Bibliothèque nationale du Canada

Services des thèses canadiennes

# CANADIAN THESES

National Library

Ottawa,/Canada K1A QN4

Canadian Theses Service

of Canada

# The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming Every effort has been made to ensure the highest quality of reproduction possible.

NOTICE

If pages are missing, contact the university which granted the degree

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

# THÈSES CANADIENNES

# AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage Nous avons tout fait pour assurer une qualité supérieure de reproduction

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aíde d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure

Les documents gui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30

# THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE



DISSOLVED AND PARTICULATE' LIPID CLASSES IN THE AQUATIC ENVIRONMENT Christopher C. Parrish Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy di P at Dalhousie University March, 1986,

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the theses nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission. L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de, vendré des exemplaties du film.

L'auteur (titulaire du droit. d'auteur) se réserve les autres droits de publication; ni lá thèse ni de longs extraits de celle-ci ne doivent être, imprimés ou autrement reproduits sans son autorisation écrite.

ISBN@ 0-315-33061-9

**Lesli**e

iv

# TABLE OF CONTENTS

ix

xi

xi1i

xiv

xvi

List of Figures

List of Tables Abstract

Abbreviations, symbols, and formulae

Acknowledgements

GENERAL INTRODUCTION

Chapter 1

# A SURVEY OF THE LITERATURE PERTAINING TO DISSOLVED AND PARTICULATE MARINE LIPID CLASSES

	1.1 Introduction	. 4
	1.2 Dissolved and particulate matter	7
	1 3 Herdrogorhong	17
	1.3.1. Bisson the descention	17
	1.3.1 Biogenic hydrocarbons	1/
	1.3.2 Hydrocarbon pollution	19
	1.3.3 Dissolved and particulate marine hydrocarbons	20
	5 te 41	
	1.4 Free and esterified fatty acids	22
	1.4.1 Analytical techniques .,	32
	1.4.2 Total fatty acids	23
	41.4.3 Free fatty acids	25
	1.4.4 Wax esters and sterol esters	26.
	1.4.5 Short-chain esters	28
	1.4.6 Acvlated olycervl ethers	.29
۲	1 4 7 Triglycerides diglycerides and monogly Tarides	30
	1.4.7 Chlorenlack ligida	27
	1.4,0 Ontotoptast lipius	34
		34
	L.J Ketones	34
		<b>.</b>
	1.6 Phthalic acid esters ,	35
		1
	1.7 Free aliphatic alcohols	36
	1.8 Conclusion	39
	•	

#### TABLE OF CONTENTS - II

'Chapter

# SEPARATION OF AQUATIC LIPID CLASSES BY CHROMAROD THIN-LAYER CHROMATOGRAPHY WITH DETECTION AND MEASUREMENT BY THE IATROSCAN FLAME, IONIZATION DETECTOR

2.1 Introduction		8	41
· · · · · · · · · · · · · · · · · · ·	я <sup>4</sup> .	£	• •
2.2 Methods		*********	45
2.2.1 Sample workup		•• • • • • • • • • • • •	45 <sub>°N</sub>
2,2.2 Analytical apparatus and operating condit	tions		57
2.2.3 Ständards and calibration			58
2.2.4 Standard and sample application,		*********	65
2.2.5 Chromarod developments		*********	66 *
2.2.6 Chromarod conditioning and cleaning			66
	- 1		•
2.3 Results and discussion		• • • • • • • • • •	, 71
2.3.1 Extraction and average of seguritar limits	•	1 ,	72

2.3.2	Chromarod ser	parations and	part	ial scar	nning .			* * * * *	'7
2.3.3	Quantitative	determination	of	aquatic	lipid	classes			8
		•					1	۸ ک	•

2.4 Conclusions ...

# Chapter 3

ĥ

# - PARTICULATE AND DISSOLVED LIPID CLASSES IN CULTURES OF <u>Phaeodacty1um tricornutum</u> GROWN IN CAGE CULTURE TURBIDOSTATS WITH A RANGE OF SUPPLY RATES OF INORGANIC NITROGEN

3.1 Introduction			• •		
3.2 Methods	, , , , , , , , , , , , , , , , , , ,		• • • • • • • • • • • •		
3.3 Results	· ·		*°°	109	
3.4 Discussion	۔ دور کی کی کو	• •	• • • • • • • • • •		,
3.4.1 Intracellular lip: 3.4.2 Dissolved lipid p	id classes, roduction rate	,	• • • • • • • • • • • •	135 138	•
3.5 Summary and Conclus	Íon		, , , , , , , , , , , , , , , , , , , ,	1,39	

ŕv

# TABLE OF CONTENTS ÷ 111

# Chapter 4

# TIME-SERIES OF DISSOLVED AND PARTICULATE LIPID CLASSES DURING SPRING PHYTOPLANKTON BLOOMS IN BEDFORD BASIN, A MARINE INLET

•	•		· ·	• <sup>°</sup>	,	" , V
4.1 Introduct	10n	••••		; 		141
		0	•	*	*	110
4.2.1 Spring	bloom, 1982		· • • • • • • • • • •	• • • • • • •	* * * * * * * * * * * * * * * * * * *	140
4.2.2\Spring	bloom, 1984			· · · · · · · · · · · · ·		151
	6 <b>6</b>	a 		- 1.	۰.	150
4.3 Results a	nd Discussio	)n		• • • • • • • • •	* * * * * * * * * *	152
4.3.2 Spring	bloom, 1982			· •,• • • • • • • • • • •	••••••••••••••••••••••••••••••••••••••	164
4.3.3 Lipids	before and a	fter the cl	nlorophyĺj	a maximum	1   0 • • • • • • • • • • • • •	178
4.3.4 Lipids	and inorgani	ic nitrate	• • • • • • • • • • •		~ ************************************	181
4.4 Conclusio		د • • • • • • • • • • •	s===================================	• • • • • • • • • •	، پې د • • • • • • • • • • •	184

# "Chapter 5

# VERTICAL PROFILES OF DISSOLVED AND PARTICULATE AQUATIC LIPID CLASSES IN SURFACE WATERS OVER THE SCOTIAN SLOPE, IN BEDFORD BASIN,

ά .	AND "IN	LAKE	HURÔN	٠	1	•	u .	_ L _
5-1 Introduction		c						186
,	ر د			a			e	
5.2 Methods					• • •, •	• • • • • • • •	· · · · · · · ·	,188
5.2.1 Scotian Slope		•••••	* * * * * * *	•••	• • • • •	•••••		188
5.2.3 Lake Huron	• • <b>E</b> • • • •	, , , , , , , , , , , , , , , , , , ,			* * * * *	• • • • • • • •		192
	1		' <b>、</b>		ş	•		1.02
5.3 Results and Discussion		· · · · · · ·		*** * *	а <sub>р</sub> а а с	• • • • • • • • •		195 195 ·
5.3.2 Bedford Basin				• • • •			64 8 <b>8 9 8 8 8 4</b>	221
5.3.3 Lake Huron	• • • • • • • •			• • •	• • • •			222
5.4 Conclusion	. • ( • • • • • • • •			<b></b>				224

vii



# LIST OF FIGURES

<ul> <li>1.1 The principal lipid classes of seawater and the principal</li> <li>representatives of those classes</li></ul>	, 9
2.1 Marine lipid class chromatograms from extracts of operationally defined dissolved and particulate matter	y 48
2.2 Lipid class chromatograms from sediment trap and invertebrate samples	<sup>51</sup>
<ul> <li>2.3 A comparison of gravimetrically determined lipid weights</li> <li>with the sum of the latroscan-measured classes</li></ul>	53
2.4 One-step development and scań of marine zooplankton lipid	55
. 2.5 Circuitry for scan interruptors	59
2.6 Iatroscan calibration data for nine different lipid clagses	ģ1
3.1 Schematic representation of phytoplankton culturing systems	99
3.2 Histograms of the nitrogen budget for cage cultume turbidostats	1 Ó7
3.3 TLC/FID chromatograms of particulate lipids from cells in cage cultures of <u>Phaeodactylum tricornutum</u>	14 I
3.4 TLC/FID chromatograms of dissolved lipids in the ingoing medium and the effluents from cage cultures	117
3.5 Growth rate and lipid per cell as a function of the rate of nitrogen use per cell	122 <sup>°</sup>
3.6 Lipid classes as a function of the rate of nitrogen use per cell	124
3.7 Lipid production per cell as a function of mitrogen use per cell	126 <sup>°</sup>
3.8 Lipid per unit chlorophyll <u>a</u> as a function of nitrogen concentration	128
3.9 Lipid classes as a function of growth rate	130

ix

# LIST OF FIGURES - II

4.1 Sampling stations in Bedford Bay, Bedford Basın and Halifar	x
Harbour	. 148
4.2 Spring bloom 1982; chlorophyll, nutrient and total lipic	đ
concentrations at the bottom of the surface mixed layer in	n .
Bedford Basin	. 155
4.3 Spring bloom 1982; dissolved and particulate lipid class	s
concentrations at the bottom of the surface mixed layer in	n
Bedford Basin ',	• 157
4.4 Spring bloom 1984; chlorophyll, carbon and nutrient	t
concentrations at 3 m and 5 m in Bedford Basin	, 166
4.5 Spring bloom 1984; particulate lipid concentrations at 3 m	n ° °
and 5 m in Bedford Basin	. 170
4.6 Dissolved and particulate lipids from two blooms and three	∍
stations	174
•4.7 Lipids versus dissolved inorganic nitrate concentrations for	r
the 1982 and 1984 spring bloom samples	. 176
<ul> <li>5.1 Station locations. (1) Scotian Slope, 1985; (2) Scotian</li> <li>Slope, 1982; (3) Bedford Basin, 1984; (4) Lake Huron, 1984</li> </ul>	1 , 190
5.2 Vertical profiles of density and total lipid concentrations	₃ ~~
over the Scotian Slope in 1982	, 198
5.3 Vertical profiles of the concentrations of particulate and	1
dissolved lipid classes over the Scotian Slope in 1982	200
5.4 Vertical profiles of density and concentrations of phosphate	è
and lipids over the Scotian Slope in 1985	, 210
5.5 Ve cal profiles of salinity, temperature and total lipids	\$
in Bedford Basin in 1984	, 212 ~
5.6 Vertical profiles of the concentrations of particulate and	1
dissolved lipid classes in Bedford Basin in 1984	.214 ,
5.7 Vertical profiles of the ratio of particulate lipid classes to particulate organic carbon in Bedford Basin in 1984	216
5.8. Vertical profiles ot the ratio of particulate lipid classes to particulate organic carbon in Lake Huron in 1984	218
X	

# S LIST OF TABLES

- <sup></sup>	2.1 Standards used routinely for identification and calibration of aquatic lipid classes in the latroscan	5 <b>1</b>
•	2.2 Developing and conditioning sequences used routinely for the separation of aquatic lipid classes on Chromarods	52 .
	2.3 Model compounds representing aquatic lipid classes that can be resolved in a three-step separation	53 <sup>°</sup>
4	<ul> <li>2.4 Average precision of integrator areas obtained from scanning</li> <li>0.4 to 20.0 μg of compounds with low FID responses</li> </ul>	່ : 69 ຼ
3	2.5 Average precision of integrator areas obtained from scanning 0.4 to 20.0 µg of compounds with high FID responses	، ہ 70°
ay t	3.1 Non-volatile organic classes detected at the pg/cell level and at the µg/l level in extracts of particulate and digsolved matter from cage culture turbidostats	08
	3.2 Pearson product moment linear correlation coefficients for the complete range of nutrient conditions offered	13
	3.3 Lipid class composition of nitrogen-replete P. tracornutum.	14
•	3.4 Percentage lipid class composition of <u>P. tricornutum</u> in chaine form	15
مۇرىيە ئ	3.5 Production rates of particulate and dissolved lipid classes 1	19:
10	4.1 Dissolved and particulate lipid class concentrations and proportions at the bottom of the surface mixed layer during the 1982 Bedford Basin spring bloom	,, 5 <b>2</b> ,
	4.2 Pearson product moment linear correlation coefficients for lipid class concentrations during the 1982 spring bloom 1	63.
, 9	4.3 Particulate lipid and nutrient data from before and after the maximum measured chlorophyll a concentrations at the centre of Bedford Basin in 1982 and 1984 1	80
	· · · · · · · · · · · · · · · · · · ·	*
, ,		

xi

# LIST OF TABLES - II

ł

ن ب ب	5.1 Lipid class concentrations in dissolved matter and suspended particulate matter from Bedford Basin and near the edge of the Scotian Shelf, and in particulate matter from sediment traps deployed in Lake Huron	194
		<b></b> ,
	5.2 Dissolved marine lipids from profiles in or near shelf	· 207 -
		494.7
	5.3 Particulate marine lipids from profiles in or near shelf	
	regions	203
	A.1 The distribution of particulate lipids during the 1984 spring bloom	236
	A:2 The distribution of dissolved lipids during the 1984 spring	

The distribution of dissolved lipids during the 1984 spring

xií

ABSTRACT

Lipids are composed primarily of atoms of carbon and hydrogen. They can be subdivided into several classes according to their structure. Most classes have known functions in cells or known anthropogenic sources. Groups of classes can thus be used to indicate the presence of certain types of organisms and their physiological state. Other classes can provide a strong signal of lipid degradation or pollution.

The measurement of the complete suite of lipid classes in both dissolved and particulate matter in this thesis is a new, informative approach to research into oceanographic processes. A new comprehensive analytical technique was used: Chromarod thin-layer chromatography (TLC) with latroscan flame ionization detection (FID). Development schemes were designed for maximal separation of marine lipid classes on Chromarods. Procedures were also elaborated to optimise the detection and measurement of lipid classes in the latroscan: An intercalibration showed that FID measurements were close to true gravimetric values.

The principal environmental processes studied were those occurring during spring phytoplankton blooms and those occurring at pycnoclines. Laboratory culture data were collected under carefully controlled conditions and several time-series and profiles of field data were also collected in Bedford Basin, over the Scotian Slope and in Lake Huron.

The production rates of intracellular and extracellular algal lipid classes were examined as a function of the rate of supply of nitrogen. A new system was used to provide a highly controlled algal environment: the cage culture turbidostat. The intracellular synthesis of a storage component, triglyceride, was triggered by nitrogen stress; under these conditions other lipid classes were released extracellularly.

The results of the culture studies reflected observations made during phytoplankton blooms in Bedford Basin. Triglyceride became the major lipid class measured in particulate matter from the time of the chlorophyll maximum onwards. This class was also observed to have maximal values in the vicinity of pycnoclines in a variety of environments. The temporal and spatial location of this energy-rich storage component may reflect a survival mechanism used by autotrophs and is important to the metabolism of heterotrophs.

Dissolved lipid class data indicate decreased exudation and increased "bacterial uptake at the time of the chlorophyll maximum during blooms. There is also evidence for the uptake of dissolved phospholipids by algae. These lipids could be used by autotrophs as a source of nitrogen and phosphorus when inorganic nutrient levels are depleted.

	ABBREVIATIONS, SYMBOLS AND FORMULAE
	لن من ۲ <sup>۲</sup> من من ۲ من ۲ من
ALC -	Free aliphatic alcohol.
AMPL	Åcetone-mobile polar lipids.
CHC13	Chloroform.
$CH_2C1_2$	Dichloromethane, methylene chloride, methylene dichloride.
CHL	Chlorophyll a
DG	Diglyceride.
FFA	Free fatty acid.
FID o	Flame ionization detector.
GC	Gas chromatography.
GE	Acylated glyceryl ether.
HC	Hýdrocarbon.
HPLC	High performance liquid chromatography.
KET •	Ketone.
μΕ	Micro-Einstein.
, µg 、	Microgramme.
μ1 ື	"Microlitre.
• ME '	Methyl ester.
MeOH	Methanol.
<u>n</u> -	Normal, unbranched
<u>n</u> -C <sub>n:x</sub>	Unbranched lipid chain with n carbon atoms and x double bonds. This is a combination of traditional systems used by lipid chemists (Gurr and James, 1980) and is similar to that used by Saliot (1981) for marine hydrocarbons.

ß

xiv

ABBREVIATIONS, SYMBOLS AND FORMULAE - II

Polycyclic aromatic hydrocarbon.

÷2.

(Density - 1) X 1.000.

PAH

pg 🖏

PL

 $\sigma_{t}$ 

TG

TLC

∕ ,₩E

Triglyceride. Thin-layer chromatography.

Wax ester.

7, \* , \* \* \*

, . , . , .

ب ب

é ©

. XV

Ð

KNOWL MENTS

I thank the members of my committee headed by Dr. P.J. Wangersky. for their interest and support in this endeavour. Drs. Ackman; Moore, Ragan, and Wangersky have provided useful comments and friticisms<sup>1</sup> throughout the course of my research and writing.

'I am grateful to Dr. R.Q. Fournier for the opportunity to participate in a Scotian Shelf cruise in 1982 and for the use of chlorophyl'l, temperature and salinity data. R.P. Delmas shared the experience of working in a shipboard lipid laboratory on that cruise, and was also responsible for the analysis of the samples we brought back. pr. M.R. Lewis permitted me to use some of his water samples from the 1982 Bedford Basin spring bloom study; he also made his nutrient and chlorophyl'l data available to me. Dr. W.S. Gardner extracted the Laurentian Great Lakes samples and Dr. K. Dunham provided the organic carbon data. Thanks are also due to the captain, officers and crew of the C.S.S. <u>Dawson</u>, the M.V. <u>Sigma T</u> and the M.V. Tudlik.

Financial support came from grants from the Natural Sciences and Engineering Research Council of Canada to P.J. Wangersky and R.G. Ackman. I am also grateful for support by Izaak Walton Killam Memorial Scholarships, a Dælhousie Graduate Fellowship, and Dalhousie Teaching Assistantships.

xvi

1,\*\*\*

### ACKNOWLEDGEMENTS - II

Special thanks to friends and collegues at Dalhousie for the heated discussions and their assistance and companionship in sampling programmes and laboratory work. They include R. Gershey, B. Johnson, R. Maass, S. Roy, B. Schofield, N. Sharp, and D. Wallace. I would also Tike to thank my non-scientifc friends and family for enduring tales of 'Iatrastats', 'Turbidoscopes', etc. and for being quite un-understanding when I needed it most. Finally I thank the far too many 'Coburg Road' immates, and 'last and mostly, Leslie Fram.

xvii

GENERAL INTRODUCTION

Lipids are hydrophobic compounds composed primarily of atoms of carbon and hydrogen. In environmental sciences the adjective 'biogenic! is often tacitly implied whenever the term is id is used. In addition, this term is sometimes further restricted to only those molecules bearing acyl groups (R-C=0): principally the free fatty, acids and their esters. These esters can be subdivided into the following classes: wax esters, sterol esters, methyl esters, acylated glyceryl ethers, triglycerides, diglycerides, monoglycerides, glycolipids, and phospholipids. These classes include essential components of membranes, energy storage compounds, and certain compounds that are the products of lipid degradation.

The operational definition of lipids, however, can result in the inclusion of all hydrophobic classes that are extracted with organic solvents. Several of these additional classes are also biogenic; others can contain a significant proportion of pollutants. Compounds in the additional classes include aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, aliphatic ketones, aromatic ketones, phthalate esters, aliphatic alcohols, alicyclic (aliphatic + cyclic) alcohols, glyceryl ethers, and pigments. It is the broader range of extractable lipid classes that will be considered in this study (Fig. 1.1). The principal aim of this research was to study the distributions a of marine lipid classes in time-series and in profiles. The processes that were considered to be of particular interest for a study of this kind were those occurring during spring phytoplankton blooms, and those occurring near frontal regions.

Traditionally, lipids in the natural environment have been investigated by measuring the total lipid content or the fatty acid moleties of several lipid classes after hydrolysis. With this approach, however, it is difficult to elucidate the nature and source of the original lipid material

At the time this project began, a promising new analytical technique for Tipid measurement, the Chromarod-Iatroscan system, was starting to receive consrderable, attention in the biomedical literature (Ackman, 1981a). This technique employed a combination of two well-established analytical tools: thin-layer chromatography and the flame ionization detection of carbon. The technique became known as TLC/FID. The first step in this research project then became the development of lipid class separation procedures suitable for aquatic samples, as well as the establishment of calibration and quantification procedures. The technique matter, but also for use in studies of marine dissolved and particulate matter, but also for use in the analysis of lipid classes in freshwater samples, in " phytoplankton cultures, and in aquatic invertebrates, Samples were taken from these of sources to complement field studies undertaken in Nova Scotian marine, waters, At the time of the development of the TLC/FID procedure, the only studies of the complete suite of lipid classes in seawater were those of Jeffrey (1966) and Larsson <u>et al</u>. (1974). It was thus necessary to examine the recent literature on aquatic lipids in order to estimate the likely contribution of each lipid class to a seawater sample, and to establish suitable representative compounds for use as standards in the TLC/FID procedure. This literature review forms the basis of Chapter 1.

There have been only two other studies involving time-series and profiles of dissolved and particulate marine lipid classes during spring blooms. Kattner <u>et al.</u> (1983a) measured dissolved free fatty acids and fatty acid esters in time-series and profiles during a spring plankton bloom in the North Sea. Morris <u>et al.</u> (1983) measured the proportions of lipid classes in particulate matter from a diatombloom in an enclosed ecosystem bag. These studies are discussed further in Chapters 1 and 4.

After developing TLC/FID procedures for aquatic lipid classes (Chapter 2), dissolved and particulate classes were measured in diatom cultures grown with varying rates of nitrogen supply (Chapter 3). The diatom study, conducted with cage culture turbidostats (Wangersky <u>et al.</u>, submitted), gave further insights into distributions of lipid classes that were observed during spring blooms in Bedford Basin, Nova Scotia (Chapter 4). Profiles of lipid classes from Bedford Basin, the Scotian Slope and Lake Huron are compared in Chapter 5.

3,

# 1.1 Introduction

The ease with which hydrophobic compounds can be extracted from magine samples and concentrated using organic solvents, together with, the widespread use of gas chromatography, has led to the accumulation of considerable information on intact lipids and especially the components of lipid molecules. These molecules can be broadly divided into those that are associated with the metabolism and structure of cells and those associated with pollution.

Biogenic Lipids are important energy sources in the marine environment. The fatty acid moieties of these lipids have been useful, tracers in food-web studies (Lewis, 1969; Lee <u>et al.</u>, 1971; Morris and Culkin, 1976; Paradis and Ackman, 1977; Velimirov, 1982). For an understanding of lipid metabolism, however, the measurement of constituent classes is more useful (Lee <u>et al.</u>, 1971, 1972; Morris and Culkin, 1976).

Chapter 1

A SURVEY OF THE LITERATURE PERTAINING TO

DISSOLVED AND PARTICULATE MARINE LIPID CLASSES

Hydrophobic pollutants are also extracted from seawater samples into organic solvents (Ware and Addison, 1973; Giam <u>et al.</u>, 1978; Whittle <u>et al.</u>, 1982). The measurement of pollutants in combination with biogenic lipids would be useful in studying the distributions of pollutants since all lipids can potentially act as solvents, transporters or sinks for pollutants (Olsen <u>et al.</u>, 1982; Sullivan <u>et</u> al., 1982; Florence <u>et al.</u>, 1983; Simkiss, 1983).

As with studies of marine lipids in invertebrates (Morris and Culkin, 1976), it would seem that the measurement of constituent lipid classes in dissolved and particulate seawater samples would provide very useful information. The detection of a pollutant class, or of classes of the degradation products of biogenic lipids would be important in assessing water 'quality. High levels of classes associated with cell membranes would be a good indication of growth in a microbial community. An accumulation of high-energy storage compounds would give an indication of the origin, the nutritional state and the energy value of particulate material. An accumulation of an energy class associated only with heterotrophs would indicate that energy has been transferred in the food-web. Information of these kinds can be derived only from the measurement of constituent lipid classes.

Since the first measurements of lipid classes in seawater by Jeffrey (1966) there have been few attempts at quantifying a range of individual classes, Recently, however, there has been renewed interest in this approach to studying dissolved and suspended

particulate matter (Hennion <u>et al</u>., 1983; Kattner <u>et al</u>., 1983a; Morris <u>et al</u>., 1983; Parrish and Ackman, 1983a; Delmas <u>et al</u>., 1984; Wakeham <u>et al</u>., 1984). The purpose of this review is to examine the potential value of lipid class measurements in seawater. A secondary but more practical purpose of this review is to establish which compounds should be used as model compounds for qualitative and quantitative work with marine lipid classes.

Figure 1.1 summarises the results of this review. It shows what are thought to be among the chief non-volatile representatives of the principal lipid classes present in seawater. They are arranged in an approximate order of increasing polarity. As one proceeds down the list the compounds tend to contain a greater proportion of oxygen. nitrogen and phosphorus. However, a significant proportion of each molecule is composed only of carbon and hydrogen atoms. This is what makes all these compounds lipids, and it is this hydrophobic part of the molecule that prevents any of these compounds from being substantially soluble in water.

The order in Figure 1.1 is also significant in analytical terms. It is the approximate order in which individual classes would be eluted from a silica gel column with solvents of increasing polarity. Silica gel chromatography, either column chromatography or thin-layer chromatography (TLC), is the traditional means of separating lipid classes.

### 1.2 Dissolved and particulate matter

The primary concern of this survey is with marine dissolved matter and with marine suspended particulate matter. The distinction between these two fractions is entirely operational, and has been causing problems for many years (Whitehouse <u>et al.</u>, 1984). The reason for wanting to make a distinction is clear: it' is an attempt to distinguish between living and recently living material, suspended solids, and micelles, and that material which exists as single molecules, dimers or oligomers. The means of making the distinction is also obvious: a filter with very small pores is used. Particulate material is retained by the filter, dissolved material is that which passes through the filter. The problem has been that no one filter has been found that will adequately separate dissolved and particulate matter and also that no one filter has ever been used universally by, oceanographers.

Whatman, GF/F and GF/C glass-fibre fifters are commonly used in work with marine organic compounds (Wangersky and Zika, 1978). In lipid work, Gelman A/E glass-fibre tters have also been used (Boehm, 1980; Kennicutt and Jeffrey, 19 b; Bates et al., 1983; Delmas et al., 1984; Wakeham et al., 1984). The reasons for using glass-fibre filters are that they can be easily and thoroughly cleaned and that they are also relatively inexpensive. There are, however, several problems associated with the use of these filters. Firstly, the above three filter types have three different nominal pore sizes: 0.7, 1.0 and 1.2 µm. These differences may not be signifficant, however, in light of the fact that the manufacturers state that the particle size for which the filters offer absolute retention "... is generally several times the diameter of their nominal rating." This situation could be exacerbated by the most common method of cleaning glassfibre filters: heating overnight in an oven at 400 - 450 °C.

. Data presented by Bates et al. (1983) suggests that although 25% of 1 µm particles pass a precombusted Gelman A/E (1 µm nominal pore size) filter, at 3  $\mu$ m the filter is 85% efficient and at 4  $\mu$ m it is .90% **Af**ficient. It is necessary to estimate how this might affect lipid chemists' perceptions of 'dissolved' and 'particulate' matter. Filtration studies by Iturriaga and Zsolnay (1981) indicate that approximately 85% of autotrophs and 55% of heterotrophs were greater than 3 µm in size in coastal water. Taking an extreme case where all autotrophs and heterotrophs are assumed to be either significantly smaller than the median retention of a Gelman A/E or else exactly 3 µm in size, then a precombusted Gelman A/E should retain about 70% of autotrophs and about 45% of heterotrophs. In another study involving filtration of coastal waters, Larsson and Hagstrom (1982) indicate that more than 75% of autotrophs are greater than 3 µm, while only 40% of heterotrophs are larger than 1 µm. Again, taking the extreme case, this would indicate a retention of 65% of autotrophs and only of 30% of heterotrophs by an A/E.

## Figure 1.1.

The principal lipid classes of seawater and the principal representatives of those classes. The compounds are subdivided into three groups according to the three TLC separations obtained for these model compounds on silca-coated Chromarods (Parrish and Ackman, 1983a; Parrish, Chapter 2). Each compound structure is drawn with the most hydrophobic part of the molecule pointing towards the right of the Figure. The hydrophobic portions of the molecules have been drawn close together, as they might be expected to be oriented in seawater.

(a) The least polar classes: the hydrocarbons and simple esters. Wax esters and sterol esters are difficult to separate by silica gel TLC. The hydrocarbon molecules are approximately equally hydrophobic everywhere in each structure.

(b) Ethers, esters, acids, and alcohols.

(c) The most polar and most complex classes. Monoglycerides, glycolipids, and pigments often migrate together in TLC separations on silica gel.



r 2

\*





It would seem that in coastal waters, 70% of the autrotrophs and 40%, of the microbial heterotrophs are reasonable minimal estimates of the proportion of each population extracted from filters by lipid chemists: Although absolute retention of living matter is clearly not feasible with glass-fibre filters, an advantage of having a nominal pore size of the order of 1 µm is that truly dissolved material is less likely to be adsorbed on the filter than would be the case with smaller pore sizes (Johnson and Wangersky, 1985). As would be expected, however, the adsorption of lipids on filters is still a significant problem. Schultz and Quinn (1972) suggested the term 'retained organic matter' for samples filtered through glass-fibre filters. They found that 20% of the fatty acids retained by glassfibre filters actually belonged to the dissolved fraction of seawater (Schultz and Quinn, 1973; Schultz, 1974). A value of around 20% seems to be an accepted level for adsorption of fatty acids in near-shore waters (Schultz and Quinn, 1977; Goutx and Saliot, 1980). This suggests that from a quantitative perspective, adsorption should create only a small bias; however, there is clearly a need for further research on the effect of filtration on dissolved hydrophobic compounds.

From a quantitative perspective it also would appear that inclusion of dissolved fatty acid compounds in particulate lipid samples should generally not have a great effect on the interpretation of particulate lipid data. Dissolved and particulate fatty acid compounds present in relatively unpolluted coastal samples have been

found to be similar except for a greater range of compounds in particulate lipid samples (Goutx and Saliot, 1980; Kennicutt and Jeffrey, 1981a,b). This is not the case for dissolved and particulate hydrocarbons which seem to have quite different sources (Boehm, 1980; Goutx and Saliot, 1980). There is, however, only 3 to 10% adsorption of dissolved hydrocarbons on filters (Schultz, 1974; Boehm, 1980). Given that bacterial carbon has been estimated to constitute only about 10% of the total particulate organic carbon in coastal waters (Douglas, 1985), in samples where chlorophyll <u>a</u> is a significant proportion of the lipid mass measured, it can be assumed that particulate lipid class data are a reasonable reflection of autotrophic activity in the water column.

The next question to be addressed is what is meant by 'dissolved' lipids and whether the contribution of 'picoplankton' (Sieburth <u>et</u> <u>al.</u>, 1978) to this fraction should seriously affect our perception of this fraction. The maximum value of particulate organic carbon measured in a <1  $\mu$ m fraction in coastal water by Smith <u>et al.</u> (1985) was 84  $\mu$ g/1. Kennicutt and Jeffrey (1981b), estimated that lipids accounted for about 20% of the particulate organic carbon retained by Gelman A/E filters. Bacteria, which pass glass-fibre filters should not be expected to synthesize any more lipid than the phytoplankton that are retained by these filters, since most bacteria do not store triglycerides (Fulco, 1983). Neither should photosynthetic 'picoplankton contribute excessive amounts to what are thought of as dissolved lipids. Glover <u>et al</u>. (1985) found that coastal

picoplankton fixed less of their carbon into lipids than >3  $\mu$ m plankton. The maximum incorporation they measured was 20%.

1

Using 20% as a reasonable proportion of <1  $\mu m$  cells that is lipid, and using the maximum particulate organic carbon value of Smith et (1985), this suggests a maximal concentration for lipids a1. associated with living cells that pass a glass-fibre filter would be around 20 µg/1. The minimum gravimetric value for dissolved lipids measured by Kennicutt and Jeffrey (1981a) is 60  $\mu$ g/l. Thus the maximum likely contribution of planktonic lipid to the dissolved fraction would be around a third. The remaining lipids are presumably. present in true solution or else they may be held in a colloidal dispersion (Cauwet, 1978; Yarıv and Cross, 1979; Sigleo et al, 1982). Although lipids are hydrophobic compounds, all lipids are soluble to some degree in water. There are few data on the aqueous solubility of lipids and virtually none on their solubility in seawater. Examination of data from two sources (Ralston and Hoerr, 1943; Stephen and Stephen, 1963) suggests that 100  $\mu$ g/1 would be a conservative estimate of the solubility of individual compounds from a variety of lipid classes. This value applies to lipids dissolved in N 1 litre of distilled water at around 20 °C. It is necessary to estimate how applicable this value might be to seawater.

Decreasing the temperature from about 20°C to about 0°C, which is closer to the global average seawater temperature, decreased the solubility of free fatty acids and polynuclear aromatic hydrocarbons (PAH) in distilled water by about 50% (Ralston and Hoerr, 1942;

Whitehouse, 1983). A change in salınity from about 0%, to 35%, which is close to the global average, decreased the aqueous solubility of PAH by about 50% (Whitehouse, 1983). The presence of more hydrophilic organic compounds dissolved in segwater may have the opposite effect (Hassett and Anderson, 1979; Gjessing and Berglind, 1981). Whitehouse (1983) found that the inclusion of naturally occurring organic matter in seawater increased the solubility of some PAH by about 50%. The effect of lipid compounds on each other, however, should be negligible. At seawater temperatures most non-volatile marine lipids (Fig. 1.1) are likely to be solids. Although mixtures of these solids in water should not interact and change the solubilities of the components, in practice some deviations do, occur (Eganhouse and Calder, 1976; Banerjee, 1984). The deviations are usually small; however, in one quaternary system a deviation of 65% was observed . (Eganhouse and Calder, 1976). "Using a value of 100  $\mu$ g/l as the solubility of an individual lipid in distilled water, and using worst-case values in the translation to an equivalent solubility in seawater, 1  $\mu$ g/1-seems to be a reasonable minimum solubility for lipid compounds in seawater. Since individual lipids are present in unpolluted coastal water in the ng/l range (Kennicutt and Jeffrey, 1981a) it would appear that many lipids in seawater could be in true solution. This is supported by the . measurement of significant levels of a complete range of lipid classes in dialyzed seawater (Chapter 3). Some lipids, however, are undoubtedly associated with colloidal-sized particles (Sharp, 1973;

Sigleo <u>et al.</u>, 1982; Means and Wijayaratne, 1984) which would pass a glass-fibre filter. Some may be adsorbed onto inorganic surfaces, and some may occur as micelles which are also of colloidal dimensions (Yariv and Cross, 1979).

Although the actual physical state of the operationally defined 'dissolved' lipids is unclear, it is clear that processes occurring in this fraction are different from those occurring in the operationally defined 'particulate' fraction. Temporal and spatial distributions of dissolved and particulate lipids have been found to be very different (Boehm, 1980; Morris <u>et al.</u>, 1983; Parrish, Chapters 4,5). The range of compounds present in the two fractions has also been found to be significantly different (Boehm, 1980; Goutx and Saliot, 1980; Kennicutt and Jeffrey, 1981a,b).

#### 1.3 Hydrocarbons

Hydrocarbons in seawater, are often thought of as being an indicator of pollution. Hydrocarbons can, however, account for a significant proportion of the lipids of aquatic organisms.

### 1.3.1 Biogenic hydrocarbons

The unicellular green alga <u>Botryococcus braunii</u> is an example of a freshwater and brackish water organism which is predisposed to hydrocarbon synthesis. It can produce hydrocarbons in amounts of between 20 and 80% of its dry mass (Wake and Hillen, 1981; Bachofen, 1982). This is exceptional, however, as hydrocarbons are usually

present in much smaller quantities in microorganisms (Tornabene. 1981). They occur in all marine organisms and normally account for 1% or less of the total lipid content of the organisms (Sargent et al., 1976). In marine microalgae, however, a 10% hydrocarbon content is quite common, with the majority of this being composed of nheneicosahexaene (Lee et al., 1972; Holz, 1981). This is an unbranched alkene with 21 carbon atoms and 6 double bonds  $(n-C_{21:6})$ which is thought to be formed by decarboxylation of the characteristic marine fatty acid,  $n-C_{22:6}$  (Lee and Loeblich, 1971). Fatty acids are a common biosynthetic precursor for hydrocarbons in phytoplankton and bacteria (McInnes et al., 1980; Tornabene, 1981). Most naturally occurring fatty acids have an even number of carbon atoms. This is a result of their being built up by successive condensations of C2 units (Gurr and James, 1980). Synthesis of hydrocarbons by decarboxylation thus leads to a predominance of oddnumbered hydrocarbons in biological extracts. The major n-alkanes in bacteria and procaryotic and eucaryotic phytoplankton are in the range n-C15:0 to n-C21:0 (Materassi et al., 1980; Mironov et al., 1981; Saliot, 1981; Goodloe and Light, 1982).

18

Another common biochemical building block is the branched isoprene unit. Condensation of this unit leads to the formation of regularly branched alkenes and alkanes as well as to alicyclic (aliphatic + cyclic) compounds. Squalene ( $C_{30}H_{50}$ ) is an example of an isoprene derived alkene; it is quite commonly found in small amounts in algae and bacteria (Volkman et al., 1980a; Tornabene, 1981; Wake and

ſ

Hillen, 1981). In eucaryotes, squalene is a precursor of sterols and steroids. Pristane  $(C_{19:0})$  is the most common regularly branched alkane in marine organisms, especially zooplankton where it is believed to be derived from phytol (Sargent <u>et al.</u>, 1976; Saliot, 1981).

#### 1.3.2 Hydrocarbon pollution

Oil pollution is a source of <u>n</u>-alkanes, isoalkanes, cycloalkanes and aromatic compounds to the marine environment (Whittle <u>et al.</u>, 1982). These compounds enter the marine environment through accidental oil spills, as well as through a variety of chronic, continuous inputs. Whittle <u>et al.</u> (1982) presented data on the various sources of inputs to the marine environment. From these data it possible to estimate that 17 to 21% of the total inputs are the result of urban runoff and effluents discharged from refineries and other industrial and municipal sources.

Hydrocarbon concentrations of the order of mg/l have been measured in urban runoff (Hoffman <u>et al.</u>, 1982). Concentrations of 0.3 to 3  $\mu$ g/l of alkanes in the range <u>n</u>-C<sub>14:0</sub> to <u>n</u>-C<sub>20:0</sub> have been measured in sewage effluents (Barrick, 1982). Although the range of petroleum alkanes overlaps with biogenic <u>n</u>-alkanes, there is no preference for odd-humbered carbon chains in petroleum compounds.

Polycylic aromatic hydrocarbons are synthesized by only a few organisms in small amounts (Saliot, 1981), and thus large concentrations in the marine environment are a strong indication of
anthropogenic inputs. Concentrations of individual PAH in the ng/l range, with total PAH values up to 7.7  $\mu$ g/l, have been measured in sewage and in water from the urbanized portion of an estuary (Kyeseth <u>et al.</u>, 1982; Readman <u>et al.</u>, 1982). Phenanthrene and pyrene were the major PAH detected in these studies.

This class of compounds contains many carcinogens and mutagens; however, much of the organic material added to estuaries probably does not reach the open ocean (Wangersky, 1978). Up to 93% of petroleum hydrocarbons were found to be associated with particulate matter in urban runoff (Hoffman <u>et al</u>., 1982), and it would appear that petroleum hydrocarbons are rapidly sedimented near the point at which they enter estuarine systems (Knap and Williams, 1982). Other toxic, hydrophobic pollutants that are released into the environment are the polychlorinated biphenyls (PCBs) and the biocides DDT and pentachlorophenol (PCP). Concentrations of each of these have been found to be below 10.ng/1, even near point sources of pollution

(Murray et al., 1981a,b).

ي الي

1.3.3 Dissolved and particulate marine hydrocarbons,

With the exception of seriously polluted areas of the ocean, total dissolved or particulate hydrocarbons are resent in seawater at concentrations between 1 and 50  $\mu$ g/1 (Saliot, 1981). Zsolway (1977) suggested that the average concentration was under 10  $\mu$ g/1, and it has been suggested that uncontaminated samples should have even lower concentrations (de Lappe <u>et al.</u>, 1980).

Kennicutt and Jeffrey (1981a,b) observed that the all phatic fractions of dissolved and particulate material in the Gulf of Mexico were dominated by <u>n</u>-alkanes in the range <u>n</u>-C<sub>16</sub> to <u>n</u>-C<sub>32</sub>.0. Dissolved aliphatic hydrocarbons often showed a bimodal distribution with a maximum at C<sub>19</sub> or C<sub>20</sub> which was likely to have a source in phytoplankton, and a second maximum at C<sub>32</sub> which was likely to have a source in terrestrial plants. Concentrations of total dissolved aliphatic hydrocarbons were between 0.1 and 6.0 µg/1, with considerable variation with depth and with season. <u>n</u>-Alkanes have been found to account for 20% of the total mydrocarbons in seawater (Goutx and Saliot, 1980); Barbier <u>et al</u>. (1973) found that hydrocarbons were <u>ca</u> 20% of the total dissolved lipids.

21

Boehm (1980) and Goutx and Saliot (1980) examined the seasonal variations in dissolved and particulate hydrocarbons; they found that particulate hydrocarbons were related to biological productivity. Dissolved hydrocarbons, on the other hand, seemed to be more of an indicator of pollution.

In conclusion, it seems that the detection of total PAH at the  $\mu g/1$ level in seawater could be an indication of serious pollution, while the detection of similar levels of aliphatic hydrocarbons in particulate matter may simply be an indication of high biogenic activity. The presence of  $\mu g/1$  levels of disgolved aliphatic hydrocarbons could be indicative of either situation; however, where there are few organisms it is likely to indicate pollution. 1.4 Free and esterifed fatty acids Fatty acids are the major components of most lipid classes. Some fatty acids exist in the chemically uncombined form ('free') in nature but the most commonly employed analytical techniques do not allow them to be distinguished from the combined forms.

# "1.4.1 Analytical techniques

still be

. .

> Gas chromatography (GC) of saponified samples has been the most common way of analyzing marine lipids (Dawson and Liebezeit, 1981). The information from GC is restricted to compounds that are themselves relatively volatile, or else to compounds that have volatele derivatives. With the use of modern chromatographic technology, efforts are now being made to obtain supplementary information on intact biogenic lipids.

High-temperature glass capillary GC and GC/mass spectrometry (MS) can be used to analyse intact marine fatty acid esters (Wakeham and Frew, 1982). These techniques have been used in the measurement of wax esters, sterol esters, triglycerides and diacyl glyceryl ethers in sediment trap material (Wakeham, 1982). Most of the more polar lipids are not amenable to GC or GC/MS analyses (Kennicutt and Jeffrey, 1981a,b).

High performance liquid chromatography (HPLC) has had very little use in the analysis of marine biogenic Pipids. This is because of a lack of sensitve HPLC detectors for most lipid compounds (Aitzetmuller, 1982). Advances in MS technology (Cooks et al., 1983)

and the recent use of light-scattering mass detectors in lipid work (Christie, 1985) indicate, however, that sensitive universal detection may soon be readily available for HPLC systems.

There have been a few attempts at quantitative analyses of seawater lipids using thin layer chromatography. Ratios of lipid classes have " been measured in dissolved matter using densitometry (Rattner <u>et al.</u>, 1983a) and in a diatom population using flame ionization detection (Morris <u>et al.</u>, 1983). With the use of an internal standard (Parrish and Ackman, 1983a) it has been possible to obtain direct estimates of dissolved and particulate marine lipid class concentrations (Delmas <u>et al.</u>, 1984). These studies, together with those of Jeffrey (1966) and Larsson <u>et al.</u> (1974), have shown that there is a complete range of classes of fatty acid esters present in seawater.

1.4.2 Total fatty acids

The fatty acid moieties of esters of aquatic origin are characterized by the predominance of palmitic acid  $(\underline{n}-C_{16:0})$ , as well as the presence of several polyunsaturated fatty acids, especially  $\underline{n}-C_{20:5}$ and  $\underline{n}-C_{22:6}$  (Gunstone, 1967). The latter are particularly prevalent in marine lipids, and recently they have been found to be of interest in the fields of epidemiology and clinical and medical research because of their apparent beneficial effects on human health (Goodnight <u>et al.</u>, 1982; Glomset, 1985).

Quantitatively, palmitic acid has been found to be the major fatty acid in marine bacteria, cyanobacteria, eucaryotic algae, and krill

23

· . \*

(Parker et al., 1967; Chu and Dupuy, 1980; Materassi et al., 1980; Sargent and Falk-Petersen, 1981; Goodloe and Light, 1982; Morris, 1984). It has also been found to be the major fatty acid in sea foam,  $^{\prime}$ in the sea surface microlayer, in dissolved and particulate matter and in sewage (Larsson et al., 1974; Goutx and Saliot, 1980; Hunter. and Liss, 1981; Matsumoto, 1981; Velimirov, 1982; Kattner et al., 1983a,b). Unsaturated fatty acids were also detected in these samples. The release of these acids into seawater may be a key step in the formation of marine humic substances (Harvey et al., 1984). The most important role of fatty acid moleties in seawater, however, is undoubtedly as a source of energy. The oxidation of 1 mole of oleic acid  $(\underline{n}-C_{18,1})$ , for instance, supplies over 3 times the amount of energy supplied by the oxidation of 1 mole of glucose, and more than 138 times the amount of energy supplied by the oxidation of 1. mole of ammonia (Anderson, 1980). The release of these compounds into seawater by algae may have a more subtle ecological significance as well. It may be a means of inhibiting the growth of other species of algae or else of bacteria (Procter, 1955; Gauthier and Aubert, 1981). The toxicity of fatty acids against marine organisms may occur directly as a result of their strong surface active properties, or else it may be the result of the formation of toxic oxidation products (Hashimoto, 1979). Release of these compounds may also be a means of complexing trace metals (Mantoura, 1981).

Goutx and Saliot (1980) measured temporal variations in total fatty acids in the Mediterranean Sea and found that the dissolved and

o. 24

particulate fractions had similar fatty acid distributions. The highest total fatty acid concentration recorded in either fraction was 11.6  $\mu$ g/1. They found no correlation between fatty acid concentration and chlorophyll <u>a</u> content. They suggest, however, that the largest amounts of dissolved fatty acids were produced after a zooplankton bloom, as a result of excretion and degradation processes.

Although plankton are likely to be major sources of fatty acids in the oceans it should be noted that the concentration of fatty acids in river water can be very high in urban areas (Matsumoto (1981) recorded a value for total dissolved and particulate fatty acids as high as 1 mg/l for the Tokyo area. The concentration of particulate fatty acids was about two orders of magnitude greater than that of dissolved fatty acids, so it might be expected that, as with hydrocarbon pollution (Sect. 1.3.2), not much of this material actually reaches the open ocean.

1.4.3 Free fatty, acids '

It was thought that free fatty acids detected in biological extracts were only an artefact caused by the action of lipases released from damaged cells. It is now known that they are also normal constituents of the tissue lipid pool (Chapman, 1969). Values between 5 and 25% of the tissue lipids have been measured in phytoplankton and zooplankton (Lee <u>et al.</u>, 1971; Billmire and Aaronson, 1976; Henderson <u>et al.</u>, 1981; Sargent and Falk-Petersen, 1981; Parrish, Chapter 3). A limited

amount of work on phytoplankton cultures indicates that extracellular algal lipids do not have exceptionally high free fatty acid contents. Extracellular free fatty acids accounted for between 10 and 20% of the extracellular lipids in two experiments with cultures (Billmire and Aaronson, 1976; Parrish, Chapter 3).

Free fatty acids have accounted for 15 to 50% of the total lipids in river water, sea foam, the sea surface microlayer, and the dissolved fraction of seawater (Larsson <u>et al.</u>, 1974; Matsumoto, 1981; Velimirov, 1982; Kattner <u>et al.</u>, 1983a).

Free fatty acid values quoted for environmental samples are probably maximal values. During the sampling and extraction procedures lipases are likely to increase the proportion of free fatty acids in the extract. Poorly stored samples can also show significant increases in free fatty acid content (Sasaki and Capuzzo, 1984). For seawater lipids, a 25% free fatty acid content seems to be a reasonable value to take as a maximum proportion that is normally produced by cells. So long as there has been great care in sample handling, any reports of higher values can be taken to be an indication of in situ degradation.

1.4.4 Wax esters and sterol esters

Wax esters and sterol esters are among the most non-polar of the fatty acid esters. Wax esters generally occur on the surfaces of organisms to protect against water loss, but in aquatic animals they are used as metabolic energy reserves (Chapman, 1969; Sargent <u>et al.</u>,

1977). Sterol esters are components of membranes (Sect. 1.7). These two types of esters have been grouped together here because they are difficult to separate on silica gel (Morris and Culkin, 1975; Parrish and Ackman, 1983a). They can, however, be separated and analysed using high-temperature gas chromatography. Using this procedure, Wakeham and Frew (1982) found that the two groups were present in approximately equal proportions in sediment trap samples.

Wax esters are the major neutral lipids of numerous zooplanktonic crustacea, especially calanoid copepods, where they have been found to account for up 92% of the total lipid composition, and they often account for one third of the dry weight (Sargent <u>et al.</u>, 1976; Lee and Nevenzel, 1979). Lee <u>et al.</u> (1971) showed that copepods incorporate fatty acids from phytoplankton into their wax esters with little alteration to the source fatty acids.

Although the presence of typical wax esters or cerides (free fatty acids esterified; with unbranched alcohols) is rare in algae (Lee <u>et</u> <u>al.</u>, 1971, 1972), a significant amount (5% of the total lipids) of phytyl esters were found in a marine dinoflagellate (Withers and Nevenzel, 1977). The cells were harvested at the stationary phase of growth when the total lipid per cell had increased threefold over log-phase cells. It is possible that this unusual occurrence of branched-chain esters is a response to stress. Phytyl esters have also been found in a dinoflagellate collected from a lake (Cranwell <u>et al.</u>, 1985). but in this case they only accounted for 0.2% of the total lipids. Cerides are also uncommon in bacteria (Asselineau,

1966; O'Leary, 1967).

Wax esters can be released into the environment following the death of large numbers of zooplankton. This results in the formation of 'white' or 'milky' water. Volkman <u>et al</u>. (1980b) analysed samples of this water and found 20  $\mu$ g/1° of an oil consisting mainly of two wax esters. The major fatty acids present were <u>n-C<sub>14:0</sub></u> and <u>n-C<sub>16:0</sub></u>: They found few sterol esters present in the milky water or in the zooplankton.

28

Wakeham (1982) suggests that the major sterol esters in sediment trap material are of zooplanktonic origin, and thus a measurement of total wax and sterol esters (Chapter 2) can be taken as a reasonable indication of the zooplanktonic contribution to a sample extract. This generalization is limited to samples that do not contain significant levels of terrestrial material where wax esters are more common.

1.4.5 Short-chain estets

Ehrhardt <u>et al</u>. (1980) and Kennicutt and Jeffrey (1981a,b) demonstrated the presence of fatty acid methyl esters in seawater; both groups of workers found more in particulate matter. The dominant methyl ester in the dissolved fraction was methyl palmitate. Ehrhardt <u>et al</u>. (1980) identified 8 methyl esters in particulate matter and Kennicutt and Jeffrey (1981a,b) identified ethyl and propyl esters in addition to methyl esters in both fractions. The highest total methyl and ethyl ester concentration they measured was 0.2  $\mu$ g/1. They

Ĉ.

suggest that fat'ty acid esters are formed as a result of the degradation of humic substances. Another possibility is that they are the result of the action of yeasts on marine hydrocarbons. Significant levels of fatty acid methyl esters have been found in the medium of yeasts grown on <u>n</u>-alkanes (Blasig et al., 1984).

1.4.6 Acylated glyceryl ethers

In plate TLC, acylated glyceryl ethers appear as a spot just above triglycerides. They have a widespread occurrence in marine animals. (Malins and Varanasi, 1972; Hallgren <u>et al.</u>, 1978). Fatty alcohols are important precursors in the biosynthesis of ether bonds in marineanimals. Glyceryl ethers have a rapid turnover and may be used in buoyancy control (Malins and Varanasi, 1972).

Small amounts of acylated glyceryl ethers were detected in sediment trap samples by Wakeham (1982). The source for these ethers is unknown but they can be assumed to be derived from zooplankton since there is little evidence for their being present in significant/ quantities in algae. Some bacterial phospholipids have ether bonds as part of their structure (Comita and Gagosian, 1983; Mancuso <u>et al.</u>, 1985), but there is no evidence at present of significant levels of bacterial neutral lipids containing ether bonds.

1.4.7 Triglycerides, diglycerides, <sup>e</sup> and monoglycerides Where triglycerides are important constituents of the lipids of marine organisms their functions are similar to those of wax esters: 'they can act as energy reservoirs, buoyancy controls, or as thermal insulators (Gagosian and Lee, 1981).

Triglycerides are an important neutral lipid class in flagellates and diatoms (Lee <u>et al.</u>, 1971; Opute, 1974a; Holdsworth and Colbeck, 1976; Holz, 1981; Parrish, Chapter 3). The exact proportion of triglyceride in the cell is probably regulated by nutrient availability; it is likely to be the major cellular lipid class in stressed cells (Chapter 3).

Unfortunately, triglycerides cannot be used as a direct indicator of phytoplankton productivity. Triglycerides have also been found to be the major constituent in <u>Calanus</u> eggs, in euphausiids, and in small copepods (Larsson <u>et al.</u>; 1974; Gatten <u>et al.</u>, 1980; Henderson <u>et al.</u>, 1981; Katther <u>et al.</u>, 1981; Sargent and Falk-Petersen, 1981). In bacteria, however, neutral glycerides of any form usually represent only a minor percentage of the total lipids. Most of the glycerol-fatty acid combinations are in the form of phospholipids and glycolipids (O'Leary, 1967; Fulco, 1983).

Triglycerides have been found to be the major lipid component in the sea surface microlayer, in sea foam, and in phytoplankton and particulate matter at certain sampling periods (Larsson <u>et al.</u>, 1974; Velimirov, 1982; Morris <u>et al.</u>, 1983; Parrish, Chapters 3, 4). Algae are likely to be an important source of triglycerides to the sea

surface microlayer and to sea foam.

Monoglycerides and diglycerides are usually minor constituents in cells but they are important intermediates in anabolic and catabolic fatty acid ester pathways (Gunstone, 1967; Gurr and James, 1980; Kindl, 1984). Lipases act on ester bonds and are ubiquitous in nature. Of the three fatty acid esters being considered in this section, lipases act most rapidly on triglycerides and least rapidly on monoglycerides (Gurr and James, 1980). Thus high monoglyceride levels in marine samples would be indicative of <u>in situ</u> fatty acid ester degradation, so long as the samples were handled carefully (Sect. 1.4.3).

Monoglycerides and diglycerides have been detected in dissolved and particulate seawater samples (Kattner <u>et al.</u>, 1983; Morris <u>et al.</u>, 1983; Parrish, Chapter 4). While diglycerides are invariably minor components, it has been suggested that monoglycerides can account for as much as 40% of the lipids in phytoplankton grown in an ecosystem hag (Morris <u>et al.</u>, 1983; Morris, 1984). The identification of this component is given on the basis of TLC and GC of fatty acid methyl esters. Nonetheless this value must be taken as a maximum. No details are given of the TLC procedures, and no information is given as to where chlorophyll <u>a</u> and glycolipids occur in the lipid class separation scheme. Using the separation procedure described in Chapter 2, chlorophyll <u>a</u> was found to account for 50% of the class with which monoglycerides co-elute in extracts of <u>Phaeodactylum</u> tricornutum, (Chapter 3). Glycolipids are also likely to be an

important component of this acetone-mobile polar lipid band (Chapter
2).

High concentrations of the acetone-mobile polar lipid class have been found in the dissolved fraction of seawater (Chapters 4.5; Table A.2). It would be important to find out if the dissolved and particulate contributors to this class were related. and if monoglycerides, glycolipids and pigments are indeed major components.

1.4.8 Chloroplast lipids

Chloroplast lipids include pigments, glycolipids, sulpholipids and phospholipids. The latter are important constituents of all cell membranes but the other pigmented and non-pigmented lipids are peculiar to cells capable of photosynthesis. These lipids are actively involved in the mechanisms of photosynthesis (Benson and Shibuya, 1962; Nichols and James, 1968; Holz, 1981). Chlorophyll <u>a</u> is the principal pigment in all photosynthetic plant cells and monogalactosyl diglyceride, digalactosyl diglyceride, and sulphoquinovosyl diglyceride, are the principal glycolipids and sulpholipids in plant cells (Chapman, 1969; Kirk and Tilney-Bassett, 1978; Gurr and James, 1980). Sulphoquinovosyl diglyceride is both a sulpholipid and a glycolipid: it contains a direct C-S bond as well as a sugar moiety.

The lipids of <u>Ochromonas danica</u> were found to contain around 40% glycolipid, 20% phospholipid and 10% sulpholipid (Billmire and Aaronson, 1976). Significant amounts of these classes, particularly

sulpholipid, were also found to be released extracellularly by <u>O</u> danica (Billmire and Aaronson, 1976).

Phospholipids are mixed esters of fatty acids and phosphoric acid with an alcohol, usually glycerol. They are continuously being broken down and resynthesized in living cells (Gurr and James, 1980). Phosphatidic acid is the simplest of the phospholipids and it is the precursor of not only more complex phospholipids but also of triglycerides. Phospholipids, triglycerides and diglycerides are closely, linked biochemically (Gurr and James, 1980; Goodwin and Mercer, 1983). This has been used as the basis for an explanation of the manner in which stress is expressed in nutrient-limited phytoplankton cells (Chapter 3).

Despite the name, many phospholipids contain equimolar proportions of nitrogen and phosphorus. An example of such a compound is lecithin (Fig. 1.1c) which comprises between 30 and 40% of the phospholipids of <u>Chlorella pyrenoidosa</u> and <u>Scenedesmus obliquus</u> (Benson' and Shibuya, 1962). It is also the major phospholipid in the dinoflagellates (Holz, 1981). The amount of cellular nitrogen incorporated in phospholipids and chlorophylls is not trivial. The proportion has been found to be between 5 and 15% in <u>Scenedesmus</u> sp. and <u>Thalassiośira fluviatilis</u>, and it has been found to depend on culture conditions (Conover, 1975a; Rhée, 1978). A significant proportion (10%) of total organic nitrogen has also been found to be associated with particulate lipids in seawater samples (Conover, 1975b). Given the sensitivity of chlorophylis to oxygen, light, and

to enzymes activated by cell disintegration (Jen and Mackinney, 1970; Owens and Falkowski, 1982), lipid nitrogen might be expected to be mainly associated with phospholipids in the detrital and dissolved fractions of seawater. It should be noted, however, that significant levels of chlorophyll <u>a</u> have been measured in samples taken from sediment surfaces as deep as 4500 m (Billett et al., 1983).

There is very little direct information concerning the presence of phospholipids, glycolipids or sulpholipids in seawater. However, it would appear that the total concentrations of these compounds are in the  $\mu$ g/l range (Chapters 4,5).

# 1.5 Ketones

Aliphatic ketones are minor components of the lipids of marine organic. This is presumably the result of a rapid recycling of these molecules (Forney and Markovetz, 1971). They are interesting in the context of this review because they have been proposed as internal standards for marine lipid work (Paradis and Ackman, 1977; Parrish and Ackman, 1983a).

Small amounts of ketones have been found in various marine systems. For instance, Hayashi and Yamada (1972) found minor amounts of methyl ketones in a gastropod which feeds mainly on brown algae. Whether the algae are the source of these ketones is not discussed.  $C_{37}-C_{39}$ unsaturated ketones have been identified in a marine coccolithophorid (Volkman <u>et al</u>\*, 1981). Exudation of a hydroxy diketone has been demonstrated for a phosphate-limited dinoflagellate (Trick <u>et al</u>.

1981), and the ketone functional group has also been found in other more complex molecules produced by marine biota (Fenical, 1982).

There is evidence for the presence of saturated, funsaturated, alkyl substituted, alicyclic, and aromatic ketones in marine particulate matter (Wakeham <u>et al.</u>, 1980, 1984; Kennicutt and Jeffrey, 1981b; Ehrhardt <u>et al.</u>, 1982). In all cases the levels of ketones were either in the ng/1 range or below 5% of the total lipid mass.

Despite this evidence for small amounts of various ketones in the marine environment, the use of mono-functional, saturated, unbranched ketones as internal standards (Paradis and Ackman, 1977; Parrish and Ackman, 1983a; Delmas et al., 1984) seems to be a reasonable proposition. Most of the ketones identified in seawater would not interfere chromatographically with this type of internal standard (Parrish and Ackman, 1983a). The effect of those that do interfere is reduced by using substantially more internal standard than the maximum amount measured in seawater (Delmas et al., 1984).

## 1.6 Phthalic acid esters

Large quantities of phthalic acid esters are used in the plastics industry (Mathur, 1974). These phthalates have been detected in the marine environment in extracts of water, sediments, air and biota. In samples of seawater and sediment, phthalates have been detected at higher levels than DDT or PCBs (Giam <u>et al.</u>, 1978; Murray <u>et al.</u>, 1981b). Away from direct sources of pollution, seawater concentrations of individual phthalates have been reported in the

range 0.1 ng/1 to 200 ng/1 (Giam <u>et al</u>, 1978; Ehrhardt and Derenbach, 1980; Waldock, 1983). Concentrations in the  $\mu$ g/1 range have been detected nearer sources of pollution (Jungclaus <u>et al</u>., 1978; Murray <u>et al</u>, 1981b; Taylor <u>et al</u>., 1981). The major phthalates detected in these studies were di-2=ethylhexyl phthalate and dibudyl phthalate.

Reliable estimates of phthalate concentrations in the environment require exceptionally careful analytical work. Considerable care has to be taken not to add phthalates to samples from reagents and equipment used in the laboratory (Ishida <u>et al</u>., 1980), nor to lose phthalates from samples during storage or during sample concentration (Bowers <u>et al</u>., 1981; Karasek <u>et al</u>., 1981; Sullivan <u>et al</u>., 1981).

## 1.7 Free aliphatic alcohols

There are few data documenting the presence of free fatty alcohols.or acyclic isoprenoid alcohols in dissolved or suspended particulate matter. This is surprising because even if these alcohols are not produced in significant amounts as free aliphatic alcohols by marine biota, they should be readily available from the hydrolysis of wax esters and chlorophylls a and b. Low levels of these compounds may reflect the ability of bacteria to convert them to the corresponding acids (Gilbertson <u>et al</u>., 1981; Gillan <u>et al</u>., 1983).

Sever and Parker (1969) reported the first analyses of recent marine sediments from several environments where significant amounts of normal and isoprenoid alcohols were observed. These alcohols were

present at levels up to the same order of magnitude as fatty acids. There have recently been reports of significant amounts of free aliphatic alcohols, principally  $C_{16:0}$ ; in both marine and freshwater particulate matter caught by sediment traps (Wakeham <u>et al.</u>, 1980, 1984; Meyers <u>et al.</u>, 1984).

Kennicutt and Jeffrey (1981a,b) found evidence for the presence of free alcohols in dissolved and suspended particulate matter. The total concentration of these compounds in freshwater and in seawater would appear to be in the  $\mu g/1$  range (Meyers <u>et al.</u>, 1984; Parrish, Chapters 4.5). Free alicyclic alcohols were also present at similar levels (Meyers <u>et al.</u>, 1984; Parrish, Chapters 4.5). The major contributors to this more polar aliphatic class are the eucaryotic sterols.

There are four classes of plant lipids based on sterols: free sterols, sterol esters, steryl glycosides, and acylated steryl glycosides. All of these appear to be components of membranes (Elbein and Forsee, 1976). Sterols are thought to affect mitochondrial function, cell plasticity, and membrane permeability (Heftmann, 1971; Elbein and Forsee, 1976; Boutry et al., 1979).

Lee <u>et al.</u> (1971) found that free sterols accounted for between 10 and 17% of the lipids in three diatom species. The chemically Funcombined form seems to be the main form in diatoms and coccolithophorids (Volkman <u>et al.</u>, 1980a, 1981).

Formerly i was thought that all bacteria were unable to synthesize sterols, but it is now known that there are a few exceptions to this rule (Heftman, 1971; Bouvier <u>et al.</u>, 1976; Gurr and James, 1980). In addition, many procaryotes are now known to synthesize structural analogues of sterols (Rohmer <u>et al.</u>, 1979). These compounds are based on the alicyclic hydrocarbon, hopane, and the lipophilic ring system bears one to five hydroxyl groups. The most common type of hopanoid in procaryotes contains a polyol hydrophilic side-chain (Ourisson <u>et</u> <u>al.</u>, 1979).

38

Similarly, cholesterol used to be considered to be exclusively confined to the animal kingdom, but it is now known to be a key biosynthetic intermediate in plants as well (Heftmann, 1971). It is, however, present in only trace amounts in most algae (Goodwin, 1974). By contrast, cholesterol can be one of the most abundant lipid compounds in the water column in samples taken near the point at which, municipal discharges are released. Cholesterol levels as high as 10 µg/1 have been measured in the Delaware River (Sheldon and Hites, 1978).

In the Sargasso Sea, Gagosian (1976) found a maximum concentration of just under 0.4  $\mu$ g/l of total free sterols (unfiltered water) with cholesterol being the most abundant sterol at all depths. Above 1000 m, plant sterols, including brassicasterol, sitosterol and fucosterol, jwere present at concentrations close to that of cholesterol. Saliot and Barbier (1973) measured dissolved sterols in the eastern, Atlantic. They saponified their samples and so were measuring combined and uncombined dissolved sterols. The concentrations they measured were much higher: 2 - 14  $\mu$ g/l, but the

The .

most important compounds present were the same as those described by Gagosian (1976).

Sterol production and growth rate has been found to be strongly correlated in a turbidostat cultured diatom (Chapter 3). This suggests the possibility of using high concentrations of plant sterols as an indicator of an actively dividing phytoplankton population.

### 1.8 Conclusion

The purpose of this review has been to examine the potential value of marine lipid class measurements, and to establish suitable model compounds for use in experimental work. Figure 1.1 is a summary of the major classes and compounds that can be expected to be found in a seawater lipid extract.

The most non-polar classes, the hydrocarbons, can be used as indicators of pollution as well as of biogenic activity. Particulate aliphatic hydrocarbons are likely to be the most closely related to biogenic activity. Polycyclic aromatic hydrocarbons together with phthalic acid esters can be expected to reach µg/l concentrations near point sources of pollution. Wax esters, sterol esters and glyceryl ethers are most likely to indicate a zooplankton contribution. Esters of shorter chain lengths, free aliphatic alcohols, and high levels of free fatty acids, diglycerides and monoglycerides are most likely to be indicative of lipid degradation. Away from municipal waste outfalls, a large increase in levels of

particulate glycolipids and phospholipids would indicate an increase in living cells, whereas a similar increase in particulate sterols would be indicative of an increase in eucaryotes specifically.

Ċ

1

à

#### Chapter 2

# SEPARATION OF AQUATIC LIPID CLASSES BY CHROMAROD THIN-LAYER CHROMATOGRAPHY WITH DETECTION AND MEASUREMENT BY THE IATROSCAN FLAME IONIZATION DETECTOR

# 2.1 Introduction

The Chromarod-Iatroscan system is based on two well-established analytical tools: thin layer chromatography (TLC) and the flame ionization detector (FID) used in gas chromatography (GC). The Chromarods are reusable silica gel coated quartz rods (Ackman, 1985) on which the thin-layer separations are performed. The latroscan houses the FID and the machinery used to pass the Chromarods sequentially through its flame and under its collector electrode.

This new analytical system, known in short as TLC/FID, has received considerable attention in the biomedical literature (Ackman, 1981a), principally in the area of lipid class measurement. It is now also gaining popularity in environmental research, especially in marine lipid class work (Kellogg and Patton, 1983; Morris <u>et al</u>., 1983; Parrish and Ackman, 1983a; Vetter <u>et al</u>., 1983; Delmas <u>et al</u>., 1984; Hakanson, 1984; Harvey <u>et al</u>., 1984; Sasaki and Capuzzo, 1984; Fraser <u>et al</u>., 1985; Volkman <u>et al</u>., 1986).

TLC/FID is not limited to the measurement of lipid classes. It has also been used, for example, to estimate the relative proportions of total lipids and proteins in chloroplasts (Hirayama and Morita, 1980) as well as to quantify monosaccharide derivatives (Kondo, 1982). Nonetheless, interest in quantitative techniques for lipid classes and the complexity of the alternatives, has meant that TLC/FID has been used most intensively for lipid class measurements:

In the past five years it has become clear that TLC/FID cannot be considered as simply a combination of the traditional plate-TLC and GC/FID techniques. It is indeed an analytical system in its own right, and there has been extensive research specifically into Chromarod-TLC separations (Kramer <u>et al.</u>, 1980, 1985; Sebedio and Ackman, 1981; Parrish and Ackman, 1983a; Tatara <u>et al.</u>, 1983; Banerjee <u>et al.</u>, 1985a; Murray, 1985) and into Iatroscan-FID responses (Farnworth <u>et al.</u>, 1982; Crane <u>et al.</u>, 1983; Parrish and Ackman, 1988b, 1985; Kaimal and Shantha, 1984; Peuchant <u>et al.</u>, 1984; Banerjee <u>et al.</u>, 1985b; Harvey <u>et al.</u>, 1985; Rao <u>et al.</u>, 1985; Sebedio <u>et al.</u>, 1985).

The present study is the culmination of a series of experiments designed to establish separation and calibration procedures for the determination of marine lipid classes in dissolved and suspended particulate matter (Parrish and Ackman, 1983a,b; Delmas <u>et al.</u>, 1984; Parrish and Ackman, 1985). The methods described herein have now been used successfully in the analysis of over five hundred different samples, and the types of samples have been extended to include

freshwater samples, particulate matter from sediment traps, and benthic and pelagic invertebrate samples. The intention of this Chapter is not only to provide a detailed examination of analytical procedures, but also to supply a 'recipe' so that a reader with a minimal background in analytical chemistry could make inpld class, determinations using the Chromarod-Iatroscan system within a period of days. The procedures described here were designed specifically for complex aquatic environmental samples, but they should be readily applicable to samples from other sources, and eventually they should be easily tailored to other specific needs. Traditionally, lipids in the natural environment have been investigated by measuring the total lipid content or the fatty acid

moieties of several hydrolysed lipid classes. Gravimetry and GC have been the mainstays of this quantitative work with lipid extracts. These techniques, however, are not without their shortfalls.

Gravimetric measurements can be easily biased upward (Hopkins <u>et</u> <u>a1</u>., 1984), while total lipids obtained by GC are invariably considerably smaller than the actual lipid mass (Wakeham <u>et a1</u>., 1980; Kennicutt and Jeffrey, 1981a,b). The main reason for the bias in GC is undoubtedly the fact that these measurements include only those compounds that are both volatile and stable at temperatures used in GC ovens.

GC and gravimetric measurements are not limited solely in an analytical sense, there are also limitations to their suitability for environmental and ecological interpretation. With measurements of total lipid or fatty acid moneties it is sometimes difficult to elucidate the nature and source of lipid material. By measuring lipid classes it should be possible to distinguish energy storage classes, membrane classes, pollutants, and indicators of lipid degradation (Chapter 1).

The Chromarod-Tatroscan system is provided with a unique facility which makes it particularly suitable for the measurement of hipid classes. This feature is the ability to stop the scan' part-way along a rod; and then to be able to reuse the part that has just been scanned. By setting the scan-stop screw in the Iatroscan each rod in a frame can be stopped at a predetermined point. When the Chromarods reach this point, the rod currently being scanned is automatically moved out of the flame and the scan of the next rod is started. Passing the rods through the flame not only removes organic matter, but it also cleans and reactivates the silica layer so that it is ready for immediate reuse. Material left unburnt on the rod can thus be developed into the part of the rod that has been scanned. By combining the partial scanning facility with the use of several different solvent systems it is possible to optimise the separations between the major lipid classes in a complex sample.

It is not the intention of this Chapter to test the hypothesis that TLC/FID can in any way replace gravimetry or alternative chromatographic techniques. Rather, it is hoped that this Chapter will show that TLC/FID can have a role of its own to play: one which is entirely complementary to more conventional analytical techniques.

## 2.2 Methods

The analytical methodology leading to the measurement of aquatic lipid classes comprises several steps. These include sampling, extraction, storage, concentration, and analysis. Throughout these steps it is important to exclude contact with other tipid-containing materials, as well as to minimise exposure to light, oxygen and heat: aquatic lipids are characterised by a high degree of polyunsaturation and can thus be quite labile.

To minimise contamination, Samples came into contact only with precleaned glass, Teflon, or metal wherever solvents were involved in the sample handling. Where possible, glassware was baked overnight in a 400'<sup>7</sup>C oven, and glass or Teflon surfaces due to be exposed to samples were cleaned with chromic-sulphuric acid. The chromicsulphuric acid mixture was made with Chromer'ge (Fisher Scientific Co., Fair Lawn, NJ). All glassware was rinsed with acetone and then dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) immediately before use. High-purity solvents were used throughout: BDH 'Omnisolv' grade was found to be suitable for all purposes. Sample extracts were stored in dilute solution (<u>ca</u> 5 ml), under nitrogen, in a freezer. Nitrogen was purified by passing it through activated charcoal and molecular sieves.

2.2.1 Sample workup

Sampling for dissolved and particulate matter was usually performed with conventional 5 1 Niskin bottles. As soon as the samples were brought on deck they were transferred to precleaned and sample-rinsed

amber-glass solvent bottles. In the process of transferring, the seawater was screened through a 200 µm nylon mesh to remove larger zooplankton and particles. The bottles were closed with Teflon-lined, caps which were also precleaned and sample-rinsed, and the bottles were removed from direct sunlight as soon as possible.

In the laboratory the seawater was swirled in the glass bottles before filtering through precombusted (400 °C) glass fibre filters. The filters used were usually Gelman A/E 47 mm filters which have a nominal pore size of 1  $\mu$ m. The filter was held in a precleaned and sample-rinsed all-glass filtering apparatus. Light suction was applied via tap water aspiration. The more commonly used Millipore pumping systems were used initially, but were avoided subsequently. because of high hydrocarbon contamination. Water samples that could not be filtered immediately were kept in the dark in a coldroom ( $\underline{ca}$ ,  $2^{\circ}C$ ).

Between 500 ml and 3 1 of seawater was filtered for 'particulate' lipid measurements, and between 200 ml and 1 1 was filtered for 'dissolved' lipid measurements (Fig. 2.1). The exact amount filtered depended on location and season. Wherever possible, filtrations and extractions were done in quadruplicate, and a total system blank was run for each set of four dissolved lipid extractions, as well as for, each set of four particulate lipid extractions.

The filtrate containing the dissolved lipids was usually extracted with 20 ml of  $CH_2Cl_2$  at seawater pH (<u>ca</u> 8) and then twice with 10 ml of  $CH_2Cl_2$  after adjusting with sulphuric acid to approximately pH 2.

Particulate lipids were extracted from the filter by sonicating or grinding in  $CH_2Cl_2$ -methanol (2:1). The methanol (MeOH) and the nonlipid material was back-extracted into "Super-Q' water (Millipore) which had been redistilled and passed through an oven at 800 °C to further purify it. The two phases were separated by centrifugation. All subsequent extractions and transfers were done with 100%  $CH_2Cl_2$ to minimise the amount of MeOH in the final concentrate. The particulate material was extracted three times; the total volume of  $CH_2Cl_2$  used in the extractions was normally 20 ml.

Prior to extracting either the dissolved or particulate fractions, a solution containing 5 to 20 µg of an internal standard (<u>n</u>hexadecan-3-one) was added to each sample. The internal standard was then extracted with the lipids in the sample and the amount of internal standard measured in the final concentrate provided an estimate of lipid recovery.

The  $CH_2Cl_2$  extracts were roto-evaporated down to/1 to 2 ml and transferred to 5 ml centrifuge tubes. The  $CH_2Cl_2$  was then evaporated under purified nitrogen down to about 20 µl, and this total. concentrate was usually spotted onto a single Chromarod.

Figure 2.1

Marine lipid class chromatograms from extracts of operationally defined (Whitehouse <u>et al.</u>, 1984; Parrish, Chapter 1) dissolved and particulate latter. Each ford is scanned twice partially and once completely, resulting in three different chromatograms per sample application. The developing and conditioning sequences are given in Table 2.2 (p62). The developing direction is from right to left in the Figure, the scanning direction is from left to right. Lipid classes increase in polarity from left to right in the Figure. Lipid class abbreviations are explained in Table 2.3 (p63); NLM = non-lipid material, KET = spike added to each sample before extraction. Chromatograms were recorded at an attenuation of 16 mV full-scale deflection (FSD) unless otherwise indicated.



Extracts of benthic investebrate samples and sediment trap samples from the Great Lakes (Figs. 2.2, 2.3) were kindly supplied by Dr. W.S. Gardner (NOAA, Ann Arbor, MI). These samples had been extracted into chloroform-MeOH (2:1) using a micromethod specifically designed for these types of samples (Gardner et al., 1985). The final extract was transported and stored in sealed capillary pipettes. The same capillary pipettes were also used for directly applying the samples to the Chromarods. After breaking both ends of the pipette, the chloroform solutions was spotted onto the rod and then a few ul of CH2C12 was drawn up, into the pipette. The CH2C12 was also spotted on the rod to ensure a quantitative transfer of the sample. About half was retained in Ann Arbor for gravimetric of each sample' determinations (Gardner et al., 1985). Figure 2.3 is a comparison between these gravimetric measurements and total lipids determined with the latroscan.

The marine invertebrate sample (Figs. 2.2b, 2.4) was obtained by sonicating a 240 µm mesh net-tow sample in  $CH_2Cl_2$ . The seawater/ $CH_2CF_2$ /zoopPankton mixture was then filtered through a solvent-rinsed Whatman filter paper. The filtrate was transferred to a separatory funnel with a glass stopper and a Teflon stopcock. Purified water was added and the sample was treated as for marine dissolved lipids except that no internal standard was used and the pH was not adjusted.

Fîgure 2.2.

1º

Lipid class chromatograms from sediment trap and invertebrate samples. The developments and scans are the same as in Fig. 2.1. Lipid class abbreviations are explained, in Table 2.3; NLM = non-lipid material; no internal standard was added to any of these samples. Chromatograms were recorded at 8 mV FSD unless otherwise indicated under the chromatogram.

51

(a) Sediment trap sample from Lake Hurón.

A

(b)  $CH_2Cl_2$  extract of zooplankton from a 240 µm mesh bongo-net tow near the edge of the Scotian Shelf.

(c) CHCla-MeOH extract of Pontoporeia hoyi from Lake Michigan.



Figure<sup>2.3</sup>

A comparison of gravimetrically determined lipid weights with the sum of Iatroscan-measured classes. Filled circles represent Great Lakes benthic invertebrate samples. Crosses represent Great Lakes sediment trap samples. The slope of the linear regression line is 0.8; the broken lines are 95% confidence intervals for the regression line.

53

Ø



# Figure 2.4

One-step development and scan of marine zooplankton (Fig. 2.2b) lipid classes: peak identifications are given in Table 2.3. S and E denote the start and end of the scan, O is the point of application. Recorder attenuation was changed at X. The rod was developed from right; to left in the Figure for 40 min in hexane - diethyl ether formic acid (80:20:0.1).


2.2.2 Analytical apparatus and operating conditions Chromarods-SIR were used for lipid class separations and an latroscan TH-10 MK III analyzer was used for detection and quantification. The Chromarods and the Istroscan are supplied by Istron Laboratories of Japan via Newman Howells Associates Ltd., 'Winchester, Hants, England. Stainless steel frames to hold the rods, and glass tanks specifically designed for Chromarod developments are supplied with the latroscan. Operating conditions for the latroscan were adjusted to maximize FID response: a relatively high hydrogen flow rate (173'm1 min-1) and scan rate (4.2 mm sec<sup>-1</sup>) were used. Rods were also subjected to double developments to increase the FID response of the more mobile lipid classes (Parrish and Ackman, 1985). For recording of chromatograms and for 'peak' area integration, the Iatroscan was connected to a Hewlett-Packard 3390A computing integrator. The latroscan was also fitted with a scan-interruptor, switch (Fig. 2.5). In its original format (Fig. 2.5a) this consisted

simply of a push-button switch placed in series with the latroscan's own microswitch  $(S_1)$  ùsed to stop scans. By pushing this single-pole, single-throw switch (SPST) it is possible to stop any FID scan at any time. Electronic integrators can be stopped at the end of a partial scan by replacing the SPST switch with a double-pole, double-throw switch (DPDT). In order to stop the integrator, the signal sent from the DPDT interruptor switch (Fig. 2.5b) needs to be converted to a pulse. This is done with a small interface consisting of a resistor-capacitor circuit. Further details concerning this interface are

available from Technical Marketing Associates Ltd., Halifax. The interface is also necessary to convert the scan-start srgnal generated in the Iatroscan to a signal that will simultaneously start

## 2.2.3 Standards and calibration

Standards were obtained from a variety of suppliers (Aldrich, Analabs, Eastman, Fisher Scientific, K & K Labs, Mann Reseach Labs, Polyscience, Sigma, Supelco). Table 2.1 gives the standards used routinely for lipid class identification and calibration. These were obtained from Sigma (St. Louis, MO), K & K Labs (Plainview, NY) and Supelco (Bellefonte, PA). A stock solution of an eight-component composite standard (Table 2.1) was made up in chloroform and stored under nitrogen at -20 °C. Several different dilutions of this standard vere made and these were stored in 3 ml borosilicate glass microreaction vessels (Supelco) fitted with Teflom Mininert values (Supelco). Standards, used on a day to-day basis ranged in concentration from 0.2 µg/µ1 to 15 µg/µ1. They were regularly remewed from dilutions of the stock solution. Before making the composite standards, each component was run singly through the entire development procedure (Table 2.2) to determine purity. Only standards giving a single chromatographic peak at the 3 - 5 µg level were used in the composite standards. Table 2.3 gives the compounds that can be separated using the procedures described in Table 2.2.

# Figure 2.5

\*

١Ŷ

Circuitry for scan interruptors.

(a) Modification of part of the Iatroscan's main board circuitry: momentary, normally closed SPST push-button switch placed in series with S1.

59

., w

(b) Simultaneous stop of computing integrator with a centre-off DPDT switch.



# Table 2.1

-.....

~

aquatic lipid cla	asses in the latroscan	). <b></b> /	ð
· ·	• *	- /	
	Trivial name	Chemical name	Supplier
			Buppilei
	· · · ·	· /· **	•
Aliphatic nydrocarbon	Nonadecane	n-Nonadecane	Sigma <sub>,</sub>
	م متر اس		
lax ester	Stearyl palmitate,	OctadecyI	Sigma
		hexadecanoate 🤟	*
· 1	~	· ·	c t /t
thy1 ketone	3-Hexadecanone	<u>n-Hexadecan-3-one</u>	K & K
	۲ ۹۰ <sup>۴</sup> با بر	1 · · · · · · · · · · · · · · · · · · ·	Labs
riglyceride "	Tr <i>i</i> palmitin	Glyceryl trihexadecanòate	Sigma
5		*	P'
ree fatty acid	Palmitic acid 🔒 🏢	n-Hexadeçanoic acid .	Supelco
-	ц••	- T	e e
ree aliphatic lcohol	Cetyl alcohol	<u>n</u> -Hexadecan-1-o1	Sigma
•	, ۱	1	
ree sterol	Cholesterol	5(6)-Cholesten-3-01	Sigma
	â	۶ ۲	,
olar lipid	Lecithin	Dihexadecanoyl phosphatidy1 choline	Sigma
	۲ .	· ·	

۰.,

\*

\*

61

Ş

Developing and conditioning sequences used routinely "for the separation of aquatic lipid" classes on Chromarods. Abbreviations explained in Table 2.3.

Sequence leading to the first chromatogram (classes HC to KET):

(a) Blank scan rods twice.

Table 2.

· (b) Apply samples and standards with a Hamilton syringe.

(c) Focus twice in acetone to produce a narrow band of lipid material near the lower end of the rods.

(d) Dry and condition in constant humidity chamber for 5 min.

(e) Develop twice in hexane - diethyl ether - formic acid (99:1:0.05). The first development is for 25 min, the rods are dried at room temperature for 5 min, and then redeveloped for 20 min.

(f) Dry 5 min at room temperature in the Iatroscan.

(g) Scan to the lowest point behind the KET peak.

Sequence leading to the second chromatogram (GE to DG):

\*(a) Condition for 5 min.

(b) Develop for \$40 min in hexane - diethyl ether - formic acid (80:20:0.1).

(c) Dry and scan to lowest point behind the DG peak.

Sequence leading to the last chromatogram. (AMPL and PL):

(a) Condition for 5 min.

(b) Develop twice for about 15 min in 100% acetone.

(c) Condition for 5 min.

(d) Develop twice for about 10 min in dichloromethane - methanol - v water (5:4:1).

(e) Dry wand scan entire length of rods.

# Table 2:3

Model compounds representing aquatic lipid classes that can be resolved in a three-step separation (Table 2.2). Representatives in the second compound column cannot be completely separated from those in the same class in the first column, but they can can be completely resolved from representatives in other classes using the solvent, systems described in Table 2.2.

63

1

í "

, . .

57

÷. ت

Ċĺńsż	Abbreviation	Principal.	Other
110 Y A		representative compound	representative
e	يد. در منه	۴.	compounds * «» "
Aliphatic hydrocarbon	HC -	n-Nonadecane	n-Hexadecane
р <sub>3</sub>	* *		<u>n</u> -Eicos-1-ene Squalene
	,		by uniterie ,
Polycyclic aromatic	PAH	Phenanthrene	Anthracene
hydrocarbon			-44
Wax ester/ ,	WE ,	Octadecy1	Dodecy1
sterol ester	a.	hexadecanoate	hexadecanoate
τi	•	e k. 1	hevadecanoate
	i q ~	2 2 <sup>2</sup>	Cholestery 1 * 📚
٩.	-		octadécanoate 🔌
Methyl_ester	ME -	. Methyl docosanoate	Methyl
,			dodecanoate
4	a	۲ ۲	Methyl hexadecanoate
<b>0</b>	• *	, <b>v</b> 4	¥- • •
Ketonę,	KET	Hexadecan-3-one	Det adecan-3-one
Acylated	GE 4	-Glycery1-1-octdecy1	
glyceryl ether "	χ. τ. ζ.+. Λ	ether, 2,3 . dioctadecanoate	
Triglyceride	TG	Glycery1	Glycery1
у. 9		trihexadecanoate	tridodecandate Glyceryl
	,		trioctadecanoate



Whenever a large set of samples was to be analysed, calibration curves were compiled throughout the whole period of sample analyses. With each set of ten rods, one rod, chosen randomly, was used for standards on each day of analyses. The load applied to the rod ranged from 0.4 to 20.0 µg (Fig. 2.6a). Measurements were done mainly at low loads, near the middle of the range, and at high foads (Fig. 2.6b). It was thus possible to obtain estimates of analytical precision for individual classes at these levels, as well as at other levels where sufficient data were collected (Fig. 2.6, Tables 2.4, 2.5).

2.2.4 Standard and sample application Standards were routinely spotted onto Chromarods using Hamilton syringes fitted into Hamilton repeating dispensers. Occasionally standards were spotted with disposable µ1-pipettes. (Drummond Scientific Co., Broomall, PA).

To apply samples or standards to the rods, the frame holding the rods was placed on a warm (<u>ca</u> 30 C) hot-plate with the lower edge extending beyond the end of the hot-plate so that the point of application at the bottom of the rods was not directly over the heat source. This was to help keep the solute in a harrow band near the end of the Chromarods. Solvent focusing (Harvey and Patton, 1981; Parrish and Ackman, 1983a, b) was also employed for this purpose. Solvent focusing is useful when a large volume (>10 µl) needs to be spotted on any one Chromarod. Any solute that has spread away from the point of application is refocused into a narrow band by

developing in a polar solvent to just above the point of application (Table 2.2).

2.2.5 Chromarod developments

classes.

To perform the separations on the rods, the frames were placed in paper-lined glass tanks containing solvents. About 50 ml was required to keep the level of the developing solvent between the bottom of the rods and the point of application. Depending on use, it was sometimes necessary to replace the solvent daily. The rods were developed for a fixed amount of trme in each solvent system, and the solvent was allowed to evaporate from the rods at room temperature before redevelopment or before scanning. Table 2.2 gives the solvent systems and developing times used routinely in the measurement of lipid

2.2.6 Chromarod conditioning and cleaning

Rods were blank-scanned twice prior to use to activate them and to remove any material which had been deposited on them since they were last scanned. After spotting and before each development, the rods were conditioned for 5 min at a constant humidity (<u>ca</u> 30% at room, temperature) by placing the frame in a desiccator containing a saturated solution of CaCl<sub>2</sub>. This desiccator was also used for storing the rods between analyses.

#### Figure 2.6

Iatroscan calib<sup>\*</sup>ration data for nine different lipid classes. Compounds used to represent each class are given in Table 2.1 (p61). Additional developments and scans were performed to obtain calibration curves for steryl esters (SE). Cholesteryl palmitate was used to represent this class.

(a) Average responses from nine different compounds. Each symbol represents a different lipid class. Levels at which average response data were available have been joined by straight lines. Note that above 10  $\mu$ g, average response data are available for only seven classes; it was not possible to obtain a good separation between TG and FFA when each was present at loads above 10  $\mu$ g.

(b) Raw data from nine different compounds split into two groups. Crosses represent classes with high responses: WE, F, ST. Boxes are low-response classes: HC, KET, TG, FFA, ALC, PL. Linear regression lines are drawn through each group. Broken lines are 95% confidence intervals for the regression lines.



¥.\*

FID RESPONSE (INTEGRATOR AREA UNITS X 105)



# Table 2.4

Average precision of integrator areas obtained from scanning 0.4 to 20.0 µg of compounds with low FID responses (Fig. 2.6b).

Average coefficient of Average number of Compound

• •	•	j	••• ••	riation		نج	analy	ses at ea	ach level
a, , , , , , , , , , , , , , , , , , , ,	v (* ****	v 4 * V 7 ***	an ya da ana ana ang ang ang ang ang ang ang an	n	Г 14 в Ят	 , ,		- <u> </u>	ann ann an tar an t
HC <sup>1</sup>		* *	్, ి 7	**			4		, <i>1</i> 4
, KET 🧳		∿ 4 as	8	%		*	5	r	<i>.</i> ,
TG °	-	¢.	8	% '			4	2	7

FFA 16% 4 ALC 8% 3.

, PL 9% Pooled low-response

' calibration data 9 8%

1

**ئ**ي ا



Average precision of integrator areas obtained from scanning 044 to

**4**,

Compound Average coefficient of Average number of variation analyses at each level

WE 117 4 SE 97 2 ST 87 3

Fooled high-response 11%

At the end of each developing session, after the last complete scan of each rod, the Chromarods were removed from their frame and placed individually in numbered test-tubes containing chromic-sulphuric acid. They were left there for at least an hour before being thoroughly washed with Super-Q distilled water. They were placed back in their original positions in their frame, and the frame and the rods were rinsed with acetone. They were then either stored in the desiccator or placed in the Jatroscan for blank-scanning to activate them for further analyses.

### 2.3 Results and Discussion

The Chromarod-Iatroscan system consists of two independent parts. Up to ten rods are grouped together in frames, and each frame is usually considered as a unit. It is possible to be scanning one of these units while preparing another for development.

When applying solutions to the rods, each frame was placed on a hot-plate so that any spotting-solvent, migrating appreciably along the rod was rapidly evaporated. Solvent focusing (Table 2.2) was also performed to minimise the, spreading of solute. Without these precautions it was, found that after development (Table 2.2), petks on chromatograms could be very broad or even split in two (Parrish and Ackman, 1983b).

Development times can be quite long (Table 2.2), but it is possible o be analysing one set of samples while preparing another set for application to the Chromanods. The extraction and concentration stage

was found to be the slow step in the entire procedure leading to the determination of lipid classes. Every effort was made to simplify this stage and to make it as rapid as possible, while maintaining maximal extraction efficiencies and recoveries, together with minimal artefact formation.

2.3.1 Extraction and storage of seawater lipids.

Chloroform (CHCl<sub>3</sub>) was used fnitually to extract the lipids from both dissolved matter and suspended particulate matter. CHCl<sub>3</sub> was later replaced by duchloromethane (CH<sub>2</sub>Cl<sub>2</sub>) which has been suggested as an alternative to CHCl<sub>3</sub> in the extraction of lipids because it is less hazardous to human health (Chen <u>et al.</u>, 1981). These workers found that CH<sub>2</sub>Cl<sub>2</sub> was equally as effective as CHCl<sub>3</sub> in extracting various lipid classes from food products. Comparisons between CHCl<sub>3</sub> and CH<sub>2</sub>Cl<sub>2</sub> for seawater extractions indicated that they were equally effective at extracting marine lipid classes. Since CH<sub>2</sub>Cl<sub>2</sub> is more volatile it has the advantage of permitting a faster concentration of solutions at a lower temperature than would be feasible with CHCl<sub>3</sub>. When it is necessary to spot large volumes (>10 µl) of solutions. CH<sub>2</sub>Cl<sub>2</sub> is again preferable since the solvent evaporates more rapidly from the silica surface on the Chromared. Spotting is thus faster and the spreading of solute away from the origin is less.

All samples were stored in  $CH_2Cl_2$ , in dilute solution, under nitrogen, in the dark, and at -20 to +35 C. These conditions are very , similar to those that have been recommended recently for the storage.

of marine invertebrate lipids (Sasaki and Capuzzo, 1984). In the case of marine particulate lipids the entire filter was stored in this way until analysis. Immediately before analysis, the  $CH_2Cl_2$  was removed and the filters were ground in  $CH_2Cl_2$ -methanol. As much as possible of the methanol (MeOH) was removed with water and subsequent extractions were performed with  $CH_2Cl_2$ . All the  $CH_2Cl_2$  extracts from one sample were combined and concentrated together.

CH2Cl2-MeOH has been used previously for the extraction of lipids in phytoplankton (Volkman et al., 1980a, 1981). In the case of marine particulate matter the inclusion of MeOH was found to be essential for maximizing the recovery of lipids from filters. This was especially true for phospholipids. Repetitions of the complete extraction procedure on previously extracted samples indicated that at least 80% of the recoverable lipids in each lipid class was obtained with the procedure described in the Methods section (2.2.1). Although MeOH was found to be essential for extractions, it was found to be an understrable component in solvents coming into contact with samples for long (>1 month) periods of time. Samples stored in MeOH-containing solvents for long periods showed a significant increase in ME content (abbreviations exclained in Table 2.3). In some marine samples, levels were high enough to interfere with the quantification of the internal standard peak (KET). Thus afforts, have been made to reduce the level of exposure of lipid extracts to MeOH after the extraction process has been completed. Esterification of carboxylic acid groups with MeOH has also been found to be a serious

problem in extracts of airborne particulate matter (Clement <u>et al.</u>, 1982). This artefact formation is also a widely acknowledged problem<sup>></sup> in other fields.

The extraction procedure used for dissolved lipids entailed shaking the sample with three separate aliquots of CH<sub>2</sub>Cl<sub>2</sub>. For each class, the first extraction was found to remove 40 to 90% of the extractable lipids from the aqueous phase. The second and third extractions, performed after acidification, removed smaller proportions of the total for each class. In some instances, the proportion detected in the third extract was negligibly small.

The exact percentage extracted at each step seemed to depend on the polarity of the lipid class being extracted. At each step, the least polar and the most polar classes were generally extracted less efficiently than the rest. This lowered efficiency occurred sometimes to the extent that a repetition of the entire extraction procedure on a previously extracted seawater sample would result in the detection of significant levels of HC, DG, AMPL, or PL. However, the minimum total efficiency of the first set of extractions for these classes was still 75%. By comparison, the minimum extraction efficiency for solvent extractable classes of intermediate polarity (WE to ST) was above 80%.

Several dissolved and particulate samples were reanalysed after an extended storage period in order to assess the effects of prolonged storage on lipid class quantification. The results were, however, inconclusive: there were no clear trends in these data. In some

samples certain classes would decrease, while the same classes in other samples would remain at the same level or even increase. The only statement that can be made here with any certainty is that after' four months of storage all class values were within 30% of the original values. This four month storage period is not uncommon in oceanographic work, and it is reasonably representative of the majority of the samples measured in TLC/FID studies involving aquatic lipid classes in this thesis (Chapters 3'- 5). In order to assess the effects of storage in more detail, 1t would have been necessary to extract much larger volumes of seawater so that it would have been possible to perform several reanalyses of the same sample. To reduce the effect of sample storage on the interpretation of data, attempts were made to analyse as many as possible of the quadruplicate samples within days of sampling. These data would later be combined with the data from the remaining samples stored for extended periods so that total analytical precision values also include a storage component.

2.3.2 Chromarod separations and partial scanning "

2.2).

With the use of solvent systems of increasing polarity and by developing and scanning in opposite directions, it is possible to design a step-wise separation procedure which quantifies lipid classes of greater polarity with each step (Figs. 2.1, 2.2; Table

The most critical step in this multi-step procedure is the first one involving a double development in a hexane-based solvent system (Table 2.2). The choice of solvent system is based on detailed studies of the effect of varying the proportions of hexane, diéthy1 ether, or formic acid on neutral lipid separations (Kramer et al., 1980; Parrish and Ackman, 1983a). It is possible to measure marine FFA in the first scan (Delmas et al., 1984; Parrish and Ackman, 1985) since FFA runs ahead of TG in non-polar solvent systems, but it was eventually decided that it was better to measure it routinely in the second scan to avoid problems with cutting the scan behind large tailing FFA peaks. There are also probléms, however, with measuring FFA in the second scan. It has been found to be rarely possible to, quantify either TG or FFA accurately when both are present at loads higher than 10 µg. It is partly for this reason that a double development is not performed before the second scan (Table 2.2). While double developments improve the separation of compounds near the point of application (e<sub>r</sub>g. KET in the first scan), the more mobile compounds near the end of the rod tend to be closer together than they would be with a long single development. A brief investigation into the use of isopropanol to increase the separation of TG from other compounds (Sasaki and Capuzzo, 1984) indicated that the improvements would be negligibly small for hexane-based solvents. Thug the only recourse found for situations where there were high levels of TG and FFA was to spot a smaller amount of the same sample in a second analysis. .

Figure 2.4 is a single-step separation of the same sample that was measured in a multi-step separation in Figure 2.2(b). It can be seen that considerably more information is available from the multi-step separation, and that as a result of this it is possible to assign identities to peaks with greater confidence. There is also more information available about the wax ester component itself. WE peaks from the net-tow sample and from sediment trap samples often showed some splitting (e.g. Fig. 2.2a). This sometimes occurred to the extent that two peaks were clearly distinguishable in the wax ester region of the chromatogram (Fig. 2.2b), and could possibly indicate the presence of short-chain esters in addition to WE. However, there is probably not much ME\*\*present since the  $\underline{R}_{f}$  values (distances moved along the rod) suggest that the second component is slightly less polar than ME. Another possibility is a WE/SE split but this is also thought to be unlikely since it was not possible to achieve such a separation with standards (Table 2.3). It is probable that the splitting represents differences in unsaturation and/or chain-length within the class: both of these factors have been found to affect  $\underline{R}_{f}$ (Kramer et al., 1985). TG peaks have previously been found to be split in dissolved and particulate matter (Delmas et 'al., 1984; Parrish and Ackman, 1985). This peak splitting within classes, together with slightly different  $\underline{R}_{f}$  values depending on sample load and sample type, means that it is advisable to co-spot low loads of new sample types with authentic standards in order to confirm peak designations.

Although there is less information available with the simple approach used in Figure 2.4 it is certainly a good starting point for samples of unknown lipid content, or for a first-time user of the Iatroscan. With one-step separations of is often possible to reduce the developing time without seriously affecting the separation. Thus with a single frame of ten Chromarods it would be possible to quantify the major lipid classes in ten different samples within half an hour of spotting.

A separation such as the one in Figure 2.4 would also be adequate if, for instance, one was mainly interested in quantifying wax ester content. WE is by far the major component in the sample (note the change in attenuation in Fig. 2.2b), so that given the precision of FID responses (Table 2.5), the presence of other minor components under the same peak would not seriously affect quantification. However, for all sample types it is advisable to perform at least one extended analysis in order to gain greater confidence in peak identities.

1.10

Figures 2.1 and 2.2 also provide good examples of generations that could not be replaced by a single-step. development. In TLS separations of neutral lipids, phospholipids are often left at the origin (Fig. 2.4). An FID scan of this material leads to an estimation of the amount of 'polar lipid' in the sample. This material is given the same abbreviation as that used for phospholipids, and it is aften takitly implied that this FL measurement is directly representative of phospholipid' reatent.

However, the magerial remaining at the origin can often be subdivided into two or three major components, as can be seen from the final scans in Fighres 2.1 and 2.2.

A multi-step scheme has been used here to obtain a relatively pure band of "phospholipids on the Chromarods. It involves the use of acetone and a MeOH-containing solvent system (Table 2.2) and it is based on procedures used in 'silicic' acid column chromatography al., 1967; Christie, 1973). Acetone separates (Rouser et glycolipids, pigments and any remaining neutral lipids from phospholipids, 'and the 'polar variation of a phospholipid-developing solvent systèm used here (Table 2.2) moves phospholipids away from the point of application. The material left at the origin has been termed non-lipid material and is presumed to consist of traces of proteins, carbohydrates, humic material and particulate material. Acetone has been used previously in marine lipid class separations on Chromarods (Delmas et al., 1984; Harvey et al., 1984), but for shorter developments. The length of time used here (Table 2:2) for the final developments is probably excessive for many samples (e.g. Fig. 2.2) and could be replaced, for instance, by a single 10 min development followed by an 8 min dévélopment in the MeOH-containing solvent system. However, the particulate lipid sample in Figure 2.1, shows a situation where the extensive development times are necessary. In order that the minor classes could be quantified in this scan, a high load was applied to the Chromarod and thus broad AMPL and PL peaks are present in the final scan. In addition, both of

these classes show some splitting into their component parts, but the developments were extensive enough to permit the identification of the three major classes and to allow a summing of the various parts of these classes for their quantification.

The multi-step approach to obtaining a pure phospholipid value is not, however, foolproof. As with any peak, an absolute positive identification or quantification of the various compounds within a PL peak is not possible, but an indication of the presence of nonphospholipid compounds has been observed in particulate samples. With high sample-loadings, some blue colouration has been seen in the PL band on the Chromarod. This could well indicate the presence of a blue rearrangement product of fucoxanthin (Jensen, 1954; Hallegraeff, 1981).

Multiple development schemes (Figs. 2.1, 2.2; Table 2.2) require, extensive use of the stop-scan facility in the latroscan. In order that the scan be stopped at the right position, one of the rods which has been spotted with the sample has to be completely burnt, and measurements have to be made on the chromatogram of the distance to the desired scan-stop point. After converting this distance to a distance to be burnt on the Chromarods, the scan-stop screw can be set at the desired position for the remaining rods containing the same sample. This procedure has to be repeated whenever it is necessary to change the scan-stop position for other ceparations on other rods.

ñ.º

When using the scan-stop screw in the Patroscan, a relatively large separation between classes is required in order to avoid carry-over into the next development. This is because different rods often show slight differences in the  $\underline{R}_{\underline{f}}$  values and lengths of bands to be separated by partial scans. This can be a result of using rods of different ages or of spotting different volumes of solvent or different loads of solute. It would not be possible, for instance, to halt the scan with the set-screw facility between FFA and ALC in a Figure 2.1 and obtain complete scans of TG and FFA, and only TG and FFA, on more than a few rods. A simple way of overcoming these problems is to install a normally closed switch in series with the latroscan's microswitch for stopping scans (Fig. 2.5). Using this suitch it is possible to stop the scan at any position on any rod; the decision at which point to stop can be made according to the trace that is being produced on the chart-recorder during a scan.

2.3.3 Quantitative deterministion of aquatic lipid classes The standards used to represent aquatic lipid classes (Table 2.1) were chosen on the basis of a review of reports on lipids in dissolved and particulate matter (Chapter 1). In the belief that each rod should be considered as an isolated analytical unit, calibration curves were initially constructed for each standard on each individual rod of a set (Pelmas <u>et al</u>., 1984). Subsequently it was found that the increase in precision over the use of interrod data (Parrish and Ackman, 1985) was not enough to warrant the considerable

81 🖑

extra effort involved in calibrating each rod individually. Thus calibration curves (Fig: 2.6) and estimations of analytical precision (Tables 2.4, 2.5) are obtoined from all ten rods within a set. However, before starting any extended period of TLC/FID analyses it has been found to be very useful to group rods with similar properties together. By measuring a single compound on each rod of several sets after short developments in a polar solvent, an estimation of the magnitude of the response and the coefficient of variation (CV) can be duite quickly obtained for each Chromarod. The rods can then be rearranged into sets of ten of similar properties and each frame can then be considered as an analytical unit.

82

Previously it was found that the precision of repeated analyses of standards was usually between 10 and 15% with about seven analyses per compound (Delmas <u>et al.</u>, 1984; Parrish and Ackman, 1985). These, early CVs were obtained from Introscans operated at a lower scan rate and a lower hydrogen flow than was used in the present study, and all the standards were spotted with disposable capillary pipettes. The lower latroscan settings were used earlier in the belief that incomplete pyrolysis would be a problem at a higher scan rate (Crane <u>et al.</u>, 1983), and that rod life would be reduced at a higher hydrogen flow (Ackman, 1981a). It now appears that these precautions were unnecessary. In the present study where higher settings were used, it was possible to analyse over 500 samples on the one set of 10 rods and, except in the case of NLM, small peaks were detected in rebuths only when high loads (>25 µg) of certain classes were pyrolysed.

The higher settings used here were an attempt to increase FID response and to improve peproducibility (Ackman, 1981a). Double developments were performed for the same reason. It has previously been found that FID response could be increased by producing narrower bands, on Chromarods (Parrish and Ackman, 1983b). The use of a second development in the same solvent system reverses the normal, chromatographic process of band-broadening and thus a higher response is obtained. A double development was not performed before the second scan (Table 2.2) because of problems with the TG/FFA, separations at high loads and because precision was not improved in a previous study, where a double development was performed before this scan (Parrish and Ackman, 1985).

The use of CuSO4-impregnated Chromarods has also been suggested as a means of improving FID response (Kaimal and Shantha, 1984). A brief investigation of the use of CuSO4 indicated that there would be no particular advantage in quantification over the procedures used here, and that there might be some deterioration in separation capabilities.

Tables 2.4 and 2.5 suggest that there has indeed been some improvement in precision for individual compounds. The CV is now usually better than 10% with only about 4 analyses per compound. At least part, of the improvement observed with most compounds is undoubtedly due to the use of a syringe fitted with a repeating dispenser for sample application, rather, than the disposable capillary tubes that have been used in the past (Ackman, 1981a). Read (1985) reported that the use of a similar wringe application method could reduce the standard deviation of the latroscan measurements to as little as a third of the values obtained from the traditional method. The poor precision obtained with FFA (Table 2.4) appears anomalous and it may be related to the tendency of this peak to tail in the solvent systems used here.

By comparison with the instrument precison given in Tables 2.4 and 2.5, the total analytical precision for quadruplicate samples is usuilly between 15 and 30%. CWs even as high as 85% are not uncommon, especially for classes present in lesser amounts in extracts, or when less than four separate extractions were possible. This degree of imprecision might seem surprising at first glance, but it should be realised that even with the most entrenched or the most expensive equipment available, 20% total analytical precision is very common, (e.g. Jungclaus et al., 1978; Gourn and Saliot, 1980; Wakeham et al., 1980; Barrick, 1982). Instrument precision is clearly not the weak step in the measurement of aquatic lipid classes. Problems with replication probably arise at the extraction stage, during storage; or simply as a result of slight differences in sample handling, • Another reason for maximizing FID responses was that it was hoped that truly linear FID response curves could be obtained. It should be noted that the term 'linear' has often been employed somewhat incautiously by latroscan users when referring to FID responses. For a detector to be truly linear, not only must a straight line

reglession be obtained from regressing FID response on load, but the intercept must not be significantly different from zero-zero. In more formal terms, the detector should obey a power law equation  $(y=aX^b)$ where the exponent, known as the response index, should be between 0.98 and 1.02 (Scott, 1983). In the Istroscan literature, calibration curves whose intercepts are far from the origin are frequently used a as an indication of the linearity of the FID response. The same has even 'been said of. Iatroscan, data plotted on log-log plots where the slope is clearly different from 1.0. If a plot of response versus load curves upwards as in Figure 2.6, then the response index is greater than 1.0 and the detector response is referred to as being 'superlinear'. There are two important consequences of the superlinearity of the latroscan-FID detector. The first is that multi-level calibration curves are essential and the second is that it is possible to get differ t responses for the same amount of, compound depending on the width of the band on the Chromarod (Parrish and Ackman, 1983b, 1985; Rao et als, 1985). With a truly linear response a single-level calibration would suffice for the enture linear dynamic range, and the same amount of compound would always give the same response within this range.

It can be seen from, Figure 2.6 that despite all efforts, the câlibration curves are still distinctly curvilinear. Although the coefficients of determination  $(r^2)$  in Figure 2.6 (b) are both higher than  $0.97_{s}$  the lines clearly pass above the majority of the data points hear the centre of the range and below the majority at both

. 85

ends. As a result, these regressions could not be used for quantifying samples with class-loads below 2.5 µg. It is for this reason that quadratic, regressions have been used routinely for v calibration purposes (Delmas et al., 1984; Parrish and Ackman, 1985). An unexpected benefit of the attempts to improve FID response is the close grouping of calibration data in Figure 2.6. Previous calibration data were spread over a much broader range at each level (Delmas et al., 1984; Parrish and Ackman, 1985). Beyond about 5 µg the data tend to fall into one of two groups (Fig. 2.6), so that it, is possible to distinguish higher-response compounds (WE, SE, ST) and lower-response compounds (HC, KET, TG, FFA, ALC, PL). Within each gratp, calibration lines tend to cross and recross (Fig. 2.6a) suggesting that one calibration curve might suffice for the low response 'compounds and that another might suffice for the highresponse compounds. This is supported by comparing the singlecompound precision with the precision of all the data pooled together in the two categories (Fig. 2.6b; Tables, 2.4, 2.5). The error bars for the pooled data are not usually any wider than for data obtained from a single compound. It is not known why WE, SE, and ST give a significantly higher response than the rest, but it should be noted that the proximity of the responses of these compounds to one another is less than for the low-responding compounds: the mean precision is not improved by pooling the data despite the larger number of analyses at each level (Table 2.5).

*-* 86

Kaımal and Shantha (1984) also found that several of the classes scanned on CuSO4-impregnated Chromarods gave similar responses and had callibration curves which crossed one another. It would seem that the similarity in response to several compounds could be a fundamental property of latroscan measurements if efforts are made to maximuze FID response. This could prove to be a valuable asset in the case of lipi& classes where there may be a mixture of compound types (e.g. WE) or where the identity of the majority of the compounds is uncertain (e.g. AMPL). With both these classes there is likely to be a significant level of a compound that has a structure different from that of the main contributor to the band on the Chromarod (Table 2.3). If the responses to different compounds within a single band on , a Chromarod were very different, then it would not be possible to quantify these two particular chromatographic peaks in natural materials. This situation would be analogous to one that occurs inhigh-performance Liquid chromatography (HPLC). The use of short. wavelengh UV detection for quantification 🆓 HPLC-separated lipid classes is not possible unless there is a known or constant number of double bonds in each sample (Aitzetmuller, 1982).

In TLC/FID studies involving aquatic lipid classes (Ghapters 3 - 5) WE and AMPL have been calibrated on the assumption that the component's gave a high response. Stearyl palmitate and cholesteryl palmitate, which cannot be separated (Table 2.3), do indeed give similar high responses (Fig. 2.6), and chlorophyll <u>a</u>, which, accounts for about 50% of the AMPL peak in <u>Phaeodactylum tricornutum</u> (Chapter, 3), has a response similar to ST (Delmas <u>et al.</u>, 1984; Volkman <u>et</u> <u>al.</u>, 1986). It should be noted that undeveloped PL peaks give a high response (Delmas <u>et al.</u>, 1984), but after development, the response is lower (Fig. 2.6), as would be expected from developed Chromarod bands (Parrish and Ackman, 1983b). In terms of maximizing FID response, double developments (Table 2.2) are clearly not as effective as no developments at all. However, precision is improved after developing because the material is more evenly spaced around the rod; it is also possible to differentiate PL from NLM.

The range of responses in Figure 2.6 corresponds to compounds of videly varying structure and molecular weight. This range could be indicative of the bounds on the possible error in the estimation of the lipid class content in a sample. If the range is representative of responses from the major non-volatile lipids that could be present in an aquatic sample (Chapter 1), then the possible error through uncertainty in the actual composition of a class should be relatively small. There is, whoveen one major difference between the standards. used-(Table 2.1) and aquatic lipids in general. Aquatic lipids, and especially marine lipids, are characterised by a relatively high proportion of polyunsaturated fatty active, but because of lability none is present in Table 2.1. However, Fraser <u>et al</u>. (1985) demonstrated that this should have little effect on TLC/FID calibration.

Figure 2.3 suggests that the range of responses in Figure 2.6 is indeed a reasonable representation of responses to lipids in aquatic

samples. There, is a strong linear relationship ( $r^2=0.96$ ) between gravimetrically determined lipid weights and latroscan measurements, and the intercept is not significantly different from zero. On average, the latroscan gives 84(+9)% of the gravimetric value (n=27). There is, however, clearly a dependence on sample type (Fig. 2.3), suggesting that the quantification procedures used here are, in the more applicable to some aquatic samples than to others. end. Nonetheless, the overall comparison is favourable, and it supports the value of 88.2(+2.0)% reported by Sasaki and Capuzzo (1984) for a similar intercomparison performed on a much larger number of marine invertebrate samples. The accuracy of TLC/FID appears to be equally as good on an individual class basis (Fraser et al., 1985). A value of 93(+16)% can be calculated from the data of Fraser et al. (1985) for TLC/FID versus combined gravimetric, colourimetric and densitometric procédures for lipid classes. It is interesting that on average, TLC/FID seems to give slightly fover values' than are obtained with other techniques. This may reflect the fact that TLC/FID is strictly applicable only to non-volatile compounds: µgloads of naphthalene and methyl naphthalenes give no response. These relatively volatile compounds presumably evaporate from the rods before the band is directly over the flame and under the collector electrode.

The accurate determination of lipid classes in aquatic samples does not depend entirely on an accurate calibration of the FID. It is also sample lipids that are actually measured in a Chromarod scan. In addition, a representative blank must be measured for each sample, unless it can be shown that blanks are identical and preferably insignificant for all samples.

For dissolved and particulate marine samples (Fig. 2.1), the use of an internal standard, <u>n</u>-hexadecan-3-one, has been found to be very, useful for calculating lipid class concentrations in seawater. The term 'internal standard' is a generic one: it has been applied to ketones added to seawater samples before extraction in order to measure recovery (Paradis and Ackman, 1977; Delmas <u>et al.</u>, 1984), as well as to ketones added to Chromanods to compensate for variations in detector response (Parrish and Ackman, 1985). Keith <u>et al.</u> (1983), recommend the use of the term 'surrogate spiking' for the addition of compounds in order to determine recovery efficiency.

Figure 2.2 suggests that an aliphatic ketone should not be used as a surrogate spike in samples containing a significant proportion of invertebrate lipids. The large WE peak in Figure 2.2(a,b) yould cause problems with the quantification of KET, and there is a significant KET peak in the freshvater invertebrate chromatogram shown in Figure 2.2(c). TLC/FID analyses by Volkman <u>et al</u>. (1986) also indicate that KET could not be used for a certain class of coccolibhophorids, nor for anomic surface sediments.

This inability to use KET may not, in fact, be a problem in TLC/FID anclyses of these types of equatic samples. In the case of sediment camples, net-tow samples, and samples from algal coltures it is

usually possible to obtain much higher concentrations of lipids than is normally the case with dissolved and particulate water samples. This means that the amount of sample concentration required for TLC/FID measurements is much smaller. This an turn reduces the amount of sample handling and the possibilities for sample loss. Under these conditions, nearly quantitative recoveries during all sample transfers are a reasonable proposition: all that is needed is an accurate determination of solvent volumes at each step between extraction and application to the Chromarods.

When only small amounts of sample are available, an alternative approach that has similar results is to use microtechniques requiring ' only small volumes of solvent's (Gardner et al., 1985). "Thechromatograms in Figure 2.2(a,c) were obtained from samples handked" in this manner. This approach also permitted a direct comparison between gravimetry and latroscan measurements on these small samples (Fig. 2.3). The measurements in Figure 2.3 are not corrected for nonhypid material or blånks. NLM was assumed to have a response similar to that of a low-response lupid (Fig. 2.6), and the NLM concentration was added to that of the lipid classes to produce the Latroscanmeasured total in Figure 2.3. Not knowing the true FID response to NLM should not be a problem since the highest measured proportion of NLM in these samples was 7% and most samples had values below 4% of the total. Blank values for these samples duere also usually low. Between the blanks for the freshvater samples and the matine samples, each of the aquatic lipid classes detected in the samples
(Figs. 2.1, 2.2a,c) has been measured at least once in a blank. The level of the blanks for these classes were, however, often below 5% of the total sample measurement for the class, and it was nearly always possible to keep the levels below 15%. Values beyond this were taken to be cause for concern, and attempts would be made to eliminate, the source of contamination. This procedure would involve a stepwise elimination process that started with a complete recleaning of all glassware and Teflon surfaces. It would also include, when necessary, a check on impurity levels in solvents used in developing tanks, in extractions and in cleaning, and finally the replacement of the activated charcoal and molecular sieves used for purifying the nitrogen supply.

· 2.4° Conclusions

"uatic lipids can be efficiently extracted into dichloromethane and then rapidly concentra d by evaporating the solvent. Samples are spotted in several µl f dichloromethane onto silica gel coated 'Chromarods which are held in frames. The solute can be refocused into narrow bands with short developments in polar solvents. By spiking samples with a known amount of internal standard before extraction. it is possible to obtain an estimation of 'lipid recovery from the amount of the surrogate spike that is measured on the Chromarod.

For solvent focusing and for the separation of lipid classes the frames are placed in tanks containing pure solvents or solvent mintures. Only about 50 ml of solvent is required for each tank. After development, each frame is placed in the Iatroscan for the detection and quantification of the material separated on the rods. Each rod in a frame is passed in turn through the FID which responds to carbon ions produced from the pyrolysis of organic compounds.

In theory, any non-volatile carbon-containing compound should be measurable with this system. It should be feasible, for instance, to extend the use of the fatroscan to other environmentally and ecologically important classes like carbohydrates, proteins, and humic and fulvic acids.

Since the separation part and the detection part of this TLG#FID system are independent, and since it takes only 30 seconds to completely scan each rod, a large number of samples can be processed in a day. Several people can be working with different sets of rods and they may only require one latroscan for detection and quantification. In addition, experienced operators can work with two or three sets of rods at a time. This means that with even the mostcomplex samples and the most involved separation and scanning procedures, the processing of ten to twenty different samples per person per day is a reasonable proposition. This is more than can be processed by GC or conventional HPLC. The initial outlay and the running costs are less than with GC or HPLC, and TLC/FIP has the potential to measure a much broader range of compounds.

Having made favourable comparisons with conventional chromatographic techniques it is important that the major draubacks of TLC/FID are made equally clear. The detector is non-linear so that

calibration is more time-consuming and more complicated than in GC or HPLC. The detector is also orders of magnitude less sensitive than most detectors available for GC or HPLC, and TLC/FID analyses may be as much as an order of magnitude less precise. However, with the advent of new detector designs and new sample applicators (Patterson, 1985; Read, 1985), this may all soon change.

94

Ø

PARTICULATE AND DISSOLVED LIPID CLASSES IN CULTURES OF <u>Phaeodactylun tricornutun</u> GROWN IN CAGE CULTURE TURBIDOSTATS WITH A RANCE OF SUPPLY RATES OF INORGANIC NITROGEN

Chapter 3

3.1 Introduction

There has been considerable interest in lipid production in algal ultyres. Parsons <u>et £1</u>. (1961) grev eleven species of algaésimilar chemical and physical environments and found ratios of total lipid to carbon or total lipid to chlorophyll a to be similar for all species. They underlined the importance of environmental conditions in obtaining algae of similar chemical composition. The results they obtained suggest that, under a given set of environmental conditions, it is possible to generalise from culture data from, a few species to culture data for several more species. Ackmaniet al. (1964, 1968) found the lipids of twelve species of cultured phytoplankton to be characterised by large amounts of Gib: 6 fatty acids as well as by significant amounts of C20:5 and C22:6. The latter is an unbranched fatty acid with 22 carbons and 6 double bonds, and both the latter compounds belong to the n-3 series of fatty acids. These are acids with the final double bond ending 3 carbons from the methyl end of the molecule. These two acids are characteristic of aquatic lipids (Gunstone, 1967), and they have been receiving considerable attention recently in medical research because of their apparent beneficial effects on human health (Goodnight et al. / 1982; Glomset, 1985).

Lipid production in álgae has been examined in terms of the nutritional requirements of oyster farvae (Chu and Dupuy, 1980). Algae have long been accepted as a direct source of nutrition for humans (Ackman, 1981b; Aaronson and Dubinsky, 1982; Pirt, 1984), and attempts have been made to increase algal lipid content with a view to providing a suitable food source for the inhabitants of space vehicles (Richardson et al., 1969); Lipid production has also been proposed as a means of providing renewable energy fuels, either by direct production of hydrocarbons (Tornabene, 1981; 1982; Pirt, 1984), or after processing other algal lipids (Shifrin and Chisholm, 1980). Algal lipids may also be a source of specific compounds for industry (Aaronson and Dubinsky, 1982).

It can be seen that culture studies involving lipids are of interest in themselves, as well as in terms of projecting the results topaquatic ecosystems. An understanding of factors affecting lipid production by microalgae in the laboratory should give insights into the dynamics of the production of compounds with high calorific value in the oceans. This information could also be used to manipulate the lipid content of algae grown in large-scale cultures to provide optimal lipid production for commercial applications.

This study is a new approach to the examination of lipid production by cultured algae. A new culturing unit has been used: the cage culture turbidostat. This system was recently introduced as a device for the rapid determination of algal growth rate (Skipnes <u>et al</u>., 1980). In the present study a complete range of lipid classes has been measured in both the particulate and the dissolved fractions from turbidostats. Most studies have determined only the total lapid content of the phytoplankton or the fatty acids of some of the particulate lipid classes. We have analytical technique has been used to perform the lipid class measurements: the Chromarod-latroscan (TLC/FID) system which has recently seen considerable use in biomedical research (Ackman, 1981a). Chapters 1 and 2 give a detailed description of the analytical technique used here and its relationship to other techniques. Figure 3.1 shows the relationship between the cage culture turbidostat and more commonly used culturing

devices.

Batch cultures are the most common type of culture used in experimental work with phytoplankton (Fogg, 1975). In this type of culture, cells are ineculated in a limited volume of vater in glass or plastic containers (Fig. 3.1a). The great advantage of batch cultures is their simplicity, their disadvantage is that conditions are constantly changing in the culture vessel. Growth rate changes with time, and so does the physical and chemical environment of the cells (e.g. light, nutrients and toxic agents). Free transport between the inside of the culture vessel, and a supply of fresh medium would stabilize the chemical environment of each cell. This can be achieved by growing microorganisms inside dialysis bags (which are exposed to a large reservoir of medium. Nutrients diffuse through a semipermeable membrane to the microorganisms, and the exudation products pass, to the outside, With largen phytoplankton it is possible to use material that has pores with diameters of the order of microns, so long as it is transparent. The largest possible poresize should produce the closest possible similarity between the inside and the outside of the enclosure. Under these conditions dialysis is no longer an important process and the generic term 'cage culture' is used (Sakshaug and Jensen, 1978). Figure 3.1(b) shows a

The use of cage cultures does not, however, overcome the problem of variable growth rates and an increasing degree of self-shading by the algae (Sakshaug and Jensen, 1978). This can be achieved only with 'continuous' cultures. Here, not only is the environment of each cell stabilized by periodic exposure to fresh medium, but there is alse 4 mechanism for regularly removing cells from the culture vessel. This can be just a simple overflow system. In this way a culture can be held at some chosen point, on its growth curve. A further attraction of this type of culture is that it is amenable to automation. There are two types of continuous cultures that are regularly used in experimental work with phytoplankton, the 'chemostat', and the

'turbidostat'.

gure 3. Schematic representation of phytoplankton culturing systems. (a) <sup>°</sup> Batch culture. (b) Dialysis or cage culture. (c) Chemostat: the medium is supplied at a constant rate. te a Turbidostat: the medium is supplied to the culture by a pump (d) which is controlled by a photodetector. Cage culture turbidostat: the medium is supplied at a constant (e) rate. Medium is removed from inside the culture by a pump which is controlled by a \*photodetector.



Chemostats are supplied with medium at a constant rate (Fig. 3.1c). The population density adjusts itself to the rate of supply of the 'limiting' nutrient. Turbidostats (Fig. 3.1d) are similar to chemostats except that the population density in the culture is monitored by a photosensitive device. This measures the turbidity in the culture vessel. If the density increases beyond a predetermined point, the photodetector causes more medium to be passed through the vessel. One of the problems with this type of system is that at low growth rates the algae spend most of their time under conditions resembling those found in a batch culture: any further input of fresh medium has to avait the replication of enough cells to change the turbidity of the culture.

2.0

A recent innovation has been to combine the cage culture technique with the turbidostat means of °density regulation (Skipnes <u>et al</u>, 1980). Figure 3.1(e) is a diagrammatic representation of such a system. With this system a flow of fresh, medium is maintained continuously, and the population of phytoplankton is kept constant by removing cells as soon as the turbidity in the culture exceeds a threshold. Cage culture turbidostats provide a very stable environment and healthy cultures can be maintained for periods of months.

The supply of nutrients plays a key role in algal grouth in cultures and in the natural environment. In most aquatic environments the supply of not rogen, phosphorus, or silicon is at least periodically grouth-limiting. There are several species of marine and

freshvater phytoplankton which accumulate total lipid as a result of nitrogen or silicon starvation (Collyer and Fogg, 1955; Shifrin and Chrisholm, 1981). However, an examination of data presented by these authors, and by Richardson <u>et al</u>. (1969), indicates that members of the same genus respond to nitrogen starvation differently, and that even members of the same species may not accumulate lipid as a result of nitrogen starvation in one type of culture system, while accumulating substantial amounts in another type. It seems likely that a factor other than simply a lack of nitrogen may be involved. It is also likely that since lipids are a heterogeneous group of compounds of varying structures and function's (Chapter 1) the measurement of total lipids may be masking different trends occurring among the individual lipid classes.

There have been few studies on the effects of nutrients on lipids within phytoplankton cells. There have been even fewer on lipids released extracellularly by living cells (Hellebust, 1974). Nutrient saress is believed to be one of the more important factors influencing extracellular release of organic compounds (Wangersky, 1978; Jensen, 1984). In the present study a complete range of intracellular and extracellular lipid classes has been measured in both nitrogen-stressed and nitrogen-replete cultures.

The alga used in this study is the marine diatom <u>Phaeodactylum</u> <u>tricornutum</u>. A survey by Ansell <u>et al</u>. (1964) of the first publications containing, lipid data for this alga indicated that the intracollular lipid content was very variable. Values from 2 to 343.

of the dry weight were published between 1930 and 1964. Ansell <u>et al</u>. (1964) suggested that the available nitrogen supply was 'the most influential single factor causing this variation'. Recently, Thomas <u>et al</u>. (1984) found that although they could increase the lipid content of <u>P. tricornutum</u> to 30% of the dry weight under extreme nitrogen deficiency, there was no increase in the lipid yield (the product of the lipid composition and the growth rate). The present work with cage culture turbidostats examines the yield under a range of nitrogen supply rates.

## 3.2 Nethods

Three cage culture turbidostat units were used to grow <u>Phaeodactylum</u> <u>tricornutum</u> with varying supply rates of nitrogen. The units used were modifications of that described by Skipnes <u>et al.</u> (1980). Figure 3.1(e) is a diagrammatic representation of such a unit; it is described in detail by Wangersky <u>et al.</u> (submitted).

The volume of the culture vessels was 400 ml and approximately 3 1 of medium passed through each of the vessels per day. The medium supplied to the phytoplankton was a modified f/2 (Guillard and Ryther, 1962). Dialyzed seawater was used as the base of the medium. The relative proportions of the nutrients in the medium were adjusted so that at low nutrient levels nutrogen would be growth-limiting. NH4Cl was used as the nitrogen source and the amount entering and leaving the turbidostats was monitored regularly. Cell concentrations were also measured regularly with a Coulter Counter (Model 1015 ZB).

With these measurements it is possible to calculate the picogrammes (pg) of nitrogen offered to each cell per 24 hours (Fig. 3.2a), and the amount taken up by each cell each day (Fig. 3.2b). The turbidostat cultures were held at a variety of hitrogen supply rates. The different values of the supply rates were evenly, spaced for most of the experiment, but were more closely spaced at the lowest, end of the scale (Fig. 3.2a).

1 64

The temperature of the cultures was held at 20 the light intensity at 70  $\mu$ E m<sup>-2</sup>s<sup>-1</sup> with a light-dark cycle of 16:8 hr. The threshold at which the turbidity-controlled pump removed cells from the medium was maintained at the same level for all three culture vessels throughout the experiment. Growth rate was calculated as the proportion of the volume of the turbidostat that was removed in a day. The specific growth rate, u, is used throughout this Chapter (doubling times or the number of divisions per day involve a logarithmic relationship). The product of the specific growth rate and the lipid class composition of the cells was used to estimate particulate yields. Yields of dissol ad lipid classes were obtained by measuring the lipid class concentration in the in Aming medium and in the effluent media from the turbidostats. The analyses of the lipid classes were performed on dichloromethane extract's on an. Iàtroscan MK III (Iatron Laboratories, Tokyo) using modifications of previously described procedures (Delmas et al., 1984; Parrish and Ackman, 1985), as detailed in Chapter 2. Table 3.1 shows the organic classes detected in the extracts.

Dissoluted lipids were extracted from between 250 ml and 1 1 of seawater. Particulate samples were usually obtained by filtering 50 ml of philoplankton culture (10<sup>6</sup> cells/ml). These samples were extracted by grinding the filter, initially in dichloromethanemethanol (2:1), and subsequently in 100% dichloromethane. The extracts were washed with purified water (Whitehouse, 1988; Parrish, Chapter 2). Samples were spiked with an internal standard, heradecan-3-one, immediately after filtration, and total procedure blanks were run on each day of analysis. Blank levels for most lipid classes were usually below the detection limit of the analytical procedure. Several samples were extracted without the addition of the internal standard to 'check that there were no significant level's of any compounds that could interfere chromatographically with hexadecan-3one.

Yields of dissolved lipids were obtained for cultures near the two extremes of nitrogen supply rates used here. Particulate lipids were measured at the extremes of nitrogen-stressed and nitrogen-replete conditions and at several points in between. Chlorophyll <u>a</u> (CHL) was measured daily in a Turner fluorometer.

1-95

Figure 3.2

Histograms of the nitrogen budget for cage culture turbidostats.

(a), The amount of nitrogen offered to each cell.

(b) The amount of nitrogen used up by each cell. -

(C) The amount of surplus mitrogen in the effluent in microgrammeatoms per litre.

(d) Nitrogen used as a percentage of thet offered; the histogram cells, are reversed so that high percentages correspond to low rates of nitrogen supply and nitrogen uptake in the prededing histograms.



• · · · · ·

Non-volatile organic classes detected at the picogramme per cell level (pg/cell) and at the µg/l level in extracts of particulate and dissolved matter from cage culture turbidostats.

108

\*\* ~ Organic class Abbreviation Aliphatic hydrocarbons HC Sterol esters and wax esters WE Methyl esters and other short-chain esters ME Ketones\* KET Triglycerides TG Free fatty acids FFA Free aliphatic alcohols ALC Free sterols ST**Diglycerides** DG Acetone-mobile polar lipids\*\* AMPL Phospholipids and other acetone-immobile polar lipids  $\mathbf{PL}$ Non-lipid material

\*Samples were usually spiked with an internal standard, hexadecan-3-

AMPL includes chlorophyli a (CHL).

Table 3.1

n 1.

3.3 Results

The amount of introgen offered to the cells, the amount used by each cell, and the surplus in the effluent from the turbidostats were all closely correlated (Table 3.2). The cells continued to take up more and more nitrogen per day as the supply increased, but at the same time a greater and greater proportion of the nitrogen supplied was passing through the culture vessel unused. At low rates of mitrogen supply the proportion of the nitrogen in the incoming medium used by the cells was much higher than at high supply rates. Most cultures either used 80 to 90% of the nitrogen supplied o else only between 5 and 10%. This suggested a means of categorisang the cells grown in this experiment. On the basis of the histogram in Figure 3.2(d) the dividing line between the two categories has been. taken to be around 45% mitrogen used. Those cells using more than this have been assumed to be nitrogen-stressed, while those using, less than 20% have been assumed to be growing under nitrogen-replete conditions.

TLC/FID chromatograms for the two types of cells were different in one major respect: triglyceride was a minor constituent of cells grown under nitrogen-replete conditions, but a major constituent in nitrogen-stressed cells (Fig. 3.3). The pigment-containing AMPL peak (abbreviations are explained in Table 3.1) was significant in all chromatograms, as was the PL peak. HC, FFA, different types of alcohols and different types, of esters were also detected in smaller amounts in most samples. Table 3.3 gives the mean amounts of the

various lipid classes detected in P. tricornutum grown under nutrient-replete conditions The variability in lipid class amounts per cell among the ten different cultures being supplied with different batches of media was quite small for the major lipid classes; the coefficient of variation (CV) was usually less that 35%. The variability was greater for the minor lipid classes but this is. as likely to be associated with the analytical procedures as it is an indication of differences among the cultures. Because of the large differences between the amounts of the major and minor classes, the latter were often analysed at levels close to the detection limit of the latroscan. For more accurate and precise quantification, a second set of analyses would have had to be performed using higher concentrations of lipids. Occasionally, P. tricornutum growing in nitrogen-deficient media would change from its normal unicellular forms to a chain-form. The lipids in this chain-form were measured on two separate occasions (Table 3.4). TLC/FID chromatograms for the chain-form were very similar to those obtained from nitrogen-deficient, unicellular, P. tricornutum (Figs. 3,3b,c). Since it is not possible to count cells. in the chain-form, no internal standard was added (Fig. 3.3c), and lipid classes could only be given as percentage composition (Table 3,4). The nitrogen supply rate could not be calculated on a per cell basis either, and so only the incoming nitrogen concentration is given in Table 3.4.

Figure 3.3®

scale deflection.

TLC/FID chromatograms of particulate lipids from cells in cage cultures of <u>Phaeodactylum tricornutum</u> grown in nitrogen-deficient and nitrogen-replete media. Abbreviations explained in Table 3.1. P Recorder attenuation is given under each chromatogram in mV full-

111

(a) Unicellular organisms grown under nitrogen-replete conditions.
(b) Unicellular organisms grown under nitrogen-deficient conditions.
(c) Phaeodactylum tricornutum in chain-form obtained from a nitrogen

deficient culture (19 µg-at N/1 in the incoming medium). No internal standard was added to this sample. Its percentage composition is given in Table 3.4.



Table 3.2

Λ¢,

2

Pearson product moment linear correlation coefficients for the' complete range of nutrient conditions offered. Abbreviations explained in Table 3.1.

	•	N`offer	red		,	I	ı		
	, (* :			-	*				* "
*	N used (pg/cell/day)	0,92	N used	s. 7 7	-		, r	.' •	
•	%N used '	-0.74	-0:68	78N use	d.		a F	4 U	
	Surplus N (µg-at/1)	0.97	0.84	-0.80	, Surplu	ts N <sub>-st</sub>	rt Sin r	er P <sup>art</sup> ga <sup>rts</sup>	*
Ŷ	Growth (day <sup>-'</sup> )	0, 75	0.80 :	- <b>0.</b> 70 "	0.71	Growth	and a star water	**************************************	(M
	CHL (pg/cell)	0.92°	0.88	,0.86 ~ ,	0.92 *.	0~ 82	CHL.	~ etc. "	* -5;*
	, (pg/cell)`	-0.65	-0.66 "	.0: 89	-0, 67	NS*	-0.71	LIG	μ * Ι *
	Sterols (pg/cell)	0.63	- 0.73	NS	NS	0.73	<b>0.6</b> 8	NS	~
	AMPL () (pg/cell) ~~	0.72	0.77.	-0.76	0.72	0.80	0.*84	-0.66 -* ,	-
	PL , (pg/cell)	ÑS	NS Č	∿-0.79 ≁	NS	NS	0.61	-0.71	
	Total lipids <sup>°</sup> (pg/cell)	NS 7	NS <sup>*</sup>	-0.67	NS	Q.73 -	0.68	NS #	
2		,	÷ ,	4	١.			*	

Not significant at the 9% confidence level (n=17).

113 -

Table 3.3

و مور پيد ه

Lipid class composition of nitrogen-replete Phaeodactylum tricornutum in picogrammes per cell (<u>+</u>95% confidence intervals, n=10).

114

Hydrocarbons.	0.09 ( <u>+</u> 0.06)
Sterol esters and wax esters	0.04 ( <u>+</u> 0.04)
Methyl esters	0,02 ( <u>+</u> 0,01) <sup>3</sup>
Triglycerides	0.11 ( <u>+</u> 0.04)
Free fatty acids,	° 0.21 ( <u>+</u> 0.08)
Free alcohols	0.06 ( <u>+</u> 0.02)
, Free sterols	`0;06 ( <u>+</u> 0.02)
Diglycerides	0.03 (+0.03)
Acetone-mobile polar lipids	0.95 ( <u>+</u> 0.16) ,
(AMPL - CHL):	0.41 ( <u>+</u> 0.13)
Phospholipids	0.98 ( <u>+</u> 0.19)
Total lipids	2.54 ( <u>+</u> 0.29) ·

×...

"The difference between AMPL and the CHL value obtained from a Turner

fluorometer,

. Table 3.4

Percentage lipid class composition of <u>Phaeodactylum</u> tricornutum in chain-form in two different nitrogen deficient cultures (analytical precision given as <u>+</u> 1 standard deviation of the mean, n=3).

19 µg-at N/1: , 47  $\mu$ g-at N/1 Hydrocarbons . 0.3 (+0.1) 0.2 (+0.1) 0.2 (+0.1) Sterol esters and wax esters 0.3 (+0.1) 0.3 (+0.1) 0.4 (+0.2) Methyl esters, 48 (+3) 24 (+2) **Triglycerides** 0.7 (+0.6) 0.8 (+0.4) Free fatty acids 1.1 (+0.9) 0.2,(+0.1) Free alcohols () Free sterols 1.4 (<u>+</u>0.1) 2.2 (+0.6) \* 0.8 (+0.3) " Diglycerides 2.3, (+0.8)\_ Acetone-mobile polar lipids 19 (<u>+</u>4) 31 (+5) 28 (+2) 39 + (+5) Phöspholipids

\*Concentration of nitrogen supplied to the turbidostat; chromatograms for this culture given in Figure 3.3(c).

**T**¥5

TLC/FID chromatograms of the dissolved lipid classes from nitrogendeficient and nitrogen-replete cultures were quite similar, and not very different from those of the medium supplied to these cultures (Fig. 3.4). For several of the classes one-way analysis of variance . -indicated no significant difference in lipid class concentration before and after passing through the turbidostats. Where there was a significant difference the production rate per cell has been calculated (Table 3.5). The variability in nitrogen supply per celk in Table 3.5 reflects small différences in cell numbers and flow rates among the turbidostats. The variability in the particulate lipid production rates for the three cultures being supplied with the same medium was usually less than 30%. Dissolved lipid production rates were more variable. Again, this may be simply an analyfical problem. There was a significant input of most lipid classes to the turbidostats (Fig. 3.4a), so that the differences between input and output concentrations were sometimes quite small by comparison with the total amounts measured. One way around this would be to slow down the flow of medium, so that the concentration of dissolved lipid classes in the effluent would be higher. Nonetheless, even with small differences between input and output, the CV for most dissolved lipid class production rates was still below 35%.

Figure 3.4

TLC/FID chrömatograms of dissolved lipids in the ingoing medium and the effluents from cage cultures of <u>Phaeodactylum tricornutum</u> grown under nitrogen-deficient and nitrogen replete-conditions. The attenuation for all 3 final scans is 32 mV full-scale deflection. The preceding scans are all st a lower attenuation (mostly 16 mV).

(a) Dissolved lipid class composition of the ingoing medium:

(b) Dissolved lipid classes in the effluent from a nitrogen-replete

(c) Dissolved lipid classes in the effluent from a nitrogen-

Ø



ble 7 4

Production rates of particulaten and dissolved lipid classes per Phaeodactylum tricornutum cell under nitrogen-deficient and nitrogenreplete conditions. All values in this Table are in picogrammes per cell per day. The precision is "1 standard deviation of the mean, of 3 turbidostat measurements, \*

Nit rocent gunni ve	· · · · · · · · · · · · · · · · · · ·	05) 7	- + 315 (+3	5)
wrr rogen arhbry	• • - ·			
Production rate:	Particulate	"Dissolved	Particulate	Dissolved
Alfiphatic	0.02+.008	NS*	- 0 <sub>4</sub> 0'2 <u>+</u> .006	NS (
7 m 7 m 7 m		Antin Lusia		
Sterol esters and that esters	MDate	NS	ND .	0.02 <u>+</u> .QI
Methy,1',esters	ND	0.02+.008	ND	NS
		¥ 9	a	*
Friglycerides	0.27 <u>+</u> .01	NS	Q.07 <u>+</u> .01	NS f
έια' <sup>6</sup> ″" <sup>1</sup> <sup>1</sup> <sup>χ</sup>	تى <sup>10</sup> بە بە	*1 2	21 F 3 N -	¥ F
* Free fatty acids	0.03.+.0,1	0,03+.01	0.16 <u>+</u> .05 ' ;	'NS
Free alcohols	0.014.004	0.02 <u>+</u> .015	0.04 <u>+</u> .02	0.03 <u>+</u> .01
Sterols	0.01+.003-	ŗ NS 🌷	0.02+.01	NS
A and the second			£ <sup>3</sup>	~
Diglycerides	0.01 <u>+</u> .005	NS	0,02 <u>+</u> .001	0.03+.002
	т. Б. с. т. т. т. с.	· · · · · · · · · · · · · · · · · · ·	दे	۰
Acetone-mobile	0.15+.03	Ű. 07 <u>+</u> 02	0.52+.12	0.06 <u>+</u> .01
polarlipids	a a t	······································	*	
Phospholipids	0.07+:05	-0.01+.005	0:45+.008	ns
γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ	م میں والی میں اور		1 2 2	
.Total.lipids	ີ 0. ີ5.9 + . ີ 0ໍຣໍ້ໍໍ	0.15+.01	1.29+.29	0.14+.01

"The difference in lipid class concentration between the incoming medium and the effluents from the 3 turbidostats was not significant at the 95% confidence level (one-way analysis of variance).

. . . Not derected in any FID scans of these 3 samples.

٠,

F19

The growth rate of the cells continued to increase over the entire range of rates of nitrogen offered and nitrogen used (Fig. 3.5a). The 'datafdo, however, seem to fall into two groups with a change of slope close to 2 pg N used/cell/day. The distribution of the data suggests a Michaelis-Menten; curve would give a good fit; however, -considerably more data would be needed to test this. For the purpose of this study, two linear regressions have been used to approximate the distribution of data wherever there seemed to be a break in the slope. The choice of data points to include in the two regressions is based on Figure 3.2(d): data from cells using more than 45% of the incoming nitrogen have been grouped together, as have cells using less than 20% of the incoming nitrogen. While the plotting of data as a function of percentage N-used rather than picogrammes N-used would have been preferable in some ways, the distribution of data points prevented this (Fig. 3.2d): the distribution of % N-used data results in all parameters grouping into two clusters at each end of the 7% Nu.ed axis. In the case of the growth rate data' versus pg Nused/cell/day the correlation and the slope of the nutrient-replete data points were both significant in this experiment (95% confidence level 'n-10). This explains the continuous increase rn mitrogen uptake mates across the entire range of supply rates (Figs. 3.2a,b). The lipids per cell or per unit chlorophyll a were different in that their/distributions tended to reach a plateau: none of the slopes or correlations of the nutrient replete lipid data as significant in-Figures 3.5 and 3.6. Thus, beyond an uptakes rate of around

2 pgN/cell/day the lipid composition appears to be independent of the rate of nitrogen use. The calculation of mean Aevels of lipids per cell in Table 3.3 is therefore justified. Evidence of a plateau, or even a change in slope uss/not, however, clearly apparent in all aspects of this data set (Figs. 3.7 - 3.9). although triglycerides per unit chlorophyll a did repeat the pattern of a significant negative slope followed by a levelling off (Fig. 3.8b).

121

Of the twelve lipid classes measured, chlorophyll <u>a</u>, AMPE, and sterols, were the only classes correlated with growth at the 99% confidence level (Table 3.2. This relationship with growth seems to be quite linear (Fig. 3.9), and in the case of sterols the intercept

is not significantly different from the origin.

Figure 3.5

. .

Growth rate and lipid per cell as a function of the rate of nitrogen use per cell. Abbreviations explained in Table 3.1. Crosses represent nutrient-replete cells, boxes represent nutrient-deficient cells.

(a) Growth rate, μ, versus nitrogen use.

(b) The sum of the particulate lipid classes versus nutrogen use.

(c), Acetone-mobile polar lipids versus nitrogen use.







126 Figure 3. Lipid production per cell as a function of nitrogen use per cell. Broken lines are 95% confidence intervals for the regression lines. A, 1°~ (a) - Chlorophyll a production rate. The sum of the latroscan-measured particulate lipid classes. (Ъ) Triglyceride production rate. Er



ž
Figure 3.8

Lipid per unit chlorophyll a as a function of nitrogen concentration in the effluent from the turbidostats. Broken lines are 95% confidence intervals for the regression lines.

(a) The ratio of the sum of the Iatroscan-determined lipid classes ' to fluorometrically-determined chlorophyll <u>a</u>.

The ratio of triglyceride to chlorophyll a.

0.0

(c) The ratio of the sum of triglyceride and phospholipid to chlorophyll a.



**š**834 47

X

4-

Figure 3.9

Lipid classes as a function of growth rate. Broken lines are 95%, . confidence intervals for the regression lines.

(a) <sup>°</sup>Chiorophyll <u>a</u> per cell.

(b) Acetone-mobile polar lipids per cell.

(c) Sterols per cell:



## 3.4 Discussion

The decrease in total lipids per cell and in total lipid productivity vith increasing nitrogen-stress (Figs. 3.5b; 3.7b) appears to be in direct opposition to currently held views concerning lipid production in nitrogen-limited phytoplankton. The lack of any significant increase in the production of total dissolved lipids (Table 3.5) does not seem to support currently held views concerning the production of dissolved organic matter by nitrogen-stressed phytoplankton. What i clear from these data, houever, is that there is a change in the types of lipids produced as P. tricornutum makes the transition &rom groving in nitrogen-replete media to nitrogen-deficient media Different classes of dissolved lipids are produced under these two conditions (Table 3.5). Particulate triglycerides per 'čell and triglycerides per unit chlorophyll a both increase significantly the amount of nitrogen used per cell decreases (Fig. 3.6b, c) By comparing total lipids to chlorophyll a and plotting these data as a function of the concentration of nitrogen left in the medium (Fig. 348a) it is possible to make a direct comparison with more conventional measurements made in cultures and in the field Chlorophyll a 1s often used as a reference for measurements, if not numerically, at least conceptually. The course of physoplanktor blooms is usually followed by measuring chlorophyll a concentrations. in the water column, and other organic classes are offen expressed in Zakio to these concentrations. The concentration of surplus nitrogen the effluent from the turbidostats most nearly approximates

**,132** .

concentration that would be measured in a sample of seawater taken from a culture or from the euphotic zone during a bloom. Under these conditions it can be seen that total lipids do indeed increase with increasing nitrogen-stress (Fig. 3.8a). This is evidently the result of chlorophyll a levels in cells being more sensitive to nitrogen depletion than are other lipids.

133

The maximum level of votal lipid was seven times the chlorophyll a concentration (Fig. 3:8a). Sinde ratios of two orders of magnitude and more can be calculated from measurements made during diatôm blooms in enclosed ecosystem bags (Morris <u>et al</u>., 1983) and in the field (Chapter, 4) the dilemma is still unresolved. Neither is the answer simply that <u>P. fricornutum</u> is a species that does not respond dramatically to nitrogen deficiency. Similarly high lipid to chlorophyll <u>a</u> ratios can be calculated for <u>P. tricornutum</u> from data presented by Thomas <u>et al</u>. (1984). The answer probably lies in the culturing system used here. Richardgon <u>et al</u>. (1969) were also unable to increase lipid content in their continuous cultures. Both Shifrin and Chisholm (1981) and Thomas <u>et al</u>. (1984) employed combinations of batch and continuous cultures and found the highest lipid contentoccurred after the longest period of time in nitrogen free batch

In this cage culture study it was found that a supply rate of '' 'hitrogen that was any lower than the lowest used would result in the halting of cell division. Similarly, Richardson etoal. (1969) found that they could not further reduce the nitrogen supply to their

cultures.

chemosynts without losing the cultures. It would seem therefore, that physoplankton cells have to stop dividing before there can be massive accumulation of lipid. Microscopic observations by Op (1974b) Suggest that diatom cells accumulate large levels of lipids at the end of the stationary phase in batch cultures. Opute (1974b) also presented some evidence to suggest that an autotoxin may esponsible for the prevention of cell divisions at this stage vould be consistent with the general observation that it is not possible to produce large accumulations of lipids in algae grown continuous cultures, and that the greatest accumulation of lipids occurs when algae have been in the same medium for the greaters length of time, Cage culture turbidostats could be useful in deciding whether the dilution of entracellular products is an important factor in inducing physiological or morphological changes. By continuously supplying medium with no nitrogen to a cage culture furbidostat it would be possible, to observe, a population of highly stressed diatoms without fear of culture loss as would be the case in a chemostat." By halting the flow of this medium the cage culture turbidostat would revert to operating like a conventional furbidostat, or in the case of extreme nutrient deficiency, like a batch gulture in its stationary phase Under these circumstances, 'extracellular products yould build up in the medium, and their effects on the cells could be monitored.

3.4.1 Intracellular lipid classes

The strong negative correlation between triglycerides and phospholipids, (Table 3.2) suggests a simple mechanism that would lead to the accumulation of triglycerides in nutrient-depleted cells. The major pathway of CO, fixation in P. tricornutum is into 3-1 phosphoglycenate (Holdsworth and Colbeck, 1976). This is a direct precursor of phosphatidic acid, which is itself a precursor of both complex phospholipids and triglycerides (Gunr and James, 1980). Lecithins are the major phospholipids synthesized in P. tricornutum (Heidsworth and Colbeck, 1976). These phospholipids contain equimolar proportions of phosphorus and nitrogen, and so their synthesis in nitrogen-limited cells is likely to be hindered. In addition, the fitrogenous base cytidine is an essential cofactor in the biosynthésis of all complex phospholípids from phosphatidic acid Gurr and James, 1980; Goodwin and Mercer, 1983). This importance of nitrogen in phospholipid biochemistry probably explains the decrease in phospholipid content in nitrogen-limited cultures (Fig. 3.6). Since phospholipids and triglýcerides have common precursors it is reasonable to assume that the common intermediates are likely to be channeled towards triglyceride, synthesis if phospholipid synthesis is hindered. If this were the only reason for the synthesis of tr,iglycerides then sthe sum of the triglyceride and phospholipid contents should remain reasonably constant over the entire range of nitrogen supply rates. Figure 3.8(c) indicates that this is not the The increase in triglyceride content is greater than the

decrease in phosphölipid content. This could be the result of an inability to synthesize other mitrogen containing compounds and of having other intermediates channeled towards triglyceride synthesis. The synthesis of triglyceride under conditions of low nitrogen supply and low nitrogen use is such a clear signal (Figs. 3.3, 3.6b,c, 3.7c, 3.8b; Tables 3.2, 3.4, 3.5) that it may be possible to use triglyceride measurements to help indicate whether natural phytoplanktom populations are stressed. High levels of triglycerides have indeed been found after the time of the maximum measured chlorophyll <u>a</u> concentrations during spring blooms in Bedford Basim (Chapter 4). Thus the determination of lipid class concentrations could provide a useful addition to the biochemical indices used to assess nitrogen deficiency and growth rate in natural populations. (Dortch et <u>a</u>1., 1985).

The acetone-mobile polar lipids also gave a clear signal, but one that was different from that observed with triglycerides. The decrease in total lipid per cell with increasing nitrogen-stress (Fig. 3.5b) reflects the pattern observed for AMPL which was, on average, the major component of the lipids (Fig. 3.3). This class of lipids accounted for  $34(\pm 4)$ % of the lipids over the complete range of nitrogen supply rates. Chlorophyll <u>a</u> was clearly a component of this band on the Chromarods, and by comparing fluorometric chlorophyll <u>a</u> values with AMFL values it would appear that CHL accounted for about 50% of the AMPL peak in nitrogen-replete cells (Table 3.3). It is thus not surprising that these two values are correlated (Table 3.2),

and that if one is correlated with growth so is the other (Fig. 3.9a,b). The strength of the relationship with each other and with growth suggests, however, that the other 50% of the AMRL peak is also closely related to cell replication. The remaining components of this peak undoubtedly include glycolipids, the so-called 'chloroplast lipids' (Gurr and James, 1980). Specific types of glycolipids, the galactosyldiglycerides, are usually the predominant lipid in chloroplasts; they have been shown to be synthesized by the membranes of the chloroplast envelope (Goodwin and Mercer, '1983). These glycolipids have also been found to be the most important products of lipid synthesis in <u>P. tricornutum</u> (Holdsworth and Colbeck, 1976).

The strong relationship between sterols, which are also membraneassociated, and growth (Fig. 3.9) is also of interest. In Bedford Basin, sterol concentrations were found to be significantly higher at the time of spring blooms than at other times (Chapter 4). Sterols may thus be another useful indicator of actively growing microbial cells.

In two different cultures <u>P. tricornutum</u> was observed to change from its normal unicellular Forms to a chain-form first reported by Coughlan (1962). It was unfortunately not possible to measure the amount of lipid per cell in this chain-form, but the percentage composition was similar to that of the unicellular form taken from other cultures being supplied with the same amount of nitrogen but at a different time during the experiment. Thus, a chain-form from a culture that had a slightly higher supply of nitrogen had less

triglyceride and more polar lipid than the chain-form from a more stressed ćulture (Table 3.4). The chain-forms occurred when the two turbidostats that were in operation at the time were being supplied with two different enrichments of nitrogen in dialysed seawater from the same source. It does not seem to be the exact amount of nitrogen that, is critical in the formation of chains, nor were both these cultures belêw a threshold of nitrogen supply since it. was subsequently possible to maintain normal unicellular forms for extended periods at even lower nitrogen supply rates. It would seem that some other component in the base-medium was responsible for this transformation.

<sup>°</sup>138

3.4.2 Dissolved lipids, the absolute production rate of total dissolved lipid per cell does not seem to be influenced by nitrogen-stress (Table 3.5). However, it does become a significantly larger proportion of the total particulate lipid produced. The proportionrose from about 10% to about 25% under nitrogen-stress. These values are quite consistent with most estimates of the percentage of fixed carbon that is released extracellularly by natural populations of phytoplankton growing under nutrient-replete and nutrient-stressed conditions (Wangersky, 1978). Increases in concentrations of dissolved lipids after spring blooms (Morris <u>et al.</u>, 1983; Kattner <u>et</u> <u>al.</u>, 1983a; Chapter 4) could simply reflect the large numbers of cells that were in the water columns immediately prios to this point. Kattner <u>et al.</u> (1983b) found large accumulations of polars lipids after blooms. In Bedford Basin, phospholipids were found to be a significant contributor to the increase in polar lipids after the bloom \*(Chapter 4). Elevated levels of dissolved phospholipid production as a result of nitrogen-limitation (Table 3.5) might seem incongruous. However, if nitrogen-limitation hinders the production of complex particulate phospholipids from phosphatidic acid (which does not contain nitrogen) then a build up of intracellular phosphatidic acid is likely to occur. Part of this could go to the production of, triglycerides, the rest could presumably be released extracellularly.

The production of free fatty acids was also significantly higher in nitrogen-stressed cells (Table 3.5). This might be an artefact related to the significant increase in phospholipid production, since it is possible that a portion of the extracellular phospholipids are rapidly hydrolysed to produce free fatty acids.

3.5 Summary and Conclusion

In <u>Phaeodactylum</u> <u>tricornutum</u> synthesis of a storage component, triglycerides, is clearly triggered by nitrogen-stress. The yield of triglycerides is higher at the lower levels of nitrogen supply and nitrogen use per cell despite lower growth rates.

The membrane-associated polar lipid classes are also affected by nitrogen-stress. Their levels, however, are reduced. This results in the total lipid per cell decreasing with a decreasing supply of b

' nitrogen to the cage cultures. The production rate of total dissolved lipids does not seem to be affected by stress; however, there are differences in the types of dissolved classes produced under. nutrient-stressed and nutrient-replete conditions.

## Chapter 4

TIME-SERIES OF PARTICULATE AND DISSOLVED LIPID CLASSES DURING SPRING PREFORLANKTON BLOOMS IN BEDFORD BASIN, A MARINE INLET

4.1 Introduction.

Large increases in numbers of microscopic plants are common in midlatitude and high-latitude waters in the spring. Enhanced sunlight, fewer storms and high nutrient levels expedite the conversion of carbon dioxide to cellular material in the photic zone. Radiant energy is used by photoautotrophs to reduce  $CO_2$  to organic molecules composed of carbon, hydrogen and oxygen. These elements, together with others obtained from the assimilation of inorganic nutrients, are combined in various proportions to form the basic classes of compounds necessary for life.

As the plant bloom proceeds, inorganic nutrients become depleted while light intensity increases. Under such conditions it is reasonable to assume that photoautotrophs favour the synthesis of compounds composed solely of C, H and O: these elements are always in plentiful supply in marine environments. Most lipids and carbohydrates are composed only of these three elements. Both of " these organic classes comprise essential energy storage and atructural compounds. They differ in that lipids are considerably more reduced, and as a result, lipids can be oxidised to yield more than twice the amount of ATP per gram. Lipids are also more heterogeneous: they lack a well-defined monomeric unit.

 Members of different lipid classes can be distinguished according to the types and proportions of elements present in the molecule. Lipid classes vary in the proportion of oxygen in the molecule, from - hydrocarbons with none, to glycolipids which contain monosaccharide units. Triglycerides, which are important energy storage compounds, are about half-vay in between. Phosphorus and nitrogen are also common components of the more polar lipids, which include chlorophylls and phospholipids. P and N often appear together in phospholipids which are essential constituents of all biological membranes. The one feature that unifies biogenic lipid classes is the presence of a high proportion of CH2 groups (Chapter 1). Since there are considerable differences in both the structure and function of the various lipid classes (Chapter 1), it would seem likely that the synthesis of individual classes would be favoured at different times during spring phytoplankton blooms. Thus, not only 🔩 can one expect to see variations in the principal biochemical classes according to nutrient availability, but also in individual lipid

Experiments with a diatom-dominated bloom in a large enclosure showed that when nitrate became almost undetectable, there was a large change in C:N atomic ratios in particulate matter: they increased from 3.5 to 17.5, (Antia <u>et al.</u>, 1963; Banse, 1974). Carbohydrate levels have also been found to increase significantly in such experiments (Antia <u>et al.</u>, 1963; Strickland <u>et al.</u>, 1969; Morris <u>et al.</u>, 1983). Total lipids, however, have not shown a uniform

classes.

response. Cellular lipid levels have remained virtually unchanged after nutrients have become depleted (Antia <u>et al.</u>, 1963), or they have shown a small (<30%) increase (Strickland <u>et al.</u>, 1969), or else there has been a substantial change (Morris <u>et al.</u>, 1983), one that would amount to more than a 100% increase per cell. These differences could simply reflect the responses of the different species present in each enclosure. Indeed, given the degree of variability in gross biological parameters among replicate enclosed ecosystems (Cooke, 1977; Hurlbert, 1984) one might not expect much similarity in lipid results from different studies. Also, different analytical methods were used to measure total lipids in the enclosure experiments. The problem with this is that rapid or simplified methods for total lipid content often respond only to certain functional groups, and thus are more representative of one lipid class than another.

1.2.7

With the use of more sophisticated analytical techniques it should be possible to overcome any disparities in the results of enclosed ecosystem experiments that are a consequence of methods for total lipids. In addition, a technique that measures individual lipid classes should reveal trends otherwise masked in total lipid measurements. One such technique is the Chromarod-Jatroscan (TLC/FID) system, which has been used to measure percent lipid class composition during a diatom bloom in an experimental enclosure (Morris <u>et al.</u>, 1983). It was found that the dominant particulate lipid class was originally triglyceride, then it changed to phospholipid during the maximum period of growth, and finally to

triglyceride again after nutrients had become depleted. TLC/FID was also developed (Parrish and Ackman, 1983a) for the measurement of absolute lipid class concentrations in both the particulate and the dissolved 'fractions' (Whitehouse <u>et al.</u>, 1984; Parrish, Chapter'l) of seawater. The present study involves the use of modifications (Chapter 2) of previously described TLC/FID procedures to measure dissolved and particulate lipid class concentrations during diatom blooms. The measurement of dissolved 'lipids during blooms is also of particular interest because it is believed that increased extracellular release of organic compounds occurs as a result of nutrient stress (Wangersky, 1978; Jensen, 1984), and it would seem that when N and P are lacking, lipid compounds, could be preferentially released.

To examine further the patterns in lipid levels during bloom's and specifically in response to varying nutrient concentrations it is possible, to use environments that are both simpler and more complex ' than are found in enclosed ecosystems. By growing a single species of phytoplankton in 'a highly controlled environment it should be' possible to examine the rate of production of intracellular and 'extracellular lipids as a function of the rate of supply of nutrients, provided all other variables are kept constant. This degree of control can be obtained with cage culture turbidostat's (Uangersky et al., submitted), which provide a means of maintaining a constant population density while preventing the accumulation of

found that the rate of production of intracellular triglycerides in the diatom <u>Phaeodactylum tricornutum</u> increased with increasing nitrogen-stress, while the rate of production of total lipids actually decreased (Chapter 3). The rate of production of total dissolved lipids, houever, did not change. What did change was the type of lipid compound that was released (Chapter 3).

In the present, study of patterns in lipid levels a relatively complex environment has been chosen. Samples were taken at regular intervals and at different depths during spring diatom blooms in Bedford Basin. Nova Scotia. Although this environment is vastly more complicated than any that might be experienced in a laboratory setting. it is still relatively simple by oceanographic standards: there is only a small freshwater input to Bedford Basin and there is only a limited exchange of seawater with the open ocean. The Bedford Basin spring bloom has previously been found to have <u>P. tricornutum</u> as the fifth most abundant species in surface waters (Platt <u>et</u> <u>al.</u> 1973) and thus it was anticipated that the laboratory experiments (Chapter 3) would be at least in part directly relevant to the studies an Bedford Basin.

Until now, the only comprehensive field measurements of dissolved and particulate lipids during a spring phytoplankton bloom have been those of Kattner <u>et al</u>. (1983a,b). Their study, a detailed description of fatty acid moieties in North Sea samples, documents changes in the types of fatty acids that occur during a bloom. They also measured dissolved acy1 (R-C=O) lipid classes in surface vaters

during the bloom period (Kattner <u>et al.</u>, 1983b). They found that free (not esterified) fatty acids were the major dissolved acyl lipids present, and they observed a maximum concentration of free as well at total fatty acrds just after the period of maximum phytoplankton growth. Morris <u>et al.</u> (1983) also recorded a peak in total dissolved lipids just after the chlorophyll <u>a</u> maxima in an ecosystem bag. The present study is the first to document changes in concentrations of the complete range of dissolved and particulate lipid classes during a phytoplankton bloom.

A.2 Methods.

To examine distributions of marine lipid classes during spring, phytoplankton blooms, samples were collected in Bedford Bays Bedford Başın and Halifax Harbour, Nova Scotia (Fig. 4.1), Nost of the samples were collected in Bedford Basin and (17 km²) marine inlet connected via Halifax Harbour to the Atlantic Halifas Harbour is generally shallower than Bedford Basin and there is only limited seawater exchange between the two. This exchange occurs through The Narrows, which provides an effective sill depth of 20 m and a minimum total width of 375 m. The major freshwater input to Bedford Basin is from a single shall river, the Sackwille River, via Bedford Bay. The sampling station used in Bedford Basin was located at its centre in 70 m of water. This station has been occupied for other studies directly concerned with spring phytoplankton blooms, Freshet et cl., 1973; Conover, 1975; Conover and Mayzaud, 1984). The present study is concerned with seawaver, samples, taken during the spring blooms of 1982 and 1984, and it concentrates on measurements that yere common to both years. The determinations were made with bottletrapped, samples, and the studies focussed on both the dissolved and particulate fractions of organic matter as well as, on dissolved inorganic salts. M.R. Leuis and S. Roy (both of the Department of Oceanography a Dalhousie University) kindly provided nutrient and fluorometric chlorophyll <u>a</u> data, together with information on <u>in situ</u> fluorescence and species composition.

Samples vere collected for lipid analyses in the early afternoon every 2'- 3' days over a period of 27 days in March and April. Sampling was conducted from the M.V. <u>Sigma T</u> in the top 10 m of the vater column at the centre of Bedford Basin (Fig. 4.1). Samples were trapped in a conventional 30 1 Niskin bottle at the bottom of the surface mixed layer as discerned from the temperature trace of a Guildline CSTD. The CSTD temperature profiles and the exact sampling depths are given by Livis et al. (1984) On Julian day 91 an

additional' sample was collected at 0.5 m.

4.2.1 Spring bloom, 1982

1-\$/





• •

њ с.4

Once the water sample was on board it was transferred via silicone tubing to precleaned and sample-rinsed 4 1 amber glass solvent bottles. In the laboratory the samples were swirled in the bottles and screened through a metal sieve ( $\underline{ca}$  250 µm mesh) before filtering through precombusted Gelman A/E filters. Between 600 ml and <sup>#</sup>2 1 (usually 1.75 1) was filtered for particulate lipid measurements, and 200 - 275 ml (usually 250 ml) was used for dissolved lipid measurements. All'filtrations and extractions were done in triplicate or quadruplicate, and a total system blank was run for each set of dissolved lipid extra tions, as well as for each set of particulate extractions.

All extractions were performed with cold dichloromethane that had been freshly redistilled under purified nitrogen. Immediately before extracting, all glassware was rinsed with this  $CH_2Cl_2$ . Dissolved lipids were then extracted with a total of 40 ml of  $CH_2Cl_2$ , particulate lipids with a total of 20 ml. All extractions were completed within five hours of sampling; during this period exposure of samples and extracts to light, heat and oxygen was minimised. Chromarod thin-layer chromatography and latroscan flame ionization detection were used to measure the lipid classes in the extracts. TLC/FID was performed with Chromarods SII and an latroscan MK III (latron Laboratories, Tokyo) using modifications of previously described procedures (Delmas <u>et al.</u>, 1984; Parrish and Ackman, 1985), as detailed in Chapter 2. For the 1982 spring bloom samples, the total analytical precision (CV) of both the dissolved and particulate lipid class determinations was normally between 15 and 30%, and blank levels were usually below 10%.

151

4.2.2 Spring blocm, 1984

The same station that was occupied in 1982 was occupied again in 1984. Samples for lipid class measurements were taken around noon, once or twice a week. This 1984 study was part of a larger programme described in detail by Roy (1986). The present study is mainly concerned with particulate samples collected at 5 m from Februrary to April in the centre of Bedford Basin. Further details concerning the 1984 sampling stations are given in the Appendix.

The 5 m Bedford Basin samples provided the most comprehensive timecorries of lipid measurements in 1984. These samples were collected in 5 1 Go-Flo bottles and processed for parbiculate lipid measurement as described for the 1982 spring bloom and in Chapter 2. Particulate organic carbon (POC) and nitrogen (PON) concentrations were also measured in these samples. POC and PON were determined after combustion in pure oxygen in a Perkin-Elmer CHN analyser (Model 240B). Carbon and nitrogên values were standardized using cyclohemanone-2:4-dinitro-phenylhydrazone (BDH). 4.3 Results and Discussion

Diatom blooms were observed within the top 10 m of the water column at the centre of Bedford Basin in March of 1982 and 1984. The <u>in situ</u> fluorescence maximum usually coincided with the beginning of the pycnocline, which was almost always found within the top 10 m<sup>9</sup>, throughout both periods of study.

4<sub>a</sub>3.1 Spring bloom, 1982

In 1982, water samples were taken from the bottom of the mixed layer, which was found to be between 3 m and 9 m, and was usually around 5 m. The bloom that was observed during this month-long sampling period was dominated by representatives of the genus <u>Melosira</u>. The chlorophyll <u>a</u> data (Fig. 4.2a) suggest that this bloom was close to the classic concept of a spring bloom. The maximum observed chlorophyll value coincides with a period in which there was the sharpest decrease in nitrate (Fig. 4.2b). Nutrient levels then remained low for the next two weeks, and chlorophyll <u>a</u> concentrations generally declined. There was, however, a second chlorophyll peak observed during this period and the value from the last sampling day suggests that there might have been a third peak in this series.

Two of the peaks in total particulate lipid concentrations (Fig. 4.2c) coincided with the chlorophyll <u>a</u> maxima (Fig. 4.2a), but the highest total particulate lipid value occurred 10 days after the highest chlorophyll value. This pattern is almost identical to that observed by Morris <u>et al.</u> (1983) in 3 m samples from a spring diatom

bloom in an enclosed ecosystem bag. The pattern for total dissolved lipids (Fig. 4.2c) is also similar to that at 3 m in the ecosystem bag' (Morris et al., 1983). The lowest dissolved fipid concentre long occurred near the time of the chlorophyll maxima, and the highest values were measured before and after this period. Kathaer et al, (1983a) also measured a trough in total dissolved fatty. acid , concentrations, that was coincident with the maximum in phytoplankton growth during a North Sea bloom. These decreases in dissolved lipid entrations could be the result of a greatly decreased rate of exudation on a per algal cell basis and/or a greatly increased rate of uptake by bacteria and possibly even by phytoplankton. Table 4.1 gives the mean composition of the dissolved and particulate lipids during the 1982 bloom. From the 95% confidence intervals it can be seen that the concentrations of the dissolved lipid classes were more wariable than those of the same class in the particulate fraction. The mean concentration; however, use usually quite similar for the two fractions. This similarity in concentrations (Table 4.1) was not anticipated; neither was the general decrease in total dissolved lipids after the first feu sampling days (Fig. 4.2c). This led to some analytical problems because the volume of water required for extraction was chosen at the beginning of the sampling period, when the concentration of dissolved fipids was significantly higher than that of particulate lipids. It was thought that the levels of dissolved lipid classes would increase as the bloom progressed as a result of exudation and decomposition,

153

٩. ۲

in fact The levels of most classes generally decreased until after the chlorophyll maxima (Fig. 4.3). This meant that total ranalyrical precision values as high as.35% were not uncommon for the three to four 250 ml dissolved lipid extractions that were used. This is especially true for classes, found in lesser amounts in the extracts. It also meant that blank values vere somètimes as high as 20%. These analytical considerations do not, however, alter the general patterns seen in Figure 4.3, because of the large differences between the peaks and troughs in the time Serie's. The problems associated with fou dissolved lipid class concentrations were álléviated dúring the 1984 study by expracting; the dissolved lipids from up to four times the amount of sequater per Chromarod analysis There were two exceptions to, the general similarity in concentrations of lipid classes in the dissolved and particulate fractions. The concentrations of ALC and AMPL (abbreviations explained in Table 4.1) were consistently higher in the dissolved raction. It is not known which compounds are present in these two classes, but it is likely that phytol and monoglycerides are among the major components (Chapter 1), These two contributors would be expected to occur during bloom periods as a result of the hydrolysis of chlorophylls and acyl lipids.

Figure 4.2 Spring, bloom 1982; chlorophyll, nutrient and total lipid concentrations at the bottom of the surface mixed layer in Bedford Basin. (a) Fluorometric chlorophyll <u>a</u> 'Nitrate and phosphate. ). (Ъ) Total dissolved and total particulate lipid. (c) N .\* .



## Figure 4.3

 $\langle \rangle$ 

Spring bloom 1982; dissolved and particulate lipid class concentrations at the bottom of the surface mixed layer in Bedford Basin.

, 157

- (a) Dissolved and, particulate phospholipid.
- (b) Dissplved and particulate triglyceride.

(c) Dissolved and particulate hydrocarbon.



-%

## Table 4.1,

Dissolved and particulate lipid class concentrations ( $\mu$ g/1) and proportions (%) at the bottom of the surface mixed layer during the 1982 Bedford Basin spring bloom. The values given are the mean TLC/FID measurements for the 11 sampling days, and the limits are the 95% confidence intervals.

Class	Abbreviation	Dissolved	lipıds	Particula	te lipi	ds				
Aliphatic hydrocarbon	HC .	7.8+3.4	4+2%	9.5 <u>+</u> 3.2	12 <u>+</u> 5%					
Wax ester/ sterol ester	WEa	4.1 <u>+</u> 2,4	3 <u>+</u> 2%	1.9+0.9	2 <u>+</u> 1%					
Triglyceride	TG	14 <u>+</u> 11	7 <u>+</u> 4%	23,+9 -	25 <u>+</u> 6%	x				
Free fatty -	FFA _	13 <u>+</u> 4	7 <u>+</u> 2%	11 <u>+</u> 5	12 <u>+</u> 4%	ø				
Free aliphatic alCohol ·	AL C <sup>b</sup>	19 <u>+</u> 9	11 <u></u> 8%	5.2+2.5	6 <u>+</u> 2%	<i>•</i>				
Free sterol	"STC	9.6 <u>+</u> 6.7	4+1%	5.1 <u>+</u> 2.4 4	* 6 <u>+</u> 2%	`				
Acetone- mobile polar lipids	AMPLd (	88 <u>+</u> 36	46 <u>+</u> 10%	24 <u>+</u> 5	28 <u>+</u> 5% 、	Ŀ				
Phospholipil	PL:	<sup>,</sup> 39 <u>+</u> 36	17 <u>+</u> 8%、	6 <u>,9+</u> 3.1	8 <u>+</u> 3%					

<sup>a</sup>A small peak adjacent to the UE peak with the  $R_F$  of methyl esters (Parrish and Ackman, 1983a; Chapter 2) was detected in a few samples.

<sup>b</sup>Often composed of two peaks (Chapter 2).

CA'small peak adjacent to the ST peak with the Rf of diglycerides (Chapter 2) was detected in a few particulate samples.

<sup>d</sup>This TLC/FID lipid class may contain monoglycerides, glycolipids, and chlorophylls (Chapters, 1, 2).

ALC and AMPE together with PL were, on the major vage, components of the dissolved lipids. This is in contrast-to the results of Kattner et al. (1983a) who found FFA and TG to be th major contributors during a spring blobm in the North Sea. The dat in Table 4.1 and those given by Katiner et al. (1983a) are not directly comparable since Kattner et al. measured only acyl lipids and only in term's of carbon. However, by using representative compounty (Chapter 1). it is possible to estimate a fortal weight for each acyl lipid class. Also, with the knowledge that 'TLC/FID lipid class measurements give values that are close to 'true' gravimetric, values (Fraser et al., 1985; Parrish) Chapter 2), the values in Table 4.1 can be used to estimate the relative proportions of the acyl lipid classes in Bedford Basin. With these corrections made, FFA still accounts for over 40% by weight, of the acyl lipids-during the North Sea bloom, while remaining under 10% by weight, of the lipids during the Bedford Basin bloom. It would appear, however, that the absolute concentrations of FFA\_were similar during the two blooms what was significantly different in terms of concentration was the amount of polar lipid (AMPL + PL) present: the concentrations of polar lipids were an order of magnitude higher in Bedford Basin. It is poscible to speculate on environmental of even analytice 🏣 easons for the differences between the two studies, but since these are the only two budies of discolved lipid class concentrations during blooms, a detailed consideration of these differences will, have to  ${\mathbb Q}$ avait further field measurements of this type.

The comparison of the particulate lipid values (Table 4.1) with those in the literature (Morris et al., 1983) is better than was the case with the dissolved lipids. HC was not included in the calculation's of percent composition by Morris et al. (1983), and so the values in Table 4.1 have to be adjusted slightly to be comparable. TG, which is an important storage class in phytoplankton, uas a major contribut/or in both studies. 'TG was also a significantly 'larger proportion of the particulate lipids than it was of the . a dissolved lipids (Table 4.1). AMPL, which is undoubtedly equivalent to 'monoglycerides' in the ecosystem bag study (Morris et al., 1983), vas also an important contributor to the particulate lipids. The particulate AMPL band on the Chromarods clearly contains chlorophyll and a comparizion of particulate AMPL concentrations with a fluorometric chlorophyll a concentratio a suggests that chlorophylls account for about 2/3 gf this band.

Figure 4.3 shous the classes with the clearest trends in both the particulate and the dissolved fractions in 1982. These time-series suggest an approximately reciprocal relationship for class concentrations in the two fractions of seauater. The dissimilarity in the patterns for the dissolved and particulate lipid classes is emphasized by the fact that there was only one significant correlation between dissolved and particulate measurements of the same class (Table 4.2). These results lend credence to the accertion that measurements of lipids made on the operationally defined dissolved and particulate information on processes

that are different, and perhaps even somewhat independent. (Chapter 1). Particulate lipid classes contrast with their, dissolved.

counterparts by having at least one peak coincidents with the chlorophyll manima (Julian days 86 and 91), while the dissolved classes have their louest values around this time (Fig. 4.3). This pattern of low values extends to time-series of dissolved FFA, ST and AMPL. Kattner <u>et al</u>. (1983a) also recorded low dissolved TG and FFA concentrations at the lime of maximum phytoplankton growth. Both these classes contain energy rich compounds that may be used by bacteria which are presenably also highly active at this time during blooms.

The concentrations of many dissolved lipid classes shoued a significant increase after the lou values associated with the chlorophyll maxima. The highest ALC, ST, AMPL, and PL, concentrations for the 1982 bloom were measured during the period of declining chlorophyll values. The wide range of chemical structures involved (Chapter 1) would suggest a non-specific source for these dissolved compounds. Cell lysis, either autolysis or mechanical rupture as a result of zooplankton grazing, could be at least partly the cause of such a large input of dissolved lipids to the water column. Table 4.2

Pearson product moment linear correlation coefficients for lipid class concentrations (µg/l) during the 1982 spring bloom. Abbreviations are explained in Table 4.1; prefixes 'd' and 'p' denote dissolved or particulate, 'TOT' is the sum of the lipid classes.

ŭ	<b>CHL</b>	pTOT	°pHC ⊸	pTG	pFFA	pALC	pST "	pAMPL	pPL	*
ں ب		<u> </u>			······			*	*	
dTOT	-*	- ,	-			<u>م</u>	-	- `	· ,	
҉҄ӯӈӈ҉҇С	-	<i>⊷</i>		1_ <b>^</b>	_	- ,	,	. <b>-</b> *	• •	
, dho	· -	- `	-	• 	-		•		-	
.pTG	0.62	0.85	 0	y .	<b>_</b> °	0.82	0.78	-, *-	0.71	P
dTG	-0.70	<u>-</u> ،	-		, <b></b> * >		-	, —	*	
<b>pFEA</b>		0.68₀		-	د ب	',	/-	ء و خسر	-	7
``dF‡A	-	`_ `	-	)			'- ·	- "		
° pALC	۳۳ ۲۵۲ ۲	0.71	<u>`</u>	0.82	ð 	1) Đ	⁄ 0.79	-	0.64	۲ ۲
· dALC	- -	-	۵_	0.76	<del>ہ</del> ۔۔۔	ົ0.70	0.83	• ^	*	1
pSŢ	· - , ·	0.74	r _	0.78	2 ,	0,79		、	0.71	2
dST	- · ·	<u>م</u>	· —	- ,	-	ه ه د ا	م ••••	-	q´	4
, pAMPL,	<sup>م</sup> ريم 	0.65	* 	, <b>-</b> ,	<sup>1</sup> ,	ت • ج • '	s'		-	0
o dampl	· · · · ·	-	· _ *		·		م م	-	~ ~	
pPL	0.72	0.79	(	0.71		0.64	0.71	<sup>2</sup> ب م م	`	ь , <sup>4</sup> ,
dPL dPL	0		° 4	۲ ۲ ۲ ۲ ۲ ۲ ۲ ۲	· - · č	* ~ * ~ ~		_1 		°
°,` ≏Not s	, , , ignifica	nt at 't	he 958	confide	ence fer	vel (n=,1	.1),	054 05 0	<b>, '</b> '	ų
o • •	9 .00 .	"		•		* ,	*	.9		-
Not all dissolved lipid classes had maximal or even high concentrations after the chlorophyll maxima. Dissolved TG and HC tended to decrease throughout the entire sampling period (Fig. 4.3b, c). This pattern contrasts with particulate TG, FFA, ALC, ST and PL which tended to increase through the times of the chlorophyll maxima as well as afterwards for at least a few days. Particulate HG, however, shares some features with its dissolved counterpart: both have high values at the beginning of the sampling period, as well as low values at the end (Fig. 4.3c). These results suggest similarities with observations made by Gordon <u>et al.</u> (1978) at the centre of Bedford Basin. They performed a temporal study of hydrocarbons using unfiltered water samples; their total <u>m</u>-alkane concentrations in March were among the highest observed during the year. The elevated levels at that time were suggested to be the result of anthropogenic inputs (Gordon <u>et al.</u>, 1978).

4.3.2 Spring blocm, 1984.

The 5 m sample depth used in 1984 was found to be within or close to whatever halocline's or thermoclines could be detected with a Beckman RS5-3 electrodeless induction salinometer. The 5 m bottle sample was also found to be usually very close to the depth of the <u>in situ</u> fluorescence maximum. On one day, Julian day 68, a very distinct <u>in</u> <u>situ</u> fluorescence maximum was observed at 3 m, and so on that day the water sample was taken at that depth rather than at the usual depth. The 1984 bloom was different from that of 1982 in that it was

dominated by <u>Chaetoceros</u> spp. and the chlorophyll <u>a</u> values (Fig. 4.4a) did not reach the levels a sained in 1982 (Fig. 4.2a). In 1984 the maximum chlorophyll <u>a</u> and POC levels were observed at 3 m on Julian day 68 (Fig. 4.4a,b). These levels were accompanied by the lowest measured nitrate and phosphate concentrations (Fig. 4.4c). This particular sample also gave the highest and lowest walues when compared with the complete data set obtained during this two month period. (Roy, 1986), This data set consists of twice-weekly samples obtained from five different depths.

After Julian day 68 there was a substantial increase in nutrients at 5 m (Fig. 4.4c). This is probably the result of increased runoff as well as mixing with deeper water. Five of the seven days between Julian, days 70 and 76 were characterized by wind speeds that were above 'normal' for March (Environment Canada, 1984) as well as above be mean for March 1984. On two consecutive days the wind speeds were ire than 50% higher than the mean for the month and their prevailing direction was parallel with the longest dimension of the Basin. On these two days the Halifam-Dartmouth area received one third of the month's rainfall (Environment Canada, 1984), and the end of the seven day period (March 16) was marked by a high daily discharge value for the Sackville River (Water Survey of Canada, 1985). This value was in fact the highest measured during all of 1984.

Figure 4.4 Spring bloom 1,984; chlorophyll, carbon and nutrient concentrations at 3 m and 5 m in Bèdford Basin. Tic marks on the x-axis are spaced one week apart. (a) Fluorometric chlorophyll a. Particulate organic carbon. (b,) & (c) Nitrate and phosphate.



What is surprising about the data in Figure 4.4 is that despite the partial recovery in nutrient levels and a long period of relative stability in the vater column (Roy, 1986; Parrish, Chapter 5), there, vas no major second bloom. Following the storm, chlorophyll <u>a</u> and POC levels continued their downward trend, albeit more gradually. Nutrient levels also showed a decrease over this period.

Although there are distinct differences between the 1982 and 1984 blooms in both absolute concentrations and the timing of peaks and troughs, there are also, clear, similarities. There was a steep increase in chlorophyll a levels in March of both years followed by an equally steep decrease and then two secondary increases in chlorophyil levels. Nutrients did not remain low after the first. chlorophyll maximum in 1984, but nonetheless there are still some indications of nutrient stress. C:N atomic ratios as high as 7.2 were measured after Julian day 68 at 5 m. Evença value as high as 10.0 úas measured at 1 m on Julian day 75. Atomie ratios between 7 and 10 were also observed in surface waters by Copover (1975b) during the late stages of Bedford Basin spring blooms when concentrations of, various ' nitrogen-containing nutrients had become markedly reduced. High HPLC-measured chlorophyll c : chlorophyll a ratios together with high carbon : chlorophyll a ratios provide further evidence for phytoplankton stress after Julian day 68 in 1984 (Roy, 1986). It is possible that in addition to sub-optimal nutrient conditions, the surface-layer phytoplankton vere experiencing sub-optimal light conditions following Juljan day 68. In the three week period after

Julian day 68 when chrorophyll levels generally declined, the average number of hours of "bright sunshine" (Environment Canada, 1984); per day was more than 2 hours lover than during the period of rapidly increasing chlorophylli concentrations (Julian days 61 to 68). The ' three week period of lower light levels included six consecutive days uhen 0.0 Hours of bright sunshine were recorded for this area. Concomitant uith the differences in chlorophyll a and nutrient levels, differences were also, observed in particulate lipid distributions in 1982 and 1984 (Figs. 4.3, 4.5; Table A.1), However, given the dearth of available lipid class data from blooms and the complexity of the processes governing their distributions, it was felt that a detailed discussion of inter-annual variability should attait further field measurements. The principal aim of this discussion 15 thus to examine the similarities in hipid distributions during the bloom periods in order to make generalizations that should be applicable to other blooms. As in the 1982 spring bloom, total particulate inpid levels were much higher following the chlorophyll maximum than before (Fig. 4.5a; Table A.1), Obut the effect was much more pronogneed, and much more prolonged. This, may possibly be related to the presence of significant levels of nutrients during this period. Alternatively it may be related to the fact that sampling was terminated much sooner after the chlorophyll maximum in 1982 than in 1984.

Figure 4.5

Spring bloom 1984; particulate lipid concentrations at 3 m and 5 m in .

170

(a) Total particulate lipid and triglyceride.

(b) Phospholipid and free fatty acid.

(c) Free aliphatic alcohol and free sterol.



TG sgain became the major lipid class from the time of the chlorophyli maximum onwards, and again the maximum in TG per unit chlorophyli or per unit carbon occurred quite a while after the chlorophyll maximum (three useks). This has considerable implications for the quality (in terms of energy) of particulate material to grazers. It is interesting that the highest zooplankton activity measured by Conover and Mayzaud (1984) during a spring bloom in Bedford Basin was after the chlorophyll maximum. The highest zooplankton ingestion rates in terms of particle volume or in terms of organic carbon occurred from 4 to 18 days after the maximum of chlorophyll in the top 5 m of the water column. The highest gross and net zooplankton growth efficiencies also occurred during that time period (Conover and Mayzaud, 1984).

172、

The pattern for PL was also similar to that observed in 1962. PL levels were higher after the chlorophyll maximum than before it, except in 1984 the effect was much more marked (Fig. 4.5b). The higher levels of this membrane indicator class (Chapter 1) suggests greater numbers of organisms, possibly bacteria and/or microzooplankton. The lack of any increase in ST during this period would tend to favour bacteria in this regard, since bacterial cells contain few sterols. It should be noted that bacteria do not store TG either. FFA and ALC (Fig. 4.5c) increased steadily through the block and afterwards and are probably indicative of the degree of lipid degradation in particulate matter.

Dissolved lipid classes were also extracted from 5 m samples but , only on five occasions (Table A.2), so the time-series are not shown here. Some of these data can be found in Figure'4.6 and Chapter 5. Samples of dissolved and particulate lipids were also taken from 1 m and again, some of these data can be found in Figures 4.6 and 4.7 and in Chapter 5. In addition to sampling over'a larger time period and from more depths then in 1982, sampling ih 1984 was not restricted to the central Bedford Basin station. On two occasions during the spring 1984 study (Julian days 72 and 103) measurements were made at two additional stations & One station was near the location of the small freshvater input to the Basin the other was on the seaward side of the Basin's narrow connection with the open ocean (Fig. '4.1). Both stations were more than 3 km away from the centre of Bedford Basin. They vere sampled in an attempt to estimate the effects of mixing. with water outside the basin on the interpretation of time-series-and profiles from the centre of Bedford Basin. There were, houever, feu consistent patterns either vertically or horizontally for the three stations. For most of the parameters measured in this study there were either no significant unidirectional gradients or else the differences between the same sampling depths at the three stations were significantly smaller than the differences between depths within each station. Except, perhaps, for the case of hydrocarbon data (Fig. 4.6a), it can be seen that lipid data from the two additional stations fir vell with the Bedford Basin data collected in 1982 and 1984 (Figs. 4.6, 4.7).

0

Figure 4.6

Dissolved and particulate lipids from two blooms and three stations. Tic marks on the x-axis are spaced at weekly intervals from the 1982 and 1984 Bedford Basin chlorophyll <u>a</u> maxima. There are four symbols that have been used to distinguish samples from different years, different depths and different stations. The beres represent Bedford Basin 1982 samples taken from the bottom, of the surface, mixed layer. The, crosses are 1984 Bedford Basin 3 m and 5 m data. The plusses are 1984 Bedford Basin 1 m data. Finally, the diamonds are data from the remaining surface mixed layer depths and stations from both the 1982 and 1984 spring bloom studies.

(a) Dissolved Hydrocarbon concentrations.

(b) Particulate sterol concentrations.

(c) The ratio of total particulate lipids to chlorophyll a.



Lipids versus dissolved inorganic nitrate concentrations for the 1982 and 1984 spring bloom amples. Symbols as in Figure 4.6.

. Ata .

Figure 4.7

studies.

176

ر گذ

(a) Dissolved phospholipid concentrations from before the 1982 chlorophyll a maximum.

(b) Particulate phospholipid concentrations from after the chlorophyll a maximum in both 1982 and 1984.

(c) The ratio of total particulate lipid to chlorophyll f for all the surface mixed layer samples taken, during both spring bloom



Measurements of salinity and nitrate were a clear exception to the generalization that the three stations were similar in most regards. On Julian day 103 there was a gradient of 3, 7, from Bedford Bay to Halifax Harbour; this was accompanied by a reverse gradient of about 1.5 µg-at/1 of nitrate.

4.3.3 Lipids before and after the chlorophyll a maximum Banse (1974, 1977) has detailed the importance of grouping carbon, nitrogen and chlorophyll data into temporal or spatial assemblages before regression analysis. The most obvious point of reference for the grouping of data in this study is the maximum observed chlorophyll a concentration in the time series.

All the data collected from the surface mixed fayer in 1982 and 1984 there pooled together to see if there was any significant difference between samples collected before the collorophyll maximum in Bedford Basin and data collected after it. Data collected on the day that the chlorophyll maximum was observed were not included because it is not known whether the samples are more representative of 'before' or 'after', (Table, 4.3). The data were examined statistically using a t-test that did not require approximately equal variances (Ryan <u>et al.</u>, 1976). This was necessary because 'lipid data collected before the chlorophyll maximum were considerably less, variable than data collected after it. Table 4.3 ghows the concentrations and ratios that were significantly different, and it can be seen that there is no overlap of the error baro in these data.

Dissolved lipid concentrations are given only for comparison, because it was only in 1982 that dissolved lipid samples were collected before the chlorophyll maximum.

170

It can be seen that concomitant with the significant differences in nutrients there are significant differences in lipids. There were generally, much higher levels of lipids after the nutrients had become depleted. Using the interpretations of lipid class data discussed in Chapter 1, Table 4.3 indicates that there were more storage products, more degradation, and more cells after the chlorophyll maximum. Several classes showed no significant difference between before and after the chlorophyll maximum. Figure 4.6(a,b) demonstrates two distributions of data that would give such a situation. For comparison, Figure 4.6(c) shows a situation where there was a dignificant difference.

Dissolved HC (Fig. 4.6a) bear no relationship to the chlorophyll <u>a</u> distribution, although there does appear to be generally less HC after day zero than beford, presumably as a result of the decreased use of fuel orls (Gordon <u>et al</u>., 1978). Samples taken from Halifax. Harbour vere generally higher in HC than those taken at the other, stations (Fig. A.1). This, is to be expected because the station was adjacent to a large container pier, and this area is exposed to a considerable level of chipping traffic.

Table 4	3.	3
---------	----	---

Particulate lipid and nutrient data from before and after the maximum measured chlorophyll a concentrations at the centres of Bedford Basin in 1982 and 1984. The data are taken from the top 10 m of the water column from three stations (Fig. 4.1); only those data which were significantly different (95% confidence) are shown in the Table. Dissolved lipid concentrations from after the chlorophyll a maximum in both years, are also given for comparison. All limits are 95% confidence intervals.

Ø

00

77* *	43			• •
9، خر	BEFORE (n=10)	AFTER (n=22)	ಭೆಗ್	47 -
Dissolved inorganic • nitrate (µg-at/1)	, 6.6 <u>+</u> 2.3	2.6 <u>+</u> 0.6	Dissolved 1i	pids '
Dissolved inorganic phosphate (µg-at/1)	1.1 <u>+</u> 0.3	0.6 <u>+</u> 0.1	the chioroph maxima (n=17	yll <u>a</u>
Total particulate 1mpids (µg/1)	62 <u>+</u> 18	116 <u>~+</u> 27	103-54	TOTb
(Total lipids)/CHL	1 <u>5+10</u>	4.9423	1 A.	ب ج جن م
Particulate triglyceride (µg/1)	12+4	30+10	5.2 <u>+</u> 2.2	` d͡ᡗG ```_``
TGŸĆHI.	2.5 <u>+</u> 1.9 '	12 <u>+</u> 7	n 4 N	** *2 ~ 0 *2 ~ 4
Particulate fréé ∴fatty acid (µg/1) .	8.2+3.4	- 19 <u>+</u> 4	* <sup>*</sup> 8.9 <u>+</u> 4 <sup>*</sup> .1	offa j
FFA/CHL	1.7 <u>+</u> 0.7	8.3 <u>+</u> 3.5.	±* `,	چ بر ا
*' Particulate* * phospholipid (µg/I)	4.5 <u>+1</u> .7	16 <u>+</u> 9 <sup>*</sup> ´	22 <u>+</u> 22	dPL Y,
) PL/CHL	0.940.5	7 • 5 <u>+</u> 5.5	· _ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	وسم بیٹریہ ا
	• 40 <sup>0</sup> es + 21	<b>e</b>	* Prie- * -	a , ", ",
د بالا هد <sup>الر</sup> ماؤي ۱۵۰ کو سند اد			-	۶. ۲. م
the state of the s	: ۲۰۰۰ ۲۰۰۰ ۲۰۰۰ ۲۰۰۰ ۲۰۰۰ ۲۰۰۰	۰ مطالع	* · · ·	، ر ۲

180 , ,

745

C

For both blooms and all three depths, the highest particulate ST concentrations occurred within two weeks of the chlorophyll maximum (Fig. 4.6b). ST consistenvly shows a sharp increase around day zero. This result, doupled with the linear relationship shown between sterols and growth rate in turbidostat cultures (Chapter 3), suggests that where chlorophyll <u>a</u> values are high, enhanced particulate sterol levels can also be used as an indicator of healthy, actively dividing cells. The second EL peak observed in 1982 (Fig. 4.6b) occurred when chlorophyll fevels were low (Fig. 4.2a), but when PL levels were high (Fig. 4.3a). This combination would suggest the presence of a large microspoplankton population.

"levels that are below the detection limit. That this should significantly affect algal physiology and metabolism is without question. The manner in which these effects are expressed in terms of differences in dissolved and particulate lipid class concentrations has been a primary concern of this discussion.

Although there are clear and coincident differences in lipids and nutrients before and after the chlorophyll makinum (Fable 4.3), a causal relationship does not necessarily have to exist. A stronger test of such a relationship is one that involves a regression between situate and concentrations of a lipid.class that could be expected to be directly related to nutrient devels. PL is obviously the best contender for such a relationship.

182

The two significant linear regressions that were obtained with PL are shown in Figure 4.7(a, b). The relationship with dissolved PL before, the chlorophyll maximum is significant at the 99% level. The relationship between dissolved PL and nitrate after the 1982 chlorophyll maximum was very different. The correlation between the two was not significant at the 95% confidence level, and the sign for the Pearson product moment correlation coefficient was negative. A regression involving all the pairs of dissolved PL and nitrate data from after the chlorophyll maximum in both 1982 and 1984 produces in correlation coefficient othat is again negative. This suggests that the factors controlling dissolved PL concentrations before.'

Figure 4.7(a) indicates that dissolved phospholipido may be assimilated for the same reasons that desolved inorganic nutrients are. Phospholipids are thought to be taken up by algae in order to provide a source of phosphorug (Langovska, 1982; Admiraal and Werner, 1983). The action of cell-surface phosphatases would make the phosphate available to the algae, and the remainder of the phospholipid molecule would presumably re-enter the discovered fraction of seawater. This new lipid material entering seawater is likely to be composed primarily of partial glycorides, especially diglycerides. The ubiquitous lipases tend to act more efficiently on

λ.

diglycerides than on monoglycerides (Gurr and James, 1980) and so monoglycerides might be expected to accumulate in the environment. The high levels of dissolved AMPL that have been found in surface vaters (Debmas <u>et al.</u>, 1984; Parrish, Table 4.1, Chapter 5, Table A.2), provide evidence that this does indeed occur. The <u>Rf</u> value of monoglycerides is identical to that of AMPL in the solvent systems used for the Chromarod TLC separations (Chapter 2).

183

There is further evidence to support the idea that dissolved monoglycerides are produced from dissolved phospholipids as a result of their use by phytoplankton. Before the 1982 chlorophyll <u>a</u> maximum, the correlation between AMPL and nitrate produces a negative coefficient. The correlation between dissolved AMPL and dissolved FL also produces a negative coefficient. The sign of these Pearson product moment correlation coefficients is consistent with AMPL being produced as the concentrations of inorganic nutrients and dissolved PL decrease.

The linear relationship with particulate PL (Fig. 4.7b) is significant only at the 95% level, though clearly two linear regressions or a non-linear regression would give an even better fit. Figure 4.7(b) suggests that membrane material may be rapidly synthesized only beyond a certain threshold of nutrient levels in the water column. Very high FL concentrations occurred only beyond 2 µgat/l of mitrate. Such a threshold relationship could be important For the synthesize of all particulate lipids: Figure 4.7(c) suggests that there is a marrow range of mitrate concentrations over which lipid synthesis is maximized. For both blooms and all three sampling stations, the highest values per unit chlorophyll <u>a</u> occurred between 2 and 4  $\mu$ g-at/1. Beyond 4  $\mu$ g-at/1 it is likely that the algal cells favoured the synthesis of blochemical classes other than lipids.

4.4 Conclusion

There vere significant variations in the concentrations of dissolved and particulate lipid classes during spring phytophinkton blooms in Bedford Basin. There was a greater variability in lipid class concentrations after the maximum observed chlorophyll <u>a</u> value during the bloom; dissolved lipid class concentrations were nearly always more variable than those of the same class in the particulate fraction.

Dissolved and particulate méasurements of the same class were poorly correlated. This indicates that different processes were controlling the concentrations of the lipid classes in the dissolved and particulate fractions. Particulate lipid class concentrations were almost invariably high during periods when chlorophyll levels were at their highest. This is undoubtedly the result of a close association between particulate lipid classes and phytoplankton productivity. By contrast, several dissolved lipid classes were at their lowest levels at that time. This could be the result of heterotrophic assimilation of dissolved lipid classes.

' Several discolved and particulate lipid classes show maximal

**№**184

supply of high-energy compounds far exceeds demand by heterotrophs at that time. The concentrations of some classes appear to be unrelated to chlorophyll or nutrient levels, suggesting the possibility of an anthropogenic contribution.

ġ,

Chapter 5%

VERTICAL PROFILES OF DISSOUVED AND PARTICULATE AQUATIC LIPID CLASSES

5.1 Introduction' 🗤 –

The first profiles and areal studies of lipid classes in seawater were those of Jeffrey (1966). Using a combination of column chromatography, thin-layer chromatography (TLC), infrared analysis, gas chromatography (GC), and various chemical tests, Jeffrey (1966) was able to separate and identify eight lipid classes in seawater extracts.

Any further attempts at surveys as detailed as Jeffrey's have avaited the arrival of more comprehensive individual analytical techniques. The techniques used in contemporary aquatic lipid work are generally at two extremes in terms of analytical complexity. The most favoured technique for dissolved or particulate matter is now GC with mass spectrometric (MS) detection (Wakeham <u>et al.</u>, 1980, 1984a, b; Kennicutt and Jeffrey, 1981a, b; Saliot <u>et al.</u>, 1982; Wakeham, 1982, 1985; Meyers <u>et al.</u>, 1984). This technique also happens to be highly complex and thus it is more expensive and more labour intensive than simpler techniqueb. GC/MS would also be inappropriate for shipboard work.

186 . /

TLC with either densitometric or flame ionization detection (FID) has also been used formarine dissolved or suspended particulate matter (Kattner <u>et al.</u>, 1983a; Morris <u>et al.</u>, 1983; Parrish and Ackman, 1983a; Delmas <u>et al.</u>, 1984; Volkman <u>et al.</u>, 1986). This approach is much simpler and is workable under shipboard conditions (Delmas <u>et al.</u>, 1984). The values obtained with quantitative TLC are also much closer to true gravimetric weights (Sasaki and Capuzzo, 1984; Fraser <u>et al.</u>, 1985; Gardner <u>et al.</u> 1985) than can be obtained with <u>GC</u> measurements (e.g. Wakeham <u>et al.</u>, 1980; Kennicutt and Jeffrey, 1981a,b).

The fact that only total class values can be obtained from TLC measurements is not necessarily a limitation. Total class values may be valuable as indicators of organic processes occurring in the water polumn. The use of lipid classes in this context is discussed in detail in Chapter 1.

Although the original lipid class studies involved the dissolved fraction of seawater (Jeffrey, 1966), most studies since then have been concerned with particulate matter, especially that which has been caught in sediment traps. In the few studies, where both dissolved and particulate lipids have been measured, the lipids in each fraction have usually been considered separately (e.g. Kennicutt and Jeffrey, 1981a,b; Kattmor <u>et al.</u>, 1963a,b). In the present study, the lipid classes from the two fractions of seawater are considered together.

۵

Chromarod TLC with Iatroscan FTD has been suggested as a useful tool for studying variability in the distribution of lipid classes in the oceans (Parrish and Ackman; 1923a). The present study reports on the Iatroscan-measured lipid class profiles obtained at four different locations.

5.2 Methods

Samples yere collected from up oto seven discrete depths vithin the top 300 m of the vater column in four different locations (Fig. 5.1). Dissolved and particulate lipids were extracted with dichloromethane " (CH<sub>2</sub>Cl<sub>2</sub>), concentrated, and spotted onto silica gel coated Chromarods. These were developed, dried, and then scanned in an latroscan. Peak areas obtained from TLC/FID chromatograms were calibrated with lipid class standards. Unless otherwise indicated below, all lipid samples were separated on Chromarods-SII and pyrolysed in a slightly modified latroscan MK III (latron Laboratories, Tokyo) as described in Chapter 2.

5.2.1 Scotien-Slope

Sampling was performed near the edge of the Scotian Shelf in June of 1982 and in April of 1985. In 1982 the seawater\_uas collected in conventional 5 1 Niskin bottles, passed through a 250 µm metal screen and processed as detailed by Delmas er al. (1984). R.O. Fournier (Department of Oceanography, Dalhousie University) kindly provided CTD, nutrient and fluorometric chlorophyll a data from this cruise.

In 1985, the lipid samples were collected with a Seastar in situ vater sampler (Seastar Instruments Ltd., Sidney, B.C.). The sampler was attached to a hydrowire and used to pump 5 to 10 1 of seawater through a filter and an extraction column. The filter-holder and columns were made of Teflon and were supplied with the instrument. The extraction material in the columns was XAD 2. Prior to the cruise each of the 5 columns was cleaned with about 1 1 of CH2C12 and isopropanol. Blanks were measured using TLC/FID and GC. Solvents were passed through the columns until the latroscan-measured total lipid content was below 5 µg per column. The columns were rinsed with about 1 1 of purified (Chapter 2) 'Super-Q' water (Millipore) immediately before use. The seawater passed through a 200 µm screen and a precombusted (400°C) Gelman A/E glass fibre filter before passing through the XAD column. On recovery each filter was wrapped in precombusted (400 °C) aluminium for and placed in a freezer (-20 °C). Each KAD column was capped and also placed in the freezer.

In the laboratory each column was ortracted with 200 ml of methanol-(Analabs) and then with 200 ml of C <sub>1</sub>Cl<sub>2</sub> (Analabs). The MeOH-seawater minuture was back extracted with CH<sub>2</sub>Cl<sub>2</sub> and the CH<sub>2</sub>Cl<sub>2</sub> portions. We find obtained, and roto-evaporated down to a few mls at room temperature. Further details describing the cleaning and use of these columns, (together with GC and GC/MS analyses of extracts, are given by Gershey (1986).

Figure 5.1

Station locations. Scotian Slope (1) in April, 1985 in 2750 m of water (41° 39.9'N, 64 33.2'W), and (2) in June, 1982 in 1280 m off water (42° 23.1'N; 64° 18.5'W). Bedford Basin (3) during the spring of 1984 in 70 m off water. Lake Haron (4) during the summer of 1984 in 95 m of water (43° 59.7'N, 82° 04.5'W).



Bottles vere closed at depths near the levels at which the pump, samples, vere collected in order to determine temperature, salinity, and phosphate concentrations. Salinity was measured with a Guildline Autosal model 8400 salinometer. Phosphate determinations were made with a Carlo Erba, autoanalyzer.

5.2.2 Bedford Basin

6

Samples were collected from five different depths at the centre of Bedford Basin during the spring bloom of 1984 (Chapter 4). Complete profiles were obtained on March 29 and April 5, three and four weeks after the maximum chlorophyll value had been measured at the same station (Roy, 1986; Parrish, Chapter 4). The seawater was collected in 5 1 Go-Flo (General Oceanics, Inc., Miami) bottles and processed as described in Chapter 4.

Temperature and salinity profiles vere obtained úsing a Beckman RS5-3 electrodeless induction salinometer. The salinity data vere calibrated against bottle salinities measured with a Guildline Autosal model 8400 salinometer. Particulate organic carbon was determined after combustion in pure oxygen in a Perkin-Elmor CHN Analyzer (Model 240B).

5.2.3 Lake Huron

An array of sediment traps was deployed in the southern part of Lake Huron (Fig. 5.1). from July 16 to September 17, 1984. The sediment traps were set at five different depths. The shallowest were at 10<sup>m</sup>. thermocline (35 m), and the deepest traps (83 m) were deployed 10 m above the bottom. The sediment trap design and the sample handling procedures are the same as those described by Eadie <u>et al</u>. (1984). Subsamples from the five depths were extracted with chloroform-MeOH (2:1) and washed with 0.9% MaCl solution using microtechniques. detailed by Gardner <u>et al</u>. (1985). Samples were stored under nitrogen in capillary tubes at -20°C for a maximum period of two months before analysis. The storage tubes were also used for applying the lipid organic, carbon data for the sediment trap samples were kindly provided by K. Dunham (University of Michigan, Ann Arbor).

in the surface mixed layer, the next traps were at the bottom of the

5.3 Results and Discussion-

Table 5.1 lists the classes detected by TLC/FID that were common to all the samples from all the sampling sites. This Table also provides a summary of all the lipid class concentrations that were observed in the surface mixed layer and below the pycnocline at all four stations. The concentrations of lipid classes below the pycnocline vere on dverage half the values observed in the surface mixed layer for dissolved or suspended particulate matter. In the case of sediment trop particulate matter and suspended particulate matter expressed as a ratio to total particulate organic carbon, the values in surface waters are on average 1.5 those in deeper values.

11 .

Ø

Table -5

F

Lipid class concentrations in dissolved matter (underlined) and suspended particulate matter from Bedford Basin and near the edge of t 1 040 the Scotian Shelf, and in particulate matter from sedimont traps deployed in Lake Huron. Station locations are given in Figure 5.1. The concentrations are averages and their units are µg per litre or + µg lipid class per mg total particulate organic carbon. There are two averages for each particulate or dissofved class for each station. The upper concentration given for each class is for the surface mixed layer; the lower concentration is for samples, taken below the pycnocline.

Station numbers (Figure 5.1)						
Lipid class	Abbrev <del>,</del> iation	(1) -µg/1	(2) * µg/1	(3) µg/1	(4) µg/mgPOC	I
∽ Aliphatic hydrocarbon ∿	HC	$\begin{array}{c c} 0.35 \\ 2.0 \\ \frac{12}{5} \\ $	$2.8 + \frac{10}{6.9}$	8:1 6.4 $\frac{1.0}{6.7}$	37 385 39 92	
Wax ester and stêro1 ester	WE	$\begin{array}{c} 0.17 \\ 0.56 \\ \hline 0.73 \\ \hline \end{array}$	$\begin{array}{c} 2.0 \\ 0.6 \\ 1.0 \\ 1.0 \\ 1.0 \\ 3 \end{array}$	$\begin{array}{c} 1.0 & \underline{1.3} \\ 1.7 & \underline{4.8} \\ \end{array}$	7.3 21 20 42	ø `
Triglycerıde	TG	$\begin{array}{c} 0.35 & \underline{5.4}^{2} \\ 0.20 & \underline{2.6} \end{array}$	1.7 <u>14</u> 1.8 <u>9.7</u>	$ \begin{array}{c}             11.2 & \underline{1.1} \\             6.4 & \underline{0.5} \\             \end{array} $	51 54 36 16	2-1 <u>-</u> -**  
Free-fatty	FFA	$\begin{array}{c} 0.30 & \underline{1.5}, \\ 0.37 & \underline{1.2} \end{array}$	$\begin{array}{c} 17 & \underline{30} \\ 2.8 & \underline{2.4} \end{array}$	$\begin{array}{c} 6.0 & \underline{3.1} \\ 7.2 & \underline{7.0} \\ \end{array}$	· 27 . · 23 41 27:	5 m
Free sterol⊽	ST, 	$\begin{array}{c} 0.19 \\ 0.09 \\ 0.51 \end{array}$	$\begin{array}{c} 0.8 \\ 0.2 \\ \end{array}  \frac{1.0}{0.8} \end{array}$	$\begin{array}{c c} 2.0 & \underline{1.4} \\ 1.3 & \underline{0.5} \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & &$	9. <sup>*</sup> 2 11 7.9 8.	,5 , ··`,
Acetone- mobile ~polar lipid	AMPL	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	19 <u>16</u> 9.4 <u>23</u>	88 62 54 56	ی در بر ۱ بر ۲ بر ۲
- Phospholipid	PL .	$\begin{array}{c} 0.50 & \frac{1.8}{1.5} \\ 0.27 & \frac{1.5}{1.5} \end{array}$	1.8 <u>15</u> 0.3 <u>3.6</u>	$\begin{vmatrix} 3.0 \\ 1.8 \\ 1 \end{vmatrix} \frac{1.1}{12}$	14,   18 11 <sup>3</sup> 5,	.0 ້

times greater than the particulate lipid concentrations in the same deavater sample. There seems to be a trend in this ratio from station (1) where there were large differences between dissolved and particulate lipid concentrations to station (3) where there were small differences in concentrations. In order to accortain if this trend could be related to the proximity of the sampling location to the shore, it would be necessary to occupy similar stations a second time and dise identical sampling and workup procedures at each station.
By comparing all the dissolved or all the particulate data for each class in Table 5.1 it is possible to suggast results that appear anomalous. These data are the surface particulate HC values for surface waters at station, (2). An analysis of new samples from the same be decided if

The concentrations of dissolved lipid classes were on average four

these values are real or artefacts of sample handling.

5.3.1 Scotian Slope

'T-S diagrams from the region where the 1982 samples were collected (Fig. 5.1) indicate the influence of three water types, and suggest that this region was in the vicinity of a front. The temperature descended to a minimum around 50 m (Delmas <u>et ar</u>, 1984); at this , " depth nutrient levels reached a maximum. The top 30 m of the water column was well-mixed. The closest grouping of isophalines and

isopycnals starts at around 75 m, where there was the most abrupt change in  $O_{1}$  (Fig. 5.2a).

196

Total lipid concentrations (Fig. 5.2b,c) were obtained from the sum of the TLC/FID peak areas when corrected for FID response as u for recovery using an internal standard or 'surrogate spike' (Keith et al., 1983). TLC/FID chromatograms of dissolved and particulate 'lipids obtained from shipboard analyses during the 1982 cruise are given by Delmas et al. (1984), and Parrish and Ackman (1985).

To a first approximation, the distributions of total lipid concentrations appear to be related to both biological and physical processes. The particulate lipid distribution (Fig. 5.2b) would seem to be related to that of chlorophyll <u>a</u> (Fig. 5.3a), while high concentrations of dissolved lipids appear at the chlorophyll maximum and also immediately above the largest step in  $O_t$  (Fig. 5.2a,c). There are few studies with which these TLC/FID data can be compared, but this is partly compensated by the fact that there are two-studies where the data are very suitable for comparison, both in terms of area sampled and in terms of the analytical workup procedures used.

Tables 5.2 and 5.3 are a comparison with detailed temporal and spatial studies by Kennicutt and Jeffrey (1981a,b) and Bhosle <u>et al.</u> (1983). The filtration and extraction procedures that were used in the Gulf of Mexico and the Arabian Sea were very similar to those used by us over the Scotian Shelf and Slope (Delmas <u>et al.</u>, 1984). except they were manipulating up to 90 1 while we were using only 4 1. All three studies were undertaken in or very close to shelf waters which in at least two of the sites were reasonably unpolluted. This assessment is made on the basis of hydrocarbon data (Keizer et el., 1977; Kennicutt and Jeffrey, 1981a,b).

\* 197 -

Although our data and those of Bhosle <u>et al</u>. (1983) are considerably more sparse than those of Kennicutt and Jeffrey (1981a,b), the three data sets compare favourably. For instance, Kennicutt and Jeffrey's temporal gange of 60 to 160 µg/l for surface vater dissolved lipids is very similar to our spatial range of 49-190 µg/l. The particulate lipid values compare equally well (Taple 5.3).

Kennicutt and Jeffrey found the majority of the lipids were in their polar fraction (Tables 5.2, 5.3). Their separation scheme included neutral lipids in this fraction. Taking this into account, the comparison with our data is again very good. Bhosle <u>et al</u>. based their fractionation scheme for dissolved lipid classes on that of Kennicutt and Jeffrey (1981a); their 'polar'lipid' percentages fall within the range reported by Kennicutt and Jeffrey (1981a).

Dissolved lipids seem to be characterized by a high degree of variability in space and time (Table 5.2) suggesting considerable patchiness and a high rate of anabolism and catabolism. There is also a considerable range in the ratio of dissolved to particulate lipids (Table 5.2). This range guggests that the distributions of dissolved and particulate lipids are not closely coupled and thus their production and destruction rates may not be either.

.198 Figure Vertical profiles of density and total "lipid" concentrations over the Scotian Slope in 1982. The exact station location is given in Figure " 5.1<sub>e</sub> (a) Density. (b) The sum of the Istroscan-determined particulate 11pid classes. (c) \* The sum of the latroscan-determined dissolved lipid classes.



1

.
Figure 5.3

¥.

Vertical profiles of the concentrations of particulate and dissolved lipid classes over the Scotian Slope in 1982. The exact station location is given in Figure 5.1:

200

V

(a) Fluorometrically determined chlorophyll <u>a</u> and Iatroscandetermined acetone-mobile polar lipids.

(b) Iatroscan-determined particulate phospholipid and triglyceride.

(c) Iatroscan-determined dissolved phospholipid and triglyceride.

PARTICULATE (Hg/L) PARTICULATE (Hg/L) DISSOLVED (Hg/L)

З



·Dissolved marine lipids from profiles in or near shelf regions.

Table 5.2

This study Kennicutt and Jeffrey, Bhosle et al. (1981a). (1983).Surface Waters Location Edge of Shelf of northeast Scotian Gulf of Mexico, 1977 of the Arabian Shelf, 1982. and 1978. Sea, 1983. 5-Degree of pollution Relatively, 'Relatively assessed from unpolluted' unpolluted' hydrocarbon data Kennicutt and Jeffrey, Keizer et al., (1977). (1981a,b). 60-160 · 49-190 Range of dissolved 165-295 lipids, in, the surface . miked layer (µg/l). Values in deepest 29 61-116 , vater (µg/1). 72-93% 55-95% 57-85% Proportions of total attributable to 'polar lipids' (TG, FFA, ST, AMPL, 🔊, PL) 🛹 5 <sup>(2)</sup> 5 Maximum variability: the largest ratio of maximum to minimum concentrations within a 10 m'interval or a 12 hr period. Ration of discolved to. 2-7 (3) 3-10(4.5)particulate lipids (median in brackets). nkormation unavailable.

Table 5.3

Particulate marine lipids from profiles in or near shelf regions.

203

		2	
	This study	Kennicutt and Jeffrey, (1981b).	من <sup>2</sup> مع چیم الکی D پر مع
Location	Edge of Scotian Shelf, 1982.	Shelf of northeast Gulf of Mexico.	້ •\$5 
Degree of pollution assessed from hydrocarbon data	'Relatively'. unpolluted	'Relatively'	۰. ۲
Range in surface ' mixed layer (ug/1).	28-58	12-70	r - 4 
Values in deepest vater (µg/1).	8.	9 <del>-</del> 52	۵۵ - ۲۵ ۱۳۵۳ - ۲۳ ۱۳۵۹ - ۲۳ ۱۳۵۹ - ۲۳ ۱۳۵۹ - ۲۳
Proportions of total attributable to 'polar lipids'.	59-90%	*4.9-85% ***	
Maximum variability: the largest ratio of maximum to minimum within a 10 m interval or a 12 hr period.	2 " <sup>*</sup> ~ ~ ~	5 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	
	10		
			At

C,

e de la

Boehm (1980) has already provided evidence that dissolved and particulate hydrocarbons are decoupled spatially and temporally. He sampled at stations close to the Scotian Slope stations sampled in the present study. The results in Table 5.2, together with the dissimilarity in the time series of dissolved and particulate lipid classes in Bedford Basin (Chapter 4), indicate that the decoupling of dissolved and particulate lipid classes may be more general in terms of the types of lipid classes and more widespread in terms of

The apparent lack of correlation between dissolved and particulate lipto distributions reflects other differences in the lipids measured /in each fraction of seawater. A comparison between data for dissolved lipids (Table 5.2) and data for particulate lipids (Table 5.3) gives similar results in both studies where measurements were made on both fractions of the same sample. In each of the categories in each Table, the walues for particulate lipids are generally lower than those for dissolved lipids. The absolute concentrations are lower, the ranges of concentrations are lower, there is less 'polar' material, and there may also be a lower variability.

environment.

Figure 5.3 shoul the distributions of chlorophyll <u>a</u> and some of the individual. Istroscan-measured Lipid classes. Other lipid class profiles from the same station are given by Delmas <u>et al</u>. (1984). For the dissolved lipids, each data point is the mean of 3 or 4 independent analyses. The total analytical precision is around 20%, which is the value one might expect from other chromatographic

E a

techniques, even GC. Unfortunately the precision for the particulate lipid analyses was not as good; the average CV was 35%. This is the result of being unable to collect and process enough water to do more than duplicate extractions and analyses. Because of the lack of precision in particulate lipid data at this station, the discussion will concentrate on features of these profiles that were repeated at other stations.

Most particulate lipid classes, especially the pigment-containing AMPL peak, follow the chlorophyll distribution. (Fig. 5.3a). The concentration of particulate AMPL and that of chlorophyll <u>a</u> we're s'trongly correlated (99% confidence). The AMPL level was, however, about 2 ug/l higher than that of chlorophyll <u>a</u>. This difference is probably caused by the inclusion of other pigments in this TLC/FID peak, as well as glycolipids which are also thought to be involved in the photosynthetic process. In addition to pigments and glycolipids, the AMPL peak probably contains diols, especially monoglycerides. Three particulate divid classes did not follow the chlorophyll distribution. These were wax esters, hydrocarbons, and trigfycerides. The first of these could be associated with microzooplankton. The pamples were prescreened at 250 µm, so it is less likely that larger zooplankton were contributing to the UE maxima found both at the chlorophyll maximum and at 75 m.

The maximum in particulate triglycerides at 75 m is interesting because it occurs at a level in the water column where there were low "" chlorophyll a values. Again, a heterotrophic contribution to this TG

peak is clearly a possibility. Another, though, is that this material has a purely algal origin. Phytoplankton exposed to low light levels for extended periods have been found to increase the amount of  $^{14}C$ assimilated into lipids, both in the laboratory and in the field (Harding et al., 1985). Thus the TG maximum at 75 m could be indicative of the presence of highly stressed algal cells, or detritus derived from such cells. In addition to light-stress, this TG-peak could also be indicative of algal nutrient-stress. A decrease rin nitrogen availability has been shown to cause an increase in algal TG synthesis (Pohl and Zurheide, 1982; Parrish, Chapter 3). A spring ( + bloom near the frontal region over the Scotian Slope could result in ... a large increase in the ratio of TG to chlorophyll a in surface vaters as nutrients become depleted, Large increases in this "tatio have been observed in the surface vaters of Bedford Basin during spring blooms (Chapter 4). If such a bloom sank then there is clearly a source for TG in deeper waters. Any large increase in density in ' the water column could act at least as a partial barrier to further sinking. In this way a TG-rich 'false bottom' (Sieburth, 1983) might be created.

The appearance of high Lipid levels either directly above the pycnocline or in it's immediate vicinity is a consistent pattern (Figs. 5.2-5.8). This points to the generality of large defisity differences acting as an impediment to further sinking of lipidcontaining material out of the surface mixed layer. This observation completes the spectrum of biochemical classes that have previously

<sup>-</sup> 206

been found to accumulate at discontinuity layers. These include particulate carbohydrates (Biggs and Wetzel., 1968), chlorophyll <u>a</u> (Goering <u>et al.</u>, 1970), and dissolved free amino acids and carbohydrates (Leibezeit <u>et al.</u>, 1980).

207 ،

For most lipid classes, the concentrations measured in the vicinity of the pychocline are anomalously high by comparison with measurements made at shallower depths (Figs. 5.3-5.8). This is also generally true for comparisons with measurements made at sampling levels that are markedly deeper than the surface mixed layer. Because of this, hone of the measurements made in the vicinity of the pychocline is included in the calculation of averages in Table 5.1. The high lipid values associated with discontinuities are not considered to be representative of the water types either above or below them.

Smetacek (1985) has argued that the rapid sinking of cells following diatom blooms represents the transition of these cells to a resting stage. Any viable phytoplankton cells present at 75 m at the 1982 Scotian Slope station could presumably have maintained their basal metabolism with the stored triglycerides while warting for a mixing event. Such a mixing process might take the form of very high winds, an upwelling event or a large amplitude internal wave. Each of these would result in the transport of some cells to levels in the water column where there was greater light. At the same time, nutrient levels would be replenished in the surface waters as a result of mixing. Thus the phytoplankton would be able to grow

- 208

rapidly until nutrient levels were again depleted. At this point TG would start to accumulate in the cells again and the cycle could be

Frgure 5.3(c) shows dissolved lipid classes from the 1982 statuon. The most striking feature of these profiles is the large peak in concentrations at '75" m. It is possible that these dissolved lipid peaks were associated with elevated levels of bacterial activity. Douglas (1985) measured bacterial numbers and activity at the station immediately preceeding the station where the lipid samples were taken (Figs. 5.2, 5.3). At station 55 of the 1982 Scotian Shelf cruise . Douglas (1985) measured a subsurface maximum in both bacterial numbers and bacterial activity on the same "isohaline that the elevated lipid levels were observed. Since the two stations were very close to one another in both time (<1.5 hrs) and space (<10 nautical males) it is possible that there were similar levels of bacteria and lipids on'the same is chaline at both stations. Liebezeit et al. (1980) found that glevated levels of bacteria and dissolved freeamino acids and carbohydrates occurred together at the upper boundary of the pycnocline in the Sargasso Sea, The increase in particulate phospholipid at 75 m (Fig. 5.3b) would suggest that microheterotrophs · of some form were present at that depth. PL is an indicator of biological membranes, and without any increase in chlorophyll <u>a</u> (Fig. 5.3a) it is probable that the PL peak is the redult.of the presence of heterotrophs smaller than 250 µm. Again, the association of high levels of PL and TG with pycnoclines is a pattern that is

repeated (Figs. 5.4-5.8). Figure 5.4 shows some of the data obtained from the second Scotian Slope cruise. At the station where the lipid samples were taken in 1985 it was possible to make only four vemperature measurements, and only three of these had salinity values for the same depth. Thus some of the densities in Figure 5.4(a) have had to be calculated by interpolation of salinity or temperature and there is more error than usual associated with these values. Nonetheless, the broad picture should be close to reality, and the water column can be assumed to be vell-mixed down to about 90 m. At this point there was also a change in the gradient of phosphate concentrations (Frg. 5.4b).

209

Elevated levels of lipids were again found at the bottom of the surface mixed layer (Fig. 5.4c, d). The classes with maximum concentrations at the pycnocline included particulate phospholipid and both particulate and dissolved trigyceride (Fig. 5.4c, d). The dissolved TG concentrations reached those observed immediately above the largest increase in  $\sigma_t$  in 1982 (Fig. 5.3c).

Although much more vater was sampled with the <u>in situ</u> sampler than was possible with bottle samples, during the 1982 cruise, the improvement in the analytical precision of lipid class concentrations was less than had been hoped. The reason for this is that significantly lover concentrations of lipids were measured at the 1985 station. Figure 5.4.

Vertical profiles of density and concentrations of phosphate and lipids over the Scotian Slope in 1985. The exact station location is given in Figure 5.1.

. . 210

(a) , Density.

В,

à

(b) Dissolved inorganic phosphate.

(c) Particulate phospholipid and the sum of the latroscan-measured particulate lipid classes.

(d) Dissolved and particulate triglyceride.



Figure 5.5

**X**2

7

Vertical profiles of salinity, temperature and concentrations of total lipids in Bedford Basin in 1984. The exact station location is given in Figure 5.1.

212

 $\tilde{U}$ 

(a) Salinıty.

(b) Temperature.

\*<sup>f<sup>2</sup>etr</sup>

170

(c) Total dissolved and total particulate lipids.

Υ.



Figure 5,6

Vertical profiles of the concentrations of particulate and dissolved lipid classes in Bedford Basın in 1984.

(a) Particulate free alcohol and acetone-mobile polar lipid.

(b) \* Dissolved phopholipid and acetone-mobile polar lipid.

(c) Dissolved triglyceride and free fatty acid.

Ø





BEDFORD BASIN PARTICULATE LIPID CLASSES UG LIPID CLASS/MG ORGANIC CARBON



Vertical profiles of the ratio of particulate lipid classes to particulate organic carbon in sediment trap samples from Lake Huron

218

" \* (a) \* Hydrodarbon and wax ester + sterol . ester.

TURO

(b) Triglyceride and acetone-mobile polar lipid.

(c). Phospholipid, and free fatty acid.



The intention had been to re-sample in a 'similat Hydrographic region in 1985 in the hope that there would be similarities in lipid distributions. The water 'sampled, however, was both warmer and more saline'than in 1982, and the concentration's of individual lipid classes vere .on average, six times lover (Table.5.1). This, coupled with the fact that half of each sample was used for GC analyses, meant that the Vevels of the lipids measured on Chromarods in 1985 uere similar to those measured in 1982. Nonetheless, the precision' Sor, the 1985 samples was still more acceptable. The precision for most dissolved lipid classes was under 20% CV, "while for the particulate classes it was usually between 5 and 35%. The concentrations of particulate lipids at the 1985 station (Table. 1) were similar to those observed by Volkman et al (1986) at an oceanic station. Volkman et al. also used TLC/FID; they report the concentrations of six particulate lipid classes at five depths in the Tasman Sea. Inspection of their data suggests further similarities with the data presented here. For two of their lipid classes, the thighest concentrations measured were at 90 m, and for another two the second highest concentration was measured, at this depth. The highest concentration of the sum of their lipid classes (10.7 ug/1) occurs also at 90 m. Volkman et al. (1986) state that the top 90 m of the vater column at their station was well mixed, and that their deepest sampling level (120 m) was below the thermocline. This suggests that in the Tasman Sea there was also an accumulation of lipid material

immediately above the pychocline.,

## 5.3.2 Bedford Basin

The lipid class profiles were very similar on the two days that dissolved and particulate samples were obtained from five different depths in Bedford Basin during the 1984 spring bloom. Most of the observed differences were less than 5 mg/1 for dissolved or particulate lipid class concentrations measured in samples taken from the same depth on both days. Data from these two profiles are summarised with other Bedford Basin data in the Appendix.

Once again, maxima in dissolwed and particulate lipids were observed to be coincident with the beginning of the sharpest changes in salinity and temperature in the profiles. Both salinity and temperature profiles have been presented in Figure 5.5 because the RS5 temperature readings were not properly calibrated and thus accurate density values could not be obtained. However, at the temperatures and salinities in question the density profile should. Resemble that of salinity. The observed variations in salinity (Fig. 5.5a) would produce an order of magnitude greater change in  $\sigma_t$  than yould the observed changes in temperature (Fig. 5.5b).

The March 29 profiles of salinity and temperature were loss noisy than those obtained on April 5, and so it is the lipid profiles obtained in March that are presented here (Figs. 5.5-5.7). The particulate lipid class concentrations have been normalized to POC values in Figure 5.7 in order to make them directly comparable with the sediment trap data in Figure 5.8. Table 5.1 gives the averages for Bedford Basin particulate lipids in both units.

Typical chromatograms of lipid class separations from Bedford Basin dissolved and particulate samples can be found in Chapter 2. The precision of replicate analyses for most dissolved lipid classes was under 20% CV, while for particulate classes it was usually under 30% CV. The lipid class profiles (Figs. 5.6, 5.7) are in most respects similar to those observed at other stations. The only major difference is the marked increase in some dissolved lipid classes below the pycnocline (Table 5.1, Fig. 5.6b,c). This increase is, likely to be the result of degradation following the spring bloom. These profiles were obtained three weeks after the maximum chlorophyll a concentration was measured in Bedford Basin (Roy, 1986; Parrish, Chapter 4). Just after the peak of the bloom a substantial storm is thought to have spread a large number of diatoms below the level of the pycnocline in Figure 5.5(a,b). This material then continued sinking (Roy, 1986) and it presumably started decaying at the same time.

5.3.3 Lake Huron

Two to four subsamples were obtained from each of the five traps deployed in Lake Huron; each subsample was extracted and measured independently. Each extract was applied to one or more Chromarods so that two to ten Iatroscan determinations were made per depth. The total analytical precision for four extracts from the same trap, but measured on four different. Chromarods was better, than ±20% CV, for each lipid class. This is the same level of precision as obtained by

Wakeham <u>er al.</u> (1980) vorking with capillary & analyses on marine sediment trap material.

223

TLC/FID lipid class determinations were also made on several total procedure blanks. A maximum of five lipid classes was detected in the blanks; these classes mormally accounted for less than 15% of the amounts measured in samples.

Sypical TLC/FID chromatograms from sediment trap extracts are shown and discussed in Chapter 2. The units used for the sediment trap data (µg lipid class / mg organic carbon) are somewhat awkward but are the same as those used by other workers with GC data (e.g. Meyers et al., 1984; Uakeham et al. 1984b). A factor of about 20 is needed to convert these units to percent by weight. Thus latroscan-measured hydrocarbons could have accounted for as much as 20% of the organic matter in the near-surface sediment traps in Lake Huron (Table 5.1, Fig. 5.8). This value is extremely high and very different from the values observed in Bedford Basin suspended particulate.matter (Table 5.1. Fig. 5.7b). If such a high value is close to reality for the Great Lakes area it would suggest that there is a massive source of hydrocarbon pollution. Perhaps such an input would occur in the form of macroparticulates: there was no prescreening of these samples. Surprisingly, once the values in the vicinity of the pycnocline are excluded, there was only one other major difference between the obpervations made with sediment trap material and with suspended particulate matter. A comparison of the last two columns of Table 5.1 suggests that UE levels were significantly higher in the Lake Huron

samples. A comparison of Figures 5.7(c) and 5.8(a) shows that this yas especially true in the vicinity of the pycnocline. Again, macroparticulates could be responsible for this difference. It seems likely that the large WE peak in Figure 5.8(a) is the result of zooplankton debris entering the 35 m trap. An active zooplankton population could be taking advantage of high energy compounds present in the thermocline. Once again, the maximum TG and PL content of particulate matter appears to be associated with the density discontinuity (Fig. 5.8b).

224

Terrestrial material could obviously be partly responsible for the high lipid class values observed at the pycnocline. The problem with this interpretation, however, is that the land-derived material would have to somehow bypass the 10 m trap. While this would not be impossible, there is evidence to suggest that this does not happen. Meyers <u>et al</u>. (1984) found that the organic material in midwater particles in Lake Michigan was predominantly of aquatic origin.

The dissolved and particulate lipid class data collected near the edge of the Scotian Shelf compare well, both qualitatively and quantitatively, with other lipid data collected in other shelf regions (Kennicutt and Jeffrey, 1981a,b; Bhosle <u>et al.</u>, 1983). Dissolved lipid concentrations were characterized by a high degree of variability and there was a considerable range in the ratio of dissolved to particulate lipids. The lipid class data in these shalf

5.4 Conclusion

studies, together with the work of Boehm (1980) and Parrish (Chapter 4), suggest that the lipid class concentrations measured in the dissolved and particulate fractions are the result of somewhat independent processes occurring on significantly different space and time scales.

High dissolved and particulate lipid class levels were observed either directly above the pycnocline or in its immediate vicinity in the four environments sampled in the present study. The presence of certain lipid indicator classes suggests that bacteria and zooplankton take advantage of the energy-rich compounds that were concentrated at the discontinuities in the water columns.

<u>a</u>.

The spatial heterogeneity observed in lipid classes in the upper water column in the present study adds weight to arguments against the treatment of dissolved or particulate organic carbon as a single variable in oceanographic processes. Harvey (1983) has summarized the literature data on the extensive variability in the concentrations of three of the major dissolved organic classes in seavater. This variability is not reflected in dissolved organic carbon measurements, and thus DOC cannot be truly representative of organic processes occurring in the upper water column.

The same argument obviously applies to particulate organic matter. The use of a single chemical formula (Redfield <u>et al.</u>, 1963) or a single calorific conversion factor for phytoplankton (Platt and Irvin, 1973) that been an accepted practise in oceanography for many years. One argument in support of this practise has been that these

single values are 'reasonable' global averages; 'houever, the general applicability of even the ratio given by Redfield <u>et al</u>. (1963) is being brought into question (Jones <u>et al</u>., 1984; Takahashi <u>et</u> <u>(al</u>., 1985).' In both these latter studies there is some question as to what is a reasonable lipid content of decaying biogenic material in the water column. Observation<sup>(2)</sup> made in the present study show that within just the top 100 m of the water column the highest lipid values can be expected to be several times larger than the lowest values both in terms of concentrations per litre and in terms of the proportion of total organic carbon. Clearly it will be a while before we can decide on a reasonable global average lipid content for particulate matter.

There are, however, indications in this Chapter and in Chapter 4 that within certain depths in the water column and during certain times of the year it would be possible to estimate an average "particulate or dissolved lipid content. The importance of hydrography in determining the lipid conter of a seawater sample is confirmed by ' the surprising similarity between lipid data obtained in this study. and data from other studies in similar water macces.

226

CL)

## General Conclusion

Lipids can be categorized into as many as twenty classes according to their chemical structures. The compounds in most of these classes have known functions in various types of cells or known anthropogenic sources. It is possible to distinguish energy storage classes, membrane classes, pollutants, and indicators of lipid degradation. Individual classes or groups of certain classes can thus be used to indicate the presence of certain types of organisms as well as their physiological state and activity. The presence of other classes can provide a signal of poor water quality.

673

The measurement of the complete suite of lipid classes in both dissolved and particulate matter in several different studies in this thesis represents a new approach to research into important oceanographic processes. A new analytical technique wagused to make the lipid class measurements: Chromarod thin-layer chromatography (TLC) with latroscan flame ionization detection (FID). In order to make the seawater determinations, development procedures were designed for the separation of marine lipid classes on the silica gel coated Chromarods. The latroscan was slightly modified to facilitate these development schemes. Procedures were also elaborated to optimize the detection and measurement of lipid classes in the latroacan. An intercalibration was conducted with an independent laboratory using a different analytical technique. This comparison between TLC/FID and gravimetry showed that the two techniques gave very similar results.

227

Ð

The TLC/FID procedures developed during the course of this thesis work were used successfully in the measurement of over 500 dissolved and suspended particulate seawater samples. The separation and calibration schemes were also found to be applicable to extracts of freshwater samples, sediment trap material, samples fromphytoplankton cultures, benthic invertebrates and net-caught zooplankton samples. The procedures developed here are of value in fields other than the aquatic sciences. There has already been considerable interest from other lipid chemists, especially those working in the field of medicine, where the sample matrix can be as complicated as that of aquatic samples.

\*228

In addition to the development of TLC/FID techniques, it was also necessary to investigate the other steps in the analytical process. It was important to ensure that sampling, extraction, storage and sample concentration procedures were all compatible with the TLC/FID analyses. Dichloromethine was found to be an efficient extracting solvent, and due to it clative volatility, its use is an advantage over other solvents at the concentration stage and when applying samples to Chromarods. Samples could be rapidly concentrated at relatively low temperatures; it was also possible to minimize the spreading of material along the Chromarods during spotting.

The environmental research in this thesis has centred on processes occurring among the unicellular organisms which form the base of the food-webs in the oceans. Until recently, lipid research in this field has been conducted with either rough total lipid measurements or with Satty acid measurements which gave no indication of the lipid classes to which the fatty acids belonged. In this thesis, TLC/FID has been used to investigate the production and distributions of dissolved and particulate lipid classes in two complementary types of study. Laboratory culture work has been conducted under highly controlled conditions, and several field studies were conducted to see how the laboratory data relate to what is occurring in the natural environment.

229

The laboratory work was conducted with cultures of the marine diatom <u>Phaeodactylum</u>, tricornutum. The value of such a study is twofold. An understanding of factors affecting lipid production by algae in the laboratory should give insights into the dynamics of the production of compounds with high calorific value at the base of food-webs. This information could also be used to manipulate the lipid content of algae grown in large scale cultures to provide optimal lipid production for commercial applications.

The culture studies focused on the effect of changing the rate of nitrogen supply on algal lipid class production. Both dissolved and particulate lipid production were investigated using cage culture turbidostats for continuous automated culture. This new culturing technique circumvents the major problems that occur with microcosme. and batch cultures: conditions are not constantly changing in the culture vessel. Under a given set of experimental conditions, growth rate and the physical and chemical environment of the cells remain relatively constant. In principle, any changes that occur, in the cage culture turbidostats are the result of some alteration caused by the researcher. In practise, there are, of course, deviations from this ideal behaviour. One example given in Chapter 3 is the unexplained change from the normal unicellular forms of <u>Phaeodactylum tricornutum</u> to its chain-form. It is possible to minimise any effects associated with whatever unknown variables there are in the system by repeating the experiments with each turbidostat. In this way, cause and effect can be undeniably linked.

In the study described in Chapter 3, up to three culturing units were supplied with medium of known nutrient content simultaneously, and the intracellular lipid content and the lipid content in the effluent media were monitored. It was found that the intracellular synthesis of a storage component, triglyceride, was clearly triggered by nitrogen-stress. This result parallels observations made during spring blooms in Bedford Basin (Chapter 4). Triglyceride became the major lipid class measured in particulate matter from the time of the chlorophyll maximum onwards, a period when there were significantly lower nutrient levels in the water column.

The directing of anabolism towards an energy-rich storage component. under conditions of nutrient stress has important ecological implications. It would suggest that the most efficient transfer of energy to the next level in the food-web during a bloom would be after the maximum chlorophyll a concentration occurred. Measurements of zooplankton activity by Conover and Mayzaud (1984) during spring. blooms in Bedgord Basin indicate that zooplankton do indeed take.

advontage of energy-rich particles present after the chlorophyll:

aaximum.

The accumulation of triglyceride under conditions of stress can also be construed as an algal survival mechanism. The sinking of cells out of surface waters after a bloom should provide a gource of triglyceride to the bottom of the surface mixed layer. where the density difference would act as an impediment to further sinking. The fact that this occurs is supported by the observation that particulate triglyceride levels were consistently at their highest in the vicinity of the pychocline in a wide variety of environments. (Chapter 5). This triglyceride could possibly be important in maintaining the basal metabolism of algae while they await a mixing event which would transport them back to surface waters with replenished nutrient levels.

The lipid-rach particles present at pychoclines (Chapter 5) could be an important component of a form of 'false benthos' where elevated levels of bacterial and zooplanktonic activity would occur. This elevated activity would explain the elevated dissolved lipid concentrations that were measured in the vicinity of pychoclines (Chapter 5).

There were other interesting observations made during blodms in Bodford Basin. The major particulate lipid classes present after the chlorophyll maxima suggested that in addition to increased energy. storage, there was also a greater degree of degradation of lipids and a higher level of microheterotrophs. Particulate sterols shoued a very clear signal both during the phytoplankton blooms and in the turbidostat cultures. It would seem that the level of particulate sterol present in a marine sample could be directly linked to phytoplankton growth rate, provided chlorophyll a fewels were high and anthropogenic or terrestrial sterol

<u>232</u>

-contributions vere lous

During the 1982 Bedford Basin spring bloom, the concentrations of dissolved lipid classes were not correlated with those of the particulate lipid classes. An unexpected observation was that the lowest concentrations of dissolved lipids were measured near the time when the maximum chlorophyll a value was measured. This suggests that tipid release by algae is decreased at this time and that uptake by bacteria is increased. In addition, Chapter A provides evidence that when dissolved inorganic levels of phosphorus and nitrogen become depleted, algae use dissolved phospholipids as a source of these elements.

The various time series and profiles described in this thesis indicate that the processes controlling dissolved lipid class concentrations may be independent of those controlling particulate lipid class concentrations. The spatial and temporal heterogeneity that appears to be characteristic of dissolved and particulate lipid class concentrations varns against assuming any uniformity in the composition of total dissolved matter or total particulate matters There has never been any logical reason to suppose that DOM or POM can be treated or would behave like an autonomous unit in the photic zone, yagit is still often thought of in this manner. This thesis supports the contention that this is not, in fact, arreasonable assumption.

The results of the measurements made in this thesis together with their interpretation suggest several fruitful areas for further research. Firstly, more culture work with other species would be useful to examine the universality of the algal responses to stress observed in Chapter 3. It would be worthwhile to extend the stressing factors to include deprivation of phosphorus, silicon and light. Combinations of stressing factors could also be examined. An investigation into the uptake of phospholipids under stressed conditions may provide some important information on algal survival strategies in oligotrophic waters.

With the results described here, field work could now become much more focused, and with the right equipment and sufficient workers, much less arduous. The key to'a successful sampling programme in the future would be not to use the labour and time intensive survey approach used predominantly in this thesis. It would be important to use continuous recording, in situ devices as much as possible; good GTD data are essential for sampling at discontinuities. Important acceanographic processes could be followed by measuring in situ fluorescence and back-scatter. In this way it would be possible to sample for lipids at strategic points in space and time: superfluous measurements would be avoided and important 'events' would not be

) s

## . Wishful thinking?

• • • • • • •

() ``

\$~~ \$}

.

234

1 • • •

## APPENDIX

This appendix gives a summary of the dissolved and particulate lipid class data obtained during the 1984 Spring Bloom in Bedford Basin. The data were obtained from three stations one in each of Bedford Bay, Bedford Basin, and Halifax Harbour, Nova Scotia. The exact locations of the stations are given in Chapter 4 (Fig. 4.1). The tables on the following two pages give the day sampled in Julian days and the depths and stations sampled on each day.

A dash indicates a depth that was not sampled. All the data are for Bedford Basin unless otherwise indicated. The principal figure given for each depth is the total lipid concentration in µg/1. The figure below the concentration is the total number of lipid classes detected; the abbreviation following this number indicates the lipid class that was the major contributor to the dichloromethane extract. An explanation for the abbreviations is given in Chapter 4 (Table 4.1). In the case of dissolved lipids (Table A.2) AMPL was almost always the major contributor and so the second largest component has also been given.
s ,	Ŷ		0	Ş -		<b>N</b>
		, T <sub>a</sub>		×	1 D	
1 A	6 90 90 90	۵	<b>1</b>			236 ,
	Table A.	". 1 , , , , , , , , , , , , , , , , , , ,	articul	ate lipids during	v the 1984 spring	به ب bloom.
	, , ,		1	"Lulian day		
	Depth	۲47 <sup>5</sup> 4	61 <sup>°</sup>	68 , 72 , 75	89 96	103
. <i>G</i>	1 m	- 45 <sup>**</sup> 8,FFA	42 8, Ampl	123 <sup>1</sup> *94 61 10, TG , 9, FFA 8,	. 53 80 <sup>,</sup> TG 9, AMPL 9, TG	72
•	Bedford Bay		 ·		_ ~ _ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	91 8, AMPL
- , v -	Halifax Harbour	· · · ·	-	* · · · · · · · · · · · · · · · · · · ·	`-;`-`````````````````````````````````	108
۔ م س	° ^ ^ 3 m	÷ - ·	- •	114 – – 10, AMPL	ۍ ۲۰۰۰ ۲۰۰۰	<sup>ال</sup> حو الحو
- ( 1. cz. ) 	5 m	3,2 43 10,TG 9,AMPL	107 9 <sub>D</sub> AMPL		54 231 191 TG 10,TG 10,TG	156 10,AMPL
* ~ * ~	Bedford Bay	 a	24 — 7 7	82 - 		89 <sup> </sup> 9, FFA
	Halifax Harbour		,	48		67 `` 8, FFA
٥	'8 m	23 - ' 10,HC	-	• • • • • • • • • • • • • • • • • • •	•	-
، ۲ م ۱ م	15 m		 	· · · · · · · · · · · · · · · · · · ·	, 50 30 8, AMPL 8, AMPI	79 .9,HC ,
€ س <i>د ≴ب</i>	35 m	`	٭ ۹		44 41 9,AMPL 9,HC	- &, ,
с <sub>т</sub>	с 55 ш		-	۲ ۲ ۳ ۳۰ می معم ۲۹	22 27 8. AMPL: OFAMPL	- * •
		۰ ر		, , , , , , , , , , , , , , , , , , ,	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ہ , 2 <sup>6</sup>
. /	<b>τ</b>	•		, <b>,</b>	n	· .

Table Ä.2

The distribution of dissolved lipids during the 1984 spring bloom. 1 7 1 ¥ 3 Julian/day 89⁄ 72 96 103 ្ Depth 🗸 68 128 79 25 \* 36 1 m

23

8, AMPL, FFA 8, AMPL, FFA 7, AMPL, FFA 8, AMPL, PL , 160. 3 m 7, AMPL, FFA ŝ 34 🕈 5 m 76 86 🚬 🦿 88 ۍ <del>ک</del>ې 5, AMPL, FFA 7, AMPL, FFA 8, AMPL, TG 7, AMPL, PL Bedford 108 🕻 52 8, AMPL, FFA Bay 4, AMPL, FFA Halifax 81 -54 Harbour 7, AMPL, FFA 8, AMPL, HC 25 15 m 39 5, AMPL, ALC 6, AMPL, FFA

.35 m - 31 59 - 5, PL, AMPL 9, AMPL, FFA 55 m - 26 31 - 4, AMPL, PL 6, FFA, AMPL

## BIBLICGRAPHY.

Aaronson, S., and Dubinsky, Z., 1982. Mass production of microalgae. Experientia 38: 36-40.

Ackman, R.G., 1981a. Flame ionization detection applied to thin-layer , chromatography on coated quartz rods, pp205-252 in: Methods in Enzymology, J.M. Lovenstein (ed), Academic Press, London, Vol. 72.

Ackman, R.G., 1981b. Algaç as sources for edible lipids. pp189-220 in: Wew sources of fats and oils, E.H. Pryde, L.H. Princeu, and K.D. Mukherjee (eds), American Oil Chemists' Soclety, 340pp.

3 2

ረኈ

翁

Ackman, R.G., 1985. Siliça gel particle size and coating thicknesses

Ackman, R.G., Jang, ard, P.M., Hoyle, R.J., and Brockerhoff, H., 1964. Origin of marine fatty acids. I. Analyses of the fatty acids produced by the diatom <u>Skeletonema costorum</u>. J. Fish. Res. Board Cant 21: 747-756.

Ackman, R.G., Tocher, C.S., and McLachlan, J., 1968. Marine phytoplankter fatty acids. J. Fish. Res. Board Can. 25: 1603-1620.

Admiraal, W., and Werner, D., 1983. Utilization, of limiting concentrations of ortho-phosphate and production of extracellular organic phosphates in cultures of marine diatoms. J. Plankton Res. 5: 495-513.

Aitzetmuller, K., 1982. Recent progress in the high performance liquid chromatography of lipids. Prog. Lipid Res. 21: 171-193.

\*Anderson, J.W., 1980. Bioenergetics of autotrophs and heterotrophs. Eduard Arnold, London, 59pp.

Ansell, A.D., Coughlan, J., Lander, K.F., and Loosmore, F.A., 1964. Studies on the mass culture of <u>Phaeodactylum</u>. IV. Production and nutrient utilization in outdoor mass culture. Limnol. Oceanogr. 9: 334-342.

Antia, N.J., McAllister, C.D., Parsons, T.R., Stephens, K., and Strickland, J.D.H., 1963. Further measurements of primary production using a large-volume plastic sphere. Limnol. Oceanogr. 8: 166-183.

Asselineau, J., 1966. The bacterial lipids. Holden-Day, San Francisco, 372pp.

238

" \*

Bachofen, R., 1982. The production of hydrocarbons by <u>Botryococcus</u> braunii. Experientia 38: 47-49.

Banerjee, A.K., Ratnayake, U.M.N., and Ackman., R.G., 1985a. Effect of omalic acid impregnation of Chromarods on the separation of phospholipids for determination by the Iatroscan TLC/FID. Lipids 20: 121-125.

Beherjee, A.K., Ratnayake, W.M.N., and Ackman, R.G., 1985b. Enhanced response of more highly unsaturated lipids on thin-layer, chromatography - latroscan flame ionization detection after exposing the developed Chromarods to iodine vapour. J. Chromatogr. 319: 215-217.

Banse, K., 1974. On the interpretation of data for the carbon-tonitrogen ratio of phytoplankton. Limnol. Oceanogr. 19: 695-699.

Banse, K., 1977. Détérmining the carbon-to-chlorophyll ratio of natural phytoplankton. Mar. Biol. 41: 199-212.

Banerjee, S., 1984. Solubility of organic mixtufes`in water. Environ... Sci. Technol. 1,8: 587-591.

Barbiers M., Joly, D., Saligt, A., and Tourses, D. (1973). Hydrocarbons from seawater. Deep-Sea Res. 20: 305-314.

Barrick, R.C., 1982, "Flux of aliphatic and polcyclic aromatic hydrocarbons to central Puget Sound from Seattle (Westpoint) primary sevage effluent. Environ. Sci. Technol. 16: 682-692."

Bates, T.S., Hamilton, S.E., and Cline, J.D., 1983. Collection of suspended particulate matter: continuous flow centrifugation <u>vs.</u> filtration Estuar. Coast. Shelf Sci. 16: 107-112.

-Benson, A.A., and Shibuya, I., 1962. Surfactant lipids. pp371-383 in: Physiology and biochemistry of algae, R.A., Lewin (bd), Academic Press, New York.

Bhosle, N.B., D'Silva, C., and Reddy, C.V.G., 1983. Lipid content in surface water of Arabian Sea. Indian J: Mar. Sci. 12: 242.

Biggs, R.B., and Wetzel, C.D., 1968. Concentration of particulate carbohydrate at the halocline in Chesapeake Bay. Limnol. Oceanogr. 13: 169-171.

Billett, D.S.M., Lampitt, R.S., Rice, A.L., and Mantoura, R.F.C., 1993. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature 302: 520-522.

r.1

Billmire, E., and Aaronson, S., 1976. The secretion of lipids by the freshvater phytoflagellate <u>Ochromonas</u> <u>danica</u>. Limnol. Oceànogr. 21: 138-140.

Blasig, R., Schunck, U.-H., Jockisch, W., Franke, P., and Muller, H.-G., 1984. Degradation of long-chain n-alkanes by the yeast <u>Lodderomyces elongisporus</u>. I. Products of alkane oxidation in whole cells. Appl. Microbiol. Biotechnol. 19: 241-246.

Boehm, P.D., 1980. Evidence for the decoupling of dissolved, particulate and surface microlayer hydrocarbons in northwestern Atlantic continental shelf waters. Mar. Chem. 9: 255-281.

Boutry, J.L., Saliot, A., and Barbier, M., 1979. The diversity of. Marine sterols and the role of algal bio-masses; from facts to hypothesis. Experientia 35: 1541-1543.

- Bowers, W.D., Parsons, M.L., Clement, R.E., and Karasek, F.W., 1981. Component loss during evaporation-reconstitution of organic environmental samples for gas chromatographic analysis. J. Chromatogr. 207: 203-211.
- Cautiet, G., 1978. Organic chemistry of sea water particulates, concepts and developments. Oceanol. Acta 1: 99-105.
- Chapman, D., 1969. Intoduction to lipids. McGraw-Hill, London, 141pp.
  - Chen, I.S., Sheh, C.-S.J., and Sheppard, A.J., 1981. Comparison of methylene chloride and chloroform for the extraction of fats from food products. J. Amer. Oil Chem. Soc. 58: 599-601.

Christie, W.W., 1973. Lipid analysis. Isolation, separation, fdentification, and structural analysis of lipids. Pergamon, Oxford, 338pp.

Christie, W.W., 1985. Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (lightscattering) detection. J. Lipid Res. 26: 507-512.

Chu, F.-L.E., and Dupuy, J.L., 1980. The fatty acid composition of three unicellular algal species used as food sources for larvae of the American oyster. Lipids 15: 356-364.

240 .

.0

Clement, R.E., Sweetman, J.A., and Karasek, F.W., 1982. Esterification of carboxylic acids present on airborne particulate matter during methanol extraction and storage. Intern. J. Environ. Anal. Chem. 11: 97-104.

Collyer, D.M., and Fogg, G.E., 1955. Studies on fat accumulation by algae. J. Exp. Bot. 6: 256-275.

- Comita, P.B., and Gagosian, R.B., 1983. Membrane lipid from deep-sea hydrothermal vent methanogen: a new macrocyclic glycerol diether. Science 222: 1329-1331.
- Conover, R.J., and Mayzaud, P., 1984. Utilization of phytoplankton by. zooplankton during the spring bloom in a Nova Scotia inlet. Can. J. Fish. Aquat. Sci. 41: 232-244.
- Conover, S.A.M., 1975a. Partitioning of nitrogen and carbon in cultures of the maring diatom <u>Thalassiosira</u> <u>fluviatilis</u> supplied with nitrate, ammonium, or urea. Mar. Biol. 32: 231-246.
- Conover, S.A.M., 1975b. Nitrogen utilisation during spring blooms of marine phytoplankton in Bedford Basin, Nova Scotia, Canada. Mar. Biol. 32: 247-261.
- Cooke, G.D., 1977. Experimental aquatic laboratory ecosystems and communities. pp59-103 in: Aquatic microbial communities, J. Cairns (ed), Garland, New York, 695pp.
- Cooks, R.G., Busch, K.L., and Glish, G.L., 1983. Mass spectrometry: analytical capabilities and potentials. Science 222: 273-291.
- Coughlan, J., 1962. Chain formation by <u>Phaeodacty1um</u>. Nature 195: 831-832.

Crane, R.T., Goheen, S.C., barkin, E.C., Rao, G.A., 1983. Complexities in Nipid quantitation using thin layer chromatography/ for separation and flame ionization for detection. Lipids 18: 74/ 60.

Cranvell, P.A., Robinson, N., and Eglinton, G., 1985. Esterified. lipidb of the freshwater dinoflageblate <u>Peridinium</u> <u>lomnickii</u>. Lipids 20: 645-651.

Davson, R., and Liebezeit, G., 1981. The analytical methods for the characterisation of organics in seavater. pp445-496 in: Marine organic chemistry, E.K. Duursma and R. Davson (eds), Elsevier-Scientific, Amsterdam.

Delmas, R.P., Parrish, C.C., and Ackman, R.G., 1984. Determination of , lipid class concentrations in seawater by thin-layer chromatography with flame ionization detection. Anal. Chem. 56: 1272-1277.

242

Dortch, Q., Clayton, J.R., Thoresen, S.S., Cleveland, J.S., Bressler, S.L., and Ahmed, S.I., 1985. Nitrogen storage and use of biochemical indices to assess nitrogen deficiency and growth rate in natural plankton populations. J. Mar. Res. 43: 437-464.

- Douglas, D.J., 1985. Spatial association and trophic coupling of heterotrophic bacteria with phytoplankton. Ph.D. dissertation, Dalhousie University, 200pp.
- Eadie, B.J., Chambers, R.L., Gardner, W.S., and Bell, G.L., 1984. Sediment trap studies in Lake Michigan: resuspension, and chemical fluxes in the southern basin. J. Great Lakes Res. 10: 307-321.
- Eganhouse, R.P., and Calder, J.A., 1976. The solubility of medium molecular weight arometic hydrocarbons and the effects hydrocarbon co-solutes and salinity. Geochim. Cosmochim. Acta
  - Ehrhardt, M., Bouchertall, F., and Hopf, H.-P., 1982. Aromatic ketones concentrated from Baltic seawater. Mar. Chem., 11: 449-461.
  - Ehrhardt, M., and Derenbach, J., 1980. Phthalate esters in the Kiél Bight. Mar. Chem. 8: 339-346.
  - Ehrhardt, M., Osterroht, C., Petrick, G., 1980. Fatty-acid methyl esters dissolved in seawater and esociated with suspended particulate material. Mar. Chem. 10: 67-76.
  - Elbein, A.D., and Forsee, W.T., 1976. Diosynthesis of steryl glucosides and acylated steryl glucosides in plants. pp345-368 in: Glycolipid methodology, L.A. Witting (ed), American Oil Chemists' Society, Illinois.
  - Environment Canada, 1984. Monthly meteorological summary, February -April, 1984. Halifax - Dartmouth, N.S. (Shearwater A.). Environment Canada, Atmospheric Environment.
  - Farnworth, E.R., Thompson, B.K., and Kramer, J.K.G., 1982. Quantitative determination of neutral lipids on Chromdrods. J. Chromatogr. 240: 463-474.
  - Fenical, W., 1982. Natural products' chemistry in the' marine environment. Science 215: 923-928.

Florence, T.M., Lumsden, B.G., and Fardy, J.J., 1983. Evaluation of some physico-chemical techniques for the determination of the fraction of dissolved copper toxic to the marine diatom <u>Nitzschie</u>.
 <u>Closterium</u>. Anal. Chim. Acta 151: 281-295.

243

\*\*

Fogg, G.E., 1975. Algal cultures and phytoplankton ecology. 2nd ed. Univ. Wisconsin. 175pp.

- Forney, F.W., and Markovetz, A.J., 1971. The biology of methyl ketones. J. Lipid Res. 12: 383-395.
- Fraser, A.J., Tocher, D.R., and Sargent, J.R., 1985. Thin-layer chromatography - flame ionization detection and the quantitation of marine\_neutral lipids and phospholipids. J. Exp. Mar. Biol. Ecol. 88: 91-99.

Fulcó, A.J., 1983. Fatty acid metabolism in bacteria. Prog. Lipid Res. 22: 133-160.

Gagosian, R.B., 1976. A detailed vertical profile of sterols in the Sargasso Sea. Limnol. Oceanogr. 21; 702-710.

Gagosian, R.B., and Lee, C., 1981. Processes controlling the distribution of biogenic organic compounds in seawater. pp91-123 in: Marine organic chemistry, E.K. Duursma and R. Dauson (eds), Elsevier Scientific, Amsterdam.

Gardner, U.S., Frez, W.A., Cichocki, E.A., and Parrish, C.C., 1985. Micromethod for lipids in equatic invertebrates. Limnol. Oceanogram 30: 1099-1105.

Gatten, R.R.; Sargent, J.R., Foresberg, T.E.V., O'Hara, S.C.M., and Corner, E.D.S., 1980. On the nutrigion and metabolism of zooplankton. XIV. Utilisation of lipid by <u>Calanus helgolondicus</u> during maturation and reproduction. J. mar. biol. Ass. U.K. 60: 391-399.

Gauthier, M.J., and Aubert, M., 1981. Chemical telemediatory in the marine environment. pp225-257 in: Marine organic chemistry. E.K. Duursma, and R. Dawson, jeds) Elsevier. Scientific, Amsterdam.

Gerchey, R.M., 1996. Compassion of TLC-FID and high resolution gas chromatography for the analysis "Escavater lipid& NRC contract DSS 098C. 31028-4-5304Y.

Giam, C.-S., Chan, M.S., Neff, G.S., and Atlas, E.L.; 1978. Phthalate ester plasticizers: a new class of marine pollutant. Science 199: 419-421.

- 17

Gilbertson, J.R., Crout, R.J., and Gilbertson, J.D., 1981. Utilisation of polyunsaturated long-chain alcohols by <u>Clostridium</u> <u>butyricum</u>. Lipids 18: 601-603.

Gillan, F.T., Nichols, P.D., Johns, R.B., and Bavor, H.J., 1983. Phytol degradation by marine bacteria. App. Environ. Microbiol. 45: 1423-1428.

Gjessing, ET., and Berglind, L., 1981. Adsorption of PAH to aquatic humus. Arch. Hydrobiol. 92: 24-30.

Glomset, J.A., 1985. Fish, fatty acids, and human health. N. Engl. J. Med. 312: 1253-1254.

Glover, H.E.F. Phinney', D.A., and Yentsch, C.S., 1985. Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations, in various water masses in the Gulf of Maine. Biol. Oceanogr, 3: 223-248.

- Gering; J.J., Wallen, D.D., and Nauman, R.M., 1970. Nitrogen uptake by phytoplankton in the discontinuity layer of the eastern subtropical Facific Ocean. Limnol. Oceanogr. 15: 789-796.
- Goodloe, E.S., and Light, R.J., 1982. Structure and composition of hydrocarbons and fatty acids from a marine, blue-green alga, <u>Synchococcus</u> sp. Biochim. Biophys. Acta 710: 485-492.
- Goodnight, S.H., Jr., Harris, W.S., Connor, U.E., Illinguorth, D.R., 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. Arteriosclerosis 2: 87-113.
- Gooduin, T.U., 1974; Stérols. pp266-280 in: Algal physiology and biochemistry, W.D.P. Stewart (ed), University of California Press, California.

Goodvin, T.U., and Mercer, E.I., 1983. Introduction to plant biochemistry, Pergamon, Oxford, 677pp.

Gordon; D.C., Keizer, P.D., and Dale, J., 1978. Temporal variations and probable origins of hydrocarbons in the water column of Bedford Basin, Nova Scotia. Estuar. Coast. Mar. Sci. 7: 243-256.

244

G.

-Guillard, R.R.L., and Ryther, J.H., 1962. Studies. of marine planktonic distoms I. <u>Cyclotella</u> <u>nana</u> Hustedt, and <u>Detonula</u> <u>confervacea</u> (Gleve) Gran. Can. J. Microbiol. 8: 229-239.

Gunstone, F.D., 1967. An introduction to the chemistry and biochemistry of fatty acids and their glycerides. Chapman and Hall, London, 209pp.

Gurr, M.I., and James, A.T., 1980. Lipid biochemistry: an introduction of dea, Chapman and Hall, London, 247pp.

Nakanson, J.L., 1984. The long and short term feeding condition in field-caught <u>Galanus</u> pacifidus, as determined from the lipid content. Limnol. Oceanogr. 29: 794-804

Hallegraeff, G.N., 1981. Seasonal study of phytoplankton pighents and species at a coastal station off Sydney: importance of diatoms and the manoplankton. Mar. Biol. 107-118.

Hallgren, B., Stallberg, C., and Boeryd, B., 1978, Occurrence, synthesis and biological effects of substituted glycerol ethers. Prog. Chem. Fats other Lipids 16: 45-58

Hording, L.U., Meeson, B.U., and Fisher, T.R., 1985. Patterns of photosynthetic carbon metabolism in light-limited phytoplankton. Mar. Biol. 89: 121-133.

Marvey, G.R., 1985. Dissolved carbohydrates in the New York Bight and . . the wariability of marine organic matter. Mar. Chem. 12: 333-339,

Harvey, G.R., Borán, D.A., Piotrovicz, S.R., and Weisel, C.P., 1984.
 Synthesis of marine humic substances from unsaturated lipids.
 Nature 309: 244-246.

Harvey, M.R., and Poeton, J.S., 1981. Solvent focusing for rapid and sensitive quantification of total hipids on Chromarods. Anal. Biochem. 116: 312-216

Natvey, "M.R., Richardson, M.D., and Ratton, J.S., 1984. Lipid composition and vertical distribution of bacteria in acrobic sediments of the Venezuela Basin. Deep-Sea Res. 31: 403-413.

Harvey, N.R., Rigler, M.L., and Patton, J.S., 1985. The use of the Introscan TH-10 analyzer to quantify total lipids in a variety of cample types and lipid classes in human gallbladder bile, Lipids 20: 542-545.

Illachinoro, Y., 1979. Marine torius and other bioactive marine Detabolites. Japan Scientific Societies, Tokyo.

Hassett, J.P., and Anderson, M.A., 1979. Association of hydrophobic organic compounds with dissolved organic matter in aquatic systems. Environ. Sci. Kechnol. 13: 1526-1529.

Hayashi, K., Yamada, M., 1972. Studies on the lipids of shell-fish -I. On the visceral lipid composition of Abalone, <u>Haliotis discus</u> <u>hannai</u> (INO). Bull. Japan. Soc. Sci. Fish. 38: 255-263.

Heftmann, E., 1971. Functions of sterols in plants. Lipids 6: 128-, 133.

Hellebust, J.A., 1974. Extracellular products pp838-836 in: Algal physiology and biochemistry, W.D. Stewart (ed), Univ. California.

Henderson, R.J., Sargent, J.R., and Falk-Petersen, S., 1981. Lipogenesis in the Arctic euphausiid <u>Thysanoessa</u> <u>inermis</u>. Mar. Biol. 63: 235-240.

Hennion, M.C., Thieblemont, J.C., Rosset, R., Scribe, P., Marty, J.C., and Saliot, A., 1983. Rapid semi-preparative separation of organic compounds from marine lipid extracts by high-performance 'liquid chromatography, and subsequent quantitative analysis by gas chromatography. J. Chromatogr. 280: 351-362.

#« Hirayama, O., and Morita, K., 1980. A simple sensitive method for the quantitative analysis of chloroplast lipids by use of thin layor chromatography and flame ionization detector. Agric. Biol. Chem. 44: 2217-2219. .

Hoffman, E.J., Latimer, J.S., Mills, G.L., and Quinn, J.G., 1982. Petroleum hydrocarbons in urban runoff from a commercial land use area. J. Uater Pollut. Control Fed. 54: 1517-1525.

Holdsworth, E.S., and Colbeck, J., 1976. The pattern of carbon fixetion in the marine unicellular alga <u>Phaeodactylum</u> tricornutum. Mar. Biol. 38: 189-199.

τ<sup>τα</sup> τ. τ. τ. τ.

Nolz', G.G. Jr., 1981. Non-isoprenoid lipids and lipid metabolism of " ('maxime.flagellates.'pp301-332 in? Biochemistry and physiology of "" protozoa. 2nd ed., Vol. 4, academic Press.

Ropkins, C.C.E., Seiring, J.V., Nyholmen; O., and Hermannsen, A., 1984. Ecological energetics from total lipid and total protein; fact and artifact using a gravimetric method for lipid and a biuref - method for protein. Oceanogr, Mar. Diol. Ann. Rev., 22: 211-261.

Hunter, K.A., and Lipo, P.S., 1981. Organic sea purface films. pp259-. 298 in: Marine organic chemistry, E.K. Duursna and R. Davson (eds), . Elsevier Scientific, Amsterdam.

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.

Iturriaga, R., and Zsolnay, A., 1981. Differentiation between auto- and heterotrophic activity: problems in the use of size fractionation and antibiotics. Bot. Mar. 24: 399-404.

Jeffrey, L.M., 1966. Lipids in sea water. J. Amer. Oil Chem. Soc. 43: 211-214.

Jen, J.J., and Mackinney, G., 1970. On the photodecomposition of chlorophyll in vitro - L. Reaction rates. Photochem. Photobiol. 11: 297-302.

Jensen, A., 1964. Algal carotenoids IV. On the structure of fucoxanthin. Acta Chem. Scand. 18: 2005-2007.

Jensen, A., 1984. Excretion of organic carbon as a function of nutrient stress. pp61-72 in: Marine phytoplankton and productivity, O. Holm-Hansen, L. Bolis, and R. Gilles (eds), Springer-Verlag, Berlin.

Jones, E.P., Dyrssen, D., and Coote, A.R., 1984. Nutrient regeneration in deep Baffin Bay with consequences for measurements of the conservative tracer'NO and fossil fuel CO<sub>2</sub> in the oceans. Can. J. Fish. Aquat. Sci. 41: 30-35.

Johnson, B.D., and Wangersky, P.J., 1985. Seawater filtration: particle flow and impaction considerations. Limnol. Oceanogr. 30: 966-971.

Jungclaus, G.A., Lopez-Avila, V., and Hités, R.A., 1978. Organic. compounds in an industrial vastevater: a case study of their . environmental impact. Environ. Sci. Technol. 12: 88-96.

Kaimal, T.N.B., and Shantha, N.C., 1984. Quantitative analysis of / lipids on copper(II) sulphate-impregnated Chromarods. J. Chromatogr. 288: 177-185. ~

Karasok, F.U., Clement, R.E., and Sveetman, J.A., 1961. Preconcentration for trace analysis of organic compounds. Anal. Chem. 53: 1050A 1058A

Kattner, G., Gercken, G., and Hammer, K.D., 1983a. Development of lipids during a spring plankton bloom in the northern North Sea. II. Dissolved lipids and fatty acids. Mar. Chem. 14: "163-173.

Kattner, G., Gercken, G., and Eberlein, K., 1983b. Development of ·lipids during a spring plankton bloom in the northern North Sea. I. Particulate fatty acids. Mar. Chem. 14: 149-162.

Kattner, G., Krause, M., and Trahms, J., 1981. Lipid composition of some typical North Sea copepods. Mar. Ecol. Prog. Ser. 4: 69-74.

Keith, L.H., Crummett, W., Deegan, J., Libby, R.A., Taylor, J.K., and Wentler, G., 1983. Principles of environmental analysis. Anal. Chem. 55: 2210-2218.

Keizer, P.D., Gordon, D.C., and Dale, J., 1977. Hydroce bons in eastern Canadian marine waters determined by fluorescence spectroscopy and gas-liquid chromatography. J. Fish. Res. Board, Can. 34: 347-353.

Kellogg, R.B., and Patton, J.S., 1983. Lipid droplets, medium of energy exchange in the symbiotic anemone <u>Condylactis</u> <u>gigantea</u>: a model coral polyp. Mar. Biol. 75: 137-149.

Kennicutt, M.C. II., and Jeffrey, L.M., 1981a. Chemical and GC-MS characterization of marine dissolved lipids. Mar. Chem. 10: 367-387.

Kennicutt, M<sub>0</sub>C. II., and Jeffrey, L.M., 1981b. Chemical and GC-MS<sup>1</sup> characterization of marine particulate lipids. Mar. Chem. 10: 389-407.

Kindl, H., 1984. Lipid degradation in higher plants. pp181-204 in: Fatty\_acid metabolism and its regulation. S. Numa(ed), Elsevier Scientific.

Kirk, J.T.O., and Tilney-Bassett, R.A.E., 1978. The plastids. Their chemistry, structure, growth and inheritance. Elsevier/North-Holland Biomedical, Amsterdam.

 Knap, A.H., and Uilliams, P.J. LeB., 1982. Experimental studies to determine the fate of petroleum hydrocarbons from refinery effluent
 on an estuarine system. Environ. Sci. Technol. 16: 1-4.

Kondo, Y., 1982. Partial tosylation of 1,5-anhydroxylitol. Carbohydrate Res. 103: 154-157.

Kramer, J.K.G., Fouchard, R.C., and Farnworth, E.R., 1980. Effect of solvents on the resolution of neutral lipids on Chromarods. J. Chromatogr. 198: 279-285.

Kramer, J.K.G., Fouchard, R.C., and Farnworth, E.R., 1985. The effect of fatty acid chain length and unsaturation on the chromatographic behavior of triglycerides on latroscan Chromarods. Lipids 20: 617-619.

Kveseth, K., Sortland, B., and Bokn, T., 1982. Polycyclic aromatic hydrocarbons in sevage, mussels and tap water. Chemosphere 11: 623-639.

Langouska, I., 1982. Utilization of organic phosphates by selected bactoria and algae. Water Res. 16: 161-167.

de Lappe, B.U., Risebrough, R.W., Springer, A.M., Schmidt, T.T., Shropshike, J.C., Letterman, E.F., and Payne, J.R., 1980. The sampling and measurement of hydrocarbons in natural vaters. pp29-68 in: Hydrocarbons and halogenated hydrocarbons in the aquatic environment, B.K. Afghan and D. Mackay (eds), Plenum, New York, 588pp.

- Larsson, K., Odham, G., and Sodergren, A., 1974. On lipid surface films on the sea. I. A simple method for sampling and studies of composition. Mar. Chem. 2: 49-57.
- Larsson, U., and Hagstrom, A., 1982. Fractionated phytoplankton m primary production, exudate release and bacterial production in a Baltic eutrophication gradient. Mar. Biol. 67: 57-70.
- Lee, R.F., Hirota, J., Nevenzel, J.C., Sauerheber, R., Benson, A.A., and Levis, A., 1972, Lipids in the marine environment. Calif. Mar. Res. Comm., CalCOFI Rept., 16: 95-102.

Lee, R.F., and Loeblich, A.R., 1971. Distribution of 22:6 hydrocarbon and its relationship to 22:6 fatty acid in algae. Phytochem. 10: 593-602.

Lee, R.F., and Nevenzel, J.C., 1979. Wax esters in the marine environment: origin and composition of the Wax from Bute Inlet, British Columbia. J. Fish. Res. Board Can. 36: 1519-1523.

Lee, R.F., Nevenzel, J.C., Paffenhofer, G.-A., 1971. Importance of vox esters and other lipids in the marine food chain: phytoplankton and copepods. Mar. Diol. 9: 99-108.

Lewis, M.R.; Horne, E.P.W., Cullen, J.J., Oakey, N.S., and Platt, T., 1984. Turbulent motions may control phytoplankton photosynthesis in. the upper ocean. Nature 311: 49-50.

Levis, R.W., 1969. The fatty acid composition of arctic marine phytoplankton and zooplankton with special reference to minor acids. Limnol. Oceanogr. 14: 35-40.

Liebezeit, G., Bolter, M., Broun, T.F., Dauson, R., 1980. Dissolved free amino acids and carbohydrates at pycnocline boundaries in the Sargasso Sea and related microbial activity. Oceanol. Acta 3: 357-362.

Malins, D.C., and Varanasi, U., 1972., The ether bond in marine 7, lipids. pp297-312 in: Ether lipids chemistry and biology, F. Synder (ed), Academic Press, New York

Mancusco, C.A., Odham, G., Westerdahl, G., Reeve, J.N., and Uhite, D.C., 1985, C<sub>15</sub>, C<sub>26</sub>, and C<sub>25</sub> isoprenoid homologues in glycerol diether phopholipids of methanogenic archaebacteria. J. Lipid Res. 26: 1120-1125.

Mantoura, R.F.C., 1981. Organo-metallic interactions in natural uatars. pp179-223 in: Marine organic chemistry, E.K. Duursma and R. ¿Davson (eds), Elsevier Scientific, Amsterdam.

Materassi; K., Paoletti, C., Balloni, W., and Florenzano, G., 1980. Some considerations on the production of lipid substances by macroalgae and cyanobacteria. pp619-626 in: Algae biomass, production and use, G. Shelef; and C.J. Soeder (eds), Elsevier/North-Holland Biomedical Press, Amsterdam.

Mathur, S.P., 1974. Phthalate esters in the environment: pollutants or natural products? J. Environ. Qual. 3: 189-197.

Matsumoto, G. 1981. Comparative study on organic constituents in polluted and unpolluted inland aquatic environments - II. Features of fatty acids for polluted and unpolluted waters. Water Res. 15: 779-787.

 McInnes, A.G., Ualter, J.A., and Uright, J.L.C., 1980. Biosynthesis
 of hydrocarbons by algae: decarboxylation of stearic acid to nheptadecane in <u>Anacystis nidulans</u> determined by <sup>13</sup>C- and <sup>2</sup>H \* labeling and <sup>13</sup>C nuclear magnetic resonance. Lipids 15:, 509-615.

Means, J.C., and Wijnyaratne, R.D., 1984. Chemical characterization of estuarine colloidal organic matter: implications for adsorptiveprocesses. Bull. Mar. Sci. 35: 449-461. Meyers, P.A.; Leenheer, M.J., Eadie, B.J., and Maule, S.J., 1984. Organic geochemistry of suppended and settling particulate matter in Lake Michigan. Geochim. Cosmochim. Acta 48: 443-452.

 $\mathcal{L}$ 

251

Mironov, O.G., Shchekaturina, T.L., and Tsimbal, I.M., 1981. Saturated hydrocarbox's in marine organisms. Mar. Ecol. Prog. Ser. 5: 303-309.

Morris, R.J., 1984. Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. II. Changes in the component fatty acids and sterols. J. Exp. Mar. Biol. Ecol. 75: 59-70.

Morris, R.J., and Culkin, F., 1976. Marine lipids: analytical techniques and fatty acid ester analyses. Oceanogr. Mar. Biol. Ann. Rev. 14: 397-433.

Morris; R.J., McCartney, M.J., and Robinson, G.A., 1983. Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient.chemistry of water. J. Exp. Mar. Biol. Ecol. 70: 249-262.

Murray, ~D.K., 1985. Improved reproductibility and quantitative analysis of phospholipids by flame ionization detection. J. Chromatogr. 331: 303-312.

Murray, H.E., Ray, L.E., and Giam, C.S., 1981a. Analysis of marinesediment, water and biota for selected organic pollutants. Chemosphere 10: 1327-1334.

Murray, H.E., Ray, L.E., and Giam, C.S., 1984b., Phthalic acid esters, total DDTs, and polychlorinated biohenyls in marine samples from Calveston Bay, Texas. Bull, Environ. Contam. Toxicol. 26: 769-774.

Nichols, B.W., and James, A.T., 1968. Acyl lipids and fatty acids of photosynthetic tissum .Prog. Phytochem, 1: 1-48

O'Leary; U.M. 1967. The chemistry and metabolism of microbial lipids. The World Publishing Company, Cleveland.

Olsen, C.R., Cutchall, N.H., and Larsen, I.L., 1982. Pollytantparticle associations and dynamics in coastal marine environments: a review. Mar. Chem. 11: 501-533.

Opute, E.I., 1974a. Lipid and farty-acid composition of diatoms. J. Exp. Bot. 25: 823-834.

Opure, F.I., 1974b. Studies on fat accumulation in <u>Nitzschia palea</u> Kuts. Ann. Bot. 38: 889-902. Ourisson, G., Albrecht, P., and Rohmer, M., 1979. The hopanoids. Balaeochemistry and biochemistry of a group of natural products. Pure Appl. Chem. 51: 709-729.

252

Ouens, T.G., and Falkouski, P.G., 1982. Enzymatic degradation of chlorophyll <u>a</u> by marine phytoplankton <u>in vitro</u>. Phytochem. 5: 979-984.

Paradis, M., and Ackman, R.G., 1977. Influence of ice cover and man on the odd-chain hydrocarbons and fatty acids in the wayers of Jeddore Harbour, Nova Scotia. J. Fish. Res. Board Can. 34: 2156-2163.

Parker, P.L., Bhalen, C. van., and Maurer, L., 1967. Fatty acids in eleven species of blue-green algae: geochemical significance. "Science 155: 707-708.

Parrish, C.C., and Ackman, R.G., 1983a. Chromarod separations for the analysis of marine Lipid classes by latroscan thin-layer chromatography - flame ionization detection. J. Chromatogr. 262: 103-112.

Parrish, C.C., and Ackman, R.G., 1983b. The effect of developing solvents on lipid class quantification in Chromarod whin layer chromatography/flame ionization detection. Lipids 18: 563-565.

Parrish, C.C., and Ackman, R.G., 1985; Calibration of the latroscan-Chromarod system for marine lipid class analyses. Lipids 20: 521-530.

Parsons, T.R., Stephens, K., and Strickland, J.D.H., 1961. On the chemical composition of eleven species of marine phytopfankters. J. "Fish. Res. Board Can. 18: 1001-1016.

Patterson, P.L., 1985. A specific detector for nitrogen and hologen compounds in TLC on coated quartz rods, Lipids 20: 503-509.

Peuchant, E. Covi, G., and Jensen, R., 1984. Faecal lipid chromatography I. Quantitative determination with Chromarods. J. Chromatogr. 310: 297-305.

Pirt, S.J., 1984. Algal photosynthésis: the Aladdin's Cave of biotechnology. Chém. Ind. (London), 843-849,

Platt, T., and Irvin, B., 1973. Caloric content of phytoplankton. Limnol. Ocganogr. 18: 306-310.

253

Platt, T., Irwin, B., and Subba Rao, D.V., 1973. Primary productivity Thd nutrient measurements on the spring phytoplankton bloom in Bedford Basin, 1971. Fish. Res. Board Can. Tech. Rep. No. 423, 45pp.

Pohl, P., and Zurheide, F., 1982: Fat production in freshwater and marine algae. pp65-80, in: 'Marine algae in pharmaceutical science, Vol. 2, Hoppe and Levring (eds), Walter de Gruyter & Co., Berlin. .

Procter, V.W., 1957. Studies of algal antibiosis using <u>Haematococcus</u> and <u>Chlamydomonas</u>. Limnól. Oceanogr. 2: 125-139.

Ralston, A.W., and Hoerr, C.W., 1942. The solubilities of the normal saturated fatty acids. J. Org. Chem. 7: 546-555.

Rao, G.A., Riley, D.E., and Larkin, E.C., 1985. Comparison of the thin layer chromatography/flame ionization detection/system with other methods for the quantitative analysis of liver lipid contents in alcohol-fed rats and controls. Lipids 20: 531-535.

Read, H., 1985. Improved sample application methods for the [latroscan. Lipids 20: 510-515. .

Readman, AW., Mantoura, R.F.C., Rhead, M.M., and Brown, L., 1982. Aquatic distribution and heterotrophic degradation of polycyclic aromatic hydrocarbons (PAH) in the Tamor Estuary. Estuar. Coast. Shelf Sci. 14: 369-389.

Redfield, A.C., Ketchum, B.H., and Richards, F.A., 1963. The influence of organisms on the composition of sea-water. pp26-70.in: The Sea, VOL. 2. M.N. Hill (ed), Robert E. Krieger Publishing. Company, 554pp.

Ree, G.-Y., 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. Limnol. Oceanogr., 23: 10-25.

"Richardson, B., Greutt, D.M., Schwertner, H.A., Martinez, C.L., and Uickline, H.E., 1969. Effects of nitrogen limitation on the growth and composition of unicellular-algae in continuous culture. App. Microbiol. 18: 245-250.

Rohmer, M., Bouvier; P., and Ourisson, G., 1979. Molecular evolution. 'Of biomembranes: structural equivalents and phylogenetic precursors of sterols. Proc. Matl. Acad. Sci. U.S.A. 76: 847+851. Rouser, G., Kritchevsky, G., Simon, G., and Nelson, G.J., 1967 Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids 2: 37-40

254

Roy, S., 1986. Marine chloropigments: chromatographic measurement in, a phytoplankton and transformations in algal cultures and a spring bloom event in a marine bay. Ph.D. dissertation, Dalhousie University, 172pp.

Ryan, T.A., Joiner, B.L., and Ryan, B.F., 1976. Minitab student handbook. Durbury Press, Massachusetts, 341pp.

Sakshaug, E., and Jensen, A., 1978. The use of cage cultures in studies of the biochemistry and ecology of phytoplankton. Oceanogr. Mar. Biol. Ann. Rev., 16: 81-106.

Saliot, A., 1981. Natural hydrocarbons in sea water. pp327-374 in: 'Marine organic chemistry, E.K. Duursma and R. Dawson (eds), 'Elsevier Scientific, 'Amsterdam.

Saliot, A., Goutr, M., Fevrier, A., Trusseau, D., and Andrie, C., 1982. Organic solimentation in the vater column in the Arabian Sea: relationship between the lipid composition of small and large-size, surface and deep particles. Mar. Chem. 11: 257-278.

Sargent, J.R., and Falk-Petersen, S., 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norvay: lipids and fatty acids in <u>Meganyctiphanes</u> <u>norvegica</u>. <u>Thysanoessa</u> <u>raschi</u> and <u>T. inermis</u> during mid-winter. Mar. Biol. 62: 131-137.

Sargent, J.R., Gatten, R.R., and McIntosh, R., 1977: Wax esters in the marine environment - their occurrence, formation, transformation and ultimate fates. Mar. Chem. 5: 573-584.

Sargent, J.R., Lee, R.F., and Nevenzel, J.C., 1976. Marine waxes., pp49-91 in: Chemistry and biochemistry of natural waxes, P.E. Kolattukudy (ed), Eléctier, Amsterdam, 459pp.

Sasaki, G.C., and Capuzzo, J.M., 1984. Degradation of <u>Artemia</u> lipids under storage. Comp. Biochem. Physiol. 78B: 525-531.

Schultz, D.M., 1974. Source, formation and composition of suspended lipoidal material in Natragan sett Bay, Rhode Island. Ph.D. & dissertation, University of Rhode Island, 205pp.

Schultz, D. M., and Quinn, J.G., 1972. Fatty acids in surface particulate-matter from the North Atlantic. J. Fish. Res. Board Can. 29: 1482-1486. Schultz, D.M., and Quinn, J.G., 1973. Fatty acid composition of organic detritus from <u>Spartine</u> <u>alterniflora</u>. Estuar. Coast. Mar. Sci. 1: 177-190.

- Schultz, D.M., and Quinn, J.G., 1977. Suspended material in Narragansett Bay: fatty acid and hydrocarbon composition. Org. Geochem. 1: 27-36.
- Scott, R.P.W., 1983. Column chromatography. J. Chromatogr. 22A: A137-A160.
- Sebedio; J-L:, and Ackman, R.G., 1981. Chromarods-S modified with silver nitrate for the quantitation of isomeric unsaturated fatty acids. J. Chromatogr. Sci. 19: 552-557.
- Sebedio, J.L., Farquharson, T.E., and Ackman, R.G., 1985. Quantitative analyses of methyl esters of fatty acid geometrical isomers, and of triglycerides differing in unsaturation, by the Iatroscan TLC/FID technique using AgNO<sub>3</sub> impregnated rods. Lipids 20: 555-560.
- Sharp, J.H., 1973. Size classes of organic? carbon in seawater. Limnol. Oceanogr. 18: 441-447.
- Sheldon, L.S., and Hites, R.A., 1978. Organic compounds in the Delaware River. Environ. Sci. Technol. 128. 1188-1194.
- Shifrin, N.S., and Chisholm, S., 1980. Phytoplankton lipids: environmental influences on production and possible commercial applications. pp627-645 in: Algae Biomass, G. Shelef and C.J. Soeder (eds), Elsevier/North-Holland Biomedical, Amsterdam.
- Shifrin, N.S., and Chrisholm, S.U., 1981. Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. J. Phycol. 17: 374-384.
- Sieburth, J.McN., 1983. Microbiological and organic-chemical processes in the surface and mixed layers. pp121-172 in : Air-see exchange of gases and payticles, P.S. Liss and W.G.N. Sinn (eds), D. Reidel Publishing Company.
- Sieburth, J.McN., Smetacek, V., and Lenz, J., 1978; Pelagic ecosystem structure: heterotrophic compartments of the planking and their relationship to plankton size fractions. Limnol. Oceanogr. 23: 1256-1263.
- Sigleo, A.C., Hoering, T.C., and Helz, G.R., 1982. Composition of estuarine colloidal material: organic components. Geochim. Cosmochim. Acta 46: 1619-1626.

Simkiss, K., 1983. Lipid solubility of heavy metals in saline solutions. J. mar. biol. Ass. U.K. 63: 1-7.

Skipnes, O., Eide, I., and Jensen, A., 1980. Cage culture turbidostat: a device for rapid determination of algal growth rate. App. Environ. Microbiol. 40: 318-325.

Smetacek, V.S., 1985. Role of sinking in diatom life fistory cycles: ecological, evolutionary and geological significance. Mar. Biol. 84: 239-251.

Smith, J.C., Platt, T., Li, W.K.W., Horne, E.P.W., Harrison, W.G., Subba Rao, 4 D.V., and Irwin, B.D., 1985. Arctic marine photoautotrophic picoplankton. Mar. Ecol. Prog. Ser. 20: 207-220.

()

Stephen, H., and Stephen, T., 1963. Solubilities of inorganic and organic compounds. Pergamon Press, Oxford.

Strickland, J.D.H., Holm-Hansen, O., Eppley, R.W., and Linn, R.J., 1969, The use of a deep tank in phytoplankton ecology.(I. Studies of the growth and composition of phytoplankton crops at low nutrient levels, Limnol. Oceanogr. 14: 23-34.

Sullivan, K.F., Atlas, E.L., Giam, C.-S. 1981. Loss of phthalic acid esters and polychlorinated biphenyls from seawater samples during storage. Anal. Chem. 53: 1719-1721.

Sullivan, K.F., Atlas, E.L., and Giam, C.-S., 1982, Adsorption of phthalic acid esters from seawater. Environ., Sci. Technol. 16: 428-432.

Takahashi, T., Broecker, W.S., and Langer, S., 1985. Redfield ratio based on chemical data from isopycnal surfaces. J. Geophys. Res. 90: 6907-6924.

Tatara, T., Fujii, T., Kawase, T., and Minagawa, M., 1983. Quantitative determination of tri-jedi-, monooleins and free oleic acid by thin layer chromatography-flame ionization detector system using internal standards and boric acid impregnated Chromarod. Lipids 18: 732-736.

Taylor, E.T., Curry, R.W., and Corcoran, E.F., 1981. Potential for biodegradation of phthalic acid esters in marine regions. Appl. -Environ. Microbiol. 42: 590-595.

Thomas, U.H., Seibert, D.L.R., Alden, M., Neori, A., Eldridge, P., 1984. Vields, photosynthetic efficiencies and proximate composition of dense marine microalgal cultures. I. Introduction and <u>Phaeodactylum tricornutum</u> experiments. Biomass 5: 181-209. Tornabene, T.G., 1981. Formation of hydrocarbons by bacteria and algae. pp421-438 in: Trends in the biology of fermentations for </ fuels and chemicals, A. Hollaender et al. (eds), Plénum, New York.

浵

Tornabene, T.G., 1982. Microorganisms as hydrocarbon producers. -; Experientia 38: 43 $_{
m V}$ 46.

- Trick, C.G., Harrison, P.J., and Andersen, R.J., 1981. Extracellular secondary metabolize production by the marine dinoflagellate <u>Prorocentrum minimum</u> in culture. Can. J. Fish. Aquat. Scil 38: 864-857.
- Velimirov, B., 1982. Sugar and lipid components in sea foam near kelp beds. Mar. Ecol. 3: 97-107.

Vetter, R.D., Hodson, & E., and Arnold, C., 1983. Energy-metabolism in a rapidly developing marine fish egg, the red drum (<u>Sciachops</u> <u>ocellata</u>). Con. J. Fish. Aquat. Sci. 40: 627-634.

Volkman, J.K., Eglitton, G., Corner, E.D.S., 1980a, Sterols and fatty acids of the marine diatom <u>Biddulphia</u> <u>sinensis</u>. Phytochem. 19: 1809-1813.

Volkman, J.K., Everitt, D.A., and Allen, D.I., 1986. Analysis of lipid classes in marine organisms, sediments and seavater using thin layer chromatography-flame ionisation detection (TLC-FLD). J. Chromatogr. 356: 147-162.

Volkman, J.K., Gatten, R.R., and Sargent, J.R., 1980b. Composition

Volkman, J.K., Smith, D.J., Eglinton, G., Forsberg, T.E.V., and Corner, E.D.S., 1981. Sterol and fatty acid composition of four marine Haptophycean algae. J. mar. biol. Ass. U.K. 61: 509-527.

Wake, L.V., and Hillen, L.U., 1981. Nature and hydrocarbon content of blooms of the alga <u>Botryococcus braunii</u> occurring in Australian freshvater lakos. Aust. J. Mar. Freshvater Res. 32: 353-367.

Wakehom, S.G., 1982. Organic matter from a sediment trap experiment in the equatorial North Atlantic: wax esters, steryl esters, triacylglycerols, and alkyldiacylglycerols. Geochim. Cognochim. Acta 46: 2239-2257.

Wakeham, S.G., 1985. Wax ebters and triacylglycerols in binking particulate patter in the Peru upwelling area (15°%, 75%W). Mar. Chem. 17: 213-235.

Wakeham, S.G., Farrington, J.U., and Gagosian, R.B.; 1984a. Variability in lipid flux and composition of particulate matter in the Peru upvelling region. Org. Geochem. 6: 203-215.

- Vakeham, S.G., Farrington, J.W., Gagosian, R.B., Lee, C., DeBoar, H., Nigrelli, G.E., Tripp, B.W., Smith, S.O., and Freu, N.M., 1980. Organic matter fluxes from sediment traps in the equatorial Atlantic Ocean. Nature 286: 798-800.
- . Wakeham, S.G., and Frew, N.M., <sup>ℓ</sup> 1982. Glass capillary gas chromatography-mass spectrometry of wax esters, storyl esters and triacylglycerols. Lipids 17: 831-843.

Wakeham, S.G., Gagosian, R.B., Farrington, J.W., and Canuel, E.A., 1984b. Stemenes in suspended particulate matter in the eastern tropical North Pacific. Nature 308: 840-843.

Waldock, M.J., 1983. Determination of phthalate esters in samples from the marine environment using gas chromatography mass spectrometry. Chem. Ecol. 1: 261-277.

Wangersky, P.J., 1978. Production of dissolved organic matter. pp115-220 in: Marine ecology, O. Kinne (ed) Vol. 4, Uiley, London.

Wangersky, P.J., Wangersky, G.P., and Maaşs, R.L., 1986. A modified cage culture turbidostat for ecological and physiological research with phytoplankton. J. Exp. Mar. Biol. Ecol. (submitted).

Wangersky, P.J., and Zikar R.G., 1978, The analysis of organic complunds in sea water. Mar. Andl. Chem. Stands. Prog., Rep. 3, Nat. Res. Coun. Can. No. 16566

Ware, D.M., and Addison, R.F., 1973; PCB residues in plankton from the Gulf of St. Laurence. Nature 46: 519-521.

Water Survey of Canada, 1985. Surface, Water data, Atlantic provinces. Inland Waters Directorate, Water Resources Branch, Ottawa, p103.

Whitehouse, B.G., A983. The partitioning of polynuclear aromatic hydrocarbons into the dissolved phase of the aquatic environment. Ph.D. dissertation, De<sup>1</sup>housie University, 153pp.

Whitehouse, B.G., Parrish, C.C., and Wangersky, P.J., 1984. The analysis of organic compounds in seawater, 1978-1983. Mar. Anal. Chem. Stands. Prog., Rep. 9, Nat. Res. Coun. Can. No. 23609.

O

Whittle, K.J., Hardy, R., Mackie, P.R., and McGill, A.S., 1982. A quantitative assessment of the sources and fate of petroleum compounds in the marine environment. Phil. Trans. R. Soc. Lond. B 297: 193-218.

Withers, N.U., and Nevenzel, J.C., 1977. Phytyl esters in a marine dinoflagel fate. Lipids 12: 989-993.

Variv, S. Valld Cross, M. 1979. Geochemistry of collected systems for a corthing ciegrists. Springer-Verlag, Berlin, 450pp

Zsolney, A., 1977. Inventory of nonvolatile facty acids and .
 hydrocarbons in the oceans. Mar. Chem. 5: 465-475.