coated with SE-54 (film thickness 0.15 μm) was used. Hydrogen was used as the carrier gas (50 mL/min) and the temperature was programmed from 60 to 300 °C at 6 °C/min. Before analysis, the sample was processed by filtration, extraction, XAD-2 column adsorption, hydrolysis, and esterification with 10% BF_3/MeOH. The limits of detection for hydrocarbons and fatty acids was 0.0l ng/L and 0.1 ng/L, respectively. The recoveries and standard deviations were not mentioned.

Until now, there have been few reports on the analysis of phospholipids in seawater. However, results from investigators working in a related matrix may provide a very useful basis for developing a GC method that can be used in the analysis of lipid

profiles in seawater. Kuksis (1975) and Myher *et al.* (1984) determined the content of fatty acids, alcohols, cholesterol, triglyceride, and phospholipid in blood plasma by GC after the dephosphorylation and trimethylsilylation of samples.

At present, some lipids classes (hydrocarbons, sterols/alcohols, fatty acids) can be analyzed by GC. However, most GC methods necessitate the preparation of derivatives for some lipid classes (fatty acids and sterols) and the fractionation of other lipid classes. These procedures are usually labour-intensive and prone to contamination.

1.2.3 The TLC-FID method

Since Iatroscan thin-layer chromatography with flame ionization detection (TLC-FID) became available in 1970, it has been used extensively for the analysis of simple and complex lipids in biology and medicine. In the early 1980's, TLC-FID was used for the analysis of lipids in marine samples (Parrish and Ackman, 1983). This method combines the separation efficiency of TLC and the detection sensitivity of FID. This method is much simpler than GC-FID. Unlike GC-FID, TLC-FID does not necessitate the preparation of volatile derivatives and it can be also used for shipboard analysis (Delmas *et al.*, 1984).

Procedures used for sampling, extraction, storage, concentration, sample application, development, detection, and calibration by FID have been described by Parrish (1987). Seawater samples were screened through a 200 μm nylon mesh to remove

larger particles. Then a precombusted (400 °C) glass fibre filter was used to fractionate seawater into dissolved and particulate matter. Dissolved lipids were extracted into dichloromethane and particulate lipids were extracted into CH_2Cl_2/MeOH (2:1). Before extraction, hexadecan-3-one was added to each fraction as an internal standard. The extracts were concentrated by evaporation under a gentle nitrogen flow. A few microlitres of a concentrated extract was spotted onto the Chromarod using a syringe.

Complex lipid mixtures may be separated in 3-step separations with partial scanning between developments. Hexane-dimethyl ether-formic acid (HDF 99:1.0:0.1) was usually used as the first development system (Parrish and Ackman, 1983). The operating conditions for the Iatroscan TLC-FID was adjusted to maximize FID response. The hydrogen flow rate was between 170 and 190 mL/min, the air flow rate was 2000 mL/min, and the scan speed was between 3.1 and 4.2 mm/s for routinely use.

A stock solution of an eight-component standard was used to make calibration curves. In order to obtain a precision that was better than 10% coefficient of variation (C.V.), extensive calibration was necessary, especially in trace analyses. Lower loads tend to give higher C.V. values.

1.3 Carbohydrates

As the primary product of photosynthesis, carbohydrates are an important form of energy storage for autotrophs as well as for the heterotrophs which consume them. Carbohydrates may be released into seawater by living organisms during growth periods

or after death (Mague *et al.*, 1980). Because of the low content of carbohydrates in the very complex seawater matrix, characterizing carbohydrates is rather difficult. Isolation and purification steps are usually needed before any chromatographic procedure, with consequent losses and changes in constituents. Because of these problems, various analytical methods have been investigated in order to develop a reliable routine method to analyze carbohydrates in seawater.

1.3.1 Chromatographic methods

Paper chromatography suffers from low sensitivity. Gas chromatography is a highly sensitive technique for analyzing volatile compounds. Although it is possible to convert non-volatile carbohydrates to volatile trimethylsilyl derivatives (Modzeski *et al.*, 1971) or acetylated derivatives (Sakugawa *et al.*, 1985; Ochiai *et al.*, 1988), desalting and concentration of seawater samples are required before derivatization can be carried out. These procedures are laborious and it is difficult to avoid contamination.

Partition chromatography of sugars on anion-exchange resins also has serious shortcomings when applied to the analysis of marine samples. There is interference from other compounds in samples after preconcentration, leading to a rapid deterioration in the performance of the anion-exchange column (Mopper and Degens, 1972). Although the chromatography of the borate complexes of carbohydrates on strong anion-exchange resins with a sensitive colorimetric regent (Cu^{2+}/aspartic acid/sodium 2,2'-bicinchoninnate) overcomes the above drawbacks (Mopper and Gindler, 1973), the

seawater sample still needs to be desalted and concentrated using ion-exchange, electrodialysis and freeze drying (lyophilisation). As with gas chromatographic analyses, the separation of trace amounts of carbohydrates from salts in seawater was impractical for routine analysis (Mopper and Gindler, 1973). However, because GC can be used with MS, it was a very important tool for identifying individual monosaccharides and short oligosaccharides. Nine oligosaccharides in dissolved fraction of seawater were quantified with a range of 0.5-28 μg/L by GC, GC-MS, and ^{1}H-NMR (Sakugawa *et al.*, 1985a, b).

Attempts to employ fluorometric locating reagents for the detection of the carbohydrates in seawater after ion-exchange chromatography have not resulted in any significant improvement in sensitivity and precision (Mopper *et al.*, 1980; Mopper and Johnson, 1983). HPLC determination of carbohydrates has few applications because of the lack of a suitable fluorescent reagent for sugars. It is noteworthy that Compiano *et al.* (1993) analyzed the monosaccharide composition in seawater samples by HPLC and fluorescence detection after dansylhydrazine precolumn derivatization (65 °C, 20 minutes) and obtained good precision. The detection limit varied from 5.5 nmol/L for rhamnose to 30 nmol/L for glucose, and reproducibility varied from 1% for glucose to 6% for arabinose.

Because the composition of monosaccharides can be analyzed by HPLC methods, HPLC is a potential method to be fully developed. Its development depends on the availability and sensitivity of sugar-specific fluorochrome reagents for high salt matrix samples.

1.3.2 Colorimetric methods

Many earlier colorimetric methods employing *N*-ethylcarbazole, anthrone, and phenol sulphuric acid (Dubois *et al.*, 1956; Gerchakov and Hatcher, 1972) have three fundamental problems for measuring total carbohydrate concentrations (Burney *et al.*, 1977): the calibration curve is different for each carbohydrate; some reagents do not detect sugar alcohols; and the use of strong acids can produce other degradation products affecting detection (Josefsson *et al.*, 1972).

These shortcomings may be overcome by the method of Johnson and Sieburth (1977). They made some modifications to minimize contamination of samples in assaying procedures. The method includes reduction of free monosaccharides to sugar alcohols with KBH_4 or $NaBH_4$, periodate oxidation, and the determination of derivatives of formaldehyde with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and $FeCl_3$ reagent. The reaction of formaldehyde with MBTH and $FeCl_3$ produced a coloured complex, which dissolved in 50% acetone. The molar absorptivity of this complex (43,600 $\mu M^{-1}cm^{-1}$ at 635 nm) was not affected by changes in salinity. The modified procedure decreased the standard deviation by 50% from older methods (from 5.3 to 2.7 $\mu g/L$) and allowed a single analyst to determine 15 instead of 8 samples in the same two day period. The limit of detection was 180 nmol/L for formaldehyde when a pathlength of 1 cm was used (Eberhardt and Sieburth, 1985). Analytical precision for total carbohydrate analyses was 10% (Henrichs and Williams, 1985).

The MBTH method is thus a good procedure because of its sensitivity and it shows no salinity dependence, and needs no preconcentration and separation steps. The other advantages of this method are that both total dissolved monomeric carbohydrate (MCHO) and total carbohydrate concentrations (TCHO) can be determined when a hydrolysis step is used to hydrolyse combined carbohydrate. Hence, this method has been widely accepted by many laboratories to determine total carbohydrate fractions in seawater. Full details of this method and its modifications may be found in the following papers (Burney and Sieburth, 1977; Johnson and Sieburth, 1977; Johnson *et al.*, 1981; Henrichs and Williams, 1985; Pakulski and Benner, 1992).

However, this method does suffer from some shortcomings. It is a time-consuming method and cannot be used to determine the composition of monosaccharides. Obviously, this method cannot distinguish between true monosaccharides and any substances which possess a terminal glycol group. As a result, the total dissolved carbohydrate concentrations perhaps do not closely reflect biological availability of carbohydrates (Senior and Chevolot, 1991). As well, the hydrolysis of combined carbohydrates with diluted hydrochloric acid may not be effective enough. Pakulski and Benner (1992) found that sulphuric acid was more effective than dilute hydrochloric acid in hydrolysing oligosaccharides. For example, dissolved carbohydrates determined by the MBTH method after 10% HCl hydrolysis accounted for 6-10% of the DOC, whereas when sulphuric acid was used the carbohydrate fraction accounted for substantly more of the DOC (10-28%). Benner *et al.* (1992) also applied a modified MBTH method with

H_2SO_4 hydrolysis to determine the amount of carbohydrate in the colloidal size fraction (1-200 nm) of seawater samples. The results of such a modified MBTH method were similar to the those determined independently using a ^{13}C-NMR method. Hence, more studies using the modified MBTH assay should yield a more reasonable estimate of total carbohydrate content in seawater.

The only drawback in the modified method is the requirement that the sample be specially dried before addition of concentrated H_2SO_4. It was found that sample evaporation using a rotary evaporator resulted in loss of carbohydrate trapped in the inorganic salts. Hence, this study did not adopt the modified MBTH method of Pakulski and Benner *et al.* (1992) but, because of the lack of the required evaporator, the diluted HCl hydrolyzed step was used.

2.0 OBJECTIVES

DOM exerts a significant influence on biogeochemical processes. The dynamics of the DOM pool is a key factor in understanding carbon flux in the ocean. After the report of high concentrations of DOC (Suzuki *et al.*, 1985, 1988, 1992) and of the high number of colloidal particles (Koike *et al.*, 1990; Well and Goldberg, 1991) found to exist in seawater, more and more marine scientists realized the importance of marine colloids in biogeochemical cycles in the oceans. This is particularly true since marine colloids can aggregate and then can be utilized or, settle out of the water column (Well and Goldberg, 1993).

Because of the large solid/liquid interface, marine colloids were believed to be very important in interfacial processes such as adsorption, polymerization, complexation, and coagulation. These interfacial processes play a key factor in the transfer of dissolved substances into particulate matter.

In spite of the importance of marine colloids, relatively little progress has been made on the chemical composition or structure of marine colloids. At present, less than 35% of DOM has been characterized, owning to the limitations of collecting sufficient amounts of unaltered OM and of the lack of sufficient analytical methods. Thus, the characterization of the chemical composition of OM, especially of marine colloids, continues to challenge many marine chemists.

The overall objective of this study was to understand further the chemical features and roles of marine colloids in biogeochemical cycles by characterizing the chemical composition of marine colloids associated with different particle size classes. To reach this objective, CFF was used to concentrate marine colloids from microalgal culture and from seawater samples because CFF can rapidly filter a large volume of seawater and effectively collect marine colloids. Water samples were obtained from laboratory algal cultures in order to develop methods for waters with high concentrations of colloids. Seawater samples were then collected in a time course following the spring phytoplankton bloom in Conception Bay, Newfoundland.

The relatively precise technique of TLC-FID was employed to analyze the lipid classes because it does not necessitate the preparation of volatile derivatives as GC does. Total carbohydrate concentrations in seawater and algal cultures was determined using the established and widely used MBTH method. Analyses of both the lipids and the carbohydrates using accurate measurements were used (i) to evaluate the efficiencies of CFF by calculation of the mass balance on different filters, (ii) to illustrate partitioning of organics between truly dissolved and colloidal fractions, and (iii) to explain some biogeochemical processes.

The specific objectives were:

(a) to assess cross-flow filtration as a means of separating and concentrating marine colloids by studying:

(1) The blank lipid values of the CFF system for measuring lipid classes;

(2) a mass balance for all the lipid classes in all the particle size classes;

(b) to apply cross-flow filtration to collect marine colloids present in algal cultures and in local seawater samples. The two types of culture samples analyzed came from laboratory cultures of phytoplankton (*Chaetoceros muelleri*, and *Isochrysis galbana*);

(c) to measure lipid and carbohydrate concentrations in different filtration fractions and to apply linear regression to analyze the data. The results of the analyses of lipid classes and of total carbohydrates might help explain the role of marine colloids in biogeochemical cycles.

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Analysis of lipids

For the analysis of lipids, the representative compounds in **Table 3.1** were used as standards to identify and quantify lipid classes. These compounds are representative of the marine environment. All of these compound were purchased from Sigma Chemical Co. In order to make a calibration curve, a stock standard solution of these compounds was made in chloroform and stored under nitrogen at -20 °C. Because there is very little ketone present in most marine samples, hexdecan-3-one was usually used as an internal standard. Development solvents used for separation of lipid classes are chloroform, formic acid, acetone, diethyl ether and hexane.

Analysis of carbohydrates

Glucose was used as a standard for the spectrophotometric measurement of carbohydrates. The other reagents include hydrochloric acid, sodium hydroxide, sodium borohydride, periodic acid, sodium arsenite, 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH), ferric chloride, and acetone. All of these chemicals were analytical grade reagents. MBTH and sodium borohydride were kept in a desiccator in a refrigerator.

Table 3.1 Marine lipid classes and standards used for their identification and calibration by TLC-FID

Marine lipid Class	Abbreviation	Model compound Name	Model compound Formula	Stock solution mg/mL
Aliphatic hydrocarbon	HC	*n*-Nonadecane[1]	$C_{19}H_{40}$	0.5834
Sterol ester	SE	Cholesteryl hexadecanoate[2]	$C_{43}H_{76}O_2$	0.5200
Ketone	KET	3-Hexdecanone[1]	$C_{16}H_{32}O$	0.8235
Triacylglycerol	TAG	Glyceryl trihexadecanoate[2]	$C_{51}H_{98}O_6$	0.9855
Free fatty acid	FFA	Hexadecanoic acid[3]	$C_{16}H_{32}O_2$	0.4074
Free aliphatic alcohol	ALC	1-Hexadecanol[3]	$C_{16}H_{34}O$	0.3658
Sterol	ST	Cholesterol[1]	$C_{27}H_{46}O$	0.5115
Acetone-mobile polar lipid	AMPL	Glyceryl 1-monohexadecanoate[2]	$C_{19}H_{38}O_4$	1.0006
Phospholipid	PL	Phosphatidyl choline[2]	$C_{40}H_{80}NO_8P$	0.7622

[1] Stored in desiccator at room temperature
[2] Stored in desiccator at < 0 °C
[3] Stored in desiccator at 0-5 °C

1. Sodium borohydride solution

$NaBH_4$ (100 mg) was dissolved in 5.0 mL of chilled doubly distilled water (about 4 °C). This solution was prepared for immediate use.

2. Periodic acid solution

Periodic acid (0.57 g) was dissolved in 100 mL of doubly distilled water. This solution was stored at room temperature in the dark.

3. Sodium arsenite solution

Sodium arsenite (3.25 g) was dissolved in 100 mL of redistilled water. The solution is stable indefinitely.

4. Ferric chloride solution

$FeCl_3$ (5.0 g) was dissolved in 100 mL of doubly distilled water. The $FeCl_3$ solution was filtered through a Whatman GF/C filter and stored at 5 °C.

5. MBTH solution

MBTH (276) mg was dissolved in 10 mL of 0.1 N HCl with warming. The solution was filtered through a Whatman GF/C filter, if any precipitate remained, and was stored in a clean, amber bottle at room temperature. This solution was prepared freshly every day.

3.1.2 Filters

Glass fibre filters

Gelman® A/E glass fibre filters (Cat. No. 61631) with nominal pore size 1 μm (liquid rating 95%), diameter 47 mm, and typical thickness of 450 μm were used. The manufacturers state that they are widely used for filtering natural water for studies of colloidal and suspended solids or the "dissolved" fraction because they provide high flow rates (550 mL min^{-1} cm^{-2}), great wet strength, and high solid-holding capacity.

Whatman® GF/C (Cat. No. 1822-047) glass fibre filters with a nominal particle retention of 1.2 μm, typical thickness of 260 μm, and a diameter of 47 mm were also used. The manufacturers state that they have a good flow rate, high loading capacity, and low water absorption (250 mL/m^2). They are widely used for the collection of suspended particles in potables, natural and industrial-waste waters.

Microporous and ultrafilter membrane

Four Mintain® microporous filter plates (Cat. No. HVLP OMP 04) were used in a cross-flow filtration apparatus. Each plate is constructed by heat-binding two hydrophillic Durapore® filter membranes to a polyvinylidene fluoride (PVDF) backing. The nominal pore size of the Durapore® filter membrane is 0.45 μm.

Four Minitan® ultrafiltration filter plates (Cat. No. PTGC OMP 04) were also used. Each filter plate consists of two polysulfone filter membranes heat-bonded to a polypropylene backing. The ultrafiltration membrane has a nominal MW cut-off of 10,000 Da. The filter area for each filter plate is 60 cm^2, and four plates were usually

used at one time, giving a total filter surface area of 240 cm^2. The maximum operating temperature is 50 °C and the maximum transmembrane pressure is 3.5 kg/cm^2. The manufacturer states the minimum cross-flow rate can reach 500 mL/min.

3.2.3 Instruments

Millipore cross-flow filtration system

The Millipore CFF system (**Figure 3.1**) was made of a Minitan® filtration unit, a Millipore pump, a pressure gauge, and vessels (glass bottle or polyethylene container). They were connected by tubing, fittings and valves. The Minitan® filtration unit (Millipore Corporation, Bedford, MA) is a tangential flow device, which was composed of Minitan® filter plates (3.2.2), open channel retentate separators, an acrylic housing, and two stainless steel plates. Flow rate and direction could be adjusted with the use of Millipore pump.

Iatroscan MK-5 chromatographic analyzer

The Iatroscan MK-5 TLC/FID Chromatographic analyzer (**Figure 3.2**) (Iatron Laboratories, Inc. Tokyo, Japan) is a complete chromatographic analyzer for the separation and subsequent detection of chemical components of complex organic mixtures. Component separation was performed on Chromarod-SIII, which is a quartz rod coated with a sintered silica gel layer. The particle size is 5 μm and the pore size of the layer is 60 Å. Detection is implemented by a hydrogen flame ionization detector

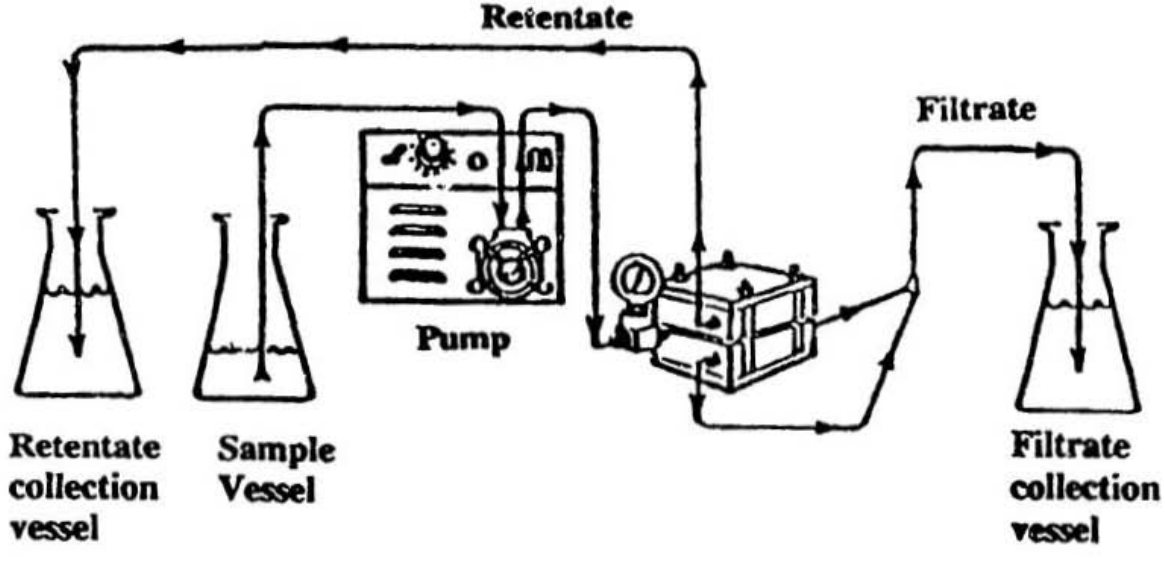


Figure 3.1 Diagram of Millipore cross-flow filtration system

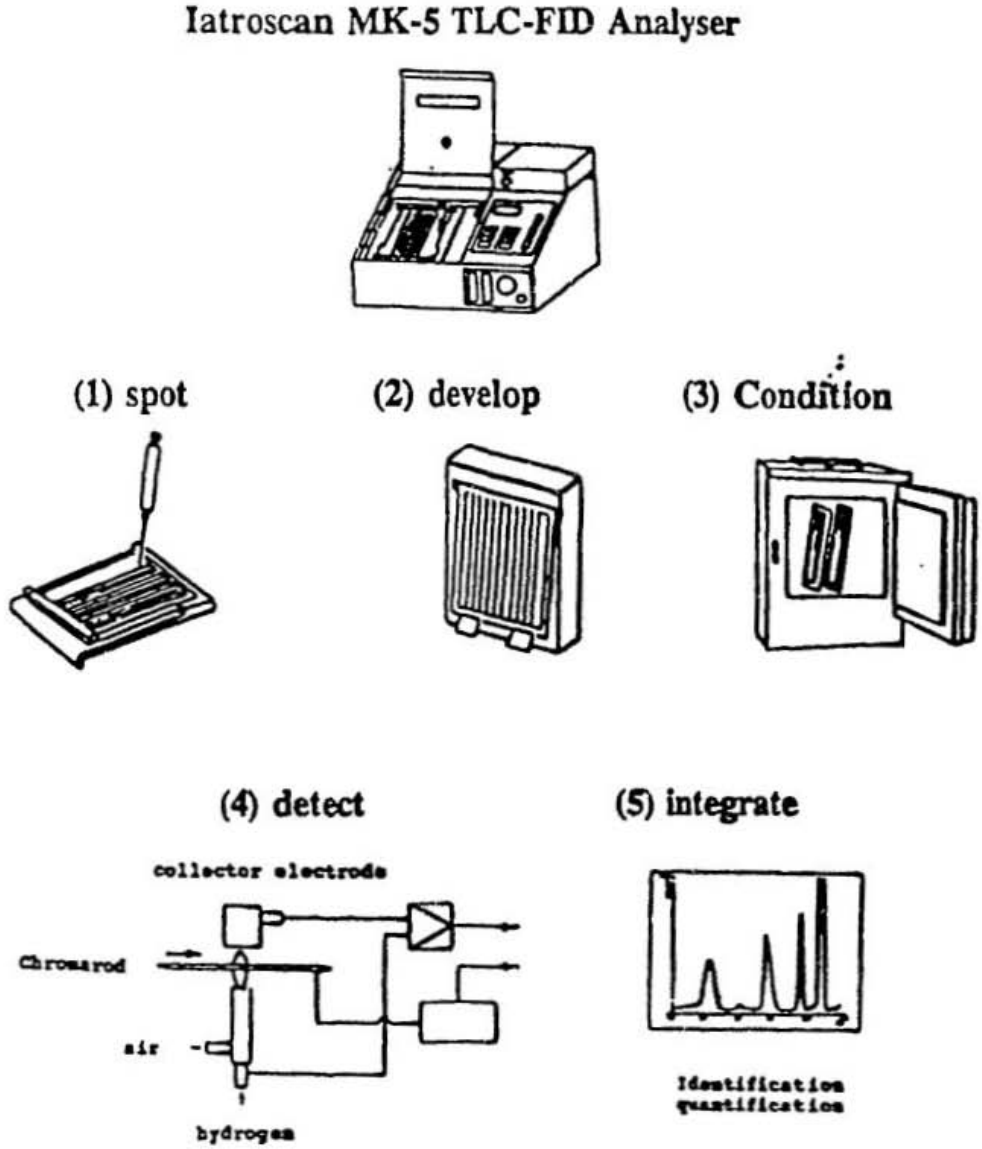


Figure 3.2 Diagrams for major operating procedures in TLC-FID method

(FID) as the Chromarods are moved automatically through the detector under microprocessor control.

Spectrophotometer

A Du®-65 spectrophotometer with memory PAC™ module (Beckman Instruments, Inc.) was used for the spectrophotometric measurement of total carbohydrate concentration.

3.2 Methods

3.2.1 Sampling

Isochrysis galbana (T-Iso) and *Chaetoceros muelleri* culture samples were taken from mass (100 L) cultures. The microalgal cells were grown in nutrient-replete semi-continuous cultures. The density at which the sample were collected was ~4-6 x 10^6 cells/mL and the growth rate varied from 0.24 to 0.90 div./day. The sample volume is about 1-2 L for each sample. The detailed information for each culture is shown in **Table 3.2**. Before the culture samples were collected in a 1 L dark Teflon container, the containers were rinsed three times with culture and the density (cells/mL) and temperature of culture was determined. After sample collection, each sample was immediately filtered.

Four seawater samples were taken from the surface mixed layer (10-28 m) of the ocean at the Brigus-Long Pond (BRLP4) station by Niskin bottle or Go-Flo sampler. Hydrographic conditions at the sample site are shown in **Table 3.3**. BRLP4 (latitude

Table 3.2 Microalgal culture conditions

Species	*I. galbana*	*I. galbana*	*C. muelleri*
Conditions	Semicontinuous, replete	Semicontinuous, replete	Batch, replete
Density (cells/mL)	4.2×10^6	5.8×10^6	4.4×10^6
Division rate (div./day)	0.90	0.61	0.24
Temperature (°C)	22	21	23

Table 3.3 Station BRLP4 hydrography

Date	May 19	June 24	August 2	October 5
Depth (m)	25	10-25	25-27	25-26
Temperature (°C)	1-2	3-4	8-9	10-11
Overall depth (m)	215	200-215	197-215	215
Salinity (‰)	35	35	35	35

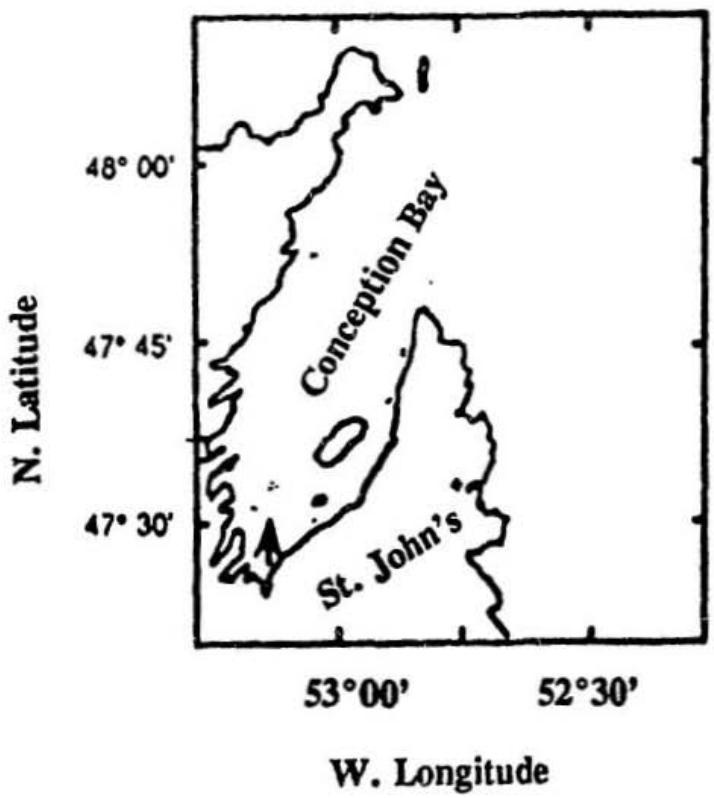


Figure 3.3 Map showing the location of the BRLP4 sampling station.

47°32.2'N and longitude 53°06.0'W) is in Conception Bay, Newfoundland in eastern Canada. Conception Bay is about 70-100 km long and 20-40 km wide. It is a fjord-like embayment opening directly onto the Newfoundland Shelf (**Figure 3.3**). The bay has a 170 m deep sill at the mouth and a maximum depth of 300 m in the central basin. The depth at the sampling site is 215 m. In order to remove larger particles, the sea water samples were passed through a 75 μm copper sieve. Then about 15-35 L of seawater was collected in 20 L high density polyethylene containers. The containers were precleaned with distilled water for several days. Immediately before seawater samples were collected, the containers were rinsed with seawater three times. All of the seawater samples were transported to the laboratory within 2 h of collection and then immediately filtered.

For both seawater and culture samples, care was taken to avoid exposure to light, and samples were stored at low temperatures. Processing of culture samples was started within 10 min, while processing of seawater samples occurred within 2 h of sampling. All lipid analyses were conducted within one week.

3.2.2 Handling of filters

Before the sample was filtered, glass-fibre filters were put in a Muffle furnace and combusted at 450 °C for 20 h to remove organic impuries. All glassware used in the filtration process was washed with purified distilled water and baked at 450 °C for 3 h.

Purified distilled water was recycled through a hydrophillic Durapore® filter

membrane (0.45 μm) and an ultrafilter membrane (10K NMWL cut-off) in the CFF system for several days. In order to check the efficiencies of the water cleaning procedure, the lipid and organic carbon blanks in the CFF system were determined. The CFF lipid blank was obtained by recycling 500 mL of purified distilled water through the 0.45 μm filter or 10 kDa filter and then analyzing the lipid content in the water. The organic carbon blank was also obtained by filtering 500 mL of artificial seawater and then analyzing the organic carbon content.

A cleaning procedure including use of methanol was compared with a water-only cleaning procedure by measuring the lipid blank in the CFF system after cleaning. Immediately after filtering culture samples, the Durapore membrane (0.45 μm) in the CFF system was cleaned by pumping 2 L of 50% methanol solution for about half an hour, recycling 1 L of purified water for 20 h, and then filtering 1 L of purified water for about 2 h. After cleaning, 500 mL of purified water was recycled to pass the filter 5 times, then analyzed in order to determine the efficiency of the cleaning procedure.

3.2.3 Treatment of samples

Conventional filtration

All culture samples and only one seawater test sample were prefiltered through glass fibre filters (Whatman GF/C or Gelman A/E) before the filtrates were ultrafiltered with the cross-flow filtration system. Filtration with glass fibre filters was completed by the conventional pass-through method under a slight vacuum. Samples were protected

from oxidation with a nitrogen flow. In order to reduce filter clogging, less than 50 mL of culture, or less than 2 L of seawater sample was used for each filter. Just before filtration, the samples were slightly swirled. During the filtration process, exposure of samples to light was avoided. The filtration of culture samples was completed within 10 minutes, while the filtration of seawater was completed within 20 minutes.

Cross-flow filtration

Cross-flow filtration was conducted in a covered chamber (~ 180 L) in which the whole Millipore CFF system (**Figure 3.1**) was placed. When filtering, the temperature in the chamber was held at 5 °C. A gentle nitrogen flow was delivered to the mouth of each vessel: the sample, retentate, and filtrate collection vessels. The chamber was kept dark. The filtration pressure was less than 1.5 kg cm^{-2}.

Before the sample was filtered, the Millipore CFF system was thoroughly cleaned with purified water and then a small volume of sample, usually about 40 mL for culture and about 150 mL for seawater samples, was filtered and discarded. Usually, the microporous filters with 0.45 μm rated pore size were used first in cross-flow filtration. The filtrate of the 0.45 μm filter was then passed through the ultrafiltration membrane with a 10,000 NMWL cut-off (pore size ~3 nm). In this way, different fractions were collected and each fraction contained different particle size classes.

In order to calculate mass balance (%), the volume in all fractions including prefiltered, retentate, and filtrate fractions was measured and recorded. The filtration flow diagram for one culture sample is shown in **Table 3.4.** The filtration of other

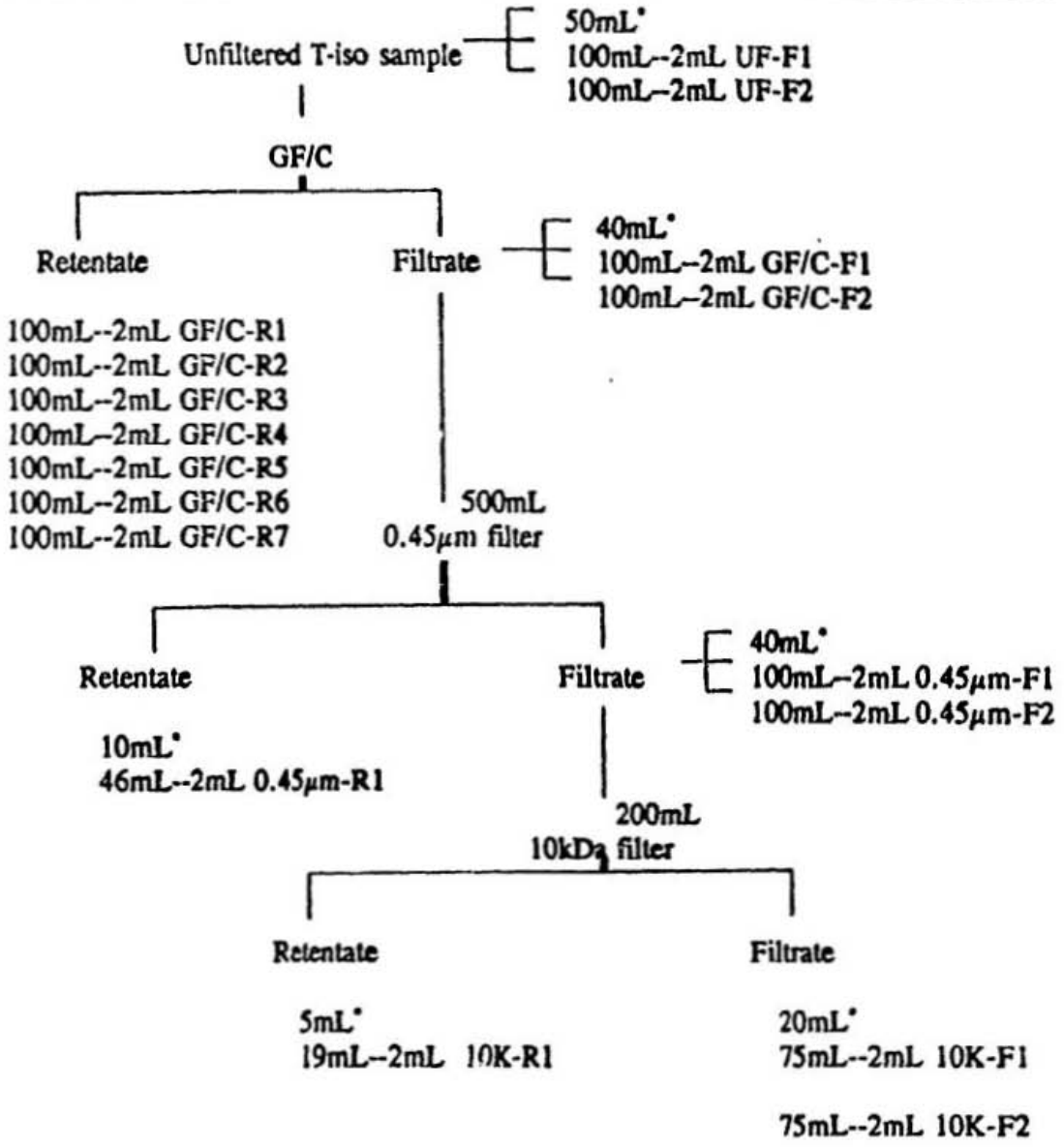
culture and seawater samples is similar to the sample in **Table 3.4** except that large volumes of seawater samples were used.

Extraction, concentration and storage

Culture and seawater samples for measurement of carbohydrates were collected in 20 mL glass vials after filtration. The vials were filled with nitrogen and stored in a freezer (-20 °C). Culture and seawater samples for measurement of lipid classes were extracted and concentrated before storage for later analysis. The total lipids on glass fibre filters (GF/C or A/E) were extracted with a cold chloroform/methanol (1:1 v/v) solution according to the Bligh and Dyer (1959) method.

Usually, one or two glass fibre filters with their retentates were placed in a 10 mL glass centrifuge tube. Two millilitre of ice-cold chloroform/methanol (1:1 v/v) was added to the tube to begin passive extraction of the lipids on the filter. The sample in the tube was stored in a freezer (-20 °C) for two days, after which the glass fibre filter was ground with a metal rod. One millilitre of chloroform and 1 mL of chloroform-extracted pure water was added to the tube. The centrifuge tube was flushed with nitrogen, capped immediately, vortexed on a Deluxe mixer, and then centrifuged for two minutes at 4,000-5,000 rpm. The organic layer (bottom) was removed by a double pipetting technique, in which one pipette is placed inside another, and placed in a prerinsed vial (10 mL). After this, 2.0 mL of chloroform was added to each centrifuge tube to extract residual lipids on the filter by repeating the above operations. Usually, filters were extracted 4 times. All extracts were combined in the vial.

Table 3.4 Filtration flow diagram for an *Isochrysis galbana* (T-ISO) culture sample.



* samples for measurement of carbohydrates

The chloroform extracts in the vial were evaporated to exactly 2.0 mL under a gentle nitrogen flow and stored in a freezer. This sample was usually designated GF/C-R or A/E-R, according to the filter and filtration fraction.

The other filtration fractions of culture samples were extracted 4 times with chloroform in a 1 L separatory funnel. For each fraction (<200 mL), 60 mL (20 mL, 20 mL, 10 mL, and 10 mL) of chloroform was used. The filtration fractions of seawater samples were extracted 4 times with chloroform in a 4 L amber, glass bottle. The volume of chloroform used in the extraction depended on the volume of the seawater sample. Usually, 60 mL of chloroform was used to extracted a 1 litre of seawater sample. Before extraction, 5 drops of methanol were added to each sample to inhibit enzyme reactions. After the first extraction, 6 drops of sulphuric acid per litre of sample was added to adjust the pH to facilitate the extraction of free fatty acids.

The resulting lipid extracts were concentrated to about 2.0 mL in a Rotavapor under vacuum at <30 °C. All the concentrated extracts were transferred to a small vial, concentrated to exactly to 2 mL under a gentle nitrogen flow, and then stored in a freezer until measurement of lipids.

3.2.4 Measurement of lipid classes

The Chromarod-Iatroscan thin-layer chromatography (TLC) with flame ionization detection (FID) method was used in measuring marine lipid classes. Most of the

procedures used in this study are based on those of Delmas *et al.* (1984), Parrish and Ackman (1985), Parrish (1987).

Outline of TLC-FID method

This method involves two important techniques, separation and detection. Separation of lipid classes was completed on Chromarod-III. The basis of separation is that different compounds have different polarities. The procedures include Chromarod cleaning, sample spotting, conditioning and developing.

Detection of lipid classes was conducted with a hydrogen flame ionization detector. The detection process involves optimization of a series of parameters such as hydrogen flow rate, air flow rate, scan rate, data sampling rate, and scan length.

The identification and quantification of lipid classes in samples was performed by a comparison with standard lipid compounds. The position and area under the peak were used to identify and quantify lipid classes.

The detection limitation of the TLC-FID method is 0.1-0.5 μg for lipids, with a coefficient of variation for most marine samples of about 15-20% after analysis of 3 or 4 replicate samples.

Chromarod cleaning

Before spotting lipid extracts on Chromarods, the rods were washed with purified water and acetone, and then checked by passing them through the hydrogen flame ionization detector. If the response signal was larger than 0.5 mV, the rods were scanned several times to burn off any remaining lipids or organic material. If the signal was still larger than 0.5 mV after rods were scanned several times, the rods were put into concentrated sulphuric acid for 4-8 h. The rods were removed from the acid, washed with water, then acetone, and then rescanned. This was repeated until the signal from the rod was less than 0.5 mV.

Sample spotting

The extracts or standards were spotted on Chromarods using a Hamilton syringe fitted into Hamilton repeating dispenser with divisions of 0.5 μL. The volume of sample usually was in the range of 1 to 20 μL. When spotting samples or standards on the rods, the rack holding the rod was placed on a warm hot-plate and nitrogen was blown over the rods so that the chloroform evaporated quickly.

Conditioning

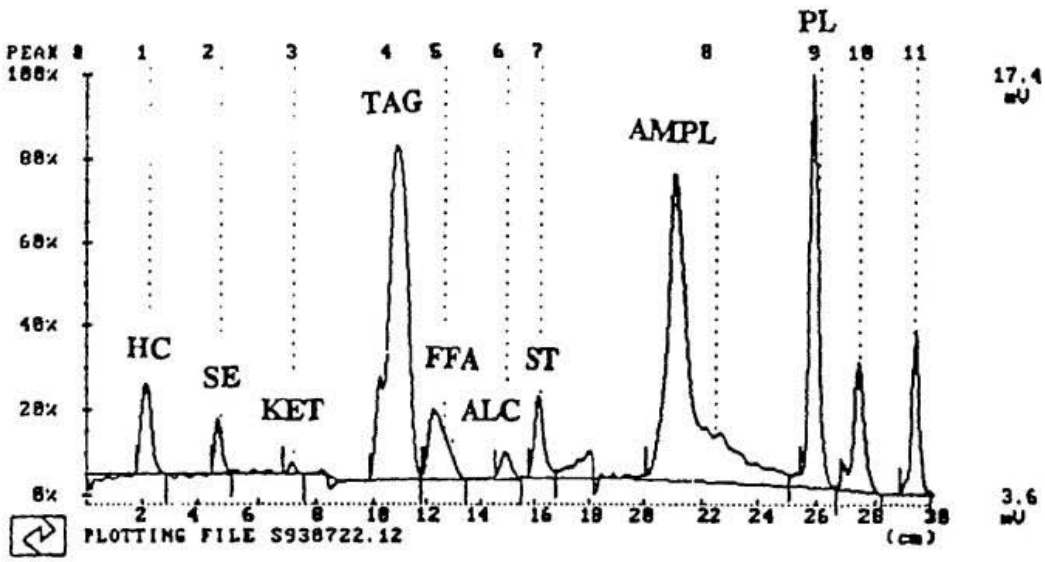
After spotting was finished, the rack holding the rods was put in a constant humidity ($\sim$30%) chamber. Before each development, the rods were conditioned for 5 min.

Development

Before separation of lipid classes, the rods were put into a developing tank containing acetone to narrow and align the spotted band. The rods were then conditioned for 5 min. Usually, four development solvent systems were used to separate the lipids on the rods and the rods were scanned three times. The scanner may be programmed to scan a part of a rod, and then the remaining unscanned lipids can be well separated in the next development solvent system. The first solvent system was hexane/diethyl ether/formic acid (99:1:0.5 v/v/v). After a 25 min and then second a 20 min development, the rods were scanned to the position behind the ketone peak to obtain the first chromatogram. The second chromatogram was obtained after a 40 min development in the second solvent system, hexane/diethyl ether/formic acid (80:20:0.1 v/v/v), and scanning to the lowest point behind the cholesterol peak. The remaining lipids were developed twice in acetone for 15 min and then twice in chloroform/methanol/water (50:40:10 v/v/v). The third chromatogram was obtained by scanning the entire Chromarod.

Chromatograms

The three chromatograms obtained by development in the four different solvent systems were combined by computer to form a single chromatogram (**Figure 3.4**) using T data scan software (RSS Inc., CA). Signals detected in millivolts were converted to peak areas. Peak retention (mm), area (mV·mm), and height (mV) were listed for chromatograms. **Figure 3.4** illustrates a typical seawater lipid chromatogram.



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Peak No	Peak Retention	Width	HalfHigh Width	Height	%Height	Area (mVmm)	%Area
1	21.5 mm	10.4 mm	3.9 mm	3.042 mV	5.4	12.22	3.7
2	46.8 mm	6.9 mm	2.6 mm	1.821 mV	3.2	4.968	1.5
3	71.6 mm	7.2 mm	2.3 mm	0.3564 mV	0.6	0.8862	0.3
4	109.3 mm	19.3 mm	7.6 mm	11.07 mV	19.7	95.72	28.6
5	123.8 mm	16.3 mm	7.3 mm	2.267 mV	4.0	17.05	5.1
6	149.0 mm	9.3 mm	3.9 mm	0.8993 mV	1.6	3.783	1.1
7	161.3 mm	10.4 mm	3.5 mm	2.841 mV	5.1	11.68	3.5
8	211.4 mm	49.[illegible] mm	5.9 mm	10.19 mV	18.2	104.3	31.2
9	258.7 mm	12.8 mm	3.4 mm	13.81 mV	24.6	49.64	14.8
10	275.0 mm	13.4 mm	3.3 mm	4.281 mV	7.6	17.93	5.4
11	294.7 mm	10.8 mm	2.7 mm	5.493 mV	9.8	16.36	4.9

TOTAL HEIGHT	TOTAL AREA	%TOTAL AREA
56.07 mV	334.5 mVmm	100.0

Figure 3.4 Typical TLC-FID chromatogram for lipid analysis in seawater samples. No internal standard was added to this sample.

Detection conditions

Operating conditions used in this study are listed in **Table 3.5**. Stable and maximum FID response to lipid classes can be obtained using these detection conditions (Parrish, 1987).

Table 3.5 Operating conditions for the Iatroscan MK-5 TLC-FID analyzer

Parameters	Settings
Hydrogen flow to detector	200 mL/min
Air flow to detector	1 L/min
Data sampling rate	18.6 samples/second
Scanning time	30 second/rod
End position of first scan	25 mm from lowest point
End position of second scan	11 mm from lowest point
End position of third scan	whole rod

Calibration curve

A stock solution of nine standard compounds (**Table 3.1**), representing marine lipid classes, was made up in chloroform in order to create calibration curves for measurement of lipid classes by the TLC-FID method. Various known volumes of stock solution were spotted, separated and detected. Calibration curves (**Figure 3.5**) for each marine lipid class were obtained through linear regressions on lipid amount (μg) versus peak area (mV·min) (**Table A1**).

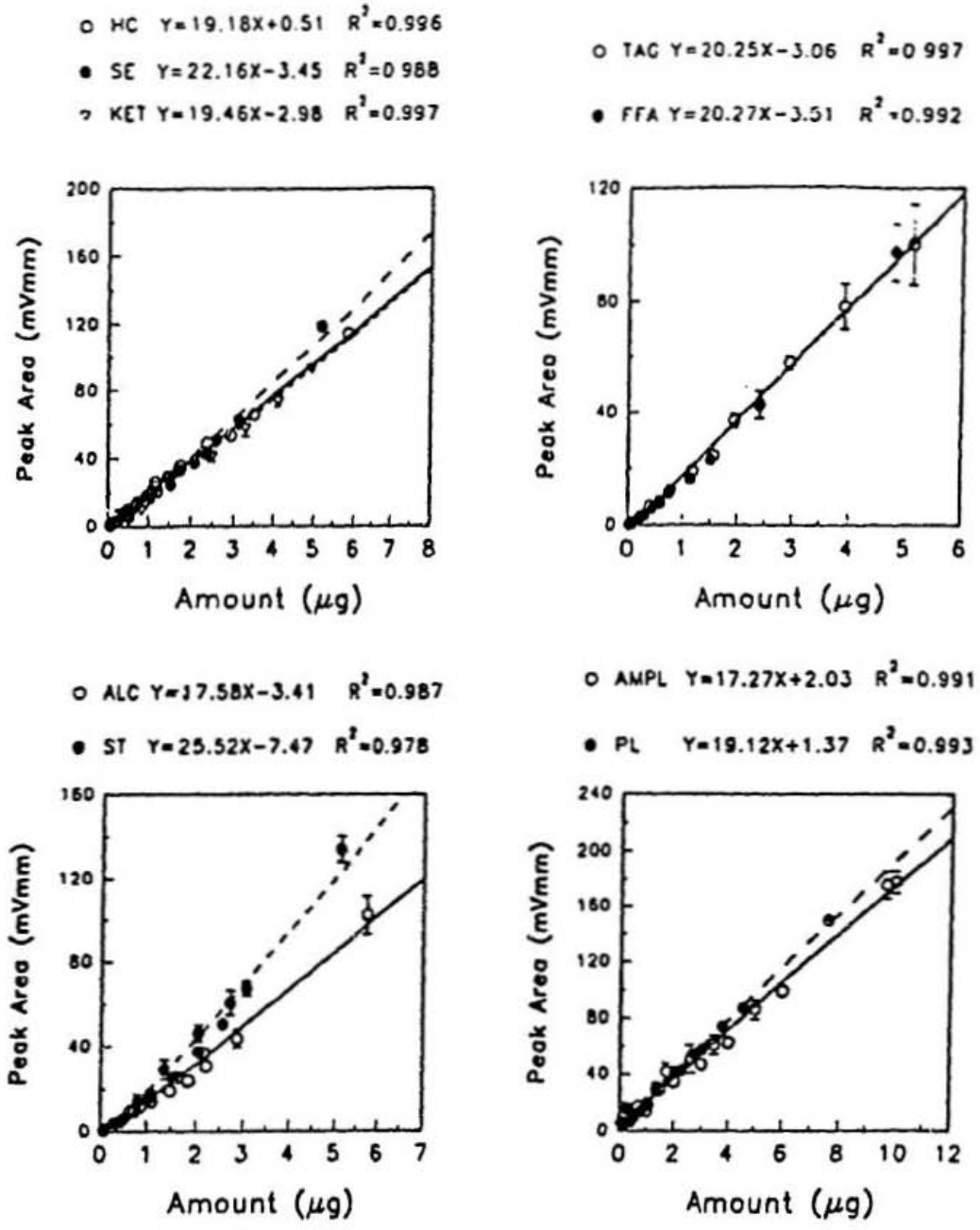


Figure 3.5 Calibration curves for each lipid class measured by TLC-FID

Blanks

In order to improve the precision of the TLC-FID method for the measurement of colloidal lipids, the blanks in each operational process were analyzed according to the detection conditions of **Table 3.5**. The blank values in the Chromarod-Iatroscan system, the extracting solvents, and the Millipore CFF system were studied.

1. Chromarod-Iatroscan system blank

The lipids in the Chromarod-Iatroscan system blank came mainly from the development solvent system. When the rods are put into the development solvent systems, impurities, including lipids in the solvents, can be absorbed onto the rod and then developed by the solvents. To obtain the Chromarod-Iatroscan system blank data, no sample was spotted on the rod. Then the rods were developed in the four solvent systems and scanned three times with the Iatroscan instrument.

2. Extracting Solvent Blank

Sixty millilitre of chloroform was concentrated to about 2 mL with a rotary evaporator. The concentrate was quantitatively transferred to a small vial, and evaporated to about 20 μL under a gentle nitrogen flow. All of the concentrate was then spotted onto Chromarods, which were developed and scanned normally. The results included the Chromarod-Iatroscan system blank, so the extracting solvent blank was obtained by subtracting the Chromarod-Iatroscan system blank.

3. Water blank

Distilled and deionized water was extracted three times with chloroform. The extracted water was bubbled with nitrogen until no chloroform could be detected by smell. Purified water (500 mL) was extracted 4 times in the same way as the extracting sample. The total volume of chloroform used was 60 mL. The extracts were combined and concentrated to about 20 μL. The extracts were spotted on the Chromarod, developed, and scanned. The results obtained include the Chromarod-Iatroscan system blank and the solvent blank. The water blank can be obtained by subtracting the Chromarod-Iatroscan system blank and the solvent blank.

4. Millipore CFF system blank

The Millipore system was cleaned with extracted water and then 500 mL of purified water was recycled through the system 5 times. The water was extracted, concentrated, and analyzed according to the methods used for the water blank. The results include the Chromarod-Iatroscan system blank, the solvent blank, and the water blank. Since two kind of filters, 0.45 μm pore size and 10,000 NMWL cut-off, were used in CFF systems, two blanks were calculated by subtracting from the other blank values.

Analysis of marine samples

The separation and detection procedures for measurement of lipid in marine samples was exactly same as for standards. Preventing contamination and minimizing

exposure to light, oxygen, heat and correcting various blanks are important in obtaining reliable data. The data obtained after correction for various blanks are discussed in the next section.

3.2.5 Measurement of total carbohydrates

Analyses for dissolved monomeric carbohydrates were conducted using the MBTH method of Johnson and Sieburth (1977) and Johnson *et al.* (1981). Briefly, the MBTH method involves some well established chemical reactions (**Figure 3.6**). These reactions entail the reduction of monosaccharide with sodium borohydride to alditols, periodate oxidation to formaldehyde, formation of a ligand by formaldehyde reacting with 3-methyl-2-benzothiazolinone hydrazone, and complexation of the ligand with ferric ions to form a coloured complex. This compound can be detected colorimetrically at 635 nm.

Particulate and dissolved total carbohydrates can be analyzed after hydrolysis with 1N HCl to form monomeric carbohydrates. The coefficients of variation (per cent standard deviation of the mean) for seawater samples was usually less than 10 %. Theresults are reported in glucose equivalents (1 μM glucose is equivalent to 6 μM carbon) after subtraction of a blank value and comparison with a standard curve (using glucose as standard).

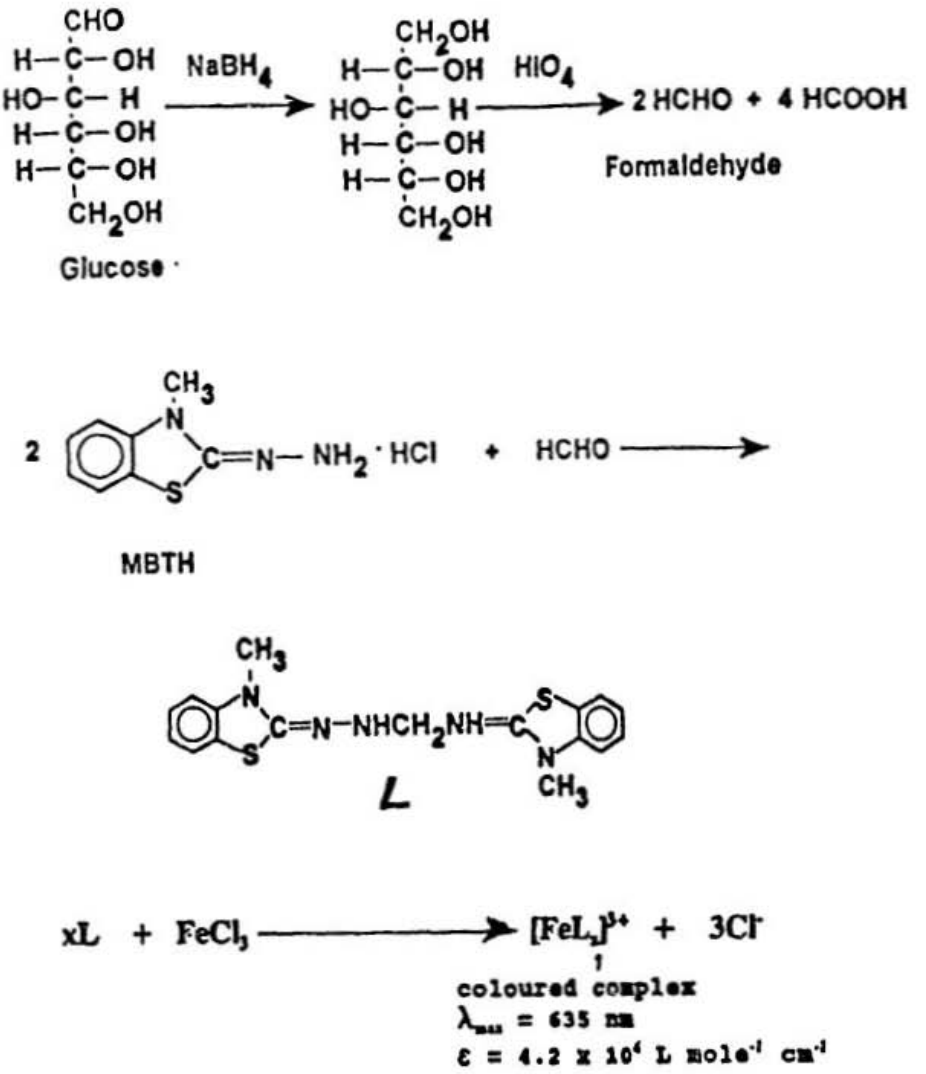
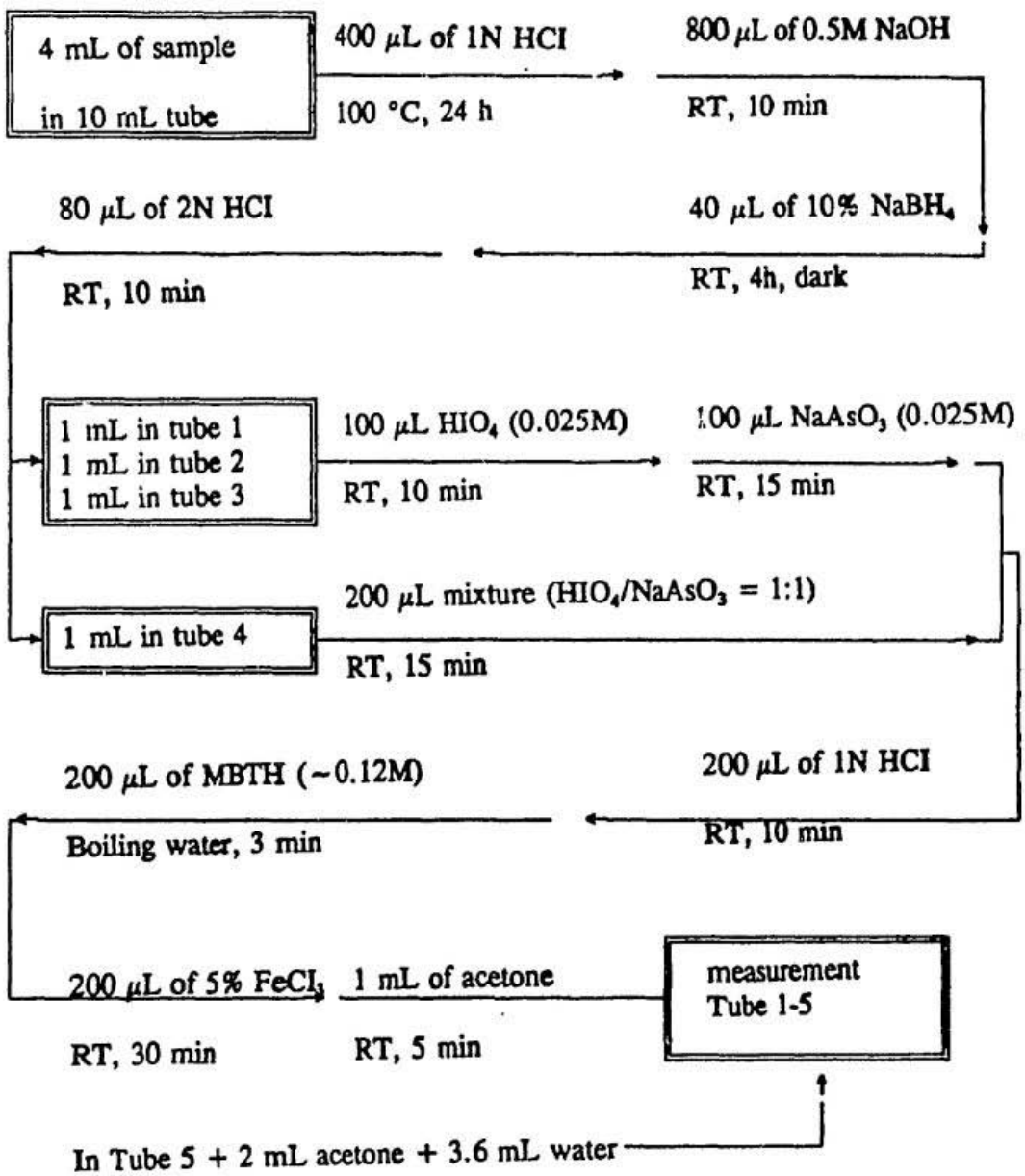


Figure 3.6 Chemical reactions for determination of carbohydrate by the MBTH method

Table 3.6 Flow chart for determining carbohydrates by the MBTH method



RT: Room temperature

Operational procedures

The operational procedures for measurement of total carbohydrate using the MBTH colorimetric method are shown in **Table 3.6**. The addition of analytical reagent was performed with two Eppendorf® autoclavable pipettes with adjustable volume ranges method between 10 and 100 μL, and 100 to 1000 μL. A Du®-65 spectrophotometer (Beckman Instruments) was used for detection.

If monosaccharides were determined, the hydrolysis step was unnecessary because glucose standard solutions were measured without hydrolysis. A dilution factor (1.3) resulting from addition of the acid reagent was considered when total carbohydrate concentrations were calculated.

Calibration curve

Glucose was used as the standard to make a calibration curve because glucose was a major monosaccharide in samples from the marine environment (Romano *et al.*, 1993). Prior to making the standard solution, glucose were dried at 102 °C for 4 h. Five different known concentrations of glucose standard solution were measured strictly according to the procedures in **Table 3.6** but without a hydrolysis step. The calibration curve for carbohydrates (**Figure 3.7**) was obtained through linear regression on glucose concentrations (μM) versus absorbence values at 635 nm.

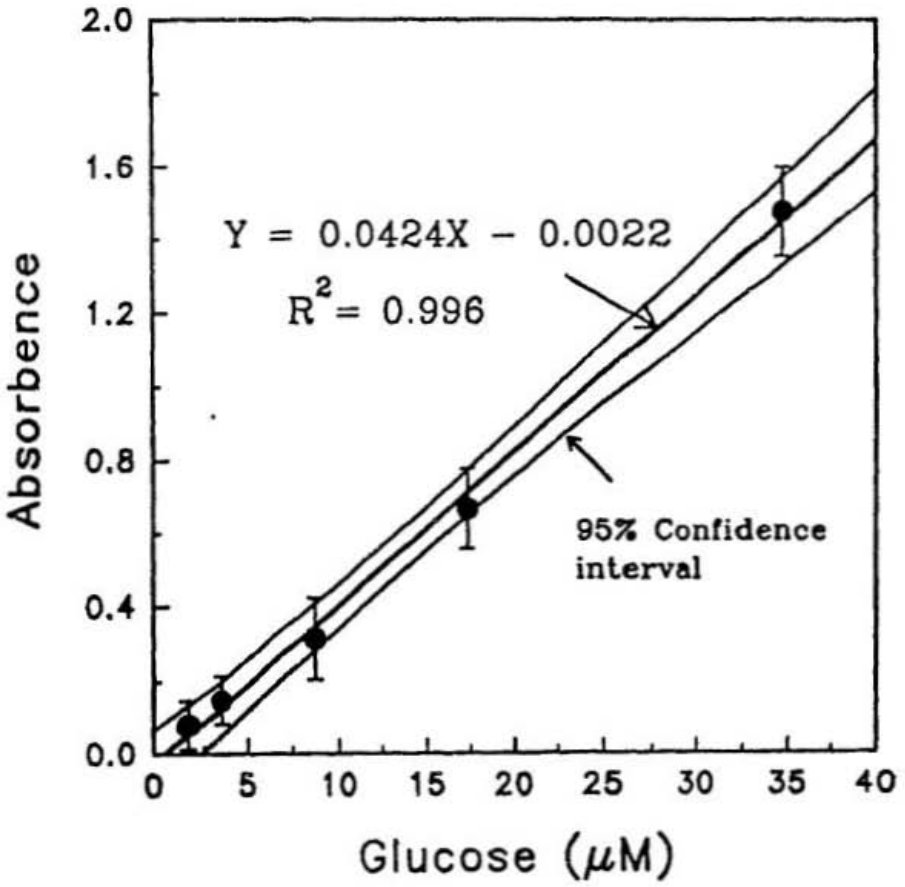


Figure 3.7 Calibration curve for carbohydrate measured by the MBTH colorimetric method

4.0 RESULTS AND DISCUSSION

4.1 Blank for measurements of lipid classes

Sometimes, disagreements on analytical data can be caused by blanks in analytical processes. For example, after he recognized a problem with his blank, Suzuki (1993) modified his DOC data (Sugimura and Suzuki, 1988) that had caused some arguments in the oceanographic community. Therefore, a relatively low blank value is an important factor in improving analytical precision. However, there are very few publications that discuss and report blank values with respect to analytical processes dealing with experimental data.

CFF is a good method to concentrate colloids for various studies (**Table 1.2**). TLC-FID has widely been used to analyze lipid classes (**Table 4.1**) in the marine environment because of its simplicity, flexibility, rapidity and sensitivity. However, at present, no publications exist which investigate lipid classes on marine colloids. This study not only focuses on lipid analysis but also on the use of blanks in analytical processes.

4.1.1 Chromarod-Iatroscan system blank

A series of blank values (**Table 4.2**) were obtained using the Iatroscan Mark V chromatographic analyzer. From **Table 4.2**, the total lipid blank value in the Chromarod-

Table 4.1 Total lipid concentrations in dissolved and particulate fractions of seawater in different locations

Location	Sampling Date	Depth	Filter	Method	"Dissolved" (µg/L)	Particulate (µg/L)	Reference
Gulf of Mexico	Nov., 1977	near surface	A/E (1.0 µm)	Gravimetric	69.9–81.3	12.2–22.2	Kennicutt and Jeffrey (1981)
	Feb., 1978	near surface			103.0–165.6	23.6–70.2	
	Nov., 1977	near bottom			62.6–116.3	12.8–18.1	
	Feb., 1978	near bottom			60.9–86.2	28.8–51.8	
Funka Bay, Japan	Jan. 1983–Jan. 1984	84-92 m	GF/C (1.2 µm)	Spectroscopic	15–287[a]	–	Yoshida (1984)
Bedford Basin, NS							
--Halifax Harbor	March–April, 1982	10 m	A/E (1.0 µm)	TLC–FID	195 ± 40	87 ± 13	Parrish (1987)
--Bedford Basin	Feb.–April, 1984	5 m			–	30–250	
Near Scotian Shelf							
--Bedford Basin	June, 1982	surface	A/E (1.0 µm)	TLC–FID	49–190	28–58	Parrish et al. (1988)
--Scotian Shelf	April 1985	Deep			29	9	
Coast of Nova Scotia							
--Northwest Arm	July 17–August 2, 1989	12 m	A/E (1.0 µm)	TLC–FID	87 ± 33	156 ± 59	Parrish et al. (1992)
--Ship Harbor	August 3, 1989				158	83	
Norrby Archipelago		0-14 m	1.0 µm	Gravimetric	105	104	Andersson et al. (1993)
		surface	0.2 µm	Gravimetric	77	132	
Northern Baltic Sea	March–June	surface	1.0 µm	Gravimetric	130	35–66	
Mediterranean Sea	May, 1991	0-100 m	GF/F (0.7 µm)	TLC–FID	15–75[b]	10–50[b]	Gerin and Goutx (1994)
		0-1600 m			9–113	3–84	
Conception Bay, NF	May 19, 1993	25 m	0.45 µm[c]	TLC–FID	63.9 ± 1.7	3.5 ± 2.6	This study
	June 14, 1993	10-25 m	0.45 µm		94.5 ± 16.9	1.1 ± 1.6	
	August 2, 1993	24-26 m	0.45 µm		87.6 ± 3.0	1.3 ± 1.2	
	October 5, 1993	25 m	A/E (1.0 µm)		26.5 ± 1.8	10.1 ± 1.7	

Note: [a] Data coverted from lipid carbon to total lipids assuming 75% C by mass.
[b] Data were estimated from vertical profiles
[c] Polyvinylidene fluoride membrane (Durapore, nominal pore size 0.45µm)

Table 4.2 Contributions to the blank by each step in the procedure

Lipid Classes	Chromarod & Iatroscan (n = 6-16) g/rod	Extracting solvent (n = 10-20) g/60mL	Distilled water (n = 6-15) g/L	Cross-flow Filtration 0.45 μm (n = 5-9) g/500mL	Cross-flow Filtration 10 kDa (n=2-9) g/500mL
HC	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.3	0.4 ± 0.6	0.5 ± 0.2
SE	N.D.	0.5 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.3
KET	N.D.	0.2 ± 0.0	0.0 ± 0.0	N.D.	0.1 ± 0.1
TAG	0.4 ± 0.0	2.9 ± 2.0	7.6 ± 2.8	3.8 ± 7.3	4.7 ± 5.4
FFA	0.2 ± 0.0	0.3 ± 0.2	0.6 ± 0.4	0.1 ± 0.6	-0.2 ± 0.6
ALC	N.D.	0.5 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	-0.2 ± 0.1
ST	N.D.	0.6 ± 0.0	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.2
AMPL	1.4 ± 0.3	1.0 ± 0.5	0.2 ± 0.8	1.4 ± 1.5	1.8 ± 1.1
PL	1.5 ± 0.3	-0.2 ± 0.4	0.1 ± 0.4	0.2 ± 0.4	0.2 ± 0.4
TOTAL	3.6 ± 0.4	6.0 ± 2.1	8.8 ± 3.0	6.1 ± 7.5	7.2 ± 5.6

Iatroscan system is 3.6 ± 0.4 μg. Polar lipids such as AMPL and PL contribute about 80% to this blank. In the analytical process, polar lipid peaks are obtained in the third development with a mixed solvent system (acetone: methanol: water 5:4:1). Polar lipids in solvents can be absorbed on the Chromarods and then developed in this solvent system. Hence, the use of pure development solvents is a key to decreasing the blank value in the Chromarod-Iatroscan system.

After using ultrapure water extracted with chloroform instead of unextracted distilled water as the development solvent, the blank value was decreased by 2-3 μg. This indicates that polar lipids (AMPL and PL) in the Chromarod-Iatroscan system came

mainly from the development procedure rather than during the conditioning or the spotting procedures.

4.1.2 Extracting solvent blank

During sample handling, chloroform was used to extract lipids in the sample. A blank of the extracting solvent was studied. Unlike the Chromarod-Iatroscan system blank, acetone-mobile lipids (AMPL), triacylglycerols (TAG), but not phospholipids (PL), were the major lipid classes in the extracting solvent blank (**Table 4.2**). The data indicate TAG has a high coefficient of variation, and this suggests that some TAG may be coming from sample handling procedures (concentration and transfer) rather than the extracting solvent. Analytical results from seawater samples also indicate TAG measurements have high S.D.. Why was TAG easily introduced from sample handling procedures? Is the peak that appears in the TAG position really a "TAG" lipid? If not TAG, what is the exact contamination source? These questions will be answered only after further research.

4.1.3 Cross-flow filtration system blank

The blank in the cross-flow filtration procedure was obtained by recycling 500 mL of distilled water through the filters. So, in order to calculate a CFF blank, a water blank was needed to be obtained first. **Table 4.2** shows that TAG was major lipid class in the water blank.

The total lipid in the cross-flow filtration system (**Table 4.2**) for both filters is approximately 6-7 μg. The pattern of the lipid class distribution in the CFF system blank is almost same. TAG and AMPL contribute about 90% of the total lipids found in the CFF blank. Since both filters were made of different materials, it seems unlikely that TAG and AMPL came from the filters.

In order to estimate the DOC amount in the CFF system, organic carbon in the filtrates of artificial seawater was measured with a new solid state NDIR detector by Dr. Kepkay at the Bedford Institute of Oceanography, NS. The DOC concentrations in the filtrates for both 0.45 μm and 10 kDa filters in the CFF system were less than 14.5 μM C, approaching the detection limit.

The CFF lipid blank may be partly explained by the filter's "memory" effect (Whitehouse *et al.*, 1990) because high concentrations (20-30 mg/L lipid) of cultures had been filtered before determining the CFF blank. It is possible that lipids in the culture samples were adsorbed on the filters and then released slowly to contribute the CFF blank.

Although such "used" filters may be used to filter culture samples for measuring high concentration of lipid classes, it seems unsuitable to use these filters to filter small volumes of low lipid concentration seawater samples (20-90 μg/L) because of the possible "memory" effect. After recognizing this problem, new filters were used and a large volume (15-35 L) of seawater samples were filtered to obtain relatively accurate data.

In order to decrease the filter blank, immediately after the culture sample was

filtered (**3.2.2**). methanol/water (1:1 v/v) solution was filtered to clean the CFF system. In order to know the efficiencies of the cleaning procedure, the total blank (Chromarod & Iatroscan, solvent, water, and CFF system) was determined. The total lipid blank (20.5 μg) obtained after cleaning the CFF system is slightly lower than that (21.3 μg) before filtering culture sample (**Table 4.3**), which means the cleaning procedure is effective and no lipids from cultures remain on the filter after cleaning. Although the cleaning procedure was effective, pure methanol was not used to clean the CFF system, in case of change of pore size of the filter.

Table 4.3 Comparison of total blank (μg) before filtering culture samples with after cleaning the CFF system with methanol-water (1:1 v/v)

lipid classes	Total blank before filtering culture samples	Total blank after cleaning CFF system with filters installed
HC	1.4	3.9
SE	0.4	0.6
TAG	9.1	11.5
FFA	4.1	1.4
ALC	3.8	1.4
ST	0.7	1.9
AMPL	1.6	0.7
PL	0.6	0.7
TOTAL	21.3	20.5

Total blank includes the Chromarod-Iatroscan blank, solvent blank, water blank, and CFF system blank.

To filter seawater samples, new filters were used. Before they were used, they were completely cleaned with ultrapure distilled water for four days. Analytical results of lipid classes from the filtered fraction indicates that ultrapure water as a cleaning solvent is enough to clean filters that are used only for lipid in seawater samples.

However, filter cleaning for four days with water is still not enough for measuring carbohydrates with the MBTH method. New filters are usually stored in 5% aqueous formaldehyde solution. In the MBTH method, formaldehyde as an intermediate in the measurement procedure. Formaldehyde in the filter pores can get into the filtrate fraction during filtration. From some carbohydrate data, the concentration of carbohydrate and DOC in the filtrate is indeed higher than that in the retentate. Therefore, the new filters need to be cleaned further to remove formaldehyde from the filter pores. New filters that have been cleaned continuously with water for at least two weeks were found to have reasonable carbohydrate values.

Formaldehyde is easily oxidized to formic acid. Procedures containing oxidants as cleaning reagents can perhaps be effective to remove formaldehyde. Whatever the specific procedures that are applied, the cleaning solvent in last step of the procedure should be ultrapure distilled water.

4.2 Cross flow filtration

4.2.1 Characteristics of CFF

In order to characterize the composition of the low concentration OM in seawater, the first step is to collect sufficient quantities of unaltered OM. Adsorption on XAD resins and filtration are commonly used methods to concentrate OM in aquatic environment.

Adsorption on XAD resins suffers from shortcomings such as low recoveries, the necessity of adjusting pH, and preferential adsorption of hydrophobic constituents. Traditional flow-through ultrafiltration also has some disadvantages. During filtration, particles are kept on the filter. Thus, particles not only clog filter pores but also greatly decrease the filtration rate (mL/h). Because of pore clogging, nominal sizes of filters change greatly during filtration. It is obvious that traditional flow-through methods are not suitable for filtering large volumes of water samples and for isolating marine OM into particulate, colloidal, and truly dissolved fractions.

CFF differs from traditional flow-through filtration in that the flow is parallel rather than perpendicular to the filter. Therefore, tangential flow removes particles from the surface of the filter membrane, and thus reduces the clogging of filter pores, increasing filtration rates, and making particle cut-offs consistent with nominal pore size. Whitehouse *et al.* (1990) confirmed that colloids do not accumulate on the filter membrane. They found there was a very good linear relation between flux and increasing pressure when seawater was filtered by CFF.

CFF techniques allow the use of multiple filters in parallel to increase filtration area, thus filtration rates can be further increased. CFF is equipped with facilities for backflushing, which removes clogging particles and cleans the filter membrane. Another advantage of CFF is that it requires relatively lower filtration pressures than gel filtration. Higher pressure easily ruptures biological cells and causes some changes in the biochemical concentrations in filtrate fractions. The CFF apparatus has a relative small dead volume and little OM is lost.

The advantages mentioned above make the CFF method an important tool used in various studies (**Table 1.1**). However, there are few studies focused on CFF itself. There are very few papers available to report mass balances or recoveries of different substances on CFF filter membranes (Carlson *et al.*, 1985) as well as analytical blanks. This is partly attributed to the imprecisions in retentate characterization.

When concentrations in the unfiltered fraction are nearly equal to those in the filtrate, the characterization of the retentate fraction is difficult because of the small difference between the filtrate fraction and the original fraction. This sometimes results in large errors or unreasonable results because of the propagation of errors associated with each step in the analytical procedures (Caulcutt and Boddy, 1983). Thus, in order to improve precision on characterization of the retentate fraction, it is necessary to make direct measurement on the concentrated retentate fraction by a relatively sensitive analytical method, rather than to make an estimate by subtraction.

4.2.2 Optimization of CFF system

In order to optimize the CFF system and avoid contamination from system materials, Teflon fittings, valves and tubing were used. Before filtering samples, the CFF system was cleaned with purified water for about 4 days. After that, the total lipid blank and the DOC blank in the CFF system were determined by recycling purified distilled water and artificial seawater, respectively. The total lipid blank for the CFF system is about 6-7 μg. The DOC blank for artificial seawater is < 10 μM C which approaches the detection limit. From these data, we know the CFF system has a low organic blank value.

In order to avoid the chemical transformations of OM, the whole CFF system was placed to a ~ 180 L of covered box which contained a refrigerator, a fan, and a temperature control unit. The cold box was filled with nitrogen from a cylinder. In order to reduce filter clogging, the retentate fractions were not recycled through the filters.

4.2.3 Mass balance

Calculation of mass balance

In these studies, the volumes of all fractions, including the unfiltered fraction, were recorded to calculate the concentration factor, F ($F = V_i/V_r$ where V is volume, and i, r, indicate initial and retentate fractions). The concentration factors in these studies varied from 3.4 to 69 for seawater samples and from 7.0 to 16.7 for culture samples. Because the filtrate is closely related to a specific filter, the initial fraction was defined

as the unfiltered fraction for a specific filter. In a sequential (stepwise) filtration, the initial fraction in a second filtration is the filtrate fraction of the first step filtration. The detailed filtration sequence is shown in a filtration diagram (**Table 3.5**).

The concentrations (μg/L) of lipid classes in unfiltered (C_u), initial (C_i), retentate (C_r) and filtrate (C_f) fractions were obtained by subtracting blanks (Iatroscan system, solvent and filter). The concentration (μg/L) of lipid classes on particles (C_p) was obtained by applying the formula $C_p = (C_r - C_f)/F$. Mass balance, or recovery percentage, was calculated as $R(\%) = 100\ (C_p + C_r)/C_i$. Recoveries more than 100% indicate that there was some contamination from the CFF system. Recoveries less than 100% indicate that some materials were lost during cross flow filtration.

Mass balance of culture samples

From the mass balance obtained in the culture samples (**Table C1-C3**), recoveries of most lipid classes are close to 100%. The coefficient of variation (C.V.) for lipid classes varies from 5% to 50% for both 0.45 μm filter and 10K NMW cut-off filters. High C.V. values can be attributed to imprecisions in the measurement of trace materials. Individual lipid classes such as HC and SE sometimes have higher uncertainties in recovery because of their low concentrations and large C.V.'s in measurement near the detection limit. The recoveries for major lipid classes approach 100% with relatively low C.V. Therefore, recovery data obtained suggest contamination and adsorption from CFF system is minimal for filtering cultures having high

concentration of lipids. There were no obvious differences between the two kind of filters.

Mass balance of seawater samples

Recoveries obtained from seawater samples vary from 80% to 120% for most lipid classes (**Table D1-D4**). However, abnormally high recoveries of 756% (**Table D3**), 176%, 202% (**Table D2**) were obtained for TAG, FFA, and ALC neutral lipid classes. Apparently, these abnormal values result from contamination or possible decomposition of some other materials. This kind of situation sometimes occurs in blank results. Why is TAG in seawater easily contaminated? What processes cause contamination? The answers to these questions are not clear at the moment. How are the high recoveries for FFA and ALC explained? A possible explanation is the decomposition of TAG, WE or other materials during the CFF process. Low recoveries of HC (63%) appear to result from loss of lipids during CFF or absorption onto the 0.45 μm filter.

From recoveries obtained from seawater samples (**Figure 4.1**), the S.D. on 10K NMW cut-off filters is usually larger than that on 0.45μm filters. Seawater samples are more prone than cultures to contamination because of low lipid contents. However, the C.V. of the culture samples is usually larger than that of seawater. Thus, it may be attributed to the higher biological activity in the algal samples. There is no obvious relationship between recoveries and concentration factors. These results obtained with

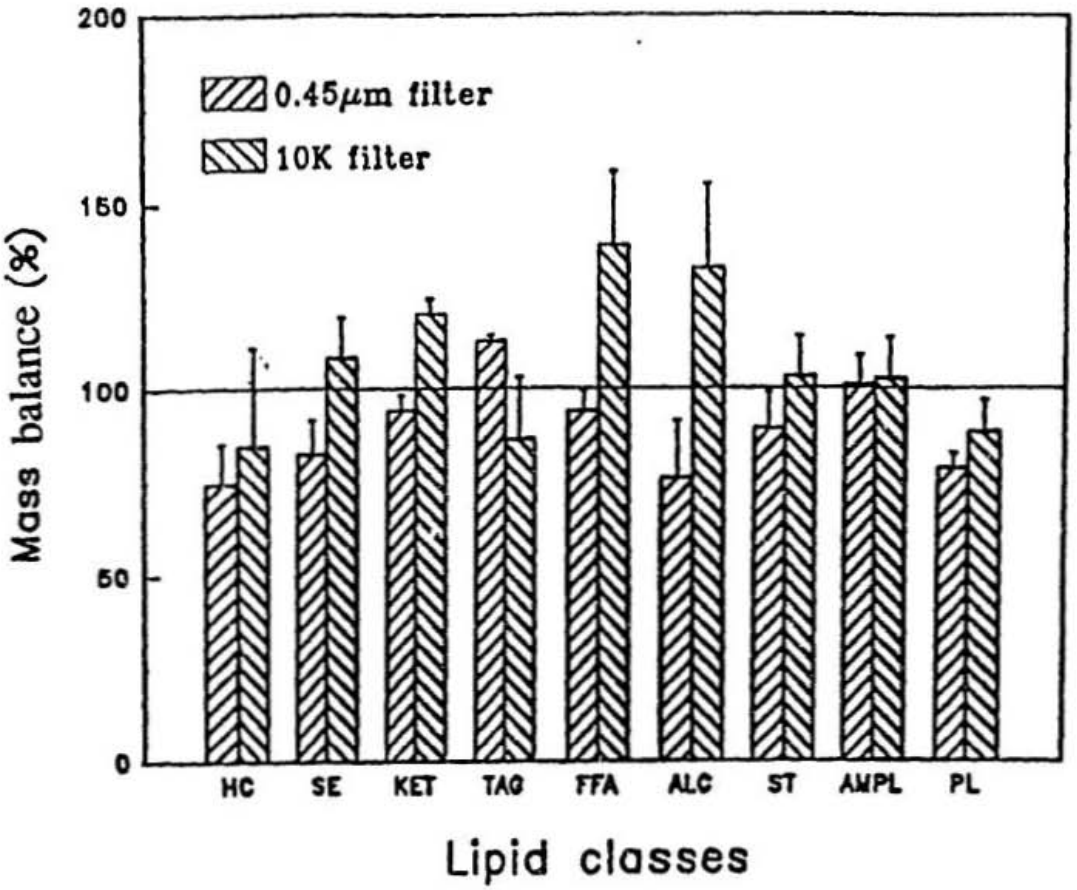


Figure 4.1 Mass balance (%, mean ± S.E.M., n = 3-4) of lipid classes in seawater samples for two filters

CFF are consistent with the results of Whitehouse *et al.* (1990). They obtained recoveries of organic carbon and trace metals in seawater and river water by CFF.

From the discussion above, it is possible to avoid contamination from CFF materials by cleaning the CFF system and optimizing operation procedures. Possibly, decomposition of some organic materials can occur during CFF filtration. For most lipid classes, especially major lipid classes, the mass balance approaches 100%. Therefore, after thorough cleaning, CFF is suitable for studies of lipid class concentrations.

4.3 Lipid class concentrations in seawater samples

Lipids are a group of hydrophobic compounds easily soluble in organic solvents. The structure, form, and characteristics of lipids are very complex. They are present in dissolved, colloidal and particulate form in the oceans. Chemical transformations can occur among different lipid classes. The concentration and composition of lipids can be affected by biological, geochemical and anthropogenic factors.

In order to give a general idea about lipid classes in the ocean, total lipid class concentrations will be discussed firstly. After that, lipid classes and carbohydrates in particulate, dissolved, and colloidal fractions will then be discussed.

4.3.1 Total lipids

From the literature (**Table 4.1**), the total lipid concentrations for seawater samples in different locations are in the range of 15-267 μg/L. The total lipid concentrations in

this study (**Table 4.4**) were found to be 34-99 μg/L, which are comparable with data in the literature. Total lipid concentrations were obtained by measuring lipids in prescreened (75 μm) seawater samples. Because no contamination came from filtration procedures, these data are relatively accurate and precise. The coefficient variation for these data is lower than 10%.

Table 4.4 Lipid class concentrations (μg/L, mean $\pm$ S.D., n= 4-8) in unfiltered seawater samples collected in Conception Bay, Newfoundland.

Sample	May 19	June 14	August 2	October 5
HC	1.5 $\pm$ 0.2	3.3 $\pm$ 0.2	2.9 $\pm$ 0.4	4.4 $\pm$ 0.6
SE	0.5 $\pm$ 0.2	0.2 $\pm$ 0.1	0.5 $\pm$ 0.1	1.8 $\pm$ 0.4
KET	0.7 $\pm$ 0.2	1.0 $\pm$ 0.0	0.9 $\pm$ 0.1	1.6 $\pm$ 0.0
TAG	25.2$\pm$ 4.0	30.9$\pm$ 2.1	6.5 $\pm$ 0.1	5.5 $\pm$ 0.3
FFA	5.8 $\pm$ 0.0	6.0 $\pm$ 0.7	5.6 $\pm$ 0.1	3.9 $\pm$ 0.7
ALC	1.0 $\pm$ 0.4	1.6 $\pm$ 0.2	1.0 $\pm$ 0.0	1.5 $\pm$ 0.1
ST	2.5 $\pm$ 0.1	2.7 $\pm$ 0.1	1.3 $\pm$ 0.0	1.6 $\pm$ 0.2
AMPL	23.1$\pm$ 3.7	40.5$\pm$ 1.3	23.1$\pm$ 1.0	9.9 $\pm$ 0.3
PL	4.4 $\pm$ 1.7	12.8$\pm$ 0.2	3.0 $\pm$ 1.5	3.3 $\pm$ 0.0
Nonpolar[a]	2.8 $\pm$ 0.3	4.5 $\pm$ 0.2	4.3 $\pm$ 0.4	7.8 $\pm$ 0.7
Neutral[b]	34.5$\pm$ 4.1	41.1$\pm$ 2.2	14.4$\pm$ 0.1	12.5$\pm$ 0.8
Polar[c]	27.5$\pm$ 4.4	53.2$\pm$ 1.4	26.1$\pm$ 1.9	13.3$\pm$ 0.4
Total	64.7$\pm$ 5.8	98.9$\pm$ 2.6	44.9$\pm$ 1.9	33.6$\pm$ 1.1

[a] HC+SE+KET
[b] TAG+FFA+ALC+ST
[c] AMPL+PL

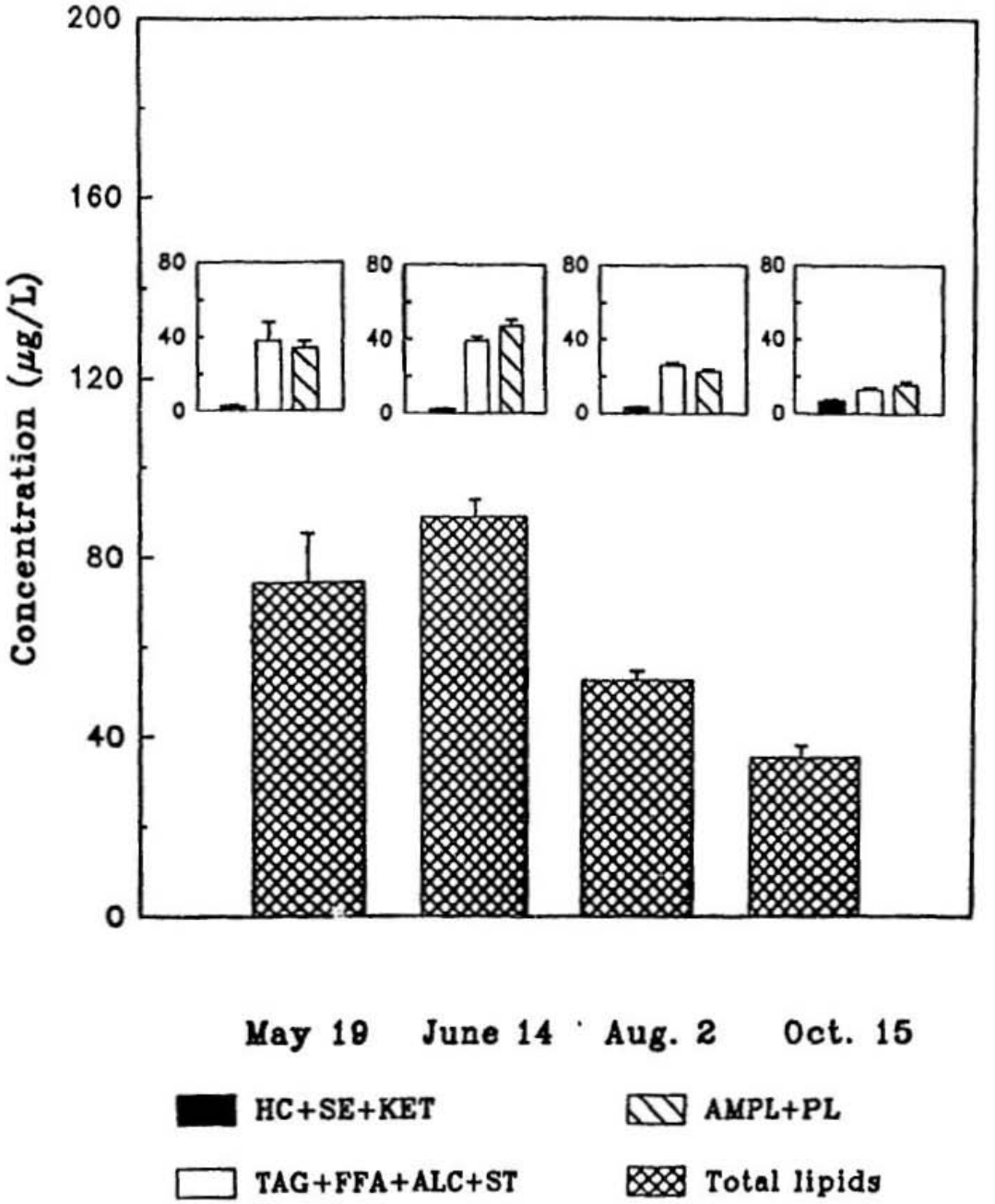


Figure 4.2 Total lipid concentrations in seawater

The lipid concentrations in May and June are higher than in August and October (**Figure 4.2**). This suggests high lipid concentrations are related to the spring bloom which usually occurs from mid-March to mid-May in Conception Bay. The maximum total lipid concentration occurred in seawater samples collected in June. It can be explained as a result of the degradation and metabolism of biomass and disruption of dead microorganisms. On the other hand, seawater collected in June was different from other samples as it was a integrated seawater from different depths (10, 15, 20 and 25 m). A large amount of biomass in the upper layer seawater apparently contributed to the maximum lipid concentration.

In four different seawater samples, major lipid classes are AMPL and TAG, which account for 29-52% and 14-38% of total lipids, respectively. Minor lipid classes are SE/WE, KET, ALC, and ST, which account for less than 6%.

4.3.2 Hydrocarbons (HC)

Hydrocarbons are conventionally grouped among lipid classes because they are easily extracted with acyl lipids into organic solvents. Iatroscan-determined HC include alkanes and alkenes. Other hydrocarbons commonly found in the marine environment include polycyclic aromatic hydrocarbons (PAH), chlorinated hydrocarbon pesticides such as DDT, and polychlorinated biphenyls (PCB). PAH, DDT, and PCB concentrations in seawater are in the order of ng/L. Long-chain alkanes in the range C_{14}-C_{37} are dominant

constituents in HC (Kennicutt II and Jeffrey, 1981, Comez-Belinchon, 1988). *n*-Alkanes have been found to account for 20% of total HC .

HC originates from both biogenic sources (microalgae) and anthropogenic inputs (urban input and oil pollution). Alkanes mainly come from biogenic sources and PAH from anthropogenic inputs. The compositional distribution of HC can be used to explain the origin of HC.

For instance, odd carbon-number n-alkanes lower than C_{21} are commonly found in algae. A C_{25}-C_{35} odd carbon-number n-alkane distribution is an indicator of the contribution from higher plants. A broad spectrum (C_{14}-C_{36}) n-alkane without an odd-even carbon number predominance may be produced from petrogenic inputs. Because PAH are rare in microorganisms, PAH can be used as an indicator of petrogenic inputs. Since hydrocarbons generally account for 1% or less of total lipids in microorganisms, HC are often thought of as being indicators of contamination.

In the literature, total hydrocarbon concentrations in seawater vary from < 1 μg/L to hundred μg/L (**Table 4.5**). Pollution at sampling sites and different methodologies for isolation and analysis are responsible for the variability of the results. Concentrations as high as 39 μg/l of HC were measured by TLC-FID found in the Northwest Arm in Nova Scotia (Parrish, 1992). Concentrations derived from gas chromatography (~0.05 μg/L) were much lower than determined gravimetrically (~4 μg/L) (Kennicult II and Jeffrey, 1981; Gomez-Belinchon *et al.*, 1988) because of the selectivity of GC.

Table 4.5 Total hydrocarbon concentrations (μg/L) in seawater. Data from the literature are the sum of dissolved and particulate concentrations.

Reference	Concentration (μg/L)	Sample information
Parker *et al.* (1972)	0.78-1.1	Off-shore seawater from bottom 2000 m
Barbier (1973)	9-56	North and equatorial seawater
Brown *et al.* (1974)	3.0	Subsurface sample from Mediterranean Sea
Hardy *et al.* (1975)	4.7±0.5	Subsurface sample from North Sea
Hardy *et al.* (1977)	< 10	
Zsolnay (1977)	< 10	
Saliot (1981)	1-50	
Kennicutt and Jeffrey (1981)	3-7	Surface and bottom, Gulf of Mexico
Parrish (1987)	17.3 ± 4	Surface mixed layer, Bedford Basin
Parrish *et al.* (1988)	2.3-13	Coastal seawater
Parrish *et al.* (1992)	23 ± 4.1	Ship Harbour, summer Coastal seawater
This study (1994)	1.5-4.4	Surface, May-Oct., Coastal seawater

The hydrocarbon concentrations in this study (**Table 4.4**) were obtained using the TLC-FID method. The lipid concentration data obtained by TLC-FID method were consistent with the results obtained gravimetrically (Parrish, 1987). The HC concentrations in seawater collected from Conception Bay are in the range of 1.5-4.4 μg/L. These results are in agreement with the data in literature (**Table 4.5**). The total HC concentration in typical unpolluted seawater usually is below 10 μg/L (Hardy *et al.*, 1977; Zsolnay, 1977; De Lappe *et al.*, 1980; Kennicult II and Jeffrey, 1981). This suggests no obvious pollution at the sampling site in Conception Bay and no sample contamination from the analytical procedures; therefore HC in Conception Bay seawater are mainly of biogenic origin. This suggestion is in accord with Goutx and Saliot's (1980) results. They studied the correlation between particulate hydrocarbon, particulate *n*-alkanes, and chlorophyll *a* contents, and showed that phytoplankton are a direct contributor to the hydrocarbons of seawater. Osterhot *et al.* (1983) found biogenic hydrocarbons are present in highly variable concentration at all seasons but always predominate over petrogenic hydrocarbons. The more stable biogenic alkanes seem to accumulate starting in the spring and to decrease during winter.

The results in this study indicate that HC concentration trends to increase slightly from May to October whereas the sum of HC and FFA concentration showed little change. This suggests that the increase of HC might come from the decarboxylation of FFA because FFA are a common biosynthetic precursor of HC in cyanobacteria, yeast,

algae, and zooplankton. The decarboxylation pathway was proved by labelling experiments (Han *et al.*, 1969). It is known that in different varieties of marine algae, including a red, greens, browns, diatoms and other phytoplankton, the hydrocarbon heneicosahexaene (C-21:6) exists in amounts inversely correlated with the abundance of the long-chain highly unsaturated fatty acid (C-22:6) (Youngblood and Blumer, 1973; Tornabene; 1981). It has been suggested that hydrocarbons in certain specific bacteria are end-products of the metabolic regulation of the fatty acid cycle (Tornabene, 1981; Suen *et al.*, 1987). Emdadi and Berland (1989) found that as much as 78% of the total lipid in *N. salina* and 48% in *P. Lutheri* are hydrocarbons in lag-phase cultures. Since microorganisms do not reproduce during lag phase, oxidation of OM and energy demands are at a minimum. hydrocarbons could be the result of the metabolism of fatty acids in microorganisms placed in a favourable environment with a large supply of nutrient salts and highly available light energy.

Parker *et al.* (1972) determined *n*-alkane content in three samples from off-shore. The total alkane concentration ranged from 0.78-1.1 μg/L. They regarded 0.78 μg/L in samples from a depth 2,000 m as a typical baseline value for unpolluted seawater. According to this baseline value, seawater in Conception Bay would seem to be heavily polluted. However, this baseline value does not apply to coastal surface seawater because a large amount of biomass exists in surface water.

4.3.3 Wax and sterol esters (WE/SE)

SE/WE represents sterol esters and wax esters. Other fatty acid ethers and short-chain fatty acid esters can be grouped into the SE/WE lipid class due to difficulty in their separation on gel layers (Romankevich, 1984). WE can serve as a metabolic energy reservoir of aquatic microorganisms. Sterol esters are components of cell membranes. Typical wax esters are rare in algae, bacteria, and viruses, although a small amount of wax esters exists in marine dinoflagellates and zooplankton. Therefore, WE has been suggested as zooplankton tracer. It was suggested that short-chain fatty acid esters such as methyl palmitate are formed from degradation of humic substances (Kennicutt and Jeffrey, 1981a, b) and the action of yeast on marine hydrocarbons (Blasig *et al.*, 1984).

SE/WE content in seawater (**Table 4.4**) obtained in this study ranged between 0.2 and 1.8 μg/L, which is toward the lower end of the literature range. From the few literature data availabie, the concentrations of SE/WE lipid classes vary from 0 to 20 μg/L (Romankevich, 1984; Parrish, 1987, 1988, 1992). Low SE/WE content can be explained by the relatively small amount of biomass in Newfoundland waters. SE/WE are minor lipid classes in seawater. They mainly originate from zooplankton. A conservative estimate of WE/SE concentrations should be not higher than 5 μg/L for surface seawater and not higher than 1 μg/L for deep seawater.

4.3.4 Triacylglycerols (TAG)

TAG is a major lipid class which accounts for 14-38% of total lipids in Newfoundland waters. Their functions are similar to those of wax esters and mainly serve as energy reservoirs. TAG is found in flagellates, diatoms (Parrish and Wangersky, 1987), algae, phytoplankton, sea-foam, and the surface microlayer of the ocean. However, only a small amount of TAG is present in bacteria. Unlike phytoplankton, zooplankton species preferentially synthesize large amounts of WE rather than TAG as an energy reservoir (Wakeham *et al.*, 1981). Nutrient availability greatly affects the TAG content in algal cells (Parrish and Wangersky, 1987, 1990; Hodgson *et al.*, 1991).

Total TAG concentrations in this study varied from 5.5 to 31 μg/L (**Table 4.4**), which is in the range of 2.8-47 μg/L of TAG concentration for the surface mixed layer in coastal seawater (Parrish, 1988). TAG concentrations decreased dramatically from 25.2 μg/L (May) to 5.5 μg/L (October). A large amount of TAG may have been biosynthesized during the spring bloom. The maximum TAG concentration may have occurred in the late period of the spring bloom as a result of accumulation under condition of nutrient limitation.

4.3.5 Free fatty acids (FFA)

Free fatty acids (FFA) are an important lipid class because they can serve as source of energy for microorganisms, as complexing agents for trace metals, and as indicators of degradation of acyl lipids. Their strong surface-active properties play a key

Table 4.6 Total free fatty acid concentrations (μg/L) in seawater. Data from the literature are the sum of dissolved and particulate concentration.

Reference	Concentration (μg/L)	Information of samples
Williams (1965)	1.25-8.84	Coastal and offshore, 20-2310 m, filtered seawater, eastern Pacific
Quinn and Wade (1972)	13-60	
Kattner (1983)	8-16	Northern North sea, 1976 spring bloom
Parrish (1987)	24 ± 6	Bottom of surface mixed layer 1982 Bedford Basin spring bloom
Parrish (1988)	1.8-47	Scotian Shelf and Bedford Basin
Gomez-Belinchon (1988)	5-44	Deltaic environment (bays, channels)
Parrish (1992)	26 ± 7 28.6 ± 2.5	Northwest Arm Ship Harbour
Gerin *et al.* (1993)	1-26 (-10) 0-18 (~5) 0-19.3 (~5.1) 2-6.5 (~3.5) 0-6.4 (~3)	20-500 m 30-700 m 25-1300 m 25-1600 m 40-1600 m
This study (1994)	3.9-6.0	Surface coastal seawater Conception Bay

role in adsorption/desorption and in inhibiting the growth of other species of algae or bacteria.

FFA are widely present in bacteria, algae, sea-foam, and the sea surface microlayer. FFA in seawater mainly comes from excretion and degradation processes of phytoplankton. From 5% to 25% of total lipids in phytoplankton are FFA. FFA accounts for 0.1-0.4% of an organism's dry weight (Zsolnay, 1977). Sometimes, anthropogenic inputs are important sources, especially in coastal seawater near urban areas.

FFA concentrations in four seawater samples collected from Conception Bay are in the range of 3.9-6.0 μg/L and account for 6-12% of total lipids (**Table 4.4**). These values appear to be low compared to literature data (0-60 μg/L) (**Table 4.6**). However, they are in agreement with William's (1965) data (1.3-8.8 μg/L). A FFA concentration for a typical seawater sample without pollution should be below 10 μg/L. From May to August, the FFA concentration changed slightly, but the FFA concentration decreased rapidly from August to October. These results suggest FFA may be degraded from other lipids such as TAG and polar lipids. On the other hand, FFA can produce HC by decarboxylation.

4.3.6 Free fatty alcohols (ALC)

The results obtained in this study (**Table 4.4**) showed that ALC was minor lipid class in seawater and that no great concentration changes occurred in 4 different samples. The concentration of ALC ranged from 1.0-1.6 μg/L. These results are similar to Gerin's

(1993), in which they found the ALC concentration ranged from 0 to 19.5 μg/L in nearly 100 samples from 6 locations with a depth range of 20-1600 m in the Mediterranean sea. Eighty percent of his data was in the range of 0.5-3.0 μg/L. Low levels of ALC perhaps suggest ALC is an active intermediate in biochemical processes.

Like FFA, ALC is also a degradation product of other lipid classes and a strong surface active material. The surface active properties affect cell plasticity and membrane permeability. It can be absorbed onto surfaces of small particles, thus modifying the particle surface and affecting aggregation and bioavailability of small particles.

4.3.7 Sterols (ST)

ST mainly comes from the organisms in surface waters (0-800 m). The majority of marine sterols contain 27-29 carbon atoms with the C_{29} sterols being predominant. Cholesterol is the most abundant sterol in the higher invertebrates. There are 0.4% sterols in phytoplankton (Boutry and Jacques, 1970), 0.47% in marine diatoms (Boutry and Barbier, 1974), 0.01% to 2% in marine invertebrates, 0.005% to 0.5% generally in marine plants, and 0.01 to 0.1% in marine yeasts (Teshima and Kanazawa 1971, 1972). Most bacteria cannot produce sterols. Free sterols account for as much as 17% of the lipids in marine diatoms (Lee *et al.*, 1971). The major sterols are C_{29} (β-sitosterol) and C_{27} (cholesterol).

There were 1.6-2.5 μg/L of sterols in seawater collected in Conception Bay (**Table 4.4**). Sterols are a minor lipid class, which account for less than 5% of total

Table 4.7 Total sterol concentrations (μg/L) in seawater. Data from the literature are the sum of dissolved and particulate concentration.

Reference	Concentration (μg/L)	Information of samples
Saliot and Barbier (1973)	2-14	Eastern Atlantic
Gagosian (1976)	0.4	Sargasso Sea
Parrish (1987)	14.7 ± 7	Bottom of surface mixed layer 1982 Bedford Basin spring bloom
Parrish *et al.* (1988)	0.6-1.1 1.0-1.8 1.8-3.4	Scotian Shelf and Bedford Basin at three stations
Parrish *et al.* (1992)	6.6 6.1	Northwest Arm Ship Harbour
Gerin *et al.* (1993)	0-2.2 0-2.9 0-5.7 0-2.5 0-1.1	20-500 m 30-700 m 25-1300 m 25-1600 m 40-1600 m
This Study (1994)	1.6-2.5	Surface mixed layer Conception Bay

lipids. Sterols are mainly derived biogenically, however, river inputs also contribute to the ST concentration in seawater. As much as 10 μg/L of ST has been detected in Delaware River (Sheldon and Hites, 1978). For a typical surface seawater sample, except for those collected from a polluted area or in the spring bloom, the sterol concentration was estimated to be lower than 5 μg/L (**Table 4.7**).

4.3.8 Acetone-mobile polar lipids (AMPL)

AMPL is an operationally defined lipid class. It includes monoglycerides, glycolipids, chlorophylls and other pigments. A small amount of monoglyceride is present in cells and seawater because it is an active intermediate. Glycolipids and chlorophylls are major constituents of AMPL. They are present in plant cells. Chlorophyll ***a*** is a principal pigment in all photosynthetic plant cells, therefore the concentration of chlorophyll ***a*** can be used to indicate the development of a phytoplankton bloom.

AMPL is a dominant lipid class which accounts for 30-50% of the total lipids in this study (**Table 4.4**). The concentration of AMPL varies from 9.9-40.5 μg/L. These results are consistent with data in the literature, 9.6-35 μg/L for the surface mixed layer, 4.8-32 μg/L for seawater below the pycnocline, and as high as 110 μg/L for seawater collected from Nova Scotia inlets in the summer and in the surface mixed layer during the spring bloom (Parrish, 1987, 1988, 1992). The AMPL concentration in the

Mediterranean Sea with various depths ranging from 20-1600 m are 4 to 70 μg/L (Gerin, 1993).

AMPL concentration in an integrated seawater sample (same amount of seawater from 10, 15, 20, 25 m depths) is twice that in seawater from only 25 m (**Table 4.4**). This could indicate that the phytoplankton in upper layer of surface water were twice as numerous as in the lower layer of surface seawater.

4.3.9 Phospholipids (PL)

A large amount of PL are present in an organism's cells. PL accounts for 38% of the total lipids in nitrogen-replete cells of *Phaeodactylum tricornutum* (Parrish and Wangersky, 1987). PL in four marine bacterial strains accounted for 81.7% of total cellular lipids and 11-82% of total extracelluar lipids when grown on different substrates (Goutx *et al.*, 1990).

The PL in seawater mainly comes from organisms. PL concentrations in this study (**Table 4.4**) are in the range of 3.0-13 μg/L. PL concentration (12.8 μg/L) in an integrated seawater sample (June 14) is about three times higher than that of the other three seawater samples (3.0-4.4 μg/L). This suggest that there is a large amount of PL in the upper layer of the surface seawater.

The PL data obtained in this study are consistent with the literature data. There are 1.8 μg/L to 46 μg/L PL in coastal seawater (Parrish, 1987, 1988, 1992). Gerin (1993) analyzed 93 seawater samples collected from 6 sites with various depths from 20

to 1600 m and found PL concentrations in the range 2.4-39 μg/L. Ninety percent of data are below 15 μg/L.

Contrary to other lipid classes, PL contains both N and P. Unlike TAG, the PL content decreases in nitrogen-limited cultures. The strong negative correlation between TAG and PL suggests TAG and PL have a common precursor, phosphatidic acid (Gurr and James, 1980; Parrish and Wangersky, 1987). However, such a correlation was not found in seawater collected in Conception Bay. This suggested nutrient limitation may not have occurred in Conception Bay during the summer. This suggestion supports the idea that hydrocarbons were the result of the metabolism of fatty acids in microorganisms placed in a favourable environment with a large supply of nutrient salts (Emdadi and Berland, 1989).

4.4 Particulate and dissolved lipids in seawater samples

The distinction between particulate and dissolved fractions is based on whether or not they can pass through a filter. In this study, the arbitrary filter is 10,000 NMW cut-off filter (pore size $\sim$3 nm). Dissolved lipid concentrations were obtained by measuring the dissolved fraction directly. CFF-determined particulate lipid concentrations were obtained by measuring the lipid concentration in the retentate fraction, subtracting the dissolved lipid concentration from the retentate lipid concentration, and then dividing by concentration factors.

Total lipid concentration in seawater for summer samples is 72.5 μg/L. About 90% of lipid is present in dissolved form and only 10% of lipid is associated with particles (**Figure 4.3**). From **Table 4.8**, 60-95% of total lipids was mainly present in the dissolved fraction. Particulate lipids accounted for 6.5-31% of the total lipids. It is clear that some lipid classes preferentially exist in the dissolved form while some other lipid classes can preferentially associate with particles. For instance, 80-98% of AMPL is present in the dissolved form whereas dissolved HC accounts for 47-83% of total HC. AMPL includes the very soluble glycolipids whereas HC includes the least soluble lipids.

Maximum dissolved lipid concentrations occurred in May and June. From June to October, total dissolved lipid concentrations decreased. These results indicate the dissolved lipid concentrations were affected by the spring bloom. The concentrations of particulate lipids did not show a trend (**Figure 4.4**). This suggests that the factors governing particulate lipids are more complex than those governing dissolved lipids. Although concentrations of total particulate lipids did not show any trends, individual particulate lipid classes such as HC did show a trend. Particulate HC increased from May to November.

An interesting observation is that dissolved FFA concentrations increased as dissolved TAG concentration decreased in June and July. This would support the view that FFA is derived from degradation of TAG.

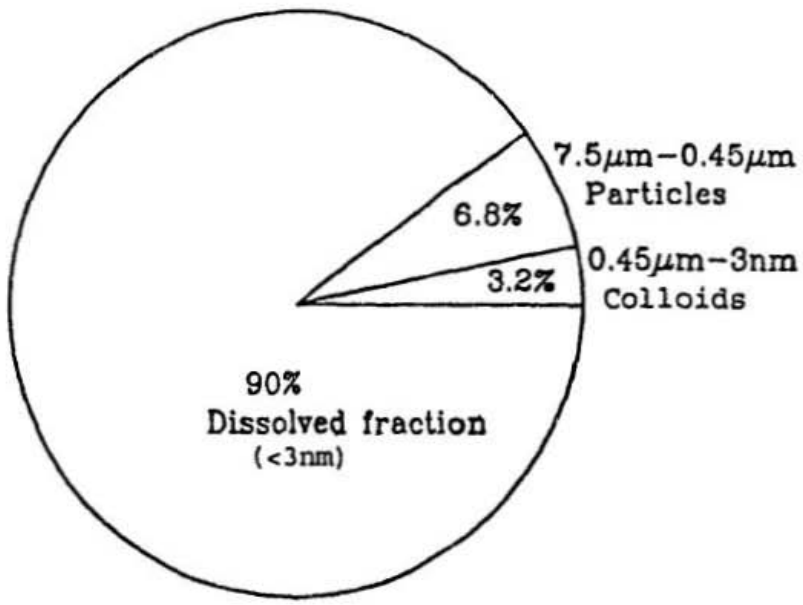


Figure 4.3 Concentration distribution of total lipid in particle pools and dissolved fraction

Table 4.8 Concentration (μg/L) of lipid classes in the particulate (Part.) and dissolved (Diss.) fractions of seawater collected in Conception Bay, NF in 1991 and 1993

Lipid Class	Sept. 17, 1991		Nov. 7, 1991		May 19, 1993		June 16, 1993		Aug. 2, 1993		Oct. 5, 1993	
	Part. >3nm	Diss. <3nm	Part. >3nm	Diss. <3nm	part. >3nm	Diss. <3nm	Part. >3nm	Diss. <3nm	Part. >3nm	Diss. <3nm	Part. >3nm	Diss. <3nm
HC	0.9	0.7	1.5	2.0	0.3 ± 0.3	1.2 ± 0.3	0.3 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	1.3 ± 0.1	3.0 ± 0.7	1.7 ± 0.3
SE	0.2	0.1	0.4	2.1	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.0	0.2 ± 0.1	0.4 ± 0.1
KET	–	–	–	–	0.1 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2
TAG	1.6	6.0	2.6	3.6	1.8 ± 2.7	27.1 ± 9.8	3.8 ± 1.4	21.1 ± 1.0	1.4 ± 0.8	8.6 ± 0.2	1.5 ± 0.7	3.4 ± 0.2
FFA	0.5	8.4	0.2	1.5	1.1 ± 0.2	5.0 ± 0.7	3.7 ± 0.9	6.5 ± 0.8	0.9 ± 0.3	12.4 ± 1.1	0.6 ± 0.3	3.3 ± 0.4
ALC	–	1.2	0.3	1.3	0.1 ± 0.2	0.7 ± 0.2	0.0 ± 0.0	1.9 ± 0.5	0.1 ± 0.1	1.3 ± 0.1	0.4 ± 0.1	1.0 ± 0.3
ST	0.2	0.7	0.1	1.8	0.2 ± 0.2	1.8 ± 0.3	0.1 ± 0.1	2.1 ± 0.2	0.3 ± 0.1	1.3 ± 0.2	0.9 ± 0.2	1.7 ± 0.9
AMPL	2.9	11.8	2.2	12.7	0.5 ± 0.4	30.0 ± 3.5	0.5 ± 0.7	37.3 ± 2.9	1.0 ± 1.3	19.7 ± 0.6	2.3 ± 1.1	9.3 ± 1.6
PL	0.5	2.0	0.8	1.6	0.7 ± 0.4	2.6 ± 0.5	1.4 ± 0.3	8.0 ± 1.6	0.2 ± 0.1	1.7 ± 0.3	1.3 ± 0.7	2.7 ± 0.3
Least polar	1.1	0.8	1.9	4.1	0.5 ± 0.3	2.3 ± 0.3	0.4 ± 0.1	2.0 ± 0.2	0.9 ± 0.1	2.6 ± 0.2	4.0 ± 0.7	3.0 ± 0.3
Neutral	2.3	16.3	3.2	8.2	3.2 ± 2.7	34.5 ± 9.9	7.6 ± 1.7	31.6 ± 1.4	2.7 ± 0.9	23.5 ± 1.1	3.4 ± 0.8	9.4 ± 1.0
Polar	3.4	13.8	3.0	14.3	1.2 ± 0.5	32.7 ± 3.5	1.9 ± 0.7	45.3 ± 3.3	1.2 ± 1.3	21.4 ± 0.7	3.6 ± 1.3	12.0 ± 1.7
Total	6.8	30.9	8.1	26.6	4.8 ± 2.7	69.5 ± 10.5	9.9 ± 1.9	78.9 ± 3.6	4.8 ± 1.6	47.5 ± 1.3	11.0 ± 1.7	24.3 ± 2.0

Note: * Data obtained from Dr. Parrish (unpublished).

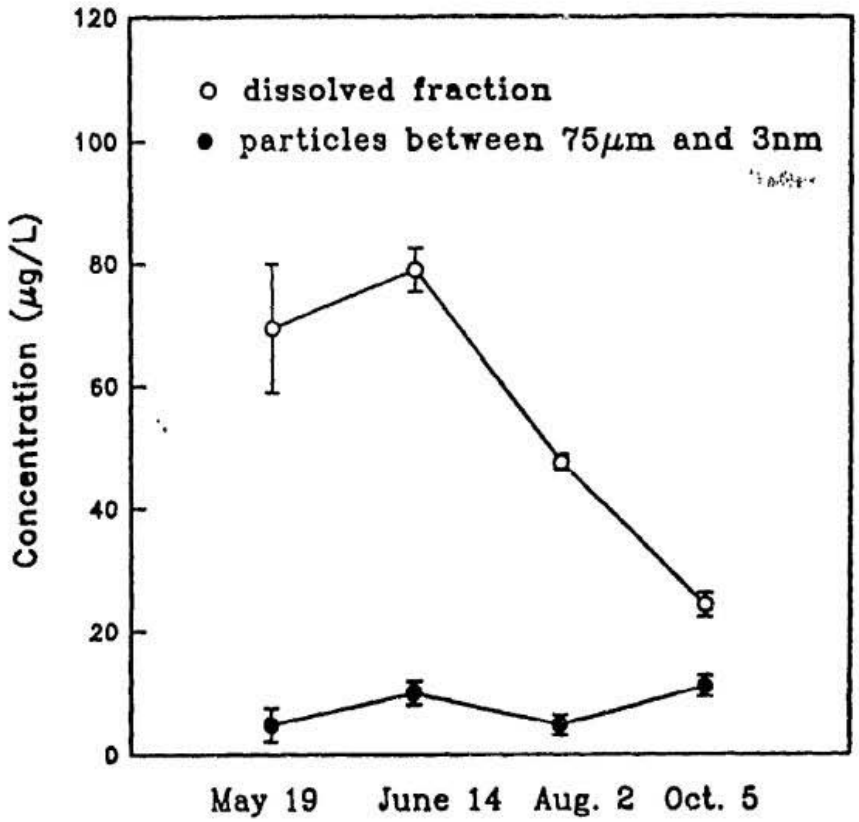


Figure 4.4 Particulate (3nm-75μm) and dissolved (<3nm) lipid concentrations in surface mixed layer

Table 4.9 Linear regression of lipid class concentration in 75 μm filtrate fraction versus ultrafiltrate fraction (10K−F)

Lipid Class	HC	SE	KET	TAG	FFA	ALC	ST	AMPL	PL	less polar	neutral	polar	TOTAL
Seawater samples	Lipid class concentration in 75 μm filter filtrate (X)												
May 19	1.53	0.51	0.75	25.16	5.84	1.00	2.47	23.15	4.36	2.78	34.47	27.50	64.75
June 23	3.33	0.22	0.97	30.86	5.98	1.58	2.72	40.45	12.77	4.52	41.14	53.22	98.87
August 2	2.91	0.53	0.90	6.52	5.63	0.97	1.30	23.05	3.05	4.34	14.41	26.10	44.85
Oct. 2	4.42	1.77	1.60	5.48	3.95	1.51	1.58	9.93	3.34	7.80	12.51	13.27	33.58
Seawater samples	Lipid class concentration in 10K filter filtrate fraction (Y)												
May 19	1.23	0.28	0.78	27.05	5.02	0.68	1.76	30.02	2.65	2.27	34.51	32.67	69.45
June 23	0.83	0.20	0.95	21.14	6.47	1.90	2.05	37.35	7.97	1.98	31.56	45.32	78.87
August 2	1.32	0.46	0.78	8.57*	12.37*	1.28*	1.29	19.75	1.70	2.56	23.51*	21.45	47.51*
Oct. 2	1.69	0.38	0.91	3.41	3.29	0.97	1.70	9.27	2.69	2.98	9.38	11.96	24.32
	Linear Regression (Y = aX + b)												
X Coefficient (slope a)	0.12	0.07	0.15	0.83	1.28	1.53	0.40	0.91	0.61	0.15	0.87	0.81	0.83
Std Err of Coef.	0.19	0.10	0.14	0.41	0.56	1.33	0.15	0.26	0.05	0.09	0.30	0.20	0.34
Intercept b on Y axis	0.90	0.28	0.69	0.22	−1.80	−0.89	0.90	2.23	0.16	1.71	−0.32	3.40	3.22
Std Err of Y Est	0.39	0.13	0.09	7.70	0.90	0.59	0.18	5.66	0.43	0.34	6.33	5.68	15.58
R Squared	0.17	0.19	0.38	0.80	0.84	0.57	0.77	0.86	0.98	0.57	0.89	0.90	0.86
[1.00−(slope a)]x100%	88%	93%	85%	17%	−28%	−53%	60%	9%	39%	85%	13%	19%	17%

Note: * These data did not take part in regression analysis because of contamination

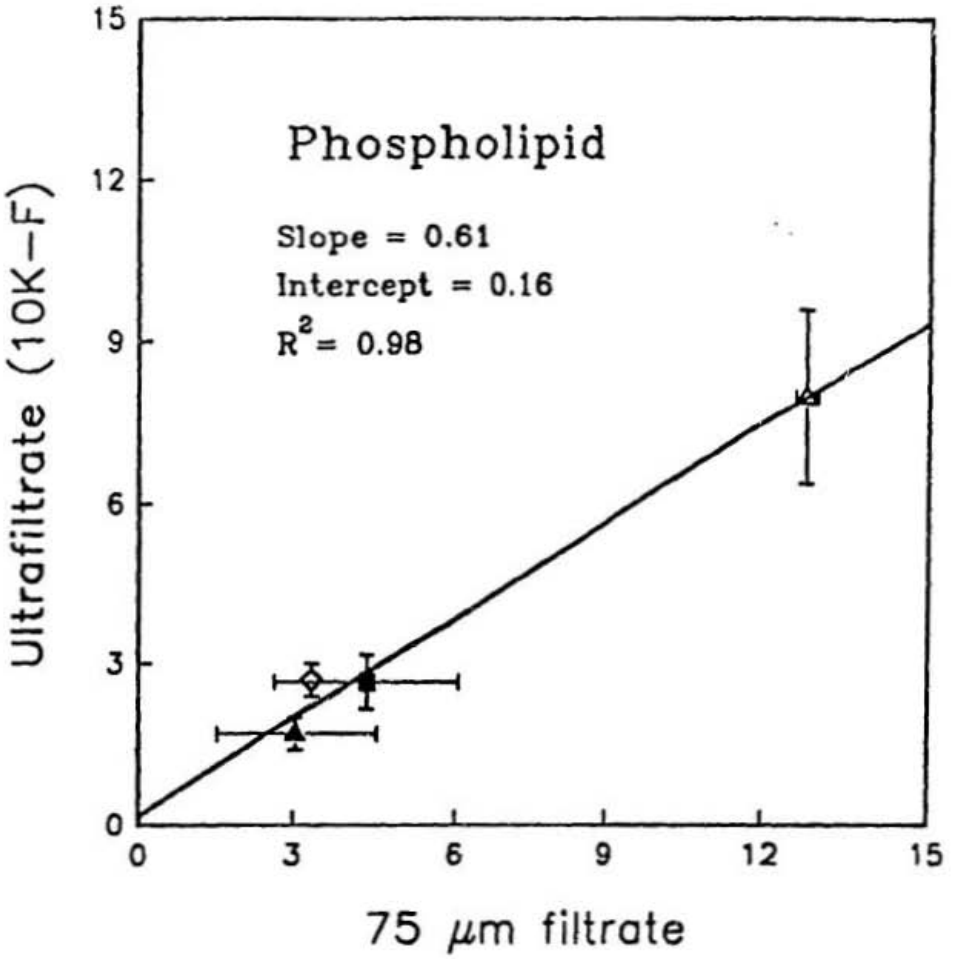


Figure 4.5 Linear regression of phospholipid concentration in 75 μm filtrate versus ultrafiltrate (10K-F) fraction.

From the discussion above, a higher proportion of AMPL than that of HC is present in the dissolved form. This conclusion is consistent with regression analysis (**Table 4.9**). **Figure 4.5** shows the regression line of the PL concentration in 75 μm filtrate fraction versus the PL concentration in the ultrafiltrate (10K-F) fraction.

The magnitude of the slopes of the regression line indicates the extent of removal of particulate lipids from the prescreened sample. If the slope is significantly less than 1.00, it indicates the ultrafiltration process removed some lipid materials. The intercept value represents the blank of the filtration process. If the intercept is a negative value, it means some material was lost during filtration.

The slopes of regression for HC (0.12, $R^2 = 0.17$), SE (0.07, $R^2 = 0.19$) and KET (0.15, $R^2 = 0.36$) are less than 1.000. Therefore, these results indicate that more than 80% less polar lipids were removed from unfiltered seawater samples after ultrafiltration. Since most of hydrocarbons are small molecules with low MW, they should pass through ultrafilter membrane. However, why are they in retentate fraction not in filtrate fraction? A possible explanation of HC removal from ultrafiltration process is they are adsorbed onto particles. Of course, the repulsion of HC by the ultrafilter material is also a possible mechanism to prevent HC molecules from passing through the ultrafilter. Because of low correlation coefficient for HC, SE, and KET and the few samples used for linear regression, the above suggestion is not persuasive enough and further research work is need.

The slopes of regression for ST and PL are 0.40 (R^2 = 0.77) and 0.61 (R^2 = 0.99) indicating 60% of ST and 39% of PL was removed from unfiltered sample after ultrafiltration. The slopes of regression for major lipid classes, TAG (0.83, R^2 = 0.80) and AMPL (0.91, R^2 = 0.86) indicate relatively less TAG (17%) and AMPL (9%) was removed. This suggested AMPL are preferential present in dissolved rather than particulate form.

It is interesting that both FFA (1.28, R^2 = 0.84) and ALC (1.53, R^2 = 1.57) have slope magnitude larger than 1. This suggested extra FFA and ALC came from degradation of other lipid classes (WE/SE, TAG, and AMPL) during or after ultrafiltration.

Unlike direct data analysis, regression analysis indicates a relatively high percentage of lipid classes were removed from unfiltered seawater samples. These discrepancies were attributed to the ultrafiltration blank. Positive values of intercepts of regression suggest the presence of an ultrafiltration blank. Intercepts of regressions for minor lipid classes such as HC (0.90 μg), SE (0.28 μg) and KET (0.69 μg) appear to be large relative to their concentration. On the other hand, both FFA and ALC have negative intercepts of regression. This suggested some of them were adsorbed on the ultrafilter.

From the discussion above, a higher proportion of HC, SE and KET than other lipid class is present on particles. Major lipid classes such as AMPL and TAG prefer to exist in dissolved fraction. FFA and ALC are different from other lipid classes because

they are strong surface materials and they can come from the decomposition of other major lipid classes. Dissolved ST and PL account for 40% of ST and 60% of PL.

4.5 Lipid classes and carbohydrates in microalgal cultures

The biochemical composition of microalgal cultures can vary, depending on many factors such as the culture species, culture growth conditions (nutrient status, temperature, salinity, pH, light intensity and quality), and growth phases (lag phase, logarithmic, and stationary phase).

Nutrient availability, light intensity, and temperature all have a great influence on biochemical composition (lipid classes, fatty acids, carbohydrates, proteins, and amino acids). Low levels of nitrogen in the culture medium causes a large decrease in microalgal proteins and a large increase in lipids and carbohydrates (Suen *et al.*, 1987). Silicate deficiency leads to an increase in the lipid composition of diatoms (Roessler, 1988). High light intensity lowers the accumulation of carbohydrates and lipids in diatoms (Varum & Mycklestad, 1984) and higher incubation temperatures causes an increase in lipid and protein concentrations.

4.5.1 Cellular lipid class concentration

Lipids in seawater come mainly from biogenic sources. In this study, two species of microalgae, used widely as food in mariculture. were selected in order to study the lipid and carbohydrate concentrations in different particle size fractions (particulate,

colloidal, dissolved). The flagellate *Isochrysis galbana* (clone T-Iso) and the diatom *Chaetoceros muelleri* were grown in continuous (Parrish and Wangersky, 1987) and semicontinuous cultures under nutrient-replete and nitrogen-limited nutrient conditions. The culture vessels were 100 L in volume and the culture medium was the f/2 type medium (Guillard and Ryther, 1962), which includes nutrient salts, trace metals, and vitamins.

The results (**Table 4.10**) show that polar lipids (AMPL and PL) were the dominant lipid classes in the two species of microalgae. Polar lipids account for 53-80% of total lipids, well within recent literature data. Dunstan *et al.* (1993) found 54-83% of total lipid in *Isochrysis sp.* are polar lipids. In fact, polar lipids predominated in most species of microalgae and account for 40-98% of total lipids (Volkman *et al.*, 1989). Therefore, polar lipid concentration in seawater may be used as an indicator of microalgae.

Besides polar lipids, the neutral lipid TAG is also a major contributor (10-28%) to total lipids in *Isochrysis galbana* and *Chaetoceros muelleri*. This result is contrary to some previous work. Brown *et al.* (1993) found TAG to be a minor lipid class (<0.1%) in *Isochrysis sp.* (clone T-ISO). However, our results are comparable with Dunstan's results (1993) in which TAG accounts for 0.6-20% of total lipids in *Isochrysis sp.*. All of these results indicate that TAG are easily affected by some physiological factors and culture conditions.

Table 4.10 Concentrations (pg/cell, mean ± S.D., n = 4–8) and proportions (%) of solvent extractable lipid classes and total carbohydrates in *Isochrysis galbana* & *Chaetoceros muelleri*

Lipids & Carbohydrates	Isochrysis galbana (0.61 doublings/day) Mean ± S.D.	%	Isochrysis galbana (0.90 doublings/day) Mean ± S.D.	%	Chaetoceros muelleri (0.24 doublings/day) Mean ± S.D.	%
HC	0.05 ± 0.03	1.1	0.05 ± 0.01	0.9	0.03 ± 0.01	0.6
SE	0.20 ± 0.02	4.2	0.01 ± 0.00	0.1	0.01 ± 0.00	0.1
KET	0.11 ± 0.00	2.4	0.15 ± 0.02	2.6	0.04 ± 0.00	0.8
TAG	1.30 ± 0.02	27.7	0.68 ± 0.12	11.8	0.53 ± 0.07	10.6
FFA	0.07 ± 0.01	1.4	0.06 ± 0.02	1.1	0.26 ± 0.04	5.2
ALC	0.21 ± 0.00	4.4	0.07 ± 0.01	1.3	0.21 ± 0.06	4.2
ST	0.24 ± 0.01	5.1	0.10 ± 0.02	1.7	0.05 ± 0.03	1.0
AMPL	1.29 ± 0.13	27.3	2.77 ± 0.69	48.4	2.18 ± 0.56	43.4
PL	1.23 ± 0.11	26.2	1.83 ± 0.44	32.0	1.71 ± 0.46	34.1
least polar lipids	0.36 ± 0.03	7.8	0.20 ± 0.02	3.6	0.07 ± 0.02	1.4
neutral lipids	1.82 ± 0.02	38.7	0.91 ± 0.21	16.0	1.06 ± 0.16	21.0
polar lipids	2.52 ± 0.17	53.5	4.60 ± 1.06	80.4	3.89 ± 0.85	77.5
Total Lipids	4.70 ± 0.17	100.0	5.72 ± 1.34	100.0	5.02 ± 0.93	100.0
Carbohyarates	7.82 ± 1.04		8.63 ± 0.16		26.96 ± 0.29	

From **Table 4.10**, cellular TAG content decreases and polar lipid increases with an increase in growth rate. This is not difficult to understand. A large growth rate means large living cells were rapidly dividing into small cells which have large surface area/volume ratio. Since the polar lipids are cell membrane materials, it seems to be reasonable that polar lipids should increase but all other lipid classes should decrease as the growth rate becomes large.

The concentrations and the distributions of the major lipid classes (AMPL, PL, and TAG) was similar in both species. However, FFA proportion (%) in *Chaetoceros meulleri* was higher than that in *Isochrysis galbana*, while KET proportion (%) is higher in *Isochrysis galbana*. These results are consistent with previous work (Brown, 1993; Dunstan *et al.*, 1993).

The cellular total lipid content in both species of microalgae was about 4.7-5.7 pg/cell which is consistent with recent literature data. Dunstan *et al.* (1993) found 4.8-7.0 pg/cell in of lipid in *Isochrysis* (Tahitian) and Brown *et al.* (1993) found 5.1-8.6 pg/cell. High cellular lipid content (7.1-10.3 pg/cell) and low cellular lipid contents (2.6-5.0 pg/cell) have also been reported (Thompson *et al.*, 1990; Renaud *et al.*, 1991). Parrish (1990) found the lipid content in *C. gracilis* was in the range of 2-9 pg/cell. The variability of cellular lipid content reflected the complexity of the factors influencing the lipid content in microalgae.

4.5.2 Cellular carbohydrate concentration

The carbohydrate content for *Isochrysis galbana* (7.8-8.6 μg/L) and for *Chaetoceros muelleri* (27.0 pg/cell) seem to be higher than that determined in previous work. In the literature, carbohydrate content in many species of microalgae including *Isochrysis galbana* and *Chaetoceros gracilis* were found to be in the range 1.0-7.0 pg/cell (Brown *et al.*, 1993; Renaud, 1991; Sukenik and Wahnon, 1991, Thompson, 1992), although concentrations as high as 20-45 pg/cell carbohydrate content was found in *D. tertiolecta* (Thompson and Harrison, 1992). However, *D. tertiolecta* also had a high lipid content (Thompson and Harrison, 1992) whereas *C. muelleri* had a lipid content (2-10 pg/cell), which was comparable to literature values. Although our carbohydrate values for C. muelleri are higher than in the literature, it should be noted that published values for *C. muelleri (gracilis)* are quite variable (Brown *et al.*, 1993; Thompson and Harrison, 1992).

4.6 Carbohydrate in seawater samples

Carbohydrates are important constituents of DOM and are reviewed as a major player in many biochemical processes occurring in the marine environment. The analyses of carbohydrate concentrations would be helpful to understand these processes.

4.6.1 Total dissolved carbohydrates

In **Table 4.11** only recent data measured by MBTH and GC methods have been tabulated because of the unreliability of earlier analytical techniques when applied to seawater samples. From these data, the following characteristics appear: (1) Most total dissolved carbohydrate concentrations vary from 30-1300 μg/L, although levels as high as 2800 μg/L have been measured. (2) The concentrations measured by GC or GC/MS were usually lower than those obtained by the MBTH method. (3) Carbohydrate concentrations were higher in the upper layer seawater. (4) Higher carbohydrate concentrations were reported in recent measurements (Benner *et al.*, 1992; Pakulski and Benner, 1992; Compiano *et al.*, 1993).

Total carbohydrate concentrations in the present study were obtained by the MBTH method with HCl hydrolysis. Values were in the range of 600 to 1200 μg/L, which is consistent with recent literature values (Benner *et al.*, 1992; Pakulski and Benner, 1992; Compiano *et al.*, 1993). The present study appears to give higher concentration values than previous studies which underestimated the carbohydrate content in seawater because of incomplete hydrolysis of the combined carbohydrate fraction and possibly, the lack of precaution in storage and handling. Pakulski *et al.* (1992) reported that dissolved carbohydrates determined after HCl hydrolysis accounted for only 6-10% of the DOC, whereas sulphuric acid-hydrolysable carbohydrate accounted for 10-28% of the DOC. If these results are true, the actual carbohydrate concentrations in Conception Bay could be twice as high as those measured with HCl hydrolysis. But one must

Table 4.11 Measurments of carbohydrates in filtered seawater

Location	Date	Depth (m)	Concentration (μg/L)	Filter	Method	Reference
Narragansett Bay	22 January	Surface	908 ± 34.9[a]	Gelman type A	MBTH	Burney (1977)
Narragansett Bay	winter and spring 1975	Surface	159 (122–226)[b]	Gelman type A	MBTH	Johson (1977)
Bermuda 64°39'N 32°16'W		3	250	0.2 μm Polycarbonate	GC	Liebezeit (1980)
Bermuda 64°39'N 32°16W		25	87.1	0.2 μm Polycarbonate	GC	Liebezeit (1980)
Narragansett Bay	August 15–30		87.3 ± 2.6	–	MBTH	Johson (1981)
Northern North Sea 58°55'N 0°32'E	March–June, 1976	0–150	250 (40–400)	GF/C	MBTH	Ittekkot (1982)
New York Bight	1–7, June	Surface	280	–	MBTH	Harvey(1983)
Funka Bay, Janpan	January, 1983 – Jauary 1984	82–94	220 (33–2800)	GF/C	MBTH	Yoshida (1984)
Thurloe Bay 28–29°N 115°W	9–19, 1981	5–15cm	468–646[a]	1 μm Polycarbonate	MBTH	Henricha(1985)
Mikawa Bay 34°37.6'N 137°16'E	May 31, 1980	1	82.7	GF/C	GC–MS	Sakvgawa (1985)
Mikawa Bay 34°37.6'N 137°16'E	May 31, 1980	6	31.1	GF/C	GC–MS	Sakvgawa (1985)
Station ALOHA 27°6.88'N 95°35W	April	10	810[d]	0.2 μm Polycarbonate	MBTH	Benner (1992)
Station ALOHA 27°6.88'N 95°35W	April	765	390[e]	0.2 μm Polycarbonate	MBTH	Benner (1992)
Gulf of Mexico 27°6.88',95°35'W	March – June,1991	105	600 – 900[f]	GF/F	MBTH	Pakulski (1992)
Conception Bay, NF	May 19, 1993	25	1203 ± 36	0.45μm	MBTH	This study
	August 2, 1993	24–26	579 ± 27	0.45μm		
	October 5, 1993	25	606 ± 162	A/E (1.0 μm)		

Note: [a] mean ± S.D.
[b] Figures in parentheses represent value ranges
[c,d,e,f] Figures obtained by calculation (1μM C = 12 μg C/L = 30 μg glucose/L)

Table 4.12 Carbohydrate concentration (μM C/L, mean $\pm$ S.D., n = 3-5) in seawater collected from station BRLP4 in Conception Bay, Newfoundland

Date	May 19.1993	August 2.1993	October 5.1993
Depth (m)	25	25-27	25-26
Temperature (°C)	1-2	8-9	10-11
Overall depth (m)	215	197-215	215
Filter Fraction	Carbohydrate (μM C/ L)		
UF	-	-	22.8 $\pm$ 2.5
75μm-F	44.7 $\pm$ 0.1	24.3 $\pm$ 0.6	22.1 $\pm$ 2.7
A/E-F	-	-	20.2 $\pm$ 5.4
0.45μm-F	40.1 $\pm$ 1.1	19.3 $\pm$ 0.9	-
10K-F	542 $\pm$ 40*	51.1 $\pm$ 3.1*	20.0 $\pm$ 3.3
0.45μm-R	69.8 $\pm$ 0.3	25.9 $\pm$ 0.9	-
10K-R	73.4 $\pm$ 13.9*	47.6 $\pm$ 4.2*	29.2 $\pm$ 5.0

* Probable contamination from a new 10 kDa cut-off filter

consider the nature and abundance of combined carbohydrates in different marine environments and seasonal differences if we are to believe that a better hydrolysis procedure is warranted.

From this study, it is clear that the total dissolved carbohydrate concentration (as glucose) in May (1203 μg/L) is much higher than in August (579 μg/L) and October (606 μg/L). These results are consistent with Senior and Chevolot (1991). They found total dissolved carbohydrate was much higher in June and suggested some polysaccharide was produced by phytoplankton lysis and excretion. The concentration change of carbohydrate concentrations is similar to that of lipid concentrations.

From **Table 4.12**, the total dissolved carbohydrate concentration (TDCHO) in retentate and filtrate fractions of the 10K filter is much higher than prefiltered TDCHO. Obviously, it is likely that some contamination occurred during the filtration procedure with the 10 kDa filter. In fact, it is known that new 10K filters are usually stored in dilute formaldehyde solution, but formaldehyde is an intermediate in measuring carbohydrates by the MBTH method. Hence, new filters must be thoroughly cleaned to remove formaldehyde before they can be used for ultrafiltration and MBTH analysis of carbohydrates.

Carbohydrate concentrations in the fraction <75 μm vary from 44.7 μM C to 22.1 μM C for surface seawater samples. TOC measurement for the same station is 98 μM C. Taking a representative DOC value of 100 μM C (1.2 mg/L), the carbohydrates would account for 20-45% of DOC. Benner *et al.* (1992) found that there are 27 μM C of carbohydrates for surface seawater samples which account for 33% of the total DOC.

4.6.2 Particulate carbohydrates

Particulate (particles >0.45 μm but <75 μm) carbohydrate concentrations can be calculated by subtracting the carbohydrate concentrations in the 0.45 μm-F fraction from those in 75 μm-F fraction. The particulate carbohydrate concentrations were found to be 4.6 μM C and 5.0 μM C in the May and August samples, respectively. These results indicate that 80-90% of carbohydrates in seawater are present as the "dissolved" form (<0.45 μm).

Particulate carbohydrate concentrations were also calculated by subtracting carbohydrate concentrations in the 0.45 μm-F fraction from those in the 0.45 μm-R fraction and then dividing by a concentration factor. The particulate carbohydrate concentrations calculated by this method were 0.43 μM C and 0.50 μM C in the May and August samples. But these levels are 10-fold lower than 4.6 μM C and 5.0 μM C obtained by direct subtraction method. The values should be the same. Why is there such a great difference caused by different ways of calculating the same thing? Although it is easier to measure carbohydrate concentration in filtrates, it seems that it is better to perform the measurement in the retentate, rather than to subtract two filtrate measurements to obtain a particulate carbohydrate concentration. This is because the filtrate concentrations are much higher than the concentrations in the colloids.

However, in terms of accuracy, the carbohydrate concentration in 0.45 μm-R fraction was easily underestimated especially because of incomplete acid hydrolysis in the MBTH measurement procedures. The adsorption of particles associated with carbohydrate materials seems the least possible cause because the lipid concentration in the retentate was not underestimated. This problem is very important and must be addressed in future studies.

It was concluded that particulate (>0.45 μm) and colloidal (1.0 μm to 10K NMW) carbohydrate concentrations are a minimal part of total carbohydrate concentrations in Conception Bay seawater. More than 80-90% of total carbohydrate is present in the dissolved (< 10k Da MW) fraction.

4.7 Marine colloids

Because a significant amount of OM is present in marine colloidal form, the importance of marine colloids in marine biochemical processes is now being recognized by more and more investigators. The development of separation techniques, especially cross-flow ultrafiltration, has greatly promoted the studies of marine colloids. Some scientists have applied these techniques in the chemical characterization of marine colloids. Also, the spatial and temporal variability of the MW distribution in OM and the elemental composition (organic carbon and some trace metals) in colloidal fractions has been widely studied. However, relatively few studies were conducted on biochemical constituents (lipids, carbohydrates, proteins, amino acids, and humic substances) of colloidal OM. The present studies show some results on colloidal lipid classes and total carbohydrates in seawater and microalgal cultures.

4.7.1 Molecular weight of colloidal lipids and carbohydrates

In 1970, Degens suggested that the bulk of DOM is in the MW range 3,000-5,000 Da. However, Maurer (1974) found only 10-15% of total DOC exists as compounds with a MW $>1{,}000$ Da. In 1984, Romankevich summarized the earlier research results on the MW distribution of OM in seawater and pointed out that the DOM for seawater in the open ocean is mainly present in the truly dissolved fraction (MW cut-off $<1{,}000$). However DOM in bays, gulfs, and highly productive ocean areas has a high proportion of small colloids (MW cut-off between 1000 and 10,000). After a detailed study of MW

distribution of OM in seawater, Carlson (1985) reported that more than 60% of OM is low MW matter (< 1000) and only 28% of OM is macromolecular with MW between 1,000-30,000.

Using gel filtration techniques, Sugimura and Suzuki (1988) showed that most organic matter in surface water in the North Pacific was in the MW range 2,000-60,000 Da, with more material in the higher range. However, the recent results obtained by ultrafiltration (Benner *et al.*, 1992; Ogawa and Ogura, 1992; Kepkay *et al.*, 1993; Guo *et al.*, 1994) showed the opposite result, most OM (>50% of DOC) has a MW less than 1000 and < 20% of OM is present in high MW (> 10,000) form. These recent results are in agreement with Carlson (1985).

From our results, at least 90% of total lipids and 80% of carbohydrates have a MW less than 10,000. Recent data show that aquatic humic substances (Thurman *et al.*, 1982) and proteinaceous materials (Tada and Maita, 1993) also have a MW less 10,000. Thus the MW of most OM constituents in seawater is probably less than 10,000. By definition (Hollibaugh *et al.*, 1991), a minimum size of marine colloid was 1 nm which is equivalent to pore size of filter with ~1,000 MW cut-off. Thus the MW of most colloidal macromolecules is between 1,000 and 10,000 Da. In other words, macromolecular matter mainly concentrates on the colloidal fraction.

4.7.2 Colloidal carbohydrate and lipid class concentrations

Colloidal lipid classes and carbohydrate concentrations (**Table 4.13** and **4.14**) can be calculated according to two methods: 1. subtraction of sequential filtrate concentrations, 2. subtraction of filtrate from retentate concentration and then division by a concentration factor. The results obtained with the two methods should be same. However, the value obtained by method 2 is usually smaller than that obtained by method 1. Sometimes, the differences are very large (**Table 4.13**). For instance, carbohydrate concentration (0.45 μm-75 μm) obtained by method 2 is 10 times lower than that obtained by method 1. The precise cause of this discrepancy is not clear, but perhaps it could be due to factors such as incomplete hydrolysis, adsorption or a greater propagation of errors associated with method 1. From the coefficients of variation, the results obtained by method 2 are better than method 1, therefore the discussion will be based mainly on the results obtained through method 2.

Colloidal lipid concentrations in the different microalgal cultures vary greatly. The colloidal lipid concentration in *Isochrysis galbana* (10 kDa-1.0 μm) is about 232-294 μg/L and in *Chaetoceros* (10 kDa-1.0 μm), it is 8.0 μg/L (**Table 4.13**). The colloidal lipid and carbohydrate concentrations in *Isochrysis galbana* are much higher than in seawater samples. Colloidal matter may come from microalgae through breakdown of cells or exudation.

Table 4.13 Total lipid concentrations (μg/L, mean ± S.D., n = 3-6) and total carbohydrate concentrations (μg/L, mean ± S.D., n = 4-8) in different size class particles for microalgal samples

Samples	Size class	Calculation method C_{F1}-C_{F2}	Calculation method $(C_{R2}$-$C_{F2})/F$
Total lipid concentration			
Isochrysis galbana[a]			
	0.45μm-1.2μm	163 ± 157	159 ± 24
	10kDa-0.45μm	143 ± 181	135 ± 38
Isochrysis galbana[b]			
	0.45μm-1.0μm	57 ± 336	78 ± 29
	10kDa-0.45μm	62 ± 134	154 ± 36
Chaetoceros[c]			
	10kDa-1.0μm	21 ± 22	8.0 ± 4.2
Total carbohydrate concentration			
Isochrysis galbana[a]			
	0.45μm-1.2μm	1512 ± 3042	108 ± 367
	10kDa-0.45μm	1296 ± 3330	743 ± 389
Isochrysis galbana[b]			
	0.45μm-1.0μm	1998 ± 558	N.A.
Chaetoceros[c]			
	10kDa-1.0μm	8100 ± 18540	N.A.

C: Concentration
F: Concentration factor
$_{F1}$: Prior filtrate
$_{F2}$: Filtrate
$_{R2}$: Retentate
[a] Density: 5.8 x 10^6 cells/mL, growth rate: 0.61 div./d.
[b] Density: 4.2 x 10^6 cells/mL, growth rate: 0.90 div./d.
[c] Density: 4.4 x 10^6 cells/mL, growth rate: 0.22 div./d.
N.A.: not available

Table 4.14 Total lipid concentrations (μg/L, mean ± S.D., n = 3-6) and total carbohydrate concentrations (μg/L, mean ± S.D., n = 3-5) in different size class particles for seawater samples collected from Conception Bay, Newfoundland

Samples	Size class	Calculation method C_{F1}-C_{F2}	Calculation method $(C_{R2}$-$C_{F2})/F$
Total lipid concentration			
May 19	0.45μm-75μm	3.9 ± 6.0	3.5 ± 2.6
June 14	0.45μm-75μm	8.7 ± 4.8	1.1 ± 1.6
August 2	0.45μm-75μm	2.7 ± 2.6	1.3 ± 1.2
October 5	1.0μm-75μm	7.1 ± 2.1	10.1* ± 1.7
May 19	10kDa-0.45μm	0.4 ± 6.2	1.4 ± 0.9
June 14	10kDa-0.45μm	13.7 ± 5.4	8.7 ± 1.0
August 2	10kDa-0.45μm	4.0 ± 2.3	3.6 ± 1.0
October 5	10kDa-1.0μm	2.6 ± 2.8	0.9 ± 0.1
Total carbohydrate concentration			
May 19	0.45μm-75μm	138 ± 33	12.9 ± 0.6
August 2	0.45μm-75μm	150 ± 32	15.3 ± 3.0
October 5	1.0μm-75μm	57 ± 181	-
October 5	10kDa-1.0μm	6 ± 183	20.0 ± 13.0

* direct analysis of particles retained in A/E filter

From **Table 4.14**, the colloidal lipid concentration in seawater (10 kDa-0.45 μm) varied from 1.4-8.7 μg/L (0.1-0.5 μM C) for May, June and August seawater samples. The colloidal (10 kDa-1.0 μm) carbohydrate concentration from the October seawater sample was 20.0 μg/L (0.67 μM C) (**Table 4.14**), which is consistent with Maurer's (1976) data (0.5-2.0 μM C) measured by the phenol-sulphuric acid method. The COC (10 kDa-0.2 μm) concentrations were reported to be about 7 μM C for most seawater samples (Kepkay *et al.*, 1993). This suggests that lipids and carbohydrates may account for less than 10% of OC on larger colloids (> 10 kDa) in this study. From Table 1.1, it is possible to calculate a representative COC (> 1 kDa) concentration of 20 μM C. From this it would appear that lipids and carbohydrates with a MW larger than 10 kDa contribution only a small part (<5%) of total COC (1 nm-1 μm). Therefore, future studies should include small colloids (1 kDa-10 kDa).

The concentrations of lipids and carbohydrates in particles with a size range from 0.45 μm to 75 μm are 1.3-3.5 μg/L (0.1-0.2 μM C) and 12.9-15.3 (0.4-0.5 μM C) μg/L respectively. This indicates that lipids and carbohydrates on particles in the size range 0.45-1 μm contribute very little to COM.

The lipid concentration (10.1 μg/L) (**Table 4.14**) for the October sample in the size class (1.0-75 μm) is about 4 times larger than those (1.3-3.5 μg/L) in the size class (0.45-75 μm). This may be attributed to the use of different filters. This perhaps means that the glass fiber filter adsorbed some low MW lipids or retained particles smaller than

the nominal pore size (Parrish, 1988). As much as 20% of dissolved fatty acids can be retained by glass fiber filters (Schutz and Quinn, 1977).

4.7.3 Composition of colloidal lipids

The composition of lipids in marine samples can be affected by many factors. Growth conditions greatly affect the composition of lipid in microalgal samples. TAG and AMPL are the major colloidal lipid classes in an *Isochrysis galbana* culture grown at 0.61 div./d (**Figure 4.6**). TAG and AMPL account for 56.2% and 30.7% of total colloidal lipids (10 kDa-0.45 μm), respectively. In the faster growing culture (**Figure 4.7**) TAG and AMPL account for less (32.2% and 24.9%, respectively), and PL and HC account for a much greater proportion (19.6% and 14.7% respectively) than in the slow growing culture.

However, for *Chaetoceros muelleri* samples, AMPL is not a major lipid class (**Figure 4.8**). The colloidal lipid concentration in *Chaetoceros* is much lower than that in *Isochrysis galbana* but is still higher than that in most seawater samples (**Table 4.13**). Therefore, this suggests that microalgae are a source of colloids.

Although AMPL (40-48%) is a major lipid class in dissolved fraction (< 10 kDa), it is not a major lipid class in the colloidal fraction of seawater (**Appendix, Table D9-D12**). TAG, FFA and PL are major colloidal lipid classes for seawater samples (**Figure 4.9**), while all others are minor lipid classes. From the discussion above, different

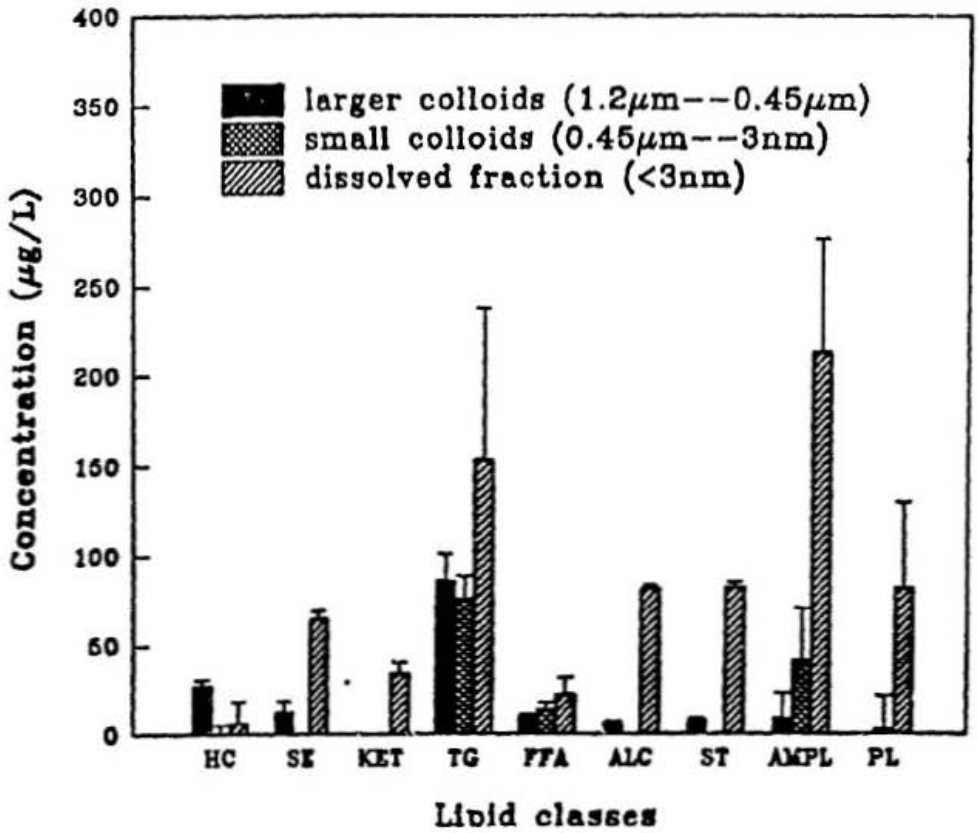


Figure 4.6 Colloidal lipid class concentrations (μg/L, mean ± S.D., n = 3-5) for *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) sample

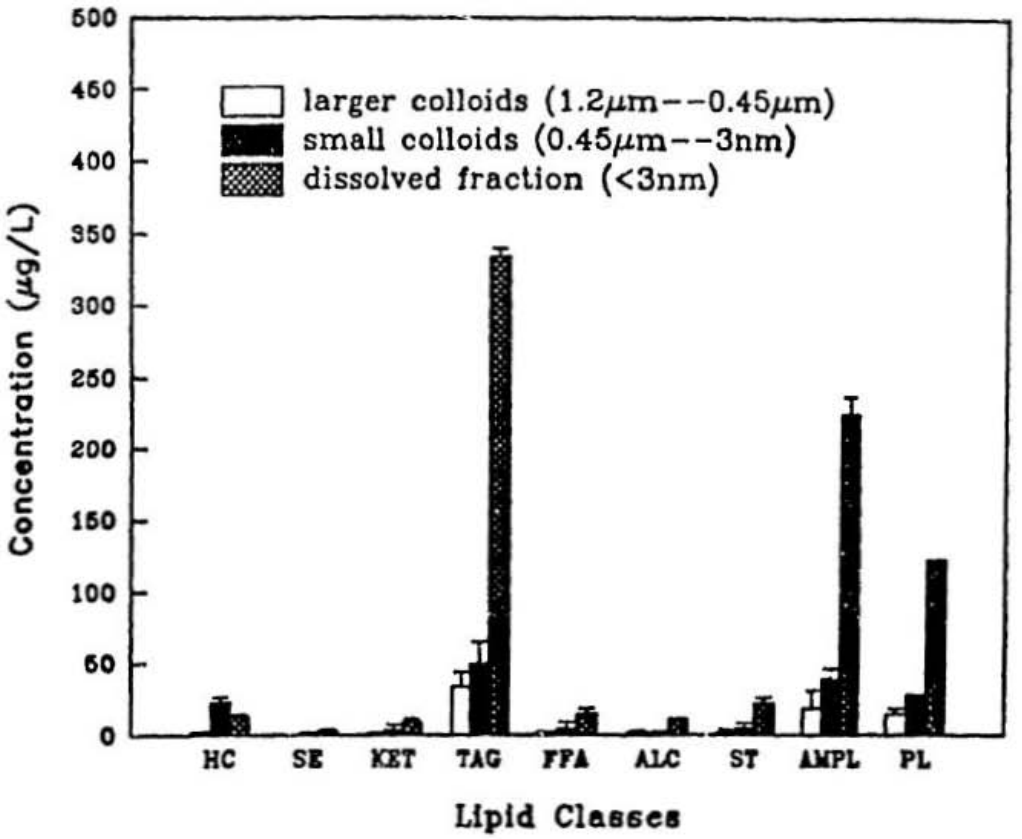


Figure 4.7 Colloidal lipid class concentration (μg/L, mean ± S.D.n = 3-5) for *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) sample

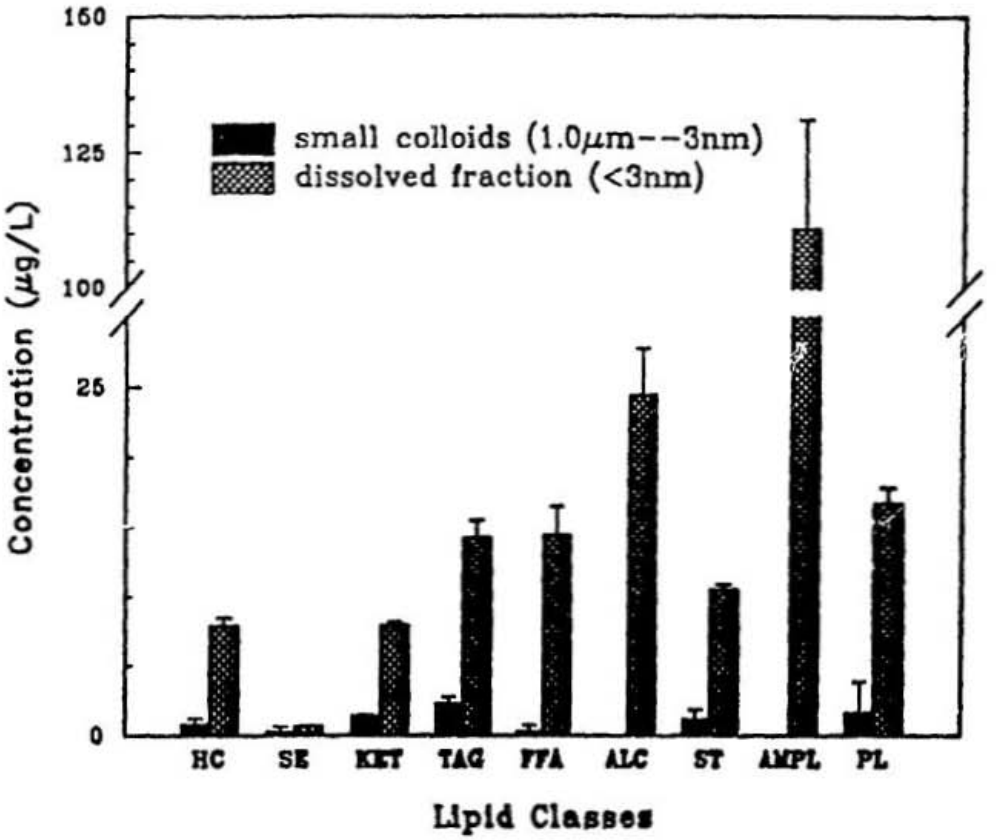


Figure 4.8 Colloidal lipid class concentration (μg/L, mean ± S.D., n = 3-5) for *Chaetoceros muelleri* (4.4 x 10^6 cells/mL, 0.24 div./day) sample

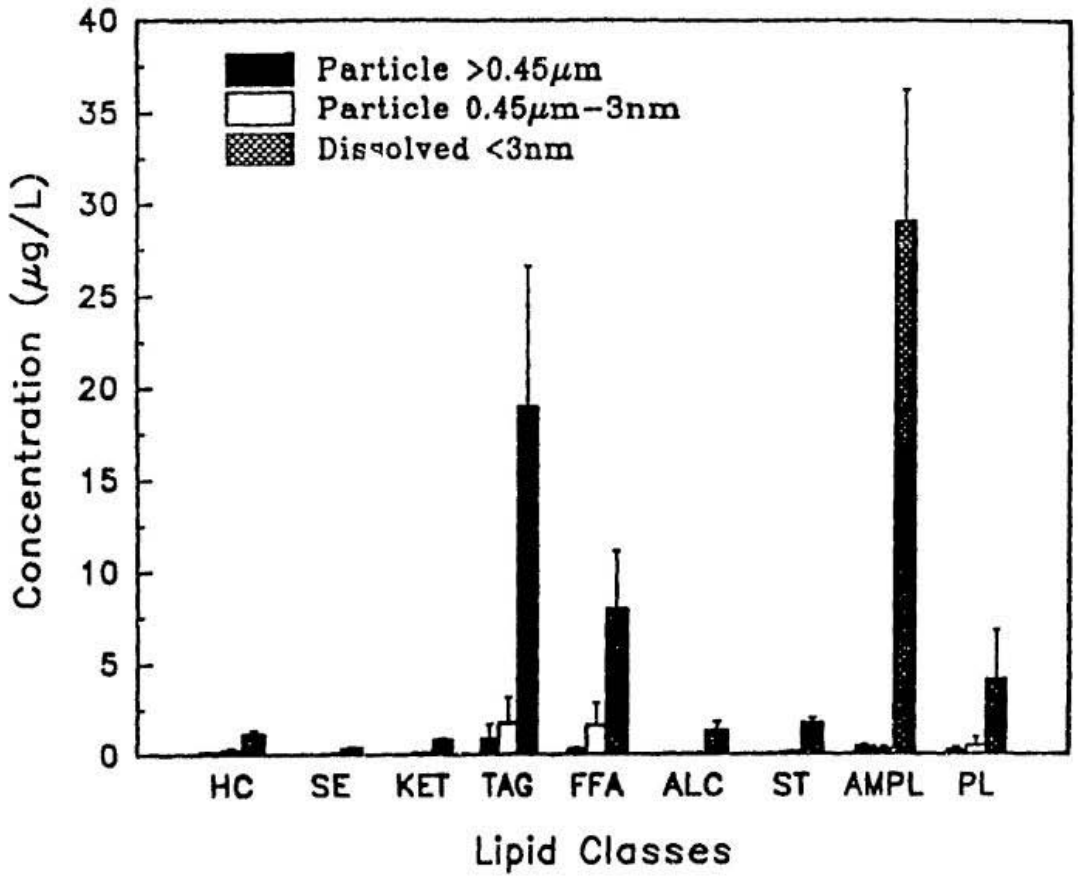


Figure 4.9 Lipid class concentration (μg/L, mean ± SEM, n = 3) in different size fraction for surface seawater sample collected from May to August, Conception Bay, NF.

species of microalgae have different major colloidal lipid classes. The composition of colloidal lipid classes in seawater is different from that in microalgae.

4.7.4 The distribution of lipid classes

The spatial and temporal distribution of colloidal matter in the ocean can be affected by many factors. For instance, there is an increasing percentage of colloidal Th from open ocean to near shore waters (Moran and Moore, 1989). The concentration of Al measured in the colloidal fraction was high in the surface water of the shelf 0.44-0.55 nM (3-4 % of dissolved Al) and decreased to a level of 0.09-0.15 nM (~ 1 % of dissolved Al) in deeper water (Moran and Moore, 1989). A strong negative correlation between colloidal carbon concentration and salinity was found (Whitehouse, 1986). Colloidal materials were thought to be enriched near a pycnocline (Means and Wijayratne, 1984). Colloidal macromolecules such as carbohydrate and proteins have several concentration layers (Maurer, 1976). All the above research indicates the complexity and variability of the distribution of colloidal material.

This study presents some results on the distribution and composition of lipid classes in particle pools and dissolved fractions for summer seawater samples. From **Figure 4.10**, AMPL contributes about 50% of the dissolved lipids. However, AMPL accounts for less than 3 % of the colloidal lipids. Although PL and AMPL are polar lipids and therefore were likely to be soluble, PL's contribution to colloidal lipids is larger than that in the dissolved fraction, suggesting solubility is not a controlling factor here. On

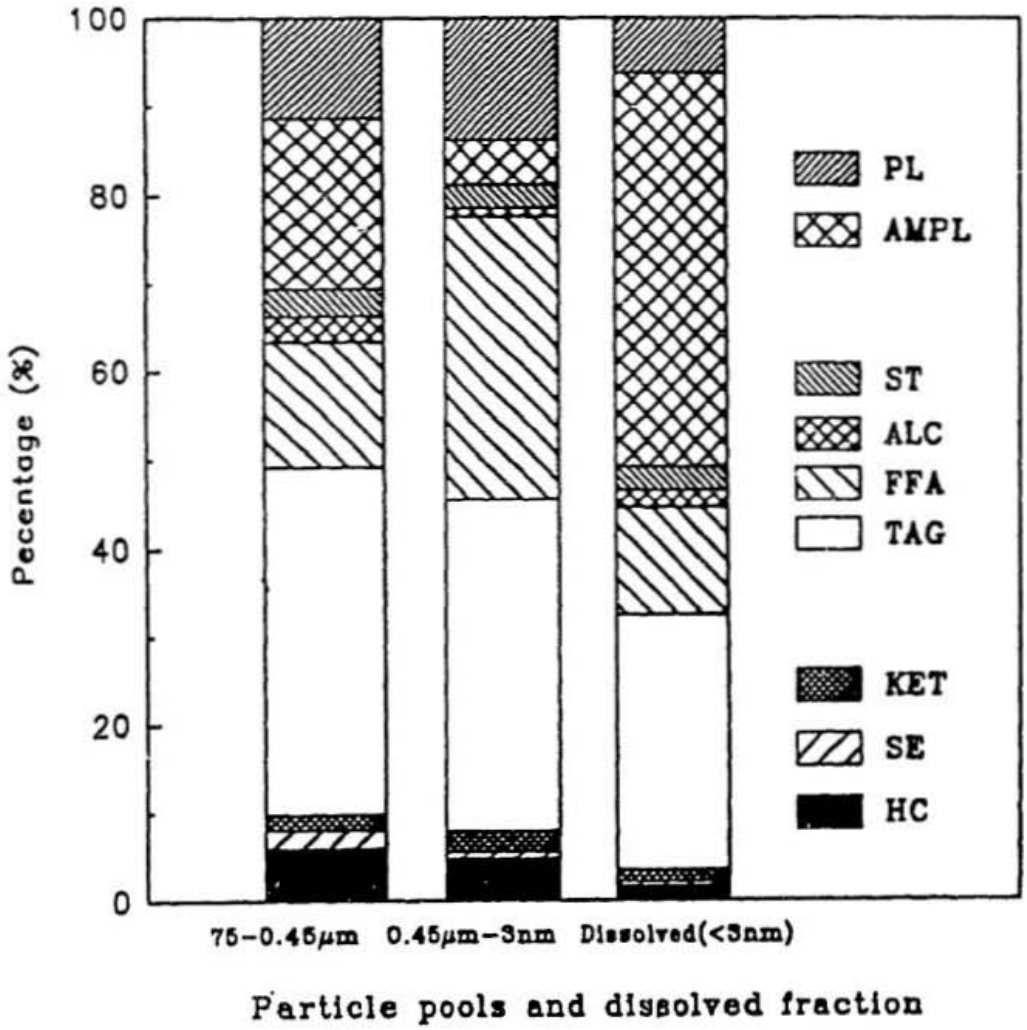


Figure 4.10 Lipid class distribution (%) in particle pools and "dissolved" fraction for seawater samples collected from May to August, Conception Bay, NF.

the other hand. the least polar lipid, HC, also has higher proportion in colloidal lipids than in dissolved lipids.

Hydrocarbons are the least polar hydrophobic substances. It is not difficult to understand that HC prefer to exist on particles rather than in seawater. However, unlike AMPL, PL is also preferentially present in colloids rather than dissolved fraction. Both AMPL and PL can contain membrane material of cells of living things. However, AMPL is quite a heterogeneous class and it seems that solubility is an important factor in governing its distribution in seawater.

Another striking feature of the lipid composition is that FFA, a strong surface active material, contributes much more to colloidal lipids than to the dissolved fraction. The other surface active material, ALC, does not show such a feature. This suggests the surface active property is not a major factor in governing the distribution of FFA on colloids. FFA molecules contain carboxylic acid groups which could react with trace metals in the seawater. Since colloids are enriched with a large amount of trace metals, the complexation of FFA with metals perhaps is a major mechanism for the enrichment of FFA on colloids.

5.0 CONCLUSIONS

In order to characterize marine colloids, CFF was used to concentrate colloids. The methods of TLC-FID and MBTH were used to measure lipid and carbohydrate content, respectively. The CFF system was optimized by changing the materials of some of the components, by modifying the flow path and by using it in an environment which minimizes artifacts. After careful cleaning of the CFF system, its performance was evaluated by determining blanks and mass balances. The total lipid blank for our CFF system was about 6-7 μg ($\sim$0.5 μM C) whereas the DOC blank for the CFF system was about 10-15 μM C. The mass balances for most of the major lipid classes were calculated to be 80-120%. This result ensured that there was no obvious filter occluding, leaking or adsorption occurring in the CFF system. For new ultrafilters, it was noted the CFF system must be cleaned continuously for at least two weeks before it can be used to collect colloids for measurement of carbohydrates by the MBTH method. The CFF system was proven to be useful, not only for seawater samples with a low colloid concentration but also for microalgal samples with high colloid concentrations.

In addition to the CFF lipid blank, the lipid blanks for the Chromarod-Iatroscan system and the extracting solvents were studied in order to improve the accuracy of the TLC-FID analytical results. Charomarod-Iatroscan system and extracting solvent blanks were 2-3 μg and 6 μg, respectively.

Microalgae were studied because they are the primary producers of lipids and carbohydrates in seawater and are widely used in mariculture. The total cellular lipid concentration is 4.7-5.7 pg/cell for both *Isochrysis galbana* and *Chaetoceros muelleri* microalgae. The carbohydrate content is 7.8-8.6 pg/cell for *Isochrysis galbana* and 27.0 pg/cell for *Chaetoceros muelleri*. More than 95% of lipids and 65% carbohydrates are present in particles larger than 1.0 μm in size. The lipid and carbohydrate concentrations in microalgal samples were 3 orders of magnitude higher than those found in seawater samples.

The total lipid and carbohydrate concentrations were found to be 34-99 μg/L and 600-1200 μg/L for seawater samples collected from Conception Bay, Newfoundland. AMPL and TAG were the major lipid classes in the seawater samples. FFA and ALC are degradation products of other lipids such as TAG and AMPL. HC in seawater samples may be end-products of FFA degradation and also come from other biogenic sources. Unlike microalgal samples, the lipids and carbohydrates in seawater are mainly present in the dissolved fraction ($<$10 kDa or $<$3 nm). Particulate lipids ($>$10 kDa) accounted for 6.5-31% of total lipid in seawater samples. A higher proportion of HC is present on particles while AMPL prefer to exist in the dissolved fraction. Although this study found that the colloidal matter is not the major component of seawater OM, its importance should not be underestimated because of its role in biogeochemical processes.

Marine colloids were obtained from microalgal and seawater samples. The colloidal (10 kDa-1.0 μm) lipid concentration in *Isochrysis galbana* cultures was found

to 232-294 μg/L while the colloidal lipid concentration in *Chaetoceros muelleri* was only 8.0 μg/L. In contrast to lipid values, colloidal carbohydrates in *Chaetoceros muelleri* cultures were found to be much higher than those found in *Isochrysis galbana*. The major colloidal lipid classes in *Isochrysis galbana* are TAG and AMPL, which account for 56-86% of total colloidal lipids, whereas the major lipid classes in *Chaetoceros* are TAG and AMPL, which account for about 50% of total lipids.

There were 0.9-8.7 μg/L colloidal (10 kDa-0.45 μm) lipids and 20 μg/L colloidal (10 kDa-1.0 μm) carbohydrates in the Conception Bay seawater samples. Colloidal lipid and carbohydrates account for only a small proportion of lipid and carbohydrates associated with colloids or macromolecules (> 10 kDa). Therefore, any future studies of colloids should use a low MW cut-off (i.e., 1 kDa filter) to ensure collection of all colloidal particles.

The major colloidal lipid classes (10 kDa-0.45 μm) in seawater are TAG, FFA and PL, which account for about 80% of the total colloidal lipids. The least polar HC, the polar PL, and the surface active FFA have a higher proportion in total colloidal lipids than in total dissolved lipids. AMPL accounts for 50% of dissolved lipids, but accounts for only 3% of total colloidal lipid in seawater. Adsorption, complexation and solubility seem to be important factors in explaining the distribution of lipid classes in colloids. Absorption perhaps is an important process in the enrichment of HC on colloids. However, absorption for FFA is not as important as it for HC. Complexation with trace metals in colloids perhaps is a major factor for enriching FFA on colloids. Solubility can

be used to explain differences in the proportions of polar lipids on colloids. Some of the components of AMPL have larger solubility than PL. Therefore, the proportion of PL in colloidal lipids is twice that in dissolved lipids while the proportion of AMPL in dissolved lipids is 20-30 times than that in colloidal lipids.

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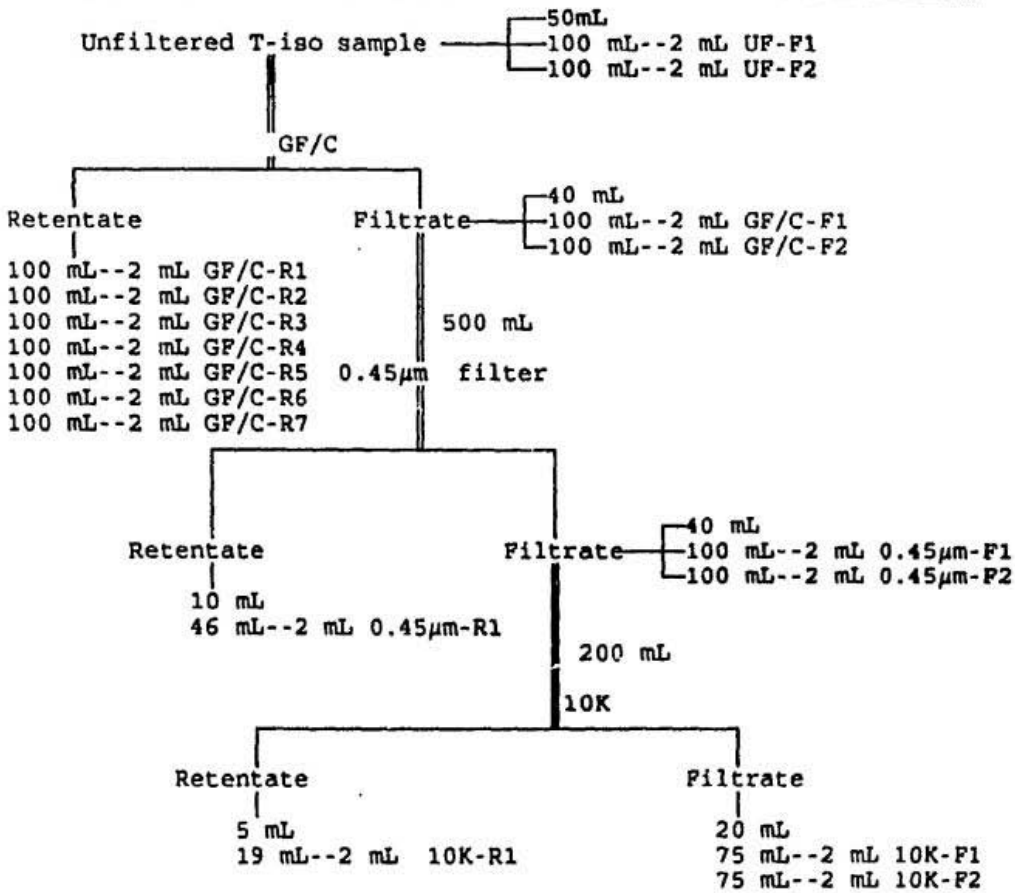
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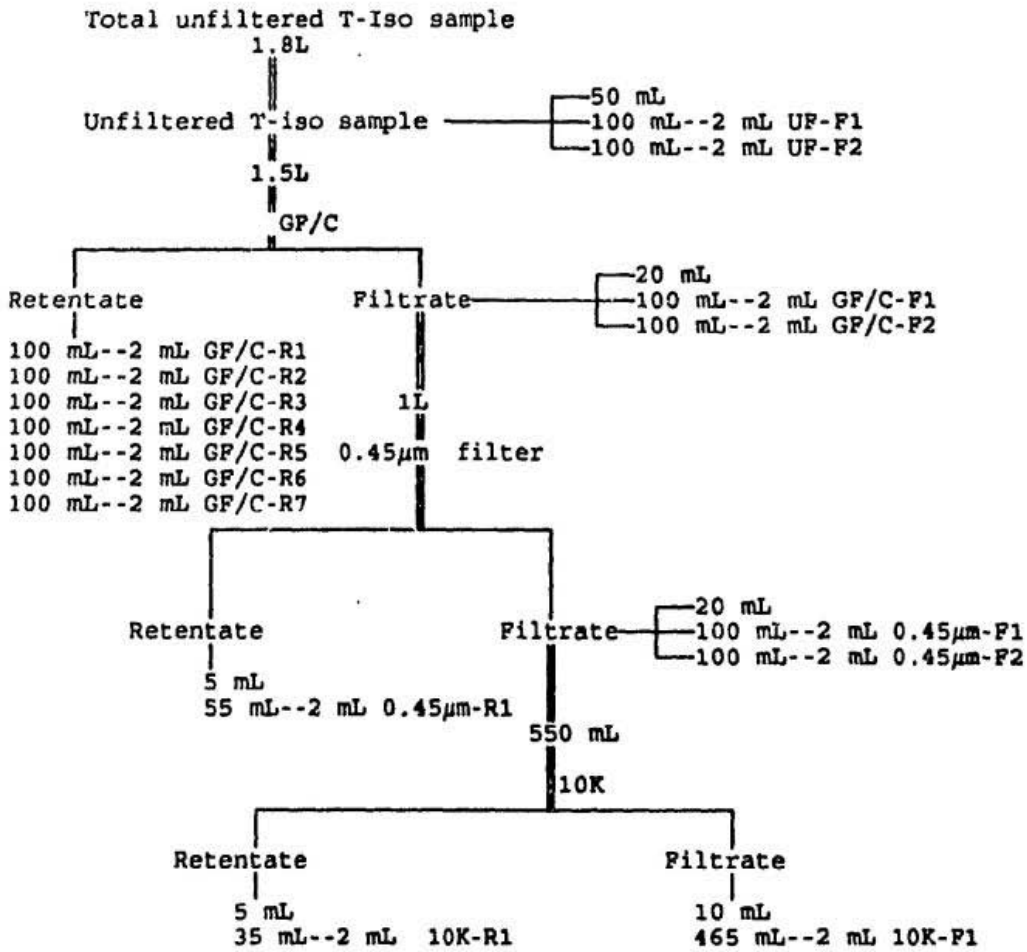
Appendix A Filtration diagrams for microalgal and seawater samples

Table A1 Flow diagram for the filtration of the *Isochrysis galbana* (T-iso) culture (5.8 x 10^6 cells/mL, 0.61 div./day) grown on semicontious and N-replete condition



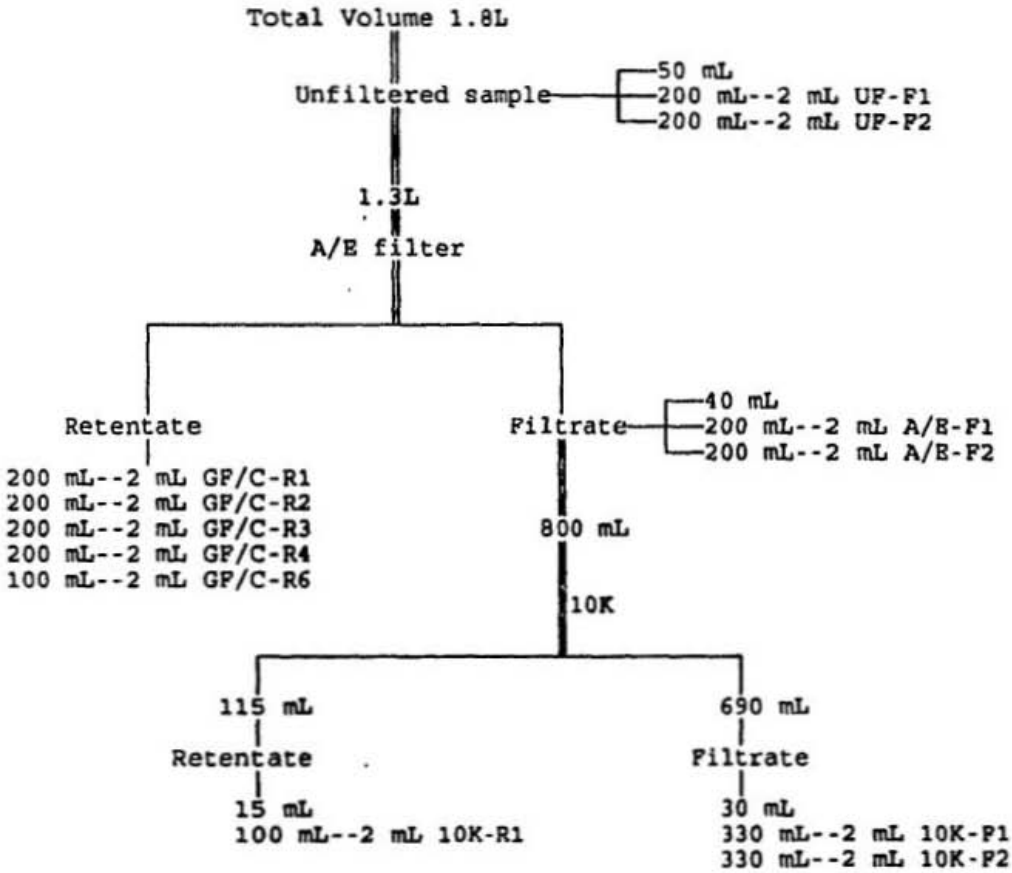
Concentration factor: $F_{0.45\mu m\ filter} = 8.9$, $F_{10KDa\ filter} = 8.3$

Table A2 Flow diagram for the filtration of the *Isochrysis galbana* (T-iso) culture (4.2×10^6 cells/mL, 0.90 div./day) grown on semicontious and N-replete condition



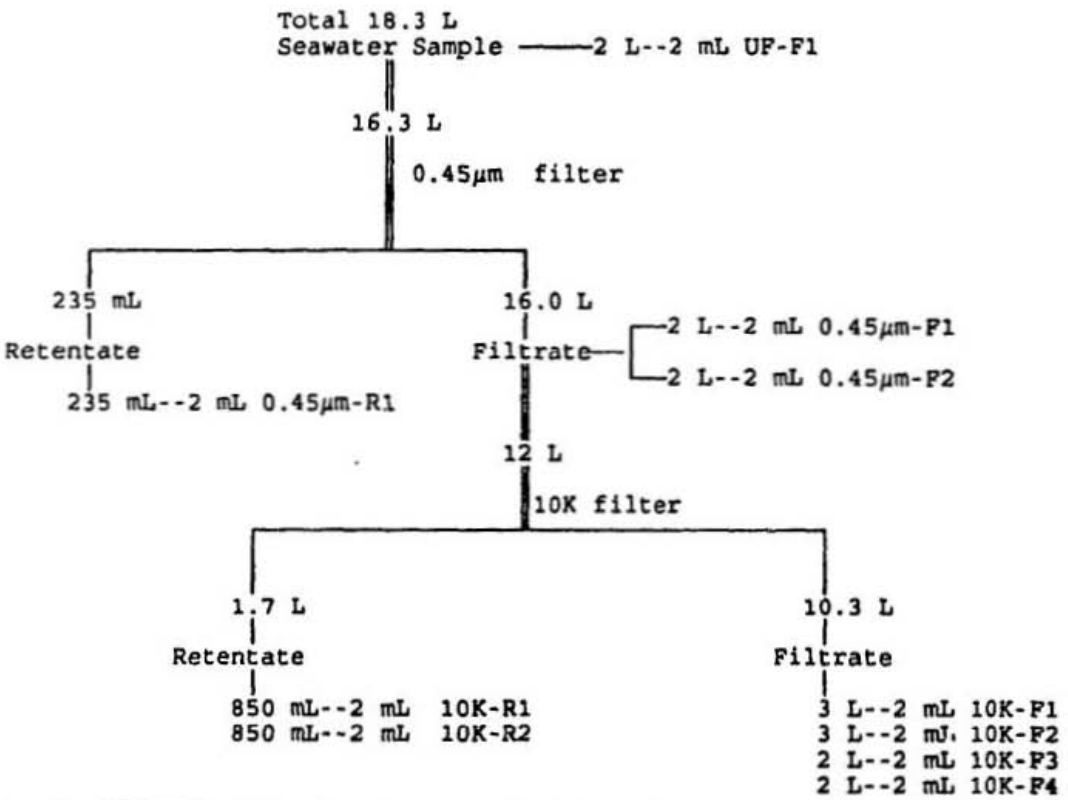
Concentration factor: $F_{0.45\mu m\ filter} = 16.7$
$F_{10KDa\ filter} = 13.8$

Table A3 Flow diagram for the filtration of the *Chaetoceros muelleri* culture (4.4×10^6 cells/mL, 0.24 div./day) grown on semicontious and N-replete condition



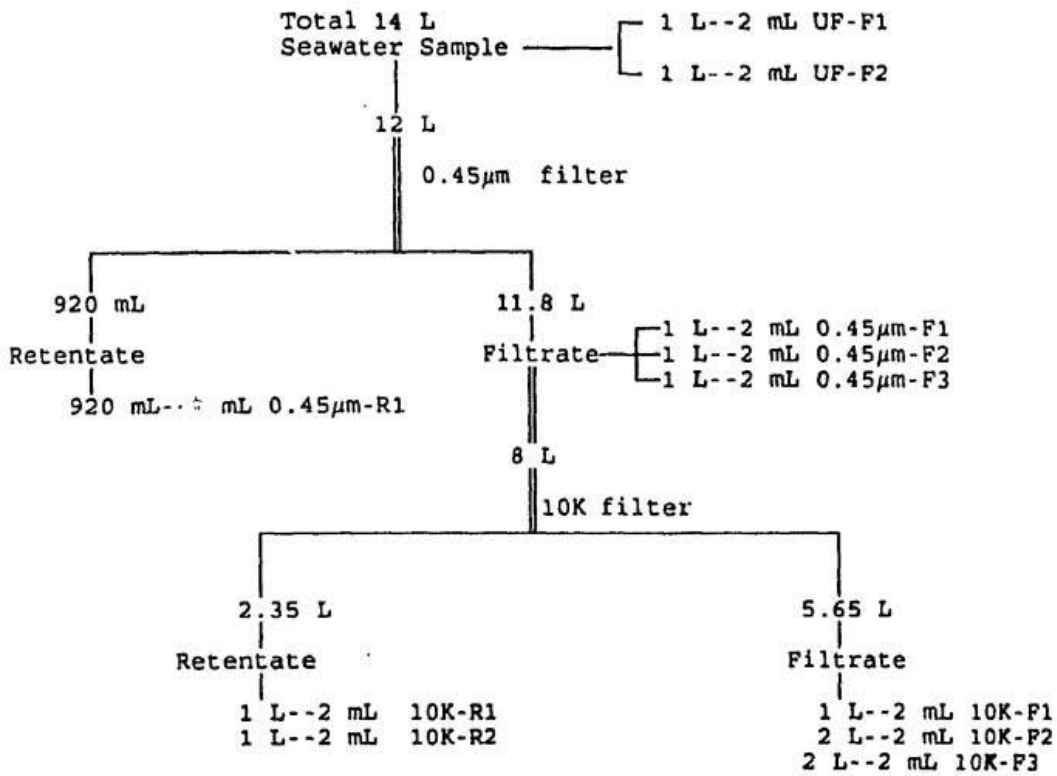
Concentration factor: $F_{10KDa\ filter} = 7.0$

Table A4 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland in May 19, 1993.



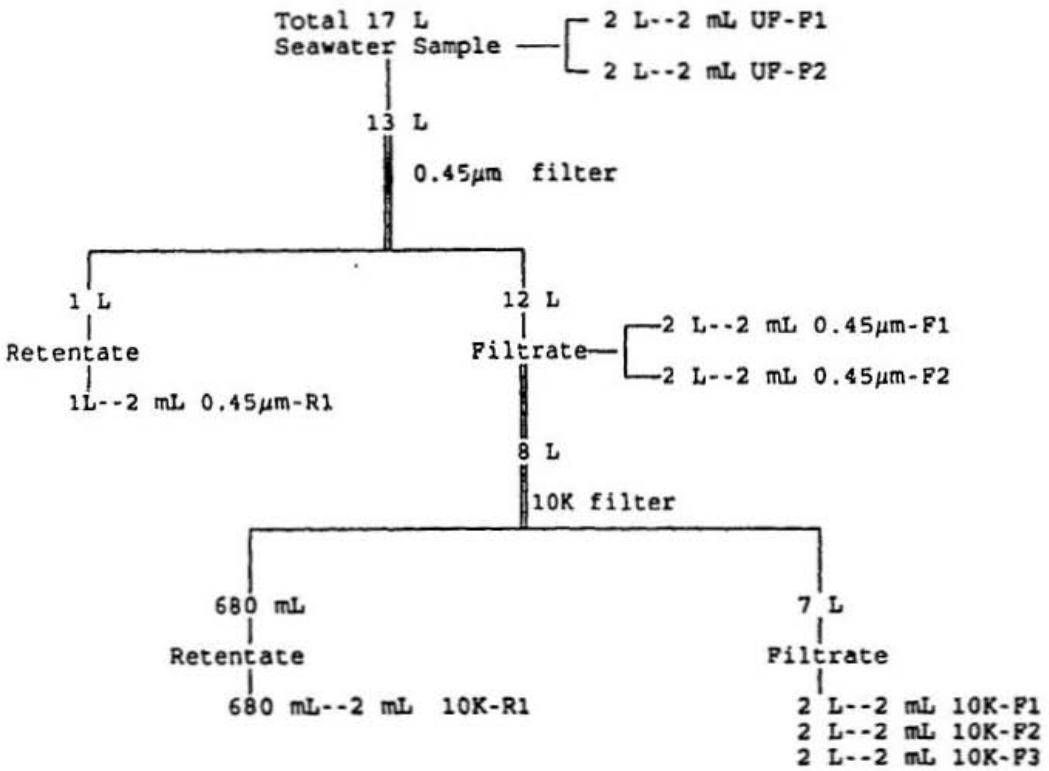
Concentration factor: $P_{0.45\mu m\ filter} = 69.4$
$P_{10KDa\ filter} = 7.1$

Table A5 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland, in June 14, 1993.



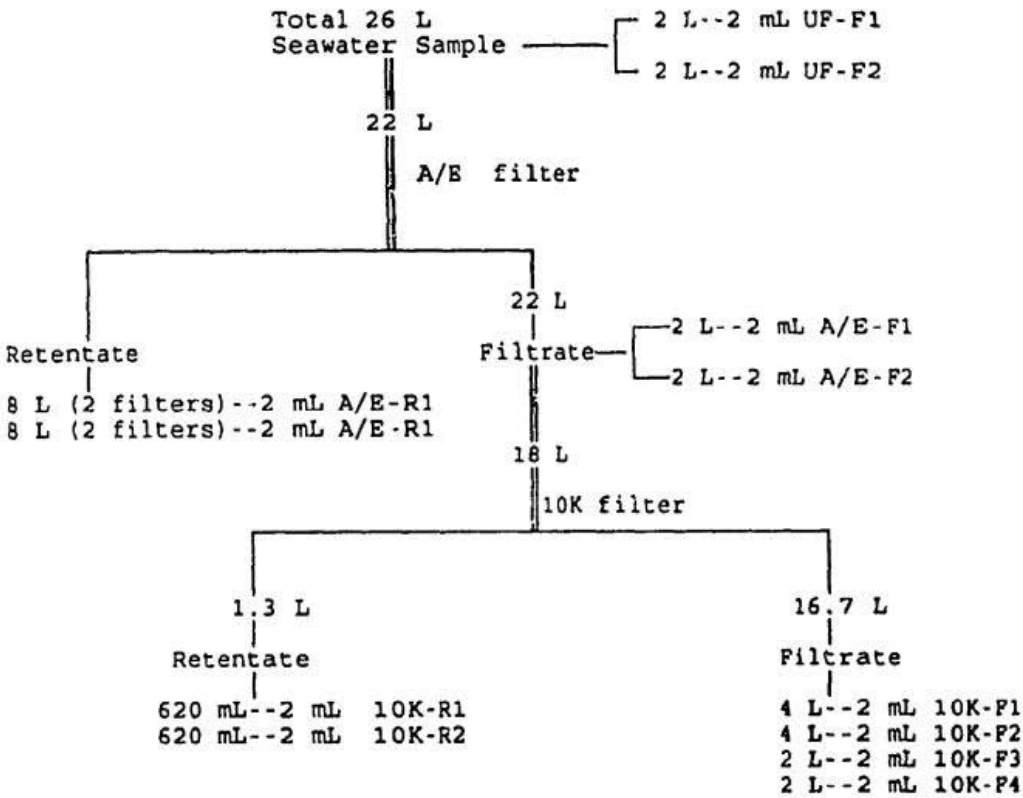
Concentration factor: $F_{0.45\mu m\ filter} = 13.0$
$F_{10KDa\ filter} = 3.4$

Table A6 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland, in August 2, 1993.



Concentration factor: $F_{0.45\mu m\ filter} = 13.0$
$F_{10KDa\ filter} = 11.8$

Table A7 Flow diagram for the filtration of seawater sample collected Conception Bay, Newfoundland, in October 5, 1993.



Concentration factor: $F_{10KDa\ filter} = 13.8$

Appendix B Analytical data for Calibration curves (lipid and carbohydrate)

Table B1 Analytical data of lipid standard solution by TLC–FID

HC				SE				KET			
Amount (μg)	Peak Area (mVmm)			Amount (μg)	Peak Area (mVmm)			Amount (μg)	Peak Area (mVmm)		
0.06	1.47	±	0.01	0.04	0.86	±	0.46	0.08	0.17	±	0.17
0.12	2.53	±	0.47	0.07	0.70	±	0.23	0.16	1.80	±	0.49
0.24	6.81	±	0.06	0.15	2.24	±	0.45	0.31	3.37	±	0.04
0.36	7.18	±	0.37	0.22	2.94	±	0.38	0.47	6.58	±	0.87
0.47	9.75	±	0.06	0.29	4.46	±	0.48	0.62	9.62	±	0.61
0.58	10.48	±	1.32	0.44	6.67	±	0.44	0.82	10.23	±	1.82
0.71	13.49	±	0.81	0.52	5.42	±	1.09	0.93	13.74	±	0.42
0.95	17.69	±	0.49	0.59	9.75	±	0.21	1.25	20.10	±	0.80
1.17	26.12	±	1.25	0.73	14.73	±	1.92	1.56	27.86	±	0.23
1.18	23.82	±	0.22	1.04	17.22	±	0.82	1.65	31.06	±	1.82
1.75	33.24	±	0.79	1.10	21.70	±	1.75	2.34	43.00	±	1.02
1.78	35.41	±	0.01	1.46	29.69	±	0.82	2.47	41.98	±	3.50
2.33	42.75	±	2.90	1.56	24.59	±	1.96	3.11	62.43	±	1.49
2.37	48.94	±	1.00	2.08	37.68	±	1.19	3.29	58.34	±	5.20
2.92	53.69	±	0.78	2.60	51.20	±	0.26	4.12	73.97	±	3.34
3.50	65.76	±	0.55	3.12	62.70	±	2.14	4.94	93.63	±	1.45
5.83	114.20	±	2.70	5.20	115.70	±	1.90	8.24	156.60	±	1.40

TAG				FFA				ALC			
Amount (μg)	Peak Area (mVmm)			Amount (μg)	Peak Area (mVmm)			Amount (μg)	Peak Area (mVmm)		
0.10	0.81	±	0.04	0.04	0.48	±	0.23	0.11	1.01	±	0.16
0.20	2.07	±	0.12	0.08	0.81	±	0.03	0.29	3.84	±	0.88
0.38	6.50	±	1.03	0.23	2.79	±	0.25	0.32	3.98	±	0.25
0.58	8.05	±	0.13	0.30	3.34	±	0.09	0.37	3.81	±	0.19
0.78	11.86	±	0.58	0.46	5.82	±	0.57	0.43	4.88	±	0.02
1.17	18.88	±	0.86	0.61	7.73	±	0.74	0.85	8.99	±	0.88
1.57	24.48	±	0.29	0.76	11.23	±	1.73	0.73	8.48	±	1.50
1.96	37.17	±	2.55	1.14	18.23	±	1.45	0.86	11.71	±	0.37
2.94	57.66	±	2.59	1.52	23.12	±	0.10	1.09	17.22	±	1.89
3.92	77.66	±	8.19	2.41	42.77	±	4.86	1.10	13.91	±	1.54
5.12	100.15	±	14.03	4.81	87.35	±	8.63	1.46	18.98	±	1.43
								1.62	25.36	±	1.25
								1.83	23.70	±	0.40
								2.15	36.63	±	6.15
								2.19	30.75	±	0.40
								2.67	43.41	±	4.05
								5.75	102.19	±	9.28

ST				AMPL				PL			
Amount (μg)	Peak Area (mVmm)			Amount (μg)	Peak Area (mVmm)			Amount (μg)	Peak Area (mVmm)		
0.07	0.85	±	0.00	0.17	5.00	±	2.56	0.07	5.55	±	4.91
0.14	1.39	±	0.27	0.35	14.72	±	4.89	0.14	4.64	±	3.78
0.27	3.37	±	1.94	0.52	9.33	±	0.81	0.27	15.37	±	9.01
0.41	4.83	±	0.88	0.69	17.14	±	2.68	0.41	8.37	±	2.07
0.51	5.92	±	0.88	1.00	14.40	±	1.14	0.55	10.40	±	2.67
0.54	6.75	±	0.81	1.04	19.13	±	2.19	0.76	14.08	±	1.00
0.82	13.82	±	3.67	1.39	26.74	±	2.67	0.82	14.25	±	1.30
1.02	15.33	±	1.47	1.74	41.66	±	6.22	1.06	18.53	±	0.66
1.09	16.21	±	1.36	2.00	34.97	±	0.97	1.36	30.16	±	3.69
1.36	29.07	±	4.58	2.61	51.06	±	9.91	1.52	29.81	±	1.14
1.53	25.26	±	1.04	3.00	46.89	±	2.25	2.04	41.76	±	3.63
2.04	46.22	±	3.76	3.47	60.80	±	6.86	2.29	42.53	±	1.02
2.05	37.52	±	2.33	4.00	62.51	±	1.89	2.73	54.73	±	0.66
2.56	50.33	±	1.33	5.00	86.05	±	6.75	3.05	57.54	±	3.04
2.72	60.81	±	5.77	6.00	98.13	±	2.27	3.81	74.00	±	2.01
3.07	67.23	±	3.12	9.99	175.40	±	10.03	4.57	87.09	±	1.84
5.12	134.10	±	6.30	10.01	177.40	±	8.00	7.62	149.75	±	1.45

Table B2 Linear regression between peak area (mVmm) and lipid class load (μg)

Regression parameters	HC	SE	KET	TAG	FFA	ALC	ST	AMPL	PL
Constant	0.510	−3.450	−2.978	−3.062	−3.512	−3.403	−7.471	2.031	1.371
Std Err of Y Est	1.792	3.448	2.240	1.963	2.758	2.839	5.222	5.267	3.196
R Squared	0.996	0.988	0.997	0.997	0.992	0.987	0.978	0.991	0.993
No. of Observations	17.000	17.000	17.000	11.000	11.000	17.000	17.000	17.000	17.000
Degrees of Freedom	15.000	15.000	15.000	9.000	9.000	15.000	15.000	15.000	15.000
X Coefficient(s)	19.177	22.163	19.456	20.245	20.269	17.582	25.522	17.267	19.123
Std Err of Coef.	0.297	0.632	0.263	0.377	0.617	0.518	0.985	0.435	0.406

Table B3 The relationship between absorbance of colour complex compound and standard glucose concentration

Glucose (μM)	1.7335	3.467	8.665	17.33	34.67
Absorbance (635nm)	0.158	0.228	0.446	0.847	1.591
	0.067	0.201	0.409	0.758	1.518
	0.017	0.101	0.274	0.651	1.420
	0.021	0.073	0.236	0.554	1.364
	0.059	0.134	0.272	0.616	1.448
	0.023	0.073	0.192	0.563	1.385
	0.150	0.195	0.386	0.679	1.512
Average	0.071	0.144	0.316	0.667	1.463
S.D.	0.056	0.060	0.089	0.098	0.075

Table B4 Regression between absorbance and glucose standard solution (μM)

Constant	−0.024
Std Err of Y Est	0.0353
R Squared	0.9971
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	0.0422
Std Err of Coef.	0.0013

Appendix C: Analytical results of lipid classes in microalgal culture samples

Table C1 Lipid class concentrations (μg/L, mean ± S.D., n = 6–12) in different filtration fractions of an *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) culture and recoveries (%) on three filters

lipid Classes	Concentration (μg/L)						Recoveries		
	UF	GF/C–F	GF/C–R	0.45μm–F	0.45μm–R	10K–F	10K–R	GF/C	0.45 μm	10K
HC	300 ± 149	36 ± 13	236 ± 172	6 ± 11	27 ± 3	6 ± 12	1 ± 5	91 ± 73	93 ± 46	105 ± 278
SE	1158 ± 101	61 ± 5	1063 ± 130	63 ± 1	13 ± 6	65 ± 4	N.D.	99 ± 14	123 ± 14	104 ± 10
KET	659 ± 11	32 ± 10	583 ± 26	34 ± 0	N.D.	34 ± 6	N.D.	93 ± 5	106 ± 33	101 ± 19
TG	7566 ± 112	366 ± 30	7146 ± 603	273 ± 119	86 ± 15	154 ± 84	76 ± 13	100 ± 7	93 ± 32	84 ± 48
FFA	389 ± 48	37 ± 7	321 ± 32	31 ± 6	10 ± 1	22 ± 10	14 ± 4	92 ± 14	111 ± 30	117 ± 46
ALC	1194 ± 14	72 ± 3	1026 ± 33	73 ± 6	6 ± 1	62 ± 2	N.D.	92 ± 3	111 ± 12	112 ± 13
ST	1404 ± 44	74 ± 6	1276 ± 31	75 ± 3	6 ± 1	62 ± 3	N.D.	96 ± 4	113 ± 10	109 ± 6
AMPL	7454 ± 736	273 ± 50	7374 ± 879	248 ± 56	9 ± 14	213 ± 63	41 ± 30	103 ± 16	94 ± 27	103 ± 37
PL	7152 ± 649	76 ± 41	7822 ± 993	81 ± 36	N.D.	82 ± 48	3 ± 18	110 ± 17	106 ± 76	106 ± 79
Least polar	2117 ± 181	129 ± 17	1903 ± 217	103 ± 11	40 ± 7	106 ± 14	1 ± 6	96 ± 13	111 ± 18	103 ± 19
Neutrual	10556 ± 131	569 ± 31	9766 ± 606	453 ± 120	111 ± 15	340 ± 86	90 ± 14	98 ± 5	99 ± 22	95 ± 31
Polar	14606 ± 963	349 ± 66	15196 ± 1328	328 ± 67	9 ± 17	296 ± 79	45 ± 35	108 ± 12	97 ± 27	103 ± 34
TOTAL	27279 ± 1009	1047 ± 74	26967 ± 1439	884 ± 196	159 ± 24	741 ± 117	135 ± 36	102 ± 6	100 ± 15	99 ± 21

Table C2 Lipid class concentrations (μg/L, mean ± S.D., n = 6–12) in different filtration fractions of an *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) cultures and recoveries (%) on three filters

Lipid Classes	Concentration (μg/L)												Recoveries (%)		
	UF		A/E–F		A/E–R		0.45μm–F		0.45μm–R		10K–F	10k–R	GF/C	0.45μm	10K
HC	205 ±	48	24 ±	2	194 ±	42	27 ±	2	2 ±	1	14 ± 1	23 ± 3	108 ± 32	119 ± 14	135 ± 18
SE	25 ±	5	12 ±	0	23 ±	5	12 ±	1	0 ±	0	3 ± 0	1 ± 1	141 ± 33	104 ± 9	36 ± 10
KET	628 ±	96	14 ±	1	565 ±	67	12 ±	0	1 ±	1	9 ± 2	4 ± 4	97 ± 18	100 ± 10	102 ± 34
TAG	2842 ±	486	327 ±	34	5726 ±	770	340 ±	84	34 ±	10	334 ± 6	49 ± 15	107 ± 33	114 ± 28	113 ± 13
FFA	272 ±	87	21 ±	1	260 ±	107	19 ±	2	2 ±	0	15 ± 3	4 ± 5	110 ± 53	102 ± 12	108 ± 30
ALC	314 ±	61	22 ±	3	260 ±	33	18 ±	3	2 ±	1	11 ± 1	1 ± 2	90 ± 20	86 ± 17	74 ± 15
ST	411 ±	77	26 ±	5	354 ±	139	23 ±	11	2 ±	2	22 ± 4	5 ± 4	93 ± 38	93 ± 45	117 ± 33
AMPL	11526 ±	2697	256 ±	54	11522 ±	1169	226 ±	66	19 ±	12	225 ± 12	38 ± 6	101 ± 27	96 ± 39	116 ± 26
PL	7686 ±	1866	173 ±	10	6930 ±	2132	143 ±	9	15 ±	3	123 ± 0	29 ± 0	92 ± 36	91 ± 7	106 ± 6
least polar	858 ±	86	49 ±	6	812 ±	140	51 ±	1	3 ±	4	28 ± 2	27 ± 4	100 ± 19	111 ± 16	104 ± 15
neutral	3838 ±	897	396 ±	147	3818 ±	803	397 ±	97	41 ±	25	382 ± 42	60 ± 48	105 ± 32	110 ± 49	111 ± 44
polar	18315 ±	4446	429 ±	80	18462 ±	2229	389 ±	152	34 ±	20	348 ± 6	67 ± 66	96 ± 26	94 ± 41	112 ± 34
TOTAL	34012 ±	8606	875 ±	810	33668 ±	4966	816 ±	188	78 ±	29	756 ± 36	154 ± 36	99 ± 31	102 ± 39	111 ± 40

Table C3 Lipid class concentrations (μg/L, mean ± S.D., n = 6–12) in different filtration fractions of a *Chaetoceros muelleri* (4.4 x 10^6 cells/mL, 0.24 div./day) culture and recoveries (%) on three filters

Lipid Classes	Concentration (μg/L)					Recoveries (%)	
	UF	A/E–F	A/E–R	10k–F	10K–R	A/E	10K
HC	125 ± 24	14 ± 3	108 ± 15	8 ± 0.5	0.9 ± 0.4	98 ± 22	64 ± 15
SE	26 ± 17	1 ± 1	13 ± 0	1 ± 0.0	0.3 ± 0.4	55 ± 36	99 ± 61
KET	167 ± 10	8 ± 0	163 ± 31	8 ± 0.2	1.4 ± 0.2	102 ± 20	120 ± 4
TAG	2345 ± 327	21 ± 2	2267 ± 293	14 ± 1.3	2.3 ± 0.5	96 ± 18	79 ± 11
FFA	1144 ± 159	12 ± 1	1325 ± 357	14 ± 2.0	0.3 ± 0.5	117 ± 35	127 ± 24
ALC	930 ± 269	23 ± 1	855 ± 306	24 ± 3.4	N.D.	94 ± 43	105 ± 15
ST	230 ± 111	12 ± 1	253 ± 31	11 ± 0.4	1.2 ± 0.7	115 ± 57	98 ± 13
AMPL	9597 ± 2458	113 ± 10	8481 ± 1730	111 ± 20.0	N.D.	90 ± 29	98 ± 20
PL	7537 ± 2020	26 ± 7	6470 ± 1104	17 ± 1.1	1.6 ± 2.2	113 ± 34	71 ± 22
least polar	318 ± 91	23 ± 3	284 ± 99	17 ± 1.7	2.6 ± 0.4	96 ± 42	85 ± 13
neutral	4649 ± 701	68 ± 6	4700 ± 1028	64 ± 5.9	3.8 ± 2.2	103 ± 27	99 ± 15
polar	17134 ± 3721	139 ± 14	16951 ± 3267	128 ± 21.0	1.6 ± 4.0	100 ± 29	93 ± 18
TOTAL	22102 ± 4089	229 ± 18	21935 ± 4040	208 ± 12.9	8.0 ± 4.2	100 ± 26	94 ± 9

Table C4 Lipid class concentrations (μg/L, mean ± S.D., n = 6–12) and distribution (%) in different particle pools and dissolved fraction of an *Isochrysis galbana* (5.8 x 10^6 cells/mL, 0.61 div./day) culture

Lipid Classes	Concentration (μg/L)					Distribution (%)			
	>1.2μm	1.2μm–0.45μm	0.45μm–3nm	Dissolved (<3nm)	Total	>1.2μm	1.2μm–0.45μm	0.45μm–3nm	Dissolved (<3nm)
HC	236 ± 172	27 ± 3	1 ± 5	6 ± 12	270 ± 172	88 ± 85	10.0 ± 6.5	0.2 ± 1.7	2.2 ± 4.7
SE	1063 ± 130	13 ± 8	N.D.	65 ± 4	1181 ± 130	93 ± 15	1.1 ± 0.5	N.D.	5.6 ± 0.7
KET	583 ± 26	N.D.	N.D.	34 ± 6	618 ± 27	94 ± 6	N.D.	N.D.	5.6 ± 1.1
TG	7146 ± 503	86 ± 15	78 ± 13	154 ± 84	7462 ± 510	96 ± 9	1.2 ± 0.2	1.0 ± 0.2	2.1 ± 1.1
FFA	321 ± 32	10 ± 1	14 ± 4	22 ± 10	367 ± 33	87 ± 12	2.8 ± 0.4	3.7 ± 1.1	6.1 ± 2.7
ALC	1028 ± 33	6 ± 1	N.D.	82 ± 2	1114 ± 34	92 ± 4	0.6 ± 0.1	N.D.	7.4 ± 0.3
ST	1275 ± 31	8 ± 1	N.D.	82 ± 3	1366 ± 31	93 ± 3	0.6 ± 0.1	N.D.	6.0 ± 0.3
AMPL	7374 ± 879	9 ± 14	41 ± 30	213 ± 63	7637 ± 882	97 ± 16	0.1 ± 0.2	0.5 ± 0.4	2.8 ± 0.9
PL	7822 ± 993	N.D.	3 ± 18	82 ± 48	7907 ± 994	99 ± 18	N.D.	0.0 ± 0.2	1.0 ± 0.6
Lasepolar	1903 ± 217	40 ± 7	1 ± 8	106 ± 14	2049 ± 217	93 ± 14	1.9 ± 0.4	0.0 ± 0.3	5.2 ± 0.9
Neutral	9768 ± 506	111 ± 15	90 ± 14	340 ± 86	10309 ± 513	95 ± 7	1.1 ± 0.2	0.9 ± 0.1	3.3 ± 0.8
Polar	15198 ± 1328	9 ± 17	46 ± 35	295 ± 79	15544 ± 1329	96 ± 12	0.1 ± 0.1	0.3 ± 0.2	1.9 ± 0.5
Total	26867 ± 1436	159 ± 24	136 ± 88	741 ± 117	27902 ± 1441	96 ± 7	0.6 ± 0.1	0.5 ± 0.1	2.7 ± 0.4

Table C5 Lipid class concentrations (μg/L, mean ± S.D., n = 6–12) and distribution (%) in different particle pools and dissolved fraction of a *Isochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) culture

Lipid Classes	Concentration (μg/L)					Distribution (%)			
	>1.0μm	1.0μm–0.45μm	0.45μm–3nm	Dissolved (<3nm)	Total	>1.0μm	1.0μm–0.45μm	0.45μm–3nm	Dissolved (<3nm)
HC	194 ± 42	2 ± 1	23 ± 3	14 ± 1	232 ± 42	84 ± 24	0.8 ± 0.5	9.7 ± 2.3	5.9 ± 1.1
SE	23 ± 5	0 ± 0	1 ± 1	3 ± 0	27 ± 5	84 ± 23	0.1 ± 0.5	3.4 ± 4.4	12.5 ± 2.7
KET	595 ± 67	1 ± 1	4 ± 4	9 ± 2	609 ± 67	98 ± 15	0.2 ± 0.1	0.6 ± 0.6	1.5 ± 0.3
TAG	2725 ± 770	34 ± 10	49 ± 15	334 ± 6	3142 ± 771	87 ± 32	1.1 ± 0.4	1.6 ± 0.6	10.6 ± 2.6
FFA	280 ± 107	2 ± 0	4 ± 5	15 ± 3	302 ± 107	93 ± 48	0.8 ± 0.3	1.5 ± 1.6	5.0 ± 2.1
ALC	260 ± 33	2 ± 1	1 ± 2	11 ± 1	274 ± 33	95 ± 16	0.9 ± 0.3	0.4 ± 0.6	4.1 ± 0.5
ST	354 ± 139	2 ± 2	5 ± 4	22 ± 4	382 ± 139	93 ± 50	0.5 ± 0.8	1.2 ± 1.1	5.7 ± 2.3
AMPL	11522 ± 1169	19 ± 12	38 ± 8	225 ± 12	11804 ± 1169	98 ± 14	0.2 ± 0.1	0.3 ± 0.1	1.9 ± 0.2
PL	6930 ± 2132	15 ± 3	29 ± 0	123 ± 0	7087 ± 2132	98 ± 42	0.2 ± 0.1	0.4 ± 0.1	1.7 ± 0.5
lesspolar neutral	812 ± 140	3 ± 4	27 ± 4	26 ± 2	868 ± 140	93 ± 22	0.4 ± 0.4	3.1 ± 0.6	3.0 ± 0.5
polar	3618 ± 803	41 ± 25	60 ± 48	382 ± 42	4101 ± 806	88 ± 26	1.0 ± 0.6	1.5 ± 1.2	9.3 ± 2.1
Total	18452 ± 2229	34 ± 20	67 ± 88	348 ± 8	18901 ± 2231	96 ± 16	0.2 ± 0.1	0.4 ± 0.5	1.8 ± 0.2
	22882 ± 4936	78 ± 29	154 ± 36	756 ± 36	23870 ± 4936	96 ± 29	0.3 ± 0.1	0.6 ± 0.2	3.2 ± 0.7

Table C6 Lipid class concentrations (μg/L, mean ± S.D., n = 6–12) and ditribution (%) in different particle pools and the dissolved fraction of *Chaetoceros muelleri* (4.4 x 10^6cells/ml, 0.24 div./day) culture

Lipid Classes	Concentration (μg/L)				Distribution (%)		
	>1.0μm	1.0μm–3μm	Dissolved (<3nm)	Total	>1.0μm	1.0μm–3nm	Dissolved (<3nm)
HC	108 ± 15	0.9 ± 0.4	8 ± 1	117 ± 15	93 ± 17	0.7 ± 0.3	6.8 ± 1.0
SE	13 ± 0	0.3 ± 0.4	1 ± 0	14 ± 0	93 ± 2	2.3 ± 2.5	5.1 ± 0.1
KET	163 ± 31	1.4 ± 0.2	8 ± 0	172 ± 31	95 ± 25	0.8 ± 0.2	4.6 ± 0.8
TAG	2267 ± 293	2.3 ± 0.5	14 ± 1	2283 ± 293	99 ± 18	0.1 ± 0.0	0.6 ± 0.1
FFA	1325 ± 357	0.3 ± 0.5	14 ± 2	1340 ± 357	99 ± 38	0.0 ± 0.0	1.1 ± 0.3
ALC	855 ± 306	N.D	24 ± 3	878 ± 306	97 ± 49	N.D.	2.8 ± 1.0
ST	253 ± 31	1.2 ± 0.7	11 ± 0	265 ± 31	96 ± 16	0.4 ± 0.3	4.0 ± 0.5
AMPL	8481 ± 1730	N.D.	111 ± 20	8592 ± 1731	99 ± 28	N.D.	1.3 ± 0.3
PL	8470 ± 1104	1.6 ± 2.2	17 ± 1	8489 ± 1104	100 ± 18	0.0 ± 0.0	0.2 ± 0.0
Lesspolar	284 ± 99	2.6 ± 0.4	17 ± 2	303 ± 99	94 ± 45	0.9 ± 0.3	5.5 ± 1.9
Neutral	4700 ± 1028	3.8 ± 2.2	64 ± 6	4767 ± 1028	99 ± 30	0.1 ± 0.0	1.3 ± 0.3
Polar	16951 ± 3267	1.6 ± 4.0	128 ± 21	17081 ± 3267	99 ± 27	0.0 ± 0.0	0.7 ± 0.2
Total	21935 ± 4040	8.0 ± 4.2	208 ± 13	22151 ± 4040	99 ± 26	0.0 ± 0.0	0.9 ± 0.2

Table C7 Lipid composition percentage (%, mean ± S.D. n = 6–12) in different particle pools, the dissolved fraction, and the unfiltered fraction for an *Isochrysis galbana* (5.6 x 10^6 cells/mL, 0.61 div./day) culture sample

Lipid Classes	>1.2μm	1.2μm–0.45μm	0.45μm–3nm	dissolved <3nm	Total
HC	0.9 ± 0.6	17.0 ± 3.3	0.4 ± 3.4	0.6 ± 1.6	1.0 ± 0.6
SE	4.0 ± 0.5	7.9 ± 3.9	N.D.	8.8 ± 1.5	4.2 ± 0.5
KET	2.2 ± 0.2	N.D.	N.D.	4.6 ± 1.1	2.2 ± 0.2
TG	26.6 ± 2.3	54.1 ± 12.5	56.2 ± 18.6	20.7 ± 11.8	26.7 ± 2.3
FFA	1.2 ± 0.1	6.4 ± 1.3	10.2 ± 4.1	3.0 ± 1.4	1.3 ± 0.1
ALC	3.9 ± 0.2	4.0 ± 0.8	N.D.	11.1 ± 1.8	4.0 ± 0.2
ST	4.7 ± 0.3	5.1 ± 0.9	N.D.	11.1 ± 1.8	4.9 ± 0.3
AMPL	27.4 ± 3.6	5.6 ± 9.0	30.7 ± 23.6	23.7 ± 9.7	27.4 ± 3.5
PL	29.1 ± 4.0	N.D.	2.4 ± 13.5	11.1 ± 6.7	28.3 ± 3.9
Least polar	7.1 ± 0.9	24.8 ± 5.6	0.4 ± 4.6	14.3 ± 3.0	7.3 ± 0.9
Neutral	36.4 ± 2.7	69.8 ± 14.1	66.4 ± 21.3	45.9 ± 13.5	36.9 ± 2.7
Polar	56.6 ± 5.8	5.6 ± 10.5	33.1 ± 27.4	39.8 ± 12.4	55.7 ± 5.6
Total	100.0 ± 6.5	100.0 ± 18.5	100.0 ± 35.0	100.0 ± 18.6	100.0 ± 6.2

Table C8 Lipid composition percentage (%, mean ± S.D. n = 6–12) in different particle pools, the dissolved fraction, and the unfiltered fraction for an *Isoochrysis galbana* (4.2 x 10^6 cells/mL, 0.90 div./day) culture sample

Lipid Classes	>1.0µm	1.0µm–0.45µm	0.45µm–3nm	dissolved <3nm	Total
HC	0.8 ± 0.3	2.5 ± 1.6	14.7 ± 4.1	1.8 ± 0.1	1.0 ± 0.3
SE	0.1 ± 0.0	0.0 ± 0.2	0.6 ± 0.8	0.5 ± 0.1	0.1 ± 0.0
KET	2.6 ± 0.6	1.6 ± 0.9	2.3 ± 2.4	1.2 ± 0.3	2.5 ± 0.6
TAG	11.9 ± 4.2	43.5 ± 20.2	32.2 ± 12.4	44.2 ± 2.3	13.2 ± 4.2
FFA	1.2 ± 0.5	3.1 ± 1.2	2.9 ± 3.1	2.0 ± 0.4	1.3 ± 0.5
ALC	1.1 ± 0.3	3.1 ± 1.5	0.7 ± 1.1	1.5 ± 0.1	1.1 ± 0.3
ST	1.5 ± 0.7	2.4 ± 2.7	3.0 ± 2.6	2.9 ± 0.6	1.6 ± 0.7
AMPL	50.4 ± 12.0	24.3 ± 18.1	24.9 ± 7.9	29.7 ± 2.1	49.5 ± 11.3
PL	30.3 ± 11.4	19.6 ± 8.2	18.7 ± 4.3	16.3 ± 0.6	29.7 ± 10.6
least polar	3.5 ± 1.0	4.2 ± 5.0	17.6 ± 4.7	3.5 ± 0.3	3.6 ± 1.0
neutral	15.8 ± 4.9	52.0 ± 36.6	38.8 ± 32.6	50.5 ± 6.1	17.2 ± 4.9
polar	80.6 ± 19.9	43.8 ± 29.9	43.6 ± 58.0	46.0 ± 2.5	79.2 ± 18.9
Total	100.0 ± 20.6	100.0 ± 47.7	100.0 ± 66.7	100.0 ± 6.6	100.0 ± 19.5

Table C9 Lipid composition percentage (%, mean ± S.D., n = 6–12) in different particle pools, the dissolved fraction, and the unfiltered fraction for a *Chaetoceros muelleri* (4.4×10^6 cells/mL, 0.24 div./day) culture samples

Lipid Classes	>1.2μm	1.0μm–3nm	dissolved <3nm	Total
HC	0.5 ± 0.1	10.8 ± 7.4	3.8 ± 0.4	0.5 ± 0.1
SE	0.1 ± 0.0	4.1 ± 4.9	0.3 ± 0.0	0.1 ± 0.0
KET	0.7 ± 0.2	17.5 ± 9.5	3.8 ± 0.3	0.8 ± 0.2
TAG	10.3 ± 2.3	29.1 ± 16.5	6.8 ± 0.7	10.3 ± 2.3
FFA	6.0 ± 2.0	3.6 ± 6.3	6.9 ± 1.1	6.0 ± 2.0
ALC	3.9 ± 1.6	N.D.	11.8 ± 1.8	4.0 ± 1.6
ST	1.2 ± 0.3	14.6 ± 11.6	5.1 ± 0.4	1.2 ± 0.3
AMPL	38.7 ± 10.6	N.D.	53.4 ± 10.2	38.1 ± 10.5
PL	38.6 ± 8.7	20.4 ± 29.9	8.0 ± 0.7	38.3 ± 8.6
least polar	1.3 ± 0.5	32.4 ± 18.0	8.0 ± 1.0	1.4 ± 0.5
neutral	21.4 ± 6.1	47.3 ± 36.8	30.6 ± 3.4	21.5 ± 6.1
polar	77.3 ± 20.6	20.4 ± 51.5	61.5 ± 10.8	77.1 ± 20.4
Total	100.0 ± 21.5	100.0 ± 65.8	100.0 ± 11.4	100.0 ± 21.3

Apendix D: Analytical results of lipid classes in seawater samples

Table D1 · Lipid class concentrations (μg/L, mean ± S.D., n = 6 – 10) in different filtration fractions of seawater collected in Conception Bay, Newfoundland on May 19, 1993 and recoveries (%) on two filters

Lipid Classes	Concentration (μg/L)					Mass balance (%)	
	75μm–F	0.45μm–F	0.45μm–R	10k–F	10K–R	0.45μm filter	10K filter
HC	1.5 ± 0.2	1.1 ± 0.1	0.2 ± 0.3	1.2 ± 0.3	0.1 ± 0.0	88.8 ± 18.4	114.7 ± 25.7
SE	0.5 ± 0.2	0.3 ± 0.1	0.0 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	70.0 ± 16.5	98.4 ± 27.2
KET	0.7 ± 0.2	0.7 ± 0.1	0.0 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	96.6 ± 16.2	118.7 ± 23.3
TAG	25.2 ± 4.0	26.3 ± 0.5	1.7 ± 2.5	27.1 ± 9.8	0.1 ± 0.8	111.4 ± 10.3	103.1 ± 37.6
FFA	5.8 ± 0.0	4.9 ± 0.1	0.3 ± 0.1	5.0 ± 0.7	0.8 ± 0.1	88.8 ± 2.9	119.1 ± 14.5
ALC	1.0 ± 0.4	0.6 ± 0.1	0.1 ± 0.2	0.7 ± 0.2	0.0 ± 0.0	71.7 ± 22.4	110.2 ± 36.0
ST	2.5 ± 0.1	1.9 ± 0.2	0.1 ± 0.2	1.8 ± 0.3	0.1 ± 0.1	79.9 ± 9.5	99.8 ± 18.7
AMPL	23.1 ± 3.7	25.1 ± 1.6	0.6 ± 0.2	30.0 ± 3.5	N.D.	110.9 ± 7.1	116.4 ± 15.9
PL	4.4 ± 1.7	2.9 ± 0.1	0.5 ± 0.4	2.6 ± 0.5	0.2 ± 0.0	76.4 ± 8.2	99.8 ± 17.6
lesspolar	2.8 ± 0.3	2.1 ± 0.1	0.3 ± 0.3	2.3 ± 0.3	0.2 ± 0.0	87.5 ± 15.7	113.7 ± 16.1
neutral	34.5 ± 4.1	33.7 ± 0.6	2.2 ± 2.6	34.5 ± 9.9	1.0 ± 0.8	104.2 ± 14.4	105.3 ± 29.5
polar	27.5 ± 4.1	28.0 ± 1.6	1.0 ± 0.5	32.7 ± 3.6	0.2 ± 0.1	105.4 ± 16.8	117.4 ± 14.4
TOTAL	64.7 ± 5.8	63.9 ± 1.7	3.5 ± 2.5	69.5 ± 10.5	1.4 ± 0.8	104.0 ± 10.4	110.9 ± 16.8

Table D2 Lipid class concentrations (μg/L, mean ± S.D., n = 6 – 10) in different filtration fractions of seawater collected in Conception Bay, Newfoundland on June 24, 1993 and recoveries (%) on two filters

Lipid Classes	Concentration (μg/L)					Mass balance (%)	
	75μm–F	0.45μm–F	0.45μm–R	10k–F	10K–R	0.45μm filter	10K filter
HC	3.3 ± 0.2	2.0 ± 1.1	0.1 ± 0.1	0.8 ± 0.1	0.2 ± 0.0	62.7 ± 31.9	51.0 ± 26.9
SE	0.2 ± 0.1	0.2 ± 0.1	N.D.	0.2 ± 0.1	N.D.	87.8 ± 51.9	103.3 ± 68.1
KET	1.0 ± 0.0	0.8 ± 0.1	0.0 ± 0.0	0.9 ± 0.1	0.1 ± 0.0	88.9 ± 7.6	125.7 ± 16.9
TAG	30.9 ± 2.1	35.2 ± 16.8	0.1 ± 1.4	21.1 ± 1.0	3.6 ± 0.1	114.5 ± 54.5	70.3 ± 33.6
FFA	6.0 ± 0.7	5.6 ± 0.5	0.4 ± 0.1	6.5 ± 0.8	3.3 ± 0.9	99.6 ± 7.9	176.0 ± 26.1
ALC	1.6 ± 0.2	0.9 ± 0.1	0.0 ± 0.0	1.9 ± 0.5	N.D.	60.2 ± 5.0	201.8 ± 56.8
ST	2.7 ± 0.1	2.3 ± 0.2	0.0 ± 0.1	2.1 ± 0.2	0.1 ± 0.1	86.2 ± 6.1	92.5 ± 10.3
AMPL	40.5 ± 1.3	36.8 ± 2.0	0.3 ± 0.5	37.3 ± 2.9	0.2 ± 0.4	91.7 ± 5.0	102.1 ± 9.6
PL	12.8 ± 0.2	10.6 ± 0.7	0.2 ± 0.2	8.0 ± 1.6	1.2 ± 0.2	84.5 ± 5.9	86.3 ± 16.2
lesspolar	4.5 ± 0.2	3.1 ± 1.1	0.1 ± 0.1	2.0 ± 0.2	0.3 ± 0.0	69.6 ± 23.9	74.9 ± 26.5
neutral	41.1 ± 2.2	44.0 ± 16.8	0.6 ± 1.4	31.6 ± 1.4	7.0 ± 0.9	108.4 ± 41.3	87.7 ± 33.6
polar	53.2 ± 1.4	47.4 ± 2.1	0.5 ± 0.6	45.3 ± 3.3	1.4 ± 0.5	90.0 ± 4.7	98.6 ± 8.3
TOTAL	98.9 ± 2.6	94.5 ± 16.9	1.1 ± 1.6	78.9 ± 3.6	8.7 ± 1.0	96.7 ± 17.4	92.7 ± 17.1

Table D3 Lipid class concentrations (μg/L, mean ± S.D., n = 6 – 10) in different filtration fractions of seawater collected in Conception Bay, Newfoundland on August 2, 1993 and recoveries (%) on two filters

Lipid Classes	Concentration (μg/L)					Mass balance (%)	
	23μm–F	0.45μm–F	0.45μm–R	10k–F	10K–R	0.45μm filter	10K filter
HC	2.9 ± 0.4	2.0 ± 0.1	0.1 ± 0.1	1.3 ± 0.1	0.4 ± 0.1	71.9 ± 3.4	88.6 ± 8.1
SE	0.5 ± 0.1	0.4 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	90.4 ± 9.8	123.4 ± 11.9
KET	0.9 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	97.7 ± 3.5	116.5 ± 12.9
TAG	6.5 ± 0.1	49.3 ± 2.3	N.D.	8.6 ± 0.2	1.4 ± 0.5	756.5 ± 37.3	20.3 ± 1.5
FFA	5.6 ± 0.1	8.2 ± 0.1	0.3 ± 0.1	12.4 ± 1.1	0.6 ± 0.3	150.6 ± 2.5	158.3 ± 13.9
ALC	1.0 ± 0.0	0.9 ± 0.1	0.0 ± 0.1	1.3 ± 0.1	0.1 ± 0.1	96.8 ± 8.5	155.2 ± 20.2
ST	1.3 ± 0.0	1.3 ± 0.1	0.1 ± 0.1	1.3 ± 0.2	0.2 ± 0.1	102.3 ± 10.1	117.7 ± 21.3
AMPL	23.1 ± 1.0	22.5 ± 1.8	0.5 ± 1.0	19.7 ± 0.6	0.5 ± 0.8	100.0 ± 9.1	89.9 ± 8.6
PL	3.0 ± 1.5	2.2 ± 0.3	0.1 ± 0.0	1.7 ± 0.3	0.0 ± 0.1	75.7 ± 10.8	79.5 ± 16.9
lesspolar	4.3 ± 0.4	3.2 ± 0.1	0.3 ± 0.1	2.6 ± 0.2	0.6 ± 0.1	79.6 ± 7.9	100.3 ± 6.2
neutral	14.4 ± 0.1	59.7 ± 2.4	0.4 ± 0.2	23.5 ± 1.1	2.4 ± 0.6	416.6 ± 16.9	43.4 ± 2.8
polar	26.1 ± 1.9	24.7 ± 1.9	0.6 ± 1.0	21.4 ± 0.7	0.6 ± 0.8	97.1 ± 10.7	89.0 ± 8.0
TOTAL	44.9 ± 1.9	87.6 ± 3.0	1.3 ± 1.0	47.5 ± 1.4	3.6 ± 1.0	196.1 ± 11.0	58.3 ± 2.8

Table D4 Lipid class concentrations (μg/L, mean ± S.D., n = 6 – 10) in different filtration fractions of seawater collected in Conception Bay, Newfoundland on October 5, 1993 and recoveries (%) on two filters

Lipid Classes	Concentration (μg/L)					Recoveries (%)	
	75μm–F	A/E–F	A/E–R	10k–F	10K–R	A/E	10K
HC	4.4 ± 0.6	2.0 ± 0.1	2.8 ± 0.7	1.7 ± 0.3	0.1 ± 0.0	109.5 ± 16.7	91.6 ± 14.1
SE	1.8 ± 0.4	0.9 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	0.0 ± 0.0	59.4 ± 7.2	45.8 ± 10.0
KET	1.6 ± 0.0	1.2 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.1 ± 0.0	122.3 ± 11.4	80.2 ± 17.3
TAG	5.5 ± 0.3	4.4 ± 0.0	1.3 ± 0.7	3.4 ± 0.2	0.3 ± 0.0	104.2 ± 12.1	82.9 ± 4.6
FFA	3.9 ± 0.7	3.5 ± 0.2	0.5 ± 0.3	3.3 ± 0.4	0.2 ± 0.0	99.8 ± 8.5	99.2 ± 12.6
ALC	1.5 ± 0.1	0.9 ± 0.2	0.4 ± 0.1	1.0 ± 0.3	0.0 ± 0.0	86.5 ± 15.1	109.0 ± 38.2
ST	1.6 ± 0.2	1.4 ± 0.2	0.8 ± 0.2	1.7 ± 0.8	0.1 ± 0.0	137.3 ± 18.8	129.4 ± 64.7
AMPL	9.9 ± 0.3	9.4 ± 1.4	2.3 ± 1.1	9.3 ± 1.6	N.D.	117.6 ± 17.9	98.3 ± 22.6
PL	3.3 ± 0.0	2.8 ± 1.0	1.2 ± 0.7	2.7 ± 0.3	0.1 ± 0.0	119.1 ± 35.9	100.6 ± 38.3
lesspolar	7.8 ± 0.7	4.1 ± 0.2	3.7 ± 0.7	3.0 ± 0.3	0.2 ± 0.0	100.7 ± 13.7	78.3 ± 8.7
neutral	12.5 ± 0.8	10.2 ± 0.3	2.9 ± 0.8	9.4 ± 1.0	0.6 ± 0.0	104.8 ± 9.3	97.1 ± 10.3
polar	13.3 ± 0.4	12.2 ± 1.7	3.6 ± 1.3	12.0 ± 1.9	0.1 ± 0.0	118.0 ± 16.6	99.0 ± 20.8
TOTAL	33.6 ± 1.1	26.5 ± 1.8	10.1 ± 1.7	24.3 ± 2.2	0.9 ± 0.1	109.1 ± 8.1	95.0 ± 10.3

Table D5 Lipid class concentration (μg/L, mean ± S.D., n = 6–10) and distribution percentage (%) in different particle pools and dissolved fraction of seawater collected from Conception Bay on May 19, 1993

Lipid Classes	Concentration (μg/L)				Percentage (%)		
	0.45μm–75μm	3nm–0.45μm	"Dissolved" (<3nm)	Total	0.45μm–75μm	3nm–0.45μm	"Dissolved" (<3nm)
HC	0.2 ± 0.3	0.1 ± 0.0	1.2 ± 0.3	1.5 ± 0.4	13.7 ± 18.4	5.8 ± 2.2	80.5 ± 28.1
SE	0.0 ± 0.1	0.0 ± 0	0.3 ± 0.1	0.4 ± 0.1	13.9 ± 17.1	5.6 ± 3	80.5 ± 25.4
KET	0.0 ± 0.0	0.1 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	3.5 ± 1.4	6.4 ± 1.0	90.1 ± 11.9
TAG	1.7 ± 2.5	0.1 ± 0.8	27.1 ± 9.8	28.8 ± 10.2	5.8 ± 9	0.4 ± 2.9	93.8 ± 47.6
FFA	0.3 ± 0.1	0.8 ± 0.1	5.0 ± 0.7	6.1 ± 0.7	5.5 ± 1.7	12.5 ± 2.5	82.0 ± 14.4
ALC	0.1 ± 0.2	0.0 ± 0.0	0.7 ± 0.2	0.8 ± 0.2	11.3 ± 22.0	1.1 ± 2.2	87.6 ± 33.1
ST	0.1 ± 0.2	0.1 ± 0.1	1.8 ± 0.3	2.0 ± 0.4	4.4 ± 9.0	6.5 ± 3.2	89.1 ± 22.8
AMPL	0.5 ± 0.2	0.0 ± 0	30.0 ± 3.5	30.5 ± 3.5	1.7 ± 0.8	N.D.	98.3 ± 16.2
PL	0.5 ± 0.4	0.2 ± 0.0	2.6 ± 0.5	3.3 ± 0.6	14.0 ± 10.9	6.3 ± 1.7	79.7 ± 21.0
Lesspolar	0.3 ± 0.3	0.2 ± 0.0	2.3 ± 0.3	2.7 ± 0.4	10.6 ± 10.5	6.0 ± 1.3	83.5 ± 16.8
Neutral	2.2 ± 2.6	1.0 ± 0.8	34.5 ± 9.9	37.7 ± 10.2	5.8 ± 6.9	2.7 ± 2.3	91.5 ± 36.1
Polar	1.0 ± 0.4	0.2 ± 0.3	32.7 ± 3.5	33.9 ± 3.6	2.9 ± 1.3	0.6 ± 0.9	96.5 ± 14.6
Total	3.5 ± 2.6	1.4 ± 0.9	69.5 ± 10.5	74.3 ± 10.9	4.7 ± 3.6	1.8 ± 1.2	93.5 ± 19.7

Table D6 Lipid class concentration (μg/L, mean ± S.D., n = 6–10) and distribution percentage (%) in different particle pools and dissolved fraction of seawater collected from Conception Bay on June 24, 1993

Lipid Classes	Concentration (μg/L)				Percentage (%)		
	0.45μm–75μm	3nm–0.45μm	"Dissolved" (<3nm)	Total	0.45μm–75μm	3nm–0.45μm	"Dissolved" (<3nm)
HC	0.1 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	1.1 ± 0.1	5.9 ± 10.9	18.1 ± 2.7	75.9 ± 13.1
SE	N.D.	N.D.	0.2 ± 0.1	0.2 ± 0.1	N.D.	N.D.	100.0 ± 41.8
KET	0.0 ± 0.0	0.1 ± 0.0	0.9 ± 0.1	1.1 ± 0.1	1.4 ± 1.5	10.6 ± 1.9	88.0 ± 13.8
TAG	0.1 ± 1.4	3.6 ± 0.1	21.1 ± 1.0	24.9 ± 1.8	0.5 ± 6	14.6 ± 1.2	84.9 ± 7.3
FFA	0.4 ± 0.1	3.3 ± 0.9	6.5 ± 0.8	10.2 ± 1.2	3.7 ± 1.3	32.8 ± 9.7	63.5 ± 11.0
ALC	N.D.	N.D.	1.9 ± 0.5	1.9 ± 0.5	N.D.	N.D.	100.0 ± 38.1
ST	0.0 ± 0.1	0.1 ± 0.1	2.1 ± 0.2	2.2 ± 0.2	2.1 ± 2.5	3.4 ± 3.1	94.5 ± 11.5
AMPL	0.3 ± 0.5	0.2 ± 0	37.3 ± 2.9	37.9 ± 3.0	0.8 ± 1.4	0.6 ± 1	98.6 ± 10.8
PL	0.2 ± 0.2	1.2 ± 0.2	8.0 ± 1.6	9.3 ± 1.6	2.3 ± 1.8	12.4 ± 3.2	85.3 ± 22.3
Less polar	0.1 ± 0.1	0.3 ± 0.0	2.0 ± 0.2	2.4 ± 0.2	3.4 ± 5.1	13.2 ± 1.4	83.4 ± 9.4
Neutral	0.6 ± 1.4	7.0 ± 0.9	31.6 ± 1.4	39.2 ± 2.2	1.4 ± 3.7	18.0 ± 2.5	80.6 ± 5.8
Polar	0.5 ± 0.6	1.4 ± 0.5	45.3 ± 3.3	47.2 ± 3.4	1.1 ± 1.2	2.9 ± 1.0	96.0 ± 9.7
Total	1.1 ± 1.6	8.7 ± 1.0	78.9 ± 3.6	88.7 ± 4.0	1.3 ± 1.8	9.8 ± 1.2	88.9 ± 5.7

Table D7 Lipid class concentration (μg/L, mean ± S.D., n = 6–10) and distribution percentage (%) in different particle pools and dissolved fraction of seawater collected from Conception Bay on August 2, 1993

Lipid Classes	Concentration (μg/L)				Percentage (%)		
	0.45μm–75μm	3nm–0.45μm	"Dissolved" (<3nm)	Total	0.45μm–75μm	3nm–0.45μm	"Dissolved" (<3nm)
HC	0.1 ± 0.1	0.4 ± 0.1	1.3 ± 0.1	1.9 ± 0.2	7.2 ± 4.1	22.2 ± 5.4	70.7 ± 8.7
SE	0.0 ± 0.0	0.1 ± 0	0.5 ± 0.0	0.6 ± 0.1	8.4 ± 8.4	12.3 ± 5	79.3 ± 11.1
KET	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	1.0 ± 0.1	7.3 ± 3.2	16.1 ± 3.3	76.6 ± 12.9
TAG	0.0 ± 0.6	1.4 ± 0.5	8.6 ± 0.2	10.0 ± 0.8	N.D.	14.4 ± 5.1	85.6 ± 7.2
FFA	0.3 ± 0.1	0.6 ± 0.3	12.4 ± 1.1	13.3 ± 1.1	1.9 ± 1.0	4.9 ± 2.1	93.2 ± 11.6
ALC	0.0 ± 0.1	0.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	3.5 ± 4.2	6.9 ± 4.3	89.6 ± 14.6
ST	0.1 ± 0.1	0.2 ± 0.1	1.3 ± 0.2	1.6 ± 0.2	4.8 ± 3.8	11.7 ± 5.2	83.4 ± 18.8
AMPL	0.5 ± 1.0	0.5 ± 1	19.7 ± 0.6	20.8 ± 1.5	2.5 ± 5.0	2.5 ± 4	95.1 ± 7.4
PL	0.1 ± 0.0	0.0 ± 0.1	1.7 ± 0.3	1.9 ± 0.3	5.6 ± 2.0	2.6 ± 2.8	91.5 ± 19.2
Lesspolar	0.3 ± 0.1	0.6 ± 0.1	2.6 ± 0.2	3.5 ± 0.2	7.4 ± 2.8	18.7 ± 3.2	73.9 ± 6.3
Neutral	0.4 ± 0.6	2.4 ± 0.6	23.5 ± 1.1	26.3 ± 1.4	1.4 ± 2.4	9.0 ± 2.3	89.5 ± 6.6
Polar	0.6 ± 1.0	0.6 ± 0.8	21.4 ± 0.7	22.6 ± 1.5	2.7 ± 4.5	2.5 ± 3.7	94.8 ± 6.9
Total	1.3 ± 1.2	3.6 ± 1.0	47.5 ± 1.3	52.4 ± 2.1	2.4 ± 2.3	6.8 ± 2.0	90.8 ± 4.4

Table D8 Lipid class concentration (μg/L, mean ± S.D., n = 6–10) and distribution percentage (%) in different particle pools and dissolved fraction of seawater collected from Conception Bay on October 5, 1993

Lipid Classes	Concentration (μg/L)				Percentage (%)		
	1.0μm–75μm	3nm–1.0μm	"Dissolved" (<3nm)	Total	1.0μm–75μm	3nm–1.0μm	"Dissolved" (<3nm)
HC	2.8 ± 0.7	0.1 ± 0.0	1.7 ± 0.3	4.7 ± 0.8	60.9 ± 18.5	2.9 ± 0.7	36.2 ± 8.0
SE	0.2 ± 0.1	N.D.	0.4 ± 0.1	0.5 ± 0.2	30.0 ± 25.1	N.D.	70.0 ± 25.8
KET	0.7 ± 0.1	0.1 ± 0.0	0.9 ± 0.2	1.7 ± 0.2	42.3 ± 9.8	4.6 ± 0.7	53.1 ± 12.6
TAG	1.3 ± 0.7	0.3 ± 0.0	3.4 ± 0.2	4.9 ± 0.7	25.5 ± 14	5.5 ± 0.9	69.0 ± 10.4
FFA	0.5 ± 0.3	0.2 ± 0.0	3.3 ± 0.4	3.9 ± 0.5	11.6 ± 7.7	4.2 ± 0.6	84.3 ± 15.1
ALC	0.4 ± 0.1	0.0 ± 0.0	1.0 ± 0.3	1.4 ± 0.3	27.2 ± 7.9	3.1 ± 0.8	69.8 ± 23.8
ST	0.8 ± 0.2	0.1 ± 0.0	1.7 ± 0.9	2.6 ± 0.9	30.3 ± 13.4	3.4 ± 1.2	66.3 ± 40.4
AMPL	2.3 ± 1.1	N.D.	9.3 ± 1.6	11.5 ± 2.0	19.6 ± 10.3	N.D.	80.4 ± 19.8
PL	1.2 ± 0.7	0.1 ± 0.0	2.7 ± 0.3	4.0 ± 0.7	30.5 ± 17.6	2.1 ± 1.1	67.5 ± 15.1
Lesspolar	3.7 ± 0.7	0.2 ± 0.0	3.0 ± 0.3	6.9 ± 0.8	53.9 ± 12.5	3.1 ± 0.5	43.0 ± 6.9
Neutral	2.9 ± 0.8	0.6 ± 0.0	9.4 ± 1.0	12.8 ± 1.3	22.4 ± 6.3	4.4 ± 0.5	73.2 ± 10.7
Polar	3.5 ± 1.3	0.1 ± 0.0	12.0 ± 1.7	15.6 ± 2.1	22.4 ± 8.9	0.5 ± 0.3	77.0 ± 15.0
Total	10.1 ± 1.7	0.9 ± 0.1	24.3 ± 2.0	35.3 ± 2.6	28.6 ± 5.2	2.4 ± 0.2	69.0 ± 7.6

Table D9 Lipid composition percentage in different particle pools, dissolved fraction, and total lipids in seawater collected from Conception Bay, Newfoundland, on May 19, 1993

Lipid Classes	0.45μm–75μm	3nm–0.45μm	Dissolved <3nm	Total
HC	6.1 ± 9.2	6.7 ± 4.5	1.8 ± 0.5	2.1 ± 0.8
SE	1.4 ± 2.0	1.5 ± 1.1	0.4 ± 0.1	0.5 ± 0.1
KET	0.9 ± 0.7	4.1 ± 2.6	1.1 ± 0.2	1.2 ± 0.2
TAG	48.7 ± 81.8	7.8 ± 60.0	39.4 ± 15.4	39.3 ± 14.9
FFA	9.8 ± 7.8	57.9 ± 37.0	7.3 ± 1.5	8.3 ± 1.5
ALC	2.6 ± 5.3	0.7 ± 1.3	1.0 ± 0.3	1.1 ± 0.3
ST	2.6 ± 5.4	9.6 ± 7.3	2.6 ± 0.6	2.7 ± 0.6
AMPL	15.4 ± 13.4	N.D.	43.7 ± 8.3	41.6 ± 7.7
PL	13.5 ± 14.3	15.9 ± 10.3	3.9 ± 0.9	4.5 ± 1.1
lesspolar	7.5 ± 10.3	8.2 ± 7.9	2.2 ± 0.7	2.6 ± 0.8
neutral	63.6 ± 87.6	75.9 ± 76.7	50.2 ± 16.1	51.3 ± 15.6
polar	28.9 ± 24.8	15.9 ± 23.9	47.6 ± 8.8	46.1 ± 8.2
Total	100.0 ± 91.6	100.0 ± 80.7	100.0 ± 18.3	100.0 ± 17.7

Table D10 Lipid composition percentage in different particle pools, dissolved fraction, and total lipids in seawater collected from Conception Bay, Newfoundland, on June 24, 1993

Lipid Classes	0.45μm–75μm	3nm–0.45μm	Dissolved <3nm	Total
HC	5.8 ± 13.1	2.3 ± 0.3	1.1 ± 0.1	1.3 ± 0.2
SE	N.D.	N.D.	0.3 ± 0.1	0.2 ± 0.1
KET	1.4 ± 2.3	1.3 ± 0.2	1.2 ± 0.2	1.2 ± 0.1
TAG	11.7 ± 127.6	42.1 ± 5.1	27.1 ± 1.8	28.4 ± 2.4
FFA	33.7 ± 46.9	38.7 ± 11.2	8.3 ± 1.1	11.6 ± 1.5
ALC	N.D.	N.D.	2.4 ± 0.7	2.2 ± 0.6
ST	4.1 ± 7.2	0.8 ± 0.8	2.6 ± 0.2	2.5 ± 0.2
AMPL	25.4 ± 58.4	2.7 ± 4.7	47.9 ± 4.2	43.2 ± 3.9
PL	18.8 ± 29.1	13.4 ± 2.9	10.2 ± 2.0	10.7 ± 1.9
lesspolar	5.8 ± 14.3	2.3 ± 0.5	1.3 ± 0.2	1.5 ± 0.3
neutral	50.0 ± 143.7	81.6 ± 14.1	40.5 ± 2.6	44.7 ± 3.2
polar	44.2 ± 77.6	16.1 ± 5.6	58.2 ± 4.9	53.8 ± 4.5
Total	100.0 ± 163.9	100.0 ± 15.2	100.0 ± 5.5	100.0 ± 5.5

Table D11 Lipid composition percentage in different particle pools, dissolved fraction, and total lipids in seawater collected from Conception Bay, Newfoundland, on August 2, 1993

Lipid Classes	0.45μm–75μm	3nm–0.45μm	Dissolved <3nm	Total
HC	11.4 ± 11.9	12.1 ± 4.2	2.8 ± 0.3	3.6 ± 0.3
SE	4.1 ± 5.4	2.1 ± 1.0	1.0 ± 0.1	1.1 ± 0.1
KET	6.3 ± 6.2	4.8 ± 1.5	1.7 ± 0.2	2.0 ± 0.2
TAG	N.A.	42.2 ± 18.0	18.3 ± 0.7	19.5 ± 1.7
FFA	21.3 ± 22.0	19.0 ± 9.3	26.5 ± 2.4	25.9 ± 2.4
ALC	4.2 ± 6.1	2.9 ± 1.9	2.7 ± 0.3	2.8 ± 0.3
ST	6.3 ± 7.4	5.3 ± 2.6	2.8 ± 0.5	3.0 ± 0.5
AMPL	43.5 ± 90.9	15.0 ± 23.4	42.3 ± 1.8	40.5 ± 3.2
PL	9.1 ± 8.7	1.4 ± 1.5	3.6 ± 0.6	3.6 ± 0.5
lesspolar	15.5 ± 21.2	14.2 ± 5.9	3.8 ± 0.4	4.8 ± 0.5
neutral	31.9 ± 58.2	69.4 ± 24.7	50.3 ± 2.8	51.1 ± 3.4
polar	52.6 ± 94.8	16.4 ± 23.5	45.9 ± 1.9	44.1 ± 3.3
Total	100.0 ± 113.2	100.0 ± 34.8	100.0 ± 3.4	100.0 ± 4.8

Table D12 Lipid composition percentage in different particle pools, dissolved fraction, and total lipids in seawater collected from Conception Bay, Newfoundland, on October 5, 1993

Lipid Classes	1.0μm–~75μm	3nm–~1.0μm	Dissolved <3nm	Total
HC	30.4 ± 8.6	16.6 ± 3.1	7.2 ± 1.2	13.9 ± 2.4
SE	1.7 ± 1.3	N.D.	1.6 ± 0.4	1.7 ± 0.5
KET	7.8 ± 1.7	9.7 ± 0.9	3.9 ± 0.8	5.1 ± 0.7
TAG	13.5 ± 6.9	33.7 ± 3.4	14.6 ± 1.4	14.7 ± 2.2
FFA	4.8 ± 3.0	20.1 ± 1.8	14.1 ± 2.0	11.6 ± 1.6
ALC	4.0 ± 1.0	5.3 ± 1.0	4.1 ± 1.1	4.1 ± 0.8
ST	8.3 ± 2.5	10.8 ± 1.4	7.3 ± 3.6	7.7 ± 2.6
AMPL	24.2 ± 11.7	N.D.	39.6 ± 7.4	34.3 ± 6.1
PL	13.0 ± 6.9	10.3 ± 4.8	11.5 ± 1.6	11.9 ± 2.3
lesspolar	32.2 ± 9.7	19.9 ± 3.5	8.9 ± 1.7	15.6 ± 2.7
neutral	30.7 ± 8.9	69.8 ± 5.4	40.1 ± 5.2	38.2 ± 4.5
polar	37.2 ± 14.1	10.3 ± 4.8	51.1 ± 7.9	46.2 ± 6.8
Total	100.0 ± 19.3	100.0 ± 8.0	100.0 ± 9.6	100.0 ± 8.6

Table D13 Lipid class concentration ($\mu g/L$, mean ± S.E.M, n = 3) in different filtration fractions of seawater collected in Conception Bay, Newfoundland on May 19, June 14, August 2, 1993 and mass balance (%) on two filters

Lipid Classes	Concentration ($\mu g/L$)					Mass balance (%)	
	75μm−F	0.45μm−F	0.45μm−R	10k−F	10K−R	0.45μm filter	10K filter
HC	2.6 ± 0.8	1.7 ± 0.4	0.1 ± 0.1	1.1 ± 0.2	0.2 ± 0.1	74.5 ± 10.8	84.8 ± 26.2
SE	0.4 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	82.8 ± 9.1	108.4 ± 10.8
KET	0.9 ± 0.1	0.8 ± 0.1	0.0 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	94.4 ± 3.9	120.3 ± 3.9
TAG	28.0 ± 2.9	30.8 ± 4.4	0.9 ± 0.8	24.1 ± 3.0	0.8 ± 0.7	112.9 ± 1.6	86.7 ± 16.4
FFA	5.8 ± 0.1	5.2 ± 0.4	0.3 ± 0.1	5.7 ± 0.7	0.7 ± 0.1	94.2 ± 5.4	138.7 ± 19.6
ALC	1.2 ± 0.3	0.8 ± 0.1	0.0 ± 0.0	1.3 ± 0.5	0.0 ± 0.0	76.2 ± 15.2	132.7 ± 22.5
ST	2.2 ± 0.6	1.8 ± 0.4	0.1 ± 0.0	1.7 ± 0.3	0.1 ± 0.0	89.5 ± 9.4	103.3 ± 10.6
AMPL	28.9 ± 8.2	28.2 ± 8.2	0.4 ± 0.1	29.0 ± 7.2	0.2 ± 0.2	100.9 ± 7.9	102.8 ± 10.8
PL	6.7 ± 4.3	5.2 ± 3.8	0.3 ± 0.1	4.1 ± 2.8	0.5 ± 0.5	78.9 ± 4.0	88.5 ± 8.5
lesspolar	3.9 ± 0.8	2.8 ± 0.5	0.2 ± 0.1	2.3 ± 0.2	0.4 ± 0.2	78.9 ± 7.3	96.3 ± 16.1
neutral	37.8 ± 3.3	38.9 ± 5.2	1.0 ± 0.8	29.9 ± 4.7	3.5 ± 2.6	106.3 ± 2.1	96.5 ± 8.8
polar	35.6 ± 12.5	33.4 ± 10.0	0.7 ± 0.2	33.1 ± 9.8	0.7 ± 0.5	97.5 ± 6.3	101.6 ± 11.8
TOTAL	81.8 ± 17.1	79.2 ± 15.3	2.0 ± 1.1	65.3 ± 13.1	4.6 ± 3.1	100.3 ± 3.6	101.8 ± 9.1