GRAZER-MEDIATED CHLOROPIGMENT DEGRADATION AND THE VERTICAL FLUX OF SPRING BLOOM PRODUCTION IN CONCEPTION BAY, NEWFOUNDLAND

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GRAZER-MEDIATED CHLOROPIGMENT DEGRADATION AND THE VERTICAL FLUX OF SPRING BLOOM PRODUCTION IN CONCEPTION BAY, NEWFOUNDLAND

by

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DEDICATION

I dedicate this thesis to the one who has taught me most about the meaning of commitment and the rewards of perseverance.....my mother.

ABSTRACT

The fate of spring phytoplankton production at subzero temperatures in Conception Bay, Newfoundland, was traced using chloropigments as biomarkers of phytoplankton biomass and flux, and as indicators of biological processes. The study included conventional fluorometric and HPLC analyses of seston, zooplankton and bivalve gut tracts and faeces, trap collections, and sediments. Chloropigment conservation during copepod grazing was examined in experiments conducted at 0°C for 24 hr, using various concentrations of natural seston and cultured diatoms. Transformation to nonfluorescent products was negligible or low (<35%) at seston concentrations >3 μg chlorophyll *a*/l. At lower food levels, chloropigment destruction was high (>80%). Losses were primarily due to post-starvation feeding and digestive processes. *In situ* gut and faecal pigment levels in copepods and oikopleurids were highest during mid-bloom production. The dominant copepod grazers exhibited diel feeding rhythms, while oikopleurids showed near-continuous feeding activity. Pyrophaeophorbide *a* was the primary fluorescent degradation product of copepod grazing; more complex compositions of chlorophyll *a* derivatives were found in oikopleurid and mussel faeces. Considerable amounts of undegraded chlorophylls were also commonly found in oikopleurid faecal pellets. The contribution of copepod and oikopleurid faecal pellets to the vertical flux of bloom production was highest following the mass sedimentation of primarily senescent diatoms (20-23 m/d) during early May. POC flux to bottom waters was 30-40% of estimated primary production, and 75% of this flux occurred during May. The relative contribution of zooplankton grazing products increased from 35% at 40 m to 67% at 240 m. Relatively constant carbon/chloropigment ratios at all depths throughout the bloom, and comparable losses of POC and pigment flux between surface and bottom waters, support the use of chloropigments as reliable tracers of vertical flux. C/N ratios of cumulative fluxes were low (7.0-8.8), indicating little decay of the bulk of sedimenting material. High quality particulate material (20% POM) reached near-bottom waters during the terminal phase of the bloom, resulting in

elevated carbon and chloropigment levels in the surface sediments. Chloropigment concentrations decreased with sediment depth in the uppermost 5 cm, but composition remained uniform down to 10 cm, presumably due to the bioturbating activities of abundant polychaetes, and pigment preservation processes following burial.

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Some of the data in this thesis was provided by Dr. D. Deibel and Dr. R.J. Thompson, who sampled the water column and deployed sediment traps during the COPE study in Conception Bay. In particular, I have made use of their CTD, inorganic

nutrient and POC/PON measurements in the water column, and their data on the weight and C/N composition of sediment trap contents. Dr. D. Deibel also assisted with the copepod grazing studies and made Coulter Counter measurements of the particle spectra in the plankton wheel experiments.

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LIST OF ABBREVIATIONS

C	carbon
C/N	carbon/nitrogen ratio
chl or chlor	chlorophyll
chlid or chllde	chlorophyllide *a*
CIV, CV, CVI	copepod development stages IV, V and VI
ESD	equivalent spherical diameter
EXP	experiment
FSW	filtered seawater
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
IP	injection peak
ND	no data
phae or phaeo	phaeopigments of chlorophyll *a*
pho or phor	phaeophorbide *a*
phy or phyt	phaeophytin *a*
POC	particulate organic carbon
PON	particulate organic nitrogen
POM	particulate organic matter
RCF	relative centrifugal force
RFU	relative fluorescence units
SCM	subsurface chlorophyll maximum
SD	standard deviation
TChl	total chloropigments (chlorophyll *a* and fluorescent derivatives)
TD10	Turner Designs Model 10 fluorescence detector
TPM	total particulate matter

Chapter 1

GENERAL INTRODUCTION

The spring phytoplankton bloom is the most important annual event in the carbon dynamics of many fjordic environments (review in Wassmann 1991). In coastal waters at high latitudes, the spring bloom period is often characterized by low recycling, the uncoupling of primary production and zooplankton grazing, and the mass sedimentation of primarily intact cells (Smetacek 1980, Wassmann 1983, Davies and Payne 1984). In bays and fjords of temperate regions, zooplankton grazing plays a more important role in the cycling and transport of carbon, and is the primary contributor to the vertical flux of phytoplankton production (Welschmeyer and Lorenzen 1985a,b; Peinert et al. 1989).

1.1. Tracing the Fate of Spring Bloom Production

Several investigators have used phytopigments as biomarkers of primary production in order to investigate the fate of spring phytoplankton blooms (Welschmeyer and Lorenzen 1985a, Smith and Lane 1988, Head and Horne 1993). In particular, fluorometric determinations of chlorophyll *a* and its fluorescent derivatives, collectively referred to as chloropigments, have been used to trace algal biomass, to determine standing stocks and vertical fluxes of phytoplankton, and to estimate *in situ* rates of phytoplankton growth and grazing in coastal waters (Welschmeyer and Lorenzen 1985a,b). More recently, the application of High Performance Liquid Chromatography (HPLC) to pigment analysis has allowed the identification of specific degradation products of feeding and digestion by various pelagic and benthic grazers (e.g. Hawkins et al. 1986, Downs 1989, Strom 1993). These studies have shown that some derivatives of chlorophyll *a* (e.g. phaeophorbide *a*-type pigments) are commonly produced by many grazer types. Various pigment transformation products which are formed in the digestive tract may be taxon-specific, and therefore of use in the identification of the primary grazers contributing to the flux of phytogenous material. Chloropigment transformation

products found in pelagic and benthic environments may also serve as indicators of biological and diagenetic processes.

The degree to which chloropigments are useful tracers of ingested phytoplankton will depend on the extent to which chloropigments are modified by the digestive processes of grazers, and on the limitations of the methods of detecting the transformation products of ingested chlorophyll *a*. Their use as quantitative and qualitative biomarkers may also depend on the stability of chloropigments in samples which are stored in a frozen state for various periods of time, prior to analysis.

The molecular structures of chlorophylls *a*, *b* and *c* and commonly encountered fluorescent derivatives of chlorophyll *a* are shown in Figure 1.1. Some potential chemical pathways and products of chlorophyll *a* degradation include losses of the central Mg atom (phaeophytin *a*) and the phytol tail (chlorophyllide *a*), the loss of both Mg and phytol (phaeophorbide *a*), and various substitutions on the porphyrin ring (Figure 1.2). These products are known to result from the action of enzymes and/or acids (review in Hendry et al. 1987). Further degradation, leading to the cleavage of the porphyrin ring, results in colourless products which are undetectable by standard spectrophotometric and fluorometric methods for pigment analysis. Colourless products are known to form as a result of photo-oxidation of pigments in senescent algae and sinking faecal pellets (Jeffrey 1974, Gieskes et al. 1978, Gowen et al. 1982, Tett 1982). Coprophagy is also a potentially important mechanism for chloropigment destruction (Nelson 1986, Downs 1989, Strom 1993). Much controversy over the use of chloropigments as tracers of copepod grazing has been stimulated by a growing number of reports of high and variable losses of ingested chloropigments to undetectable products (Conover et al. 1986, Dagg and Walser 1987, Lopez et al. 1988, Penry and Frost 1991). Other investigators, however, maintain that chloropigment destruction during copepod grazing is low (Kiørboe and Tiselius 1987) or negligible (Pasternak and Drits 1988).

1.2. Primary Objectives and Outline of Thesis

The main goal of this study was to determine the fate of the spring phytoplankton bloom in Conception Bay, Newfoundland. This is a fjord-like bay at mid-latitude, with subzero water temperatures at depths within and below the subsurface chlorophyll maximum during spring. The series of studies that follow involve an examination of chloropigments as biomarkers of phytoplankton biomass and flux, and as indicators of biological processes. This work was conducted as part of the Cold Ocean Productivity Experiment (COPE), a program initiated to investigate the effect of low temperatures on the heterotrophic utilization of the spring phytoplankton bloom in Conception Bay.

Chapter 2 outlines the two methods (standard fluorometric technique and HPLC) used throughout this work to measure chlorophyll *a* and its fluorescent derivatives in the extracts of phytogenous material. It also details the effects of short- and long-term sample storage at -20°C on chloropigment composition and conservation in various sample types. Methodological comparisons of chloropigment measurements, conducted to examine the reliability of the standard fluorometric method, are presented with the data shown in each chapter.

A study of the effect of food concentration on the ingestion rates of the copepod, *Calanus finmarchicus*, was conducted in closed grazing chambers incubated at 0°C with a range of concentrations of natural phytoplankton assemblages collected during the spring bloom (Chapter 3). This study includes an investigation of the fluorescent degradation products of copepod grazing, and an examination of the extent to which chlorophyll *a* is transformed to nonfluorescent products as a result of copepod feeding and digestive processes at low to high food levels.

Chapters 4, 5 and 6 describe the temporal and spatial distribution of phytoplankton, and the grazer-mediated degradation and vertical flux of chloropigments,

during the development and decline of the bloom. Chapter 4 provides a general description of some physical, chemical, and biological features of the bay during this period. It includes a series of time-depth plots of temperature and density, nutrients, particulate organic carbon and nitrogen, and chlorophyll *a*, and a description of the taxonomic composition and relative abundance of phytoplankton throughout the spring bloom.

Determinations of the *in situ* concentrations and compositions of the chloropigments in the gut tracts and faeces of some common particle and suspension feeding zooplankters and bivalves, collected throughout the bloom period, are reported in Chapter 5. This study also examines the diel feeding periodicities of the dominant mesozooplankton grazers during the peak of bloom production.

Vertical flux measurements of particulate organic matter, particulate organic carbon and nitrogen, and chloropigments were determined throughout the development and decline of the bloom and are presented in Chapter 6. Carbon flux is used in this study to estimate the sinking rates of settling particles during the onset of mass sedimentation. This chapter includes an examination of the composition of chloropigments in sediment trap collections, the surface sediments and the overlying flocculent layer. The schematic in Figure 1.3 illustrates some potential pathways of chloropigment flux, and processes contributing to the utilization and vertical transport of the spring phytoplankton bloom in Conception Bay.

An overview of the conclusions of the various studies conducted is presented in Chapter 7. It includes a discussion on the application and usefulness of chloropigments as tracers of the spring phytoplankton bloom in Conception Bay.

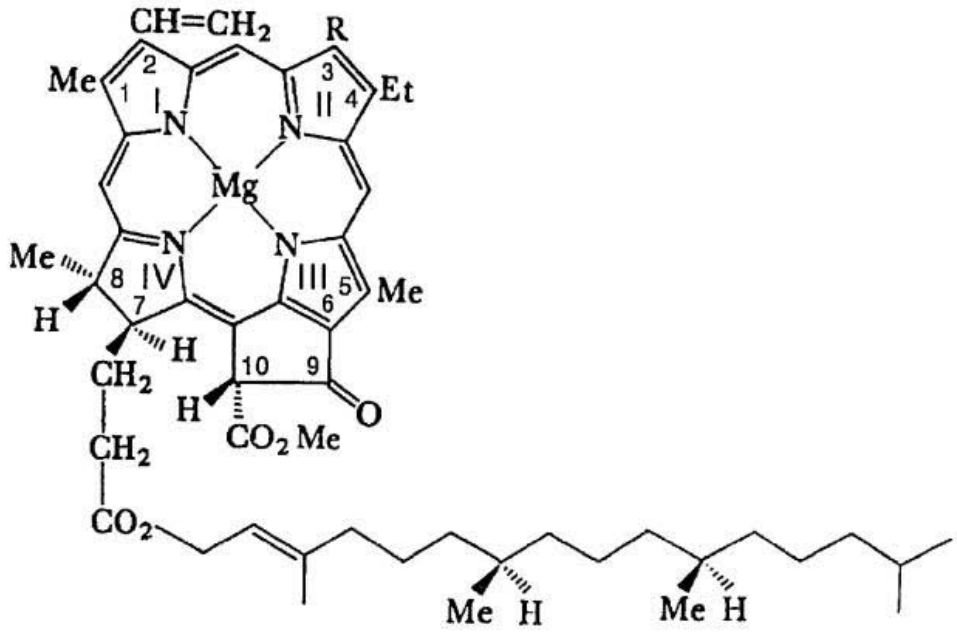

Figure 1.1. Molecular structure of the chlorophylls and common chlorophyll *a* derivatives. Figure adapted from Jackson (1976).

chlorophyll *a* : R = Me
chlorophyll *b* : R = CHO
chlorophyll *c* : IV-7 = -CH=CH-COOH; double bonds between IV-7 and IV-8
chlorophyllide *a* : IV-7 = phytol chain removed
phaeophytin *a* : Mg atom removed
phaeophorbide *a* : Mg atom and phytol chain removed
pyro-phaeopigments : loss of C-10 carbomethoxy group

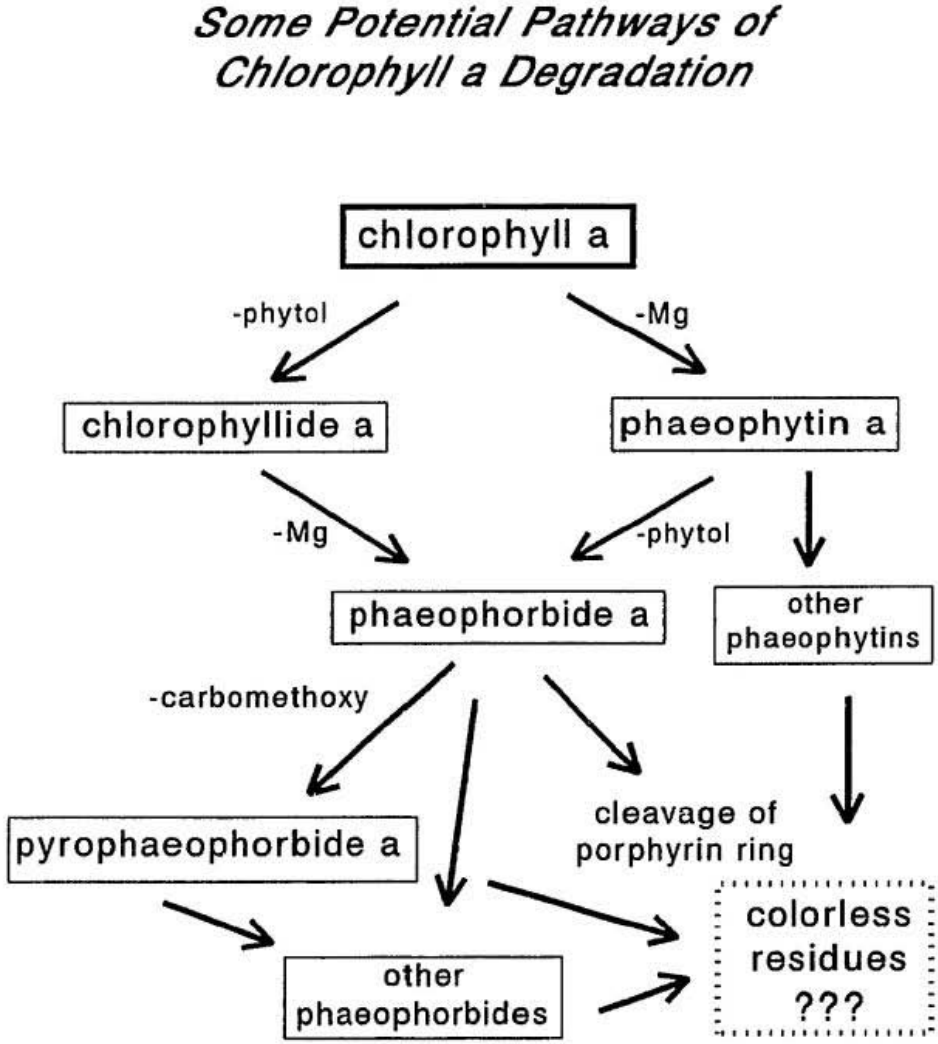

Figure 1.2. Some potential pathways of chlorophyll *a* degradation. The loss of the Mg atom occurs upon acidification, while the loss of phytol results from the action of chlorophyllase.

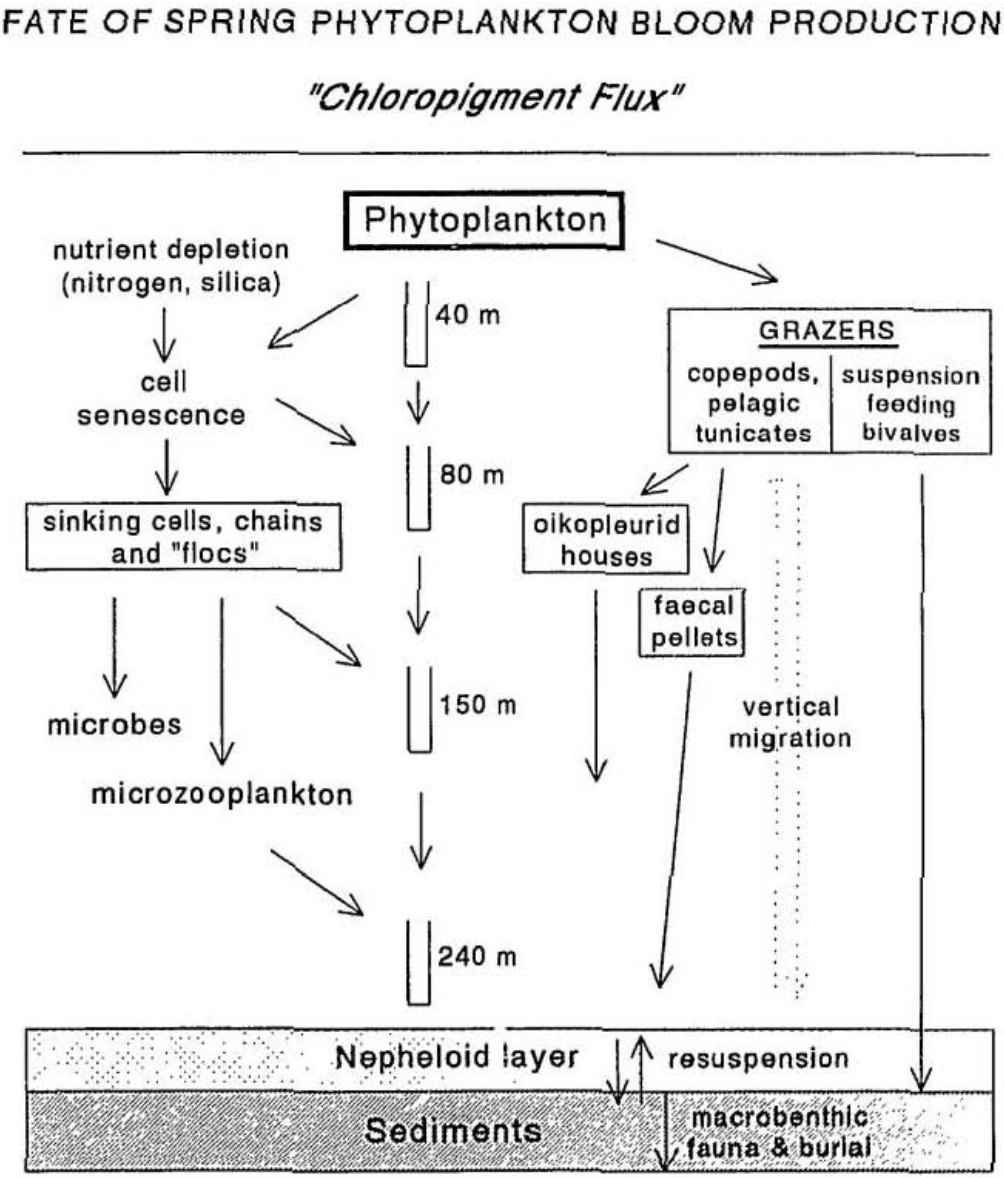

Figure 1.3. Schematic of some pathways of chloropigment flux during the spring phytoplankton bloom in Conception Bay. Chloropigment analyses were conducted on phytoplankton samples, grazer gut tracts and faecal material, sediment trap collections at various depths, surface sediments and overlying flocculent material.

Chapter 2
CHLOROPIGMENT METHODOLOGY

2.1. Introduction

Two analytical methods commonly employed for quantifying chloropigments (chlorophyll *a* and derivatives) from sample extracts are High Performance Liquid Chromatography (HPLC) with fluorescence detection, and the standard fluorometric method of Yentsch and Menzel (1963), as modified by Holm-Hansen et al. (1965). HPLC is capable of separating and individually quantifying chloropigment species, and is currently the analytical method of choice for pigment study (review in Roy 1987, Paerl and Millie 1991, Wright et al. 1991). In comparison, the standard fluorometric method, while a relatively fast and easy technique, is unable to resolve and individually quantify the chlorophyll *a* derivatives: chlorophyll *a* epimers and allomers, chlorophyllide *a*, phaeophytins and phaeophorbides. Standard fluorometric measures of chlorophyll *a* represent the sum of all chlorophyll *a*-type pigments: chlorophyll *a*, allomers and epimers of chlorophyll *a* and chlorophyllide *a*. Likewise, phaeopigment measures represent the sum of phaeophytin and phaeophorbide *a*-type pigments. The fluorometric method is also unable to resolve the accessory chlorophylls *b* and *c*. If present in large quantities, these accessory chlorophylls may interfere in the quantification of chlorophyll *a* and related phaeopigments (Loftus and Carpenter 1971, Gibbs 1979, Gieskes and Kraay 1983, Daemen 1986). Although the standard fluorometric method may lead to both quantitatively inaccurate and qualitatively misleading measures, it is a simple, convenient and relatively inexpensive technique which is widely employed for routine chlorophyll analysis.

There has been much controversy regarding the extent to which the standard fluorometric method provides reliable chloropigment measurements. Estimates of total chlorophyll *a*-type pigments in algal extracts have shown agreements with HPLC

measurements which range from good (Murray et al. 1986, Schanz and Rai 1988), to variable (Jacobsen 1978, 1982; Roy 1989, Jacobsen and Rai 1990) to poor (Gieskes and Kraay 1983, Trees et al. 1985). In most of these studies, the standard fluorometric method has overestimated chlorophyll *a* concentrations when compared to chromatographic analysis. De la Giraudière et al. (1989) suggest that differences between the methods may be more pronounced in seston samples with high organic loads. Fluorometric determinations of chloropigments in extracts of coastal sediment are also generally overestimates, presumably as a result of interference from co-extracted sediment compounds (Brown et al. 1981, Daemen 1986). Falkowski and Sucher (1981) suggest that humic compounds and other organic acids in sediment extracts may interfere with fluorometric analyses of chloropigments.

The standard fluorometric method has been used to estimate chloropigments in a variety of pigment-rich materials, including seston, digestive tracts and faeces of grazers, sediment trap collections, and sediments. I know of no methodological comparisons of chloropigment concentrations, using the standard fluorometric method and HPLC, conducted on sample types other than natural seston, cultured algae and sediments. Throughout this study, a wide variety of pigment-rich samples, collected from both field and laboratory studies, were extracted and chloropigment content determined using both HPLC and the standard fluorometric method. The sample types include:

1. seston (particles <505 μm diam.).
2. laboratory cultured algae.
3. copepod digestive tracts and faeces.
4. pelagic tunicate faeces.
5. mussel digestive gland.
6. mussel faeces.
7. sediment trap collections.
8. surface sediments.

All sample extracts were analyzed by the standard fluorometric method using the Turner Designs Model 10 fluorometer (TD10). Concurrent HPLC examinations were conducted on representative samples within large sample sets (>30 samples) and on all sample extracts within smaller sample sets.

2.2. General Methodology

Chloropigment analyses were conducted on both fresh and frozen samples. Freezing of sample material involved a quick freeze on dry ice (-79°C) and subsequent storage at -20°C in the dark. Analysis of frozen samples was usually completed within 3-6 months of initial freezing, although some samples remained in storage for up to 12 months prior to extraction. The effects of short- and long-term freezer storage on chloropigment composition and conservation were examined and are considered later in this chapter (Section 2.3).

Samples for analysis were generally extracted in 90% acetone (HPLC reagent grade) in the dark at ≤0°C for approximately 24 hours. Bowles et al. (1985) report optimal extraction of algal chloropigments in 90% acetone occurring at 24 hr. Seston and algal samples were extracted passively; all other sample types were either sonicated for 8-10 minutes in an ultrasonic bath cooled with crushed ice or were ground with a pestle. Extraction was followed by centrifugation at an RCF (relative centrifugal force) of >12000 for approximately 3 min. The chloropigment content of the supernatant from each sample extract was then determined using the Turner Designs Model 10 fluorometer. At the same time, 500 μl of the supernatant was transferred to a 1.5 ml polypropylene tube for further analysis with HPLC. All procedures were conducted in dark or subdued light conditions with a minimum of sample handling. Details on the collection and processing of specific sample types are given in subsequent chapters.

2.2.1. Standard Fluorometric Analysis

The Turner Designs Model 10 fluorometer was equipped with the recommended excitation (max. 430 nm) and emission (cut off at 670 nm) filters for chlorophyll analysis (optical kit #10-040). Sample extracts were read in 5 ml cuvettes before and after acidification with 3 drops of 1 M HCl (Riemann 1978). For extracts used as standards,

readings of the fluorescence before (Fb) and following acidification (Fa) were used to calculate an acid ratio (Fb/Fa). Acid ratios of the chlorophyll *a* standard and algal extracts were 2.12 and 2.09, respectively; complete phaeophytinization was verified by concurrent HPLC injection of neutralized calibration extracts. Chlorophyll *a* and phaeopigment concentrations were calculated following Strickland and Parsons (1972). The acid ratio for the algal calibration extracts (2.09) was applied in the calculations of chloropigments for all sample types analyzed.

Chlorophyll *a* standard (Sigma Chemical Co.) and various laboratory algal cultures (*Chaetoceros* sp., *Thalassiosira* sp., *Isochrysis* sp.), in log growth phase, were used in periodic calibrations of the fluorometer. Concentrations of chlorophyll *a* in the standard and algal extracts were determined spectrophotometrically using the extinction coefficient for chlorophyll *a* and the appropriate trichromatic equations in Jeffrey and Humphrey (1975). These extracts were then serially diluted and their respective fluorescence yields in the TD10 were used in the following calibration equation:

$$\text{chlorophyll } a \text{ conc. (ng/ml extract)} = a + b\,F$$

where a is the y-intercept (ng chlor *a*/ml extract), b is the slope, and F is the fluorescence yield using concentration multipliers (scale x door x meter reading). The coefficients (a, b) varied little over the duration of TD10 use (Table 2.1) and the detector response was linear within the range of 20-12000 fluorescence units ($r=0.999$).

The linear range of TD10 detection may not be constant for pigment extracts from highly variable sources. Sample types of interest may vary greatly in composition and in materials, other than chloropigments, which extract in 90% acetone. To determine the linearity of the TD10 response to extracts from a variety of pigment-rich compounds, representative sample types were extracted in 90% acetone, a series of dilutions prepared, and their relative fluorescence yields measured.

Common log-log plots of relative fluorescence units (RFUs) vs extract concentration showed parallel slopes below 6000 RFUs for all sample extracts (Figure 2.1, all slopes=0.94, r=0.99). Above 6000 RFUs, the extent of the linear response in the log-log plots varied greatly with sample type. It was highest for the extracts of chlorophyll *a* standard and laboratory-reared diatoms (> 10000 RFUs). The zooplankton extract showed a linear response over the entire range of concentrations prepared. but it was too dilute to determine an upper limit in linear detection. Amounts of chloropigment extracted from zooplankton gut tracts or faeces throughout this study were relatively low and produced fluorescence yields that were well within the range of linear detection.

The upper limit of linear detection was lowest for the most complex sample materials: digestive gland and sediment (about 6000 RFUs, Figure 2.1). The presence of co-extracted non-chlorophyllous material in the digestive gland and sediment extracts was suggested by an intense yellow colour in concentrated samples. Carotenoids and humic or fulvic-type substances probably contributed to this colour. Presumably, quenching effects of co-extracted compounds in these sample types reduced the d :tor range over which the fluorescence response was linear. For reliable measures of all sample types extracted, the linear range needs to be determined and not exceeded, particularly when analysing material with high lipophilic content.

2.2.2. HPLC Analysis

Separation and quantification of chloropigments was achieved with an HPLC system made up of the following components: an Eldex Model AA dual piston high pressure pump, a Valco injector valve equipped with a 100 μl sample loop, a Brownlee C18 reverse phase HPLC column (250 x 4.6 mm, ODS-5 micron particles) protected with a precolumn filter (2 μm frit) and a Brownlee guard cartridge. Chloropigments were detected with a Beckman Model 157 fluorescence detector, fitted with appropriate

excitation (max. 426-436 nm) and emission (610-660 nm) filters and a 9 μl flow cell. Linearity of fluorescence response within the range of detector use was examined and confirmed with a series of diluted extracts. The signal was fed to a chart recorder; peak areas were measured with a Jandel Scientific digitizing tablet and Sigma-Scan™ software.

Identification and peak purity of eluted chlorophylls and phaeopigments from representative sample types and standards were also confirmed using an on-line Waters 991 Series photodiode array detector, Spectroflow 980 fluorescence detector and Spectra-Physics integrator.

HPLC analyses were conducted using slight modifications of procedures outlined in Mantoura and Llewellyn (1983) and Bidigare et al. (1985). Chloropigments were eluted using the following binary solvent system:

Solvent A. 80:15:5 methanol: water: 1 M ammonium acetate

Solvent B. 70:30 methanol: acetone.

All solvents were HPLC reagent grade and were purged with helium daily prior to use. Water was purified using a Millipore Milli-Q system and ammonium acetate was A.R. grade. The stock solution of 1 M ammonium acetate (pH=7.1) was stored at 4°C to reduce bacterial growth (Nelson 1986) and was periodically filtered (0.45 μm Millipore) to remove particulate material. Initial analyses included an ion-pairing reagent, tetrabutylammonium acetate (TBAA, Fluka Chemical Co.), in the methanol: buffer solvent (Solvent A). Though TBAA was recommended as an aid in the separation of the polar pigments (Mantoura and Llewellyn 1983), it did not improve chloropigment separation in this study and was subsequently omitted. Detectable contaminants were found in one batch of TBAA, strengthening the case for discontinuing its use.

Immediately prior to sample injection, 500 μl of extract was mixed with 150 μl of 1 M ammonium acetate buffer in a 1.5 ml polypropylene tube; 250 μl of this solution was then injected for a sample loop delivery of 100 μl. Solvent flow rate was 1.7 ml

min^{-1} with an initial 6 min step gradient, over 1 min intervals, from 100% Solvent A to 100% Solvent B. An isocratic hold of Solvent B was then maintained for an additional 25 minutes. Each sample run was followed by a 5-10 min methanol wash and a 3 min re-equilibration to initial conditions prior to injection of the next sample. Coefficients of variation for peak areas of standards in replicate injections were <5%. Calibration was periodically checked with an internal standard, coproporphyrin tetramethyl ester III (Sigma Chem. Co.), dissolved in methanol.

HPLC response factors for prepared standards were calculated as follows:

$$Fs = Cs \times Iv \times Fd \times (1/As)$$

where Fs is the standard response factor (ng of standard injected per unit area of peak), Cs is the concentration of standard (ng/ml extract), Iv is the injection volume (0.1 ml), Fd is the sample dilution factor (0.769), and As is the area of the standard peak.

Concentrations of chloropigments in samples were then determined from peak areas of individual pigments and the standard response factors using the following formula:

$$Cp = Ap \times Fs \times (1/Iv) \times (1/Fd) \times Ev \times (1/Sv)$$

where Cp is the concentration of individual pigment (ng/sample volume or weight), Ap is the area of pigment peak, Ev is the extract volume (ml), and Sv is the sample volume or weight.

Chromatograms were compared with those from an automated Beckman Gold HPLC system coupled with identical column, guard column and fluorescence detector. Analytical procedures with the Beckman Gold system were similar except for a 10 min linear gradient from 100% Solvent A to 100% Solvent B and a flow rate of 2 ml min^{-1}.

Extracts of laboratory algae and mussel faeces were prepared for HPLC system comparisons. Same-day analyses of replicate samples yielded comparable profiles of chloropigment peaks and relative retention times.

2.2.3. Standards

The chlorophylls and their fluorescent derivatives were identified by comparisons with HPLC retention times and/or co-elution with authenticated standards. Standard response factors were determined for every series of samples injected (< 20 samples). A reference chromatogram (Figure 2.2), created by combining pigment standards immediately prior to injection, shows the elution order and relative retention times of prepared standards.

Chlorophylls *a* and *b* were obtained from Sigma Chemical Co. and were stored in 100% or 90% acetone under nitrogen at -20°C. Chlorophyllide *a* was prepared from a culture of *Phaeodactylum tricornutum* by extracting cells in 50% acetone (Barrett and Jeffrey 1971), and isolated by thin-layer chromatography (Jeffrey 1974). Concentrations in 90% acetone were determined spectrophotometrically using the absorption coefficients for chlorophyll *a* and *b* in Jeffrey and Humphrey (1975) and for chlorophyllide *a* in Lorenzen and Downs (1986). Phaeophytins *a* and *b* and phaeophorbide *a* were prepared from acidification of the parent pigments with HCl (Holm-Hansen et al. 1965). HPLC profiles and absorbance spectra of prepared standards were examined for chloropigment purity.

Identical molar absorption coefficients have been determined for chlorophyll *a* and chlorophyllide *a* and for phaeophytin *a* and phaeophorbide *a* (Lorenzen and Downs 1986). In addition, the fluorescence emission and excitation spectra of chlorophyll *a* and chlorophyllide *a* are the same; phaeophytin *a* and phaeophorbide *a* also share identical fluorescence properties (Bazzaz and Rebeiz 1979). Hence, dilute chlorophyllide *a* and

phaeophorbide *a* standards were routinely quantified fluorometrically using the TD10 calibration equation for the chlorophyll *a* standard and corresponding acid ratio.

Allomers and C-10 epimers of chlorophylls *a* and *b* were qualitatively prepared from a *Thalassiosira weissflogii* culture according to procedures outlined in Sartory (1985). Respective phaeophytins were prepared following acidification with 1 M HCl. Though conversion was incomplete and the derivatives were not purified, they did provide useful qualitative information on relative retention times. Because chlorophyll *a* and its isomers have identical absorption properties (Schwartz et al. 1981), chlorophyll *a* standard response factors were applied to estimate the relative contributions of chlorophyll *a* allomers and epimers in HPLC profiles.

Pyrophaeophorbide *a* and pyrophaeophytin *a* were prepared from phaeophytin *a* (Wasielewski and Svec 1980) and characterized by mass and infrared spectroscopy in the laboratory of Dr. Dan Repeta at the Woods Hole Oceanographic Institute. Purity was checked by HPLC detection of absorbance and fluorescence. Quantification was determined as for phaeophorbide *a* and phaeophytin *a*.

Chlorophyll *c* was isolated from *Chaetoceros muelleri* using a modification of the two-dimensional TLC method of Jeffrey (1981). The first dimension consisted of 2% n-propanol in light petroleum ether (boiling point 60-80°C) and the second dimension was a mixture of light petroleum ether: chloroform: acetone (75:25:0.5). Pigment concentration in 90% acetone was determined spectrophotometrically (Jeffrey and Humphrey 1975) and purity checked via absorption spectral analysis and HPLC. Phaeophytin *c* was obtained by acidification of chlorophyll *c* with HCl.

Kodak Chromagram cellulose TLC sheets #13255 (without fluorescent indicator) were used for all TLC preparations and provided excellent separation of pigments.

2.2.4. Methodological Comparisons

To allow direct comparisons between standard fluorometric and HPLC derived estimates of chloropigments, all concentrations of chlorophyll *a* fluorescent derivatives are reported in chlorophyll *a* weight equivalents. TD10 estimates of chlorophyll *a* are compared to the sum of HPLC estimates of the chlorophyll *a*-type pigments: chlorophyll *a*, chlorophyllide *a* and allomers and epimers of chlorophyll *a*. In the same manner, TD10 estimates of phaeopigments are compared to summed HPLC measurements of phaeophorbide and phaeophytin *a*-type pigments. True weights of the chlorophyll *a* derivatives can be calculated by using the pigment-specific molecular weight corrections in Lorenzen and Jeffrey (1980) and Lorenzen and Downs (1986).

2.3. Effects Of Freezer Storage

2.3.1. Introduction

Immediate processing of phytopigment samples upon collection is often inconvenient and limited to small numbers of samples. Consequently, analyses of field collections are frequently conducted on samples which have been frozen for various periods of time. In cases of delayed processing, it is generally assumed that pigment integrity is conserved during the process of sample freezing and throughout the period of storage.

Short-term storage of samples in liquid nitrogen is currently recommended to offset or minimize potential degradative effects of freezing pigment-rich material (Paerl and Millie 1991, Wright et al. 1991). Previous studies using the standard fluorometric method, however, have shown that filtered algal samples can be stored at -30°C for up to 3 mos (Gieskes and Kraay 1983) and at -20°C for 2-3 wk (Holm-Hansen and Riemann 1978, Schanz and Rai 1988) without loss of chloropigments. HPLC analysis has also confirmed conservation of pigments in seston samples frozen for up to 2 mos at -20°C

(Nelson, 1989). It has therefore been common practice to store phytopigment samples in a -20°C freezer for various periods of time (e.g. Hawkins et al. 1986, Barlow et al. 1988, Roy 1989, Leavitt and Carpenter 1990, Abele-Oeschger et al. 1992). As an aid in preserving pigment integrity, some researchers have applied dry ice or liquid nitrogen to quick-freeze samples prior to storage at -20°C (Murray et al. 1986, Nelson 1989, G. Kleppel, pers. comm.). Although several methods have been used to freeze chlorophyllous material for storage, no studies have documented the combined effects of storing samples at different temperatures for varying periods of time.

Samples collected for pigment analysis may be variable in nature (e.g. algae, faeces, sediments) and therefore variable in composition of extractable compounds. In addition, chloropigment-rich material may contain a variety of enzymes, alkalis, and acids which are known agents in the transformation and decay of algal pigments (review in Hendry et al. 1987). Conservation of pigments within frozen samples is thus subject to the combined effects of degradative agents within the sample and processes associated with initial freezing and duration of sample storage.

The following study examines the effects of short- and long-term storage of frozen samples on chloropigment composition and conservation within three markedly different sample types: lab-reared algae (diatoms), *Mytilus edulis* (blue mussel) digestive gland and *M. edulis* faeces. Homogenous suspensions of each sample type were prepared for subsequent sampling by volume or weight of sample material. Following a quick freeze on dry ice, replicate samples of each type were stored at -20°C for periods up to 12 mos. The effects of initial freezing of algal samples in a -20°C freezer, under dry ice (-79°C), and with liquid nitrogen (-210°C) were also investigated. Methodological comparisons of chloropigment estimates, using the conventional fluorometric method and HPLC with fluorescence detection, are included in this study (Section 2.3.3.3).

2.3.2. Methods

Laboratory cultures of the diatom *Thalassiosira weissflogii* were diluted with 3 litres of seawater filtered through a series of 10 μm, 5 μm and 1 μm Gelman Polypure cartridges, and uniformly mixed. Thirty-six subsamples (50 ml each) of the diluted algal stock were then removed and the cells collected on GF/C filters. Four filters were immediately extracted in 90% acetone. All other filters were folded and individually wrapped in aluminum foil prior to freezing. Four filters were frozen in liquid nitrogen; another four filters were transferred directly to a -20°C freezer. These sample sets were extracted after 1 day of storage at -20°C. The remaining subsamples were quick-frozen on dry ice (-79°C) and then transferred to a -20°C freezer. Replicates (n=4) of dry-ice treated filters were extracted following storage intervals of 1 day, 1 wk, 1 month, 3 mos, 6 mos and 12 mos.

Mussel faeces and digestive gland material were collected from adult mussels (*Mytilus edulis*) fed lab-reared *T. weissflogii* for four consecutive days. Freshly produced faeces were collected and homogenized in a mortar and pestle which was cooled with liquid nitrogen, and 24 subsamples stored in 1.5 ml polypropylene tubes. The digestive glands were removed, homogenized and similarly subsampled. Four replicates of each sample type were extracted fresh; the remainder were quick-frozen on dry ice as for the algae samples. Dry weights of all faecal and digestive gland samples were determined to the nearest 0.01 mg. Replicates (n=4) of freezer-stored faeces and digestive gland were analyzed following 1 wk, 1 month, 3 mos, 6 mos and 12 mos of freezer storage. Samples of both types were sonicated in an ice-cooled water bath for 10 min during a 24 hr extraction period.

Mean concentration and percent composition of chloropigments were determined for each set of replicate samples extracted over the 12 month time series using both the standard fluorometric method (TD10) and HPLC. Data were analyzed using ANOVA and

Duncan's multiple range test to determine whether mean chloropigment estimates, within and between methods, were significantly different over the duration of storage. Tolerance for Type I error was set at $\alpha = 0.05$.

2.3.3. Results

2.3.3.1. Laboratory Algae (*Thalassiosira weissflogii*)

A. Initial Freezing Effects

The effects of initial freezing on chloropigment composition and concentration in algal extracts are shown in Table 2.2. Chlorophyllide *a* appeared in small amounts in all samples extracted following 1 day of filter storage in liquid nitrogen, on dry ice and in a -20°C freezer. Degradative effects are shown by increases in relative amounts of chlorophyllide *a*, and chlorophyll *a* allomers and epimers. Production of chlorophyll *a* degradation products was lowest in samples stored in liquid nitrogen and highest in those stored at -20°C. Though all freezing methods resulted in minor alterations of the chloropigment composition, total chlorophyll *a*-type pigments recovered from samples extracted fresh and from those frozen for 1 day were not significantly different ($P=0.461$).

B. Short- and Long-term Freezing Effects

Total chloropigment concentrations varied little over 12 mos of sample storage at -20°C (Figure 2.3). Though the TD10 mean estimate from replicates stored for 3 mos was significantly lower ($P<0.05$) than that from all other sample sets, there were no significant differences in mean concentrations among fresh extracts of algae and sample sets frozen for 1 day, 1 wk, 1 month, 6 mos and 12 mos. HPLC mean estimates of total chloropigments were not significantly different over the entire 12 mos time series ($P=0.471$).

Between methods, mean chloropigment concentrations were significantly different ($P < 0.05$); estimates with the TD10 were consistently higher (3-18%, average 9%) than those from chromatographic analysis. Many of the TD10 analyses also indicated the presence of small amounts of phaeopigment, comprising up to 4% of the total chloropigment pool. The presence of phaeopigment was not detected in HPLC analyses.

Representative chromatograms of algae extracted fresh, and following 1 month, 6 mos and 12 mos of storage at -20°C are shown in Figure 2.4. They reveal no phaeopigment accumulation and only minor changes in the composition of chlorophyll *a*-type pigments. With prolonged storage time, sample extracts exhibited small but increasing amounts of chlorophyllide *a* (-a), and both allomers (a_1) and epimers (a^1) of chlorophyll *a*.

Table 2.3 shows the relative composition of chlorophyll *a*-type pigments in fresh and frozen sample sets. Following 6 mos of storage at -20°C, approximately 11% of chlorophyll *a* within filtered algal samples was converted to chlorophyll *a*-like derivatives (allomers and epimers) and chlorophyllide *a*. More than 60% of this transformation occurred within the first month of sample storage. Between 6 and 12 mos, the chlorophyll *a*-type pigment composition remained relatively uniform. Throughout the time series, degradation products of chlorophyll *a* were dominated by chlorophyll *a* allomers.

2.3.3.2. Mussel Digestive Gland and Faeces

A. Digestive Gland

The concentration and relative composition of chloropigments in fresh and frozen digestive gland samples are shown in Figure 2.5. Mean estimates of total chlorophyll *a*-type pigments, total phaeopigments and total chloropigments during storage, both within and between methods, showed no significant differences ($P > 0.50$).

Percent contributions of total chlorophyll *a*-type pigments and total phaeopigments remained relatively stable over the 12 month time series. However, within the phaeopigment pool, the phaeophorbide fraction increased with prolonged storage. This was also revealed in the series of chromatograms shown in Figure 2.6. An additional peak at 7.5 min, within the phaeophorbide *a*-type array (phor), appeared in extracts after 1 month, 6 mos and 12 mos of freezer storage. Furthermore, as the period of storage increased, the presence of numerous other transformation products, represented by several small peaks (11-16 min), also increased. These additional decay products are probably phaeophytin *a* (phyt) derivatives. Prolonged storage also resulted in a raised baseline between 5 and 12 min, apparently due to nonphorbin components or products of chlorophyll degradation extracted from frozen digestive gland. This baseline interference cannot be attributed to column deterioration; elevated baselines were not present in same-day chromatograms of algae and mussel faeces (compare Figures 2.4 and 2.8). Though chlorophyllide *a* was absent from freshly extracted digestive gland material, it was present in the extracts of all frozen samples.

B. Faeces

Chloropigment concentrations in extracts of mussel faeces were not uniform over the 12 month time series (Figure 2.7). HPLC analyses showed a 40-50% increase in extracted total chloropigments following 1 wk of freezer storage. Extracts from samples frozen for one month or longer exhibited overlapping mean estimates (Duncan's multiple range test, $\alpha=0.05$) of chlorophyll *a*-type pigments, phaeopigments and total chloropigments. In addition, methodological comparisons showed no significant differences between TD10 and HPLC mean estimates of chlorophyll *a*-type pigments ($P=0.448$), phaeopigments ($P=0.221$) or total chloropigments ($P=0.597$).

Percent composition of chlorophyll *a*-type pigments and phaeopigments remained relatively stable over the 12 month duration. HPLC profiles (Figure 2.8) also showed consistency in pigment composition, indicating conservation of chloropigments

throughout sample storage. This is in marked contrast to the chromatograms of extracted digestive gland material (Figure 2.6). These sample types also differ in that small amounts of chlorophyllide *a* were present in the extracts of both fresh and frozen mussel faeces, but not in digestive gland extracts.

C. Chlorophyll a-type Pigments : HPLC

Figure 2.9 shows the percent composition of chlorophyll *a*-type pigments in fresh and frozen samples of mussel digestive gland and mussel faeces. Profiles of digestive gland extracts revealed relatively greater amounts of both chlorophyllide *a* and chlorophyll *a*-like derivatives (C-10 epimer and allomers) with increased duration of storage. The contribution of these products increased from 12% to 35% of total chlorophyll *a*-type pigments during 12 mos of storage, presumably due to digestive gland enzyme activity. Throughout the time series, breakdown products of chlorophyll *a* were dominated by chlorophyll *a* allomers.

In contrast, extracts of mussel faeces showed a higher percentage contribution of chlorophyll *a*, both initially and throughout the duration of storage. Only 3-4% of chlorophyll *a* in fresh extracts was reduced to isomeric, allomeric and dephytolated forms. Compared to digestive gland extracts, transformation products of chlorophyll *a* in mussel faeces comprised a much smaller fraction (8-12%) of the total chlorophyll *a*-type pool. These decay products, however, were similarly dominated by chlorophyll *a* allomers.

D. Phaeophytin a-type Pigments : HPLC

The composition of phaeophytin *a*-type pigments, in both mussel digestive gland and faeces, changed little over the duration of freezer storage (Figure 2.10). The more polar forms of phaeophytin *a* (phyt+) represented <3% of the overall composition in

both sample types. Small increases in these polar derivatives occurred in digestive gland samples after prolonged storage at -20°C.

The less polar, pyrophaeophytin *a*-like derivatives (phyt-), comprised 66-69% of the total phaeophytin pool in the digestive gland extracts and 42-46% in extracts of mussel faeces. Differences between sample types in relative amounts of phaeophytin *a* and the less polar derivatives may be indicative of some transformation or loss of the less polar forms within the digestive tract of *Mytilus edulis*.

E. Phaeophorbide a-type Pigments : HPLC

The dominant phaeophorbide *a*-type pigment in both mussel digestive gland and mussel faeces was pyrophaeophorbide *a*. In both sample types, this form comprised >45% of the total phorbide pool throughout the duration of sample storage (Figure 2.11). Relative contributions of the phaeophorbide *a*-type pigments in both sample types changed during storage, however, with the greatest transformations occurring within the first week. Thereafter, chloropigment degradation continued but at a reduced rate. Initial freezing resulted in the formation of the less polar phorbides (pyro-) in both digestive gland and faeces. Following 1 wk of storage, the combined contributions of pyrophaeophorbide *a* and less polar forms remained relatively constant in digestive gland extracts. Changes in the relative contributions of these two components suggest that pyrophaeophorbide *a* is the precursor of the less polar derivatives. This is supported by personal observations of the partial transformation of pyrophaeophorbide *a* standard in 90% acetone to a less polar derivative following exposure to room temperature for 2 days.

2.3.3.3. Comparison of Methods : TD10 vs HPLC

Plots of TD10 vs HPLC estimates of total chlorophyll *a*-type pigments and total phaeopigments in previously frozen samples of algae and mussel digestive gland and

faeces are shown in Figure 2.12. TD10 estimates for chlorophyll *a*-type pigments in algal extracts were generally higher than those determined by HPLC. However, chlorophyll *a*-type estimates in mussel faeces extracts showed relatively good agreement. TD10 measures of phaeopigments in extracts of both mussel digestive gland and faeces tended to be slightly lower than HPLC estimates.

Mean concentrations of chlorophyll *a*-type pigments, phaeopigments and total chloropigments for all replicates within each sample type and with each method, are presented in Figure 2.13. There were small but significant differences between methods in estimates of chlorophyll *a*-type pigments in extracts of both laboratory algae ($P=0.013$) and mussel digestive gland ($P=0.001$); for both sample types, the standard fluorometric measurements were slightly higher than HPLC determinations. Neither phaeopigment nor total chloropigment measurements in digestive gland extracts showed significant differences between methods ($P>0.20$). In mussel faeces extracts, there were no significant differences between methods for chlorophyll *a*-type pigments ($P=0.530$), phaeopigments ($P=0.195$) or total chloropigments ($P=0.531$). Overall, extracts containing a mix of chlorophyll *a*-type pigments and phaeopigments showed very good agreement between methods in determinations of total chloropigment concentration.

2.3.4. Discussion

Chlorophyll *a* derivatives (chlorophyllide *a* and chlorophyll *a* isomers and allomers) were present in the extracts of all frozen filters of *Thalassiosira weissflogii*. Amounts transformed following one day of storage at -20°C comprised <2% of chlorophyll *a* present in replicates extracted immediately following filtration. Although chlorophyllase activity in *Thalassiosira* spp. is known to be very low (Jeffrey and Hallegraeff 1987), small amounts of chlorophyllide *a* were present in all sample extracts after storage in liquid nitrogen, dry ice and a -20°C freezer. Conversion of chlorophyll *a* to chlorophyllide *a* is often attributed to cellular disruption upon sample filtration

(Jeffrey and Hallegraeff 1987, Otsuki et al. 1987). In my study, chlorophyllide *a* was not present in fresh extracts of *T. weissflogii* collected on GF/C filters. Formation of chlorophyllide *a* in these algal samples is therefore attributed to degradative processes associated with freezing.

The degree of transformation to chlorophyllide *a* and chlorophyll *a*-like derivatives appears to be controlled by the temperature of initial freezing. The results in this study support the use of liquid nitrogen (-210°C) as the most effective method of preserving chloropigment composition. This method eliminates oxygen from the sample during freezing and thus prevents allomerization. Although the least effective method of initial freezing was storage in a -20°C freezer, the amount of chlorophyll *a* altered in *T. weissflogii* cells was small (1.7%). A quick freeze on dry ice (-79°C) limited the degradative effects of initial freezing. Neither of these methods showed the presence of phaeopigments or losses in total chlorophyll *a*-type pigments, measured in chlorophyll *a* weight equivalents, following a one day storage period.

During the 12 mos of storage, samples of *T. weissflogii* showed no losses of chlorophyllous material and only minor changes in pigment composition. Small amounts of alteration products were formed, primarily within the first month, and were dominated by chlorophyll *a* allomers, presumably as a result of oxidation of the parent pigment (Hynninen and Ellfolk 1973).

Standard fluorometric measurements of chloropigments in *T. weissflogii* extracts were higher than HPLC measurements; compared to HPLC determinations, chlorophyll *a*-type pigments measured using the TD10 were overestimated by an average of 9%. Similar observations have been reported by Murray et al. (1986). According to the TD10 measurements, small amounts of phaeopigment (<4% of total) were often present, even though phaeopigments were not evident in HPLC profiles of the same extracts. The acid ratio factor, predetermined with algal standards, is used to calculate the relative amounts

of total chlorophyll *a*-type pigments and phaeopigments, but it is insensitive and subject to small deviations which could lead to the observed errors in measurement.

HPLC chromatograms of extracted algae and mussel faeces were relatively constant over the 12 mos time series, indicating conservation of pigments in both sample types throughout one year of storage at -20°C. Although relative amounts of individual pigments in mussel faeces remained uniform over time, total amounts extracted increased following the first week of storage, possibly as a result of an increase in extraction efficiency following freezing. Sun et al. (1991) report greater extraction efficiency of chloropigments from sediments frozen prior to extraction; these sediments probably contained an abundance of pigment-rich faecal material. Furthermore, compared with fresh sediment samples, freeze-dried sediments yield higher amounts of extracted chlorophyll (Hansson 1988).

Total amounts of chloropigments extracted from digestive gland samples changed little over 12 mos of storage. However, there were significant transformations within the various pigment pools. Chlorophyll *a*-type pigments showed an increase in both the dephytolated (chlorophyllide *a*) and chlorophyll *a*-like fractions (allomers, epimers) with prolonged storage. Within the phaeopigment pool, the phaeophorbide *a*-type fraction increased, suggesting some conversion of phaeophytin *a* to phaeophorbide *a*. Transformation products within the phaeophorbide *a*-type pigments were most evident within the first week. Thereafter, the rate of degradation decreased. In samples of both mussel digestive gland and faeces, the composition of the phaeophytin *a*-type pigments remained relatively stable throughout the duration of storage.

Chromatograms of digestive gland extracts showed a raised baseline and an increasing number of unknown decay products with prolonged storage. Hydrolytic enzymes and acids within these samples may have induced further breakdown of chlorophyllous material during storage. In contrast, profiles of mussel faeces remained

relatively unchanged over 12 mos of storage, suggesting the absence of degradative agents within faecal material. Robinson et al. (1989) also reported numerous unidentified peaks in HPLC fluorescence chromatograms of scallop digestive gland extracts. They attributed these peaks to unknown products of chlorophyll degradation or compounds produced metabolically by the scallop. I have compared HPLC profiles of both frozen mussel digestive gland and lyophilized digestive gland and I have observed a more stable baseline and better resolution of chloropigments in chromatograms of lyophilized material (unpubl. obs.).

Differences between HPLC measures of chloropigments in extracts of mussel digestive gland and faeces were not due to the effects of freezing but were indicative of degradation and absorption of chloropigments in the digestive gland of mussels. Lower concentrations of phaeopigment, primarily phaeophorbides, were observed in faecal material, possibly as a result of ingested algae entering the intestine without prior processing in the digestive gland. There have been many reports of absorption and storage of chlorophyll degradation products within the digestive glands of various bivalves (Ansell 1974, Gelder and Robinson 1980, Robinson et al. 1984, 1989). Phaeopigments, in particular, have been identified in the lipid droplets of the digestive gland cells (Gelder and Robinson 1980). Hawkins et al. (1986) observed absorption of up to 82% of chloropigments ingested by *Mytilus edulis*.

2.3.5. Conclusions

Short-term sample storage in liquid nitrogen is the least destructive method of freezing *Thalassiosira weissflogii* chloropigments. However, in the absence of liquid nitrogen, a quick freeze on dry ice prior to storage in a -20°C freezer reduces chloropigment transformation resulting from initial freezing at -20°C. Chlorophyll *a* and its degradation products may remain relatively stable under these conditions for up to 12 mos provided that the samples are free of hydrolytic enzymes and/or acids. Alteration

of chloropigments due to processes associated with the initial freezing of samples may be greater than those due to prolonged storage at -20°C. Extraction of fresh material is preferred. However, lyophilization prior to sample storage may reduce decay and transformation of pigments to unidentifiable derivatives. Its use is supported by observations of pigment conservation during long-term storage (>300 days) of freeze-dried algal cells (Paerl and Millie 1991).

There was good agreement in chloropigment measurements between methods of analysis, particularly in samples of mussel digestive gland and faeces, both of which contained a mixture of chlorophyll *a*-type pigments and phaeopigments. In the absence of chlorophyll *b*, the conventional method appears to be reliable for estimates of total chloropigments, but with a tendency to overestimate slightly the chlorophyll *a*-type pigments in algal extracts. Methodological differences in chloropigment determinations may be much greater when samples are of natural seston origin. Use of the standard fluorometric method may be most suitable for experiments which involve cultured algae, other than chlorophytes, and in which the identification of specific chlorophyll *a*-type pigments and phaeopigments is not desired. In applications of this nature, the standard fluorometric method is robust and provides reasonably accurate measurements of chloropigments. However, because this method is quantitatively less sensitive than HPLC, the reliability of the standard fluorometric method should be determined with concurrent HPLC analysis. This is particularly important for the analysis of field-collected samples which may contain interfering pigments and extractable nonphorbin material.

Table 2.1. TD10 chlorophyll *a* calibration coefficients for 90% acetone extracts of cultured diatoms and chlorophyll *a* standard during 1987-1992. Linear equation: chlorophyll *a* (ng/ml extract) = a + b F. F is fluorescence yield (scale x door x reading).

Date	Extract (90% acetone)	a (ng/ml extract)	b (slope)	n	r
29/04/87	Mixed Diatoms	-0.627	0.0384	21	0.999
05/05/87	*Isochrysis* sp.	-0.608	0.0377	7	0.999
13/05/87	Chlor *a*	-0.757	0.0375	6	0.999
09/09/87	Chlor *a*	-0.605	0.0349	6	0.999
03/03/88	Chlor *a*	-0.423	0.0337	6	0.999
03/03/88	Mixed Diatoms	-0.487	0.0428	6	0.999
21/03/89	Chlor *a*	-0.407	0.0345	6	0.999
15/08/89	Chlor *a*	-0.383	0.0361	6	0.999
28/03/90	Chlor *a*	-0.439	0.0329	6	0.999
05/06/90	Chlor *a*	-0.222	0.0359	12	0.999
10/04/91	Chlor *a*	-0.493	0.0381	10	0.999
04/04/92	Chlor *a*	-0.388	0.0309	8	0.999

Table 2.2. Percent composition (HPLC) and total concentration (TD10) of chloropigments in freshly extracted algae and in samples stored for 1 day in liquid nitrogen, on dry ice and in a -20°C freezer prior to extraction. Proportions (%) are from duplicate samples.

	Fresh Extract %	Liquid N_2 1 day %	Dry Ice 1 day %	-20°C 1 day %
chlorophyllide *a* (-a)	0.00, 0.00	0.28, 0.39	0.69, 0.83	1.01, 0.69
chlor *a* allomers (a_1)	0.66, 0.69	0.38, 0.63	0.85, 0.94	1.25, 1.04
chlor *a* epimers (a^1)	0.62, 0.52	0.49, 0.54	0.30, 0.49	1.03, 0.87
chlor *a* (chl a)	98.7, 98.8	98.8, 98.4	98.2, 97.7	96.7, 97.4
Total Conc. (μg/l) Mean ± SD (n=4)	34.09 ± 0.42	33.86 ± 0.40	33.26 ± 0.66	33.66 ± 0.94

Table 2.3. Percent composition (HPLC) of chloropigments in freshly extracted algae and in samples frozen on dry ice prior to storage at -20°C for up to 12 mos.

	% composition (±SD)						
	Fresh	1 day	1 wk	1 mos	3 mos	6 mos	12 mos
chlorophyllide *a*	0 (0)	0.8 (0.2)	0.7 (0.1)	1.3 (0.3)	1.1 (0.2)	1.4 (0.1)	1.5 (0.3)
chlor *a* allomers	0.7 (0.1)	0.9 (0.2)	2.7 (0.3)	4.7 (0.4)	5.3 (0.8)	7.2 (1.1)	6.9 (1.7)
chlor *a* epimers	0.6 (0.1)	0.4 (0.1)	0.6 (0.2)	2.0 (0.3)	2.5 (0.4)	3.3 (0.4)	3.5 (0.7)
chlor *a*	98.7 (0.4)	97.9 (0.3)	96.0 (0.6)	92.0 (0.5)	91.1 (0.9)	88.0 (1.3)	88.1 (2.4)

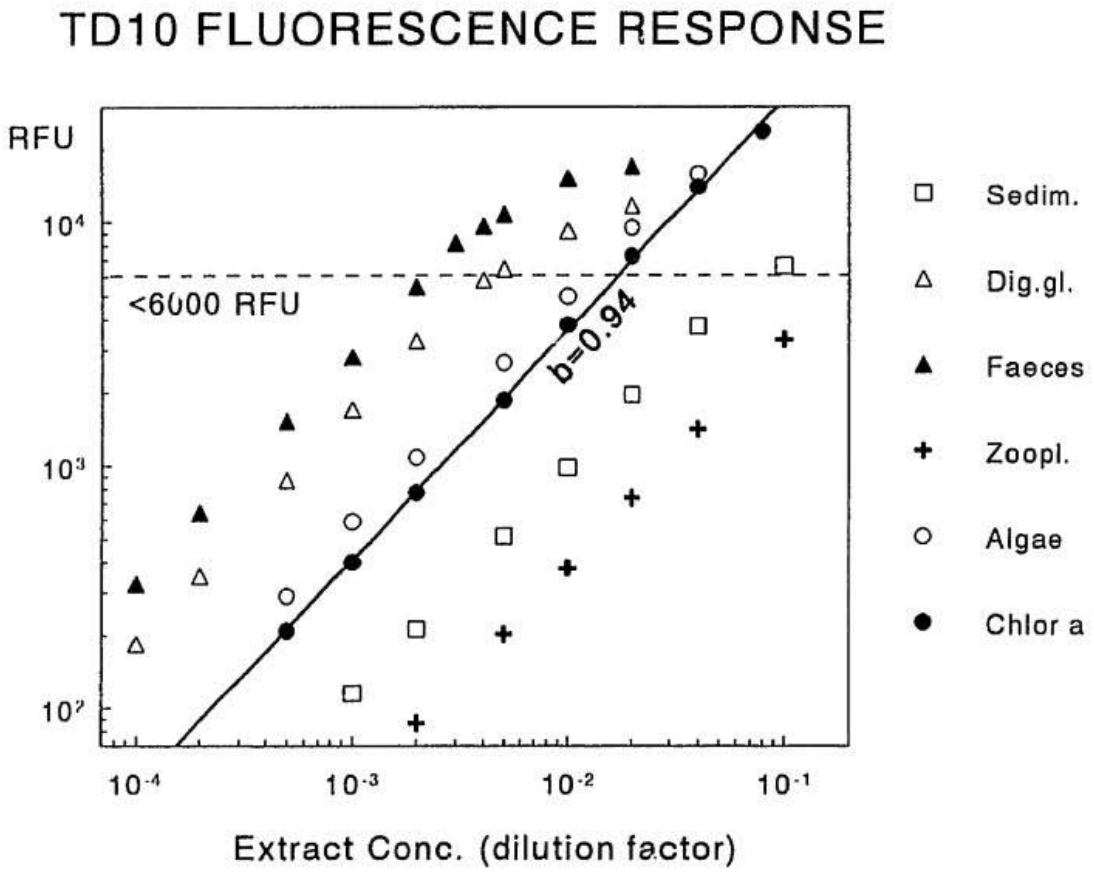

Figure 2.1. Turner Designs Model 10 fluorescence detector response for diluted extracts of marine sediment, mussel digestive gland, mussel faeces, zooplankton, lab algae (*Chaetoceros* sp.) and chlorophyll *a* standard.

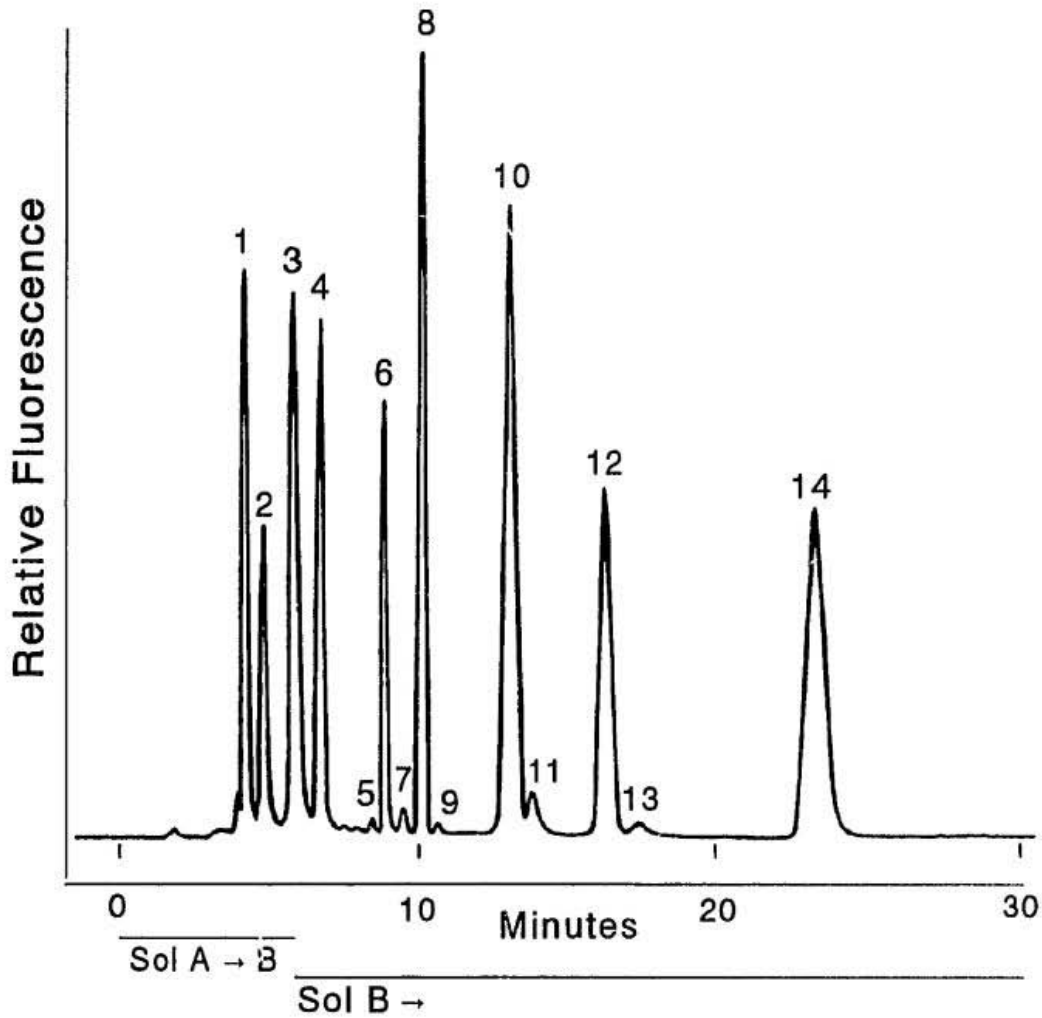

Figure 2.2. Fluorescence chromatogram of mixed standards. Peak identities are: 1-chlorophyllide *a*, 2-chlorophyll *c*, 3-phaeophorbide *a*, 4-pyrophaeophorbide *a*, 5-chlorophyll *b* allomer, 6-chlorophyll *b*, 7- chlorophyll *a* allomer, 8-chlorophyll *a*, 9-chlorophyll *a* epimer, 10-phaeophytin *b*, 11-phaeophytin *b* epimer, 12-phaeophytin *a*, 13-phaeophytin *a* epimer, 14-pyrophaeophytin *a*.

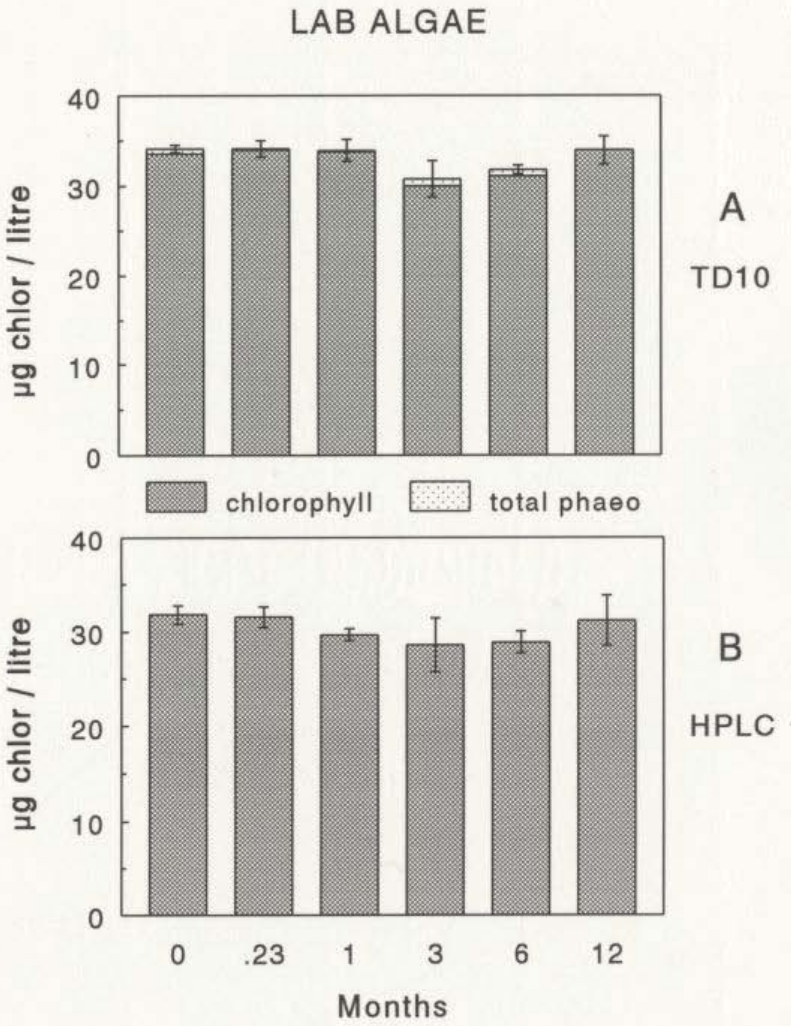

Figure 2.3. Chloropigment concentration (chlor *a* wt. equiv. $\pm$ SD of total) in laboratory algae (*Thalassiosira weissflogii*) extracts using A) the TD10 fluorometer and B) HPLC. Legend: chlorophyll (chlorophyll *a*-types), total phaeo (all chlorophyll *a*-derived phaeopigments).

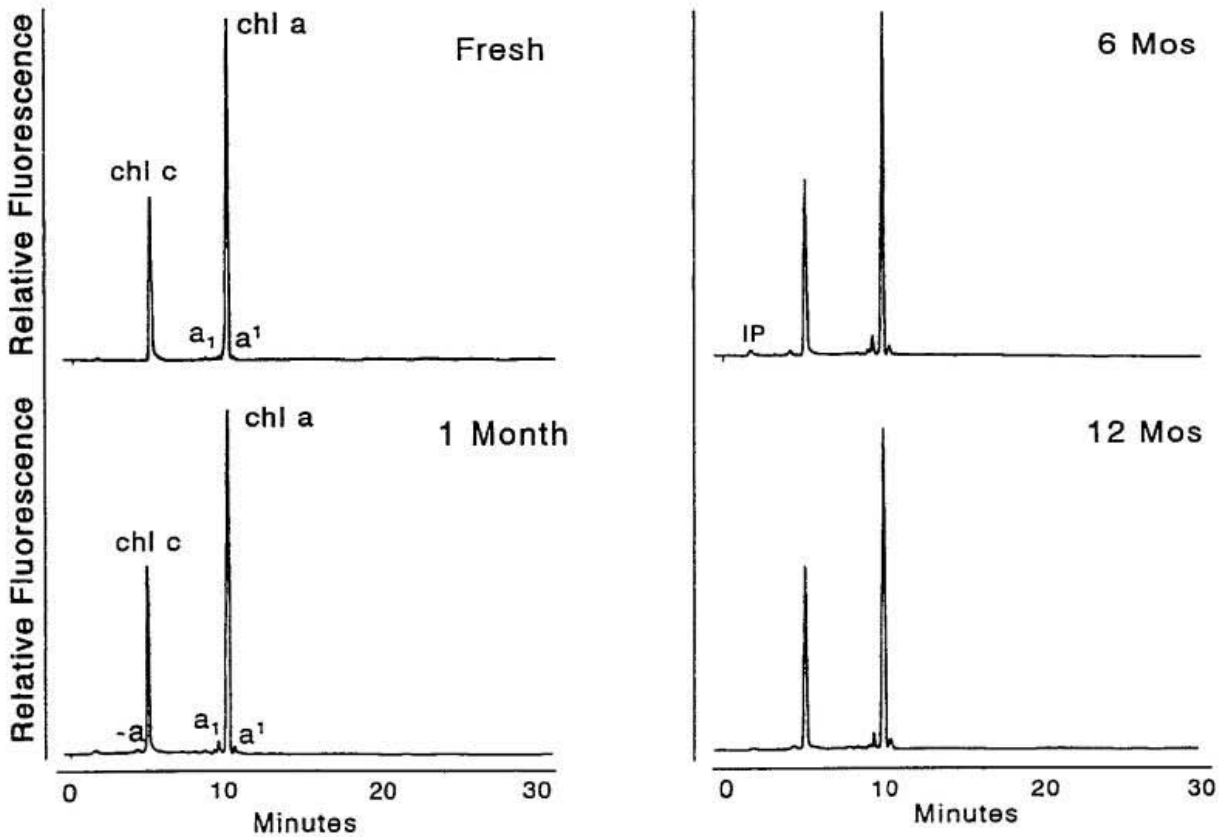

Figure 2.4. HPLC chloropigment profiles of *Thalassiosira weissflogii* extracted fresh and following 1 month, 6 mos and 12 mos of storage at -20°C. Peak identities: chl c (chlorophyll *c*), chl a (chlorophyll *a*), a_1 (chl *a* allomers), a^1 (C-10 epimer), -a (chlorophyllide *a*), IP (injection peak). Identical scaling of Y axes.

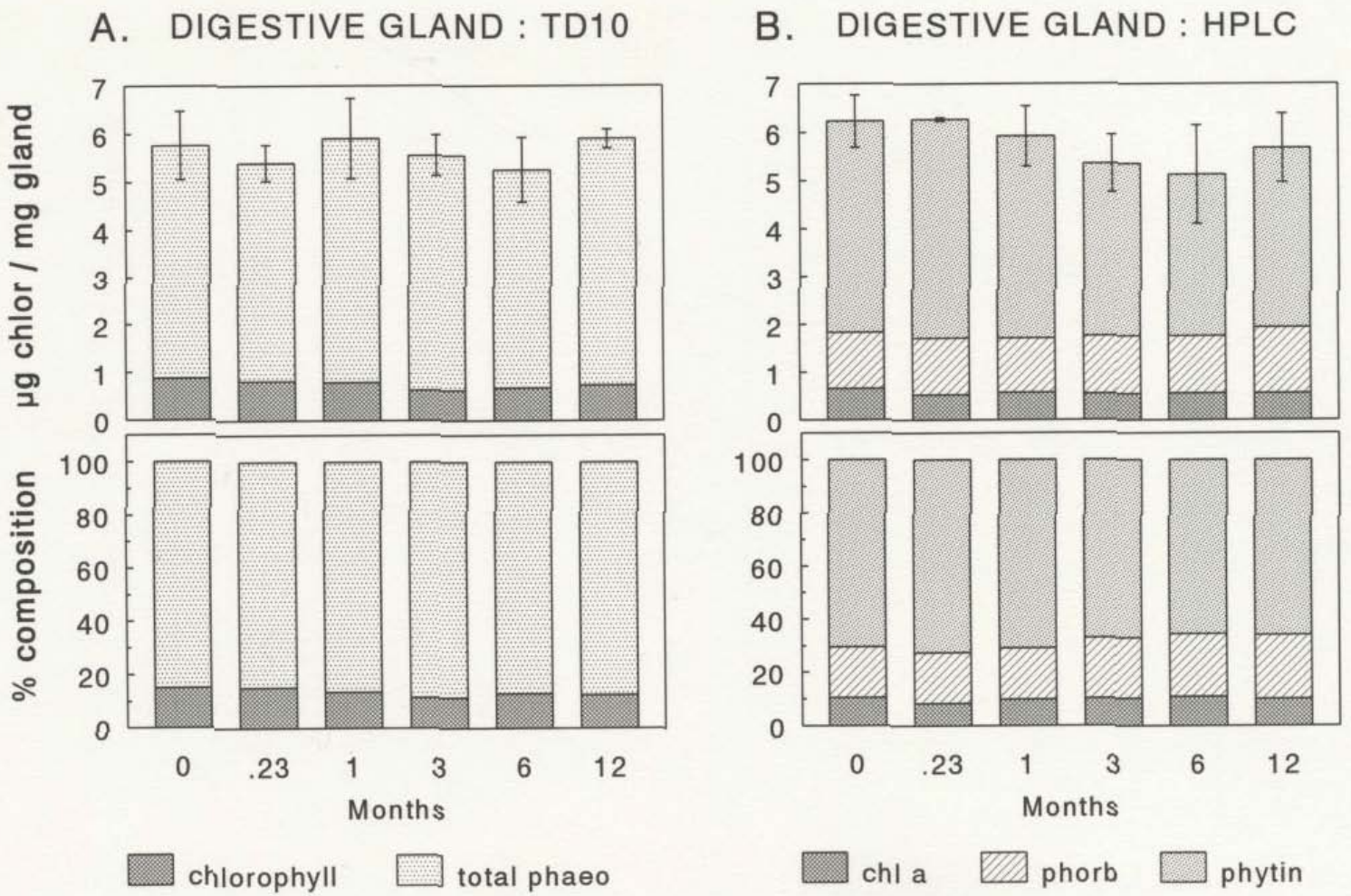

Figure 2.5. Chloropigment concentration (chlor *a* wt. equiv. $\pm$ SD of total) and percent composition in mussel digestive gland extracts using A) the TD10 fluorometer and B) HPLC. Legend: as in Fig. 2.3 plus chlor (chlorophyll *a*-types), phorb (phaeophorbide *a*-types), phytin (phaeophytin *a*-types).

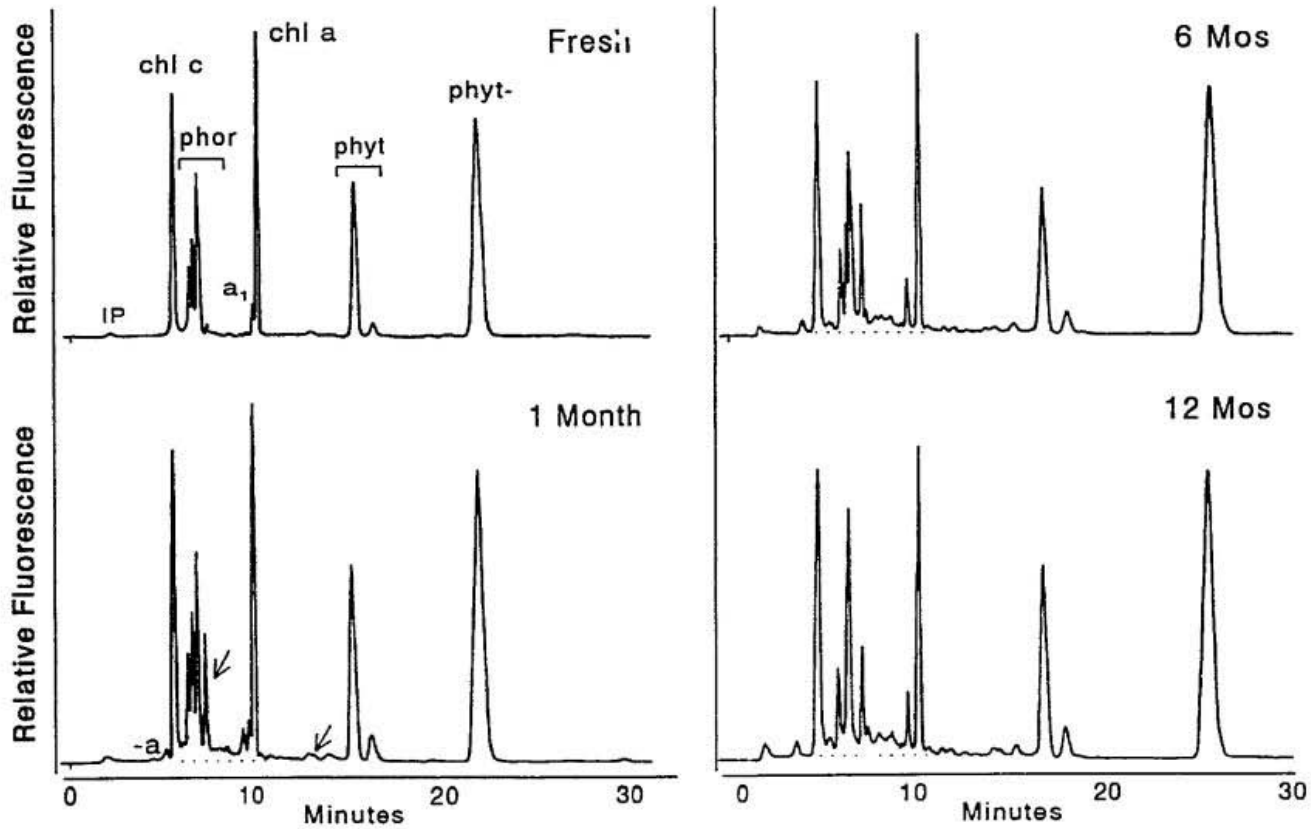

Figure 2.6. HPLC chloropigment profiles of mussel digestive gland extracted fresh and following 1 month. 6 mos and 12 mos of storage at -20°C. Peak identities as in Fig 2.4 plus phor (phaeophorbides *a* and *c*). phyt (phaeophytin *a*). phyt- (pyrophaeophytin *a*-like). Arrows point to unidentified peaks. Dotted line represents baseline. Identical scaling of Y axes.

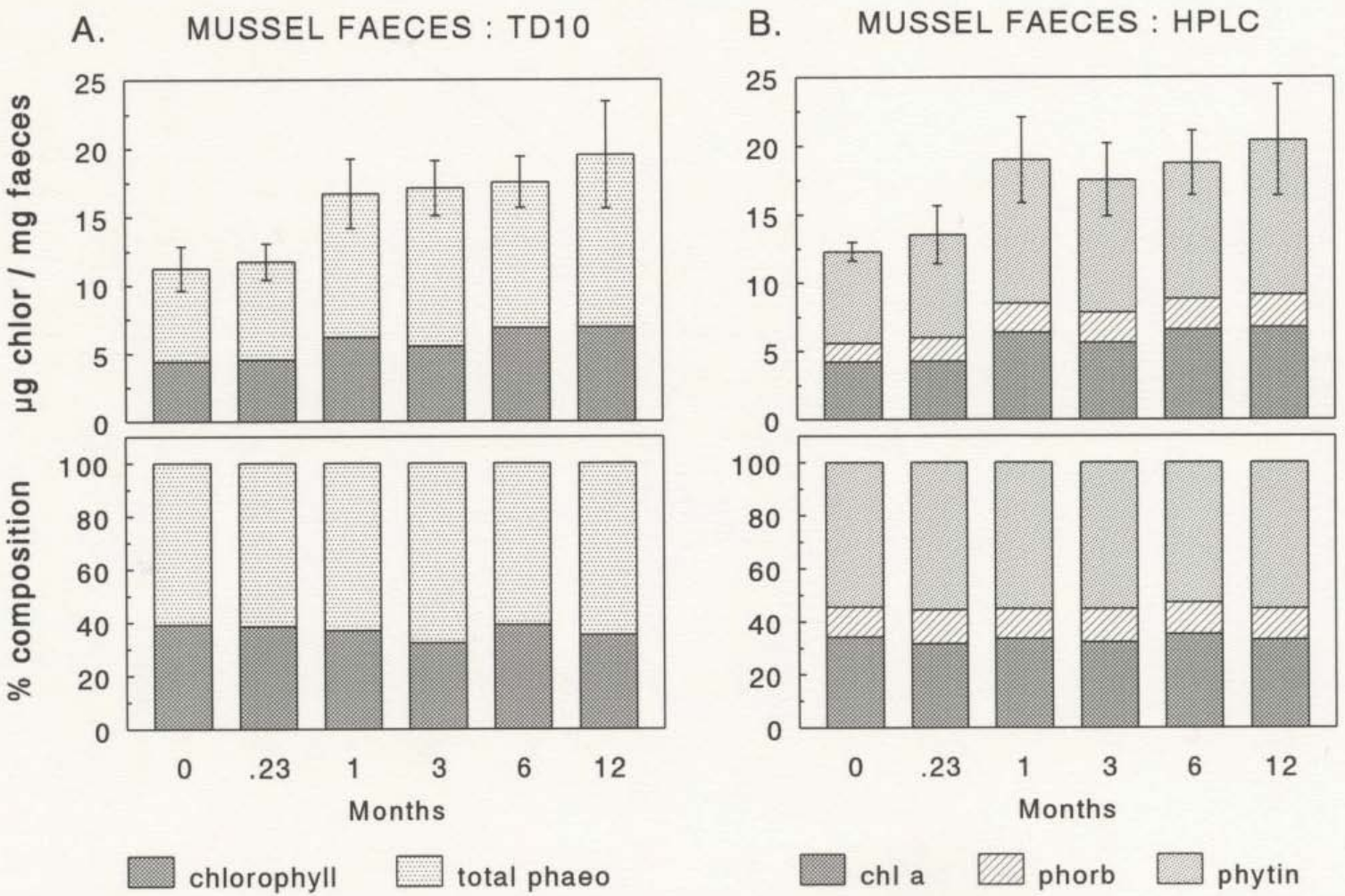

Figure 2.7. Chloropigment concentration (chlor *a* wt. equiv. $\pm$ SD of total) and percent composition in mussel faeces extracts using A) the TD10 fluorometer and B) HPLC. Legend as in Fig. 2.5.

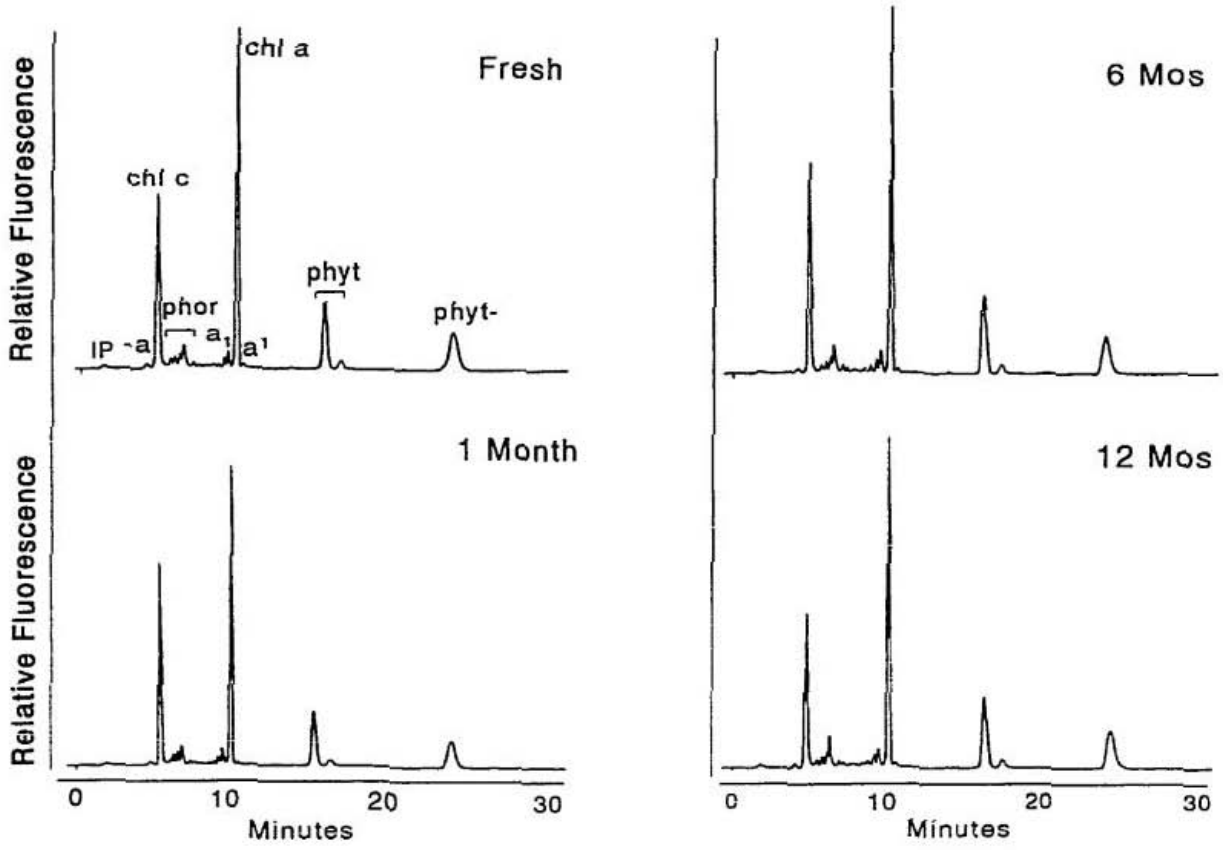

Figure 2.8. HPLC chloropigment profiles of mussel faeces extracted fresh and following 1 month, 6 mos and 12 mos of storage at -20°C. Peak identities as in Figs. 2.4 and 2.6. Identical scaling of Y axes.

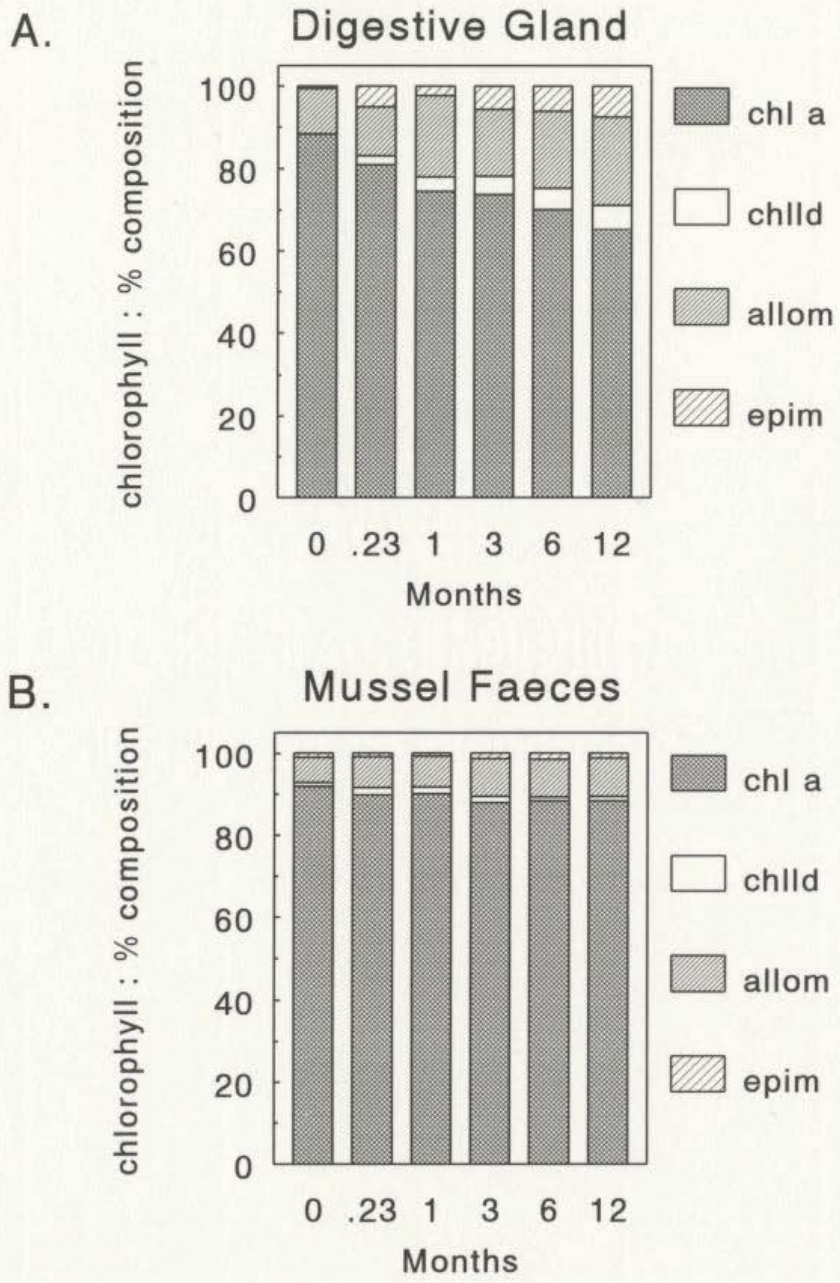

Figure 2.9. Percent composition of chlorophyll *a*-type pigments in A) mussel digestive gland and B) mussel faeces : chl a (chlorophyll *a*), chlld (chlorophyllide *a*), allom (allomers), epim (epimers).

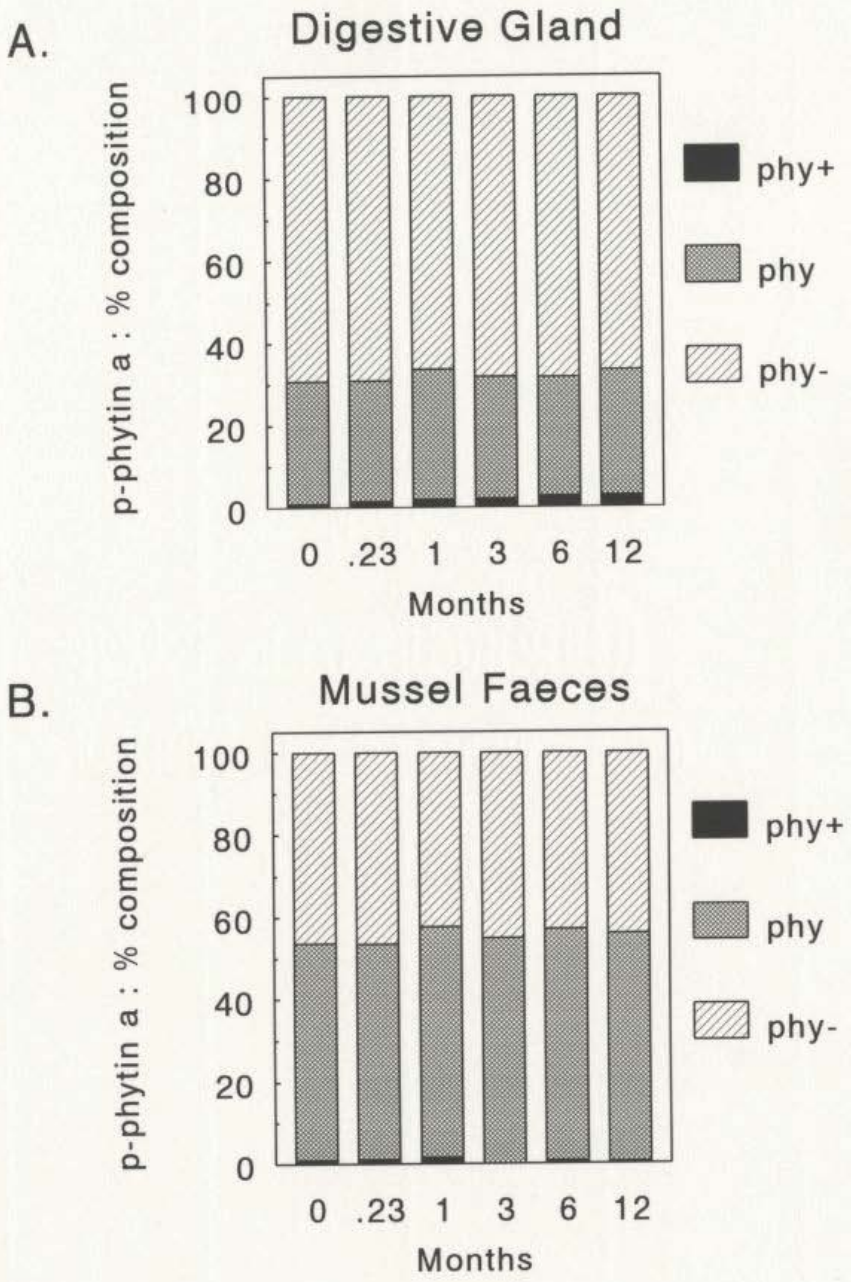

Figure 2.10. Percent composition of phaeophytin *a*-type pigments in A) mussel digestive gland and B) mussel faeces : phy (phaeophytin *a* plus isomer), phy+ (more polar types), phy- (less polar types).

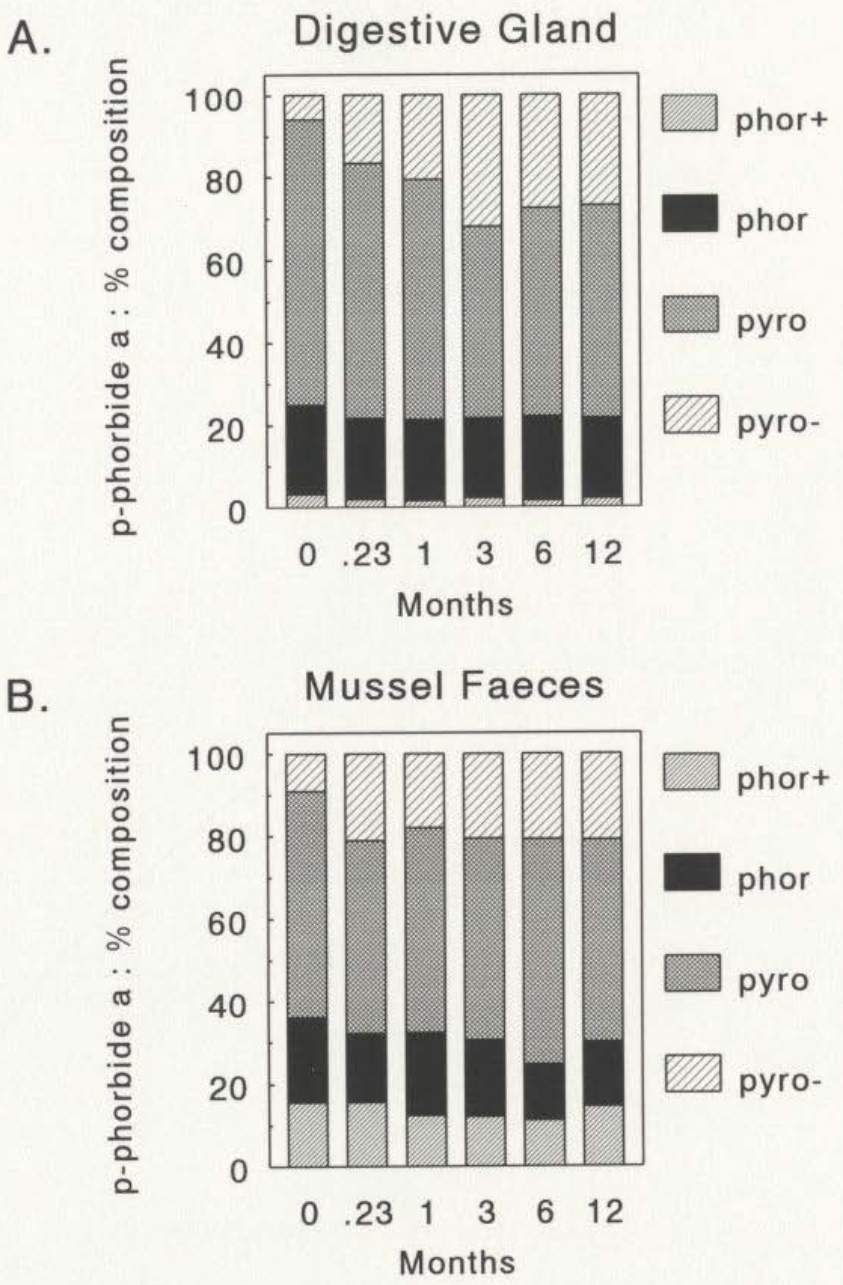

Figure 2.11. Percent composition of phaeophorbide *a*-type pigments in A) mussel digestive gland and B) mussel faeces : phor (phaeophorbide *a*), phor+ (more polar types), pyro (pyrophaeophorbide *a*), pyro- (less polar types than pyro).

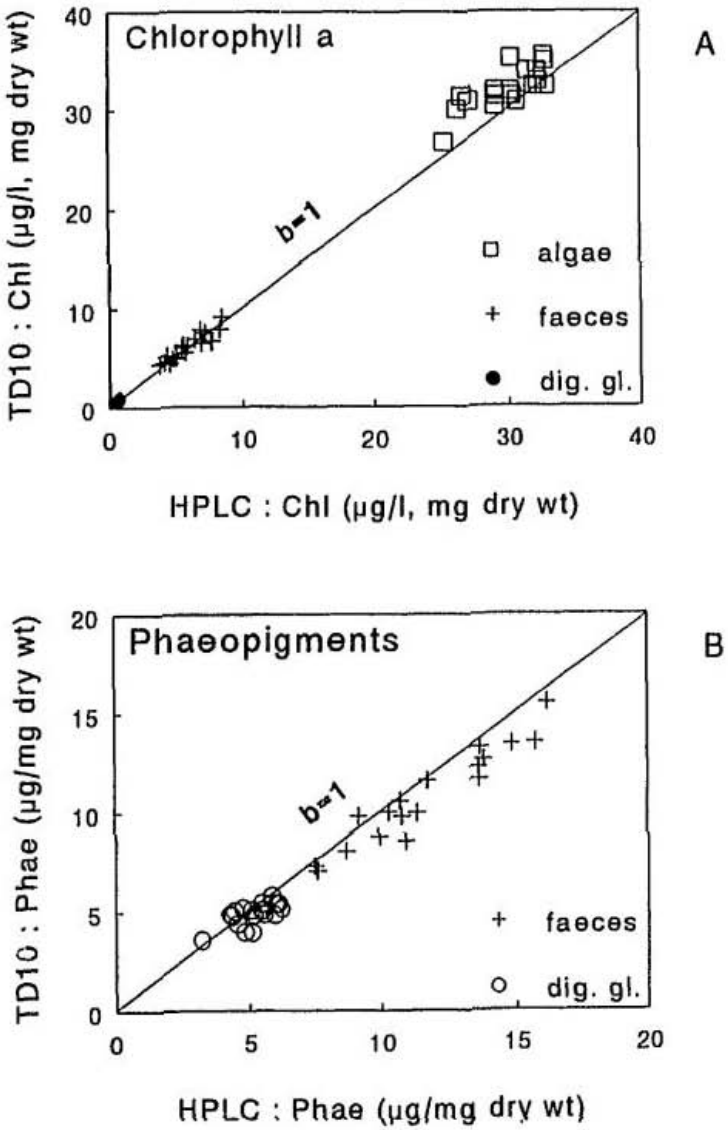

Figure 2.12. Plots of TD10 vs HPLC estimates of A) chlorophyll *a*-type pigments and B) phaeopigments in laboratory algae, mussel faeces and digestive gland extracts.

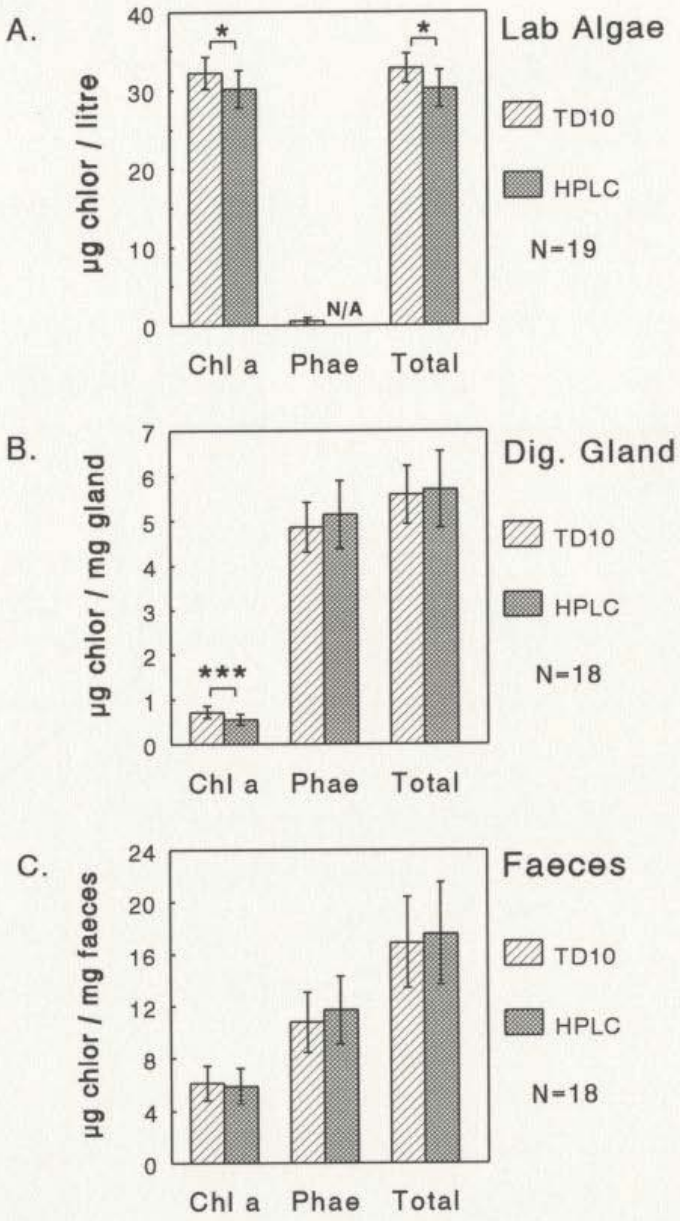

Figure 2.13. Comparative TD10 and HPLC estimates (mean $\pm$ SD) of chlorophyll *a*-type pigments, phaeopigments and total chloropigments in extracts of A) laboratory algae, B) mussel digestive gland and C) mussel faeces. ANOVA: *($p < 0.05$), ***($p < 0.001$).

Chapter 3

GRAZER-MEDIATED CHLOROPIGMENT DIAGENESIS : *Calanus finmarchicus* GRAZING EXPERIMENTS

3.1. Introduction

Zooplankton grazers are important intermediates in the transfer of energy in aquatic food webs and in the transport of phytogenous material from the photic zone to deeper waters and to the sediments (Welschmeyer and Lorenzen 1985b, Bathmann and Leibezeit 1986, Carpenter et al. 1986). The impact of zooplankton grazing and the contribution of faecal material to particle flux during the spring phytoplankton bloom are therefore of great significance to water column tropho-dynamics and pelagic-benthic coupling.

Among several techniques that have been developed to assess the rate of zooplankton grazing in coastal and marine waters, one of the most widely used is probably the gut fluorescence method of Mackas and Bohrer (1976). It was developed for the quantification of chloropigments (chlorophyll *a* and its fluorescent degradation products) in zooplankton gut tracts (Boyd et al. 1980, Dagg and Wyman 1983, Madin and Cetta 1984, Head 1986, Lopez et al. 1988, Arinardi et al. 1990), and has also been adapted for the determination of pigments in grazer faecal pellets (Downs and Lorenzen 1985, Wang and Conover 1986, Vernet and Lorenzen 1987). Ingestion rates of grazers are estimated from measurements of gut chloropigments, multiplied by an independently determined gut evacuation rate.

The simplicity and high sensitivity of the gut fluorescence technique have lead to its extensive application in the estimation of ingestion rates. It has been frequently used to estimate *in situ* grazing rates of zooplankters (e.g. Boyd et al. 1980, Kiørboe et al. 1982, Baars and Oosterhuis 1984, Hansen et al. 1990) because the method does not

require lengthy laboratory incubations which disturb feeding processes. The gut fluorescence technique, however, depends on chlorophyll *a* and its porphyrin derivatives as conservative tracers of ingested algae, and its use requires that a number of assumptions be met: 1) that all breakdown products of chlorophyll *a* are phaeopigment derivatives, 2) that these derivatives are produced with 100% molar conversion efficiency, 3) that there is no loss of chlorophyll *a* or phaeopigments to nonfluorescent residues or absorption during passage through the gut, and 4) that independently determined estimates of gut evacuation rates are representative of gut clearance rates under various feeding conditions.

There have been many laboratory observations of high and variable pigment decay to nonfluorescent products resulting from the digestive processes of various microzooplankton (SooHoo and Keifer 1982b, Klein et al. 1986, Burkill et al. 1987, Barlow et al. 1988, Strom 1993) and mesozooplankton grazers (review in Lopez et al. 1988, Downs 1989, Mayzaud and Razouls 1992). Digestive processes which cleave the porphyrin ring of the chlorophylls and their phaeopigment derivatives result in nonfluorescent products which cannot be traced by conventional pigment methodology. The fate of these nonfluorescent derivatives of chlorophyll *a*, hereinafter referred to as "lost" chloropigments, has not been well established. The phytol chain of the chlorophyll *a* molecule has been detected in copepod faeces (Prahl et al. 1984a,b), and it is probable that phytol and some of the other nonfluorescent degradation products of grazing are partially absorbed by epithelial cells in the gut wall of copepod grazers. Absorption of ingested chloropigments has been indicated in studies of the blue mussel, *Mytilus edulis* (Gelder and Robinson 1980, Hawkins et al. 1986) and the scallop *Placopecten magellanicus* (Robinson et al. 1989).

Application of the gut fluorescence method to estimate ingestion rates of herbivores has become increasingly controversial, with a growing number of reports of grazer-mediated chloropigment loss in laboratory feeding studies (e.g. Conover et al.

1986, Lopez et al. 1988, Head and Harris 1992). If chloropigments are lost to nonfluorescent products within zooplankter gut tracts, then ingestion rates determined using the method will be underestimated. Variability in chloropigment digestion efficiency within and among grazer types will also be high if grazers differ in their ability to degrade phytopigments. Pigment losses in the gut tracts of grazers preclude the use of chloropigment measurements to estimate *in situ* ingestion rates unless rates of chloropigment loss are consistent for a grazer species or taxonomic group.

Reports of chloropigment losses to nondetectable products in grazing experiments have been highly variable, ranging from 0% (Pasternak and Drits 1988) to as high as 99% (Conover et al. 1986) of ingested chlorophyll. Most reports of pigment loss, however, have indicated losses in the range of 20-40% of ingested chlorophyll *a* (Helling and Baars 1985, Dagg and Walser 1987, Head 1988, Downs 1989). It has been suggested that some of the observed losses result from the passage of pigments through glass fiber filters during the collection of particulate matter containing small, pigment-rich particles, formed from the effects of "sloppy feeding" (Roy et al. 1989) and faecal pellet fragmentation (Kiørboe and Tiselius 1987). Although chloropigments may be associated with particles smaller than the minimal retention of a GF/C filter (about 1.0 μm), the amount of pigment leakage during filtration under low vacuum is probably low. Pigment losses in short-term copepod grazing experiments have indicated some chloropigment transformation to nonfluorescent material prior to faecal pellet release (Lopez et al. 1988, Mayzaud and Razouls 1992). Digestion efficiencies and gut passage times which, in some copepods, are known to vary with both food concentration (Dagg and Walser 1987) and temperature (Kiørboe et al. 1982, Dagg and Wyman 1983, Dam and Peterson 1988), are primary determinants of the extent to which chlorophyll *a* degrades to phaeopigments and to nonfluorescent derivatives. Interestingly, most of the grazing studies which have examined the degree to which chloropigments are lost to nonfluorescent products were conducted using cultured algae, not natural phytoplankton assemblages. Head (1988), Lopez et al. (1988), Penry and Frost (1991) and Mayzaud and Razouls (1992) emphasize

the importance of feeding history and associated digestive acclimation processes in influencing the extent of pigment decay in copepod gut tracts. Grazing experiments conducted in close temporal succession, with natural particle suspensions, may thus be most appropriate for the examination of pigment losses resulting from the digestive processes of grazers.

The following study was conducted to examine *Calanus finmarchicus* ingestion rates, and the chlorophyll derivatives produced during grazing, in experiments with natural seston assemblages collected during the spring phytoplankton bloom in cold, coastal waters of Conception Bay, Newfoundland. *C. finmarchicus* is the most abundant of the large herbivorous copepods in these waters and has a diet which reflects the seasonal succession and relative abundance of phytoplankton (Urban et al. 1992). During the spring, *C. finmarchicus* faecal pellets contain primarily centric, chain-forming diatoms, with reported sinking velocities of 7-35 m/d (Urban 1992). Copepod faecal pellets are abundant in sediment traps during the decline of the spring diatom bloom (see Chapter 6), and represent an important contribution to the vertical flux of primary production and phytopigments. In addition to the examination of *C. finmarchicus* ingestion rates, this study investigates the suitability of applying chloropigment analyses to follow *Calanus* grazing events, qualitatively and quantitatively, during a period of high phytoplankton production.

The primary objectives of this study were:

1. To determine the effect of food concentration on the ingestion rate of adult *Calanus finmarchicus*. The study includes an examination of particle clearance rates, chlorophyll *a* removal, and pellet production rates following incubations of copepods with a range of natural seston concentrations at near-ambient water temperature (0-1°C).
2. To examine the degradation products of chlorophyll *a* and losses, if any, to nonfluorescent products resulting from the digestive processes of *C. finmarchicus* in

incubations with a range of natural seston concentrations. Biogenic silica is used as an inert marker as it is not absorbed during gut passage (Tande and Slagstad 1985).

3. To determine grazer-mediated chloropigment loss, if any, using ratios of particulate silica and chloropigments in food and faecal pellets of *Calanus*, following incubation with various cell concentrations of laboratory-reared diatoms, *Thalassiosira weissflogii*.

4. To investigate the potential for chloropigment loss during filtration of samples containing fragmented *Calanus* faecal pellets.

3.2. Methods

3.2.1. General Procedures

All grazing experiments were conducted in the laboratory with adult female *Calanus finmarchicus* fed with natural seston or cultured diatom food sources. Copepods and seston were collected during the 1990 spring phytoplankton bloom in Conception Bay, Newfoundland (47°32'N, 53°08'W). Adult females were selected for grazing studies because they are abundant during spring, larger than adult males and late stage juveniles, and easily identified. Furthermore, adult female *Calanus* are also known to be more active grazers than adult males (Marshall and Orr 1955, Mullin 1963). Copepods were captured using a 0.5 m diameter plankton net fitted with 333 μm Nitex mesh and a closed cod-end cup. The net was hauled vertically from 0-200 m at a rate of about 0.5 m per sec. After the net was retrieved, the cod-end contents were immediately passed through a 2 mm mesh to remove large zooplankton (amphipods, chaetognaths, gelatinous zooplankton, etc.). The remaining suspension was diluted in a 20 l bucket with seawater. Large copepods were then concentrated in a 2 l container fitted with a 333 μm Nitex mesh bottom and transferred by beaker to 20 l buckets containing seston collected from the subsurface chlorophyll maximum (SCM). Additional seston for experimental purposes was collected from the SCM and stored in 20 l carboys. Containers of animals and seston

were then transported on ice to the laboratory at Logy Bay and stored in a flow-through seawater bath at near ambient water temperature (0-3°C).

Grazing experiments and subsequent handling of chloropigment samples were conducted in darkness or under dim light conditions. Temperature during all feeding studies was maintained at 0-1°C with the aid of temperature-controlled incubators or beds of crushed ice. A Zeiss dissecting microscope, fitted with a Schott KL1500 fibre-optic cold light source and red filter (Wratten gelatin filter No.25), was used for all copepod examinations (identification, gut fullness) and for faecal pellet counts and measurements (length, width). Chloropigment sample preparation and analyses (standard fluorometric method, HPLC) followed those procedures outlined in Chapter 2. During the extraction interval, gut and faecal pellet samples were sonicated for periods of 8-10 min in an ultrasonic bath cooled with crushed ice. Gut pigment levels in whole animal extracts were determined as described by Mackas and Bohrer (1976).

3.2.2. Plankton Wheel Experiments

A. Experimental Set-up

A series of four grazing experiments, varying in the concentration of natural seston used, was conducted in closed incubation bottles attached to a rotating plankton wheel. The first three experiments took place on April 21-22, April 23-24 and April 25-26, at the time of peak phytoplankton production; the fourth experiment was conducted on May 12-13 during the decline of the algal bloom. Approximately 10 adult female *Calanus finmarchicus* were incubated with natural seston in 1040 ml polycarbonate bottles for periods of 3, 6, 12 and 24 hr. All incubations commenced at approximately 1100 hrs. The amount of food offered to grazers varied among experiments and represented a range in chloropigment concentration of 1.5-10.5 μg/l: Exp 1 (8.0 μg/l), Exp 2 (1.5 μg/l), Exp 3 (10.5 μg/l) and Exp 4 (4.5 μg/l). The first 2 experiments utilized the same seston source; for the second experiment, the suspension was diluted with

filtered seawater (FSW) to create a comparatively low food environment. Exps 1, 3 and 4 used undiluted suspensions collected from the SCM. Each study was conducted within 5 days of copepod capture and seston collection. During these intervals, copepods were maintained in a 20 l bucket in a flow-through seawater bath at near ambient water temperature (0-3°C), and were fed a diet of natural seston collected from the SCM.

Immediately before each experiment, the seston collected for incubation with copepods was passed through a 120 μm Nitex mesh into a 20 l carboy to remove large particulate matter, small copepods, nauplii and other microzooplankton. The carboy was then stored on ice. Before the incubation bottles were filled, the carboy was inverted several times to create a homogenous particle suspension.

Calanus finmarchicus collections were examined microscopically, and only those adult females with healthy appearance and showing evidence of recent feeding were selected for grazing experiments. These animals were transferred to 500 ml beakers containing FSW and starved overnight at 0°C to allow for the evacuation of gut contents. Microscopic examination revealed no detectable food in the digestive tracts of starved copepods. In order to avoid the carry-over of FSW to the incubation bottles, individual copepods were removed, by pipet, from the FSW and transferred to 200 ml beakers containing the experimental seston. This was immediately followed by a second transfer of about 10 animals to each of 12 seston-filled incubation bottles. Another 4 bottles, containing seston only, served as controls.

All 16 incubation bottles were clamped to a plankton wheel apparatus (45 cm radius), housed in a covered insulated box containing about 280 l of water. Temperature was maintained at 0-1°C. Wheel rotation was regulated at a rate of about one revolution per 90 sec. At intervals of 3, 6, 12 and 24 hr, the wheel was temporarily stopped and four bottles were removed (3 with animals, 1 control).

B. Sample Collection and Analysis

Immediately following each incubation period, *C. finmarchicus* were removed from each grazing bottle by gently pouring the bottle contents into a 505 μm Nitex mesh-lined cup, nested in a plastic beaker. Control bottles were similarly treated. Only the copepods appeared to be retained on this mesh; counts confirmed the complete recovery of all *Calanus*. The copepods from each experimental bottle were immediately transferred to a 1.5 ml polypropylene vial, quick-frozen on dry ice, and stored at -20°C to await analysis of gut pigments. The transfer from grazing bottle to storage vial was conducted within 1-2 min. The beaker contents (seston, faecal pellets) were mixed by gentle stirring and subsampled in duplicate (100-150 ml each) for subsequent determinations of chloropigment and particulate silica concentrations. Samples for pigment analysis were then collected on 25 mm filters (Whatman GF/C), quick-frozen on dry ice and stored for 4 wk at -20°C. Chloropigment content was determined using the standard fluorometric method. Representative samples from each set of replicate bottles were also analyzed using HPLC. These methods are outlined in Chapter 2.

Samples of particulate silica were collected on 1.0 μm Nuclepore polycarbonate filters and frozen in 4.5 ml polypropylene tubes until analyzed. A few replicate samples were also passed through 0.2 μm Nuclepore filters for a comparison of filter retention efficiencies. Surprisingly, silica values from samples collected on the 0.2 μm filters were 10-17% lower than replicates collected on 1.0 μm filters. This finding precluded further use of the smaller pore Nuclepore filters for sample collection. Biogenic silica was determined using a slight modification of the method of Paasche (1980). Samples and blank filters were hydrolysed with 4 ml of 0.5% Na_2CO_3, loosely capped and heated for 3 hr in a waterbath at 85°C, as recommended by Navarro and Thompson (in press). The tubes were shaken once per hour. Following cooling and neutralization, dissolved silicon hydroxide concentrations were colorimetrically determined in duplicate 2 ml subsamples according to Parsons et al. (1984). This method was also used to determine the dissolved silica concentrations in the grazing and control bottles in Exps 2, 3 and 4.

Prior to each experiment, 100 ml samples of the initial seston suspensions were fixed for microscopic examination with 1 ml Lugol's iodine, followed by the addition of 2 ml of 40% formaldehyde solution. In Exps 3 and 4, 20 ml samples were removed from the control and grazing bottles following each feeding period and were similarly preserved. These samples were examined for changes in species abundance and relative composition of ungrazed seston. All counts were conducted using the Utermöhl method and a Zeiss Axiovert 35 microscope.

Particle volume was measured in 300-400 ml samples from each incubation bottle using a Coulter Counter Model TAII equipped with a 200 μm orifice tube. These measurements were used to calculate the change in particle concentration over time in control and grazing chambers. Clearance rates were estimated using the exponential decay equations of Frost (1972). Samples from the experimental bottles, however, contained both ungrazed seston and *C. finmarchicus* faeces. To examine the effect of pellet passage through a 200 μm opening, concentrated suspensions of faecal pellets in FSW (>2 pellets/ml) were prepared and passed through the 200 μm orifice. An elevated response in those channels representing particles with equivalent spherical diameters (ESD) of >50 μm was observed. Channels used in the calculation of clearance rates were therefore limited to those representing particles in the range of 5-40 μm ESD. Although copepod faecal pellets are cylindrical, the shape factor is minimal, producing only a 1-2% error in volume estimated using the TAII (R. Sheldon, pers. comm.). Microscopic examination of the faecal pellets of adult female *C. finmarchicus* in the plankton wheel experiments revealed a length-to-width ratio of 5-7 and pellet diameters in the range of 55-140 μm.

Calanus faecal pellet production was determined from pellet counts in 100-200 ml samples fixed in 4-5% formaldehyde. Only whole pellets and those appearing to be more than 50% intact were enumerated.

3.2.3. Gut Pigment Evacuation Rate

A series of three laboratory experiments was conducted to determine the rate of chloropigment evacuation by the gut of *Calanus finmarchicus* at near ambient water temperature (0°C). Copepods were fed natural seston suspensions collected from the subsurface chlorophyll maximum, and those adult females with gut tracts >2/3 full were individually transferred by pipet to incubation chambers filled with FSW. A total of 5-10 copepods were incubated in each container. The transfer of copepods to each incubation chamber was conducted within 1-2 min. The chambers were of PVC construction (15 cm high, 7.5 cm diam; 650 ml volume) with a bottom mesh of 505 μm, nested in 1 l plastic beakers set in a bed of crushed ice. Temperature in the incubation chambers was maintained at 0-1°C. Following incubation intervals of 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90 and 150 min, the PVC chambers were removed from the beakers, and the mesh contents backwashed into a petri dish with FSW. All of the copepods from each chamber were immediately transferred to a 1.5 ml polypropylene tube and frozen at -20°C. Faecal pellets released during these intervals were collected on a 25 μm Nitex mesh, counted under red light and backwashed with FSW onto GF/C filters for pigment analysis. Chloropigmen s in both copepod gut tracts and faecal pellets were quantified using the standard fluorometric method and HPLC. Calculations of percent chloropigment evacuated were determined at each time interval by dividing gut chloropigment measurements by the summed measurements of gut and faecal chloropigments.

Numerous studies have shown an exponential decline in the gut contents of both fish (review in Elliott and Persson 1978) and copepods (Mackas and Bohrer 1976, Dagg and Grill 1980, Dagg and Wyman 1983, and others). Gut clearance rates of *Calanus finmarchicus* were therefore determined using the following exponential equation:

$$C_t = C_o e^{-kt}$$

where C_o = initial level of gut pigments, C_t = level of gut pigments at time t, k = instantaneous gut evacuation rate with units of 1/t. Estimates of gut evacuation rate were determined using 2 methods: gut chloropigment measurements (ng/copepod), and the percentage of chloropigments remaining in the gut following incubation. This latter method assumes no decay of chlorophyllous material during pellet formation (i.e. gut measurements of faecal pigments and gut pigments are comparable). It also assumes full recovery of egested pigment.

In a fourth gut clearance experiment, 20 adult female *C. finmarchicus* were fed a diet of laboratory cultured diatoms, *Thalassiosira weissflogii*. Faecal pellets produced by these copepods were examined for chloropigment content and compared to pigment levels in pellets produced by copepods feeding on natural seston.

Several attempts were made to determine gut pigment evacuation rate in continuously feeding copepods. Animals with full guts were transferred to nonchlorophyllous food suspensions (starch, milk powder, and ground freeze-dried zooplankton). All attempts, however, were unsuccessful. *C. finmarchicus* showed no evidence of grazing on non-phytogenous material immediately following a diet of natural seston.

3.2.4. Chloropigment Loss : Silica/Pigment Method

Adult female *Calanus finmarchicus* were incubated with various concentrations of cultured diatoms, *Thalassiosira weissflogii*. Suspensions were made by diluting the diatom culture with an appropriate volume of GF/C filtered seawater. Cell densities, determined with a Coulter Counter Model Z_F, ranged from 5.5-28 x 10^6 cells/l. Measurements of extracted chloropigments from these suspensions indicated a range in concentration of 3-16 μg chlorophyll *a*/l. The efficiency with which pigments were converted to nonfluorescent products was determined from the following equation:

$$\% \text{ chloropigment "lost"} = [1 - (S/C_{food} \div S/C_{faeces})] \times 100$$

where S = particulate silica concentration, and C = chloropigment concentration. Assumptions of the equation are 1) that particulate silica is not absorbed in the gut tract of copepods (Tande and Slagstad 1985), 2) that filtered algal cells do not contain significant amounts of silica in dissolved form, and 3) that both chloropigments and particulate silica are retained on GF/C and 1.0 μm Nuclepore filters with 100% efficiency.

In one set of experiments, chloropigment and biogenic silica analyses were conducted on separate samples of faecal pellets produced by individual copepods. *C. finmarchicus* were prefed a diet of *T. weissflogii* (about 4-6 μg chl/l) for >6 hr and then individually incubated in 1 l beakers with various cell concentrations for 18-24 hours at 0°C. Copepods were removed by pipet and the faecal pellets were collected on a 25 μm mesh sieve, rinsed and backwashed into a petri dish with FSW. The petri dish contents were then divided into 2 subsamples, one for chloropigment analysis and the other for the determination of biogenic silica. The pellets in each subsample were counted and individual pellet lengths were recorded. Though pellet width varied with copepod size, the widths of pellets produced by individual copepods were uniform. The lengths of all pellets in each subsample were summed and then corrected to take into account differences in total pellet length between pigment and silica samples. Three 50 ml replicates of the algal suspension in each experimental set-up were also removed for determination of the chloropigment: silica ratio in the diatom food source. Chemical analyses were conducted as described in Chapter 2 for chloropigments and Section 3.2.1 for biogenic silica.

In another series of experiments, grazer-induced loss of chloropigments to nonfluorescent material was again examined by analysis of both chloropigments and biogenic silica, but this time both measurements were made on the same samples of

faecal pellets and filtered algae. Samples were collected on 1.0 μm Nuclepore filters and frozen in 4.5 ml polypropylene tubes at -20°C. Following pigment extraction and centrifugation, a 3 ml volume of extract was removed and chloropigment content was determined using the standard fluorometric method. The remaining sample material was dried at 40°C and stored at -20°C until further analyzed for particulate silica content.

3.2.5. Faeces: Chloropigment Loss During Filtration

The efficiency with which chloropigments in copepod faeces are retained on GF/C filters was examined in 12 samples of physically-ruptured copepod faeces, suspended in FSW. Intact pellets, produced by *Calanus finmarchicus* feeding on laboratory-reared diatoms, *Chaetoceros mulleri*, were removed by pipet, collected on a 53 μm mesh and gently rinsed with FSW. They were then suspended in 0.2 μm FSW and passed through a 333 μm mesh sieve to induce pellet fragmentation. Twelve, 40 ml subsamples of the pellet suspension were removed and individually filtered under low vacuum (5-7 mm Hg) onto GF/C filters (about 1 μm pore size). The filtrate from each sample was then passed through a 0.2 μm Anapore filter. Six of the Anapore filters were immediately examined with a Zeiss Axiovert 35 epifluorescence microscope for the appearance of retained fluorescent material. The remaining 6 Anapore filters were extracted in 90% acetone for 24 hr at 0°C and examined for chloropigment content using the standard fluorometric method. Considering the hydrophobic nature of chloroplasts and the insolubility of phaeopigments in water, chloropigment passage through 0.2 μm filters was probably negligible.

3.3. Results

3.3.1. Seston Composition and Particle Clearance

Natural microphytoplankton assemblages used in all four plankton wheel grazing experiments were composed almost entirely of chain-forming, centric diatoms (Table

3.1). Seston collected in April, during the peak of the phytoplankton bloom, was dominated by *Skeletonema costatum*, *Chaetoceros socialis*, *C. debelis* and *Thalassiosira nordenskiöldii*. These four species comprised >80% of algal cells initially available to grazers in Exps 1, 2 and 3. Seston used in the fourth experiment was collected in May, during the decline of the bloom, and was composed primarily of *C. socialis*, *C. debilis* and *T. nordenskiöldii* cells as well as numerous *Rhizosolenia hebata*; *Skeletonema costatum* was absent from the May 7th collection.

Cell counts of the dominant phytoplankton taxa in grazing bottles following 3, 6 and 12 hr of incubation in Exps 3 and 4 are shown in Table 3.2. In Exp 3, *Calanus finmarchicus* grazed primarily on the most abundant species, *Skeletonema costatum*, removing 76% and 95% of *S. costatum* cells following 3 and 6 hr of incubation, respectively. As *S. costatum* cells declined in abundance, the setae-forming diatoms, *Chaetoceros* spp. and *Thalassiosira nordenskiöldii*, comprised a greater portion of the diet. There was no apparent selection for any particular phytoplankton species in the absence of *S. costatum* (Exp 4). Interestingly, microscopic examination revealed the presence of mucous masses in each of the grazing bottle samples. The source of this mucous is unknown, but it may originate from diatom cell sap released upon cell capture, and/or copepod secretions from glands located in the wall of the labrum (Mayzaud 1986). It is assumed that the observed mucous masses did not affect Coulter Counts.

Coulter Counter analyses of particle spectra indicated that *Calanus finmarchicus* ingested particles in the range of 8-40 μm ESD in all grazing bottles in each experiment. During the initial 3 hr of grazing, mean clearance rate decreased with increasing initial chloropigment concentration (Figure 3.1A). Daily estimates of water volume swept clear per copepod were highest within the first 3 hr of incubation at the lowest food level (diluted seston, 1.5 μg chl/l). Clearance rate declined following 12 hr of incubation at low seston levels but remained relatively constant with time at higher algal concentrations, even though cell concentrations were not maintained at a steady state.

Throughout the 24 hr incubation period, approximately 40-50 ml of water were cleared per *Calanus* per day in bottle incubations with high initial seston chloropigment concentrations (8.0 and 10.5 μg chl/l). In bottles provided with half as much food (4.5 μg chl/l), about 80-100 ml were cleared per day.

Because food ration within the grazing bottles was not constant throughout incubation, direct estimates of ingestion could not be obtained. Ingestion rates were therefore indirectly estimated using particle clearance rate data and the average chloropigment concentrations of available seston during each grazing interval (3, 6, 12 and 24 hr), in the following equation:

$$IR = CR \times [(F_C + F_{\Delta t})/2]$$

where IR = ingestion rate (ng chl *a* cop^{-1} d^{-1}), CR = clearance rate (ml cop^{-1} d^{-1}), F_C = food concentration (ng chl *a*/ml) in the control bottles, and $F_{\Delta t}$ = food concentration in the grazing bottles after time = t (hr). This method, however, assumes a constant rate of ingestion during each incubation interval. Further limitations of using average concentrations are discussed in detail in Marin et al. (1986) and McClatchie and Lewis (1986). Estimates of chloropigments ingested per copepod per day are shown for each experiment and grazing interval in Figure 3.1B. Ingestion rates in bottles with diluted seston (1.5 μg chl/l) were considerably lower than those at all higher seston concentrations. In the experiments with undiluted phytoplankton concentrations (4.5, 8.0 and 10.5 μg chl/l), mean ingestion rates during the first 6 hr of incubation were not significantly different ($P > 0.70$); estimates of daily ingestion were in the range of 350-500 ng chloropigment per copepod per day. At the highest food level, no significant differences in ingestion rate were shown with duration of incubation ($P > 0.70$), indicating food saturation throughout the 24 hr incubation period in bottles with initial seston levels of 10.5 μg chl/l.

3.3.2. Gut Fluorescence Method

C. finmarchicus gut chloropigment content varied with both initial seston chloropigment concentration and duration of incubation (Figure 3.2A). Variability between replicate bottles was also high. The greatest gut pigment values were observed in those copepods incubated at the highest algal concentration (10.5 μg chl/l); the maximum gut pigment, estimated from a collection of 10 animals, was 23 ng chloropigment per copepod. The lowest gut pigment levels were measured in those copepods provided with the least amount of food. Ingestion at low food levels (initial conc. = 1.5 μg chl/l) appeared to be negligible throughout most of the incubation. In each experiment, average gut chloropigment content was highest in removals within the first 6 hr of grazing. Thereafter, gut pigment levels declined, presumably due to a reduction in food ration with prolonged incubation. Other factors which may account for the observed reduction in gut pigment content following an initial period of grazing are diel or intermittent feeding (discussed in Chapter 5), and degradation of ingested pigments to nonfluorescent products. The latter possibility is examined in Section 3.3.6.

Gut pigment levels plotted against the concentration of chlorophyll *a* remaining in the grazing bottles following each incubation (Figure 3.2B) show a 5-fold range in gut chloropigments at low to high food concentrations. Though gut content generally increased with an increase in food concentration, there was no apparent relationship between *C. finmarchicus* gut chloropigment levels and food concentration within the range of 3-7 μg chl *a*/l. This suggests food saturation at seston levels of about 3 μg chl/l, and is supported by ingestion rate estimates at food concentrations $>$ 1.5 μg chl/l (Figure 3.1B).

Fluorometric measurements indicated that chlorophyll *a* comprised approximately 10% of total gut chloropigments in all incubations. HPLC determinations, however,

revealed more complete pigment degradation, with chlorophyll *a* representing only 1-5% of gut chloropigments in all 4 studies.

Gut evacuation rate determinations, following transfer of previously fed adult female *C. finmarchicus* to FSW, are shown in Figure 3.3. The amount of gut pigment, as a percentage of the sum of gut and egested chloropigments (Figure 3.3A) declined exponentially with time and shows a defaecation rate constant (k) identical to that produced using gut pigment data only (Figure 3.3B). This indicates full or near full recovery of faecal material released during incubation. The variance (r^2) was also reduced when gut pigment levels were normalized by conversion to percentage values. About 20% of gut chloropigments were evacuated within the first 10 min, and coincided with the recovery of about 1 faecal pellet per animal. Approximately 30% of pigments were evacuated following 15 min of incubation and about 50% of initial chloropigments were evacuated within 30 min. Thereafter, rate of evacuation declined, presumably due to non-feeding activity. Copepods removed after 90 min and 150 min of incubation had retained small amounts of pigment in the gut (<20% of initial pigments). Only gut contents >15% of initial content were used in the calculations of gut evacuation rate constant, as recommended by Kiørboe and Tiselius (1987), who found no difference in the gut clearance rates of feeding and nonfeeding copepods when 80-90% of the gut content was cleared. The defecation rate constant (k) of 0.020 min^{-1} (1.2 hr^{-1}) determined in this study is comparable to the gut evacuation rate constants reported for Stage V *C. finmarchicus* at -1°C (Tande and Båmstedt 1985), for *C. glacialis* females at -1°C (Hansen et al. 1990) and for *C. hyperboreus* at temperatures between -0.5 and 2°C (Head et al. 1988).

In a separate experiment, and following a diet of *T. weissflogii*, 20 *C. finmarchicus*, with full gut tracts, released 84% of all pellets produced within 60 min of copepod transfer to FSW. Both gut and faecal pigment measurements and pellet production data suggest a gut evacuation period of about 1 hr for *C. finmarchicus* adult females at 0°C.

Ingestion rates were calculated as the product of mean gut fluorescence (Figure 3.2) and the independently determined gut evacuation rate (0.020 min^{-1}). Estimates at 3 and 6 hr of incubation indicate the ingestion of about 300, 240 and 400 ng chloropigment per *Calanus* per day with seston levels of 4.5, 8.0 and 10.5 μg chl/l, respectively. Within the first 12 hr of incubation and at the 3 highest initial food concentrations, the gut fluorescence method provided ingestion rate estimates representing 51-99% (mean 79%) of daily ingestion calculated using particle clearance rate data and chloropigment measurements (Figure 3.1B). At the lowest food level (1.5 μg chl/l), ingestion rate estimates were only 23-42% of those shown in Figure 3.1B.

3.3.3. *Calanus* Faecal Pellet Production

Faecal pellet counts in samples removed from the plankton wheel grazing bottles show increasing pellet production per copepod with an increase in initial seston chloropigment concentration (Figure 3.4A). All pellet production rate data were adjusted for an initial gut filling time of 1 hr. Only counts of >5 pellets per 100 ml sample were plotted. At high food levels (10.5 μg chl/l), there was a relatively constant production of 3-3.5 pellets per copepod per hr during the 24 hr study (Figure 3.4B). Copepods released approximately 2-2.5 pellets per individual per hr when incubated with seston containing 4.5 and 8.0 μg chl/l. At the lowest food concentration, *Calanus* produced 1.5-2 pellets per hr during the first 12 hr of incubation, after which there was little or no apparent pellet production. Approximately 50% of the available particles were removed during the initial 12 hr period. Reduced feeding activity was also indicated by determinations of clearance rates (Figure 3.1A) and gut chloropigments (Figure 3.2A).

In the gut evacuation studies, *Calanus finmarchicus* produced an average of 4 faecal pellets per full gut following diets with high concentrations of both natural seston and cultured diatoms. Pellets produced by adult females contained an average ($\pm$SD) of 6.7 ($\pm$1.7) ng chloropigment per pellet (n=25 samples) with a natural seston diet and

6.0 (±0.3) ng chloropigment per pellet (n=4 samples) when offered a diet of *Thalassiosira weissflogii*. The chloropigment content of 4 pellets was comparable to the maximum gut pigment content per copepod measured in the plankton wheel experiments (Figure 3.2). This confirms the presence of full or nearly full gut tracts at high seston concentrations (10.5 μg chl/l, Exp 3), and suggests low loss of chloropigments to nonfluorescent products during gut passage.

3.3.4. Pigment Budgets and Degradation

Total chloropigments recovered in control and replicate grazing bottles, removed at 3, 6, 12 and 24 hr in the four plankton wheel experiments, are shown in Figure 3.5. Pigments recovered from the grazing bottles represent the sum of pigments in copepod guts, faeces and ungrazed seston. With the exception of Exp 3 (panel D), total pigment recovery from the grazing bottles was lower than from the control bottles. In all experiments, there was significant degradation of chlorophyll *a* and, in 3 studies, decay extended to undetectable products. A decrease in chlorophyll *a* was associated with a marked increase in phaeopigments in all experiments except Exp 2 (panel A; 1.5 μg chl/l). Though chlorophyll *a* concentration declined throughout incubation in Exp 2, there was little accumulation of phaeopigment. The difference between total chloropigments recovered in control and grazing bottles represent "lost" chloropigments, those pigments decayed beyond fluorescence detection.

HPLC determinations of the relative composition of chloropigments in bottle samples removed before and after grazing are summarized in Figure 3.6. Following 24 hr of incubation, total phaeopigments in the grazing bottles of each experiment rose from 3-16% to 44-60% of the total chloropigment pool. In all 4 experiments, the phaeopigment fraction was dominated by pyrophaeophorbide *a*, a polar derivative of phaeophorbide *a*. It was identified by co-elution with a standard and by its absorbance spectrum. Representative chromatograms of bottle samples from Exp 4 (initial conc. =

4.5 μg/l), shown in Figure 3.7, illustrate the effect of *Calanus* grazing on chloropigment composition following 12 and 24 hr of incubation. Relative amounts of pyrophaeophorbide *a* increased markedly as grazing duration increased. Copepod digestive processes also resulted in the production of small amounts of both phaeophorbide *a* and phaeophytin *a*. Gut pigment analyses from each study confirmed the formation of predominantly pyrophaeophorbide *a* in the digestive tracts of *C. finmarchicus*. Pyrophaeophorbide *a* constituted 81-98% (mean 92%) of chloropigments in whole animal extracts.

Calanus faecal pellets produced in the grazing bottles could not be isolated from the particulate suspension for examination of chloropigment content without risk of physical damage to both pellets and ungrazed seston. The difference in levels of chlorophyll *a* in control and grazing bottles was therefore used as an indirect measure of ingested pigments. This method of calculating ingestion rate is supported by the *Calanus* grazing studies of Lopez et al. (1988) and by Coulter Counter particle analyses of bottle samples following each incubation period. Determinations of chlorophyll *a* concentration and particle volume per ml, within the control and grazing bottles of all 4 experiments, were highly correlated (r^2=0.94, n=76, Figure 3.8A). There was also a strong correlation between percent volume cleared in each bottle and percent chlorophyll *a* degraded (r^2=0.90, n=48, Figure 3.8B). A slope of 1 would be expected if *C. finmarchicus* fed nonselectively and if copepod digestive processes converted 100% of all ingested chlorophyll *a* to phaeopigments and/or nonfluorescent products. There was evidence of selective feeding on *Skeletonema costatum* cells in Exp 3 (10.5 μg chl/l) (Table 3.2), and although these cells were small (4 x 4 μm) relative to other common taxa, the effect of preferential ingestion in these experiments was probably minor. The incomplete conversion of chlorophyll *a* (up to 10%) observed in copepod gut extracts may be a more important determinant of the relationship between particle volume cleared and amounts of chlorophyll *a* degraded.

The numbers of faecal pellets produced and the amount of chlorophyll *a* degraded per grazing bottle, pooled from all experiments, were highly correlated ($r^2=0.80$, $n=33$) (Figure 3.9). Average amounts of chlorophyll *a* degraded per pellet produced, however, were significantly different among experiments ($P<0.001$) (Table 3.3). The first 3 studies, conducted during a 6 day period at the height of algal bloom production, exhibited significantly lower amounts of chlorophyll *a* degraded per pellet than the study conducted in mid-May, during the decline of the diatom bloom ($P<0.05$). The lowest levels of chlorophyll *a* degraded per pellet were found in those grazing bottles provided with the lowest food concentrations. The pellet contents in these samples appeared to be less dense than those produced at higher food levels. Amounts of chlorophyll *a* degraded per pellet in low food conditions were also considerably lower than those determined from extracted faecal pellets in the gut evacuation studies (Section 3.3.3). Mean levels of chloropigments in *Calanus* pellets produced in bottle incubations at higher food concentrations were similar to values in extracts of pellets produced with diets of both natural seston (mean 6.7 ng chl/pellet) and cultured diatoms (mean 6.0 ng chl/pellet).

Estimates of chlorophyll *a* ingested per copepod per day, inferred from determinations of chlorophyll *a* degraded (Figure 3.10), decreased significantly with duration of incubation in 3 of the 4 experiments ($P<0.001$). Amounts of chlorophyll *a* degraded per *Calanus* were significantly lower in incubations with initial seston concentrations of 1.5 μg chl/l than with all other food concentrations ($P<0.001$). Mean rates of degradation (i.e. ingestion) were not significantly different at the 3 highest food levels following incubation durations of 6 hr ($P>0.60$), 12 hr ($P>0.20$) and 24 hr ($P>0.50$). Estimates of ingestion, determined from 3 and 6 hr incubations with undiluted seston suspensions (4.5, 8.0 and 10.5 μg chl/l), suggest the removal of approximately 400-550 ng chlorophyll *a* per *C. finmarchicus* per day; as food concentration within grazing bottles declined (12 and 24 hr), this value was reduced to 300-400 ng chlorophyll *a* per animal per day. These rates are comparable to those determined using particle clearance rate data (Figure 3.1B). Pigment removal estimates at 3 and 6 hr are equivalent

to the removal of 32-44 μg C per copepod per day, based on a carbon: chlorophyll *a* ratio of 80, determined from natural seston collections used in these experiments. Carbon content of adult female *C. finmarchicus* collected during the spring phytoplankton bloom was in the range of 100-160 μg C per copepod. Based on an average body carbon weight of 130 μg C, ingestion rates were estimated to be in the range of 25-34% of *Calanus* body carbon per day.

3.3.5. Comparison of Ingestion Rate Methods

C. finmarchicus ingestion rates were calculated using 3 different methods: the gut fluorescence technique, particle removal measurements (Coulter Counter), and chlorophyll *a* removal rates. Methodological comparisons, shown in Figure 3.11, show very good agreement between ingestion rates determined using particle clearance rates and those determined using measurements of chlorophyll *a* degradation. In 3 of the 4 experiments, mean estimates from these 2 methods were consistently higher, by as much as 5 times, than estimates determined using the gut fluorescence method. The discrepancies were greatest at the lowest seston concentrations and following prolonged incubation. At high food levels (10.5 μg chl/l), ingestion rates estimated with all 3 methods were not significantly different following 6 hr ($P>0.80$), 12 hr ($P>0.40$) and 24 hr ($P>0.20$) of incubation, and indicate relatively continuous grazing with little or no loss of chloropigments to nondetectable products in the guts of *Calanus* feeding at high seston concentrations.

3.3.6. Pigment Losses To Nonfluorescent Products

Both indirect and direct methods were used to measure chloropigment loss to nonfluorescent products as a result of *C. finmarchicus* grazing. Loss was indirectly determined in closed bottle experiments with natural seston using 1) total pigment budgets and 2) ratios of particulate silica : chloropigments in samples containing both

ungrazed seston and faeces. In studies with cultured diatoms, ratios of silica : chloropigments in the food source and in copepod faecal pellets provided a direct measure of loss resulting from *Calanus* digestive processes.

A. Natural Seston Diet : Pigment Budgets and Silica/Chloropigment Ratios

Estimates of chloropigments lost to nonfluorescent products, determined using pigment budgets within closed bottle incubations, are shown in Table 3.4. Incubations at the highest initial food concentration (Exp 3, 10.5 μg chl/l) showed negligible, if any, chloropigment losses. In experiments with lower food levels, chloropigment loss was evident in each of the grazing bottles. Though initial food concentration in Exps 1, 2 and 4 varied by 5 fold, mean pigment losses following 24 hr of incubation were similar (0.56, 0.61 and 0.72 ng chloropigment, respectively). At food levels of 8.0 μg chl/l, there was no cumulative increase in pigment loss with prolonged incubation; all losses to nonfluorescent products appeared within the initial 3 hr of grazing. The first 2 experiments, conducted with different concentrations (8.0 and 1.5 μg chl/l) of the same seston source, and the fourth experiment (4.5 μg chl/l) showed no significant differences in amounts of chloropigment lost per *Calanus* following either 12 hr or 24 hr of incubation ($P > 0.10$).

An examination of chloropigment loss due to fragmentation of *Calanus* faecal pellets and subsequent passage of chlorophyllous material through 1.0 μm GF/C filters was conducted by passing the filtrate of 12 samples of ruptured faecal pellets through 0.2 μm Anapore filters. Epifluorescence microscopy revealed no evidence of fluorescent material on 4 of the 6 Anapore filters examined; the other 2 filters retained only small amounts of weakly fluorescent particles. Acetone extracts of the remaining 6 Anapore filters yielded levels of fluorescence which were less than that observed for an extract of a blank filter. Based on these observations, pigment losses due to sample filtration in the *Calanus* grazing experiments were assumed to be negligible.

Figure 3.12 shows very good agreement between estimates of percent chloropigment loss determined using a total chloropigment budget per bottle and those determined from particulate silica : chloropigment ratios in samples from control and grazing bottles ($b=1.02$, $r^2=0.95$). Differences between measurements of particulate silica recovered from control (seston only) and experimental bottles (ungrazed seston plus faeces) were $<5\%$, suggesting little, if any, contribution by phytoplankton in the form of intracellular dissolved silica. Dissolved silica values were below detection limits in both control and grazing bottles.

At low food conditions (Figure 3.13, panel A), pigment losses to undetectable products increased with prolonged incubation, with $>40\%$ of total chloropigments (about 0.6 μg) unrecovered following 24 hr. Although percent loss increased with time at the lowest seston levels, gut pigment measurements (Figure 3.2) and pellet production data (Figure 3.4) indicate reduced grazing activity following an initial 3 hr of incubation. At high food concentrations (Figure 3.13, panels C & D), chloropigment losses were relatively constant over incubation intervals of 3, 6, 12 and 24 hr. Virtually no pigment losses were observed at the highest food ration, 10.5 μg chl/l (panel D). As previously shown (Figure 3.12), there was good agreement between methods in each experiment.

Pigment loss to nonfluorescent products, as a percent of chlorophyll *a* degraded (ingested), was determined for each grazing bottle. Degraded pigments include phaeopigments and nonfluorescent residues. Because no significant losses were apparent in the experiment with the highest algal densities, only incubations with initial seston concentrations of 1.5, 4.5 and 8.0 μg chl/l were examined. Within the first 3 hr of incubation, the percent chloropigment lost in all 3 experiments represented 40-90% of chlorophyll *a* degraded (Figure 3.14). At the lowest food concentration, mean percent loss rose from 65% after 3 hr to 85% following 6 hr of incubation. Thereafter, *Calanus* grazing activity was reduced (Figures 3.1, 3.2 and 3.4) and mean percent loss was maintained at $>80\%$ of ingested pigments. At higher food concentrations, the greatest

losses of ingested chloropigments to nonfluorescent products occurred within the first 3 hr of feeding activity. Percent loss decreased from 3 hr to 6 hr, and was further reduced to 20-30% of chlorophyll *a* degraded following 24 hr of incubation. As the amount of seston grazed by *Calanus* increased, the fraction of ingested pigments lost to nondetectable products decreased. The data indicate that pigment losses to nonfluorescent residues were greatest immediately following the initial 12 hr period of starvation.

B. *Thalassiosira weissflogii* Diet : Silica/Chloropigment Ratios

Highly variable chloropigment losses to nondetectable products were observed in *Calanus* grazing experiments conducted during the post-bloom period with laboratory-reared diatoms, *Thalassiosira weissflogii*. Losses determined from ratios of particulate silica and chloropigments, measured in *T. weissflogii* cells and *C. finmarchicus* faecal pellets, are shown in Figure 3.15. Estimates of grazer-mediated chloropigment losses to nondetectable residues ranged from 20% to >90% in analyses conducted on separate samples of faecal pellets (GF/C filters for pigments and polycarbonate filters for silica, n=29) and analyses of pigment and silica performed on the same pellet samples (polycarbonate filters, n=29). Chloropigment losses determined using the 2 methods were comparable. Pooled data show a mean loss of 68.0% (SD ± 22.9%) of ingested pigment determined from measurements in the same pellet samples and a mean loss of 66.8% (SD ± 21.1%) from analyses performed on separate faecal pellet samples. There was no apparent relationship between percent chloropigment lost and *T. weissflogii* concentrations representing 3-16 μg chl/l.

Chloropigment losses observed in faecal pellets of *C. finmarchicus*, following a diet of cultured diatoms in experiments conducted during the summer months, were more variable and higher than losses determined from pigment recoveries in experiments conducted during spring with natural seston concentrations of 4.5-10.5 μg chl/l. In the latter experiments, chloropigment losses following 24 hr of incubation with undiluted spring bloom seston were in the range of 0-35% of ingested pigment (Figure 3.14).

Differences in chloropigment losses observed in these two sets of experiments may reflect seasonal changes in the feeding and digestive processes of adult *C. finmarchicus*.

3.4. Discussion

The series of plankton wheel grazing experiments in this study was designed to examine the effect of food concentration on the grazing rates of adult female *Calanus finmarchicus* and the extent of chloropigment decay resulting from copepod grazing processes. Copepods were incubated with similar taxonomic compositions of spring bloom seston and temperature was maintained at 0-1 °C to simulate *in situ* conditions. To ensure that copepods experienced similar feeding histories, experiments were conducted in close temporal succession with no change in diet following animal capture. All animals were acclimated to spring bloom levels of natural seston. Chain-forming centric diatoms, dominated by *Skeletonema costatum*, *Chaetoceros* spp. and *Thalassiosira nordenskiöldii*, were the primary components of the diet both *in situ* (Urban et al. 1992) and during experimentation.

Ingestion rates were determined from chloropigment measurements in bottle incubations, assuming that: 1) phytoplankton growth within grazing bottles was not affected by *Calanus* grazing processes (e.g. nutrient release, excretion), 2) there was no significant bacterial or protozoan-induced breakdown of faecal pellet chloropigments during the 24 hr incubation period, 3) there was no significant particle generation due to copepod grazing, and 4) copepod faecal pellets were not reingested. Although this study was conducted with the assumption that the digestive processes of copepods are not altered by the pre-starvation of animals for 12 hr, the data showed that pre-starvation effects were primarily responsible for the degradation of chloropigments to colourless products.

3.4.1. *Calanus* Grazing Rates With Natural Seston

Gut pigment levels increased in response to an increase in initial seston concentration and, within each experiment, levels were greatest during the first 3-6 hr of incubation. Starvation-enhanced feeding by *Calanus* has been previously reported by Runge (1980) and by Hassett and Landry (1988) who observed a temporary hunger response, with a feeding peak at about 2 hr and a decline in ingestion rates to steady-state conditions after 6 hr of feeding. In my study, gut pigment levels in all 4 grazing experiments were reduced following 6 hr of incubation. It is uncertain whether this was caused by satiation or by the decline in food ration as incubation continued. In the experiment with the lowest initial seston levels (1.5 μg chl/l), the maximum gut pigment level measured was 4 ng chloropigment per *Calanus*. Thereafter, gut levels declined and indicated little ingestion following 12 hr of incubation. At this time, food levels were reduced by about 50%. At all higher seston concentrations, copepods grazed at relatively greater rates throughout the 24 hr incubation period. Mean gut pigment levels at seston concentrations >3 μg chl/l were in the range of 10-17 ng chloropigment per copepod. Dagg and Walser (1986) also observed considerably higher copepod gut pigment levels at algal chlorophyll concentrations >3 μg chl/l.

Although mean gut pigment levels increased in response to increased seston concentration offered to grazers, the average gut content of *Calanus finmarchicus* in replicate bottles, each containing about 10 copepods, was highly variable in each experiment and suggests intermittent feeding by at least some copepods. Mackas and Burns (1986) observed a wide variation in the gut contents of individual *C. pacificus* feeding under uniform conditions and attributed this variation to episodic feeding periods of 1-3 hr. Lopez et al. (1988) also noted considerable variability in *C. pacificus* gut fullness within grazing experiments. Ingestion periodicity, commonly observed *in situ* in both migrating and nonmigrating copepods, appears to be a natural phenomenon of copepod feeding, even during periods of high phytoplankton production (Dagg and

Wyman 1983, Baars and Oosterhuis 1984, Christofferson and Jespersen 1986, Båmstedt 1988, Morales et al. 1990). Recently, cinematography of the feeding behaviour of adult female *C. finmarchicus* has shown high individual variation in time spent feeding at a given algal cell density and no relationship between allocation of time to feeding behaviour and food concentration (Turner et al. 1993).

If daily ingestion rates are calculated using mean gut fluorescence levels at 3 and 6 hr of grazing and a gut evacuation rate of 1.2 hr^{-1}, then adult female *C. finmarchicus* ingest about 300, 240 and 400 ng chloropigment per day at seston levels of 4.5, 8.0 and 10.5 μg chl/l, respectively. These ingestion rates are lower than those estimated from both particle clearance data (350-500 ng chl per copepod per day, Figure 3.1B) and levels of chlorophyll *a* degraded (>400 ng chl per copepod per day, Figure 3.10). Head (1988) also reported higher values for ingestion rate calculated from the rate of chlorophyll *a* disappearance than determined using the gut fluorescence method.

There was good agreement between estimates of ingestion rates determined from removal rates of particles and of chlorophyll *a* in all 4 experiments. Other copepod grazing studies have also shown that the 2 methods yield comparable determinations of ingestion rate (Helling and Baars 1985, Hargis 1977). In my study, there was no apparent increase in ingestion rate with increasing food concentration at seston levels above 3 μg chl/l. This suggests food saturation at phytoplankton concentrations equivalent to about 3 μg chl/l. Based on chlorophyll *a* removal rates within the first 6 hr of incubation, and with natural seston levels in the range of 4.0-10.5 μg chl/l, the daily rate of ingestion for adult female *C. finmarchicus* was 400-550 ng chlorophyll *a*, or approximately 32-44 μg carbon per day. This represents an ingestion rate of about 25-34% of animal body carbon per day and is higher than the estimate determined by Helling and Baars (1985), who reported the ingestion of 6-11% body carbon per day by *C. finmarchicus* adult females incubated at 7-8.5°C with natural phytoplankton concentrations of 4 μg chl/l. Adult *Calanus* feeding at even higher food levels have

shown maximum daily ingestion rates of >40% of body carbon (Paffenhöfer 1971, Frost 1972, Gamble 1978). Due to the natural feeding periodicities of *C. finmarchicus*, estimates of daily ingestion in the range of 25-34% of animal body carbon are probably realistic for copepods feeding *in situ* during spring bloom conditions at near 0°C.

Although the use of chlorophyll *a* removal rates to examine grazing is supported by highly correlated determinations of percent chlorophyll *a* degraded and percent particle volume cleared (r^2=0.90, Figure 3.8), measurements of the amount of chlorophyll *a* removed in grazing chambers may underestimate ingestion if there is not 100% conversion of chlorophyll *a* to degradation products. In this study and others (Dagg and Walser 1987, Downs 1989, Nelson 1989), gut and faecal pigment extracts have shown the presence of small amounts of undegraded chlorophyll *a* (up to 10% of total chloropigments). Ingestion rates will also be underestimated if copepod diets include previously decayed material (i.e. senescent cells, detritus, faecal material). Phaeopigments were present in the natural seston collections in each experiment and comprised up to 15% of the total chloropigment pool offered as food to *C. finmarchicus*.

Faecal pellet production rates averaged 3-3.5 pellets/hr in grazing bottles with the highest initial food levels (10.5 μg chl/l), which indicates near-continuous grazing, based on the observed mean egestion rate of 4 pellets per full gut and a gut evacuation rate of 1.2 hr^{-1} at 0°C. Rate of pellet production decreased as food concentration decreased, with 2-3 pellets produced/hr at seston concentrations of 4.5 and 8.0 μg chl/l. Fewer than 2 pellets were egested/hr at seston levels of <1.5 μg chl/l. These observations are comparable to those of Raymont and Gross (1942) and Marshall and Orr (1955) who reported pellet production rates in the range of 1-5 pellets/hr for *C. finmarchicus* adult females feeding on cultured algae.

Although a constant gut evacuation rate was applied in the estimation of ingestion rates using the gut fluorescence method, gut clearance may be highly variable depending

on food quantity. Penry and Jumars (1986) observed rapid gut passage times when copepod gut tracts became saturated with food. Under these conditions, a decrease in the duration of gut retention could effectively reduce digestive efficiency and absorption of ingested material. The apparent conservation of the fluorescent decay products of chlorophyll *a* in the grazing experiment with the highest seston concentration (10.5 μg chl/l) may be a result of a high rate of gut turnover in copepods feeding almost continuously.

When food availability is low and feeding slows or ceases, food passes through the guts of copepods more slowly (Baars and Oosterhuis 1984, Simard et al. 1985), as evidenced in this study by the retention of considerable amounts of chloropigment in the gut tracts of copepods incubated in FSW for 60 min, 90 min and up to 150 min (Figure 3.3). It is likely that prolonged gut retention would result in further transformation of chlorophyll *a* degradation products. In the grazing experiment with the lowest food concentration, there was little accumulation of phaeopigment; most of the ingested chlorophyll *a* was degraded to nondetectable, nonfluorescent residues. A longer gut retention time at low food concentration may explain the observed high losses of chlorophyll *a* to nondetectable products at seston levels ≤ 1.5 μg chl/l. Faecal pellets produced in this experiment also appeared to be loosely packaged; pellets contained, on average, about half as much fluorescent material as faecal pellets produced at higher seston concentrations. Dagg and Walser (1986) and Forsyth and James (1990) also observed a decrease in the compaction of copepod faecal pellet contents at low food levels.

3.4.2. Gut Fluorescence Technique vs Other Methods

The use of the gut fluorescence method has been encouraged by reports of little or no pigment losses in the gut tracts of grazers (Kiørboe and Tiselius 1987, Pasternak and Drits 1988) and by reports of good agreement between ingestion rates determined

from gut pigment levels and removal rates of chlorophyll *a* and food particles (Kiørboe et al. 1982, 1985). However, in the study by Kiørboe et al. (1982), copepod ingestion rates, estimated using gut pigments and particle removal rates in laboratory incubations, were not determined simultaneously. Kiørboe et al. (1985) also made indirect comparisons; ingestion rates determined from *in situ* gut pigment levels were compared to chlorophyll *a* and particle volume ingestion rates determined in laboratory incubations. Because copepod feeding activity is highly variable, it may not be appropriate to examine the reliability of the gut fluorescence method by comparing it to methods conducted with different animals, at different times, and under different conditions. Comparisons between methods which estimate feeding rate should be conducted in experiments which permit concurrent determinations of ingestion rate.

In this study, ingestion rates were determined from gut pigment levels, chlorophyll *a* removal rates and particle clearance rates in the same grazing bottles. The 3 methods were only in agreement at high food concentrations (10.5 μg chl/l, Figure 3.11); *Calanus* feeding at these seston levels was near continuous. At all lower food concentrations, the gut fluorescence method gave much lower estimates of ingestion than those determined from the removal of particles and from the disappearance of chlorophyll *a* over time. The discrepancies in the estimates of ingestion rate would be even greater if a longer gut passage time was applied in calculations of ingestion at low food levels. Determinations of daily ingestion rate may be under- or overestimated if they are based on gut pigment levels, which represent only recent grazing events, and clearance rates, which are not uniform under all conditions. Ingestion rates will also be underestimated if chloropigments are lost to nondetectable residues during gut passage.

The gut fluorescence method provides only a "snapshot" of recent ingestion (< 1 hr) and it is not appropriate for use in the calculation of daily ingestion rates unless animals are continually or near continually feeding and chloropigments are conserved, or lost at some constant rate, during gut transport. My data suggest that this method may

be applicable during phytoplankton bloom periods when food is abundant and chloropigment losses to nonfluorescent products are low.

3.4.3. Chloropigment Losses During Grazing

Although initial food concentration in Exps 1, 2 and 4 varied by 5 fold, total amounts of chloropigment lost to nonfluorescent products in each experiment were similar following 24 hr of incubation (Table 4). No pigment losses were apparent at the highest food concentration (10.5 μg chl/l), and at food levels of 8.0 μg chl/l, pigment losses were concentrated within the initial 3 hr of grazing. At lower food levels, ingestion was reduced and gut throughput time may have been longer. Decay of chloropigments to nondetectable products tended to increase with duration of incubation at low seston concentrations.

The amount of chloropigment lost, as a percent of chlorophyll *a* degraded to both fluorescent and nonfluorescent derivatives, also referred to as "pigment destruction efficiency" by Mayzaud and Razouls (1992), was >40% during the first 3 hr of incubation at seston concentrations of 1.5, 4.5 and 8.0 μg chl/l (Figure 3.14). At the lowest food level, mean percent loss of ingested pigment increased to >90% within 12 hr of incubation. In contrast, the fraction lost at higher food levels decreased with an increase in incubation interval. It appears that the high levels of pigment loss observed following 3 hr of incubation are attributable to the activated digestive processes of *Calanus* following a 12 hr period of starvation. This is supported by reports of substantial pigment losses within the first hour of grazing by copepods which were previously starved (Lopez et al. 1988, Mayzaud and Razouls 1992). It is well known that copepods feed intermittently (Båmstedt 1988, Hassett and Landry 1988, and others), and during spring bloom conditions, nonfeeding activity is probably limited to durations of much less than 12 hr. The observed high losses of up to 90% of ingested pigment during the first 3 hours of incubation are probably not representative of losses incurred *in situ*

when food is abundant. The decay of 15-35% of ingested pigment to nonfluorescent products, following 24 hr of incubation at seston levels of 8.0 μg chl/l (April 21-22, mid-bloom) and 4.5 μg chl/l (May 12-13, bloom decline), is probably more characteristic of copepods feeding during phytoplankton bloom conditions.

Kiørboe and Tiselius (1987) suggested that high pigment losses in copepod gut tracts are probably not common in nature and that losses of 10-30% of ingested pigments may be typical of most herbivorous copepods. Similar reports of chloropigment destruction in other copepod grazing studies include losses of 11% (Dagg and Walser 1987) and 10-35% (Roy et al. 1989). Pasternak and Drits (1988) observed no significant pigment losses in the guts of 3 copepod species following the initial filling of the foregut. However, losses which may have occurred due to instantaneous destruction upon ingestion were not determined. Although the digestive function of mucous, secreted from glands in the wall of the labrum of copepods, has not been established (Mayzaud 1986), it seems possible that these secretions may play an important role in the partial enzymatic destruction of phytopigments upon cell capture. Rapid initial transformation of the chlorophyll *a* molecule immediately upon ingestion was suggested in this study by the presence of only small amounts of chlorophyll *a* (< 10% of the total chloropigment pool) in the gut tracts of *Calanus* feeding at both high and low food concentrations.

Calanus showed the highest levels of chloropigment decay to nondetectable products at the lowest seston concentrations, with mean losses of 80-90% following 6, 12 and 24 hr of incubation. These losses do not appear to be due to the degradative effects of coprophagy. *Calanus* faecal pellet densities were relatively low at grazing intervals of 3 hr (< 50 pellets/l) and 6 hr (< 100 pellets/l) and increased with prolonged incubation and reduction in food ration (Table 3). Presumably, reingestion of faecal material results in further digestive decay and absorption of previously grazed material. If significant numbers of pellets were ingested as incubation continued, then pellet density would have decreased and estimates of chlorophyll *a* degraded per pellet would have

increased. Estimates of the amount of chlorophyll *a* degraded per pellet produced were not different after 6, 12 and 24 hr of incubation. Furthermore, observations of *Calanus* grazing indicate that they do not readily ingest their own faecal pellets (R. Conover, pers. comm., in Head and Harris 1992). Losses due to filter leakage are also unlikely. The filtration of suspensions of fragmented faecal pellets showed little or no passage of chlorophyllous material through 1.0 μm GF/C filters. Pigment loss incurred by the production and subsequent filter leakage of small, pigment-rich particles was probably minor. It is more likely that the digestive processes of *C. finmarchicus* were responsible for the observed degradation of chloropigments to nondetectable products.

Although considerable amounts of chloropigment decay products may be absorbed in the mid-gut of copepod grazers, there is evidence that at least some residues are egested. Incomplete absorption has been indicated by an abundance of phytol (ester-linked side chain of chlorophyll *a*) and dihydrophytol observed in copepod faecal pellets (Prahl et al. 1984a,b). Transformation of phytol to pristane has also been demonstrated in copepod guts, but the specific phytol esterase has not been determined (Avigan and Blumer 1968, Prahl et al. 1984a). Although the residues of the porphyrin ring are subject to absorption in the gut, these products have not been traced and their fate is unknown. The absorption of decayed material will undoubtedly depend on gut chemistry and gut residence time.

In the second series of experiments, chloropigment losses due to *C. finmarchicus* grazing processes were determined from measurements of chloropigments and an inert marker, particulate silica, in *Calanus* faecal pellets and in cultured diatoms. Losses were highly variable (20-99% of ingested chloropigments) within most sets of replicate pellet samples and showed no relationship with *Thalassiosira weissflogii* cell concentrations in the range of 3-16 μg chl/l. Analyses of both pigment and silica in material collected on polycarbonate filters, showed a mean loss of 68% ($\pm$ 23%), which was comparable to a mean loss of 67% ($\pm$ 21%) determined from analyses conducted on separate filters

(GF/C for pigments, polycarbonate for particulate silica). In a similar experiment, Conover et al. (1986) reported losses of 90-99% of ingested chloropigments, as determined by differential recoveries of biogenic silica and chloropigments in *Calanus* faecal pellets and *Thalassiosira* sp. Copepods used in their experiments, however, were kept under laboratory conditions with very low concentrations of cultured algae for more than 2 weeks, and prior to experimentation, the animals were starved for 2 days. In the grazing studies that followed, the copepods exhibited low ingestion rates. It is likely that the natural digestive processes of these animals were altered prior to experimentation and caused the observed high levels of chloropigment decay to nonfluorescent material. Furthermore, faecal pellet analysis requires the physical separation of pellets from the food medium. This process may result in an increase in pigment loss due to handling procedures and temporary exposure to degradative agents (e.g. light, oxygen, heat) during pipet transfer, rinsing of pellets, and microscopic examination. These are potential sources of additional loss and need to be carefully controlled or eliminated.

In this study, pigment losses calculated from silica : chloropigment ratios in food and in faecal pellets were determined during the summer months (June-July). Copepods were collected from waters with low chloropigment concentrations (SCM < 2 μg chl/l) compared with spring bloom conditions. During this period, *Calanus* faecal pellet contents were diverse, consisting primarily of diatoms and diatom cysts, bacteria, choanoflagellates, cyanobacteria and dinoflagellates (Urban et al. 1992). It is possible that the efficiency of *Calanus* digestion is variable, and perhaps higher, when natural particle densities are low and the quality of food is varied. Tande and Slagstad (1981) have identified feed-back mechanisms between copepod digestion and ingestion which enable copepods to adjust digestive processes according to food quality. Recently, Navarro and Thompson (in press) have shown that the assimilation efficiency of the bivalve *Modiolus modiolus* is more variable with a diet of laboratory-cultured algae than with a diet of natural seston. Thus it may not be appropriate to compare the results from the two studies of chloropigment loss directly due to marked differences in the feeding histories,

and perhaps feeding behaviour, of *C. finmarchicus* collected during spring and summer, and due to differences in the diets offered during experimentation (natural seston vs cultured diatoms).

3.4.4. Degradation Products and Processes

The primary fluorescent decay product of ingested chlorophyll *a* in the gut tracts and faecal pellets of *C. finmarchicus* was a phaeophorbide *a*-like derivative, pyrophaeophorbide *a*. This pyrolized derivative represented 81-98% of gut and faecal chloropigments and 40-60% of all chloropigments in grazing bottles following 24 hr of incubation. Before the introduction of HPLC for pigment analyses, phaeophorbide *a* and phaeophytin *a*, detected in trace amounts in this study, were considered the primary products of chlorophyll *a* degradation in zooplankter gut tracts (Shuman and Lorenzen 1975, Hallegraeff 1981). Chromatographic analyses of gut and faecal phaeopigments of various zooplankton grazers have identified pyrophaeophorbide *a*, also referred to as phaeophorbide *a*3, as the principal fluorescent degradation product of ingested chlorophyll *a* (Vernet and Lorenzen 1987, Downs 1989, Head and Harris 1992, Head and Horne 1993, this study).

When captured cells are ruptured, algal enzymes may facilitate the process of chloropigment decay. Chlorophyllase mediates the hydrolysis of the ester-linked phytol tail of chlorophyll *a* and is known to be active in many species of centric diatoms (Barrett and Jeffrey 1964, 1971, Suzuki and Fujita 1986, Jeffrey and Hallegraeff 1987). A "magnesium-releasing enzyme" has also been reported for many centric diatoms (Owens and Falkowski 1982). The mechanisms of chloropigment degradation within zooplankton gut tracts, however, are not well understood. Copepod gut pH appears to be neutral, based on the presence of carbonate and aragonite crystals in faecal pellets observed by Honjo and Roman (1978), yet optimal digestive enzyme activity has been associated with a range in pH of 4.6-6 (Mayzaud and Conover 1976). There have been

inconsistent reports of copepod enzyme activity (in Mayzaud 1986), and although alkaline protease activity has been positively correlated with chlorophyll *a* ingestion (Mayzaud and Conover 1976), no studies have identified the specific enzymes involved in pigment transformation. Copepod digestive enzyme synthesis and secretion appear to be periodic (Mayzaud 1986) and may account for much of the observed variability in chloropigment losses to nondetectable products. Mayzaud and Razouls (1992) suggest that chlorophyll degradation may be complicated by several interacting effects, notably, phytoplankton pigment composition, ingestion rate, gut turnover time, enzyme activity and digestive acclimation processes. Food quality has also been identified as a factor influencing copepod digestive processes (Slagstad and Tande 1981) and may affect the extent of chloropigment degradation to nonfluorescent products in the gut tracts of grazers.

3.5. Conclusions

Grazing experiments with adult female *Calanus finmarchicus* and spring bloom phytoplankton, incubated at 0°C for periods up to 24 hr, showed a clearance rate of 40 to 50 ml per copepod per day at high seston levels (8.0 and 10.5 μg chl/l), and selection for *Skeletonema costatum* when available. Gut pigment levels increased in response to an increase in food and were greatest within 3-6 hr following a 12 hr period of starvation. Pyrophaeophorbide *a*, the primary product of *Calanus* grazing, and chlorophyll *a* comprised 81-98% and 1-5% of gut chloropigments, respectively. Amounts of chlorophyll *a* degraded (to phaeopigments and nonfluorescent products) were highly correlated with particle volumes cleared (r^2=0.90). At the highest food level (10.5 μg chl/l) and throughout incubation, average faecal pellet production was 3-3.5 pellets/hr. Pellet production rate declined with a decrease in initial food concentration and with prolonged incubation at low seston levels. An independently determined gut evacuation rate, following transfer of *C. finmarchicus* to FSW, was 0.020 min^{-1} at 0°C.

At high seston levels (10.5 μg chl/l), the gut fluorescence method provided ingestion rate estimates which were comparable to those determined from particle clearance rate data and from the disappearance (degradation) of chlorophyll *a*. As food concentrations declined, the gut fluorescence method yielded considerably lower estimates of ingestion than the other 2 methods, with the highest discrepancies at the lowest food levels (<1.5 μg chl/l) and following prolonged incubation. At all seston concentrations, there was good agreement between estimates of ingestion determined using clearance rate data and amounts of chlorophyll *a* degraded. Daily ingestion of spring bloom seston at concentrations >3 μg chl/l was estimated at 32-44 μg C per day, an amount representing 25-34% of *Calanus* body carbon.

Little phaeopigment accumulated in grazing bottles with low initial seston levels (1.5 μg/l); about 80-90% of ingested pigments were lost to nonfluorescent products within 6 hr of incubation. These losses were attributed to *Calanus* digestive processes and not to pellet reingestion or processes associated with sample filtration. No losses were detected in incubations with the highest food level (10.5 μg chl/l). At 4.5 and 8.0 μg chl/l, 15-35% of ingested chloropigments were lost to nondetectable products following 24 hr of incubation. Amounts lost were greatest within the first 3 hr of grazing, and were probably enhanced as a result of activated digestive processes following an overnight period of starvation.

In post-bloom experiments with cultured diatoms (*Thalassiosira weissflogii*), analyses of silica/chloropigment ratios in food and *Calanus* faeces showed highly variable chloropigment losses (20-99% of ingested pigments) within replicate samples, and no relationship between pigment loss and food concentration in the range of 3-16 μg chl/l. Because methodology and diet differed from the experiments conducted with spring bloom seston, the results of the two studies are not directly comparable. Differences in the feeding histories and the digestive activities of copepods collected during spring and summer may also influence the extent of grazer-mediated pigment degradation.

Qualitatively, chloropigments are useful tracers of ingested phytoplankton. Pyrophaeophorbide *a*, the primary chlorophyll *a* transformation product in the gut tracts and faeces of *Calanus finmarchicus*, is a signature pigment of recent grazing events. The application of chloropigments as quantitative tracers of *in situ* grazing, however, requires the conservation of chloropigments during gut passage. Pigment conservation was low (<20%) at food levels < 1.5 μg chl/l, and high (65-100%) at seston concentrations >3 μg chl/l. Losses to nonfluorescent products were minimal only when copepods fed near continuously at high food levels. The reliability of the gut fluorescence method may, therefore, be limited to periods of phytoplankton bloom production.

Table 3.1. Phytoplankton composition of seston in plankton wheel grazing bottles. Data represent initial conditions (% of total cells >4 μm): va - very abundant (>30%), a - abundant (10-30%), c - common (1-10%), r - rare (<1%).

Species / Date Seston Collected	Exp 1, 2 19/04/90	Exp 3 22/04/90	Exp 4 07/05/90
Bacillariophyceae			
Bacteriosira sp.		r	
Chaetoceros spp.			
C. convolutus		r	c
C. costatus	r		c
C. debilis	a	a	a
C. decipiens		c	
C. diadema		c	
C. furcellatus	c		c
C. socialis	a	a	a
Coscinodiscus sp.			r
Detonula sp.	r		
Eucampia groenlandica		r	c
Eucampia zodiacus	r		c
Fragillariopsis sp.	c	r	
Leptocylindrus danicus	r	r	
Navicula sp.	r	r	
Nitzschia spp.	r	r	
Rhizosolenia hebata		c	a
Skeletonema costatum	va	va	
Thalassiosira spp.			
T. hyalina	c	c	c
T. nordenskiöldii	a	c	a
Dinophyceae			
Amphidinium sp.	r		
Gymnodinium sp.		r	r
Cr ıspedophyceae			
Parvicorbicula sp.		r	
Others			
Ciliates			r
Cryptomonads	r		
Cysts	r	r	r

Table 3.2. Cell concentrations of the dominant phytoplankton taxa (cells/litre) in plankton wheel grazing experiments 3 and 4, prior to incubation (initial) and following 3, 6, and 12 hr of *Calanus finmarchicus* grazing. Percent cells removed in parentheses.

Exp.	Species	Initial	3hr	6hr	12hr
3	*Skeletonema costatum*	1.8×10^6	4.3×10^5 (76%)	9.2×10^4 (95%)	8.2×10^4 (95%)
	Chaetoceros socialis	8.7×10^5	4.9×10^5 (44%)	5.9×10^5 (32%)	2.3×10^5 (74%)
	Chaetoceros debilis	5.3×10^5	4.1×10^5 (23%)	5.8×10^5 (-11%)	3.2×10^5 (40%)
	Thalassiosira nordenskiöldii	9.7×10^4	5.3×10^4 (45%)	8.2×10^4 (15%)	4.3×10^4 (56%)
	Other cells (>4 μm)	4.3×10^4			
4	*Rhizosolenia hebata*	9.2×10^3	1.5×10^3 (84%)	2.2×10^3 (76%)	0 (100%)
	Chaetoceros socialis	3.0×10^4	1.9×10^4 (37%)	5.5×10^3 (83%)	3.2×10^3 (89%)
	Chaetoceros spp. (3)	1.1×10^5	2.6×10^4 (76%)	8.5×10^3 (92%)	1.2×10^3 (99%)
	Thalassiosira nordenskiöldii	8.4×10^4	1.0×10^4 (88%)	1.2×10^4 (86%)	1.0×10^3 (99%)
	Other cells (>4 μm)	1.7×10^4			

Table 3.3. *Calanus finmarchicus* faecal pellet production and chlorophyll *a* degraded per faecal pellet produced following 6, 12 and 24 hr of incubation at 4 seston chloropigment concentrations. ND (no data).

Exper # & Date	Natural Seston initial chloropigment conc ($\mu g/l$)	Hours fed	*Calanus* faecal pellets produced (cumulative) mean (±SD)	ng chlor *a* degraded per pellet produced mean (±SD)
	8.0	6	N.D.	N.D.
1	8.0	12	328 (60)	6.03 (1.50)
Apr 21-22	8.0	24	560 (134)	5.70 (0.52)
	1.5	6	84 (15)	4.19 (1.37)
2	1.5	12	171 (14)	3.24 (0.77)
Apr 23-24	1.5	24	212 (29)	3.59 (0.46)
	10.5	6	111 (52)	5.78 (2.00)
3	10.5	12	333 (157)	4.36 (0.94)
Apr 25-26	10.5	24	560 (118)	4.64 (0.81)
	4.5	6	128 (17)	8.68 (1.73)
4	4.5	12	244 (3)	8.07 (0.71)
May 12-13	4.5	24	433 (44)	6.10 (0.31)

Table 3.4. Estimates of chloropigments lost to nonfluorescent products per grazing bottle and per copepod at each grazing interval in each experiment.

Exp #	Initial Chl conc ($\mu g/l$)	μg chloropigment lost / bottle mean (±SD)				ng chloropigment lost / *Calanus* mean (±SD)			
		3 hr	6 hr	12 hr	24 hr	3 hr	6 hr	12 hr	24 hr
1	8.0	0.51 (0.26)	0.55 (0.06)	0.65 (0.05)	0.56 (0.04)	50.8 (26.4)	55.1 (6.0)	64.9 (5.4)	56.3 (4.5)
2	1.5	0.17 (0.02)	0.28 (0.05)	0.52 (0.12)	0.61 (0.09)	17.5 (2.3)	28.7 (3.7)	55.5 (11.4)	61.0 (8.8)
3	10.5	0.06 (0.14)	0.05 (0.21)	-0.04 (0.19)	-0.13 (0.39)	8.2 (20.5)	9.5 (34.4)	-1.7 (25.2)	-19.8 (50.4)
4	4.5	0.41 (0.17)	0.37 (0.05)	0.82 (0.14)	0.72 (0.22)	40.8 (16.8)	37.4 (5.3)	81.8 (14.1)	73.9 (7.7)

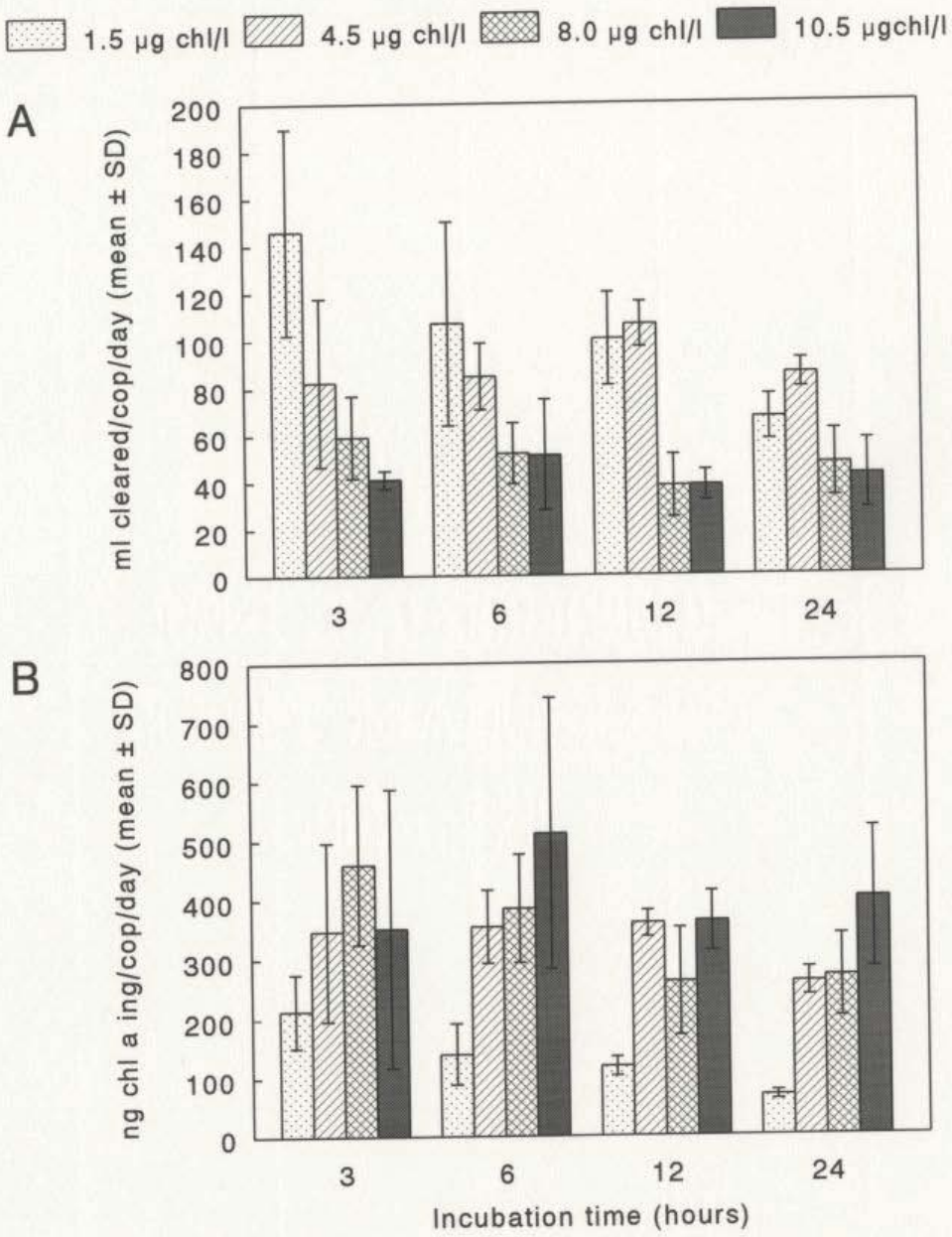

Figure 3.1. Particle clearance rates (A) and chlorophyll *a* ingestion rates (B) of adult ♀ *Calanus finmarchicus*, determined from particle spectra and chloropigment concentrations in bottle incubations at 4 initial seston chloropigment concentrations.

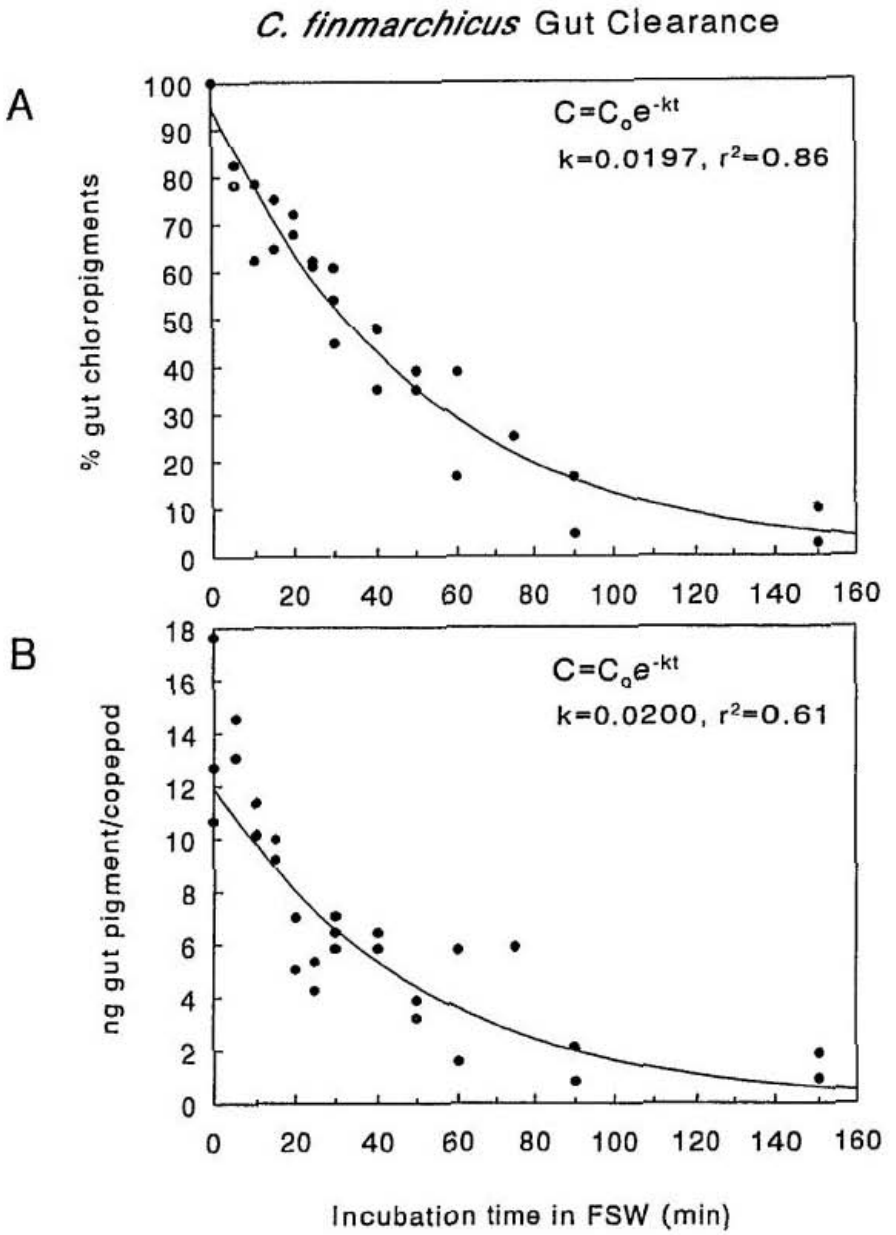

Figure 3.3. Gut clearance rates of chloropigments following transfer of adult ♀ *C. finmarchicus* to FSW. Rates determined using A) % gut chloropigments and B) gut chloropigment content at each interval. Incubations conducted at 0-1°C. Each point represents data collected from 5-10 copepods. All data fitted to an exponential curve : $C=C_0e^{-kt}$, where C = gut pigment level, k = defecation rate constant, and t = time (min).

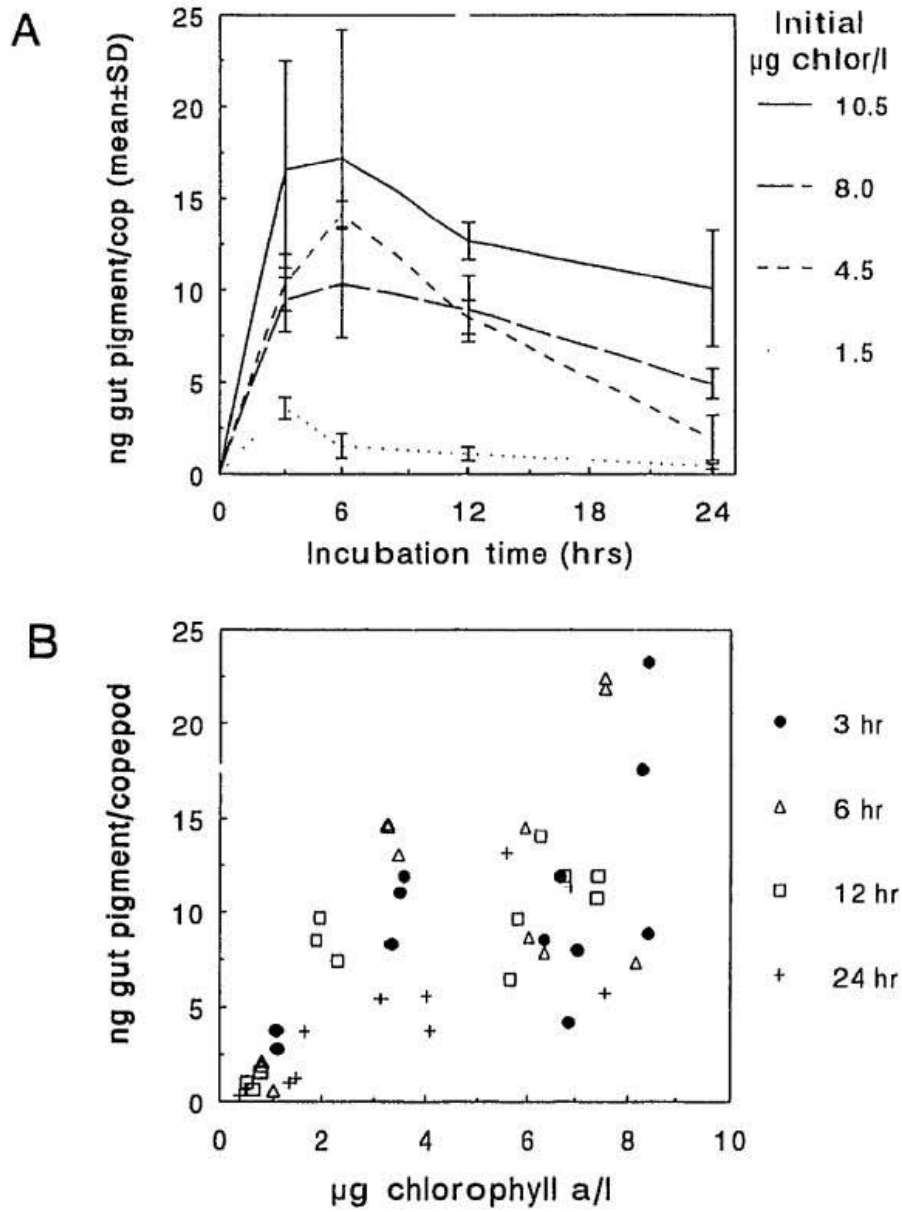

Figure 3.2. Adult ♀ *C. finmarchicus* gut chloropigments (ng/copepod) in grazing bottles following incubation at 4 initial seston chloropigment concentrations for 3, 6, 12 and 24 hr: A) gut pigments vs incubation time, and B) gut pigments vs chlorophyll *a* concentration remaining in each grazing bottle. Copepods prestarved overnight. Data represent extractions of approximately 10 animals from each of 3 grazing bottles.

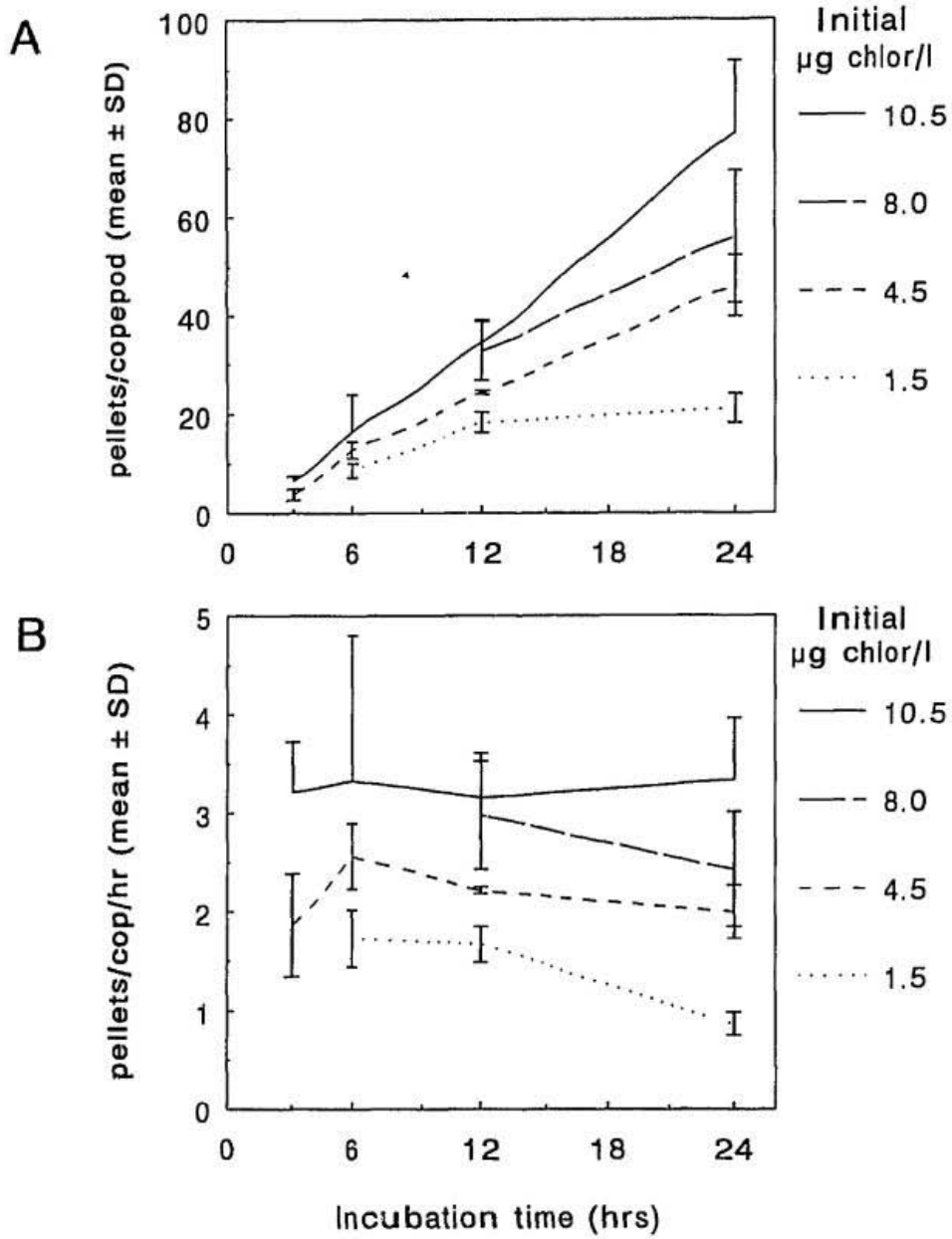

Figure 3.4. *C. finmarchicus* faecal pellet production (cumulative, A) and pellet production rate (B) following incubation at 4 seston chloropigment concentrations for 3, 6, 12 and 24 hr.

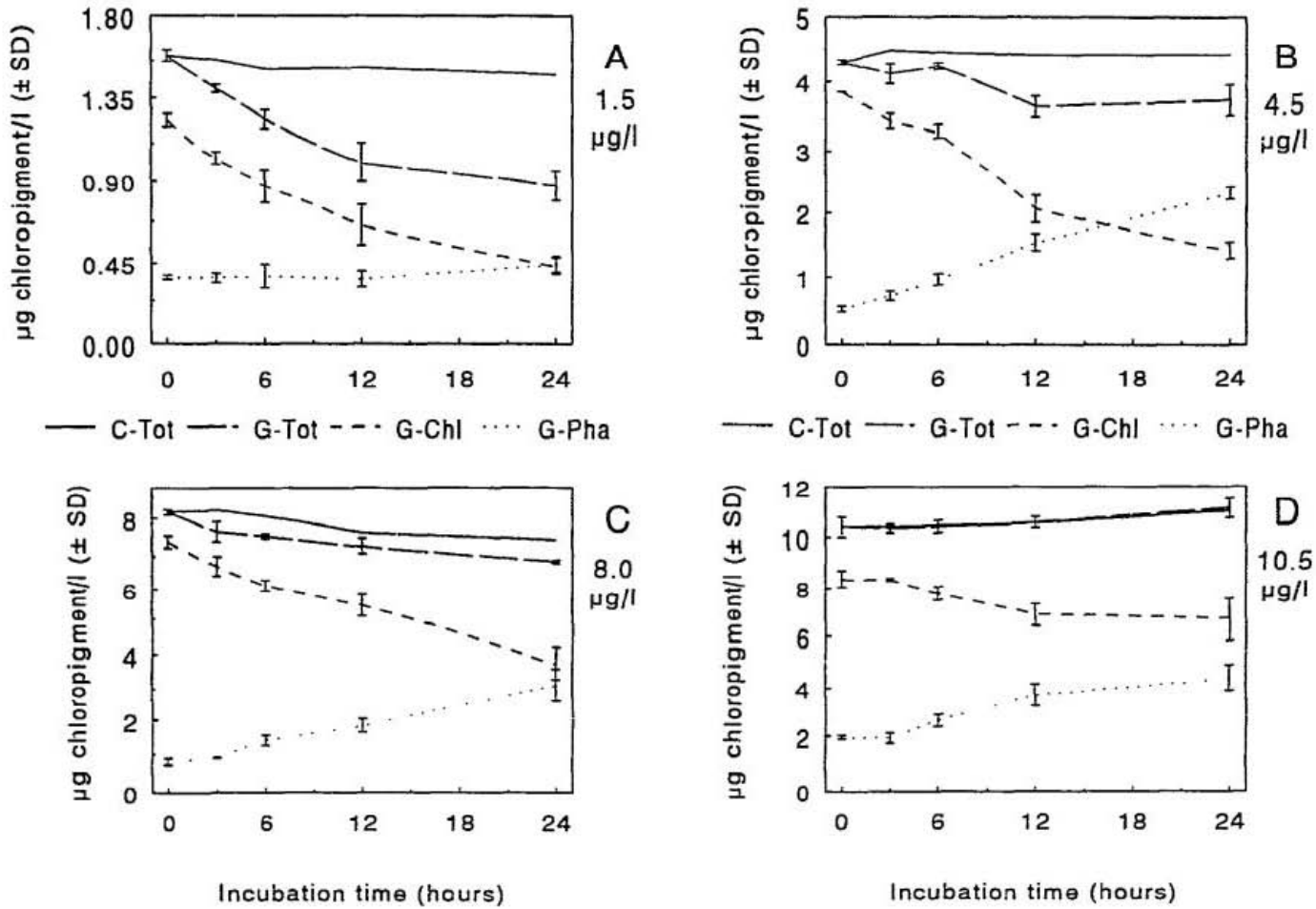

Figure 3.5. Chloropigment levels in control (C) and grazing (G) bottles following incubation at 4 seston chloropigment concentrations (A-D) for 3, 6, 12 and 24 hr. Tot-total, Chl-chlorophyll *a* and Pha-phaeopigments. Note: Y-scales differ.

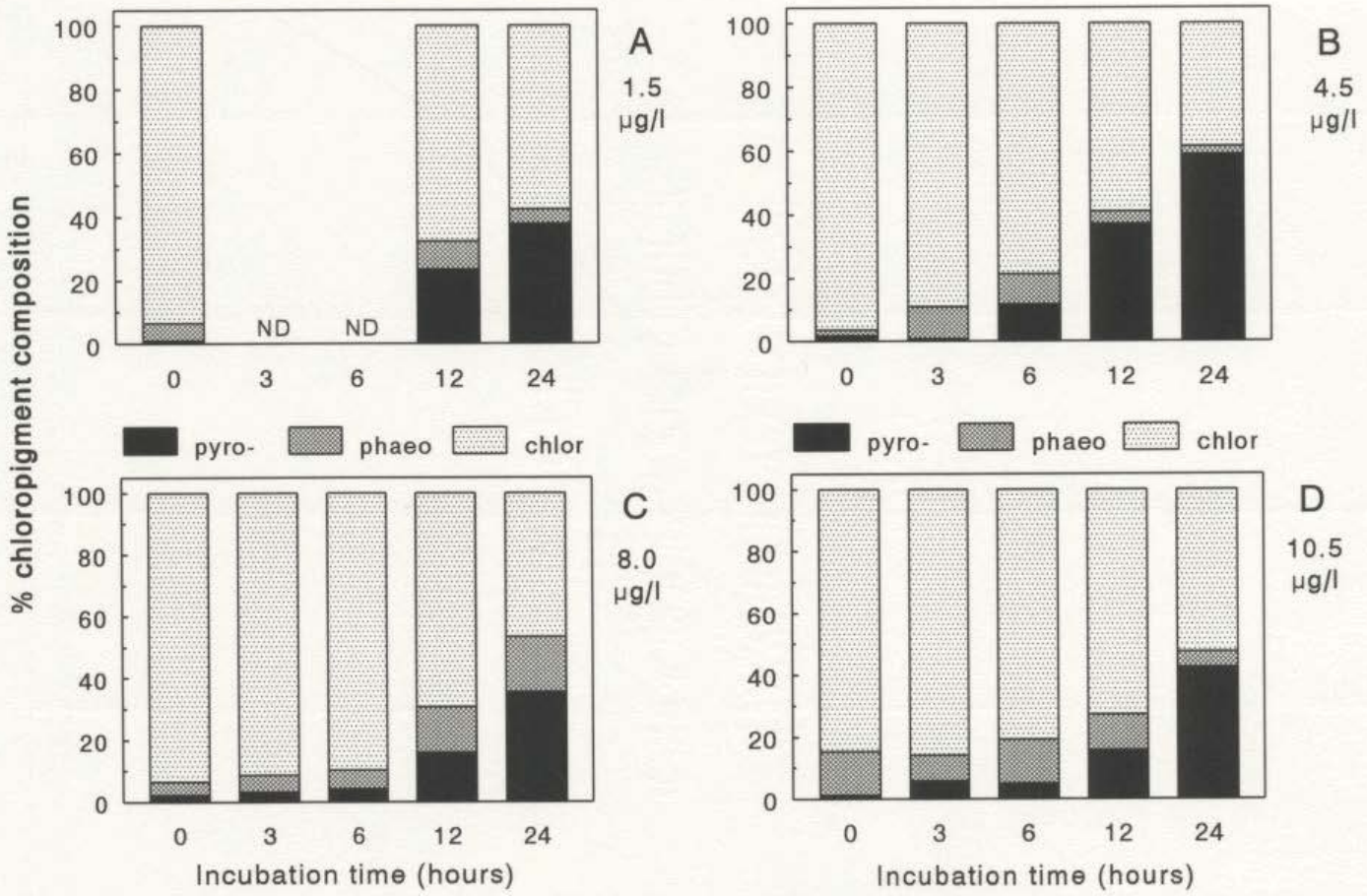

Figure 3.6. HPLC determinations of % chloropigment composition following incubation of *C. finmarchicus* at 4 seston chloropigment concentrations (A-D) for 3, 6, 12 and 24 hr. Composition includes: pyrophaeophorbide *a* (pyro-), all other phaeopigments (phaeo) and chlorophyll *a*-type pigments (chlor).

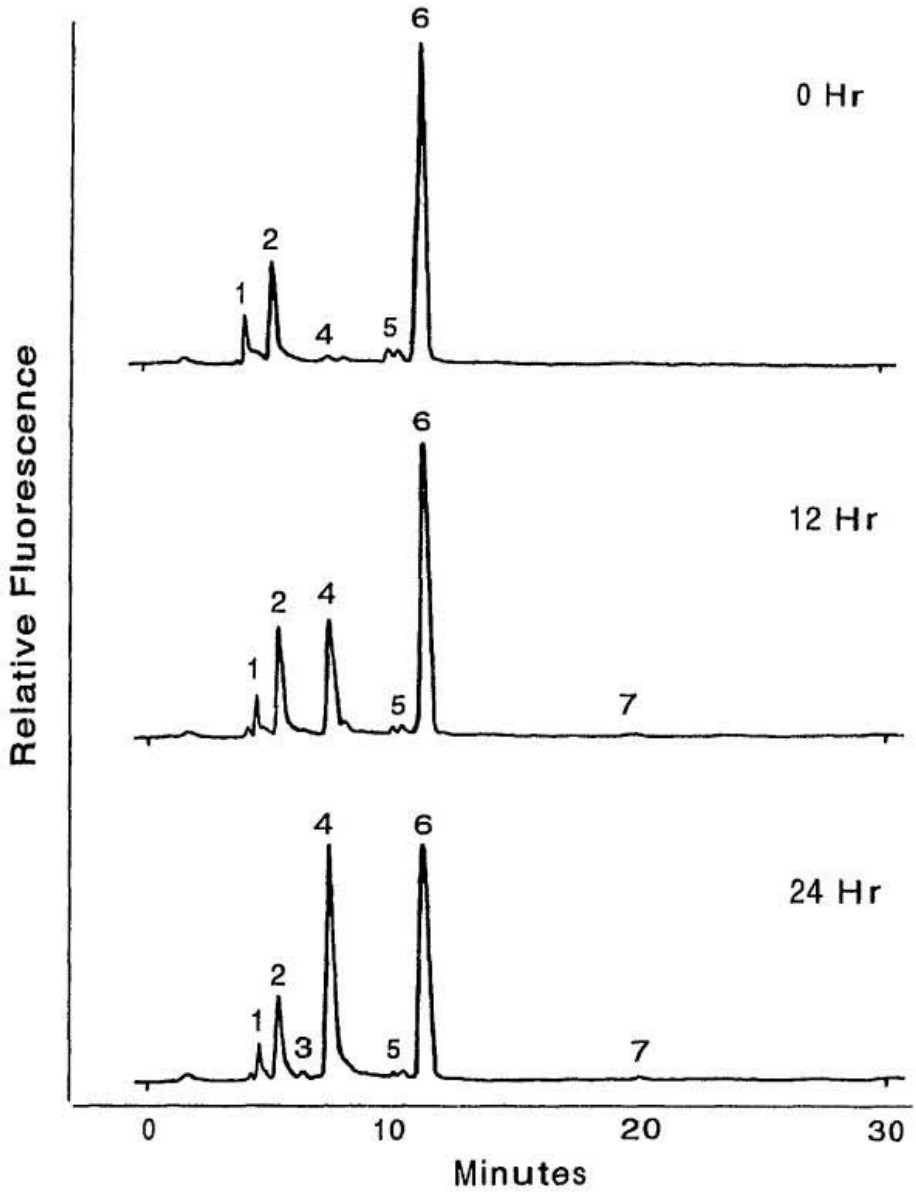

Figure 3.7. HPLC profiles of initial seston and representative bottle samples removed after 12 hr and 24 hr of incubation in *C. finmarchicus* grazing in Experiment 4. Peak identities are: 1-chlorophyllide *a*, 2-chlorophyll *c*, 3-phaeophorbide *a*, 4-pyrophaeophorbide *a*, 5-chlorophyll *a* allomers, 6-chlorophyll *a*, and 7-phaeophytin *a*. Note: Y-scales constant.

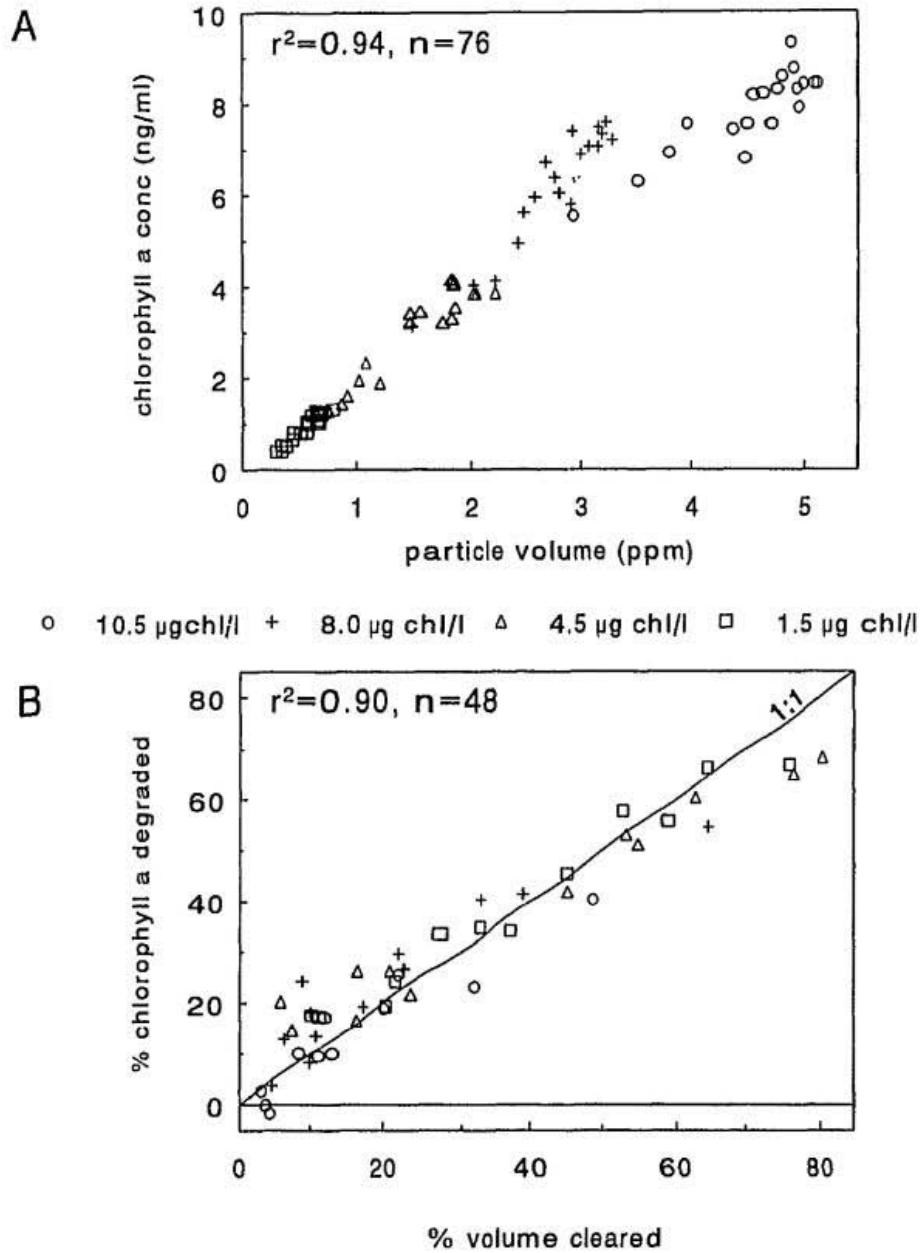

Figure 3.8. Relationships between (A) particle volume and chlorophyll *a* concentration in control and grazing bottles, and (B) % particle volume cleared and % chlorophyll *a* degraded in grazing bottles, with *C. finmarchicus* and 4 seston chloropigment concentrations.

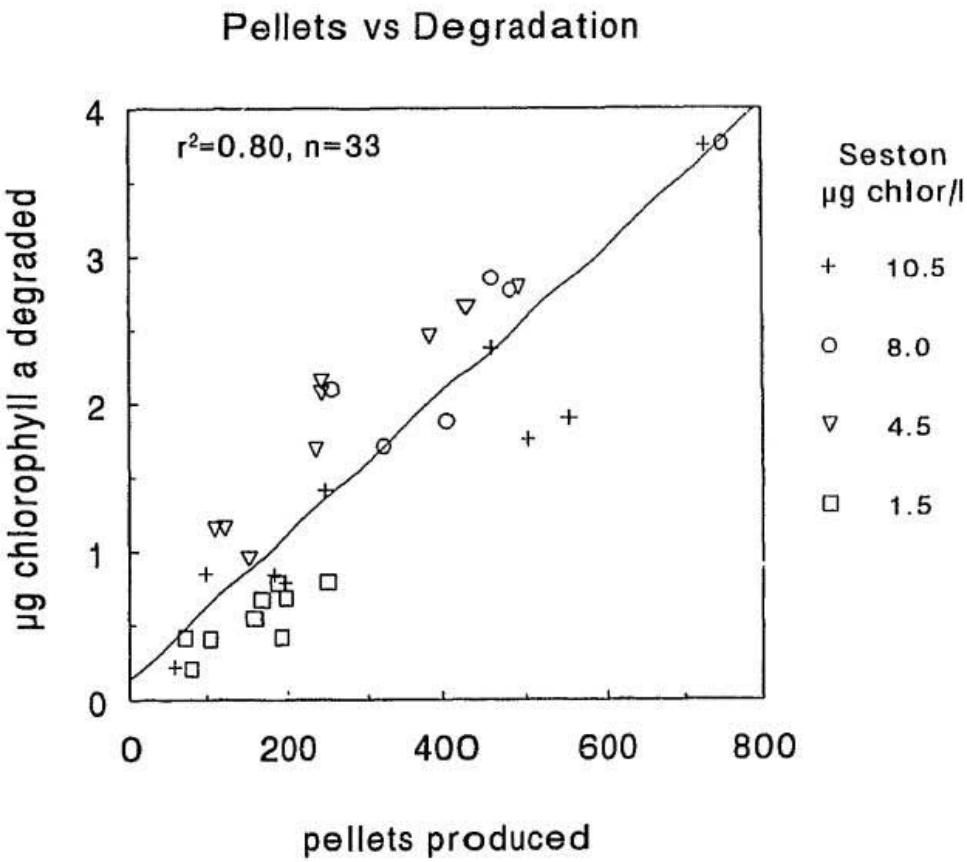

Figure 3.9. Relationship between the amount of chlorophyll *a* degraded and the number of *Calanus* faecal pellets produced per bottle in each of the 4 grazing experiments.

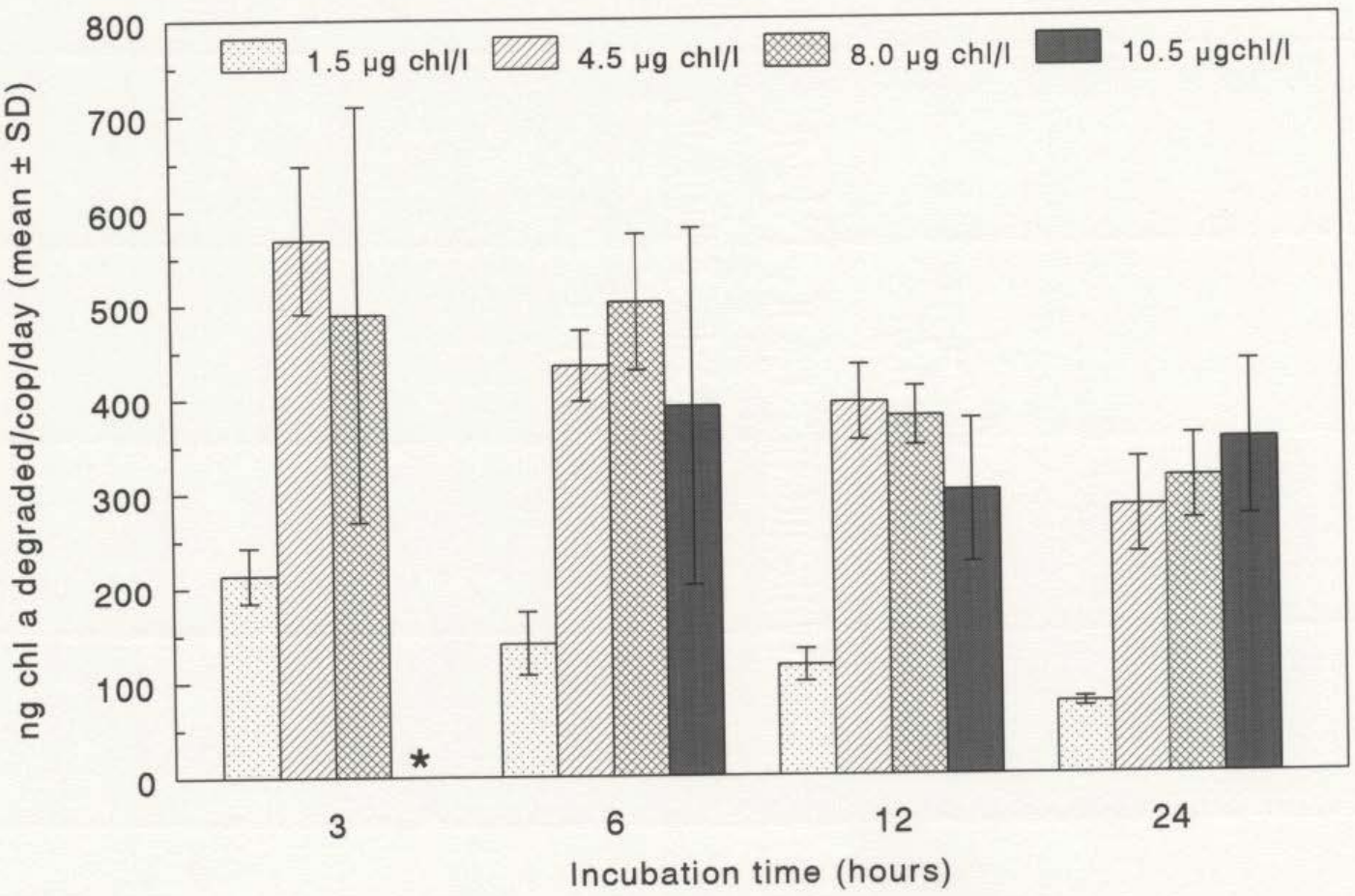

Figure 3.10. Estimates of chlorophyll *a* degraded per adult ♀ *C. finmarchicus* per day, determined from chloropigment levels in incubations at 4 seston chloropigment concentrations for intervals of 3, 6, 12 and 24 hr. (* = no degradation observed).

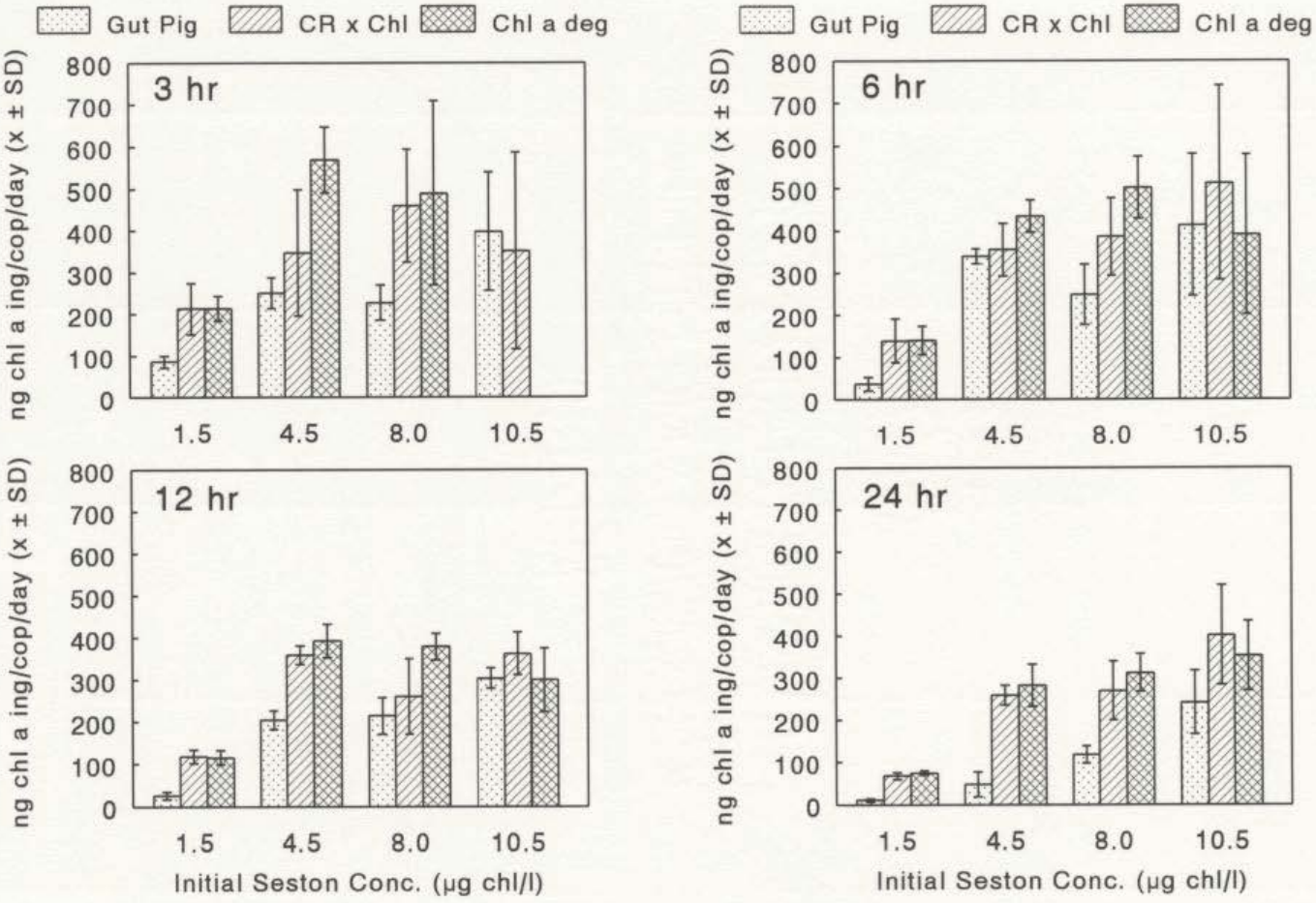

Figure 3.11. Comparison of 3 methods used to calculate chlorophyll *a* ingestion rates of *C. finmarchicus*. Copepods were incubated for 3, 6, 12 and 24 hr at 4 seston chloropigment concentrations. Methods used: Gut Pig (gut fluorescence method), CR x Chl (clearance rate x chloropigment conc.), and Chl *a* deg (rate of chlorophyll *a* degradation).

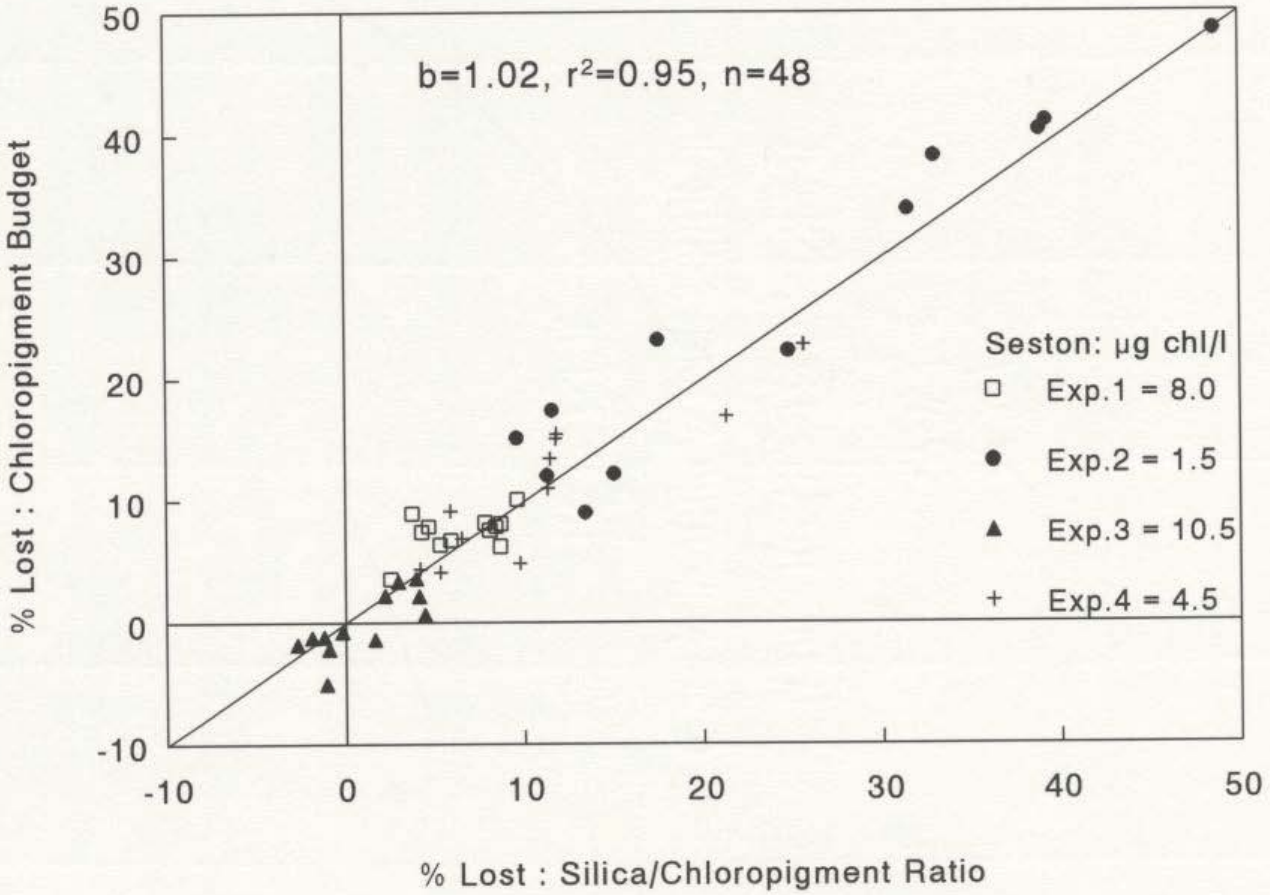

Figure 3.12. Comparison of 2 methods used to determine % chloropigment lost per grazing bottle: 1) total chloropigment budget and 2) silica : chloropigment ratio method.

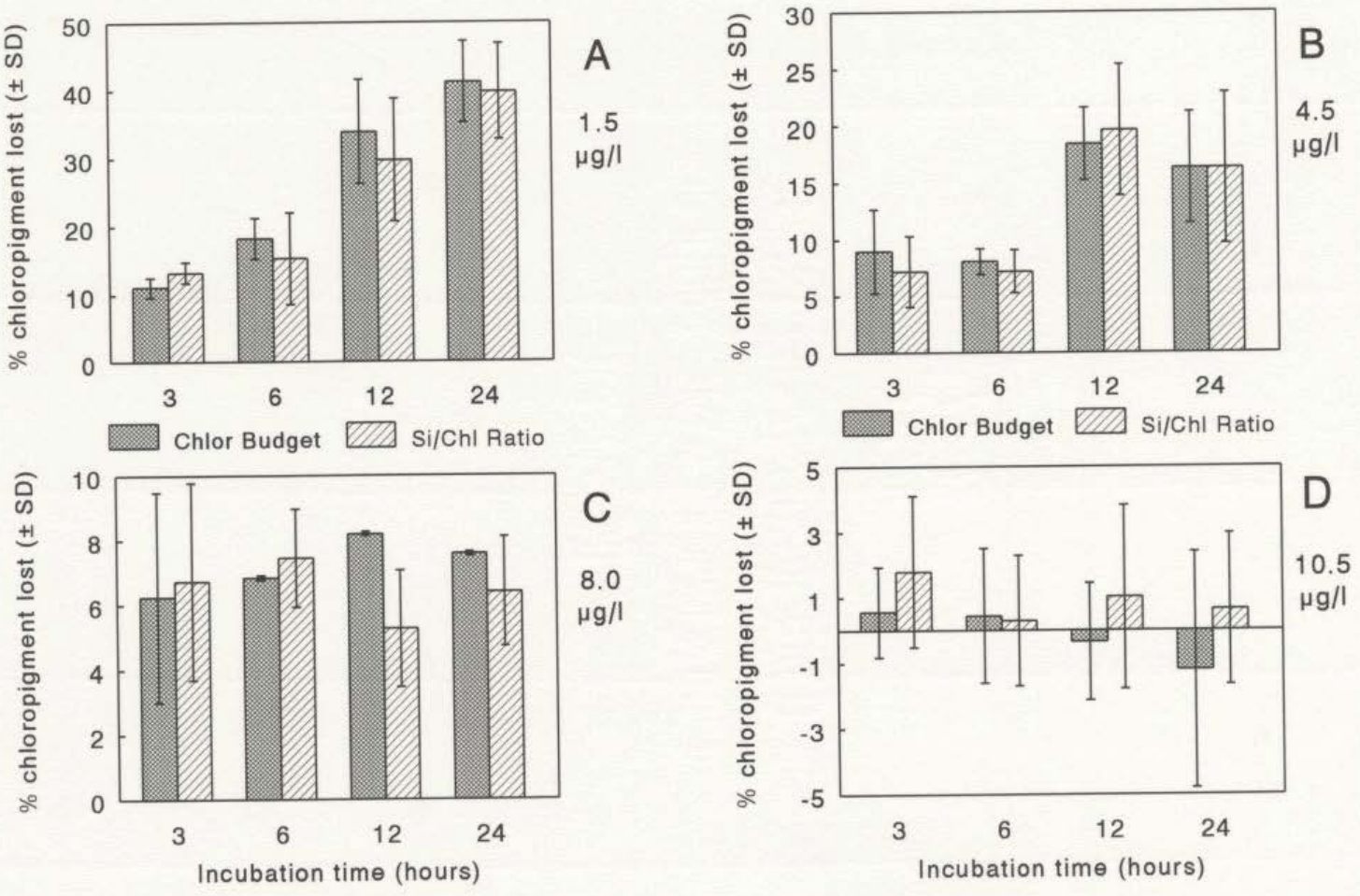

Figure 3.13. Percent chloropigment lost (of initial chloropigments) determined using total chloropigment budgets and silica: chloropigment ratios, following incubation at 4 seston chloropigment concentrations (A-D) for 3, 6, 12 and 24 hr.

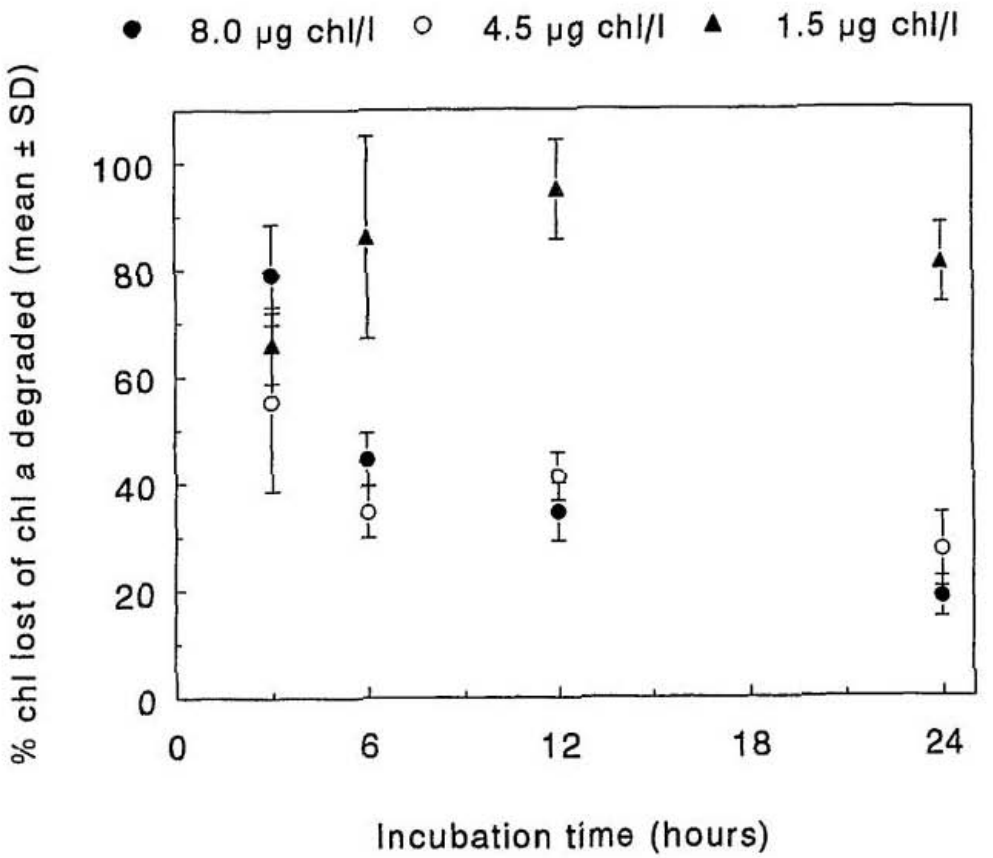

Figure 3.14. Percent chloropigment lost (of chlorophyll *a* degraded) following incubation of *C. finmarchicus* at 3 seston chloropigment concentrations (8.0, 4.5 and 1.5 µg chl/*l*) for 3, 6, 12 and 24 hr.

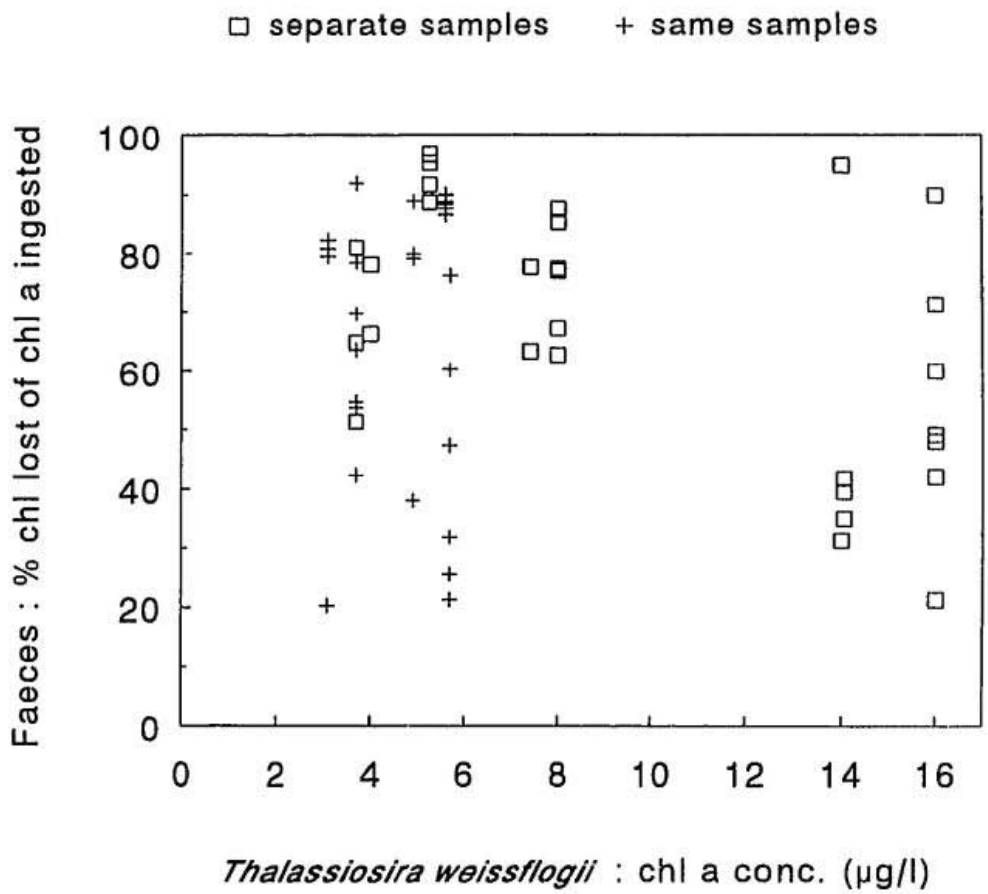

Figure 3.15. Percent chloropigment lost (of chlorophyll *a* ingested) determined using silica : chloropigment ratios in *C. finmarchicus* faecal pellets and food (*Thalassiosira weissflogii*). Copepods were incubated at algal chloropigment concentrations in the range of 3-16 μg chl/*l*.

Chapter 4

FEATURES OF THE SPRING PHYTOPLANKTON BLOOM IN CONCEPTION BAY

4.1. Introduction

Spring phytoplankton blooms in mid-latitude waters are characterized by high phytoplankton production in late winter and early spring, with diatoms dominating numbers and biomass of phytoplankton (Evans and Parslow 1985). Characteristics of bloom development are dependent on sea temperature, water column stability, available nutrients, and light (Morris et al. 1985). These relatively short periods of productivity are important sources of carbon and energy to water column heterotrophs and to the underlying benthic community. The degree to which phytoplankton carbon production is recycled within the water column or exported to the benthos may be highly variable, and is dependent on both the duration and magnitude of the bloom as well as the abundance and activity of water column bacteria and zooplankton grazers.

The development and decline of the 1986 spring bloom in Conception Bay, Newfoundland, a fjord-like embayment at mid-latitude, occurred over a 5-6 wk period (early March to mid-April) at surface water temperatures of $<2°C$; peak chlorophyll *a* levels exceeded 20 μg/l (Deibel et al., unpubl. data). During April and May, sediment traps deployed at various depths collected few faecal pellets; trap contents were composed primarily of intact diatoms, indicating little grazing impact by zooplankton (C. McKenzie, pers. comm.). Bacterial utilization of phytoplankton production at near zero temperatures was also suppressed (Pomeroy et al. 1991). The spring bloom of 1986, however, developed under unusually calm, warm and sunny conditions, resulting in rapid diatom growth, early termination of the bloom and massive cell sinking to the benthos.

Conception Bay experiences high interannual variability in meteorological conditions during late winter and spring (i.e. storms, cloud cover, ice) (Petrie et al. 1991). It seems likely that variable weather events during this period would lead to great variation in spring bloom development and in the relative utilization of phytoplankton production by pelagic and benthic heterotrophs.

The goal of this study was to describe the physical, chemical and biological characteristics of spring bloom development and decline in Conception Bay during 1988. Features examined include inorganic nutrients, water column chloropigment and particulate organic matter composition, phytoplankton abundance and biovolume, and primary production. This study provides a framework for the concurrent examination of the contribution of various grazers to the transformation and vertical transport of phytopigments (Chapter 5), and for the determination of carbon and phytopigment flux to the benthos during, and immediately following, peak periods of phytoplankton biomass (Chapter 6).

4.2. Study Area

All data presented in Chapters 4, 5 and 6 were obtained from vertical profiles and samples collected at a deep water site (250 m) located near the head of Conception Bay on the east coast of Newfoundland (Figure 4.1). This site was selected for phytoplankton production and flux studies which were initiated in 1986. It was chosen because it lies within the deep depositional area of the bay and is thus well suited for the examination of the vertical flux of spring bloom production. The station is also in close proximity to Memorial University's laboratory facilities and research vessel mooring.

Conception Bay is a long (70-100 km) and narrow (20-40 km) fjord-like embayment opening directly onto the Newfoundland Shelf. The bay has a 170 m deep sill at the mouth and a maximum depth of 300 m in the central basin. The inshore branch

of the Labrador current flows southward across the mouth of Conception Bay with a mean surface speed of about 20 cm/s (Petrie and Anderson 1983) and intrudes into the bay, causing year-round subzero temperatures below the surface solar-heated layer. Bottom water in the bay is regularly exchanged with shelf water, resulting in bottom water oxygen concentrations of approximately 7 ml/l (Ostrom 1992) and cold bottom temperatures ($<0°C$), which closely approximate those on the adjacent shelf (deYoung and Sanderson 1994).

Mean circulation in the bay is anti-clockwise and very weak, generally less than 2 cm/s. Low freshwater runoff and weak tides, with a mean range of about 1 m and constituent velocities of <2 cm/s (Aggett et al. 1987), have little effect on the circulation in the bay. Fluctuating currents are typically 10-20 cm/s near the surface and <10 cm/s at depths >25 m, becoming spatially decorrelated over distances of 4-10 km and periods of about 1 day (deYoung and Sanderson 1994). Throughout most of the bay, mean circulation is insignificant compared to transport caused by variable currents (B. Sanderson, pers. comm.). However, strong outflow at the northeast corner of the bay appears to dominate current variability locally (deYoung and Sanderson 1994). More than 50% of the current energy, with periods of 2-10 days, is forced by wind (deYoung et al. 1993). Because much of this wind forcing is nonlocal, currents and upwelling are not easily deduced from winds (B. Sanderson, pers. comm.).

Conception Bay experiences great interannual variability in the appearance of both pack ice, which forms along the Labrador Coast during mid-March to late April, and icebergs, which are carried southwards from the Arctic by the Baffin and Labrador Currents during May and June. Variability in the presence of ice in the bay is controlled by upstream conditions and local meteorological forcing; little ice is formed in the bay (Cote 1989). The sampling site shown in Figure 4.1 was chosen, in part, because it lies outside the zone of maximum occurrence of pack ice.

4.3. Methods

The development and decline of the 1988 spring phytoplankton bloom was monitored at intervals of 1-5 days from late March to early June from Memorial University's research vessel "Karl and Jackie II". More intensive sampling was conducted during April 9-17 from the Department of Fisheries and Oceans research vessel, the C.S.S. "Baffin".

Temperature, conductivity, and chlorophyll *a* fluorescence were determined with a Seabird SBE 20 CTD with SeaTech *in situ* fluorometer. The fluorometer was calibrated against acetone extracts of natural phytoplankton and with laboratory cultured algae. Contour plots used to describe the data were generated using the software package Surfer™, with the kriging interpolation algorithm. The data were averaged in 2 m bins.

Water samples for chemical and biological analyses were collected at the subsurface chlorophyll maximum (SCM) and at depths above and below the SCM using 5-l Niskin bottles, attached either to a hydrowire or to a CTD rosette frame. Nitrate, reactive phosphate and dissolved silicate were determined using standard methods (Parsons et al. 1984, Grasshoff 1976). Nitrite concentrations were below detectable levels in all samples examined.

Primary production rates were estimated routinely during the spring of 1988 using the standard ^{14}C bicarbonate technique outlined in Pomeroy et al. (1991). Deck incubations simulated *in situ* light and temperature conditions. Measurements were made over a 4 hr period between 0900 hr and 1600 hr. Downwelling photosynthetically-active irradiance was determined with a 2-pi Li-Cor quantum sensor. Hourly integrated incident irradiance was collected with a cosine-corrected Li-Cor sensor and Li-1000 integrator onboard ship, or at a meteorological station onshore. Vertically integrated daily carbon

fixation was estimated from P-I curves, irradiance depth profiles and phytoplankton biomass (Pomeroy et al. 1991).

Particulate organic carbon (POC) and nitrogen (PON) water samples (500-1000 ml) were passed through a 505 μm mesh, collected on pre-combusted GF/C filters under low vacuum (< 15 mm Hg) and frozen for up to 6 mos. Samples were analyzed using a Perkin-Elmer 240A CHN analyzer, standardized against acetanilide.

Water column samples for pigment analysis were size-fractionated using funnels fitted with various nylon meshes (505 μm, 70 μm, 15 μm). Samples of 250-500 ml were collected on GF/C filters, immediately frozen on dry ice and then stored at -20°C for up to 2 mos prior to processing. Standard fluorometric and HPLC determinations of chlorophyll *a* and fluorescent degradation products followed the procedures outlined in Chapter 2.

Cell samples (100 ml) were collected for taxonomic composition of nanoplankton and microplankton and fixed with 1 ml Lugol's iodine, followed by the addition of 2 ml of 40% formaldehyde solution. Samples were then stored at room temperature. Taxa were identified and counted with the Utermöhl method and a Zeiss Axiovert 35 inverted microscope.

4.4. Results

4.4.1. General Features of the 1988 Spring Bloom

Contours of chlorophyll *a* concentration (μg/l), as determined by *in situ* fluorometer profiles of chlorophyll *a* fluorescence, indicate development of the phytoplankton bloom in the upper 50 m of the water column prior to mid-March (Figure 4.2). Standing stock increased at depths of 20-40 m during late March, when the water

column was nearly isothermal and surface temperatures were between 0 and -1°C (Figure 4.3). A biomass peak in mid-April (>2 μg chl/l), extending to a depth of 90 m, was coincident with an increase in the depth of the upper mixed layer to 110 m (Figure 4.4), which resulted from a strong and persistent northeasterly gale during April 11-16. Subsequent peaks in chlorophyll *a* occurred during the first and third weeks of May at temperatures below 2°C. A brief northeasterly storm deepened the upper mixed layer between the second and third peaks in biomass (Figure 4.4). During the first week in May, chlorophyll *a* concentrations of 0.5-1 μg/l were detected at depths from 70-210 m, indicating massive and rapid cell sinking to the benthos. Thermal stratification by solar heating of the surface waters was underway during the third bloom event (May 12-24). Surface temperature was about 3°C. Phytoplankton biomass at this time was concentrated in water <1°C at depths of 35-50 m, with a maximum chlorophyll level of 6 μg chl/l at 45 m.

A continuous and constant concentration of chlorophyll *a* (0.5 μg/l) at depths >100 m throughout May suggests a steady flux of ungrazed phytoplankton to deep waters. Further details on vertical flux during the 1988 spring bloom are provided in Chapter 6. Termination of the spring phytoplankton bloom was marked by a rapid decline in chlorophyll *a* concentration in late May and an increase in the depth of the SCM to >50 m. This clearly coincides with the onset of thermal stratification (Figure 4.3).

Concentrations of nutrients (nitrate, reactive phosphate and dissolved silicate) showed similar patterns of decline in the upper 50 m as the phytoplankton bloom progressed (Figures 4.5, 4.6 and 4.7). Although levels of all nutrients were greatly reduced in the surface waters (0-35 m) during the first week in May, chlorophyll *a* biomass at depths of 10-40 m remained relatively high. By mid-May, declining nutrient levels in the surface layer limited algal production to depths below 30 m. Nutrient concentrations below the pycnocline remained relatively unchanged throughout the spring season. Silicate concentrations in the range of 0.2-1.2 μM/l and silicate:nitrate molar

ratios of < 1 throughout the last 3 weeks in May at depths above 50 m suggest that silicate was the most severely limiting nutrient to diatom production (Conley and Malone 1992). Silicate limitation was also suggested by levels determined from samples collected from the surface waters during the 1986 spring bloom (D. Deibel, pers. comm.).

Contours of particulate organic carbon (Figure 4.8) and nitrogen (Figure 4.9) show high concentrations of organic material within the upper 50 m during April and May. Maximum carbon concentrations (> 300 μg/l) coincided with the 2 bloom events in May. C/N ratios of 6-8, which are typical of phytoplankton (Redfield et al. 1963), were found at depths above 100 m throughout April and May (Figure 4.10). C/N ratios increased below 100 m. The highest C/N ratios appeared after peak periods of phytoplankton biomass in May, indicating grazing effects and/or physiological changes within sinking algae.

Chlorophyll *a* concentrations determined from acetone extracts of seston (< 505 μm) collected on filters (Figure 4.11) were slightly lower than concentrations determined using the *in situ* fluorometer (Figure 4.2), but indicated similar peaks in phytoplankton biomass during April and May. Data collected from sample extracts showed substantially less temporal and spatial resolution than that of the *in situ* profiles. Consequently, small scale features in the contours from *in situ* data were not always evident in the contours of extracted chlorophyll *a*.

Carbon/chlorophyll *a* ratios at the SCM showed values ranging from 53-89, with a mean of 72 during both mid-April and early May peaks in biomass. A ratio of 92 was determined during the third week in May. At peak chlorophyll levels, ratios of carbon/total chloropigments, which included both chlorophyll *a* and phaeopigments (in chl *a* wt equiv.), were 56 (mid-April), 76 (early May) and 74 (mid- to late May).

4.4.2. Taxonomic Composition of the Bloom

Fractionation of seston samples for pigment analysis showed that during mid-March to late April, most of the chlorophyll *a* in the upper 50 m of water was concentrated in particles passing through a 15 μm mesh (Figure 4.12). During this period, the phytoplankton was dominated, in both abundance and biovolume, by centric chain-forming diatoms, primarily *Chaetoceros* spp., *Skeletonema costatum* and *Thalassiosira* spp. (Table 4.1). On all sampling dates, <20% of chlorophyll *a* in surface waters was concentrated in material within a size range of 15-70 μm (Figure 4.13). From late April to late May, 50-80% of the chlorophyll *a* in the upper 50 m was contained in phytoplankton cells and aggregates >70 μm (Figure 4.14). Although small chain-forming diatoms were often numerically dominant during this time, much larger cells (dinoflagellates and many species of *Coscinodiscus*) comprised a greater fraction of the phytoplankton biovolume (Table 4.1). In early June (post-bloom), small flagellates (< 15 μm) were the primary contributors to the chlorophyll *a* pool (Figure 4.12).

Phytoplankton cell abundance (cells/l) and biovolume (μm^3/l), at selected depths above and below 35 m, are shown in Figure 4.15. Cell concentration above 35 m was highest during mid- to late April. Thereafter, phytoplankton cells were concentrated at depths below 35 m, coinciding with nutrient depletion in surface waters. Due to a shift in the species composition and cell size of common taxa, peaks in cell abundance did not always correspond with peaks in chlorophyll *a* (Figure 4.2). Biovolume, however, showed 3 distinct peaks which directly coincided with periods and depths of high chlorophyll *a* concentration.

Phytoplankton species diversity was highest during mid-bloom, with the identification of >60 species (primarily diatoms), ranging in cell size from 3 μm to >100 μm (C. McKenzie, pers. comm.). *Chaetoceros* spp. were numerically dominant throughout most of the bloom period. As the bloom progressed and nutrient levels

declined, species diversity decreased; only 11 species were identified in the June 2 sample and these were dominated by small flagellates.

Species which occurred in unusually high numbers during the spring period were *Coscinodiscus* spp. (centric diatoms, 20 to >70 μm) and a prasinophyte, *Pyramimonas* sp. (about 15 μm). These taxa, some of which were abundant in waters outside the bay (C. McKenzie, pers. comm.), were very common at the sampling site within the bay during late March and early April. Their numbers dramatically increased following the onset of a strong and persistent northeasterly gale which forced offshore water into the bay during the second week in April. Thereafter, *Coscinodiscus* spp. dominated the pool of large cells and persisted as a major contributor to phytoplankton biovolume throughout mid-April to late May (Table 4.1). *Pyramimonas* sp. dominated cells within the < 15 μm size class by the end of the storm in mid-April, but they did not persist and appeared in relatively low concentrations in samples collected the following week. *Pyramimonas* sp. were not detected in cell counts of samples collected during late April and thereafter (C. McKenzie, pers. comm.).

Throughout the sampling period, *Pyramimonas* was the only taxon which contributed significant amounts of chlorophyll *b* to the pigment pool. When *Pyramimonas* was abundant, extracts of seston < 15 μm were rich in chlorophyll *b* (often >30% of chlorophyll *a* concentration). In these samples, chlorophyll *b* interfered in the standard fluorometric measurements of chloropigments (chlorophyll *a* and related phaeopigments). Methodological comparisons with HPLC profiles indicate a TD10 overestimation of phaeopigments by > 10 fold during the peak of *Pyramimonas* abundance in mid-April (Figure 4.16), but because these small cells contributed little to the overall biomass of phytoplankton, their effect on TD10 measurements of chloropigments extracted from seston <505 μm was relatively minor. Overall, there was good agreement between TD10 and HPLC measurements of total chloropigments in particles <505 μm (Figure 4.17).

TD10 measurements, however, tended to be slightly higher than those determined by HPLC, which is consistent with methodological comparisons reported in Chapter 2.

4.4.3. Water Column Chloropigment Composition

The contribution of the various chloropigments within seston samples collected at various depths throughout the development and decline of the 1988 spring bloom is shown in Table 4.2. With the exception of one sample, collected in June, estimates of total phaeopigments determined using the TD10 were higher than those determined by HPLC. Differences between methods were generally greater in early to mid-April when chlorophyll *b* (*Pyramimonas* sp.) was present in concentrations exceeding 10% of chlorophyll *a* levels.

Percent chloropigment composition, determined by HPLC, showed that phaeophorbide *a*-type pigments, in particular pyrophaeophorbide *a*, dominated the phaeopigment fraction and generally increased with both depth and duration of the spring bloom (Table 4.2). At depths below 120 m, pyrophaeophorbide *a* typically comprised 20-50% of the total chloropigment pool. During May and early June, the percent contribution by phaeophytin *a*-type pigments also tended to be higher in deep waters. The relative amounts of total phaeopigments typically increased with decreasing particle size. In fractionated seston, collected at 70 m on April 29, phaeopigments comprised 9%, 15% and 44% of total chloropigments in particle material < 505 μm, < 70 μm and < 15 μm, respectively.

Although relative amounts of undegraded chlorophyll *a* declined with depth, samples collected at 200 m frequently showed concentrations representing 30-50% of the total chloropigment pool. The contribution by chlorophyll *a* allomers was usually $< 10\%$ of chlorophyll *a* levels during April, but in May the allomeric fraction was as high as 25% of total chloropigments. Chlorophyllide *a* was present in most all sample extracts

and generally showed a higher percent contribution in the upper mixed layer during May. On June 2, chlorophyllide *a* was absent from the flagellate-dominated surface waters, and was present at only very low levels (<2% of total) in samples collected at 85, 135 and 200 m. Chlorophyll *a*-type pigments comprised <20% of the chloropigments in deep waters following termination of the bloom.

4.4.4. Photosynthetic Carbon Production

Measurements of carbon fixed (g C $m^{-2}d^{-1}$), determined at intervals throughout the spring bloom, show the highest rates of photosynthesis during the second week of April (Table 4.3). A carbon production rate of 9.2 g C $m^{-2}d^{-1}$, measured on April 11, represents about 1 doubling of phytoplankton cell carbon per day and seems anomalously high compared with previous (April 9) and subsequent (April 13) daily carbon fixation determinations. The April 11 measurement of photosynthetic carbon production coincided with a prolonged northeasterly gale which advected water, pack ice and phytoplankton into the bay and increased the depth of the upper mixed layer to 110 m (Figure 4.4). Chlorophyll *a* fluorescence was near uniform throughout 0-100 m. Following shoaling of the pycnocline (Figure 4.4), daily carbon fixation was reduced, even though chlorophyll *a* concentrations at the SCM were often >3 μg/l. During this period, there was a decline in nutrient concentration and a change in the composition and cell size of dominant phytoplankton taxa (see Section 4.4.2).

Estimates of cumulative carbon fixed during April and May were determined from daily carbon fixation rates in Table 4.3, which were applied to periods of time between measurements (Table 4.4). The exceptionally high and anomalous value of 9.2 g C $m^{-2}d^{-1}$ on April 11 was omitted from these estimates. The calculations indicate that approximately 27 g C m^{-2} was fixed during April, about 70% of which occurred during the first 2 weeks. Small, chain-forming diatoms dominated phytoplankton biovolume at this time. An estimated 18 g C m^{-2} was fixed in May, a period during which nutrients

were depleted and large diatoms (*Coscinodiscus* spp., *Thalassiosira nordenskiöldii*) and dinoflagellates dominated phytoplankton biovolume. During April and May of 1988, total particulate carbon production at the study site was estimated at 45 g C m^{-2}.

4.5. Discussion

Initiation of the 1988 spring phytoplankton bloom occurred in mid-March, when the water column was nearly isothermal and <0°C. Periods of strong northeasterly winds during April and May led to the upward mixing of nutrients and a prolonged bloom which extended to the end of May. Biomass peaks, dominated by centric diatoms, occurred during mid-April and the first and third weeks of May, with maximum chlorophyll *a* levels of 6 μg/l during the two peaks in May. Surface water nutrients were markedly reduced by mid-May, depressing the SCM to depths below 40 m. Dissolved silicate appeared to be the most limiting nutrient for diatoms, followed by nitrate. As the surface waters became thermally stratified and nutrient depleted in late May, phytoplankton biomass and species diversity decreased. Upon termination of the bloom, small flagellates dominated the phytoplankton composition.

Highly variable meteorological conditions result in great interannual variation in both the duration and magnitude of the spring bloom in Conception Bay. The 1990 bloom developed under less severe storm activity and higher levels of incident light than in 1988, and terminated earlier (mid-May) following peak chlorophyll *a* levels of 12 μg/l (Deibel et al., unpubl. data). In contrast, the bloom of 1986 occurred during a prolonged period of calm and sunny conditions, was of much higher intensity, with a chlorophyll *a* peak magnitude of >20 μg/l, and promptly ended in mid-April following nutrient depletion (Deibel et al., unpubl. data).

Phytoplankton species composition in Conception Bay was also affected by the strength and frequency of storm events. In mid-April 1988, the influx of offshore

phytoplankton during a northeasterly gale resulted in a rapid change in the species which dominated algal biovolume. Small, chain-forming, centric diatoms (*Skeletonema costatum*, *Chaetoceros* spp., *Thalassiosira* spp.) were succeeded by large centric diatoms (*Coscinodiscus* spp.) and dinoflagellates. The prasinophyte *Pyramimonas* sp. was also advected into the bay and was abundant for a short period following the onset of the storm. During the calm and sunny spring of 1986, phytoplankton composition throughout the bloom was dominated by small, chain-forming diatoms, primarily *Skeletonema costatum* and *Chaetoceros* spp.

Carbon/chlorophyll *a* (C/Chl) ratios are known to vary with species composition (Weeks et al. 1993), and increase with light intensity (Langdon 1988), temperature (Falkowski 1980) and nutrient depletion (Sakshaug 1977). Ratios of about 70 characterized the SCM in both mid-April and early May. C/Chl ratios at the SCM increased to 92 during mid- to late May following species succession, nutrient depletion and increases in temperature and incident light. This late bloom ratio is similar to the C/Chl ratios of 100-104 reported for North Sea phytoplankton during late spring (Gieskes and Kraay 1984).

Below 100 m, the C/N ratio increased to values >10 following peaks in phytoplankton biomass, indicating cell senescence and/or utilization of bloom production by heterotrophs. Grazing products (primarily phaeophorbides) comprised up to 50% of the chloropigments in seston samples collected below the pycnocline. Mass flux of ungrazed phytoplankton cells was also evident, particularly in early May (Figure 4.2). Chlorophyll *a*-type pigments typically comprised $>50\%$, and as much as 83%, of the chloropigment pool in seston samples collected below 90 m during late April and May. Concentrations of chlorophyll *a* allomers and chlorophyllide *a*, a derivative of chlorophyll *a* commonly associated with cell senescence (Jeffrey 1980, Rideout and Morris 1985), were also high in the upper 50 m during this period. Following

termination of the spring bloom, chlorophyll *a*-type pigments comprised only a minor fraction (<20%) of the chloropigment pool in near-bottom waters.

Estimated photosynthetic production of 45 g C m^{-2} during April and May 1988, combined with photosynthetic rates measured prior to, and following, the spring bloom in Conception Bay in 1992 (Rivkin, unpubl. data), suggest an annual primary production of < 90 g C $m^{-2}yr^{-1}$. This estimate is significantly lower than production estimates of about 190 g C $m^{-2}yr^{-1}$ determined for nearby well-mixed offshore waters (Prasad and Haedrich 1993). Annual primary production rates of about 200 g C $m^{-2}yr^{-1}$ are typical of coastal and upwelling regions (Walsh 1984), and are frequently >300 g C $m^{-2}yr^{-1}$ in well-mixed coastal environments (Malone 1980). Water column stratification in Conception Bay during late spring and early summer results in limited upward mixing of nutrients and therefore low annual primary production relative to most temperate and north-temperate coastal areas (Malone 1980). Most of the annual primary production in Conception Bay seems to occur during March to late May when temperatures at depths of production are < +1°C.

At near zero temperatures, water column bacteria concentrations in Conception Bay are low (< 5 x 10^5 cells/ml) and microbial activity is suppressed, approaching the lowest published rates for marine waters (Pomeroy and Deibel 1986, Pomeroy et al. 1991). Microzooplankton, which are low in abundance during the bloom period (McKenzie, unpubl. data), also seem to play a minor role in the utilization of bloom production during April and May. Chloropigments extracted from water column samples showed low levels of phaeophorbide *a*2 (Table 4.2), a recently identified product of microzooplankton grazing (Strom 1993), suggesting low grazing impact by these heterotrophs during bloom production.

The primary chlorophyll *a* degradation product in seston samples collected below the SCM was pyrophaeophorbide *a* (also known as phaeophorbide *a*3), a decay product

commonly found in the gut tracts and faeces of copepods (Vernet and Lorenzen 1987, Downs 1989, Head and Horne 1993, see Chapter 3). Although phaeopigments were present in all seston samples, and often comprised a large fraction of total chloropigments at depths >70 m, chlorophyll *a*-type pigments generally dominated the chloropigment pool in deep waters during late April and May, suggesting cell sinking as the primary pathway of vertical flux. The impact of zooplankton grazing on the fate of spring phytoplankton production in Conception Bay is apparently limited due to a temporal phase lag in the biomass peaks of phytoplankton and zooplankton at near zero temperatures (Cushing 1975). It is expected that the effects of zooplankton grazing will be further reduced in years of early termination of the bloom. This feature was evident during 1986 when the spring bloom production collapsed in mid-April, resulting in massive sinking of intact diatoms and relatively low faecal pellet flux (Thompson et al., unpubl. data). Interannual variability in the relative contributions of sinking cells and faecal material to the vertical flux of bloom material appears to be primarily dependent on the magnitude and duration of the spring bloom.

Table 4.1. Dominant phytoplankton taxa, by abundance (cells/l) and by biovolume ($\mu m^3/l$), at or near the SCM in Conception Bay during the spring of 1988. ( ) number of species identified, * (dominant species within algal cells $< 15\mu m$), † (prolonged northeasterly gale). Species list from McKenzie (unpubl. obs.).

Date 1988	Depth (m)	Dominant Taxa (abundance)	Dominant Taxa (biovolume)
Mar 31	15	*Chaetoceros debilis* *Chaetoceros socialis* *Skeletonema costatum*	*Chaetoceros debilis* *Chaetoceros socialis* *Skeletonema costatum*
Apr 6	15	*Chaetoceros debilis* *Thalassiosiria nordenskiöldii* *Thalassiosiria gravida*	*Chaetoceros debilis* *Coscinodiscus* spp. (7) *Thalassiosira* spp.
Apr 11 †	20	*Chaetoceros* debilis *Skeletonema costatum* *Thalassiosira gravida*	*Chaetoceros decipiens* *Skeletonema costatum* *Thalassiosira gravida*
Apr 15	20	*Chaetoceros debilis* *Skeletonema costatum* *Pyramimonas* sp. *	*Coscinodiscus* spp. Dinoflagellates
Apr 27	20	*Chaetoceros debilis* *Coscinodiscus* spp. (7) *Leptocylindrus* spp. *Skeletonema costatum*	*Chaetoceros decipiens* *Coscinodiscus* spp.
May 6	20	*Coscinodiscus* spp. (4) *Thalassiosira nordenskiöldii* *Thalassiosira subtilis* Fragillaria Flagellates	*Coscinodiscus* spp. *Thalassiosira nordenskiöldii*
May 11	25	*Coscinodiscus* spp. (7) *Thalassiosira decipiens* *Thalassiosira nordenskiöldii*	*Coscinodiscus* spp. Dinoflagellates
May 24	45	*Chaetoceros* spp. *Coscinodiscus* spp. (2) Flagellates	*Coscinodiscus* spp. Dinoflagellates
June 2	25	Dinoflagellates (7) Other Flagellates	Dinoflagellates

Table 4.2. Chloropigment concentration (TD10, HPLC) and composition (HPLC) of seston $<505\mu m$ diameter at various depths during spring 1988. Identities are: chlld (chlorophyllide *a*), chl *a* (chlorophyll *a*), pho (phaeophorbide *a*), pyr (pyro-), phy+ (phaeophytin *a*-types). Note: * $<70\mu m$, ** $<15\mu m$, (*b*) abundant chl *b* (>10% of chl *a* conc.).

Date	Depth (m)	TD10 Total $\mu g/l$ (% phaeo)	HPLC Total $\mu g/l$ (% phaeo)	% chlld	% chl *a* allomer	% chl *a*	% pho 1	% pho 2	% pyr-pho (3)	% pho 4,5	% phy+
Mar 31	7 (*b*)	1.36 (30)	1.16 (6)	4.6	7.9	81.3	1.7	0	2.0	1.1	1.3
	130	0.31 (35)	0.24 (19)	4.2	7.5	68.8	3.6	0	6.1	8.8	1.0
Apr 4	25 (*b*)	1.26 (30)	0.61 (8)	5.7	10.9	75.1	2.4	1.8	1.6	2.5	0.0
Apr 13	15 (*b*)	3.00 (29)	3.35 (6)	4.9	8.4	80.4	0.7	2.0	1.2	1.7	0.7
	25 (*b*)	3.06 (28)	2.38 (6)	5.4	7.3	81.2	0.7	0	2.8	1.3	1.4
	75	2.78 (23)	2.54 (3)	5.2	8.3	83.0	0.4	0	1.2	0.8	1.0
	105	0.26 (54)	0.17 (22)	3.3	10.1	64.6	3.4	2.5	9.9	4.8	1.4
Apr 14	15 (*b*)	3.28 (30)	2.86 (8)	4.3	6.8	81.1	1.4	0	2.4	1.5	2.5
	55 (*b*)	2.62 (30)	2.40 (6)	4.5	7.7	81.9	1.2	0.2	1.6	0.7	2.3
	110	0.80 (33)	0.69 (12)	3.6	6.1	78.4	0.8	0	7.1	2.9	1.1
Apr 15	15 (*b*)	3.17 (31)	2.84 (9)	5.1	6.4	79.4	1.6	0.1	4.6	1.4	1.4
	25 (*b*)	3.39 (32)	3.16 (11)	5.2	5.6	78.2	1.6	0.4	4.4	1.3	3.2
	75 (*b*)	2.56 (23)	2.37 (9)	4.1	7.1	80.1	1.3	0.3	3.8	1.7	1.5
Apr 16	15 (*b*)	3.28 (38)	3.01 (19)	5.0	6.0	69.9	3.1	0.3	6.6	5.4	3.7
	25 (*b*)	3.06 (32)	2.75 (14)	6.4	7.9	71.3	2.3	0.2	4.2	2.4	5.2
	60 (*b*)	2.02 (25)	2.31 (8)	27.3	4.0	60.3	5.7	0.0	0.8	0.3	1.7
	90 (*b*)	1.11 (22)	0.92 (11)	6.1	5.8	77.0	1.1	0.4	5.4	1.6	2.5
	200	0.16 (84)	0.07 (66)	0	1.6	32.5	17.5	4.0	26.6	9.9	7.9

Table 4.2. continued.

Date	Depth (m)	TD10 Total μg/l (% phaeo)	HPLC Total μg/l (% phaeo)	% chlld	% chl *a* allomer	% chl *a*	% pho 1	% pho 2	% pyr-pho (3)	% pho 4,5	% phy+
Apr 25	25	1.68 (23)	1.84 (11)	3.2	7.6	78.1	3.1	0.0	4.9	1.5	1.5
	70	3.66 (19)	2.90 (7)	1.6	9.3	82.6	0.6	0.0	3.0	0.5	2.4
Apr 29	10	5.18 (5)	5.67 (4)	4.5	13.9	78.0	2.4	0.0	0.4	0.0	0.7
	45	1.20 (26)	1.29 (16)	1.7	7.1	75.2	2.3	0.5	9.6	1.8	1.8
	70	2.29 (16)	2.17 (9)	2.4	12.7	75.7	0.8	3.0	0.7	1.9	2.8
	70 *	0.85 (41)	0.76 (25)	1.6	8.9	64.2	1.2	0.5	11.1	5.5	7.0
	70 **	0.40 (55)	0.29 (44)	0.7	6.1	49.3	2.5	2.3	22.3	9.4	7.4
	95	0.96 (25)	0.85 (17)	3.6	12.1	67.0	1.8	0.2	12.5	1.6	1.2
May 9	15	3.11 (31)	2.40 (22)	9.7	7.6	61.0	18.5	0.8	0.0	1.0	1.4
	35	3.60 (29)	3.50 (21)	7.7	9.3	61.7	17.5	0.3	0.7	0.8	1.9
	85	2.02 (30)	2.01 (20)	3.5	13.6	62.5	1.8	0.4	7.0	3.7	7.5
	142	0.61 (50)	0.53 (38)	4.1	14.7	43.2	2.4	0.9	17.1	7.3	10.3
	200	0.33 (57)	0.17 (34)	3.6	14.2	48.3	3.0	0.4	19.7	8.5	2.1
May 11	25	3.30 (28)	3.21 (21)	7.3	11.3	59.9	16.8	0.0	2.8	0.9	0.9
	40	5.56 (23)	5.27 (15)	4.0	15.8	65.1	9.0	0.5	2.6	1.7	1.2
	110	0.89 (49)	0.84 (33)	2.6	9.4	55.5	3.0	0.3	20.6	5.3	3.3
	155	0.58 (58)	0.47 (34)	3.2	13.5	49.5	3.4	0.0	20.5	6.7	3.2
	210	0.32 (71)	0.16 (53)	0.9	8.4	38.1	7.1	0.0	29.7	8.7	7.1

Table 4.2. continued.

Date	Depth (m)	TD10 Total μg/l (% phaeo)	HPLC Total μg/l (% phaeo)	% chlld	% chl *a* allomer	% chl *a*	% pho 1	% pho 2	% pyr-pho (3)	% pho 4,5	% phy+
May 12	45	2.61 (27)	2.31 (15)	8.8	25.2	51.1	5.2	1.1	5.0	2.1	1.6
	70	1.64 (36)	1.34 (28)	3.8	19.6	48.9	2.5	0.0	11.5	12.1	1.7
	110	0.57 (44)	0.52 (36)	3.9	17.6	42.8	4.2	1.1	17.5	5.2	7.6
	200	0.34 (60)	0.25 (42)	3.6	15.8	38.3	6.1	1.2	25.2	6.7	3.0
May 17	35	5.24 (16)	4.58 (7)	5.9	11.4	75.2	5.2	0.5	0.6	0.9	0.2
	70	0.65 (40)	0.37 (18)	9.6	23.0	49.0	4.7	0.0	6.6	4.9	2.2
	135	0.68 (62)	0.61 (49)	3.4	12.4	35.4	3.6	0.0	28.7	7.3	9.2
May 24	45	2.68 (22)	1.76 (16)	6.1	7.8	70.5	3.9	0.2	10.3	0.8	0.4
May 26	50	1.80 (16)	1.67 (14)	4.8	9.7	71.8	2.9	0.5	7.8	1.7	0.9
	70	0.51 (36)	0.42 (31)	5.5	16.2	46.9	7.1	1.6	20.0	1.1	1.6
	95	0.35 (49)	0.32 (34)	6.2	20.5	38.9	5.7	0.0	24.0	2.5	2.2
June 2	25	0.07 (29)	0.07 (32)	0.0	3.7	64.7	0.0	0.0	31.5	0.0	0.0
	85	0.21 (64)	0.17 (52)	1.3	7.6	38.7	2.0	0.9	49.0	0.6	0.0
	135	0.32 (82)	0.21 (73)	1.9	6.9	18.5	6.5	0.0	51.1	9.4	5.6
	200	0.40 (85)	0.32 (82)	0.3	3.1	14.3	10.4	1.6	48.6	10.1	11.5

Table 4.3. Chlorophyll *a* concentration at the SCM, integrated chlorophyll *a* concentration to 40 and 100 m, and particulate carbon fixed in the water column. Data from Pomeroy et al. (1991). † (strong northeasterly gale).

Date 1988	Chl *a* max depth (m)	Chlorophyll *a* conc.			Particulate Carbon fixed ($g\ C\ m^{-2}\ d^{-1}$)
		($mg\ m^{-3}$) max.	($mg\ m^{-2}$) 0-40 m	($mg\ m^{-2}$) 0-100 m	
Mar 24	25	0.6	22	28	0.05
Apr 9	15	4.5	100	110	1.01
Apr 11 †	20	3.0	128	195	9.17
Apr 13	25	1.4	98	249	2.26
Apr 16	28	1.8	99	205	0.59
Apr 25	45	1.8	64	125	0.41
Apr 29	46	3.5	58	184	0.36
May 7	20	3.2	134	239	0.54
May 9	40	2.1	145	272	0.73

Table 4.4. Cumulative carbon production ($g\ C\ m^{-2}$) during April and May, estimated from measurements of particulate carbon fixed per day shown in Table 4.3. Measurement during storm conditions on April 11 was omitted. * (applied average of measurements determined on May 7 and May 9).

Dates 1988	Carbon Fixed ($g\ C\ m^{-2}\ d^{-1}$)	Carbon Fixed ($g\ C\ m^{-2}$)	Cumulative Carbon fixed ($g\ C\ m^{-2}$)
Apr 1-10	1.01	10.01	10.0
Apr 11-14	2.16	8.64	18.6
Apr 15-25	0.59	6.49	25.1
Apr 26-28	0.41	1.23	26.3
Apr 29-30	0.36	0.72	27.1
May 1-6	0.36	2.16	29.2
May 7-8	0.54	1.08	30.3
May 9-10	0.73	1.46	31.8
May 11-31 *	0.63	13.23	45.0

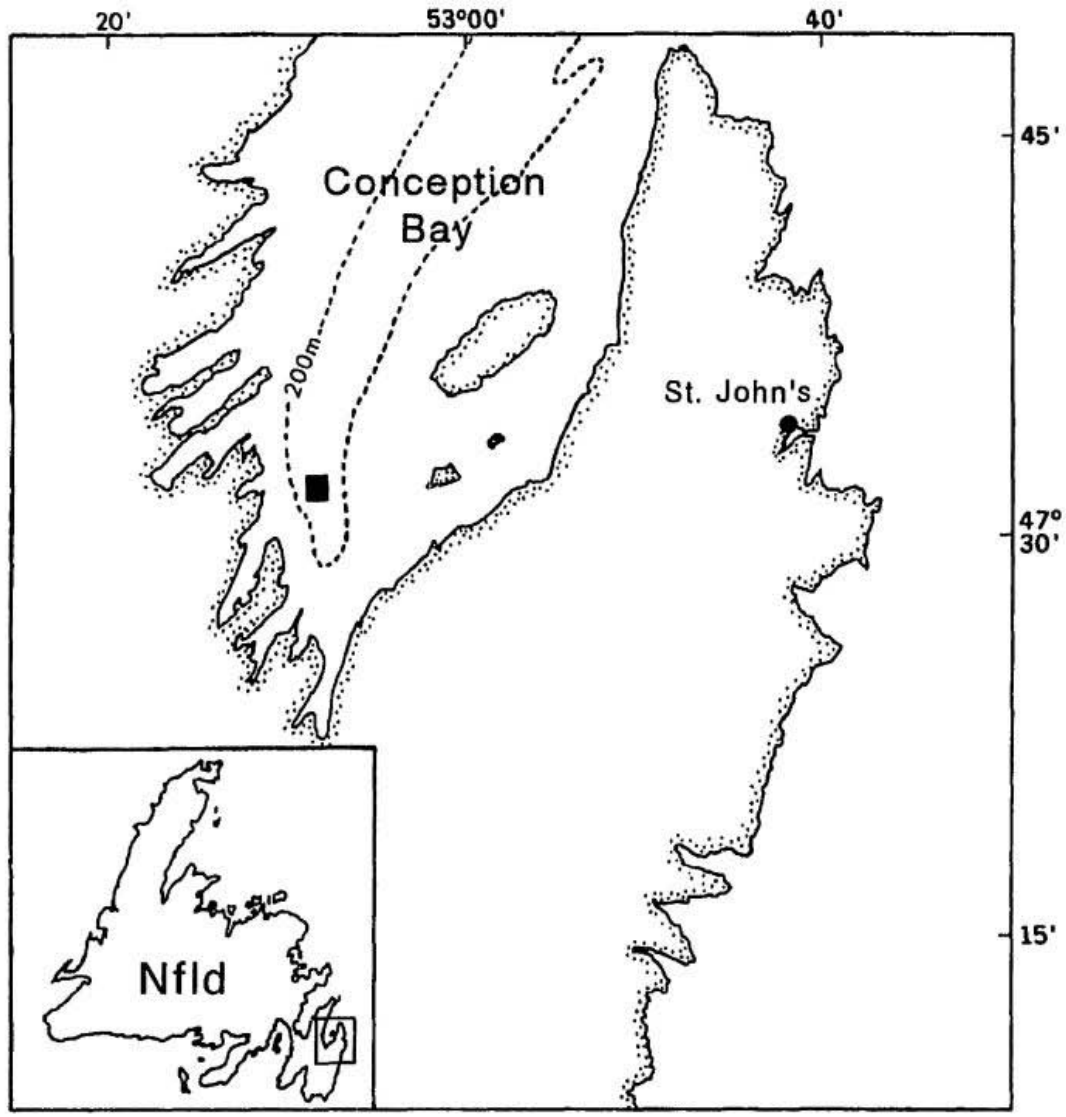

Figure 4.1. Map of Conception Bay, Newfoundland, indicating location of sampling station (47°32'N, 53°08'W). Depth at the sampling site (■) is approximately 250 m.

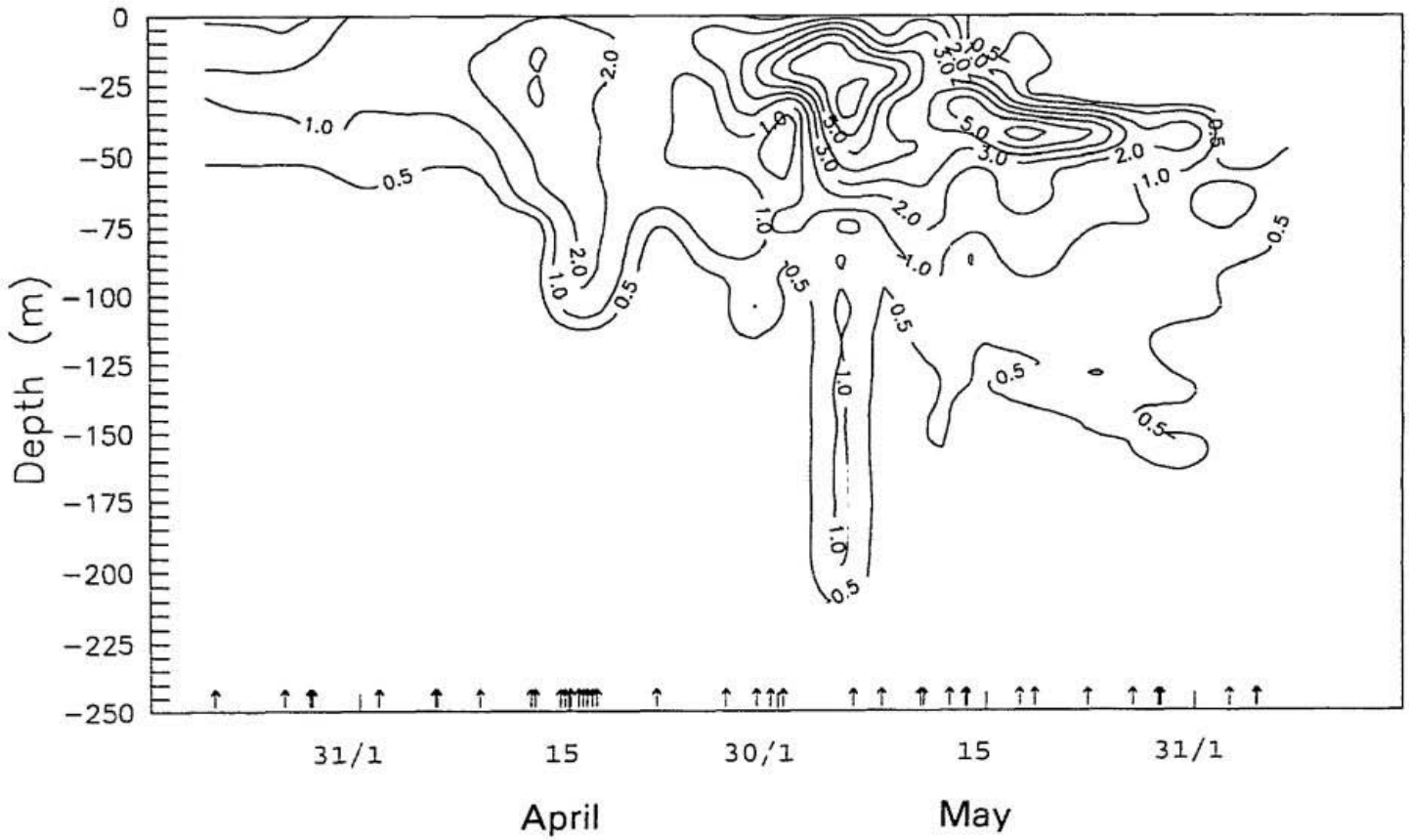

Figure 4.2. Time-depth plot of chlorophyll *a* concentration (μg/l) at the Conception Bay sampling site during mid-March to early June, 1988. Measurements were determined using a Seabird SBE 20 CTD with SeaTech *in situ* fluorometer. Arrows indicate dates of CTD casts.

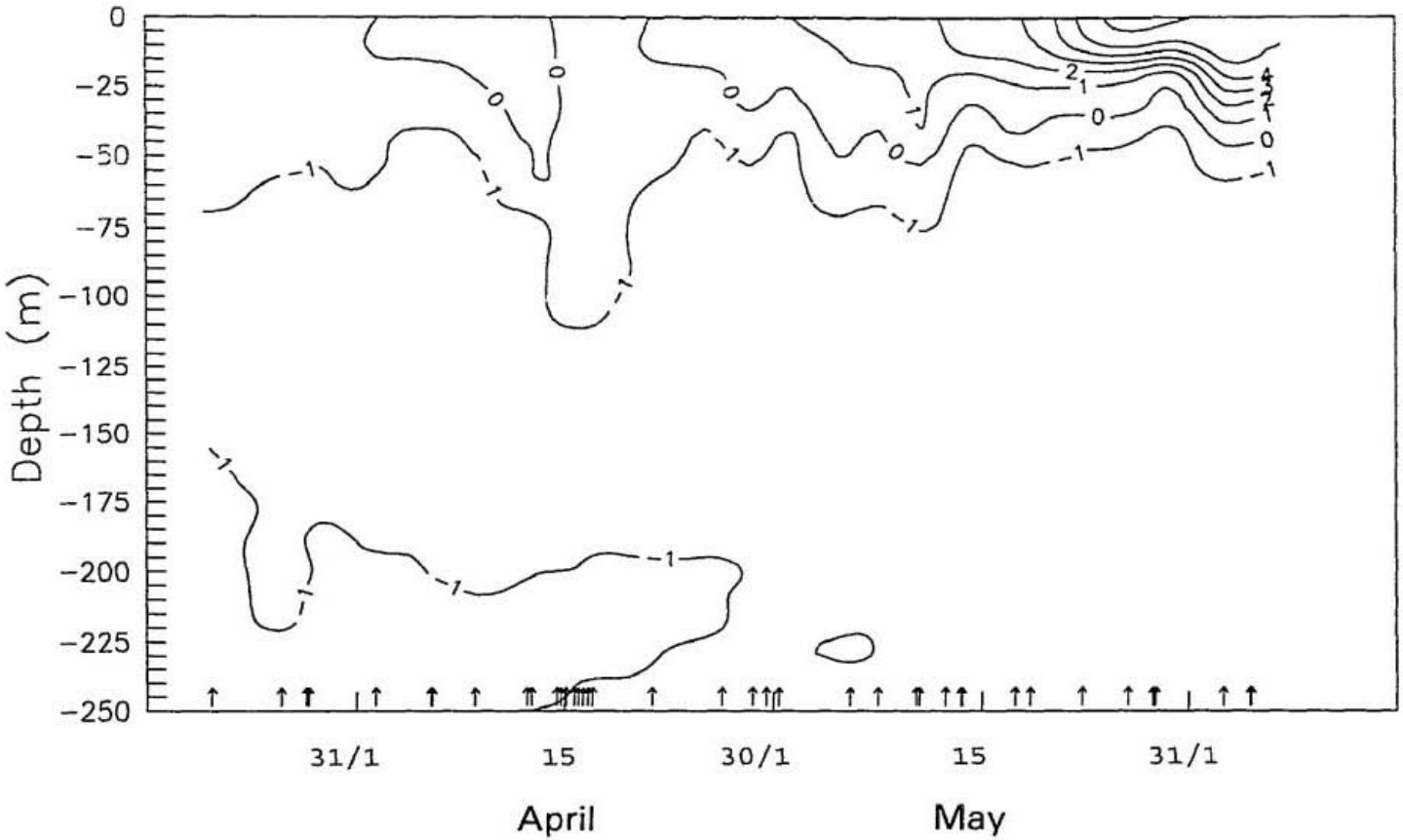

Figure 4.3. Time-depth plot of temperature (°C) at the Conception Bay sampling site during mid-March to early June, 1988. Measurements were determined using a Seabird SBE 20 CTD. Arrows indicate dates of CTD casts.

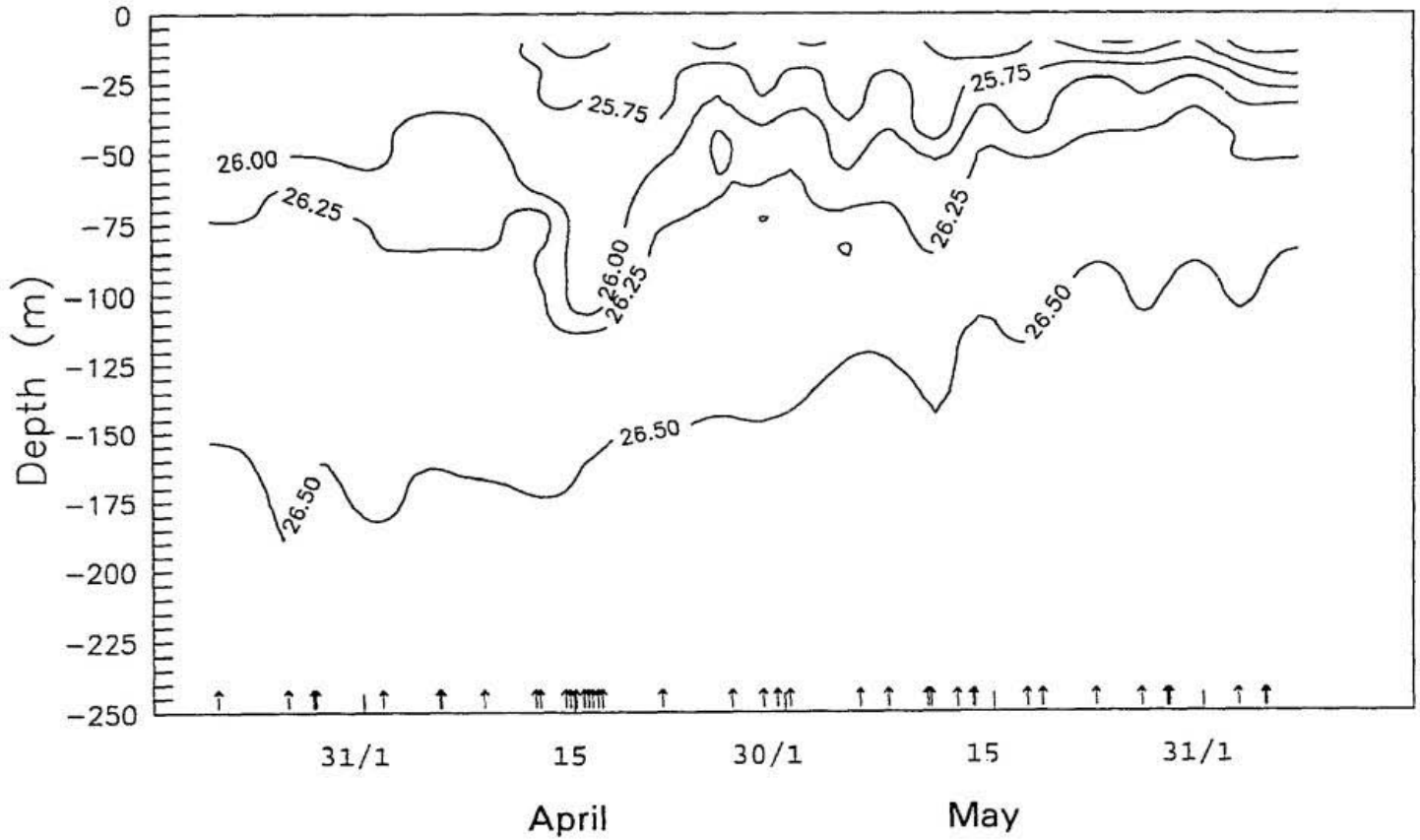

Figure 4.4. Time-depth plot of density (σ_t) at the Conception Bay sampling site during mid-March to early June, 1988. Measurements were determined using a Seabird SBE 20 CTD. Arrows indicate dates of CTD casts. Due to noisy surface measurements, the top 12 m were removed from the contour plot.

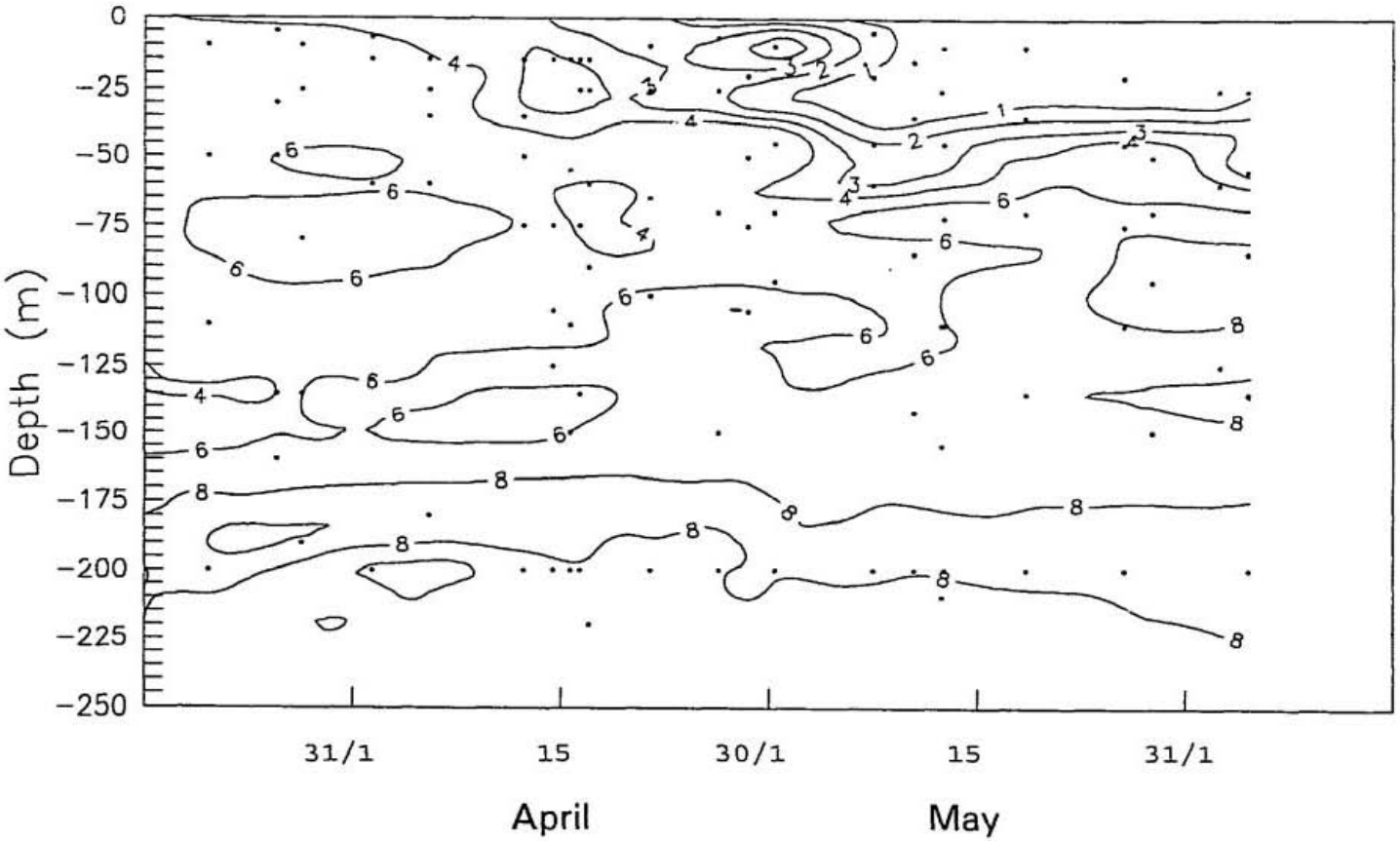

Figure 4.5. Time-depth plot of dissolved inorganic nitrogen concentration (nitrate, μM/l) at the Conception Bay sampling site during mid-March to early June, 1988.

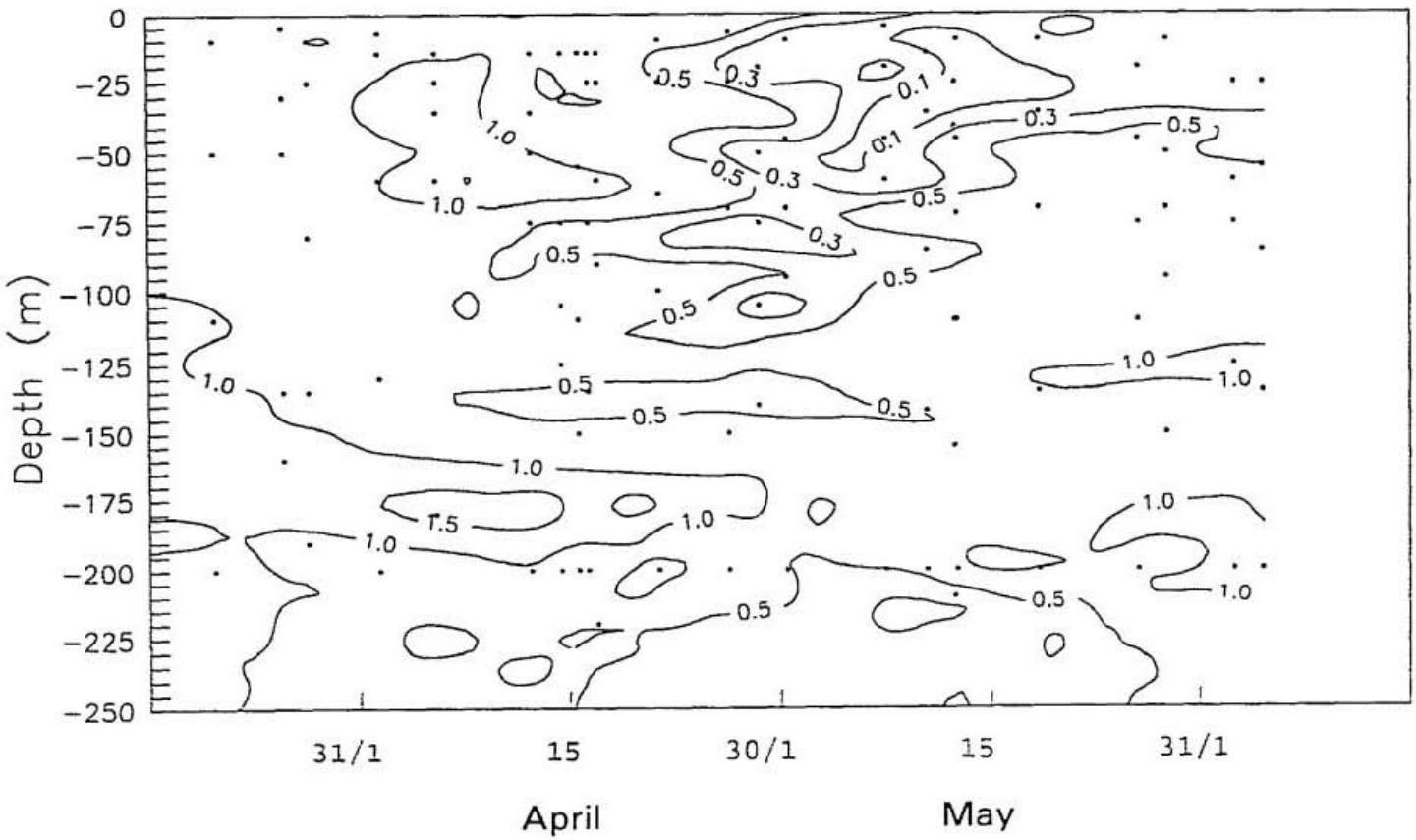

Figure 4.6. Time-depth plot of reactive phosphate concentration (μM/l) at the Conception Bay sampling site during mid-March to early June, 1988.

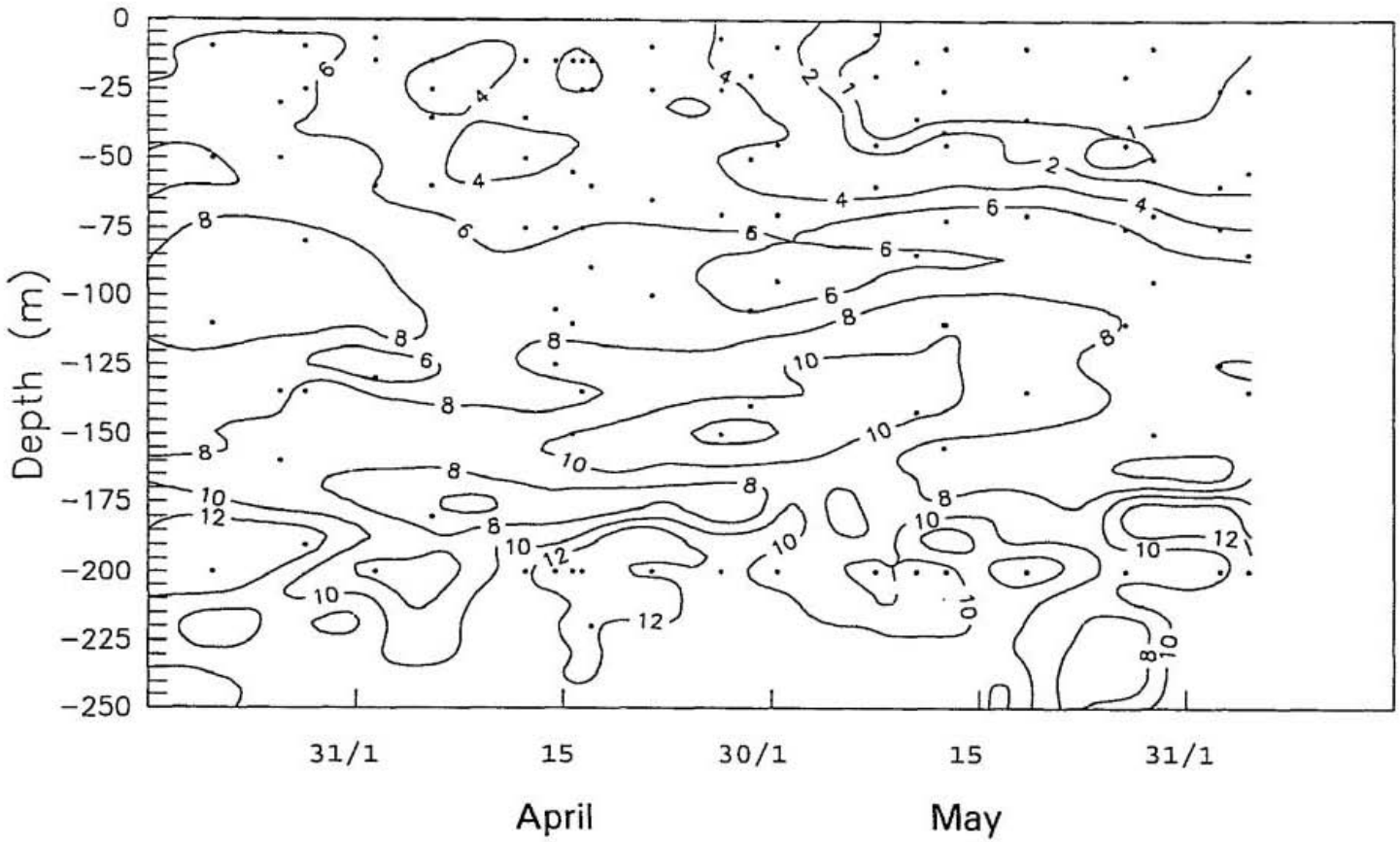

Figure 4.7. Time-depth plot of dissolved silicate concentration (μM/l) at the Conception Bay sampling site during mid-March to early June, 1988.

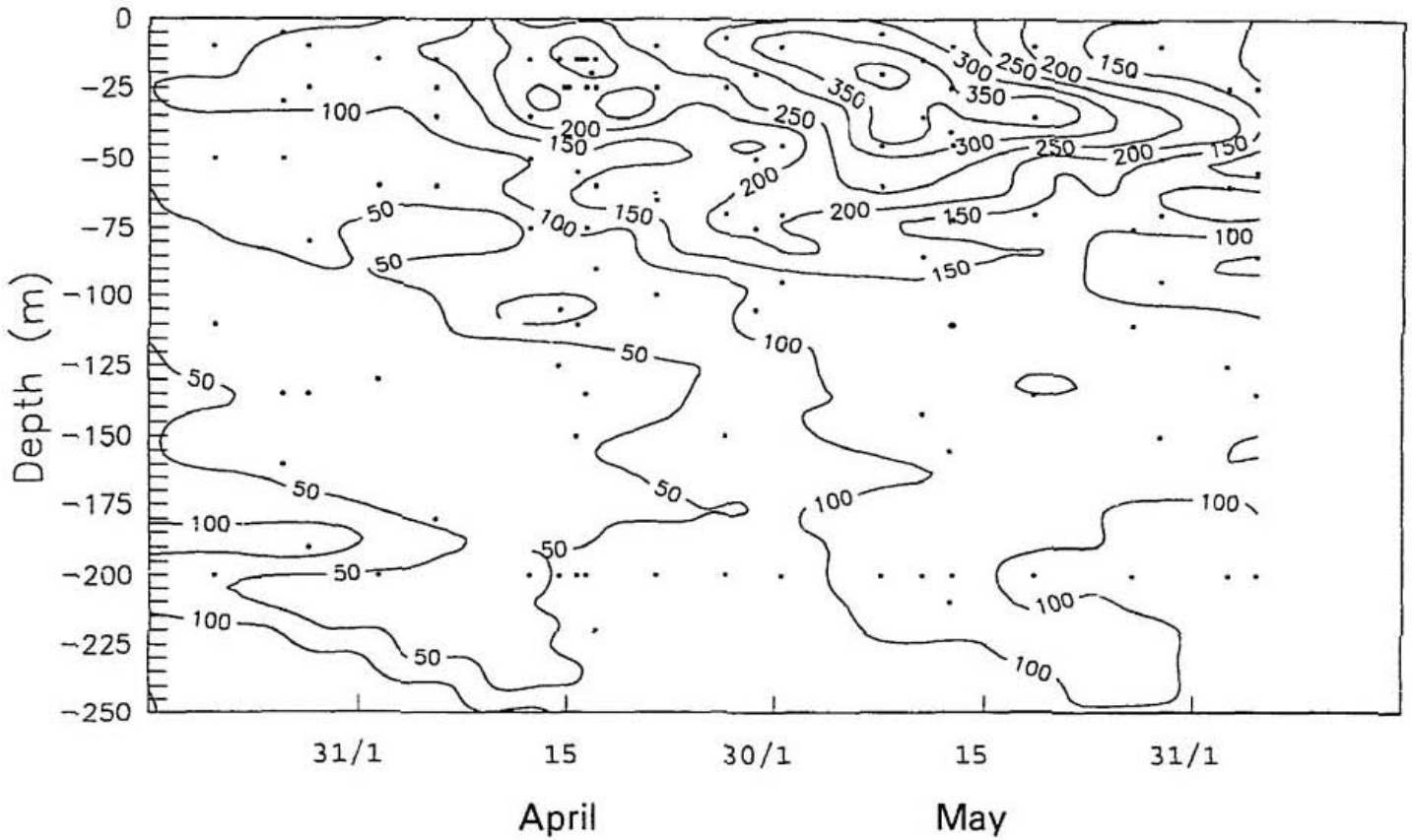

Figure 4.8. Time-depth plot of particulate organic carbon concentration (μg/l) in seston <505 μm at the Conception Bay sampling site during mid-March to early June, 1988.

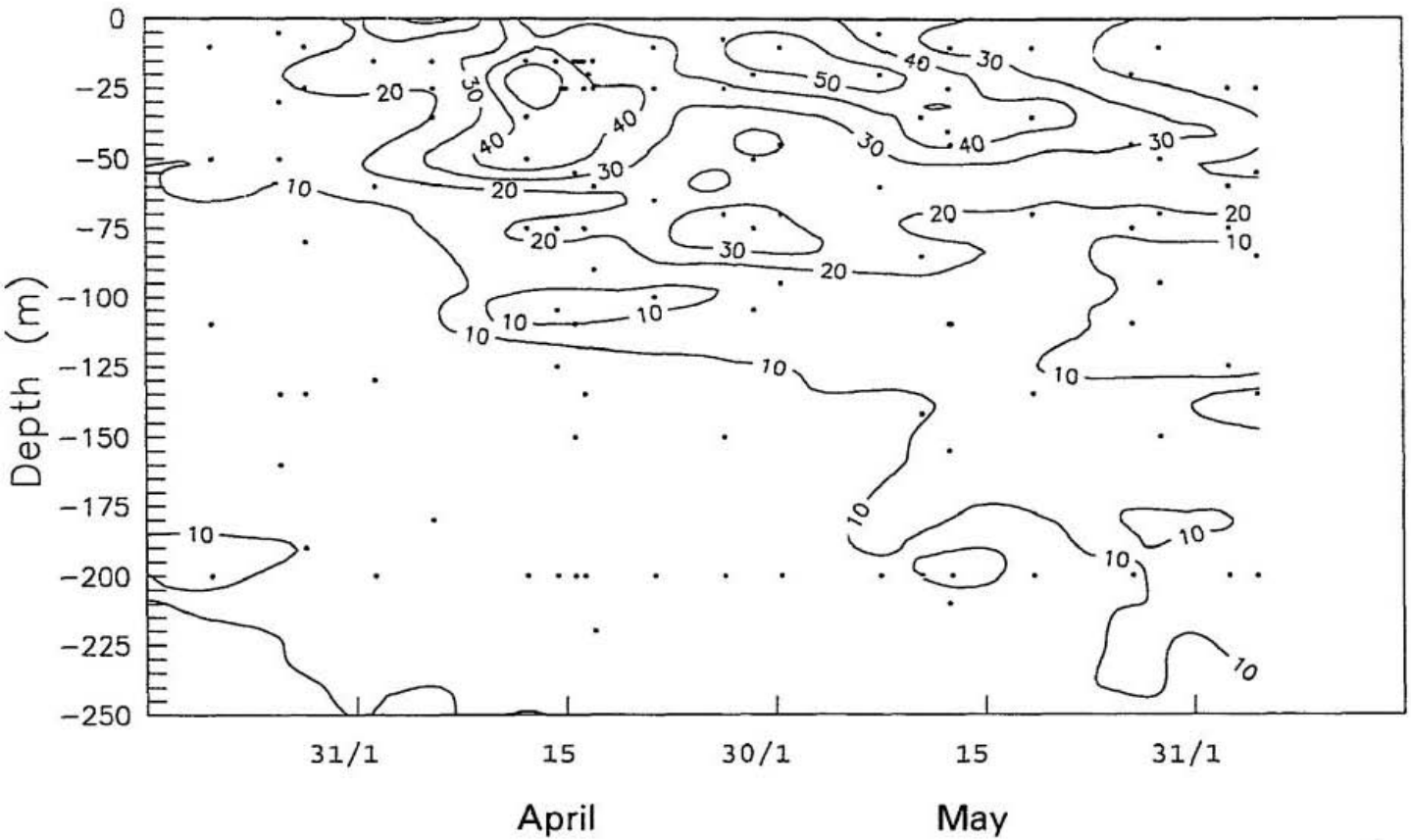

Figure 4.9. Time-depth plot of particulate organic nitrogen concentration (μg/l) in seston <505 μm at the Conception Bay sampling site during mid-March to early June, 1988.

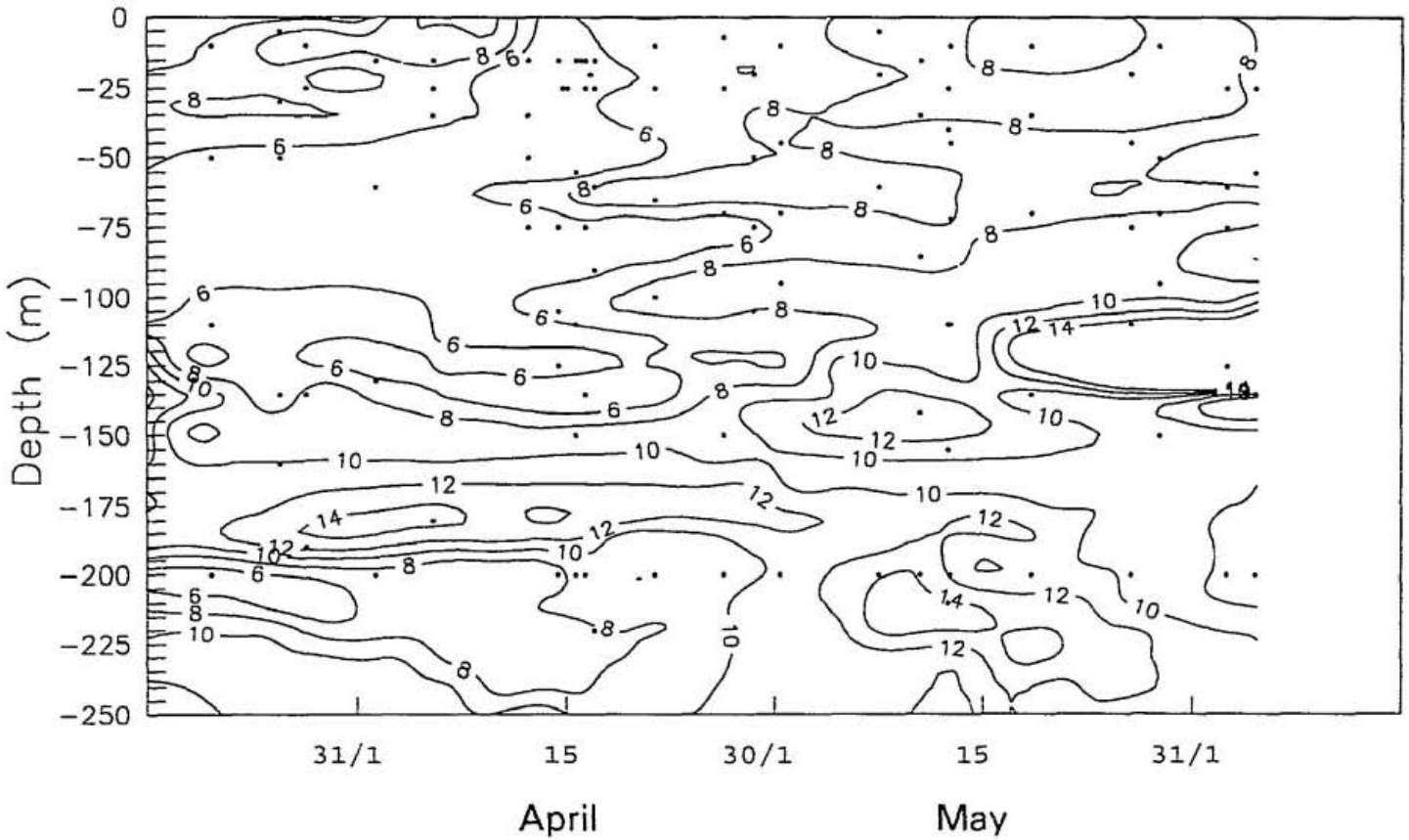

Figure 4.10. Time-depth plot of particulate carbon/nitrogen ratios in seston <505 μm at the Conception Bay sampling site during mid-March to early June, 1988.

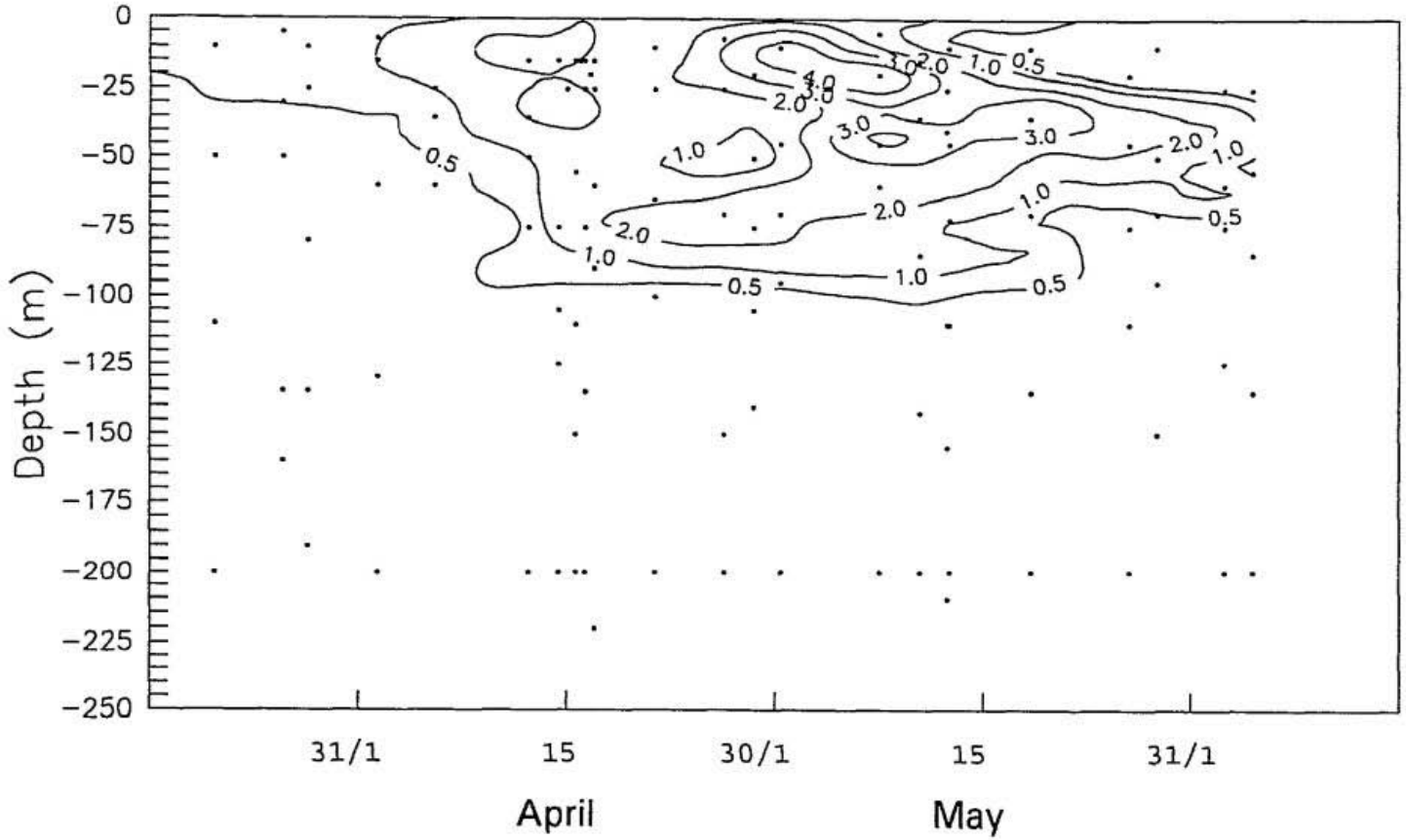

Figure 4.11. Time-depth plot of chlorophyll *a* concentration (μg/l) in seston <505 μm at the Conception Bay sampling site during mid-March to early June, 1988. Measurements determined from 90% acetone extracts of filtered samples.

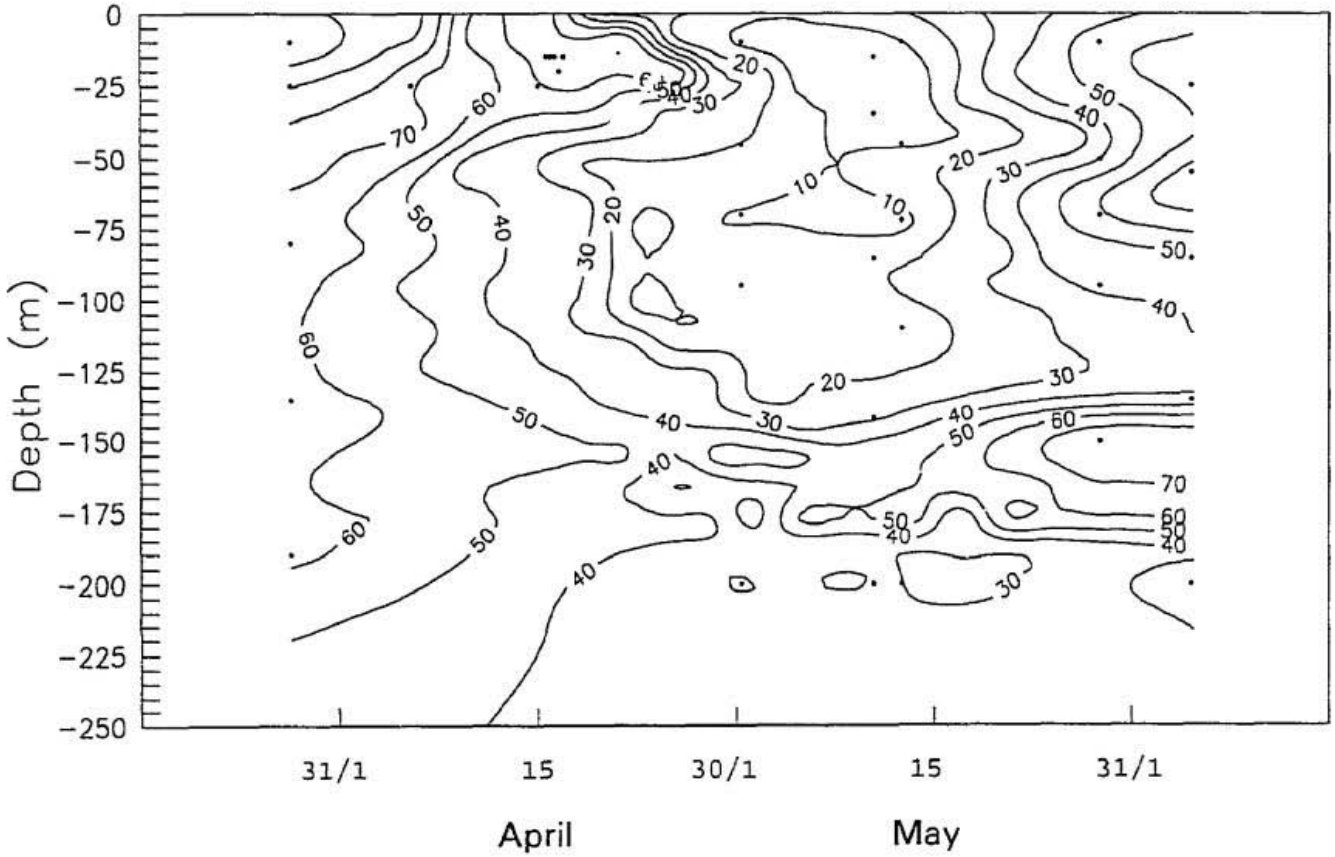

Figure 4.12. Time-depth plot of percent chlorophyll *a* in seston < 15 μm at the Conception Bay sampling site during mid-March to early June, 1988. Measurements determined from extracts of filtered samples of fractionated seston.

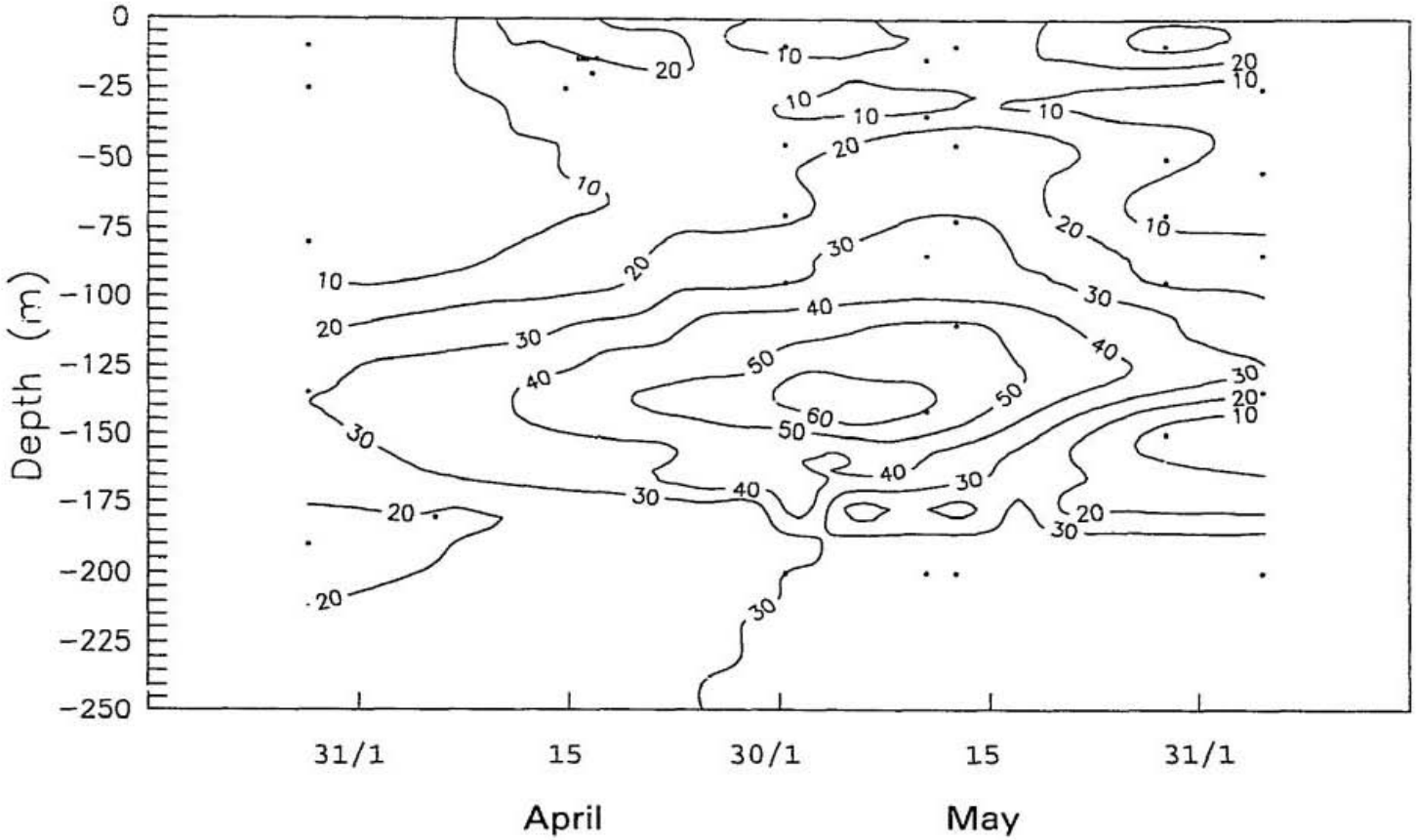

Figure 4.13. Time-depth plot of percent chlorophyll *a* in seston 15-70 μm at the Conception Bay sampling site during mid-March to early June, 1988. Measurements determined from extracts of filtered samples of fractionated seston.

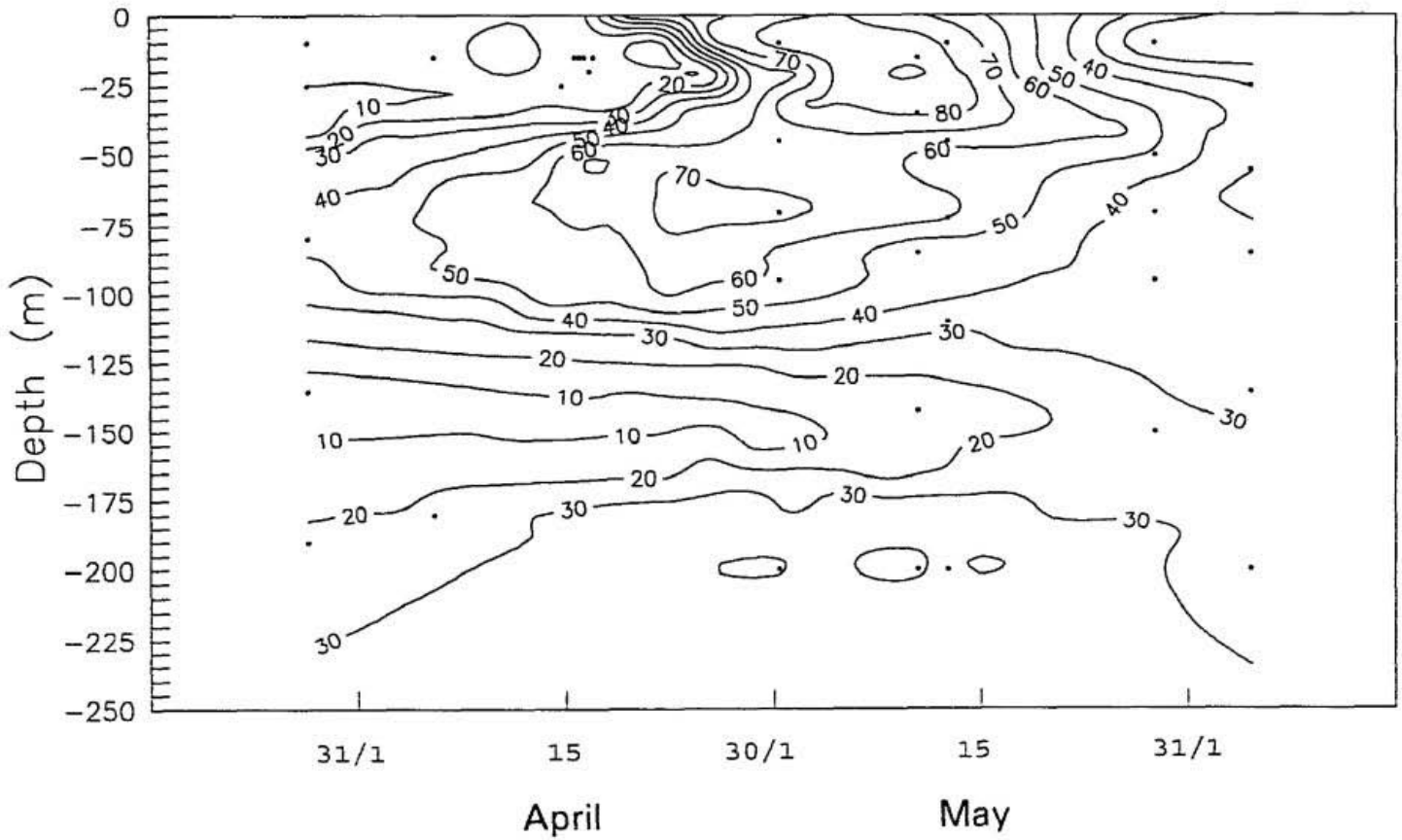

Figure 4.14. Time-depth plot of percent chlorophyll *a* in seston >70 μm at the Conception Bay sampling site during mid-March to early June. 1988. Measurements determined from extracts of filtered samples of fractionated seston.

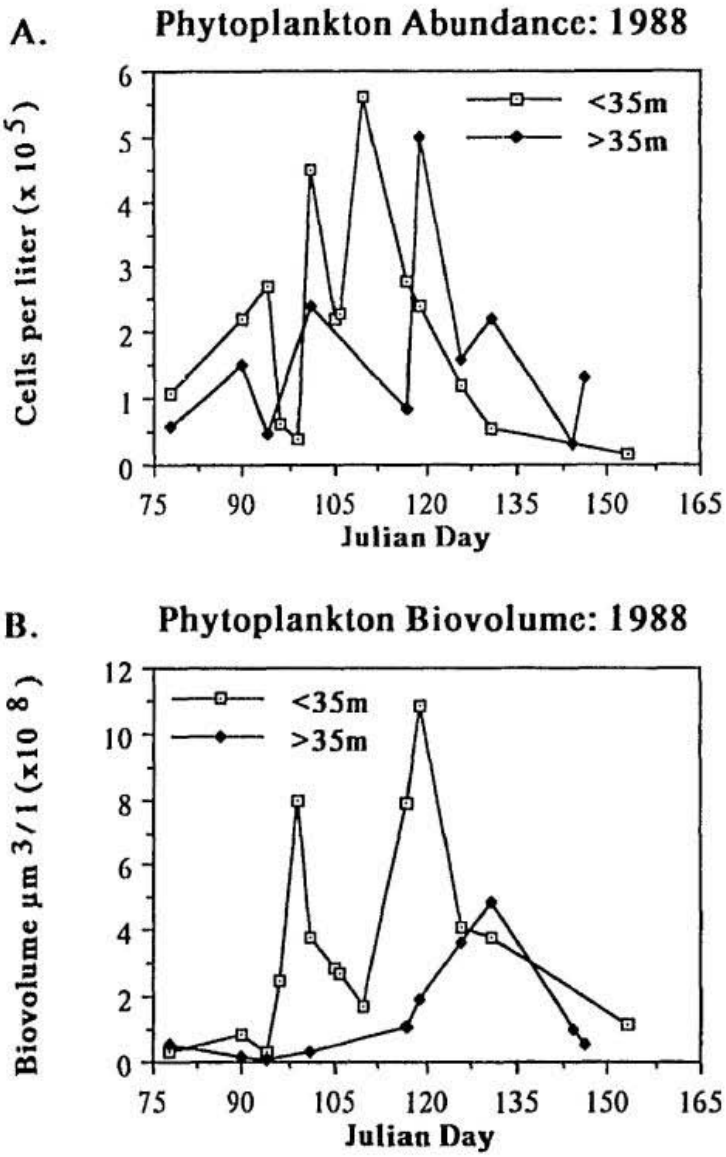

Figure 4.15. Phytoplankton abundance (cells/l) and biovolume ($\mu m^3/l$) at selected depths above and below 35 m during mid-March to early June, 1988. From McKenzie (unpub. data). Julian day equivalents: 75 (March 16), 90 (March 30), 105 (April 15), 120 (April 30), 135 (May 15), 150 (May 30).

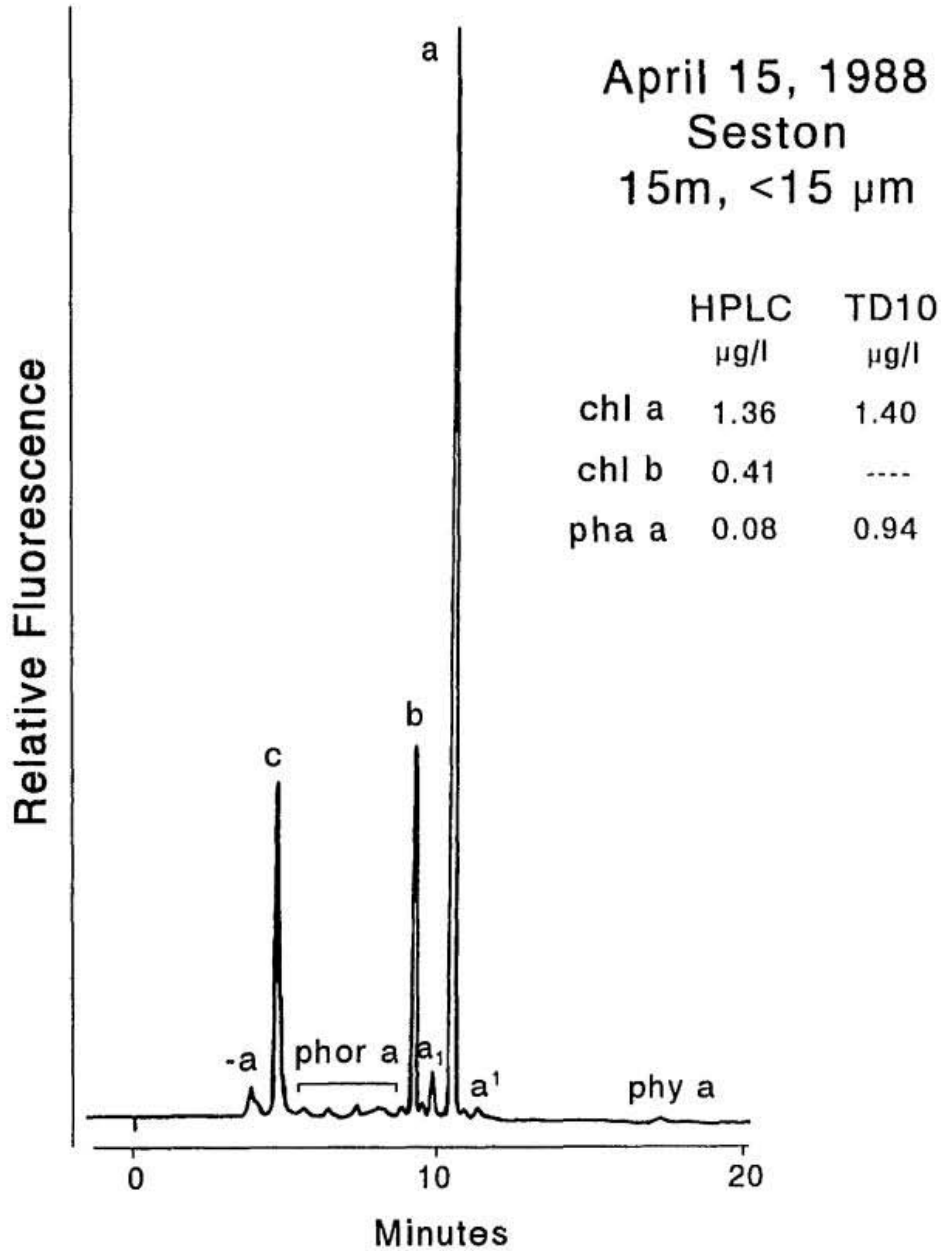

Figure 4.16. HPLC fluorescence profile of pigments extracted from seston < 15µm collected from a depth of 15 m on April 15, 1988. Peak identities are: *-a* (chlorophyllide *a*), *c* (chlorophyll *c*), *b* (chlorophyll *b*), *a* (chlorophyll *a*), a_1 (allomers of *a*), a^1 (epimers of *a*), phor *a* (phaeophorbide *a*-types), phy *a* (phaeophytin *a*). Note the TD10 overestimation of phaeopigments (pha) in the presence of high levels of chlorophyll *b*.

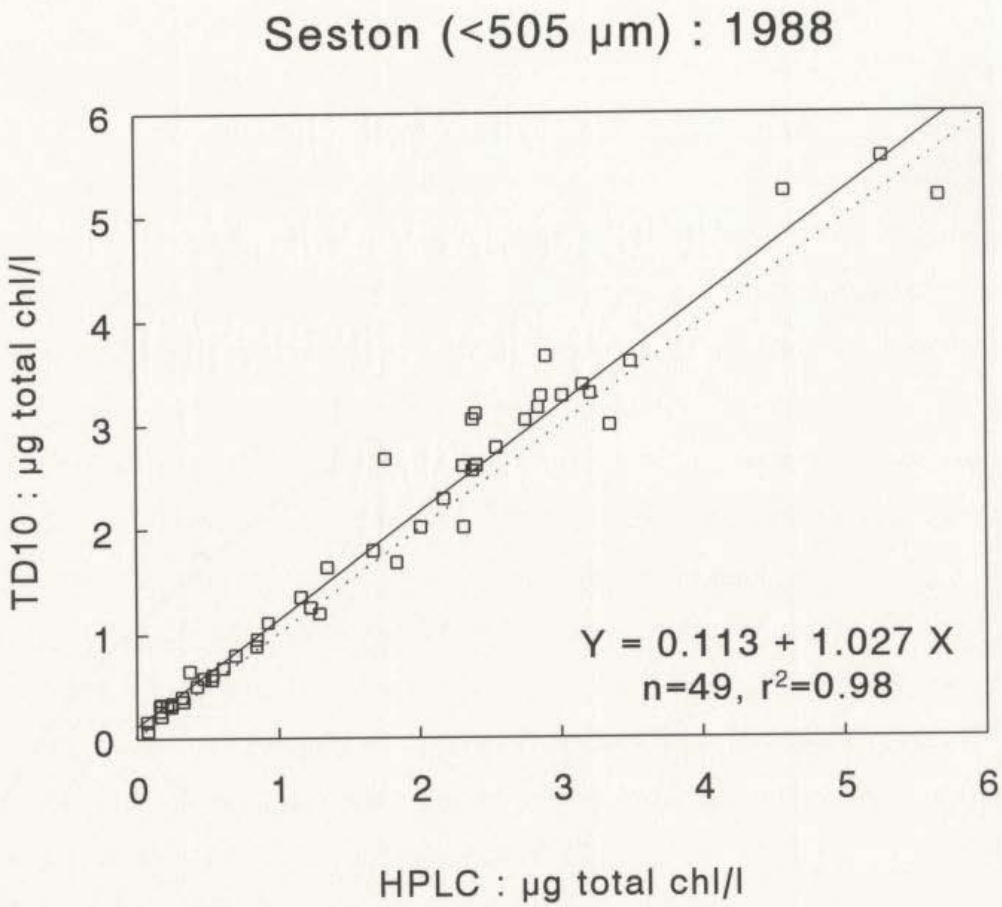

Figure 4.17. Relationship between standard fluorometric (TD10) and HPLC measurements of total chloropigments (μg/l) in seston $<505\mu$m collected during the spring 1988. Dotted line represents a 1:1 ratio.

Chapter 5

CHLOROPIGMENTS IN THE GUT TRACTS AND FAECES OF COMMON ZOOPLANKTON AND BIVALVE GRAZERS

5.1. Introduction

Herbivorous zooplankton are significant grazers of phytoplankton production in many coastal waters, and are important contributors to the vertical flux of particulate organic matter and phytopigments (Welschmeyer and Lorenzen 1985b, Bathmann and Leibezeit 1986). Copepods commonly dominate the biomass of the water column grazers and in some coastal environments they have been reported to ingest $\geq 50\%$ of the annual primary production (Dagg and Turner 1982, Welschmeyer and Lorenzen 1985b). Many copepod species exhibit both diel feeding periodicities and vertical migration rhythms to and from the upper mixed layer (Mackas and Bohrer 1976, Baars and Oosterhuis 1984, Dagg 1985, Napp et al. 1988, and many others), with most feeding and faecal pellet production occurring in the near surface layers during periods of high phytoplankton production. Faecal pellets egested in the upper water column are subject to further decay processes (e.g. reingestion, microbial attack) and to sedimentation to deeper waters. The contribution to the vertical flux of carbon and phytopigments can be enhanced by the egestion of faecal material of phytoplankton origin following the downward migration of satiated grazers (Angel 1984, Harding et al. 1988).

Pelagic tunicates can also play an important role in the utilization and vertical transport of primary production (Alldredge 1972, 1977; Paffenhöfer 1973). These suspension feeders have much higher clearance rates than do most other zooplankters, and retain smaller particles (Alldredge and Madin 1982, Deibel 1988, Deibel and Lee 1992). They occur in periodic blooms which contribute to high fluxes of particulate organic matter (Pomeroy and Deibel 1980, Taguchi 1982). The grazing impact of the pelagic tunicate, *Oikopleura vanhoeffeni*, a seasonally abundant species in coastal Newfoundland waters (Davis 1982, 1986; Mahoney and Buggeln 1983), is considerable,

with estimates of grazing comparable to that of the copepods (Knoechel and Steel-Flynn 1989).

The gut fluorescence method of Mackas and Bohrer (1976) has been commonly employed to examine *in situ* the feeding periodicities of herbivorous copepods, and to quantify zooplankton grazing impact (reviewed in Chapter 3). Limitations associated with the use of chloropigments as tracers of ingested biomass include possible losses to nondetectable products. Combined with HPLC analysis, however, the method can provide valuable information on the contribution of chlorophyll *a* and its decay products, contained in faecal pellets, to the chloropigment pools in the water column. Recent HPLC evidence has shown that specific phaeopigment derivatives of chlorophyll *a* are formed as a result of the digestive processes of various grazers (Vernet and Lorenzen 1987, Downs 1989, Abele-Oeschger and Theede 1991, Strom 1993, and others). It has been suggested that pigment decay products in settling material may serve as "signals" of the feeding activity of specific grazer types (Nelson 1989, Strom 1993). The various chlorophyll *a* derivatives detected in the water column and in sediment traps may therefore be of importance in determining the impact of various grazer types on spring bloom production, as well as their contribution to the vertical flux of particulate material.

Suspension feeding bivalves can have a considerable impact on the transformation of particulate organic material and phytopigments at the sediment-water interface (Hawkins et al. 1986, Robinson et al. 1989). These grazers are capable of processing large amounts of seston (Doering and Oviatt 1986, Smaal et al. 1986), and of altering the composition of settling particles and resuspended sediments (Muschenheim and Newell 1992, Newell and Jordan 1983). The presence of suspension feeding bivalves at the sediment-water interface can thus dramatically alter both the quantity and quality of material deposited in the surface sediments.

The primary goal of this study was to determine the chloropigment concentrations in the digestive tracts and faeces of the dominant zooplankton grazers and the suspension feeding bivalve, *Mytilus edulis*, during the spring bloom in Conception Bay. It includes an examination of the relative contributions of the fluorescent chloropigment derivatives produced by the digestive activities of these grazers to the water column and sediment-surface environments. The zooplankton component of this study includes an examination of the diel feeding periodicities of the dominant copepods. Data in this chapter, and zooplankton and phytoplankton biomass determinations (to be reported elsewhere), will ultimately be used to estimate the zooplankton grazing impact on the spring phytoplankton bloom in Conception Bay.

The main objectives of the following study were:

1. To measure the gut chloropigment concentrations of the dominant zooplankton grazers (copepods and oikopleurid tunicates) during the development and decline of the spring bloom.

2. To examine the diel feeding periodicities of the dominant zooplankton taxa during the peak of bloom production. This study includes collections from the subsurface chlorophyll maximum (SCM) during 1988, and from depths above, within and below the SCM during 1989.

3. To determine the nature of the degradation products of chlorophyll *a* in the digestive tracts and faecal pellets of the dominant zooplankton grazers.

4. To examine the concentration and composition of chloropigment decay products in the digestive gland and faeces of the suspension feeding bivalve, the blue mussel (*Mytilus edulis*), during and following the spring phytoplankton bloom of 1989.

5.2. Methods

Studies of the chloropigment concentration and composition in the gut tracts of copepods (1988, 1989), the faeces of oikopleurids (1988), and the digestive gland and

faeces of the blue mussel (1989) were conducted in Conception Bay at the study site shown in Chapter 4, Figure 4.1.

5.2.1. Zooplankton Collection

Zooplankton were collected at approximately weekly intervals at depths above, within, and below the SCM during March to June 1988, and at intervals of 4 hr at depths of 10, 25, 50 and 100 m during the peak of bloom production in April 1989.

During 1988, zooplankton were captured in a modified Tucker Trawl, with an aperture of approximately 40 x 40 cm, and a TSK flowmeter mounted above the net mouth. Most collections were conducted during daylight hours from onboard Memorial University's research vessel, the Karl and Jackie II. The net was towed horizontally at approximately 2-3 knots and was opened and closed at depth with a messenger operated double-trip mechanism (General Oceanics, Inc.). The frame was fitted with a plankton net made of 333 μm Nitex mesh, to which a closed codend cup was clamped. For the quantitative collection of zooplankton, oblique tows were conducted with a plankton net of 110 μm mesh. Because the effective operation of the Tucker Trawl was limited to low wind conditions, an open 60 cm diameter bongo frame, fitted with a 333 μm mesh plankton net, was used to collect animals during periods of high winds. Day and night drift tows, of short duration (5 min) in the surface mixed layer (0-25 m), were conducted with the bongo sampler from the C.S.S. Baffin during a prolonged northeasterly gale in mid-April.

In 1989, all zooplankton samples were collected during the peak of bloom production in April using a 0.25 m^2 multi-net plankton sampler (MOCSSY), similar in design to the BIONESS (Sameoto et al. 1977). The MOCSSY sampler was equipped with a series of 9 opening and closing plankton nets, acoustically operated from the deck of the C.S.S. Dawson. The nets were deployed at 4 hr intervals during 2-48 hr periods

(April 18-20, April 22-24). Five of the 9 plankton nets were made of 165 μm Nitex mesh and were towed obliquely for 3-5 min per net over depth intervals of approximately 0-15 m, 15-30 m, 30-45 m, 45-60 m and 60-120 m. These net collections were conducted for the estimation of zooplankton abundance and biomass, and for the determination of zooplankton vertical migration rhythms. Copepods for gut pigment analysis were collected following completion of the quantitative tows. The diel feeding periodicities of the dominant mesozooplankton taxa were examined using these net collections. Three horizontal tows, of short duration (3-5 min) and with nets of 333 μm mesh, were conducted at depths of 25, 50 and 100 m during the day, and at depths of 10, 25 and 50 m during the night. These tows followed an order that allowed a near-uniform time interval between copepod capture and shipboard processing.

Upon retrieval of all plankton nets, the cod-end contents from each horizontal tow were immediately passed through a 2000 μm mesh to remove large zooplankters (e.g. chaetognaths, medusae, ctenophores). The remaining zooplankton were poured gently into a 2 l bucket, which was fitted with a bottom mesh of 333 μm. This container was nested within a 60 l plastic tub filled with seawater collected from 200 m. Each zooplankton collection was gently diluted and rinsed with seawater to remove phytoplankton cells and diatom chains collected in the net. The zooplankters were then concentrated and collected on Sartorius membrane filters (3 or 7 μm) under low vacuum. The filters from each net tow were placed in a covered petri dish and quick-frozen on dry ice, followed by storage in a -20°C freezer.

The interval between the start of each tow and zooplankton collection on filters was generally about 15-20 min. As a result of delayed processing and possible defecation following net collection, the gut pigment concentrations measured in this study may be lower than levels present at the time of capture. Microscopic examination generally showed the presence of food only in the lower half of the digestive tracts of copepods. Although it may be appropriate to correct the gut pigment measurements with the

application of species-specific defecation rate constants for estimates of grazing impact, this was not done for the examinations conducted in this study.

5.2.2. Laboratory Analyses of Zooplankton Samples

Samples of frozen zooplankton were removed from the filters and transferred to a petri dish using a spray of ice-cold FSW. The petri dish was nested in an ice-filled glass bowl to prevent rapid warming and subsequent rupturing of the animals. A Zeiss dissecting microscope, fitted with a Schott KL1500 fibre-optic cold light source and red filter (Wratten gelatin filter No.25), was used for the identification and removal of individual copepods and oikopleurids, and oikopleurid faecal pellets. Only animals which were intact and apparently healthy upon capture were selected for analysis. Copepods and oikopleurids were removed by forceps, transferred to a glass dish containing ice-cold FSW, and gently agitated to remove adhering phytoplankton cells and debris. This was followed by a quick rinse in ice-cold distilled water to remove salts, and transfer to a 1.5 ml polypropylene centrifuge tube containing 1.25 ml of 90% acetone. The oikopleurid faecal pellets were transferred by pipet to empty 1.5 ml polypropylene tubes and centrifuged for 1 min at an RCF of 8000. The water was then decanted and the pellet sample was extracted in 1.25 ml of 90% acetone.

The number of copepods extracted in each tube was variable and depended on the size of the animal (i.e. 1-5 for large copepods, 20-50 for small copepods). Oikopleurid extractions were conducted on samples of 2-10 animals, generally containing 2-3 faecal pellets per trunk, and collections of 5-50 faecal pellets. Chloropigments were extracted over a period of 24-36 hr at temperatures between 0 and -20°C. Samples were initially ground with a motorized teflon pestle, but due to the difficulties of grinding mucous-bound oikopleurid faecal pellets, homogenization of the samples was conducted primarily by sonication for 8-10 min in an ultrasonic bath cooled with crushed ice. This method was effective in breaking apart the animal and faecal pellet contents in all tubes.

Following centrifugation for 2-3 min, 500 μl was removed and diluted with 4.5 ml of 90% acetone for the fluorometric determination (TD10) of chloropigments. Another 500 μl was removed from a representative subset of samples for HPLC analysis, as described in Chapter 2.

5.2.3. Bivalve collection

The chloropigments in the digestive gland and faeces of the blue mussel (*Mytilus edulis*) were monitored at the study site throughout late March to late May 1989. About 2 dozen mussels, 6.5-8.0 cm long, were enclosed within a mesh dive bag and fastened to a taut-wired sediment trap mooring at a depth of about 20 m. They were retrieved by divers and replaced by a new set of mussels at approximately weekly intervals. Water samples at the depth of deployment were also collected and analyzed for chloropigment content, as described in Chapter 4.

Immediately upon retrieval of the mesh bag, the mussels were emptied into a 20 l bucket filled with seawater collected from below the euphotic zone. Each mussel was brushed and rinsed to remove adhering phytoplankton and phytogenous debris. Twelve mussels were then transferred to individual 500 ml plastic beakers filled with FSW and nested inside a cooler with crushed ice. After 2-4 hr in darkness, any faeces released in each beaker were transferred by pipet to a 1.5 ml polypropylene tube. Excess water in the tubes was removed by pipet. These samples were then quick-frozen on dry-ice, followed by storage at -20°C. The digestive glands of six other mussels were removed, placed separately in covered petri dishes, and frozen in the same manner.

5.2.4. Laboratory analyses of bivalve samples

The samples of mussel faeces were extracted in 90% acetone and sonicated as described in Section 5.2.2. All samples were examined using the standard fluorometric

method and HPLC. The contents of the tubes were then dried at 60°C, and the dry weight of extracted faeces in each container determined to the nearest 0.01 mg.

The digestive glands were lyophilized to suppress enzymatic degradation during storage at -20°C. They were then weighed and homogenized in a motorized mortar and pestle, cooled with liquid nitrogen. These samples were maintained in glass vials, in the dark, at -20°C until analysis. Subsamples, of known weight, were extracted in 90% acetone and analyzed for chloropigment content using both the standard fluorometric procedure and HPLC.

5.2.5. Identification of Chloropigments

Chloropigments in the digestive tracts and faeces of all grazer types were identified based on the retention times of prepared standards, co-elution of standards with samples, and the absorbance spectra of peaks for which standards were not available. A detailed description of the methodology can be found in Chapter 2. The pigment peaks in the chromatograms presented in Chapters 5 and 6 are labelled with reference numbers which are identified in Table 5.1. This table also indicates the sample types in which the various chlorophylls and chlorophyll derivatives were detected.

5.3. Results

5.3.1. Seasonal: Zooplankton Gut and Faecal Pigments

The dominant zooplankton taxa collected in daytime net tows throughout the 1988 spring bloom in Conception Bay were *Calanus finmarchicus*, *Pseudocalanus minutus*, *Temora longicornis* and *Oikopleura vanhoeffeni*. Water column features of significance to grazing during this period (i.e. chlorophyll *a* concentration, phytoplankton composition, temperature) are detailed in Chapter 4.

A. Standard Fluorometric Measurements (TD10)

The gut chloropigment concentrations of 2 copepodid stages of *Calanus finmarchicus* (CIV, CV), and the adult stage (CVI), collected at various depths throughout the spring bloom, are shown in Figure 5.1. Adult females (CVI) were collected primarily in the surface mixed layer during the daytime in March and April, and at depths below 75 m in May and early June. CIV and CV stages were present in the net tows throughout the study period and followed a similar seasonal depth distribution as adult females (Deibel et al., unpubl. data).

Gut tract levels of total chloropigments (TChl, chlorophyll *a* and its fluorescent derivatives, in chl *a* wt equiv.) increased with developmental stage and were generally highest in animals collected in the near surface tows (10-25 m) during mid- and late April. Maximum concentrations in stages CIV, CV and CVI were 8, 24 and 28 ng TChl per copepod, respectively. TChl concentration within replicate samples of pooled animals (5-20 copepods per extract), however, was variable (up to 3 fold). The highest levels measured in *C. finmarchicus* CV were in those animals collected at depths >75 m during the daytime in late April. These animals may have been actively grazing at this depth; chlorophyll *a* concentration in water collected from 70 m was about 2 μg/l (Chapter 4, Table 4.2). Gut pigment concentrations were relatively low during the early and late phases of the bloom and reflected low levels of phytoplankton biomass during these periods (Chapter 4, Figures 4.2 and 4.15).

Pseudocalanus minutus and *Temora longicornis* adults (CVI) and late stage juveniles (CV) were most abundant at depths of 10-25 m during the day in late March and April. Both quantitative and horizontal tow collections, however, showed a predominance of *Pseudocalanus* at depths >70 m during May. *Temora* were concentrated at depths within or near the SCM during this period. Maximum gut pigment levels were detected in the surface mixed layer (0-50 m) during April. Variation in the

TChl content within replicate samples, however, was high (up to 5 fold) during this period (Figure 5.2). The gut pigment content in both species showed mean levels of about 0.5-1 ng TChl per copepod, and chlorophyll *a* concentrations representing 10-40% of TChl. These amounts of undegraded pigment were considerably higher than those found in *C. finmarchicus* extracts. High ratios of chlorophyll *a* to phaeopigments in the extracts of *P. minutus* and *T. longicornis* represent either the natural gut pigment compositions for these species, or the co-extraction of copepods and phytoplankton, possibly intertwined with the appendages of these relatively small copepods, 20-50 of which comprised a single sample extract.

Oikopleura vanhoeffeni were periodically abundant in the zooplankton tows conducted in the surface mixed layer during March and April. Peak chloropigment concentrations per animal approached 130 ng during a northeasterly gale in mid-April (Figure 5.3). The chlorophyll *a* concentration in the upper mixed layer at this time was near uniform and about 3 μg/l (Chapter 4, Table 4.2). Faecal pellets collected during this period were dark green in colour, relatively large (about 2 mm in length), and contained the highest levels of chloropigments observed in this study (up to 175 ng TChl/pellet). Variability within replicate samples during March and April, however, was high. Most oikopleurids collected in late April, and thereafter, were considerably smaller (10-15 mm tail length) and produced faecal pellets of about 1 mm in length. On May 6, juvenile *O. vanhoeffeni* were found in concentrations of $>400/m^3$ in the surface waters (2-25 m), and throughout May and early June, numerous oikopleurids and their faecal pellets were collected in plankton tows at depths throughout the uppermost 130 m. Faecal pellets collected in 333 μm mesh plankton nets during the decline of the bloom were intact, light brown in colour, and contained relatively low levels of chloropigment (generally <1 ng TChl/pellet).

B. HPLC Determinations

The fluorescence chromatograms of *C. finmarchicus* and *O. vanhoeffeni*, collected in the same net tow in mid-April, are shown in Figure 5.4. The profile of *C. finmarchicus* shows fewer pigment peaks and less complexity than the chromatogram of extracted *O. vanhoeffeni*. Pyrophaeophorbide *a* (peak 5) dominated the degradation products of ingested chlorophyll *a* in the *C. finmarchicus* sample. This feature was typical of all 3 copepod species collected throughout late March to early June (Table 5.2). Small amounts of undegraded chlorophyll *a* (peak 11) were consistently present in the gut tracts of *Calanus*, and represented 1.5-18%, although generally ≤ 10%, of TChl. These levels and TChl concentrations per copepod are comparable to those determined in the *Calanus* laboratory grazing experiments reported in Chapter 3. *Oikopleura vanhoeffeni* profiles typically showed higher levels of chlorophyll *a* (up to 32% of TChl) and a complex array of degradation products, including phaeophorbide *a*, pyrophaeophorbide *a* and 2 less polar phaeophorbide *a*-type pigments (Table 5.3). Several phaeophytin *a*-type pigments were also commonly found in the extracts of oikopleurids.

The chromatograms in Figure 5.4 also show the presence of chlorophyll *b* (9) and phaeophytin *b*-type pigments (13,14), particularly in the *O. vanhoeffeni* extract. The presence of chlorophyll *b* and its derivatives was probably the result of the ingestion of the prasinophyte, *Pyramimonas* sp., which contains chlorophyll *b*, and which was abundant for a short period in mid-April (Chapter 4, Table 4.1). Although most of the chlorophylls ingested by *C. finmarchicus* appear in degraded form, *O. vanhoeffeni* extracts show much undegraded chlorophyll *a* (11), chlorophyll *b* (9) and chlorophyll *c* (2), which suggests much lower levels of phytoplankton digestion, and thus less efficient utilization. The seasonal chloropigment compositions in the extracts of copepods and oikopleurids, and those of the blue mussel, are compared in Section 5.3.4.

5.3.2. Diel Grazing Patterns of Common Zooplankters

The diel feeding periodicities of *Calanus finmarchicus* and *Oikopleura vanhoeffeni* were examined from zooplankton tows conducted in the surface mixed layer during mid-April 1988. A more intensive investigation of the diel feeding behaviours of the dominant calanoid copepods was conducted during April 1989. The latter study included the examination of gut pigments in 3 large (3-5.5 mm) calanoids (*C. finmarchicus*, *C. glacialis* and *Metridia longa*) and 2 small (1-2 mm) calanoid species (*Pseudocalanus minutus* and *Temora longicornis*) from depths above, within, and below the SCM. Oikopleurids were not examined in 1989 due to the very low abundance of these animals at the study site during a very short sampling period (April 18-24).

A. 1988: *Calanus finmarchicus* and *Oikopleura vanhoeffeni*

Figure 5.5 shows the diel changes in concentrations of chloropigments in whole animal extracts of *C. finmarchicus* (adult females) and *O. vanhoeffeni* collected from a depth of about 20 m during April 13-16, 1988. Although high variation was evident among replicate samples, *C. finmarchicus* exhibited a marked feeding rhythm, with an increase in gut pigment levels at dusk, and peak feeding during the night. Copepods were common in the upper mixed layer during daylight hours, but gut pigment levels were considerably lower than those at night. In contrast, the TChl content in *O. vanhoeffeni* extracts indicated relatively continuous feeding behaviour in the surface waters throughout the day and night; 1-3 faecal pellets were generally observed in the trunks of oikopleurids examined.

B. 1989: Feeding Periodicities of the Dominant Copepod Taxa

Water column profiles of temperature and chlorophyll *a* fluorescence at the study site during April 19, 1989, are shown in Figure 5.6, and are representative of conditions throughout the study period (April 18-22). The SCM was located at depths between 20 and 35 m, and at temperatures below 0°C. Chlorophyll *a* concentrations at 10, 25 and

50 m were approximately 2.5, 10 and 3 μg chl/l, respectively. At depths below 80 m, chlorophyll *a* levels were <1 μg/l, and water temperatures were approximately -1°C.

The scatterplots in Figures 5.7 to 5.11 show the gut pigment concentrations in replicate samples of the 5 dominant calanoid copepod species captured at various depths. Tows at 100 m were conducted only during daylight hours; those at 10 m were limited to sampling periods between dusk and dawn. The data reported in this section are average concentrations per copepod, fluorometrically determined (TD10) from the extracts of pooled animals. Mean ($\pm$SD) values of replicate samples (N=2-6) are reported in Appendix A.

Calanus finmarchicus adult females were abundant within the upper 60-70 m of the water column during both day and night tows, but were most concentrated at depths of 0-45 m between dusk and dawn (Deibel et al., unpubl. data.). In contrast to the 1988 data, maximum gut pigment levels were found in animals collected near midday, and this peak in gut fluorescence was evident at all sampling depths (25, 50 and 100 m) (Figure 5.7). The mean ($\pm$SD) levels of TChl/copepod at 25 m (14.5 $\pm$ 2.1), 50 m (11.2 $\pm$ 1.5), and 100 m (11.0 $\pm$ 6.6) were not significantly different ($P<0.05$). Variation within replicate samples, however, was often high (up to 7 fold). Although the nighttime feeding peak, observed within the SCM layer during the 1988 study (Figure 5.5), was not shown in 1989, an increase in gut fluorescence was observed at dusk. Gut pigment concentrations were consistently lower (<3 ng TChl per copepod) at 10 m (above the SCM) during the night. The lowest chloropigment levels per copepod occurred prior to dusk, at 1600 hr, and this minimum was observed at all depths of collection. A reduction in the gut pigment content of *C. finmarchicus* was also observed 2 hr after dawn, and is consistent with the diel feeding rhythm of *C. finmarchicus* exhibited in 1988 (Figure 5.5).

Calanus glacialis, the largest calanoid examined in this study, was generally absent from the daytime zooplankton tows conducted at 25 m (Figure 5.8). Its appearance

in the surface mixed layer from dusk to dawn suggests an upward vertical migration to food-rich waters during the night and a downward movement at sunrise. This species was found throughout the day and night, immediately below the SCM at 50 m, the depth from which the highest gut pigment levels (>30 ng/ind) were observed. The chloropigment content in the digestive tracts of animals collected from 100 m was also high (up to 30 ng/ind), which is of interest because the water column chlorophyll concentration at depths below 80 m was very low (< 1 μg/l, Figure 5.6). The lowest nighttime concentrations of gut pigments in *C. glacialis* were measured in tow collections from 10 m. Similarly low levels of gut chloropigments were observed in animals collected at 1600 hr from a depth of 50 m. Reduced feeding at this time was followed by elevated gut pigment levels at dusk, and was comparable to the feeding pattern displayed by *C. finmarchicus*.

Metridia longa adult females were rarely found in the net tows conducted above 100 m during daylight hours (0600 to 1830 hr) in both 1988 and 1989, indicating vertical migration to depths below 100 m during the day (Figure 5.9). Animals appeared in net collections at 25, 50 and 100 m at dusk, and were present in the greatest abundance at night within depths of 15-45 m (55-65/m^3) (Deibel et al., unpubl. data). Gut pigment levels were generally greater than 5 ng/ind, even at 100 m, and approached 20 ng/ind at 25 m, where chlorophyll *a* levels were about 10 μg/l. The feeding periodicity of adult *M. longa* was highly coupled to its diel vertical migration rhythm, resulting in grazing within the upper mixed layer only during the night. Feeding during this time appeared to be almost continuous, as shown by gut pigment levels, and by microscopic observations of primarily full and half-full digestive tracts.

The smaller copepods, *Pseudocalanus minutus* and *Temora longicornis*, were found at all depths of zooplankton collection, but were concentrated within the uppermost 50 m during both day and night (Deibel et al. unpubl. data). The highest gut pigment levels were found in both copepod species in net collections from 25 m (Figures 5.10 and 5.11). These taxa showed little apparent ingestion at 10 m, and low gut pigment levels

at 100 m. Peak concentrations of gut pigments were found in the upper mixed layer during midday, followed by reduced levels at 1600 hr, and an increase in grazing at dusk. These feeding patterns were also demonstrated by adult *Calanus finmarchicus* and *C. glacialis*.

5.3.3. Chloropigment Products of Bivalve Grazing

Total chloropigment concentrations in the digestive gland and faeces of the blue mussel, suspended at a depth of 20 m during late March to late May 1989, are shown in Table 5.4. A 7-8 fold decrease in the total chloropigment content of both digestive gland and faecal samples was observed between late March and late May, and reflects the observed reduction in chlorophyll *a* concentrations at the depth of deployment. Throughout late March and April, the phytoplankton taxa at 20 m were dominated by chain-forming diatoms, in particular *Chaetoceros debilis*, and were succeeded by small phytoflagellates (<6 μm) during mid- to late May (C. McKenzie, pers. comm.).

The chloropigment compositions in the extracts of *M. edulis* digestive gland and faeces were not uniform throughout the bloom. During late March, when seston chlorophyll *a* concentrations exceeded 6 μg/l, chlorophyll *a*-type pigments comprised 21% of TChl in the digestive gland samples and 33% of TChl in the faeces. As the abundance of phytoplankton at 20 m decreased, the chlorophyll *a* fraction of the TChl pool in each sample type also decreased. Both phaeophorbide *a*-type and phaeophytin *a*-type pigments comprised the remaining fractions, but the relative contributions of these pigment types was altered as the bloom declined. The phaeophorbide fraction in both the digestive gland and faecal samples increased during mid-April and dominated the phaeopigment pool thereafter.

The fluorescence chromatograms in Figure 5.12 exemplify the chloropigment compositions of faeces produced during late March and late April. Although similar

degradation products were formed as a result of the digestive processes of *M. edulis*, the relative abundance of these chloropigments, in particular chlorophyll *a* and the phaeophorbide *a*-type pigments, differed greatly. The decay of ingested chlorophyll *a* was much more extensive in late April, as shown by an increase in the peak areas of the various phaeophorbides in the HPLC profiles, and by the decrease in TChl content per dry wt of faecal material (Table 5.4). Pyrophaeophorbide *a* was the dominant degradation product found in *M. edulis* faeces during late April and May (Table 5.4). The HPLC profile of faeces collected during late March showed much undegraded chlorophyll *a*, and was similar to the HPLC pigment profiles of faeces produced by mussels fed high concentrations of laboratory-reared diatoms (see Chapter 2, Figure 2.8).

5.3.4. Seasonal Chloropigment Profiles of 3 Grazer Types

The mean chloropigment compositions in the gut tracts of *Calanus finmarchicus*, and in the faeces of *Oikopleura vanhoeffeni* and *Mytilus edulis*, throughout the development and decline of the spring phytoplankton bloom, are presented in Figure 5.13. As noted previously, pyrophaeophorbide *a* was the dominant degradation product of ingested chlorophyll *a* in the gut tracts of *C. finmarchicus*, comprising up to 95 % of the TChl pool. Phaeophytins represented about 10% of TChl during the early bloom but declined to $\leq$2% during the terminal phase of the bloom. A similar reduction in the fraction of the phaeophytin *a*-type pigments was also shown in the faeces of both *O. vanhoeffeni* and *M. edulis* as the bloom declined. This phenomenon may be associated with greater digestion efficiency, and probably greater absorption efficiency, during post-bloom conditions (i.e. reduced phytoplankton biomass). It may also reflect the seasonal shift in the dominance of phytoplankton species.

Oikopleurid faecal pellets consistently contained a relatively higher content of chlorophyll *a* than did *C. finmarchicus* extracts. They also showed higher levels of the less-polar phaeophorbide *a* derivatives (*a*4-*a*6). These phaeophorbide products were also

abundant in the blue mussel faeces, and comprised 30-40% of the TChl composition throughout April and May. The suspension feeding oikopleurids and blue mussels produced faeces with similarly diverse phaeopigment compositions compared to those of *C. finmarchicus* and all other copepod taxa examined (Tables 5.2, 5.3 and 5.4).

5.3.5. Methodological Comparisons: TD10 vs HPLC

The TChl concentrations in a representative set of copepod samples, and in most of the oikopleurid extracts, were determined using both the standard fluorometric method (TD10) and HPLC. These measurements are plotted for comparison in Figure 5.14. Good agreement was found between the two methods of analysis in the extracts of the small copepods, *Pseudocalanus minutus* and *Temora longicornis*. The TD10 measurements of TChl in the extracts of *Calanus finmarchicus* and *Oikopleura vanhoeffeni*, however, were generally higher than those determined by HPLC. Elevated levels of fluorescence with the TD10 may be due to the presence of other components (e.g. astaxanthin and β-carotene) extracted from the bodies of these animals.

The standard fluorometric method also overestimated the TChl concentrations in the blue mussel digestive gland extracts (Figure 5.15A). Regression analysis, however, indicates a relatively consistent concentration of additional fluorescence, equivalent to about 0.5 μg TChl/mg dry wt of sample. This suggests interference from a constant amount of co-extracted, nonchlorophyllous material per unit dry wt of digestive gland. In contrast, the mussel faeces showed very good agreement between methods, with little or no indication of interfering compounds (Figure 5.15B). Comparable TD10 and HPLC estimates of TChl content per dry wt of *Mytilus edulis* faeces were also obtained in laboratory experiments with cultured diatoms (Chapter 2, Figure 2.13).

5.4. Discussion

The standard fluorometric method and HPLC analysis were used in this study to examine the concentrations of chloropigments in the gut tracts and faeces of copepods, oikopleurids and blue mussels during the development and decline of the spring phytoplankton bloom. High chloropigment recoveries (65-100%) in the *Calanus finmarchicus* grazing experiments with spring bloom concentrations of >3.0 μg chl/l (Chapter 3) indicate low levels of chloropigment transformation to nondetectable products in the guts tracts of copepods when phytoplankton concentrations are high. Most of the losses observed in the laboratory grazing experiments occurred primarily within a few hours of incubation; chloropigment destruction was apparently due to the activated feeding and digestive processes of copepods previously starved for 12 hr. Periods of nonfeeding activity by copepods are probably considerably shorter in nature, and may result in even lower rates of chloropigment transformation in the gut tracts of copepods feeding *in situ*.

The efficiency with which *Oikopleura vanhoeffeni* converts ingested pigments to nondetectable products is unknown. High levels of undegraded chlorophylls *a*, *b* and *c*, found in the faecal pellets in this study, and observations of intact diatoms in the faeces of oikopleurids during the spring (Deibel and Turner 1985, Urban et al. 1992), suggest that these animals have a low digestion efficiency, and egest much of their food intact. It is therefore possible that chloropigment transformation to nonfluorescent products by both copepod and oikopleurid grazers may be low during the spring bloom in Conception Bay. If losses to colourless products are reduced at high seston levels, then chloropigments may be reasonable quantitative or semi-quantitative tracers of grazing by these taxa during the spring. This is supported by comparable estimates of *Calanus finmarchicus* ingestion rates, determined by the gut fluorescence method and by measurements of particle removal, in experiments with undiluted natural seston collected during the spring bloom (Chapter 3, Figure 3.11).

In contrast, chloropigments have been shown to be poor tracers of ingested biomass in *Mytilus edulis*. Hawkins et al. (1986) reported absorption efficiencies of 46-82% of ingested chlorophyll *a* in the digestive tracts of these grazers. Chloropigment recoveries of $\leq 20\%$ of chlorophyll *a* consumed have also been observed in the faeces of the horse mussel, *Modiolus modiolus* (Navarro and Thompson, in press), and the oyster, *Crassostrea virginica* (Robinson et al. 1984). Chloropigments are obviously not suitable as quantitative tracers of ingestion by these grazer types. Their use as a quantitative biomarker should probably be limited to studies with grazers which process food quickly and in which pigment digestion efficiency is low. For zooplankton, rapid gut passage and low degradation of ingested pigments are most likely to occur during periods of high phytoplankton biomass (Penry and Frost 1991).

5.4.1. Seasonal and Diel Trends in Zooplankton

The chloropigment content in the gut tracts and faecal pellets of both copepods and oikopleurids during the development and decline of the 1988 spring bloom reflected both grazer size and water column chlorophyll *a* concentrations. The highest pigment levels per animal or pellet occurred at depths of peak phytoplankton biomass during mid-April to early May. In other studies, copepod gut pigment levels have generally shown a direct relationship with food concentration (Head et al. 1986, Ayukai 1987, Hansen et al. 1990, and others). Variability within replicate samples of pooled animals and pellets in this study was often high (up to 7 fold) and suggests that individual variability in gut fluorescence levels was even higher. This is supported by many reports of high variability in the gut pigment levels of individual copepods (Mackas and Bohrer 1976, Båmstedt 1988, Kleppel et al. 1988, Durbin et al. 1990).

A diel feeding rhythm was exhibited by *Calanus finmarchicus* in the upper mixed layer during mid-April 1988. This species showed an increase in gut pigment at dusk, and peak feeding at night followed by a decrease in gut fluorescence at dawn. This

pattern is similar to the diurnal feeding rhythms observed in many other copepod studies (Mackas and Bohrer 1976, Baars and Oosterhuis 1984, Kiørboe et al. 1985, Daro 1988). During the mid-bloom 1989, however, adult females of *C. finmarchicus* and all other dominant copepod taxa showed both day and night feeding within the surface mixed layer. On 2 consecutive days, *C. finmarchicus, C. glacialis, Pseudocalanus minutus* and *Temora longicornis* showed maximum gut pigment levels within the SCM near midday. This was followed by a decrease in phytoplankton ingestion during the late afternoon, and a subsequent increase in feeding at dusk. The feeding rhythm of these copepods may have been influenced by the absence of visually feeding predators, as very few fish overwinter in the bay and most larval fish do not appear until June (J. Anderson, pers. comm.). Egg production during this period was observed in both *C. finmarchicus* and *P. minutus*. Feeding activity may have occurred within the food-rich mixed layer during both day and night in order to enhance spawning frequency, as suggested by Runge (1985) for *Calanus pacificus*. Considerable amounts of pigment were also found in the gut tracts of *Calanus* spp. collected at 100 m, suggesting either vertical migration of satiated copepods from near the SCM, or active grazing within discrete layers of sinking phytoplankton cells. Such layers were occasionally detected in vertical profiles of *in situ* fluorescence (Deibel et al., unpubl. obs.).

During mid-bloom conditions, *Calanus glacialis* and *Metridia longa* were found in relatively low numbers at 25 m during the day. The greatest abundance and highest gut fluorescence levels of *C. glacialis* adult females were generally just below the SCM at 50 m during both day and night. *M. longa*, the largest of the copepod species examined, exhibited a diel feeding rhythm which was directly coupled to its upward migration to phytoplankton-rich waters at dusk. This species fed almost continuously during the night and migrated to depths below 100 m prior to sunrise. A strong vertical migration pattern has also been reported for *Metridia lucens* (Napp et al. 1988). The extent of vertical migration exhibited by the 5 copepod species examined decreased with decreasing body size. *M. longa* migrated to the greatest depths, followed by *C. glacialis,*

C. finmarchicus, P. minutus, T. longicornis. With the exception of *Metridia*, all copepod taxa examined showed similar diel gut fluorescence patterns during the peak of the bloom in April 1989.

A diel feeding rhythm was not observed for *Oikopleura vanhoeffeni*, which appeared to feed relatively continuously day and night. The trunk portion of the body nearly always contained from 1 to 3 pellets. Similar observations have been made during studies of oikopleurids from a subarctic polynya (J. Acuña, pers. comm.). Faecal pellets produced by these animals were much larger during the mid-bloom than at any other time, probably as a result of high food availability. Laboratory grazing experiments have shown that the size of faecal pellets produced by an individual oikopleurid can vary greatly with food concentration (A. Bochdansky, pers. comm.). Because these animals occur in blooms (Taguchi 1982, Mahoney and Buggeln 1983, Davis 1986), feed nearly continuously, and only partially digest their food, they can have a considerable impact on the standing stocks of phytoplankton, and consequently, on the vertical flux of primary production and undegraded phytopigments. It has been estimated that oikopleurids in Conception Bay can have a grazing impact comparable to that of copepods (Knoechel and Steel-Flynn 1989). Oikopleurids, however, are not always abundant, and because their distribution tends to be spatially patchy, their impact on bloom production may be highly variable from year-to-year.

The gut chloropigment profiles of the various copepod species examined were similar, and showed the degradation of chlorophyll *a* to primarily pyrophaeophorbide *a*. This derivative comprised up to 95 % of the gut chloropigments in *Calanus finmarchicus*. Although this product is probably enzymatically formed, the specific enzymes and digestive processes which enhance the production of pyrophaeophorbide *a* are unknown. Phaeophytins and other phaeophorbide *a* derivatives comprise a relatively small fraction of the total chloropigments found in copepod digestive tracts and faeces. In contrast, oikopleurids transform ingested chlorophyll *a* to various phaeophytin and phaeophorbide

derivatives, including pyrophaeophorbide *a*. Degradation within the gut tracts of oikopleurids, however, is incomplete, with up to 32% of total chloropigments represented by chlorophyll *a*. Partially-digested phytoplankton cells have also been found in the faecal pellets of salps (Silver and Bruland 1981) and several genera of pelagic tunicates (Pomeroy and Deibel 1980). Although the concentration of pigments per *O. vanhoeffeni* faecal pellet decreased as phytoplankton biomass declined, relative amounts of undegraded chlorophyll *a* remained high, suggesting that pigment digestion efficiency is unaffected by phytoplankton concentration.

5.4.2. Chlorophyll Degradation by Blue Mussels

Chloropigment concentrations in the digestive gland and faeces of *Mytilus edulis* reflected the availability of phytoplankton at the depth of mussel deployment (Table 5.4). Levels of total chloropigments at 20 m decreased by a factor of 7-8 from late March (high food) to early May (low food). High chlorophyll *a* levels in faeces produced during late March (33% of total chloropigments) indicate much unprocessed phytoplankton and therefore only partial digestion at high food concentrations. This is consistent with studies which have shown decreases in the assimilation efficiency of bivalves with increases in food concentration (Thompson and Bayne 1972, Griffiths and King 1976, Fréchette and Bourget 1987). Mussel faeces produced during the peak of the bloom in Conception Bay appear to contribute pigment-rich material of high organic content. At low seston levels, the total concentration of faecal chloropigments, and the chlorophyll *a* fraction of these pigments, were reduced. This may indicate increased digestive breakdown, and probably an increase in the absorption of chloropigments and their nonfluorescent derivatives. Interestingly, relative amounts of phaeophorbide *a* increased as food levels declined. This increase was associated with a decrease in the amounts of both chlorophyll *a* and phaeophytin *a*-type pigments in the mussel faeces and suggests an increase in the enzymatic decay of ingested pigments, which probably results from an increase in gut

retention time at low food concentrations. Changes in the chloropigment composition of the digestive gland and faeces during the development and decline of the bloom are indicative of variable digestive processes at low to high food concentrations. Nelson (1989) suggests that temporal variation in the pigment composition in grazer faeces may be due to various gut esterases, the activities of which are related to ingestion rate and gut transit time.

5.4.3. Comparison of Grazer Types

The three grazer types (copepods, oikopleurids and blue mussels) examined in this study have markedly different feeding mechanisms. Calanoid copepods are tactile particle feeders and exhibit diel and intermittent feeding periodicities. Although copepod grazing has been well studied, little is known of the digestive processes which convert chlorophyll *a* to pyrophaeophorbide *a*. In contrast to copepods, the faeces of oikopleurids and blue mussels both showed high phaeopigment diversity and very similar chloropigment decay products. They are both suspension filter feeders and ingest particles of about the same size range, but they do not mechanically rupture cells upon capture, as do copepods. Ingestion by oikopleurids and blue mussels is aided by mucous secretions, which are used to transport particulate material during the feeding process, and which may possibly serve a function in the enzymatic breakdown of ingested chlorophyll *a*.

Table 5.1. Pigment identities associated with the peak numbers on HPLC fluorescence profiles in Chapters 5 and 6. Phaeophorbide *a*-type pigment known as phaeophorbide *a*2 was not detected. Presence within each sample type (x) was variable over the sampling duration and includes both common and infrequent occurrences of the various pigments. "IP" label on chromatograms refers to the injection peak.

Peak No.	Peak Identity	Presence: Sample Types			
		Seston	Grazers gut/faeces	Sediment traps	Sediment
1	chlorophyllide *a*	x	x	x	x
2	chlorophyll *c*	x	x	x	x
3	phaeophorbide *a*1	x	x	x	x
4	phaeophytin *c*		x	x	x
5	pyrophaeophorbide *a* (*a*3)	x	x	x	x
6	phaeophytin *a*4	x	x	x	x
7	phaeophorbide *a*5	x	x	x	x
8	phaeophorbide *a*6		x	x	x
9	chlorophyll *b*	x	x	x	x
10	chlorophyll *a* allomers	x	x	x	x
11	chlorophyll *a*	x	x	x	x
12	chlorophyll *a* epimers	x	x	x	x
13	phaeophytin *b*	x	x	x	x
14	phaeophytin *b* isomer	x	x	x	x
15	phaeophytin *a*	x	x	x	x
16	phaeophytin *a* isomer	x	x	x	x
17	pyrophaeophytin *a*		x	x	x
18	pyrophaeophytin *a*-like		x	x	x

Table 5.2. Chloropigment concentration and composition (HPLC) in the digestive tracts of 3 copepod species (Stages V and VI) collected during March-June 1988. Identities are: chl *a* (chlorophyll *a*), pho (phaeophorbide *a*-types), pyro- (pyro-derivatives) and phy (phaeophytin *a*). Each sample (N) represents a pool of 10-50 copepods.

Copepod Taxa	*Date*	*Seston* SCM μg chl/l	*N*	*Tot Chlor* ng/copepod (mean±SD)	*%chl a* -types	*%pho* 1	*%pyro-* pho (3)	*%pho* 4-5	*%phy*	*%pyro* -phy
Calanus	Mar 26	0.7	3	1.18 (0.72)	17.6	6.6	55.8	10.9	2.1	7.0
	Apr 13-16	2.9-3.2	25	7.17 (8.89)	9.5	6.0	66.2	7.0	2.1	9.2
	Apr 29	5.7	7	11.83 (5.21)	1.5	0	90.2	2.7	0.2	5.3
	May 9	3.5	5	4.74 (0.78)	5.6	0.2	89.3	2.0	0.5	2.3
	May 12	2.3	9	3.27 (1.46)	1.5	0	95.0	3.5	0	0
	May 26	1.7	3	0.80 (0.40)	10.6	3.4	78.8	5.1	2.1	0
	June 2	0.4	7	0.66 (0.12)	10.0	1.7	81.2	6.4	0.5	0
Pseudo-	Mar 26	0.7	1	0.33	40.7	3.8	49.3	6.2	0	0
calanus	Apr 13-16	2.9-3.2	4	0.55 (0.22)	13.0	7.0	71.3	7.0	0	1.6
	Apr 29	5.7	3	0.74 (0.16)	18.9	0	73.[illegible]	5.4	0.4	1.5
	May 9, 12	3.5, 2.3	3	0.81 (0.05)	10.4	1.2	80.2	5.4	1.0	1.9
	May 26	1.7	2	0.30 (0.11)	4.4	0	89.5	6.1	0	0
Temora	Mar 26	0.7	4	0.68 (0.05)	30.1	6.4	50.7	5.7	2.1	4.9
	Apr 13-16	2.9-3.2	7	0.43 (0.30)	28.5	4.4	46.7	9.5	2.1	8.7
	May 9	3.5	3	0.75 (0.28)	32.4	1.7	53.7	0.7	4.1	7.4
	May 12	2.3	3	0.85 (0.50)	40.2	0	30.4	1.5	7.0	20.8

Table 5.3. Chloropigment concentration and composition (HPLC) in the digestive tracts and faecal pellets of *Oikopleura vanhoeffeni* collected during March-June 1988. Identities are: chl *a* (chlorophyll *a*), pho (phaeophorbide *a*-types), pyro- (pyro-derivatives) and phy (phaeophytin *a*). Each sample (N) represents a pool of 2-10 animals (10-25 mm tail length) or 5-50 faecal pellets (1-2 mm length).

Sample Type	Date	Seston SCM μg chl/l	N	Tot Chlor ng/ind (mean $\pm$ SD)	%chl *a* -types	%pho 1	%pyro-pho(3)	%pho 4-6	%phy	%pyro -phy
Bodies	Mar 26	0.7	7	32.1 (18.4)	20.6	22.0	25.7	18.2	8.3	5.2
	Apr 13-16	2.9-3.2	16	43.9 (18.1)	26.2	19.6	19.2	15.4	12.7	6.8
Pellets	Mar 26	0.7	3	6.5 (2.9)	29.1	19.9	18.0	18.0	8.0	7.0
	Apr 13-16	2.9-3.2	5	34.5 (28.7)	31.9	22.9	13.4	21.4	6.2	4.2
	Apr 29	5.7	2	8.1 (4.1)	7.0	16.9	18.4	43.0	8.4	6.2
	May 26	1.7	4	0.3 (0.1)	28.0	22.2	25.1	23.5	1.2	0
	June 2	0.4	5	0.6 (0.4)	26.3	14.2	40.4	17.7	0.7	0.8

Table 5.4. Chloropigment concentration (μg/mg dry wt. sample; TD10, HPLC) and composition (HPLC) in the digestive gland and faecal material of blue mussels *Mytilus edulis* suspended at a depth of 20 m during spring 1989. Identities are: chl *a* (c ophyll *a*), pho (phaeophorbide *a*-types), pyro- (pyro-derivatives) and phy (phaeophytin *a*).

Sample Type	Date	Seston <70 μm @ 20 m μg chl/l	N	Tot Chlor μg/mg (±SD) TD10	Tot Chlor μg/mg (±SD) HPLC	%chl *a* -types	%pho 1	%pyro -pho(3)	%pho 4-6	%phy	%pyro -phy
Dig.Gl.	Mar 31	6.12	4	6.02 (1.04)	5.10 (1.13)	20.7	1.3	19.3	23.0	23.9	11.7
	Apr 6	2.58	3	4.21 (0.59)	3.19 (0.66)	10.0	1.4	44.3	18.7	14.7	10.8
	Apr 14	2.53	3	4.09 (0.73)	3.56 (0.86)	5.8	2.2	56.6	20.5	10.2	4.7
	Apr 24	1.26	3	2.90 (0.22)	2.08 (0.06)	5.0	2.3	64.4	18.4	7.6	2.3
	May 1	0.34	3	3.40 (0.36)	2.52 (0.36)	5.3	1.8	65.2	16.8	9.0	1.9
	May 9	0.15	3	1.53 (0.46)	0.99 (0.30)	5.8	1.6	55.7	21.3	13.3	2.3
	May 23	0.18	3	0.90 (0.58)	0.61 (0.43)	7.5	1.8	40.1	34.7	12.7	3.3
Faeces	Mar 31	6.12	6	3.84 (2.06)	3.68 (1.80)	32.6	1.3	12.6	19.2	21.6	12.7
	Apr 6	2.58	6	6.96 (2.11)	5.97 (2.01)	19.0	1.8	11.2	32.9	19.6	15.5
	Apr 14	2.53	6	2.41 (0.41)	2.31 (0.41)	30.7	4.1	11.7	32.8	13.3	7.3
	Apr 24	1.26	6	1.21 (0.47)	1.10 (0.48)	14.7	5.7	24.9	37.8	7.6	9.3
	May 1	0.34	6	2.16 (0.79)	2.28 (0.99)	4.0	5.8	40.6	36.7	5.7	7.3
	May 9	0.15	6	1.84 (0.69)	1.60 (0.70)	2.6	3.8	43.6	38.4	6.0	5.6
	May 23	0.18	5	0.55 (0.10)	0.52 (0.11)	7.5	3.7	37.3	42.5	6.4	2.6

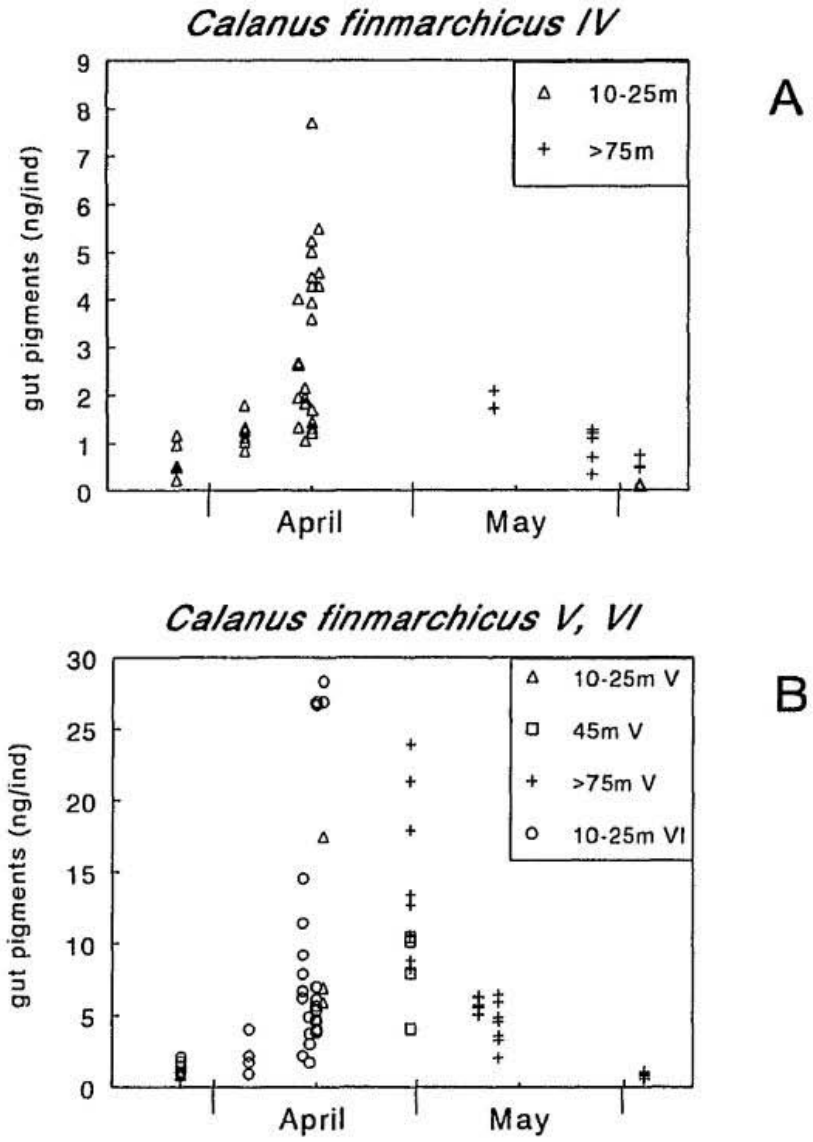

Figure 5.1. Total chloropigment concentrations (ng/ind, chl *a* wt equiv.) in the gut tracts of *Calanus finmarchicus* (Stages IV, V and VI) collected at various depths during March to June 1988. Each point represents an estimate of gut pigment per copepod determined fluorometrically (TD10) from an extract of 5-20 animals.

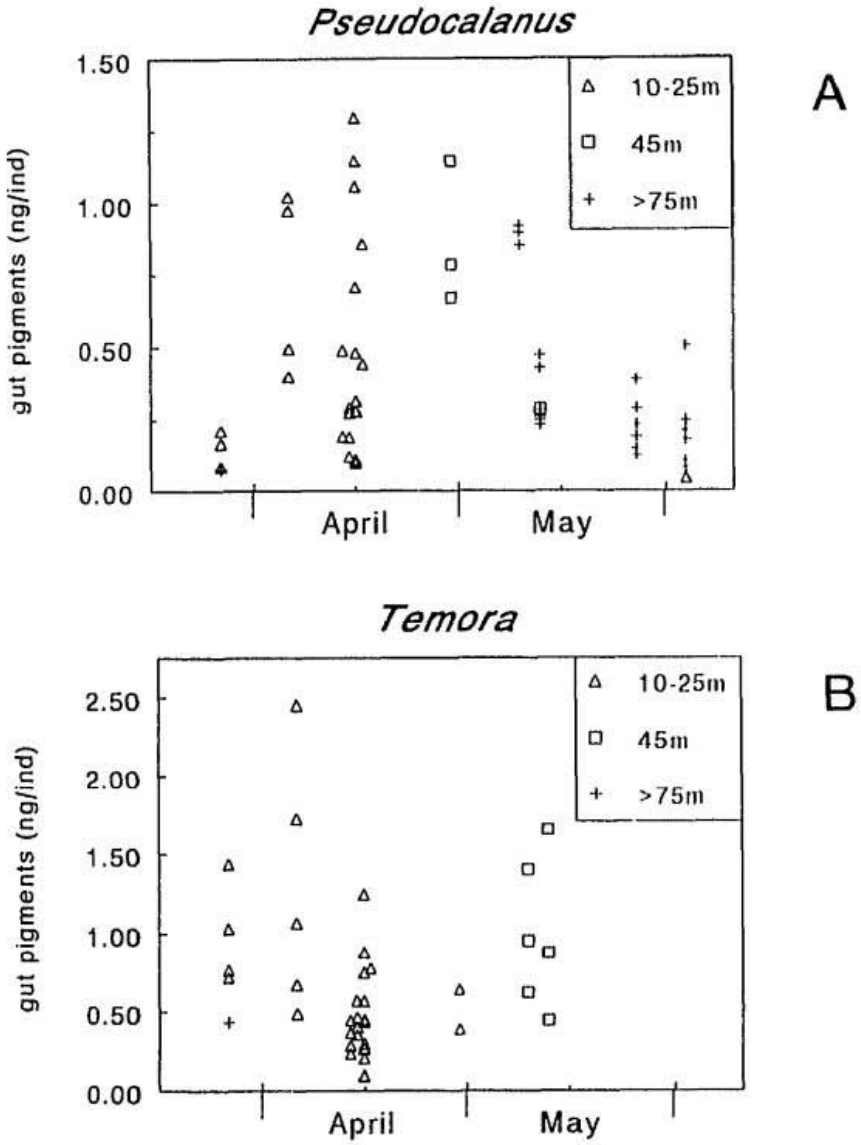

Figure 5.2. Total chloropigment concentrations (ng/ind, chl *a* wt equiv.) in the gut tracts of A) *Pseudocalanus minutus* and B) *Temora longicornis* collected at various depths during March to June 1988. Each point represents an estimate of gut pigment per copepod determined fluorometrically (TD10) from an extract of 20-50 animals.

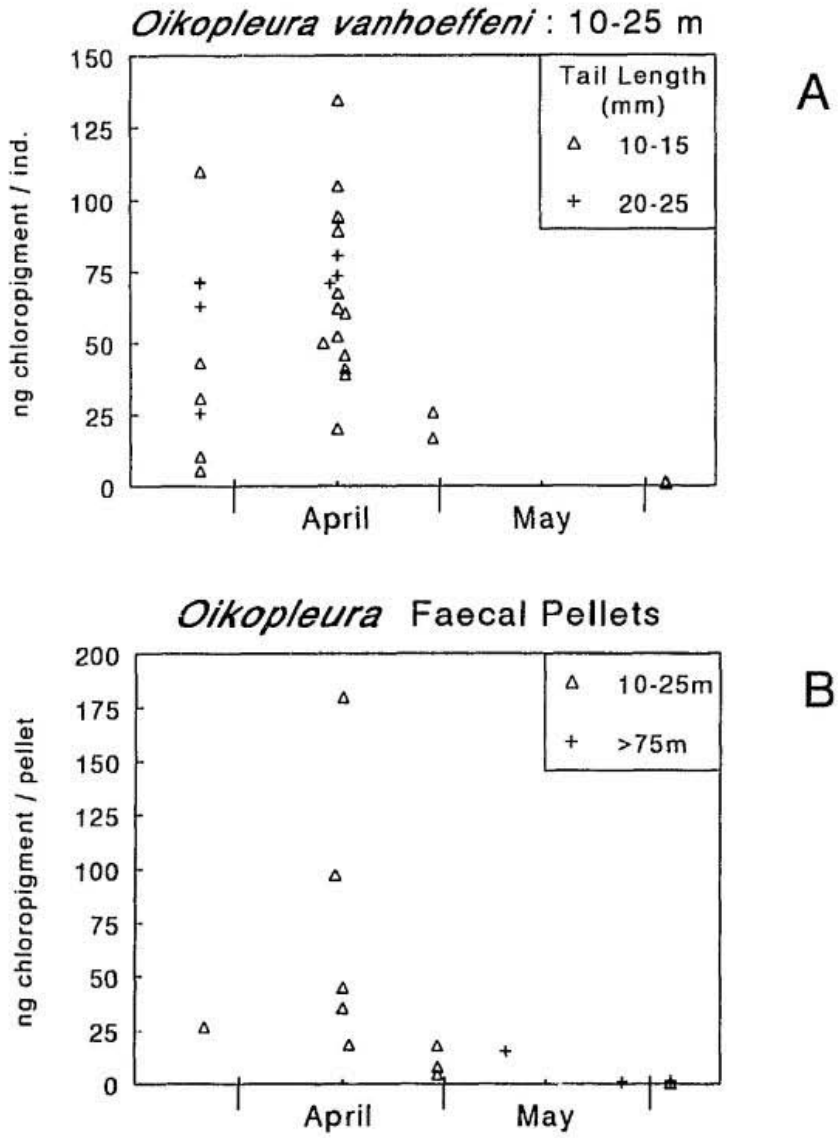

Figure 5.3. Total chloropigment concentrations (chl *a* wt equiv.) in *Oikopleura vanhoeffeni* A) digestive tracts at depths of 10-25 m and B) faecal pellets collected at various depths, during March to June 1988. Each point represents an estimate determined fluorometrically (TD10) from an extract of 2-10 animals or 5-50 pellets.

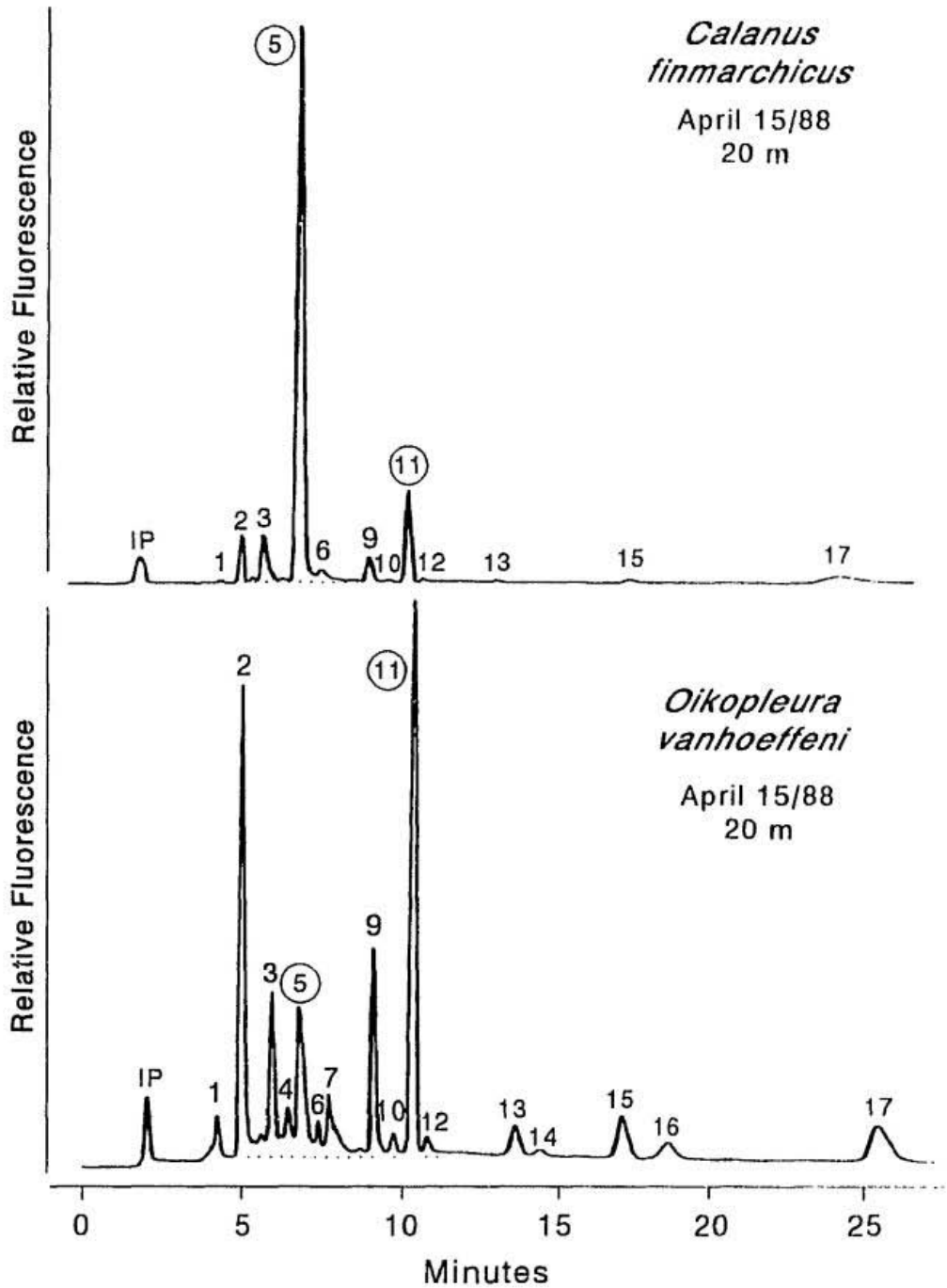

Figure 5.4. HPLC fluorescence profiles of chloropigments in the digestive tracts of *Calanus finmarchicus* and *Oikopleura vanhoeffeni*. Animals collected from the same net tow during full bloom conditions on April 15, 1988. Peak identities shown in Table 5.1.

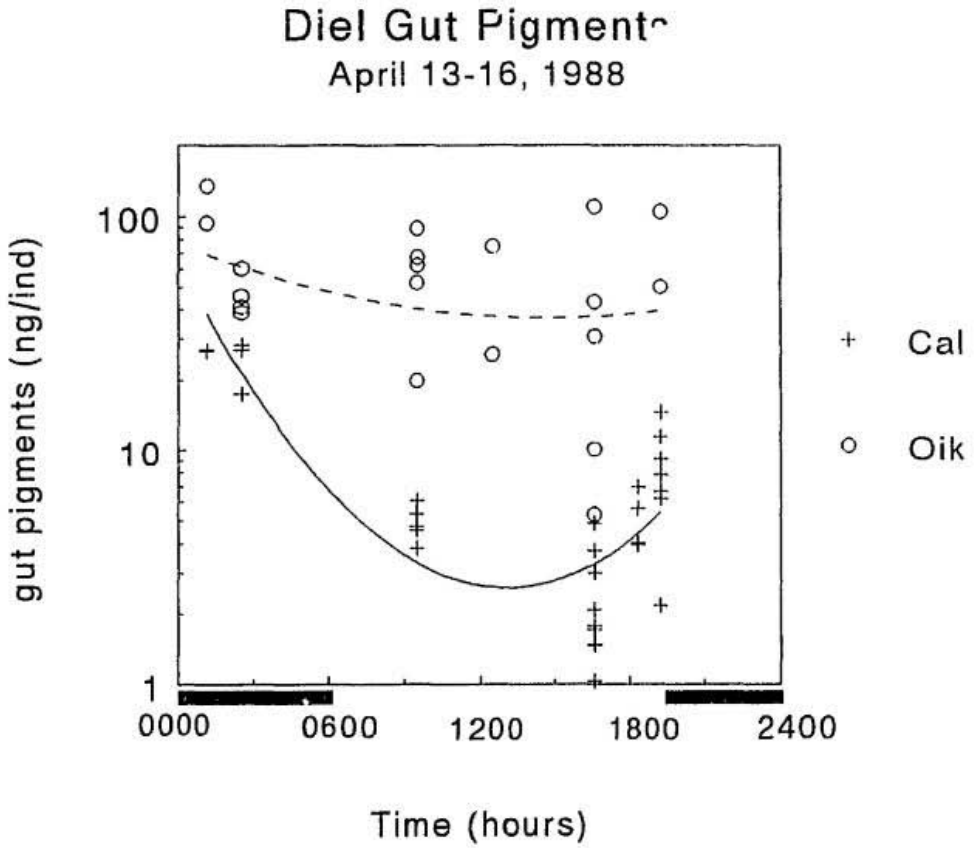

Figure 5.5. Diel chloropigment levels (ng/ind, chl *a* wt equiv.) in the digestive tracts of adult ♀ *Calanus finmarchicus* (Cal) and *Oikopleura vanhoeffeni* (Oik) collected at a depth of 20 m during April 13-16, 1988. Each data point represents an estimate determined from an extract of 10-20 copepods or 2-5 oikopleurids. Lines represent second order polynomial curve fits: solid line (*C. finmarchicus*), dashed line (*O. vanhoeffeni*).

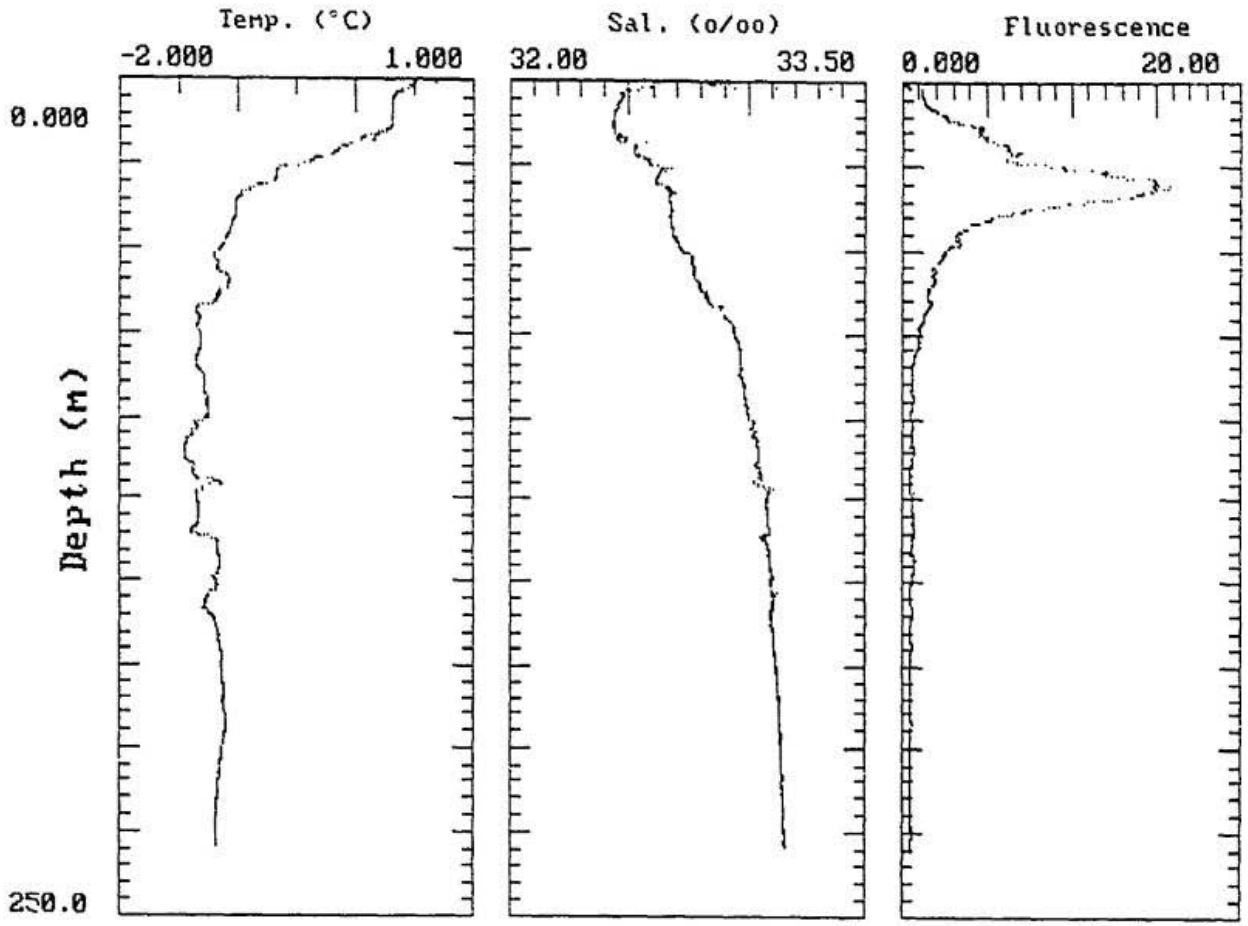

Figure 5.6. Water column profiles of temperature (°C), salinity (‰), and fluorescence (relative fluorescence units of chlorophyll *a*) at the study site at 1015 hr on April 19, 1989.

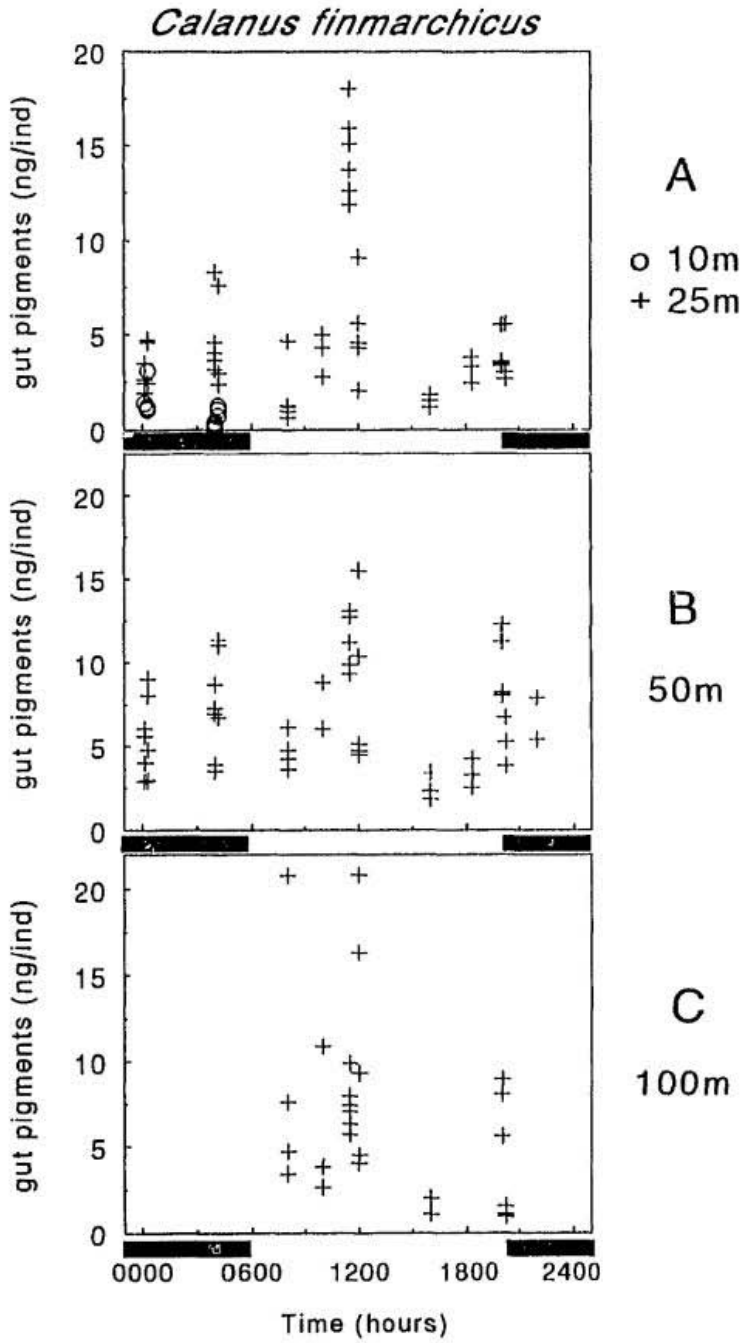

Figure 5.7. Diel chloropigment levels (ng/ind, chl *a* wt equiv.) in the digestive tracts of adult ♀ *Calanus finmarchicus* collected at various depths (A-C) during April 18-22, 1989. Each point represents an estimate determined from an extract of 5-20 copepods. See Appendix 5.1 for mean ± SD values.

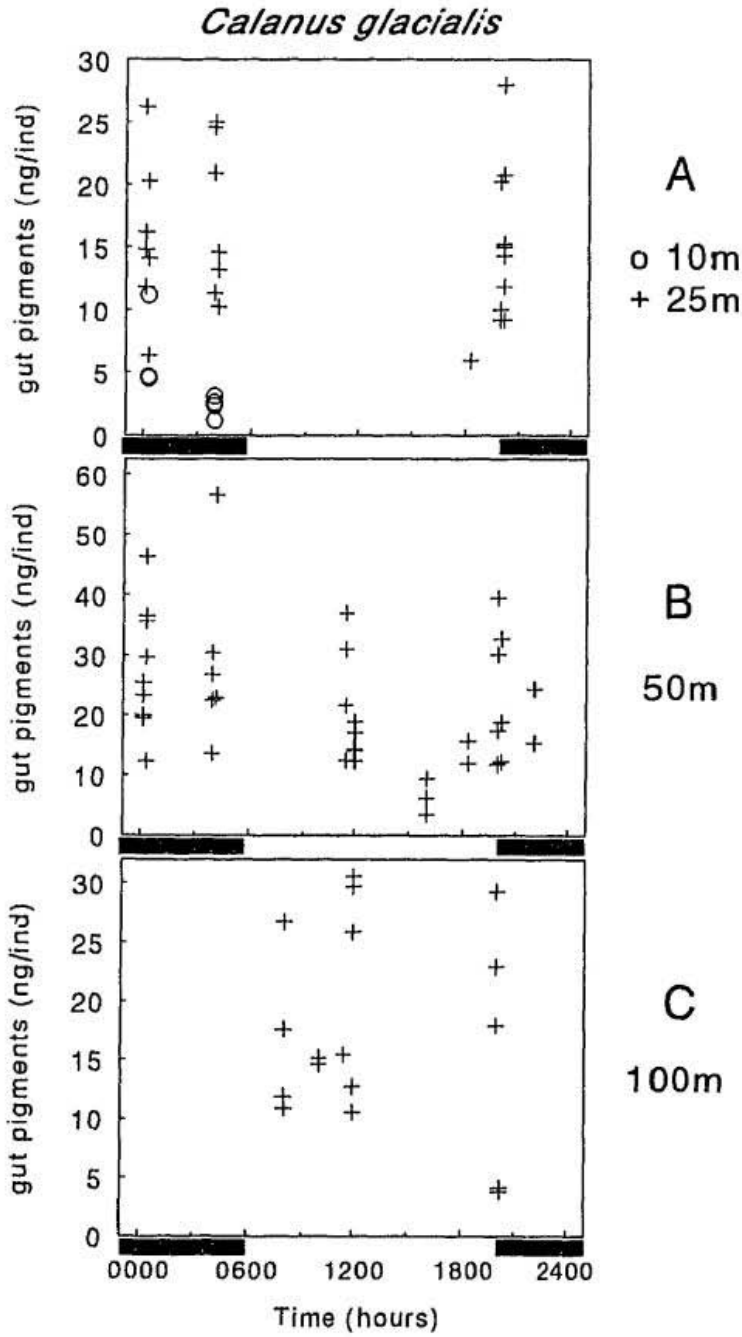

Figure 5.8. Diel chloropigment levels (ng/ind, chl *a* wt equiv.) in the digestive tracts of adult ♀ *Calanus glacialis* collected at various depths (A-C) during April 18-22, 1989. Each data point represents an estimate determined from an extract of 1-5 copepods. Note: Y-scales differ with depth. See Appendix 5.1 for mean ± SD values.

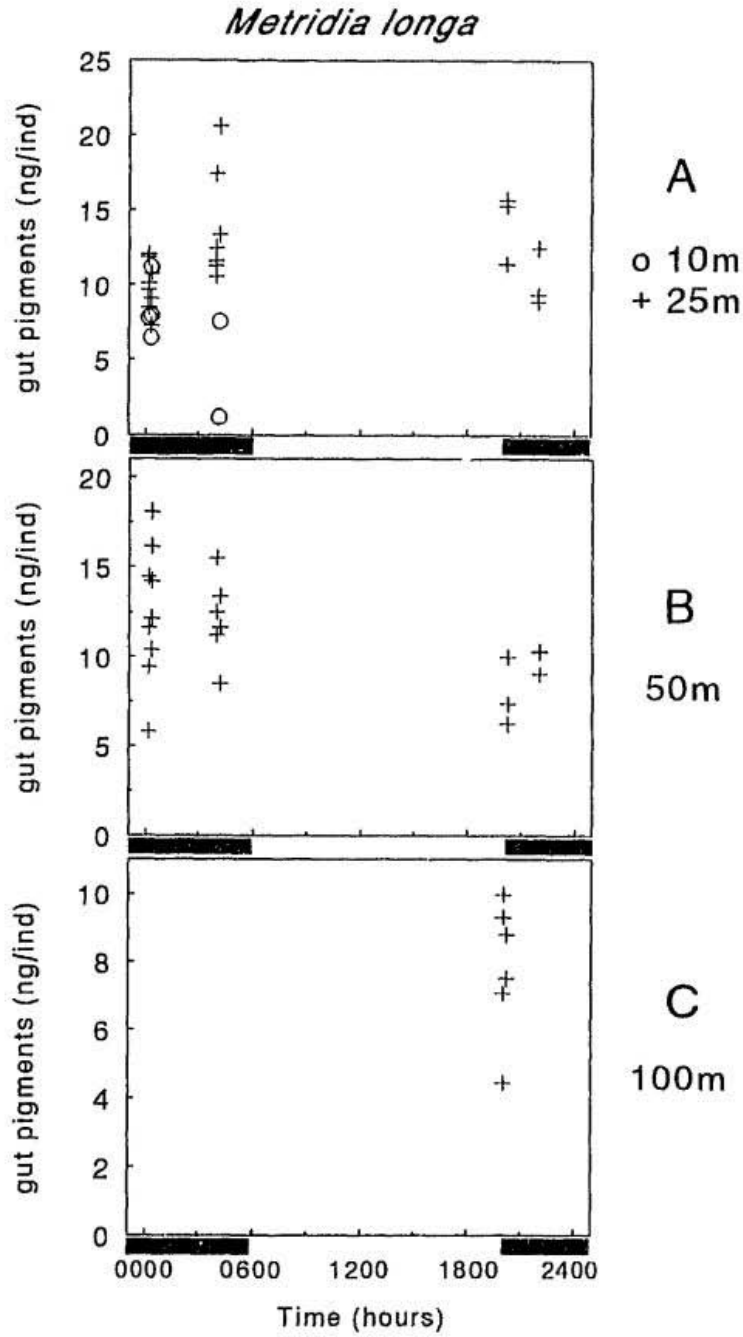

Figure 5.9. Diel chloropigment levels (ng/ind, chl *a* wt equiv.) in the digestive tracts of adult ♀ *Metridia longa* collected at various depths (A-C) during April 18-22, 1989. Each data point represents an estimate determined from an extract of 5-20 copepods. Note: Y-scale differs with depth. See Appendix 5.1 for mean ± SD values.

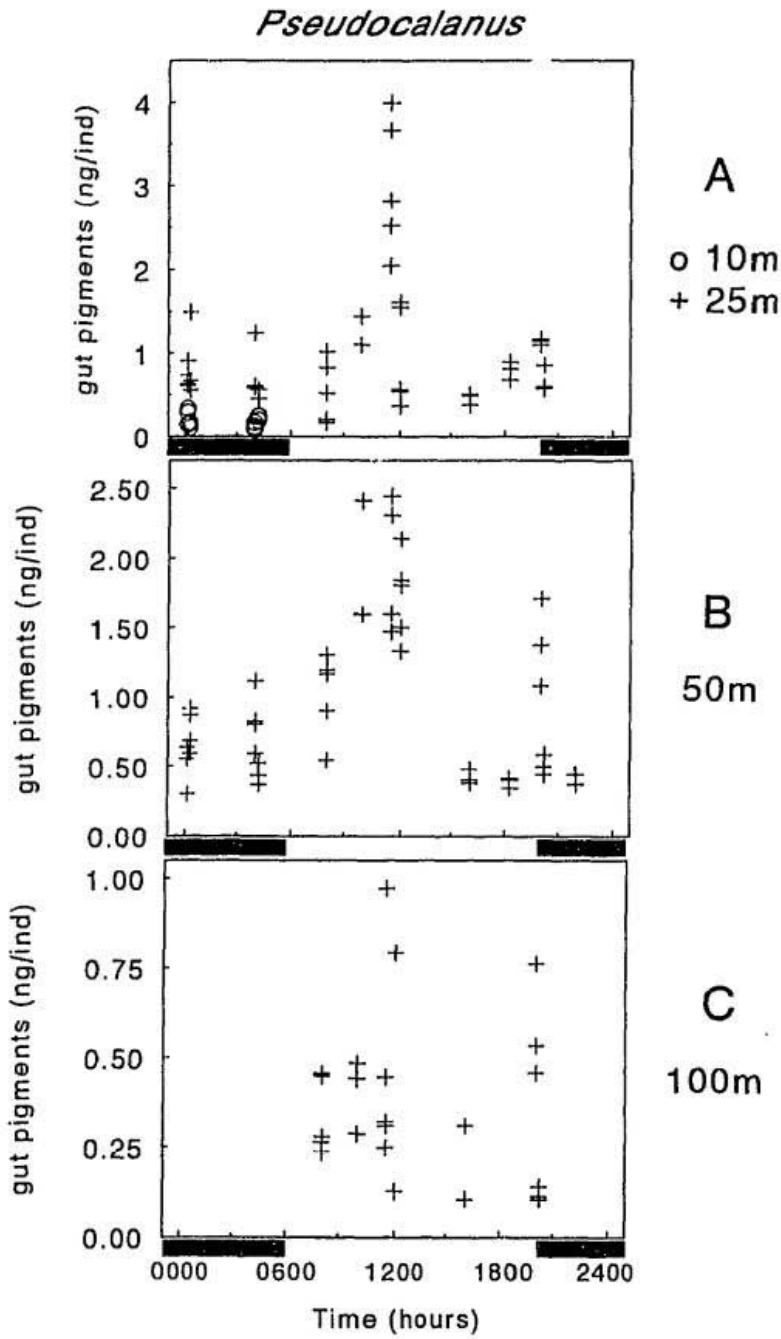

Figure 5.10. Diel chloropigment levels (ng/ind, chl *a* wt equiv.) in the digestive tracts of adult *Pseudocalanus minutus* collected at various depths (A-C) during April 18-22, 1989. Each point represents an estimate determined from an extract of 20-50 copepods. Note: Y-scale differs with depth. See Appendix 5.1 for mean ± SD values.

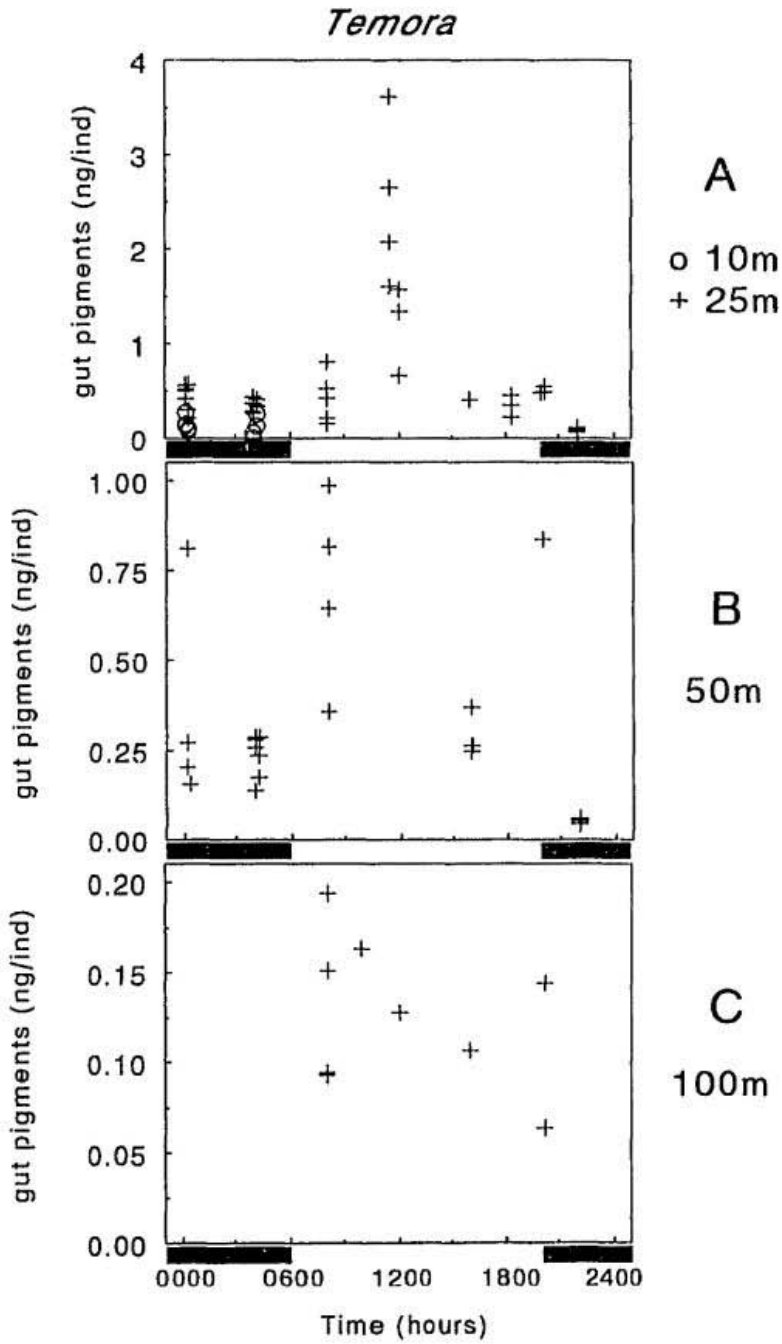

Figure 5.11. Diel chloropigment levels (ng/ind, chl *a* wt equiv.) in the digestive tracts of adult *Temora longicornis* collected at various depths (A-C) during April 18-22, 1989. Each data point represents an estimate determined from an extract of 20-50 copepods. Note: Y-scale differs with depth. See Appendix 5.1 for mean ± SD values.

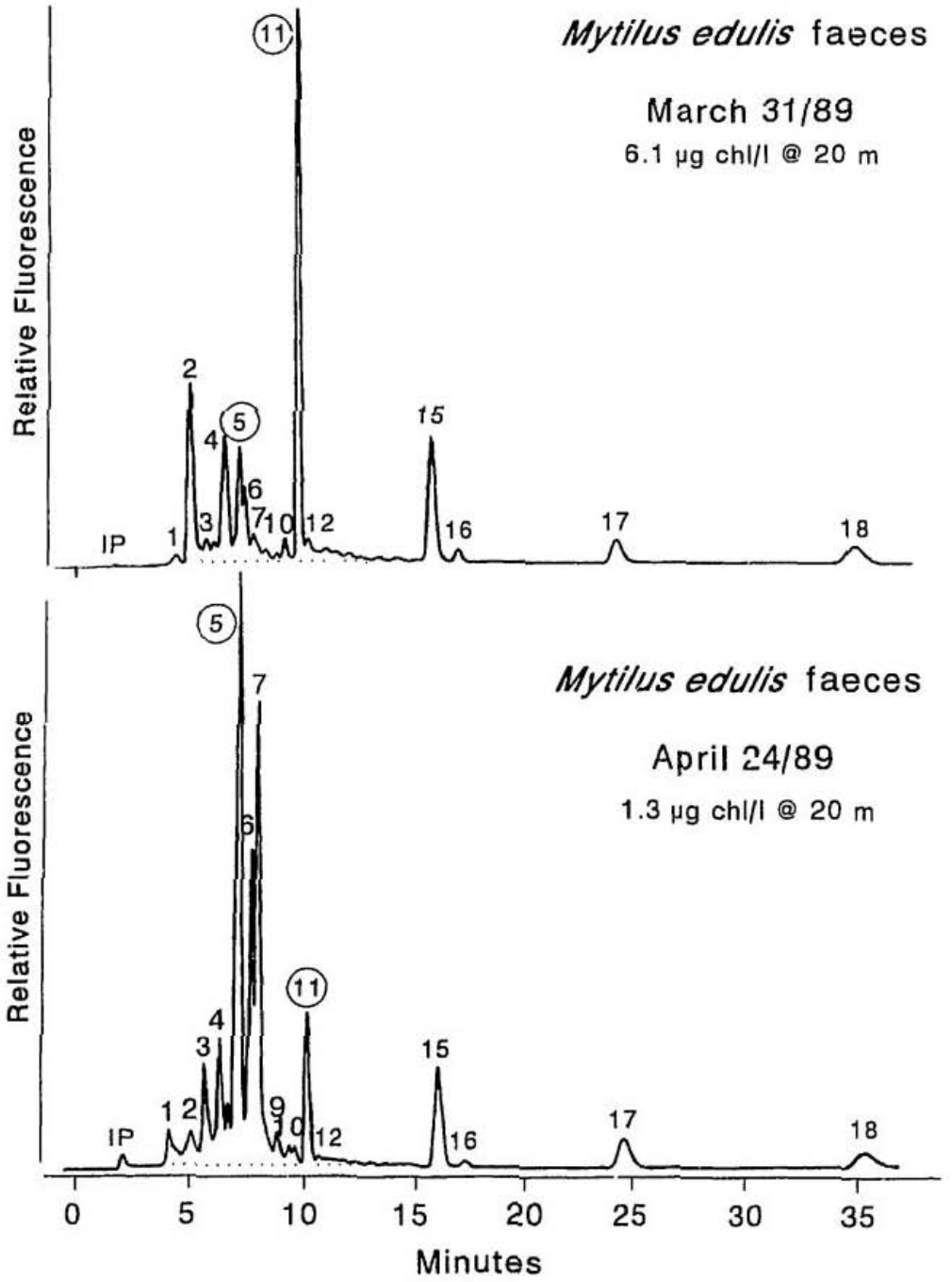

Figure 5.12. HPLC fluorescence profiles of chloropigments in the faeces of the blue mussel *Mytilus edulis*, suspended at a depth of 20 m during high and low seston chlorophyll concentrations in the spring of 1989. Peak identities are shown in Table 5.1.

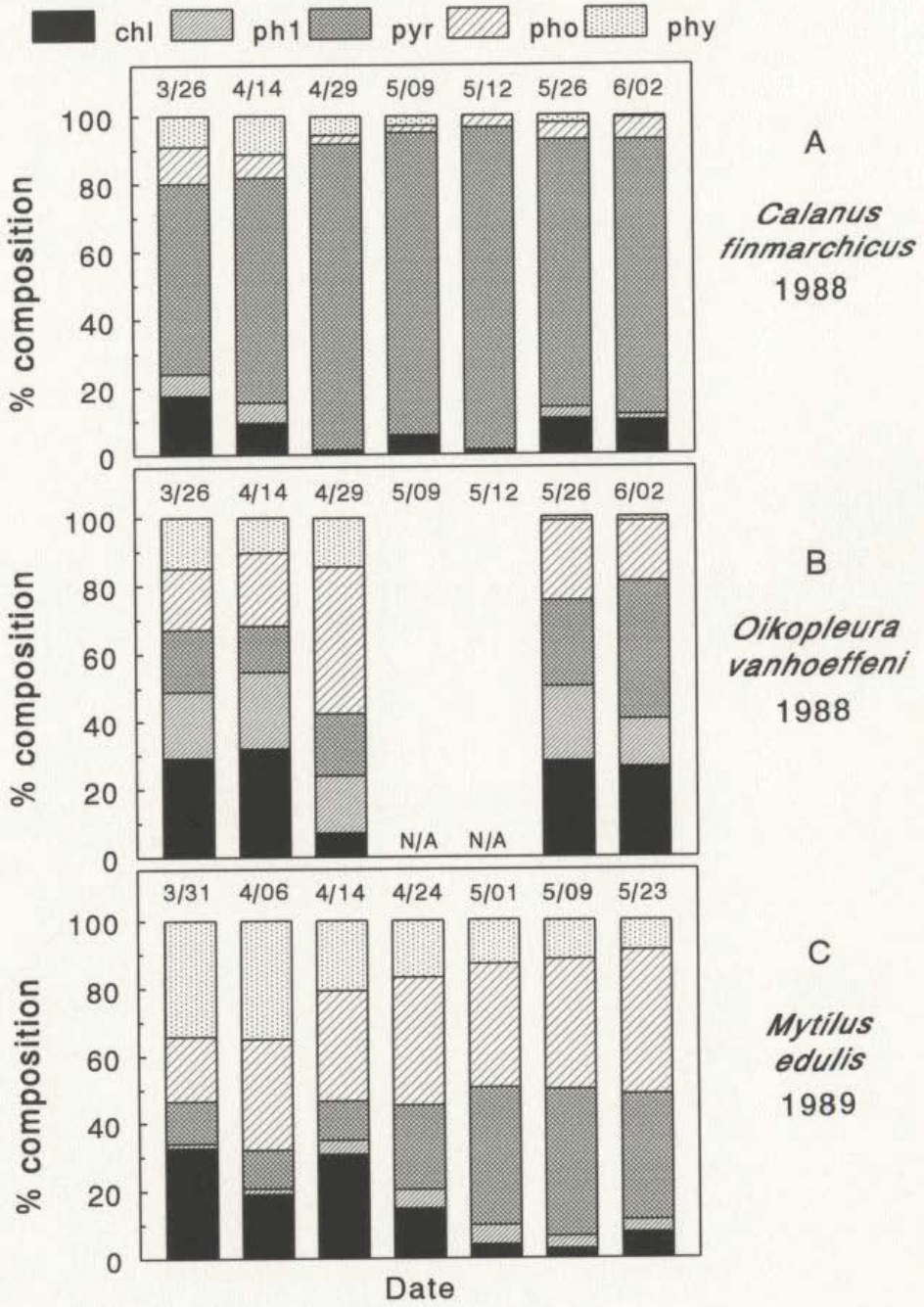

Figure 5.13. The seasonal chloropigment composition in A) *Calanus finmarchicus* digestive tracts, B) *Oikopleura vanhoeffeni* faecal pellets, and in C) *Mytilus edulis* faeces. Legend: chl (chlorophyll *a*-types), ph1 (phaeophorbide *a*), pyr (pyrophaeophorbide *a*), pho (less polar phaeophorbide *a*-types) and phy (phaeophytin *a*-types). Data represent mean values.

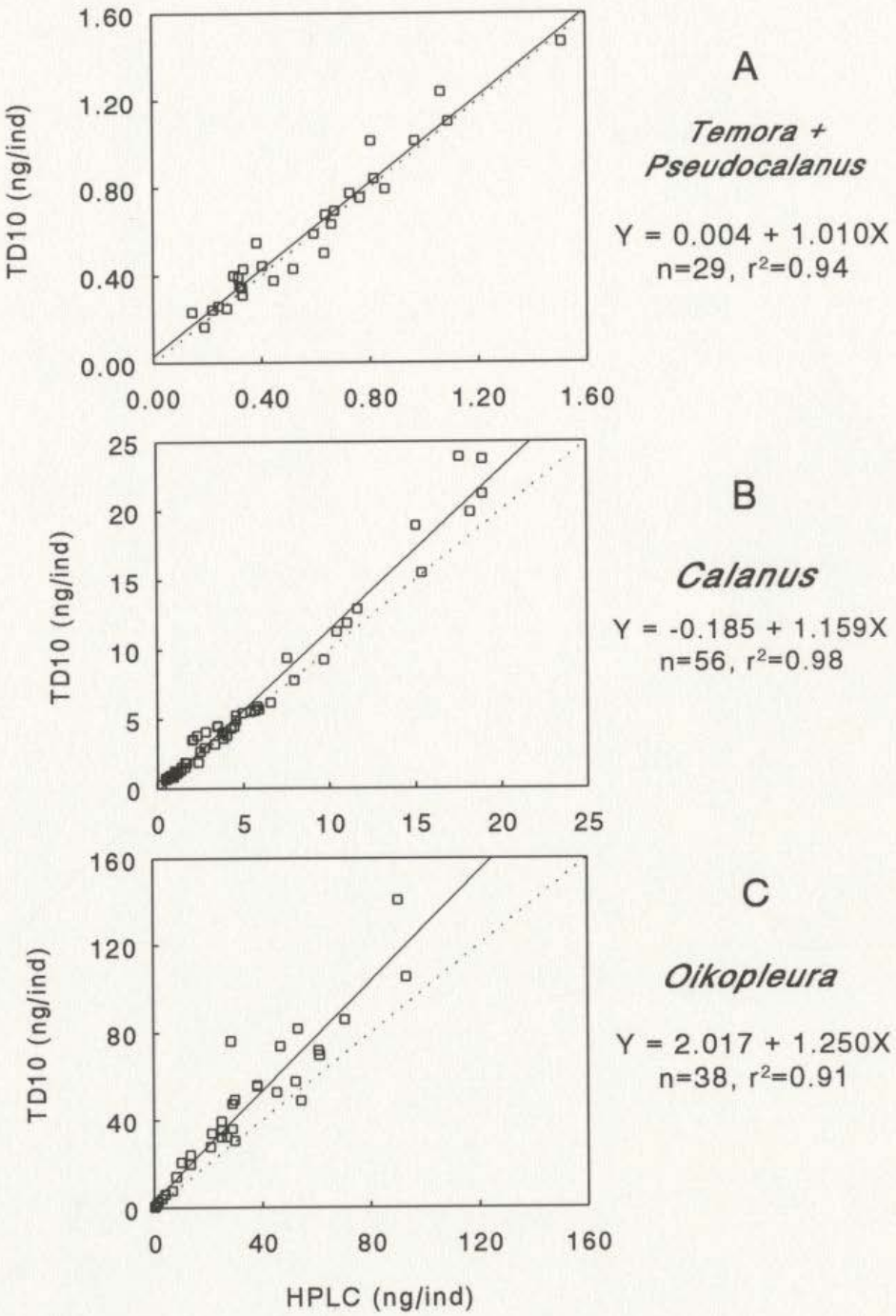

Figure 5.14. Relationships between standard fluorometric (TD10) and HPLC determinations of total chloropigment concentration (chl *a* wt equiv.) in extracts of copepods (A,B) and oikopleurids (C) collected during the spring of 1988. Dotted lines represent a 1:1 relationship.

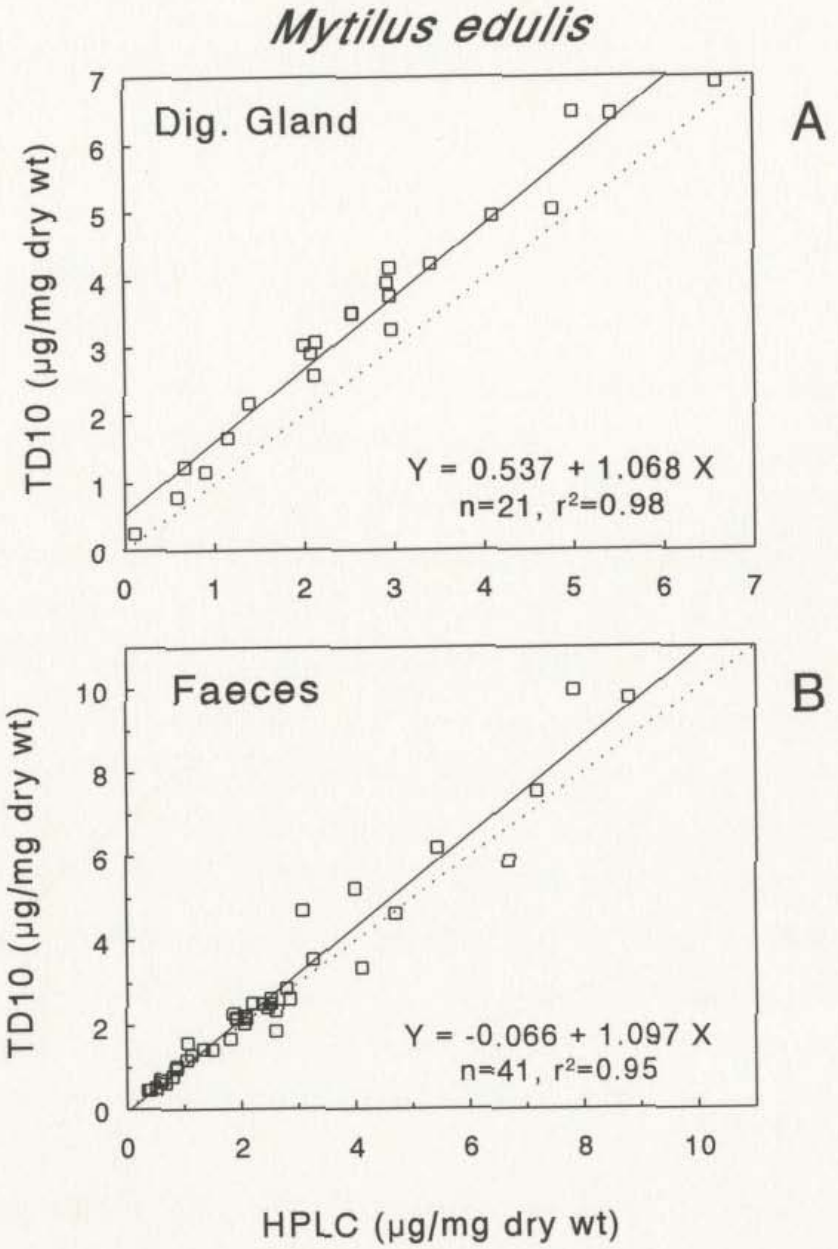

Figure 5.15. Relationships between standard fluorometric (TD10) and HPLC determinations of total chloropigment concentration in the A) digestive gland and B) faeces of the blue mussel *Mytilus edulis*. Mussels were suspended at a depth of 20 m in Conception Bay during late March to late May 1989. Dotted lines represent a 1:1 relationship.

Chapter 6

VERTICAL FLUX OF SPRING BLOOM PRODUCTION IN CONCEPTION BAY

6.1. Introduction

The mass sedimentation of spring phytoplankton production is a common feature of temperate and north-temperate coastal areas (Smetacek 1980, Bodungen et al. 1981, Peinert et al. 1982, Wassmann 1983, 1991; Davies and Payne 1984). Although faecal pellets produced by herbivorous zooplankters are important vehicles for the downward vertical flux of phytogenous material (Turner and Ferrante 1979, Urrère and Knauer 1981, Bathmann et al. 1987), sedimentation of ungrazed phytoplankton during spring blooms is often quantitatively more important than faecal pellet flux (Smetacek 1985). This phenomenon has been associated with a relatively low biomass of overwintering zooplankton, particularly in cold, northern waters (Peinert et al. 1984, Smetacek et al. 1984). A temporal phase lag in the response of zooplankton to a short period of rapid phytoplankton growth results in limited grazing impact during spring (Dagg et al. 1982, Fransz and Gieskes 1984). Consequently, a large portion of the phytoplankton biomass settles out of the euphotic zone in the form of intact cells, cysts, diatom chains, and flocs during the late senescent stage of the bloom (Smetacek 1985).

Sedimentation during spring blooms supplies significant amounts of organic matter and energy to the epibenthic and benthic environments, and thereby directly links phytoplankton production to food webs beneath the euphotic zone (Suess 1980, Betzer et al. 1984, Wassmann 1986, Pace et al. 1987). The spring pulse of organic matter is often the principal energy source for the benthic community. In temperate and high latitude regions, as much as 50% of the total annual input of organic matter can be delivered to the benthos during the spring (Townsend and Cammen 1988, Grebmeier and McRoy 1989). Flux studies in western Norwegian fjords have shown the sedimentation

of >30% and as much as 55% of the total annual POC (particulate organic carbon) production from the euphotic zone to the sea floor (Smetacek 1980, Forsskåhl et al. 1982, Wassmann 1984, 1991).

The vertical flux of spring bloom production, and the extent to which carbon fixed by primary producers is exported as particulate organic carbon, are commonly estimated from material collected in sediment traps. They yield time-integrated samples of material suspended in the water column and are most useful as quantitative estimators of flux when deployed in relatively calm waters. Phytopigments and particulate organic carbon and nitrogen are frequently used as tracers of phytoplankton biomass and flux, and as indices of the relative contributions of sinking cells and faecal material (Wassmann 1983, Downs and Lorenzen 1985, Welschmeyer and Lorenzen 1985a,b; Carpenter et al. 1988). Chloropigment composition and relative carbon and nitrogen concentrations of particulate material are suggestive of the nature of the sedimenting particles (Downs 1989, Valiela 1984). Chlorophyll *a* and its derivatives also provide an indication of water column trophic interactions and diagenetic processes (Jeffrey 1980, Vernet 1983, Downs 1989, Hurley and Armstrong 1991).

The primary goal of this study was to examine the fate of the spring phytoplankton bloom in Conception Bay, a north temperate, fjord-like bay with subzero temperatures at depths of bloom production. Sediment traps were employed to measure the vertical flux of particulate material during the development and decline of the spring phytoplankton bloom, and to estimate the overall contribution of bloom production to water column and benthic food webs. This study includes a seasonal examination of the underlying flocculent layer and surface sediments. The main objectives were:

1. To quantify the vertical flux of particulate organic matter, particulate organic carbon and nitrogen, and total chloropigments at regular intervals throughout the period of spring bloom production.
2. To estimate the portion of spring bloom production reaching the benthos.

3. To identify and quantify the various chlorophyll *a*-derived products in sediment trap collections and to estimate the relative contributions of sinking phytoplankton cells and faecal material throughout the bloom.

4. To examine the nature of the particulate material in the flocculent layer at the sediment-water interface, and the seasonal deposition and transformation of phytogenous material within the top 10 cm of sediment.

6.2. Methods

The vertical flux of particulate material was monitored at a site within the deep depositional area of Conception Bay (Figure 4.1) during the 1988 spring phytoplankton bloom. Physical, chemical and biological features of the water column at the same site were measured throughout the bloom and are reported in Chapter 4. Surface sediments and overlying flocculent material were periodically collected at the same sampling site during March to mid-September, 1989.

6.2.1. Features of the Study Area Relevant to Trap Operation

Ideally, flux measurements using sediment traps should be conducted in environments with 1) low horizontal current speeds, to avoid hydrodynamic interactions between currents and traps, 2) low vertical mixing, which reduces the amount of resuspended material, and 3) low horizontal gradients in the concentrations of sinking particles. The following combination of physical features in Conception Bay make the study site suitable for studies of vertical particle flux.

Tidal currents in Conception Bay are weak (< 5 cm/s) and cause negligible fluxes or resuspension (deYoung and Sanderson 1994). They are also unimportant for water mass exchange and general circulation; residence time for surface waters near the head of the bay is about 30 days (deYoung and Sanderson 1994). Near the western edge of the

bay, variable currents are weak and oriented mostly parallel to the shoreline. This, coupled with the steep sides of the head and western sides of the bay, suggests that advection from coastal waters into the deep depositional area of the bay is unlikely (B. Sanderson, pers. comm.). On the shallower eastern side of the bay, current flow is also usually directed away from the study site (deYoung and Sanderson 1994). Current meter records from a depth of 25 m indicate fluctuating currents typically < 10 cm/s. At about 200 m, current speed is diminished and generally <3 cm/s; these deep currents are largely oscillatory and result in little net flux (de Young and Sanderson 1993). Vertical mixing is low as a result of small mean currents and small variable currents, and should cause little interference with sediment trap operation (B. Sanderson, pers. comm.). In similarly low current velocities, sediment traps have been shown to perform well (Gardner 1980).

6.2.2. Collection and Analysis of Sedimenting Material

A series of sediment traps was attached to a taut-wired mooring at a site 270 m deep and within 500 m of the sampling station shown in Chapter 4 (Figure 4.1). The traps were 60 cm x 10 cm PVC cylinders, as recommended by Bloesch and Burns (1980), giving collection efficiency equivalent to that of funnel traps with baffles (Hargrave and Burns 1979). Arrays of 4 traps were deployed at each of four depths (40, 80, 150 and 240 m) for periods of 3-7 days, with one 13 day deployment during early April. The trap contents were retrieved a total of 10 times between March 29 and May 30, 1988. Throughout April and May, water temperatures were $< +1$°C at 40 m, and <0°C at all other trap depths.

During the first 5 trap deployments, 2 traps of each quartet at 40, 80 and 150 m were covered with plastic screens (1.8 mm mesh) to exclude larger grazers while permitting diatom chains, flocs and faecal pellets to enter. No significant differences were obtained for any variable in mean value between traps with screens and those

without (coefficient of variation $<10\%$), so the practice of using screens in later deployments was discontinued. Poisons were not used, as the duration of deployment was short and because of the interference of poisons with chemical analyses of samples.

Following recovery of the traps, the supernatant from each collection tube was slowly decanted; the remaining contents were returned to the lab in polycarbonate bottles, packed in ice. Animals were removed from the contents, which were then settled overnight on ice, decanted, and the slurry made up to 50 or 100 ml with filtered seawater (1 μm). Subsamples of the slurry were then removed for analysis.

For the determination of particulate organic carbon and nitrogen, subsamples of trap material were collected on precombusted Whatman GF/C filters under vacuum, dried at 60°C, and combusted in a Perkin-Elmer 240A CHN analyser. The same filtration process was used for the determination of dry wt and weight loss on ignition at 450°C, except that material on the filter was washed with 2 ml of 3% ammonium formate before drying.

Chloropigment samples were collected on Whatman GF/C filters under low vacuum and analyzed immediately or following storage in a -70°C freezer for up to 6 wks. Pigments were extracted overnight in 90% acetone at 4°C in darkness. Chlorophyll *a* and phaeopigments were determined by fluorometry, and one sample from each set of 4 replicates was also analyzed using HPLC. Samples for microscopy were fixed in Lugol's iodine and formaldehyde (3% final concentration), and then stored at 4°C.

6.2.3. Sediment Collection and Analysis

Surface sediment samples (0-10 cm) were collected at the sampling site on 6 occasions during 1989: March 1 and 15, April 10, May 16, July 5 and September 12. Replicate collections were made using a modified 0.5 m^2 van Veen grab equipped with

2 coring devices. The corers were fitted with plexiglass tubes 50 cm long and 6 cm in diameter, and collected sediments down to 20 cm, as well as flocculent water above the sediment surface. Only cores which showed an undisturbed sediment profile and little or no apparent resuspension in the water above the surface sediment layer were processed. Upon retrieval, the core tubes were capped, stored upright in crushed ice and the contents allowed to settle for 2-4 hr before processing, except for the September collections, which were settled overnight at 0°C before sample removal. All processing was conducted under subdued light conditions.

The highly flocculent water immediately above the sediment surface (0-15 cm), and the clearer water in the upper 15 cm of the core tubes, were siphoned into separate beakers. Particulate material in 50-100 ml samples from each water layer was collected on GF/C filters for CHN and chloropigment analysis. Pigment analysis followed the general procedures outlined in Chapter 2, and CHN determinations were conducted as described in Section 6.2.1.

Sediments within each core tube were sampled using a 60 cc plastic syringe barrel, with conical tip removed. Subsamples were collected by gently pushing the open end of the barrel down into the sediment while holding the plunger in place. The contents of the syringe were sliced at intervals of 0.2-2.0 cm, quick-frozen on dry ice and stored at -20°C prior to analysis. Subsamples of sediment from depths of 0-0.2, 0.2-1, 1-2, 4-5 and 8-10 cm were quick-frozen on dry ice and stored at -20°C prior to processing. The sediment texture at all depths was classified as mud, based on a grain size composition which showed $>90\%$ of grains in a size class of $<63\ \mu m$ (Scheibe 1991). All pigment samples were analyzed using both the standard fluorometric method and HPLC. An adjustment for a sediment pore water content of 5-7% (Scheibe 1991) was made by extracting sediment samples in 95% acetone rather than the usual 90%. Samples were extracted at -20°C for a period of 24-48 hr and were sonicated in an ice-cooled ultrasonic bath for 10 min following 12 hr of extraction.

6.3. Results

6.3.1. Daily Flux of Particulate Material

Daily fluxes of total particulate matter (TPM) into traps at 40, 80, 150 and 240 m, and percent composition of particulate organic material (POM), carbon (POC) and nitrogen (PON), of particulate flux, are shown in Table 6.1. All flux data represent the means of samples from 4 replicate traps. At depths of 40, 80 and 150 m, daily TPM fluxes were 1-3 g $m^{-2}d^{-1}$ throughout April, increasing to >4 g $m^{-2}d^{-1}$ in early May. Values of TPM flux from traps at 240 m were about 2x higher than those at 150 m in early April and during mid- to late May, suggesting that resuspension of bottom sediments was taking place. Mean percent POM content of particle flux at 40-80 m was 15-28% during April and 30-52% in May, with the highest levels occurring during May 4-10 (43-52%), coinciding with peaks in both phytoplankton biomass and vertical flux (Chapter 4, Figure 4.2). The POM content of particle flux generally declined with increasing depth.

POC comprised <7% of TPM accumulating in all traps from March 29 to April 27, and in traps at 240 m throughout April and May (Table 6.1). The POC fraction of the TPM increased at 40, 80 and 150 m after April 27, peaking at 17% in the near surface traps during the first 2 wks in May. This was followed by an apparent decline in phytoplankton production (Chapter 4) and a corresponding decrease in the POC content of TPM. The PON content of sedimenting material ranged from a low of 0.4% during the early bloom to a high of about 2% in traps at 40 and 80 m during early to late May. Trends in PON flux, with depth and duration of the bloom, followed those of POC. C/N ratios throughout April and May were low ($\leq$10), with maximum values at 40 and 80 m during the first 2 weeks in May. With the exception of the early phase of the bloom, there was no trend towards an increase in C/N ratio with trap depth. Throughout the bloom period, particulate material accumulating in traps at 240 m was not highly degraded, as indicated by a range in C/N ratios of 6.3-8.8.

Mean daily fluxes of POC at depths of 40, 80 and 150 m were <35 mg m^{-2} during the first trap deployment, suggesting little flux of bloom material prior to April (Figure 6.1). POC flux increased during April but remained at <180 mg $m^{-2}d^{-1}$ at all trap depths until early May. Mass sedimentation of bloom material was indicated by a sudden increase in the flux of POC during the first week in May, peaking at >700 mg C $m^{-2}d^{-1}$ in traps at 40 and 80 m. During mid-May, carbon flux to these depths decreased sharply, and by the end of May, POC flux was reduced to 200-300 mg $m^{-2}d^{-1}$. Maximum carbon fluxes at depths of 150 and 240 m occurred during the third week in May and were in the range of 550-600 mg $m^{-2}d^{-1}$. Sedimentation rates of >400 mg C $m^{-2}d^{-1}$ were maintained at depths of 150 and 240 m from mid- to late May. POC fluxes to traps at 240 m, however, were frequently higher than fluxes to 150 m, presumably due to TPM inputs from resuspended bottom sediments or other sources. Diel migrant zooplankton (e.g. copepods, euphausiids) may be important contributors to particle flux at these depths.

6.3.2. Trap Contents and Sinking Rate During Mass Flux

The trap contents were composed primarily of diatom cells and chains, and faecal pellets ranging in size from 20x20 μm to 700x135 μm (McKenzie, unpubl. obs.). The dominant phytoplankton taxa accumulating in the sediment traps during the mass sedimentation of bloom material in early May were *Skeletonema costatum*, *Chaetoceros debilis*, *Thalassiosira* spp. and *Fragillaria* spp. Most faecal pellets collected in the traps were intact, ovoid or cylindrical in shape, and <300 μm in length. Copepod and oikopleurid faecal pellets were most abundant in traps at 80, 150, and 240 m during mid- to late May and their relative contribution to TPM flux increased with increasing trap depth (McKenzie et al., unpubl. data).

The sinking rate of POC was calculated during the mass sedimentation of primarily intact diatom cells and chains in early May by using the slopes of cumulative

carbon flux in Figure 6.6. Separate linear fits to the early and latter parts of each time series indicate a small initial carbon flux followed by a step increase to a greater flux of carbon. The point at which the two linear fits intersect give the time for the step increase, as shown for each trap depth in Figure 6.1. These step functions indicate sinking rates of 20-23 m/d throughout the water column during May 2 to May 11 (Table 6.2). Chlorophyllide *a* and chlorophyll *a* comprised 40-80% of the chloropigments sedimenting out of the euphotic zone during this period of the bloom (Table 6.3).

6.3.3. Chloropigment Flux and Composition

Daily fluxes of total chloropigments (TChl) were <0.5 mg $m^{-2}d^{-1}$ during the first trap deployment in late March, and were 1-3 mg $m^{-2}d^{-1}$ during most of April (Figure 6.2). Peak fluxes of >10 mg TChl $m^{-2}d^{-1}$ occurred at depths of 40 and 80 m during early to mid-May, with the highest pigment fluxes often observed in traps at 80 m. In late May, chloropigment flux was reduced at 40 and 80 m to about 5 mg $m^{-2}d^{-1}$. The accumulation rates of pigments in traps at 150 m were similar to those at 240 m, where estimates of daily fluxes during May, although lower than in traps at 40 and 80 m, were less variable. Overall, the sedimentation patterns of TChl reflected those of POC flux.

HPLC fluorescence profiles of material collected in traps deployed for 7 days during April 27 - May 4 are shown in Figure 6.3. Chlorophyll *a* (peak 11) was abundant in traps at both 40 and 80 m and declined with further increases in depth. Levels of chlorophyllide *a* (1) and chlorophyll *c* (2) were also high in the traps at 40 m, indicating diatom senescence during this period. This is consistent with observations of nutrient depletion and elevated chlorophyllide *a* concentrations in seston samples during late April and early May (Chapter 4). Relative amounts of chlorophyllide *a* and chlorophyll *c* also declined with increasing trap depth. Although a bloom of *Pyramimonas* sp. appeared in the surface waters during mid-April, its presence was short-lived and there was no significant accumulation of chlorophyll *b* in the sediment traps during this period. As the

percent chlorophyll *a* of TChl flux decreased, amounts of pyrophaeophorbide *a* (5) increased, and rose from 14% of TChl at 40 m to 64% at 240 m (Table 6.3). Traps below 40 m also contained slightly higher levels of other phaeophorbide *a* derivatives (6&7) and phaeophytin *a*-type pigments (15-17).

The composition of chloropigments in material collected by traps varied with both depth and duration of the bloom (Figure 6.4). While phaeopigments comprised the largest fraction (62-88%) of TChl flux at all depths during April, chlorophyll *a*-type pigments (including allomers and chlorophyllide *a*) dominated the TChl flux at 40 and 80 m throughout May, comprising up to 85% of the pigment pool. Phytoplankton cell senescence was indicated in late April through to late May by the presence of high levels of chlorophyllide *a* in traps at 40 and 80 m. This derivative comprised >20% of TChl, and >40% of the chlorophyll-*a* type pigments, in near surface traps during mid-May. The relative contribution of chlorophyllide *a* to the pigment pool, however, was reduced in material collected at 150 m, and was lowest in traps at 240 m.

The dominant phaeopigment at almost all trap depths throughout the study was pyrophaeophorbide *a* (Figure 6.4), the primary degradation product of copepod grazing (see Chapters 3 and 5). This decay product comprised 50-64% of TChl in material sedimenting to 240 m during April, and 36-50% of TChl flux during May. High levels of phaeophorbide *a*, a decay product of chlorophyllide *a*, were also observed during the late phase of the bloom, contributing approximately 10-20% of TChl flux during mid- to late May (Table 6.3). Phaeophytins generally represented <15% of the pigment pool in traps during April and <10% during May, and were usually dominated by pyrophaeophytin *a*.

Throughout April and early May, ratios of phaeophorbide *a*: phaeophytin *a* (includes all phaeopigment derivatives) at all trap depths were in the range of 3-7 (Table 6.3). Ratios during this period generally increased with depth while those during mid-

to late May declined with depth. The phaeophorbide *a* fraction of total phaeopigment flux was also considerably higher during the decline of the bloom. Phaeophorbide *a*: phaeophytin *a* ratios of 10-50 were observed at 40-150 m during the third and fourth weeks of May. This relative increase in phaeophorbide *a* products was probably the direct result of an increase in zooplankton grazing pressure.

Carbon/total chloropigment (C/TChl) ratios in material collected in traps at all depths throughout the bloom are shown in Figure 6.5. The dotted lines plotted in each panel represent the range in mean C/TChl ratios of seston samples collected at the SCM during April and May. The lowest ratios were found in traps below 40 m during late April and early May, when chlorophyll *a* and chlorophyllide *a* fluxes were high as a result of cell senescence and massive diatom flux. Ratios of >150 were found in trap material at 240 m during early April and late May and are probably due to inputs of resuspended bottom sediments; levels of total particulate matter in the bottom traps were 2x higher than those at 150 m during these deployment periods (Table 6.1).

If digestive processes of grazers mediate the transformation of ingested chlorophyll *a* to nonfluorescent material, then an increase in the C/TChl ratio of the trap contents would be expected with an increase in grazing pressure. The products of grazing (phaeopigments, faecal pellets) were most abundant in traps during mid- to late May and were greatest in traps below 40 and 80 m. Increases in the C/TChl ratio with both depth and duration of the bloom were thus anticipated. These trends were not evident in the plots shown in Figure 6.5. C/TChl ratios did not tend to increase with depth, and ratios during the early bloom period were similar to those during the declining phase of the bloom.

6.3.4. Cumulative Flux During the Spring Bloom

Cumulative fluxes of the primary variables measured and amounts of material lost or gained between trap depths are shown in Table 6.4. The amount of TPM sedimenting

in traps at 240 m was 64% greater than that measured at 150 m, and indicates inputs to the bottom traps from sources other than sedimenting bloom material and products of water column grazing. POM and POC fluxes to 240 m were also considerably higher than those measured at 150 m. Flux measurements at 40, 80 and 150 m are therefore better estimators of the vertical transport of bloom production.

Cumulative carbon flux and total chloropigment (TChl) flux to 40, 80, 150 and 240 m from March 29 through to May 30 are shown in Figure 6.6. The trends in carbon flux were similar at all depths, and total accumulations at 40, 150 and 240 m were in the range of 16-17 g C m^{-2} for the 62 day period of trap collection. Carbon flux to 80 m was slightly lower, totalling 13.6 g C m^{-2}. Cumulative TChl flux was 260-270 mg m^{-2} at 40 and 80 m and 200 mg m^{-2} at both 150 and 240 m.

The rate of increase in TChl flux with duration of the bloom mirrored that of carbon flux, especially at depths of 40, 80 and 150 m. More than 75% of the fluxes of both carbon and chloropigments to all depths occurred during May. Although chlorophyll *a*-type pigments dominated the composition of cumulative TChl flux to 40 and 80 m (54-66%), phaeopigments comprised the largest fraction (60-67%) of the chloropigment pool sedimenting to 150 and 240 m. (Table 6.4). Overall, the contribution of undegraded chlorophyll *a*, presumably represented by intact phytoplankton cells and chains, to near bottom waters was about 33% of the cumulative TChl flux.

Carbon and chloropigment losses between 40 and 150 m were 17% and 25%, respectively. Cumulative fluxes of carbon, pigments and particulate organic matter, however, were slightly higher at 80 m than at 40 m by 3-5%, possibly as a result of phytoplankton production at depths below 40 m. Water column chlorophyll concentrations, shown in Chapter 4 (Figure 4.2), indicate significant amounts of phytoplankton biomass at depths of 40-60 m in mid-April and during May.

The percent contributions of both POM and POC flux, of TPM flux, declined with increasing depth (Table 6.4); relative amounts of both were reduced by 34% between 40 and 150 m and by 50% between 40 and 240 m. The carbon content of POM flux, however, was relatively constant (29-32%) as depth increased. C/N ratios with depth were also similar, and were low (7.0-8.8), indicating little loss of nitrogen from the bulk of sedimented spring bloom production.

6.3.5. Composition of the Flocculent Layer

Chloropigment samples from the highly flocculent layer above the sediments were analyzed using both fluorometry and HPLC. The pigment peaks in the HPLC profiles, however, were complex, weakly resolved and difficult to measure. Consequently, data presented in this section represent only those measurements determined using the standard fluorometric method.

TChl concentrations in the water layers 0-15 cm and 15-30 cm above the sediment surface were elevated during April and May, 1989 (Figure 6.7A). The highest levels were consistently detected in the flocculent layer immediately above the sediment surface. Concentrations were highly variable between core samples but indicated levels > 15 μg TChl/l during the peak months of bloom production and flux. Pigment concentrations within the clearer water, 15-30 cm above the sediment, were always < 10 μg TChl/l, and were significantly lower than concentrations within the underlying flocculent layer during March, April and May ($P<0.001$). Fall and early spring concentrations showed mean values of about 3 μg/l in the upper layer of water (15-30 cm). Within both layers, and throughout the study period, phaeopigments represented 80-88% of the chloropigment pool and C/N ratios were in the range of 7.9-9.0. The carbon content during April and May, however, was higher in the flocculent layer (3.4-3.8%) than in the overlying clearer water (2.1-2.2%). $C/TChl$ ratios of 300-500 were found in both the clear and

flocculent layers. These ratios were up to 10x higher than C/TChl ratios in near bottom trap collections.

6.3.6. Accumulation Within the Surface Sediments

Sediments within the top 10 cm showed a generally uniform carbon content with depth and season (Table 6.5). Mean carbon levels ranged from 2.6-3.2% of dry wt in samples collected during March, April, May and July, and did not appear to be altered by the apparent anoxic conditions below depths of 3-4 cm. Sediment carbon levels were slightly higher in September, and increased with increasing depth: 2.9% at 0-0.2 cm to 3.6% at 8-10 cm. C/N ratios were relatively uniform with depth and season, ranging from 7.7 to 8.1 in sediments collected throughout March to mid-September.

The highest chloropigment concentrations were observed in the top 2 mm of sediment during all sampling periods, with peak levels of >70 μg TChl/g sediment in samples collected during May and July (Figure 6.7B). Amounts in sediments 0.2-1 cm deep were slightly lower but showed a similar seasonal trend in concentration. Chloropigment levels at depths of 4-5 cm and 8-10 cm were significantly lower than those at 0-0.2 cm ($P<0.001$), and with the exception of the September collection, showed concentrations of <40 μg TChl/g sediment. Relatively high pigment levels were found at all depths during September, indicating a late summer/autumn pulse of pigment-rich material to the sediments.

The chloropigment composition of the sediments was relatively uniform with both depth and season (Figure 6.8, Table 6.6). Phaeopigments comprised about 70-80% of TChl, and were dominated by a suite of phaeophorbide *a*-type pigments. The composition of all sediment samples showed more complexity and a greater diversity of fluorescent decay products than found within the traps. The phaeophorbide *a*: phaeophytin *a* ratios were typically in the range of 2.5-3.5. The highest ratio (4.2) was observed within the

top 2 mm of sediment on May 16, and may be indicative of a high flux of recently deposited zooplankton faecal material.

HPLC profiles of chloropigments in sediments 1-2 cm deep during May and September are shown for comparison in Figure 6.9. The chromatograms are similar in both composition and relative abundance of the various pigments. The phaeophorbide *a* derivatives in the September profile, however, were not as well resolved compared to the May sample, and may suggest a higher degree of chloropigment degradation. The raised baseline in both profiles is indicative of the co-elution of other sediment components. The interference caused by these compounds is reflected in the observed differences between fluorometric and HPLC determinations of chloropigments (Table 6.5).

6.3.7. Comparison of TD10 and HPLC Measurements

Plots of total chloropigment measurements, determined using the standard fluorometric method (TD10) and HPLC, are shown for both sediment trap samples and surface sediments in Figure 6.8. The trap samples, which contained relatively fresh material and showed no elevation of the baseline in fluorescence profiles (Figure 6.3), showed very good agreement between methods. TD10 measurements of the sediment samples, however, were typically higher than those determined using HPLC; mean concentrations were approximately 20-50% higher when analyzed with the standard fluorometric technique (Table 6.5). These differences are indicative of the presence of nonchlorophyllous, fluorescent material in the sediment extracts.

6.4. Discussion

6.4.1. Trap Operation

Amounts of total particulate material collected in traps at 240 m were periodically 2x higher than concentrations found in traps at 150 m, and were probably the result of

bottom sediments resuspended from a depth of 270 m. During a preliminary study at this site in 1986, the deepest traps were deployed at the slightly greater depth of 250 m, and showed even higher additional inputs of particulate material which were believed to be the result of near bottom turbulence and sediment resuspension (Thompson et al., unpubl. data). Although circulation and vertical mixing in Conception Bay are, in general, low (DeYoung and Sanderson 1994), processes near the sediment surface in the deep depositional area of the bay appear to cause the periodic transport of bottom material from 270 m to depths above 240 m. Additional inputs to traps located close to the sediments interfere with the measurement of vertical flux. *In situ* transmissometer readings during the spring of 1989 showed elevated levels of suspended particles at depths below 200 m (Deibel et al., unpubl. data). Near-bottom traps in this area may thus require placement at a depth of about 200 m to avoid the effects of sediment resuspension. The concurrent use of downward facing traps could also be applied for estimates of upward flux.

The shallowest array of traps was placed at 40 m but accumulated 3-5% less material than traps located at 80 m, possibly as a result of phytoplankton production below the near surface traps. Significant levels of algal biomass were found within the mixed layer at depths between 40 and 50 m, especially following nutrient depletion and stratification of the surface waters in May (see Chapter 4). Flux measurements determined from the trap collections at 40 m probably underestimated the flux of total particulate matter sedimenting from the euphotic zone.

6.4.2. Features of Bloom Sedimentation

Although phytoplankton production during the 1988 spring bloom was underway during late March and continued until late May, >3/4 of the vertical flux of POC and TChl from the euphotic zone occurred in May. Mass flux was initiated following nutrient depletion and subsequent cell senescence during early May, as indicated by elevated

levels of chlorophyllide *a* in sediment traps at 40-150 m. A reduction in the daily carbon and chloropigment flux to 40 and 80 m throughout mid- to late May signalled the decline of the spring phytoplankton bloom. The faecal pellet contribution to total flux was highest during the terminal phase of the bloom, increased with depth, and was dominated by copepod and oikopleurid faecal pellets.

Phytoplankton species sedimenting throughout the bloom were representative of the numerically dominant spring bloom taxa, and included *Chaetoceros* spp., *Skeletonema costatum*, *Thalassiosira* spp. and *Fragillaria* spp. Sinking rates during the mass flux of predominantly intact phytoplankton cells and diatom chains in early May were estimated at 20-23 m/d between all trap depths. The faecal pellet contribution to this flux was low. Although the maximum *in vitro* sinking rates of nutrient-depleted cells and chains of bloom diatoms are known to be <5 m/d, and generally <1 m/d (review in Smayda 1970), higher *in situ* sinking rates typically follow the aggregation and interlocking of diatom chains (Eppley et al. 1967). Sinking rates determined in this study were similar to those reported by Passow (1991), who observed mass fluxes of *Chaetoceros* spp. in the range of 16-32 m/d during a cell senescent phase of a spring bloom in the Baltic Sea. Much higher sinking velocities, however, have been determined for large marine aggregates (up to 1 cm diam.) following peaks in spring bloom production (Kranck and Milligan 1988, Alldredge and Gotschalk 1989, Riebesell 1989). The sinking rates of faecal pellets produced by adult *Calanus finmarchicus* feeding on spring bloom phytoplankton in coastal Newfoundland waters have been estimated at 7-35 m/d (Urban 1992), and are similar to the sinking rates observed during early May in this study.

The patterns of POM, POC, PON and TChl flux were similar with both depth and duration of the bloom. POM content was high during the bloom and represented 25-35% of TPM. These values are similar to those found in polls in western Norway, but are much higher than those reported for Norwegian fjords (Wassmann 1991). Cumulative POC flux from the euphotic zone during the development and decline of spring

phytoplankton bloom production in Conception Bay was highest at 80 m (17 g C m^{-2}) and lowest at 150 m (13.6 g C m^{-2}). These POC flux values are equivalent to 30-40% of the estimated primary production during this 2 mos period (45 g C m^{-2}, Table 4.4). Furthermore, based on the chloropigment profiles, relatively undegraded phytoplankton cells and chains contributed a total of 54% of the TChl flux to 80 m, 40% to 150 m and 33% to 240 m. Volumetric data from the microscopic analyses of the trap contents are not yet available but will provide further estimates, and perhaps more accurate determinations, of the relative contributions of phytoplankton cells, faecal pellets and phytodetritus to sedimenting particulate material.

6.4.3. Composition and Condition of Sedimenting Material

The chloropigment composition in material collected in traps during deployment periods of 3-7 days directly reflected the pigment compositions in the seston samples collected from the SCM (see Chapter 4), and in the gut tracts and recently produced faeces of the primary zooplankton grazers (see Chapter 5). Cell senescence was traced throughout May by the appearance of chlorophyllide *a* and its acidified product, phaeophorbide *a*, in traps at 40 and 80 m. Pyrophaeophorbide *a* was the dominant phaeopigment encountered in samples from traps below 40 m. This product is typically found within the gut tracts and faecal pellets of many zooplankton grazers (Downs 1989, Head and Harris 1992, Chapter 5). Although there was a slight increase in the appearance of additional phaeophorbide *a*-type decay products during sinking, there was very little indication of pigment transformation as a result of containment within the traps. Most cells and faecal material were readily identifiable and observed to be intact (C. McKenzie, pers. comm.). Slow degradation rates of the peritrophic membranes of copepod faecal pellets have been observed at low temperatures (Honjo and Roman 1978, Urban 1992). In the current study, temperatures at depths of deployment were $< +1$°C at 40 m and about -1 °C at all other trap depths. Low temperatures, short trap deployment periods ($\leq$ 1 wk), and low numbers of both zooplankton grazers and microbes (Pomeroy

et al. 1991), appear to have been effective in limiting the degradation of sinking diatoms and faecal material during the spring bloom in Conception Bay.

C/TChl ratios in the range of 45-60 were frequently recorded in traps at all depths during May. These ratios were often lower than those in seston samples collected at the SCM, and appeared to be unaffected by increases in zooplankton grazing activity. A C/TChl ratio as low as 45 is uncommon in sinking bloom material, and is at the low end of the C/Chl range (45-500) reported by Smetacek (1980). Similarly low ratios, however, were recently observed during the mass sedimentation of primarily *Chaetoceros* spp. and *Thalassiosira* spp. in the central Baltic Sea (Passow 1991). Although considerably higher C/TChl ratios were expected with an increase in the grazing products of ingested chlorophyll *a* (phaeopigments and presumably, nonfluorescent products), such increases were not observed. Liebezeit and Bodungen (1987) also found ratios of 45-50 in deep traps (>300 m) containing material with high concentrations of phaeophorbide *a*. Comparable rates of carbon utilization and chloropigment loss to nondetectable products during grazing may explain the low ratios found in sedimenting phytoplankton and faecal material. Maximum observed losses of cumulative POC and TChl fluxes between near-surface and near-bottom traps were very similar (approximately 20% and 28%, respectively). It is also possible that low C/TChl ratios observed in trap collections during the mass flux of bloom material in early May were influenced by selective sedimentation of diatoms from the euphotic zone, as proposed by Passow (1991).

Particulate material collected in traps throughout the bloom was relatively undegraded, as indicated by both low C/TChl ratios and low C/N ratios (<10). The highest C/N values (9-10) were detected in near-surface traps during the peak of bloom production in May. These ratios may have been associated with microbial activity, which was low in the water column, but elevated within sediment traps during this period (Pomeroy et al. 1991). Traps at 150 and 240 m collected material with C/N ratios in the range of 6-9 throughout the bloom. These values are comparable to those determined

from seston samples collected from the euphotic zone (Chapter 4), and to C/N ratios found in faecal pellets produced by zooplankton grazers (Knauer et al. 1979, Silver and Bruland 1981, Alldredge et al. 1987). C/N ratios of the cumulative fluxes of carbon and nitrogen were similar with depth, and were low (7.0-8.8), indicating little decay of the bulk of sedimented material during the spring bloom, and a nitrogen-rich supply of organic matter to the sediments.

6.4.4. Deposition Within the Surface Sediments

There was a marked seasonal trend in the chloropigment concentrations in both the flocculent layer immediately above the sediment-water interface (about 270 m), and within the surface sediments. The flocculent layer showed maximum chloropigment levels during the spring bloom, a complex chloropigment composition similar to that of the sediments, and low Chl *a*/TChl ratios (<0.2) during both spring and summer. Microbial activity in the flocculent layer was also high, relative to that within the water column (Pomeroy et al. 1991). Although C/N ratios in this layer were in the range of 7.9-9.0, suggesting low levels of degraded material, C/TChl ratios were up to 10x higher than in material collected in traps at 240 m. These observations indicate a high degree of organic matter transformation in the flocculent layer immediately above the sediment surface. Enhanced degradation in this zone may be the result of reduced sinking velocities and entrapment within a relatively dense nepheloid layer, which contains an active microbial community. It is probable that hyperbenthic zooplankton are also very active within this layer.

Within the top 10 cm of sediment, carbon content was 2.6-3.6% of sediment dry wt. These values, uniform C/N ratios with both depth and season (7.7-8.1), and relatively constant protein concentrations (12 mg cm^{-3}) and organic matter content (18-24%) with depth (Scheibe 1991), indicate high levels of organic material throughout the top 10 cm of sediment during both spring and summer. The study site can thus be

characterized as a depositional area of high quality, nitrogen-rich phytodetritus (Valiela 1984, Walsh 1984, Grebmeier et al. 1988). It may also be an area where food is not limiting.

Seasonal inputs of phytogenous material to the sediment surface were indicated by elevated chloropigment concentrations in the top 1 cm of sediment during May, July and September. Peak concentrations, exceeding 70 μg TChl/g sediment, fall within the range of levels observed in both coastal and freshwater sediments (Tett 1982, Nalepa and Quigley 1987). Sedimentation events in the spring and late summer were also observed in the 1986 sediment trap study at this site (Thompson et al., unpubl. data), and are typical of many coastal regions (Smetacek 1980). Although TChl concentration decreased with increasing sediment depth, composition remained relatively constant within the top 10 cm, throughout spring and summer. Similar trends in phytopigment distribution have been reported by other investigators (Furlong and Carpenter 1988, Yacobi et al. 1991). Such observations have led to the conclusion that the most extensive transformation and degradation of phytogenous material occurs at the sediment surface shortly after deposition (Furlong and Carpenter 1988, Hurley and Armstrong 1991, Sun et al. 1991).

HPLC profiles of sediment extracts showed a greater number of phaeopigment decay products and more complexity in pigment composition than was observed in the deep trap collections. The dominance and diversity of the phaeophorbide *a*-type pigments in the surface sediments of Conception Bay are typical of chloropigment compositions in the sediments of both marine and fresh water environments (Carpenter et al. 1986, 1988, Furlong and Carpenter 1988). These products have been associated with the grazing activities of both pelagic and benthic invertebrates (reviewed in Chapter 5), and with diagenetic processes in the sediments (Baker and Louda 1986, Repeta and Gagosian 1987). There was little evidence, however, of further pigment transformation to detectable, fluorescent products with increasing sediment depth. Uniform chloropigment compositions within the top 10 cm during both spring and summer suggest pigment

preservation or stabilization following burial and/or mixing processes within the sediments. Several investigators have observed high levels of chloropigment preservation, particularly within anoxic sediments (Brown et al. 1977, Hurley and Armstrong 1991, Yacobi et al. 1991). In this study, concentrations within the anoxic layers (4-5 cm and 8-10 cm) were relatively constant, with levels of 20-40 μg TChl/g sediment from mid-April to mid-September. At all depths and times of sampling, 20-30% of the TChl composition was represented by chlorophyll *a*-type pigments. These high levels of undegraded material may indicate a surplus of food and/or interactions within the sediments which reduce the post-depositional degradation of chloropigments.

A rich supply of both carbon and chloropigments throughout the top 10 cm of sediment suggests mixing processes and sediment transport within the surface layers. Benthic macrofauna are primary agents of particle transport in sediments (Aller 1982). A recent survey of the macrobenthic fauna in the deep depositional area of Conception Bay has shown an abundant and diverse fauna, numerically dominated by deposit feeding, bioturbating polychaetes, in particular, *Maldane sarsi* and *Prionospio steenstrupi* (Scheibe 1991). The sea star *Ctenodiscus crispatus* was also commonly collected in benthic grabs. Its gut tract was frequently filled with mud, indicating the ingestion and processing of large volumes of sediment. Video recordings from a benthic lander, deployed during April 1990, confirmed the presence of a highly active macrobenthic community (Pomeroy et al. 1991). It is highly likely that the feeding and tunnelling activities of the benthic macrofauna were primarily responsible for the near homogenous profiles observed within the top 10 cm of sediment. The degree of homogeneity within the sediments, however, is ultimately dependent on the relative rates of sedimentation, particle mixing and decomposition (Matisoff 1982).

6.5. Conclusions

The sedimentation of spring phytoplankton bloom production in 1988 occurred primarily in May during a cell senescent phase of the bloom. More than 75% of the flux of POM, POC and TChl (chl *a* wt equiv.), in the form of intact diatom cells and chains and faecal material, occurred during this period. The sinking rate of predominantly intact phytoplankton was 20-23 m/d throughout the water column during the onset of mass sedimentation in early May. These rates were determined following the accumulation of phytoplankton biomass at the SCM, and probably represent the settling velocities of small aggregates of interlocking diatom chains.

The POC flux was 30-40% of the estimated primary production during the 1988 spring bloom (45 g C m^{-2}, see Chapter 4). The relative contribution of intact diatom cells and chains to the total flux, as determined from chloropigment concentrations, decreased with depth from 65% at 40 m to 33% at 240 m. This decrease was associated with an increase in faecal pellet abundance with depth, particularly during the terminal phase of the bloom. Pyrophaeophorbide *a* was the primary chlorophyll degradation product observed in traps below the euphotic zone, and directly reflected the abundance of copepod and oikopleurid faecal pellets. Chloropigments were shown to be good tracers of phytoplankton biomass during the spring bloom in Conception Bay based on relatively constant C/TChl ratios and comparable maximum losses of POC and TChl flux between near surface and near bottom traps (20% and 28%, respectively). A temporal phase lag in the response of grazers to bloom production, and slow faecal pellet decay rates at temperatures $\leq 0°C$ (Urban 1992), contributed to the suitability of chloropigments as tracers.

C/N ratios of the particulate material accumulating throughout 62 days of trap collection were similar with depth and were low (7.0-8.8), indicating little decay of the bulk of sedimenting material during the spring bloom and an input of nitrogen-rich,

particulate organic matter to the sediments. This is consistent with indications that little remineralization takes place in the water column during spring (Pomeroy et al. 1991). A pulse of high quality phytogenous material (20% POM) reached the near bottom waters (240 m) during mid- to late May and supports the characterization of Conception Bay as an area of high pelagic-benthic coupling.

The chloropigment content of the surface sediments and the flocculent layer immediately above the sediment-water interface reflected the seasonal production and flux of phytoplankton and faecal material. The flocculent layer appears to be a region of high organic matter degradation, based on elevated C/TChl ratios (>300) and observed high microbial activity (Pomeroy et al. 1991). Input to, and activity within, this layer is undoubtedly also influenced by the periodic resuspension of surface sediments.

Throughout March to mid-September, the top 10 cm of sediment were rich in both carbon (2.6-3.6%) and total chloropigments (>20 μg/g sediment dry wt). Although pigment concentrations decreased with depth in the uppermost 5 cm, the chloropigment composition, dominated by a complex series of phaeophorbide *a*-type pigments, remained relatively uniform down to 10 cm. The bioturbating activities of the macrobenthic taxa (primarily deposit feeding polychaetes), and diagenetic processes which enhance pigment stability, were probably responsible for the near homogenous nature of the surface sediments.

Table 6.1. Mean daily flux of total particulate matter (TPM) and percent composition of particulate organic matter (POM), organic carbon (POC) and organic nitrogen (PON), and mean daily total chloropigment flux (TChl, ±SD) to 40, 80, 150 and 240 m during April and May 1988. * traps outfitted with 1800 μm mesh.

Dates of Trap Deployment	Hrs	Depth (m)	TPM g/m²/d	% POM	% POC	% PON	C/N	TD10 TChl Flux mg/m²/d	TD10 % Phaeo	HPLC TChl Flux mg/m²/d
Mar 29 - Apr 6	193	40	1.04	16.8	2.4	0.5	4.8	0.34 ± 0.08	79	-
		80	1.05	22.8	2.3	0.5	4.9	0.29 ± 0.04	82	-
		150	1.61	17.6	2.1	0.4	5.4	0.30 ± 0.04	88	-
		240	3.42	14.5	4.1	0.5	7.7	0.49 ± 0.11	86	-
Apr 6 - Apr 19	310	40	2.25	28.1	6.5	1.2	5.5	1.67 ± 0.10	69	1.71
		80	2.54	14.6	5.3	0.7	7.2	1.81 ± 0.14	72	1.72
		150	1.81	16.0	4.9	0.6	8.7	1.15 ± 0.19	79	0.83 *
		240	2.49	11.2	5.2	0.6	8.0	1.56 ± 0.20	73	1.89
Apr 19 - Apr 22	68	40	1.74	22.6	3.6	0.7	6.0	1.55 ± 0.45	75	2.11
		80	3.00	20.4	5.2	0.6	8.5	2.94 ± 0.29	76	3.13
		150	2.25	18.9	5.1	0.7	6.4	2.06 ± 0.51	81	2.63
		240	3.44	19.6	5.3	0.6	8.8	2.11 ± 0.17	83	1.85
Apr 22 - Apr 27	122	40	1.55	22.9	5.6	0.8	7.4	1.13 ± 0.10	72	1.12
		80	2.11	19.2	6.0	0.9	7.0	2.36 ± 0.17	62	2.30
		150	1.51	20.3	5.1	0.7	7.9	1.16 ± 0.16	78	0.96 *
		240	2.60	17.5	4.3	0.7	6.3	1.46 ± 0.09	78	1.34
Apr 27 - May 4	170	40	1.73	36.5	15.7	1.6	8.9	5.61 ± 0.07	33	6.19
		80	1.35	25.4	6.9	1.0	7.3	2.98 ± 0.01	54	3.08
		150	1.26	21.7	5.6	0.8	7.0	1.98 ± 0.48	77	2.44
		240	1.89	20.2	5.2	0.8	6.7	1.74 ± 0.09	78	1.60 *

Table 6.1. continued.

Dates of Trap Deployment	Hrs	Depth (m)	TPM $g/m^2/d$	% POM	% POC	% PON	C/N	TD10 TChl Flux $mg/m^2/d$	TD10 % Phaeo	HPLC TChl Flux $mg/m^2/d$
May 4 - May 10	143	40	4.14	51.9	16.9	1.9	9.1	10.82 ± 0.15	28	11.18
		80	4.30	42.7	16.2	1.7	10.1	10.47 ± 0.09	34	10.81
		150	2.52	29.3	10.2	1.1	9.0	5.96 ± 0.05	50	6.03
		240	3.62	22.8	6.6	0.7	8.7	5.62 ± 0.80	56	4.12
May 10 - May 13	71	40	4.38	42.8	16.6	1.7	10.0	9.15 ± 0.61	25	8.32
		80	5.16	35.3	13.7	1.5	8.7	15.05 ± 0.51	32	14.72
		150	3.82	30.2	10.8	1.2	9.0	7.80 ± 0.36	43	8.38
		240	3.68	19.7	6.2	0.9	7.5	5.26 ± 0.29	60	4.92
May 13 - May 17	100	40	3.01	38.1	13.5	1.7	8.2	5.66 ± 0.30	31	4.70
		80	4.32	35.9	13.6	1.8	7.5	10.86 ± 0.80	37	12.85
		150	4.22	27.4	9.8	1.2	8.1	7.88 ± 0.23	52	7.53
		240	8.26	18.9	6.0	0.7	8.2	8.78 ± 0.25	65	9.33
May 17 - May 25	188	40	2.76	33.0	8.8	1.2	6.9	5.52 ± 0.33	34	5.75
		80	3.98	32.2	8.9	1.3	7.0	3.78 ± 0.12	54	3.84
		150	5.72	25.8	9.6	1.3	7.7	4.04 ± 0.05	61	4.19
		240	10.76	18.2	5.4	0.7	7.4	3.46 ± 0.02	69	3.22
May 25 - May 30	121	40	2.01	41.7	14.3	2.2	6.4	5.58 ± 0.62	20	6.66
		80	3.42	29.3	7.9	1.2	6.7	5.27 ± 0.12	58	5.04
		150	6.87	23.3	6.1	0.9	6.7	6.41 ± 0.08	65	6.84
		240	10.30	20.7	5.3	0.8	6.5	6.57 ± 0.09	72	7.62

Table 6.2. Estimates of particle sinking rates (m/day) during the mass sedimentation of bloom material in early May, 1988. Dates determined from the step function in Figure 6.1.

Depth Interval (m)	Dates between estimated appearance of fluxed material	Sinking Rate (m/day)
40-80	May 2-4	20.0
40-150	May 2-7	22.0
40-240	May 2-11	22.2
80-150	May 4-7	23.3
80-240	May 4-11	22.9
150-240	May 7-11	22.5

Table 6.3. Chloropigment flux and composition (chlorophyll *a* and fluorescent derivatives, as determined by HPLC) in traps deployed at 40, 80, 150 and 240 m during April and May 1988. Identities are: TChl (total chloropigments, chl *a* wt eq.), chlld (chlorophyllide *a*), chl *a* (chlorophyll *a*), pho (phaeophorbide *a*), pyr (pyro-derivative), phy (phaeophytin *a*).

Dates Traps Set	Hrs	Depth (m)	TChl Flux mg/m²/d	% chlld	% **chl *a***	% pho 1	% **pyr-pho(3)**	% pho 4-6	% phy	% pyr-phy	pho: phy
Apr 6	310	40	1.7	2.6	**32.1**	10.6	**37.6**	4.8	3.6	8.6	4.3
		80	1.8	1.9	**24.6**	5.0	**47.2**	7.4	2.5	11.3	5.0
		150	1.1	0.8	**22.6**	7.2	**47.0**	7.2	3.2	11.9	4.4
		240	1.6	1.1	**20.4**	6.6	**50.3**	9.4	3.6	8.7	5.4
Apr 19	68	40	1.6	2.5	**26.5**	3.1	**47.7**	5.7	2.9	11.6	3.9
		80	2.9	1.1	**22.8**	4.5	**52.1**	6.9	1.6	11.0	5.0
		150	2.1	2.7	**20.4**	5.0	**50.8**	6.6	2.9	11.6	4.2
		240	2.1	1.5	**16.9**	5.5	**53.6**	10.9	3.2	8.4	6.0
Apr 22	122	40	1.1	2.2	**27.9**	3.4	**45.3**	6.4	2.2	12.6	3.7
		80	2.4	2.5	**36.0**	1.5	**38.4**	5.2	3.1	13.3	2.8
		150	1.2	1.2	**19.5**	2.9	**52.4**	7.1	3.6	13.3	3.7
		240	1.5	1.2	**19.7**	3.5	**53.4**	7.9	3.6	10.6	4.6
Apr 27	170	40	5.6	21.7	**51.2**	4.1	**14.2**	1.7	1.8	5.4	2.8
		80	3.0	7.1	**42.0**	0.4	**32.3**	5.9	2.4	9.8	3.2
		150	2.0	1.8	**15.5**	0.8	**60.9**	7.2	2.2	11.6	5.1
		240	1.7	1.9	**16.4**	1.5	**63.7**	5.7	3.5	7.2	6.6

Table 6.3. continued.

Dates Traps Set	Hrs	Depth (m)	TChl Flux mg/m²/d	% chlld	% **chl *a***	% pho 1	% **pyr-pho(3)**	% pho 4-6	% phy	% pyr-phy	pho:phy
May 4	143	40	10.8	14.4	**70.2**	5.1	**4.3**	1.4	2.4	2.1	3.1
		80	10.5	16.1	**53.9**	6.6	**10.8**	5.4	2.6	4.5	3.2
		150	6.0	10.1	**42.6**	4.9	**24.3**	8.6	2.9	6.5	4.0
		240	5.6	5.7	**34.4**	0.7	**35.7**	11.7	3.7	8.1	4.1
May 10	71	40	9.1	21.5	**55.9**	12.2	**4.7**	4.4	0.8	0.5	16.4
		80	15.0	16.9	**53.5**	7.0	**13.4**	4.6	1.8	2.8	5.4
		150	7.8	9.2	**47.9**	4.6	**23.7**	8.0	1.6	5.1	5.4
		240	5.3	3.7	**36.5**	5.0	**35.6**	11.4	2.3	5.4	6.8
May 13	100	40	5.7	18.1	**57.6**	17.2	**3.1**	0.0	1.1	2.9	5.1
		80	10.9	19.6	**40.8**	14.1	**14.1**	5.3	2.4	3.7	5.5
		150	7.9	8.1	**40.1**	7.4	**26.0**	11.3	2.6	4.5	6.3
		240	8.8	3.1	**29.7**	5.6	**37.5**	14.9	2.7	6.5	6.3
May 17	188	40	5.5	26.1	**31.8**	18.8	**17.8**	4.7	0.2	0.6	51.6
		80	3.8	12.8	**25.4**	16.3	**33.9**	6.5	1.4	3.8	10.9
		150	4.0	12.4	**34.4**	16.7	**26.9**	4.8	1.3	3.6	9.9
		240	3.5	4.4	**21.0**	9.7	**41.6**	14.0	3.2	6.1	7.0
May 25	121	40	5.6	35.8	**38.8**	8.6	**13.8**	2.0	0.4	0.7	22.2
		80	5.3	13.2	**21.2**	14.5	**43.0**	4.2	0.7	3.3	15.4
		150	6.4	10.0	**22.6**	17.8	**39.3**	5.1	1.1	4.2	11.7
		240	6.6	5.8	**13.9**	14.4	**49.6**	9.0	2.6	4.9	8.7

Table 6.4. Cumulative flux of total particulate matter (TPM), particulate organic matter (POM), particulate organic carbon (POC), particulate organic nitrogen (PON) and total chloropigments (TChl = chlorophyll *a* plus phaeopigments, chl *a* wt eq.), and the percentage loss of sedimented material between 40 and 150 m and between 40 and 240 m, during March 29-May 30, 1988.

Flux (g/m^2)	Cumulative Flux March 29 - May 30				% loss between 40 m and 150 m	% loss between 40 m and 240 m
	40 m	80 m	150 m	240 m		
TPM	144.4	177.4	182.2	299.0	-26.2	-107.1
POM	51.26	52.66	42.85	55.48	16.4	-8.2
POC	16.44	17.06	13.63	16.02	17.1	2.6
PON	2.14	1.94	1.86	2.28	13.1	-6.5
TChl	0.261	0.273	0 198	0.196	24.1	24.9
% POM/TPM	35.5	29.7	23.5	18.6	33.8	47.6
% POC/TPM	11.4	9.6	7.5	5.4	34.2	52.6
% POC/POM	32.1	32.4	31.8	28.9	9.3	10.0
% Chl *a*/TChl	65.5	54.4	40.1	32.7	38.8	50.1
POC/TChl	63.0	62.5	68.8	81.7	-	-
C/N	7.7	8.8	7.3	7.0	-	-

Table 6.5. Mean values of percent carbon, C/N ratio and total chloropigment (TChl) concentration, and percent phaeopigment, as determined by the standard fluorometric method (TD10) and HPLC, in sediment samples collected during Mar-Sept 1989.

Date	Depth (cm)	No.	% C	C/N	TD10 : TChl (μg/g sed)	TD10 % Phae	HPLC : TChl (μg/g sed)	HPLC % Phae	TChl % diff
Mar 1	0-0.2	8	3.0	7.7	56.7 ± 11.8	80.6	39.8 ± 9.6	78.6	43.5
Mar 15		5	2.9	7.8	62.3 ± 5.1	78.5	42.9 ± 6.7	79.5	47.1
Apr 10		2	2.6	7.8	59.3 ± 7.2	78.1	37.9 ± 1.8	81.1	56.1
May 16		3	3.1	8.0	85.7 ± 6.5	77.9	72.8 ± 8.0	77.4	19.5
July 5		5	2.7	7.8	99.9 ± 33.4	74.5	73.7 ± 32.4	72.9	37.5
Sep 12		3	2.9	7.9	115.0 ± 29.0	79.3	64.2 ± 5.9	80.9	48.9
Mar 15	0.2-1	5	2.9	8.0	45.6 ± 5.3	76.1	29.5 ± 3.0	73.1	54.5
Apr 10		2	2.9	8.2	48.7 ± 6.1	75.5	34.4 ± 3.1	77.0	41.2
May 16		7	3.1	8.0	68.1 ± 14.8	78.8	57.1 ± 18.4	77.8	23.2
July 5		10	2.6	7.7	70.7 ± 23.4	76.9	48.4 ± 15.1	69.3	46.7
Sep 12		6	3.4	7.8	67.0 ± 12.1	76.8	57.8 ± 13.8	79.2	18.3
Apr 10	4-5	2	2.9	7.9	37.0 ± 0.1	76.6	24.4 ± 0.1	75.0	51.8
May 16		3	3.2	7.8	38.4 ± 1.7	76.3	30.3 ± 4.4	79.7	30.2
July 5		3	3.0	8.0	37.0 ± 4.2	74.4	25.2 ± 3.2	70.8	47.2
Sep 12		3	3.4	7.8	49.0 ± 14.9	74.3	39.1 ± 13.4	77.0	27.1
Apr 10	8-10	2	3.0	8.1	44.1 ± 5.8	74.5	30.0 ± 3.4	77.6	47.0
May 16		3	3.2	8.1	36.3 ± 2.8	75.0	29.9 ± 5.9	78.9	24.9
July 5		3	3.0	8.0	27.2 ± 1.1	84.9	18.8 ± 0.2	75.0	44.8
Sep 12		3	3.6	8.0	42.1 ± 13.9	77.1	34.0 ± 11.8	78.6	25.5

Table 6.6. Chloropigment composition (HPLC) in sediments collected during 1989. Legend: TChl (total chloropigments, chl *a* wt eq.), chlld (chlorophyllide *a*), chl *a* (chlorophyll *a* plus allomers, epimers), pho (phaeophorbide *a*), pyr (pyro-derivatives), phy (phaeophytin *a*). Data represent mean values.

Date	Depth (cm)	No.	TChl μg/g sed	% chlld	% chl *a*	% pho 1	% pyr-pho(3)	% pho 4-6	% phy	% pyr-phy	pho: phy
Mar 1	0-0.2	8	39.8	1.9	19.5	14.8	21.7	22.7	12.9	6.5	3.0
Mar 15		5	42.9	1.9	18.7	15.8	19.4	26.4	11.4	6.4	3.5
Apr 10		2	37.9	2.2	16.7	17.2	21.4	21.3	13.2	7.9	2.8
May 16		3	72.8	1.7	20.8	14.3	21.9	26.2	9.2	5.8	4.2
July 5		5	73.7	2.6	24.6	14.2	22.6	20.7	10.1	5.1	3.8
Sep 12		3	64.2	3.1	16.0	19.7	20.2	22.1	13.1	5.8	3.3
Mar 15	0.2-1	5	29.5	2.0	24.9	18.1	15.0	18.4	13.9	7.7	2.4
Apr 10		2	34.4	1.7	21.3	17.0	16.7	21.1	14.1	7.9	2.5
May 16		7	57.1	1.5	20.7	15.4	24.1	18.4	12.7	7.1	2.9
July 5		10	48.4	2.7	29.4	15.0	19.4	15.7	11.9	5.7	2.8
Sep 12		6	57.8	1.4	19.4	16.4	18.6	21.3	14.8	8.1	2.5
Apr 10	4-5	2	24.4	2.2	22.8	14.1	17.2	22.2	13.3	8.2	2.5
May 16		3	30.3	1.4	18.9	13.2	20.8	23.3	14.0	8.4	2.6
July 5		3	25.2	1.2	28.0	15.5	15.7	18.6	13.0	8.0	2.4
Sep 12		3	39.1	1.8	21.2	19.0	13.9	17.8	17.0	9.3	1.9
Apr 10	8-10	2	30.0	1.8	20.6	16.8	24.7	20.4	9.8	5.8	4.0
May 16		3	29.9	1.3	19.8	16.8	18.1	21.7	13.7	8.6	2.5
July 5		3	18.8	1.8	23.3	16.4	10.8	22.2	15.2	10.3	1.9
Sep 12		3	34.0	1.5	19.9	22.8	16.1	14.2	16.4	9.1	2.1

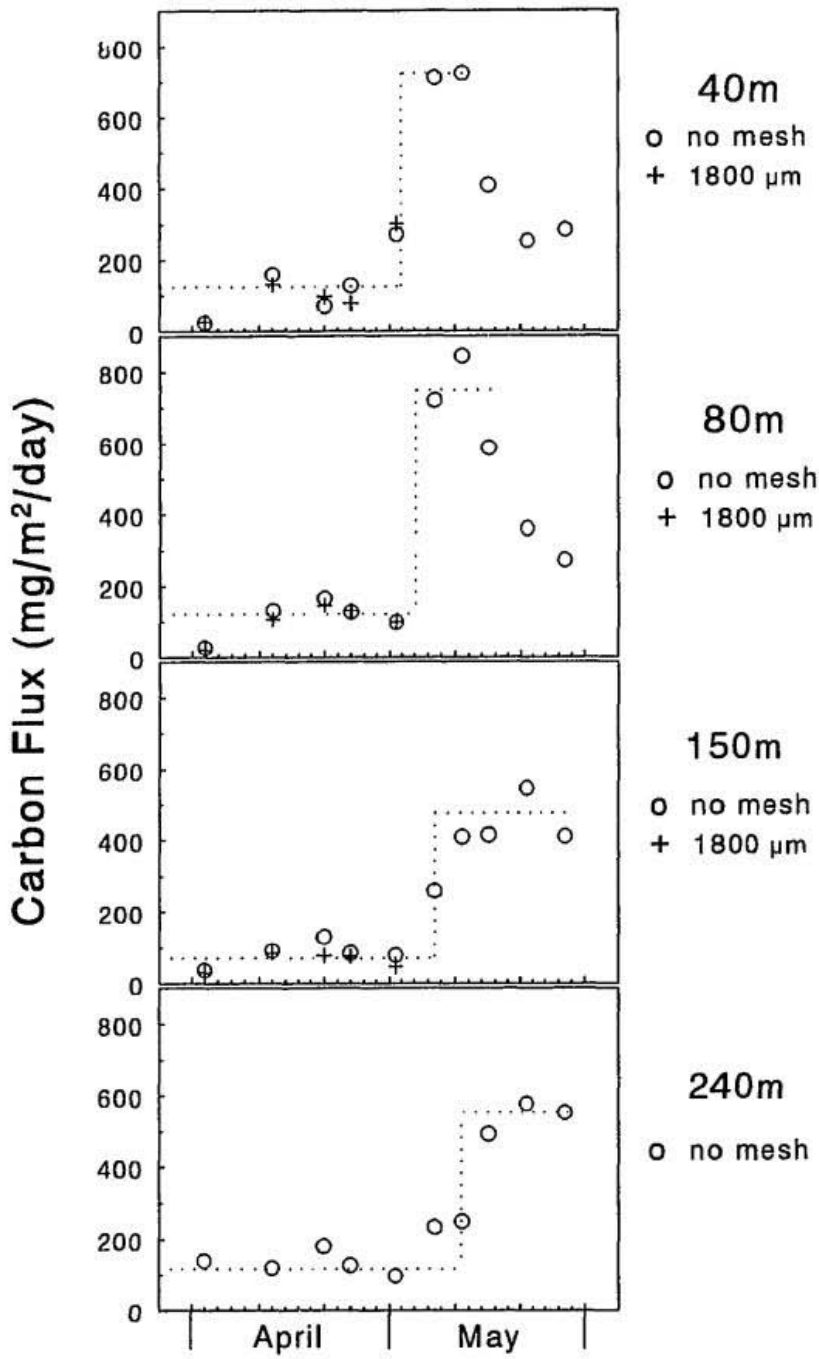

Figure 6.1. Mean carbon flux (mg/m²/day) to 40, 80, 150 and 240 m during April and May 1988, as determined by replicate sediment traps (N=4) at each depth. Step function fitted from the intercepts of the slopes of cumulative carbon flux in Figure 6.6.

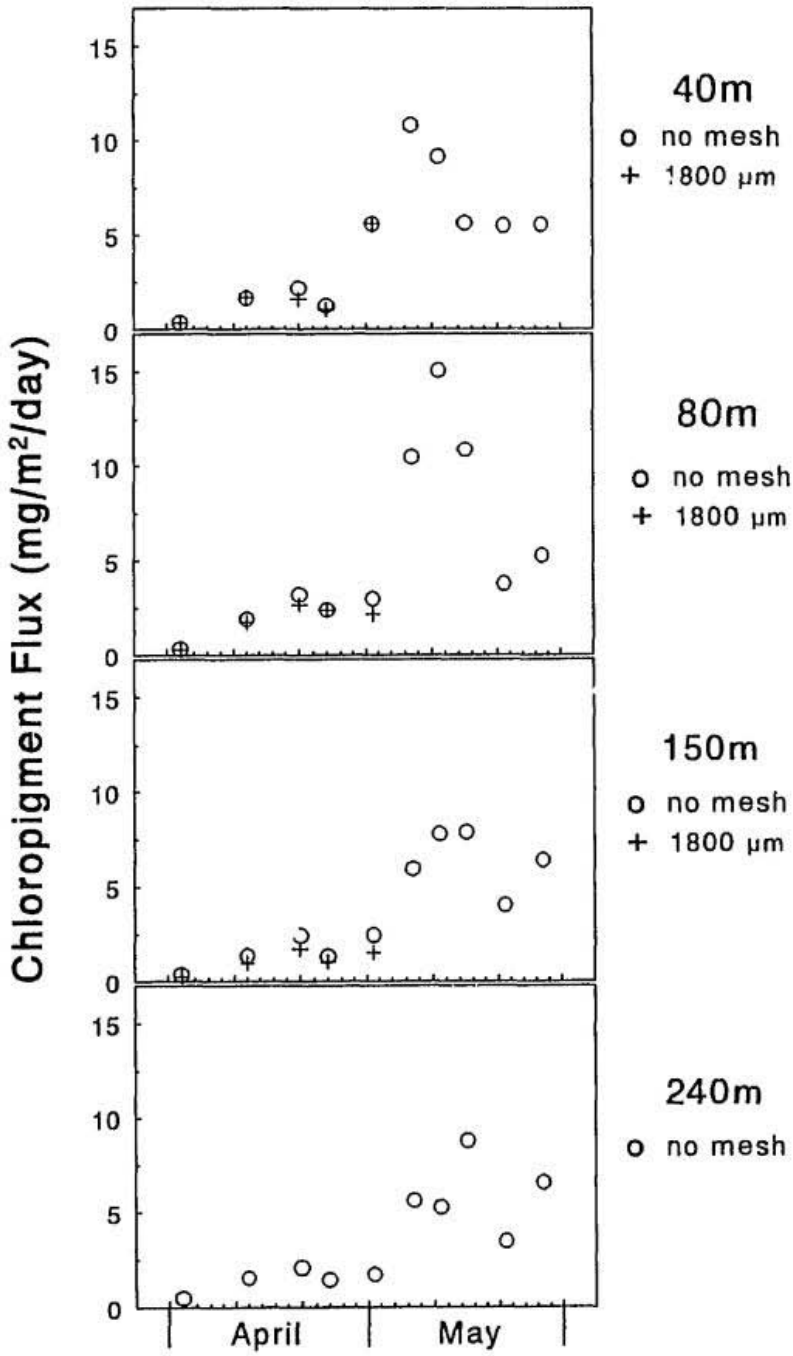

Figure 6.2. Mean chloropigment flux (mg/m²/day, chl *a* wt. equiv.) to 40, 80, 150 and 240 m during April and May 1988, as determined by replicate sediment traps (N=4) at each depth.

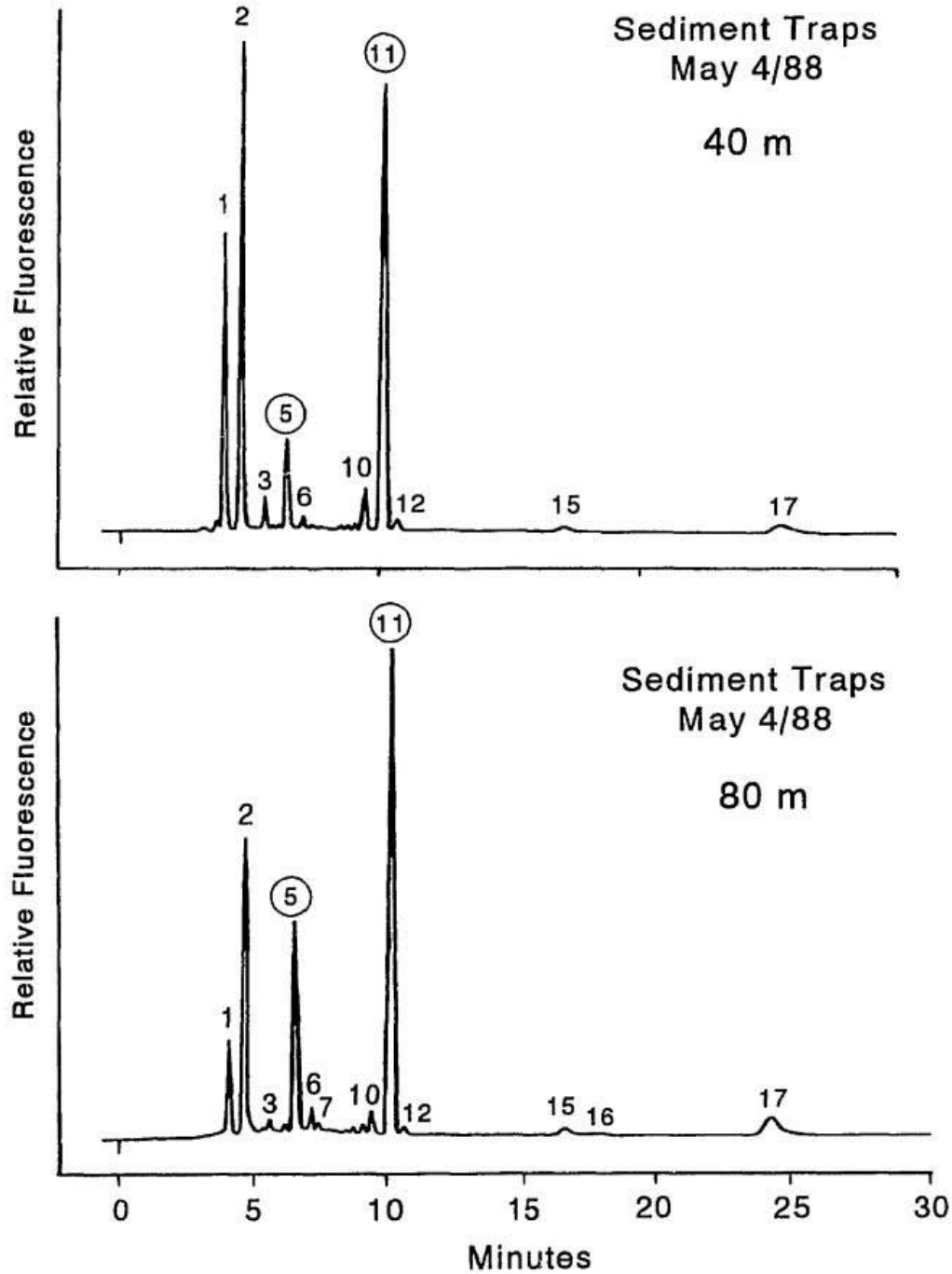

Figure 6.3. HPLC fluorescence profiles of chloropigments extracted from material collected in sediment traps at 40 m, 80 m, 150 m and 240 m. Traps retrieved on May 4, 1988 following a 7 day deployment period. Peak identities are shown in Table 5.1.

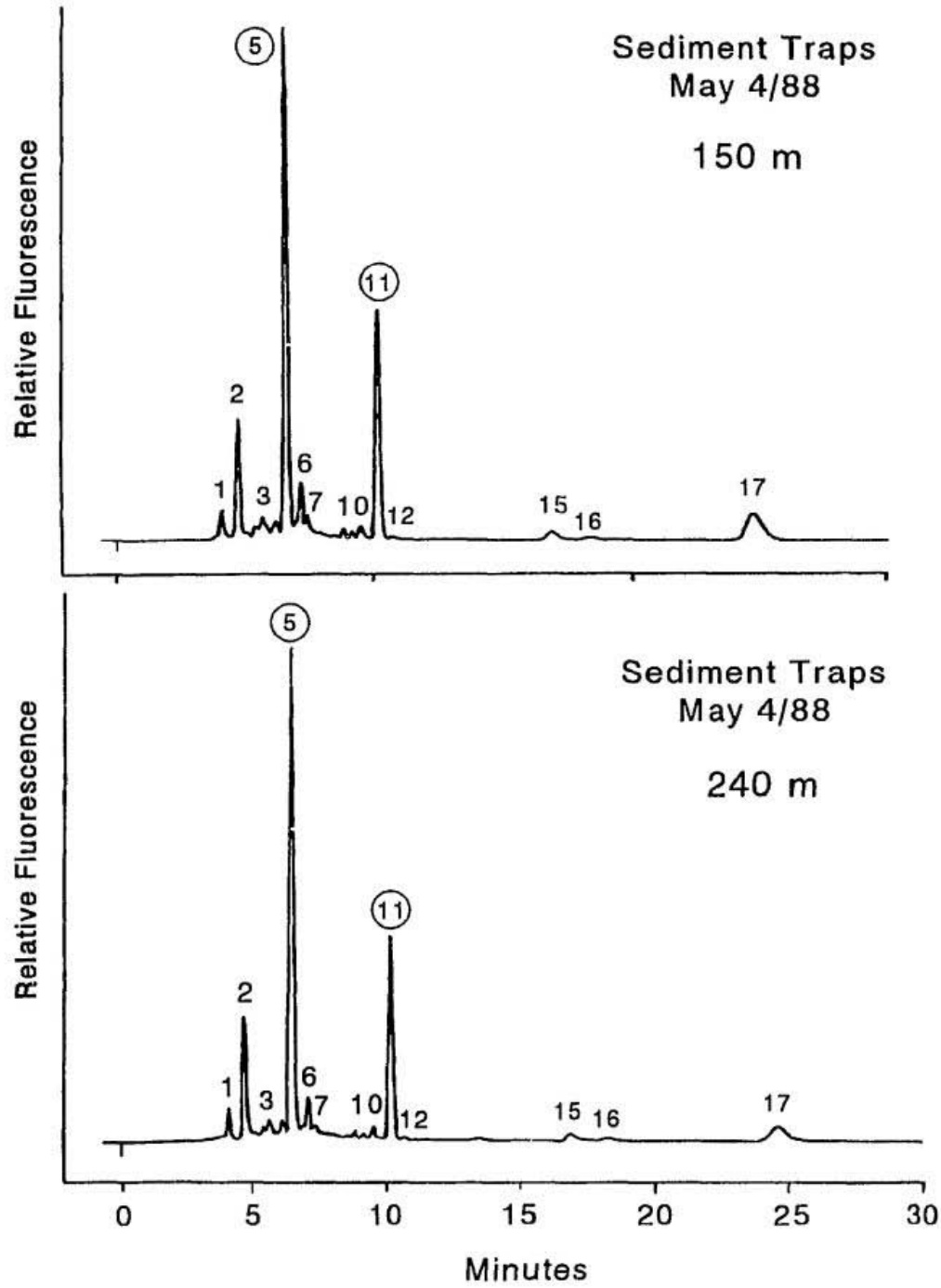

Figure 6.3. continued.

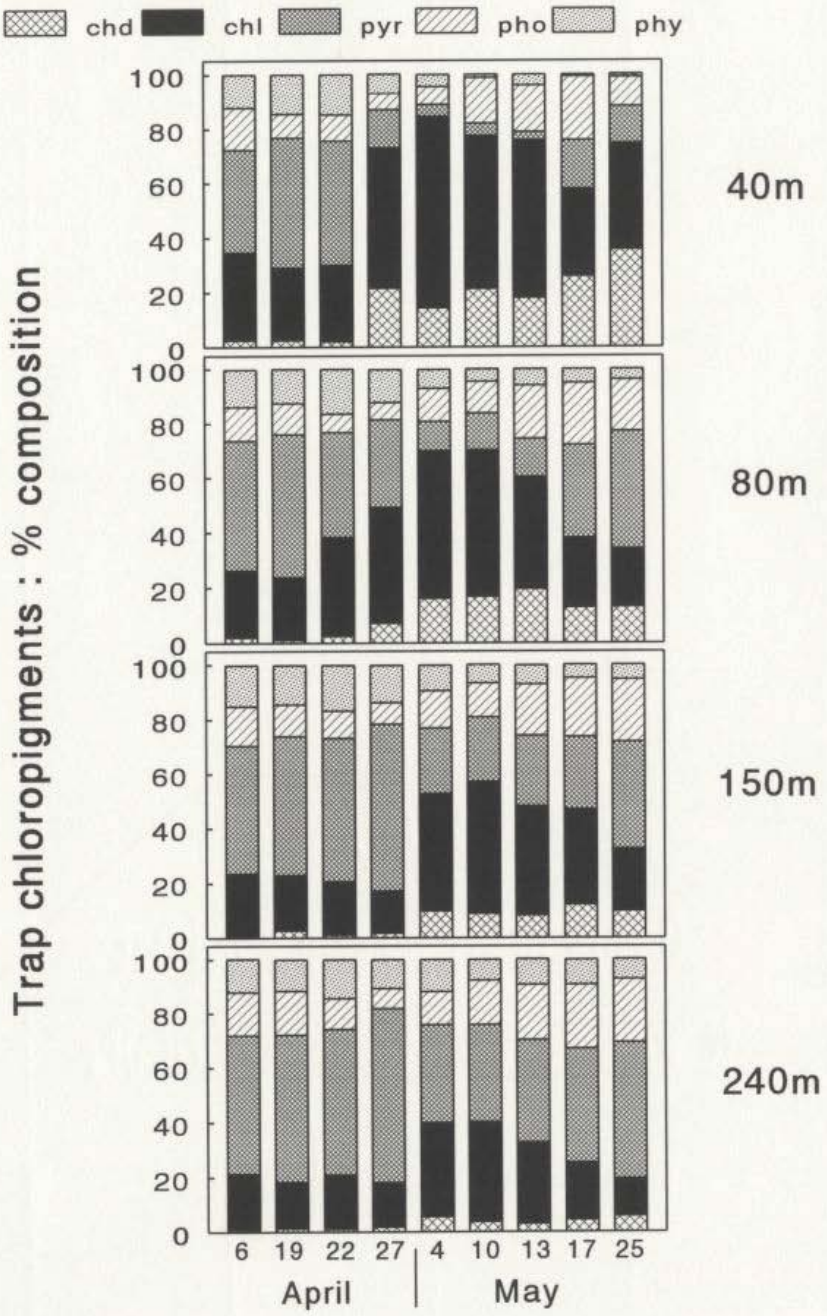

Figure 6.4. Chloropigment composition of trap contents during April and May, 1988. X-axis: dates of initial trap deployment. Legend: chd (chlorophyllide *a*), chl (chlorophyll *a*-types), pyr (pyrophaeophorbide *a*), pho (other phaeophorbides) and phy (phaeophytin *a*-types).

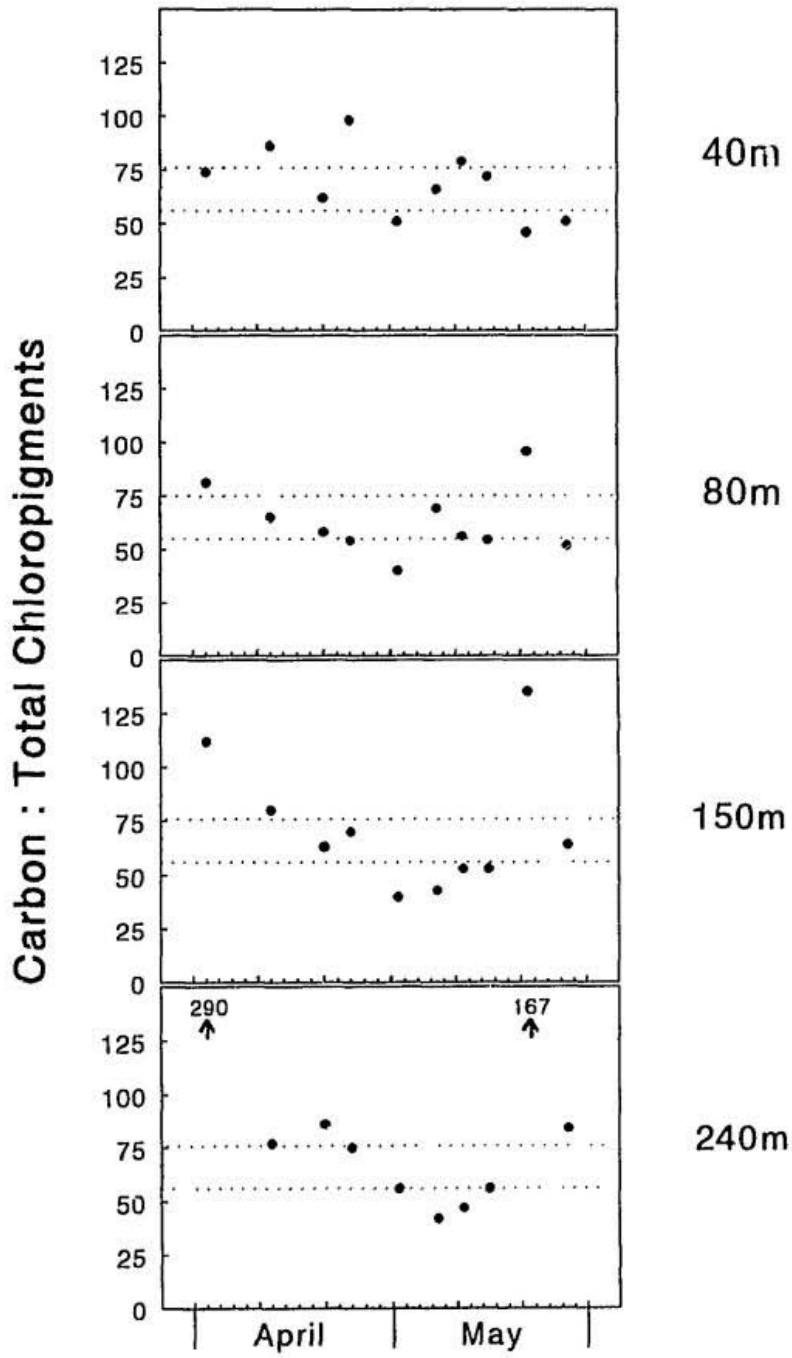

Figure 6.5. Mean carbon: total chloropigment ratios in material collected by sediment traps deployed at 40, 80, 150 and 240 m during April and May 1988. Ratios between the dotted lines in each panel represent seston values at the SCM during April and May.

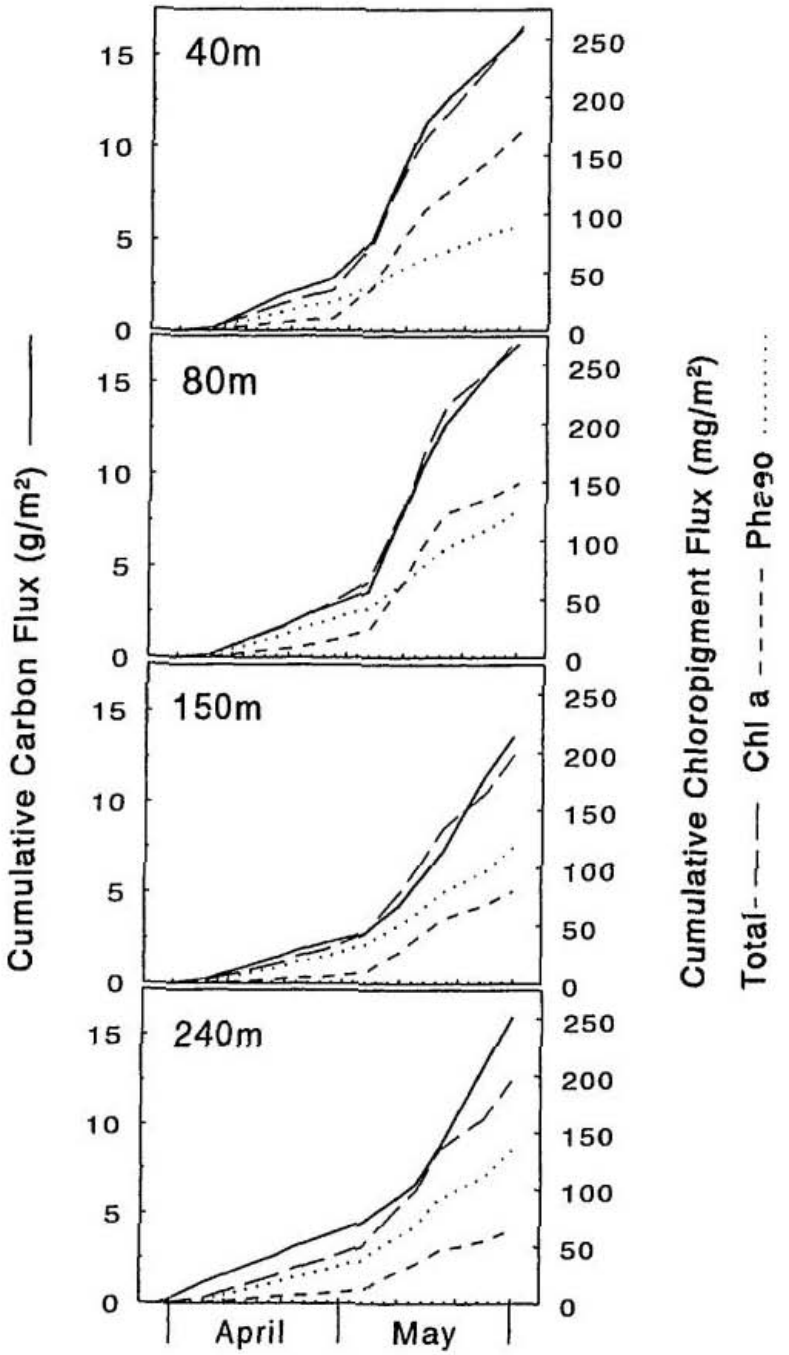

Figure 6.6. Cumulative flux of carbon (g/m^2), total chloropigments (mg/m^2), chlorophyll *a* (mg/m^2) and phaeopigments (mg/m^2) to depths of 40, 80, 150 and 240 m during April and May 1988.

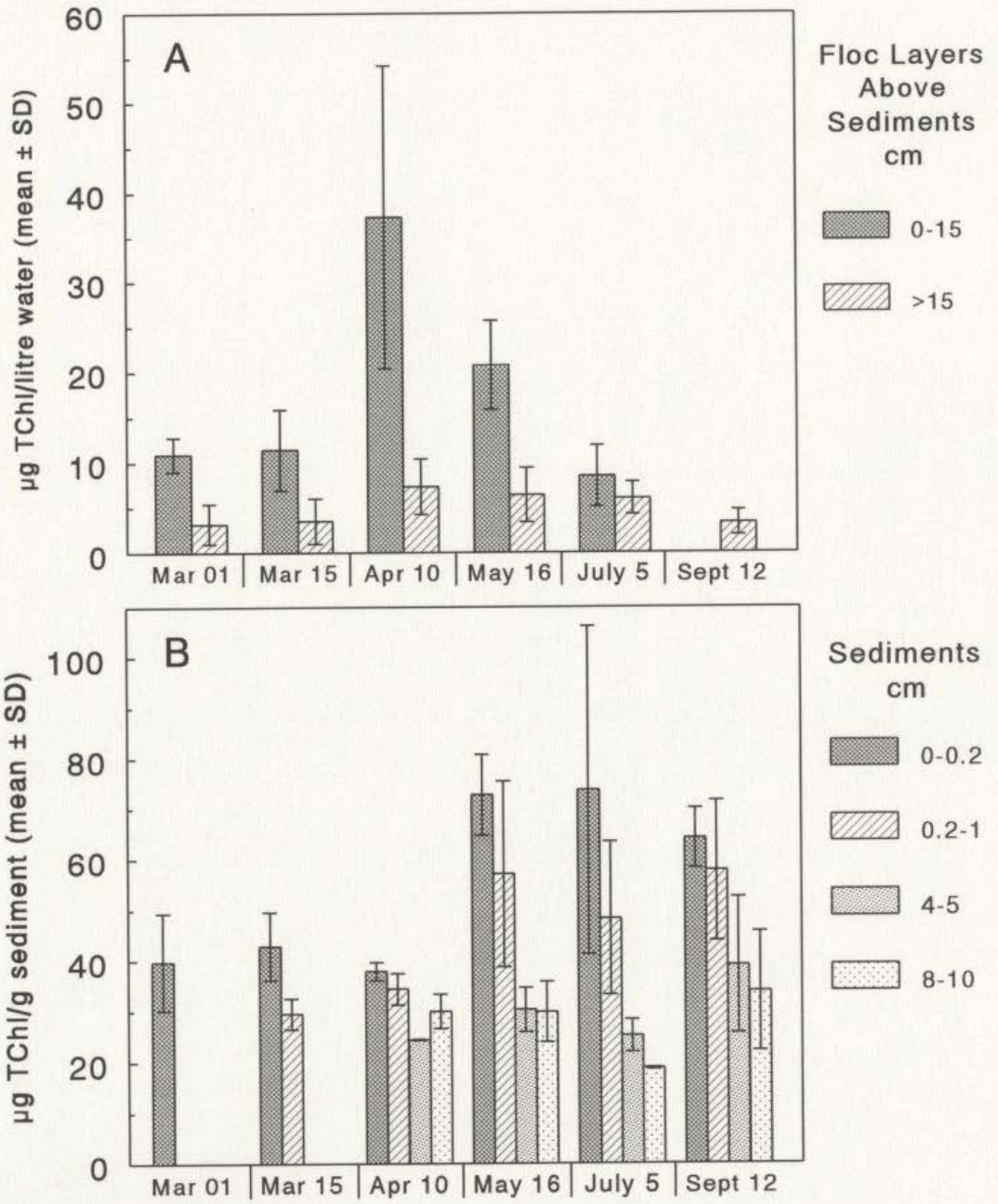

Figure 6.7. Concentrations of total chloropigments (TChl) in (A) particulate material suspended in water above the sediment surface (TD10 measurements, μg/l) and in (B) sediments at depths of 0-10 cm (HPLC measurements, μg/g sediment).

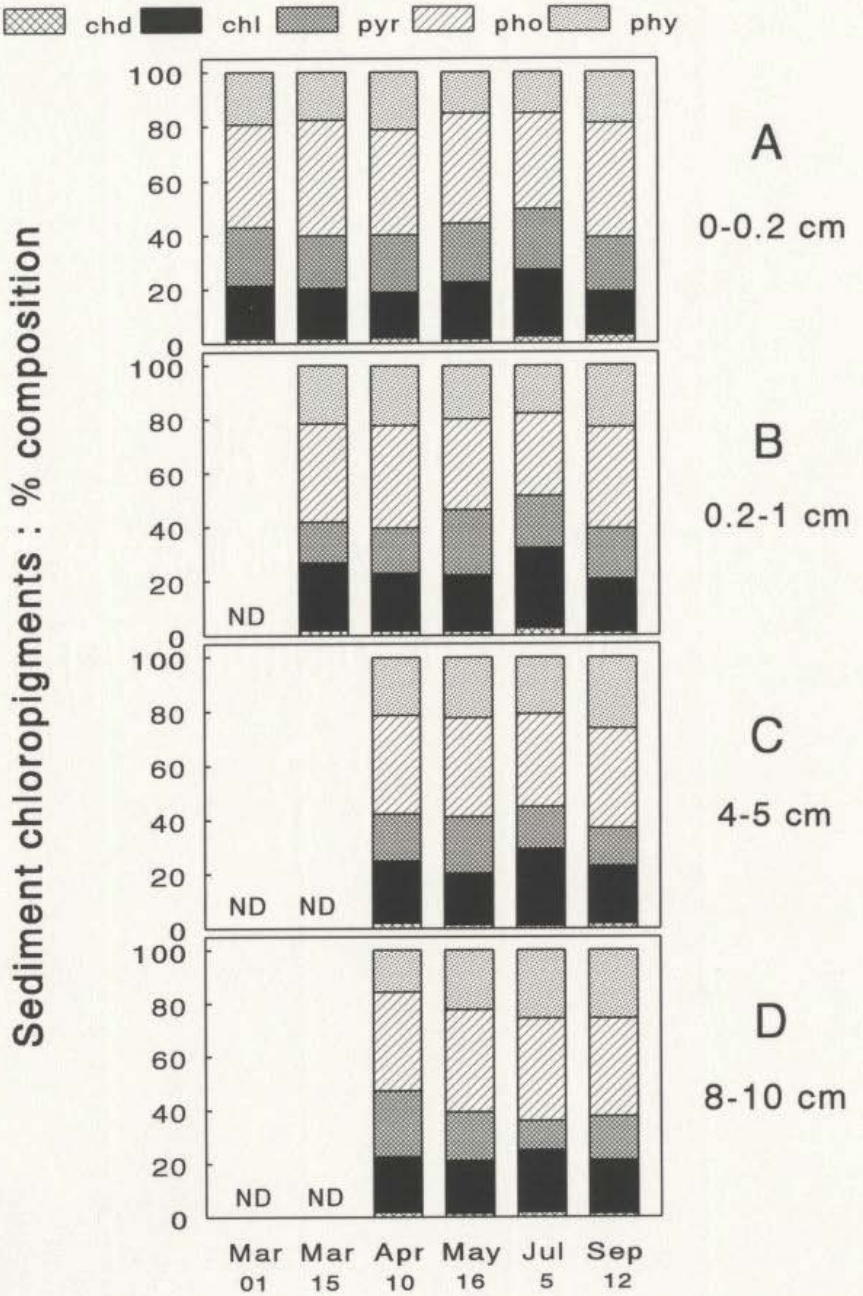

Figure 6.8. Chloropigment composition of sediments collected at depths of 0-0.2 cm, 0.2-1 cm, 4-5 cm and 8-10 cm during March to September, 1989. Legend: chd (chlorophyllide *a*), chl (chlorophyll *a*-types), pyr (pyrophaeophorbide *a*), pho (other phaeophorbides) and phy (phaeophytin *a*-types).

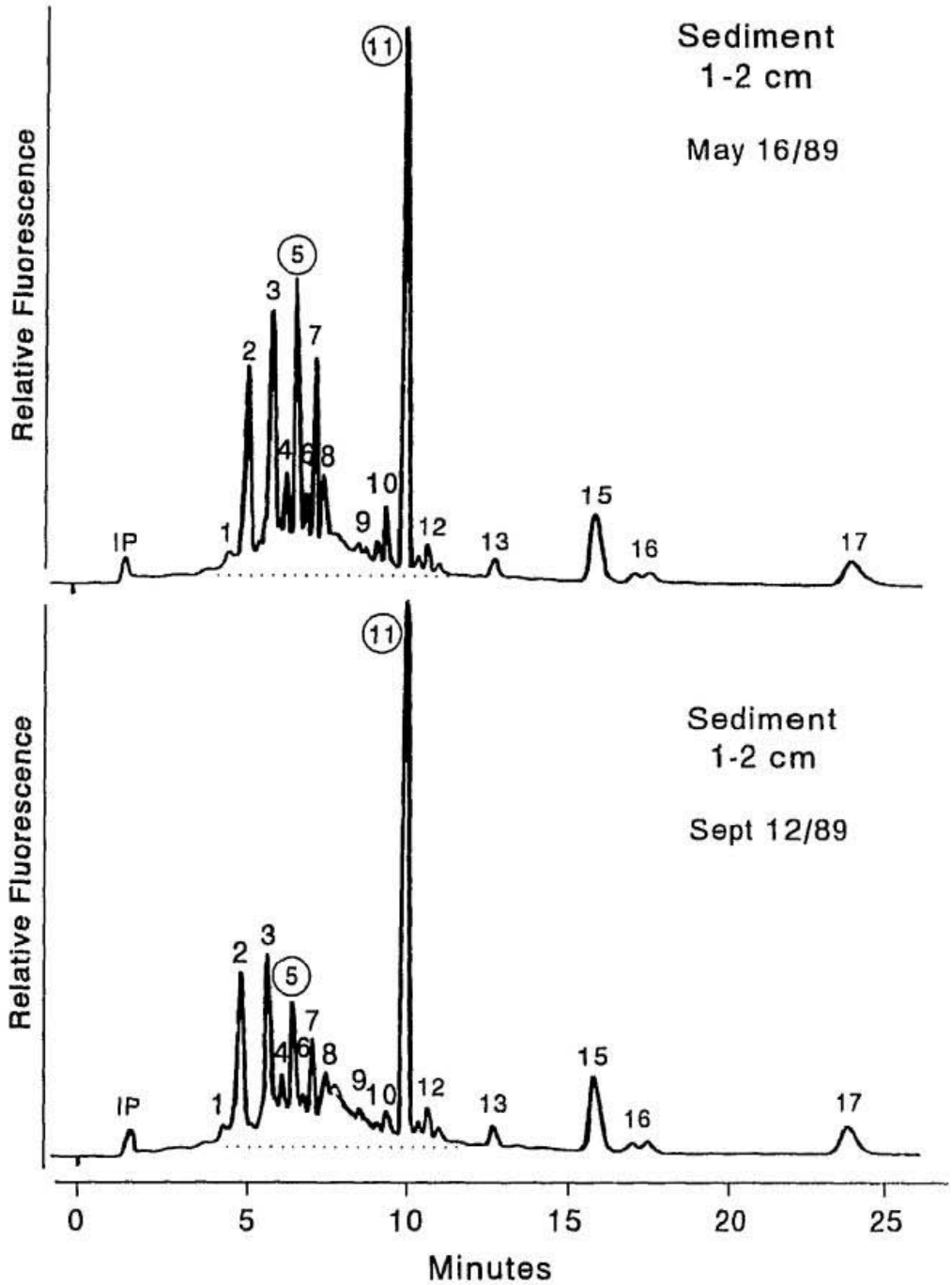

Figure 6.9. HPLC fluorescence profiles of chloropigments extracted from surface sediments (1-2 cm) during May and Sept., 1989. Peak identities are shown in Table 5.1.

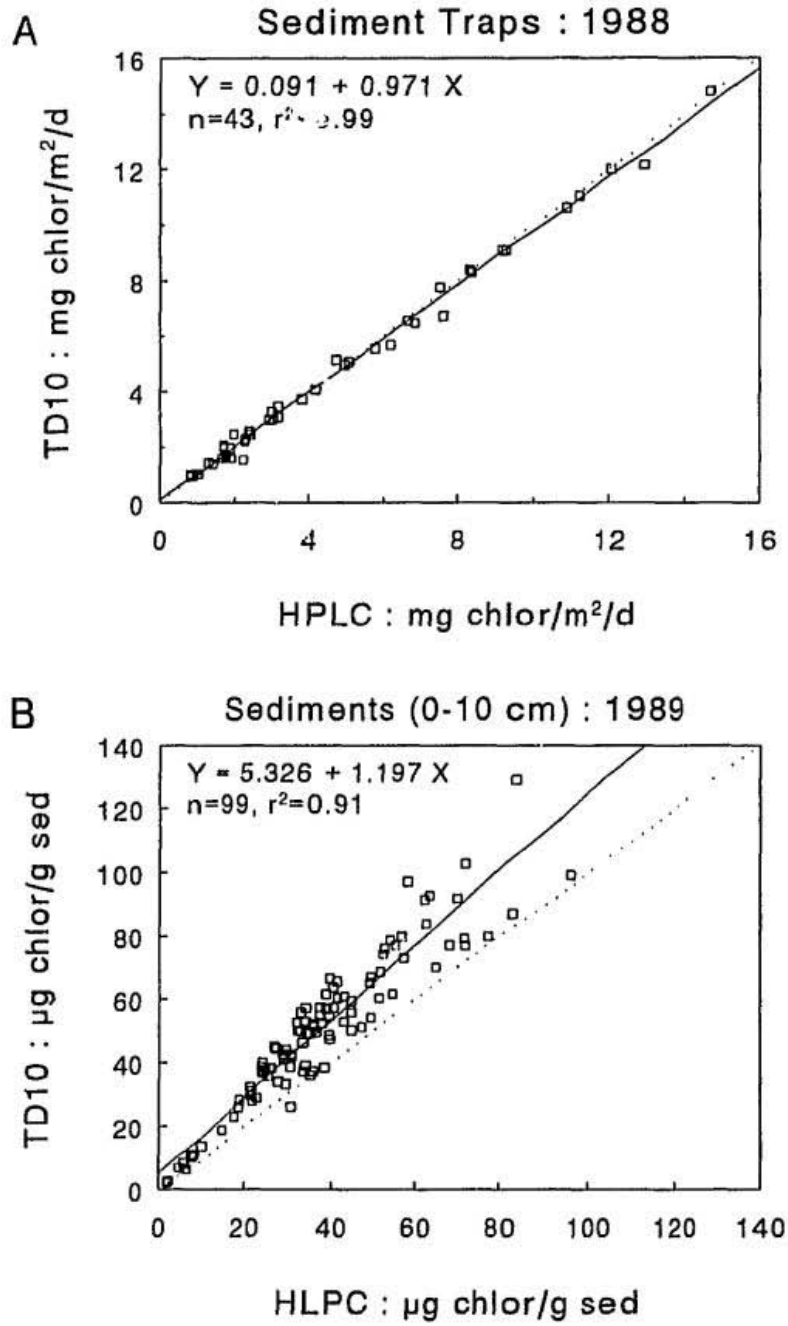

Figure 6.10. Relationships between standard fluorometric (TD10) and HPLC determinations of chloropigments in (A) sediment trap collections and (B) surface sediments. Dotted line represents a 1:1 relationship.

Chapter 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1. Chloropigments as Biomarkers

Chlorophyll *a* and its fluorescent derivatives were used as tracers of phytoplankton biomass and flux, and as indicators of biological processes throughout the spring bloom. This study included an examination of a wide variety of sample types, all of which were frozen for various periods of time prior to sample analysis. The use of chloropigments as tracers of phytogenous material requires the conservation of the porphyrin ring of chlorophyll *a* and its fluorescent derivatives throughout the duration of sample storage. An examination of the effects of freezing (-20°C) on the chloropigment concentration and composition within various sample types indicated that chlorophyll *a* and its degradation products are stable for up to 12 mos in samples that are relatively free of digestive enzymes and/or acids. Only minor alteration products were formed during sample storage, and these appeared to be generated by processes associated with the initial freezing of the sample material.

The use of chloropigments as tracers requires reliable and sensitive methods of detection. In this study, two different methods for determining chloropigment concentration were employed. The standard fluorometric method was used for the quantitative analysis of chlorophyll *a* and phaeopigments (in chl *a* wt equiv.). A representative set of samples was analyzed by HPLC for determinations of both the concentration of chloropigments and the relative contributions of the various chlorophyll *a* derivatives. The estimates of total chloropigment concentration determined by these methods were in good agreement for most of the sample types examined, supporting the use of the fluorometric method as a reliable and robust technique when chlorophyll *b* levels are low or negligible. The co-extraction of nonchlorophyllous compounds in the samples of sediment and digestive gland, however, caused interference with the standard

fluorometric determination of chlorophyll *a* and phaeopigments, and resulted in the overestimation of total chloropigments. The conventional fluorometric method is therefore not recommended for use with these sample types.

Biomarkers remain quantitative in tracer studies providing that they are not transformed to undetectable products. In this regard, the reliability of chloropigments as tracers of phytoplankton biomass has been highly questionable. In this study, chloropigment destruction to nonfluorescent products was examined in a series of grazing experiments with *Calanus finmarchicus* and various concentrations of spring bloom phytoplankton in laboratory experiments conducted at 0°C, for periods up to 24 hr. Losses to undetectable products were high (>80% of ingested chlorophyll *a*) following 6 hr of incubation at low initial food concentrations (< 1.5 μg chl/l), but were low and in the range of 0-35% of ingested pigment at seston concentrations of >3 μg chl/l. The highest rates of destruction were found within the first 3 hr of grazing, and appeared to have been enhanced by the activation of copepod feeding and digestive processes following an overnight period of starvation. Post-starvation digestive processes may have also caused the high and variable losses observed in many other grazing studies (Conover et al. 1986, review in Lopez et al. 1988). Chloropigments were not transformed to colourless products in incubations with high initial food levels (10.5 μg chl/l) and near-continuous copepod grazing. At high cell concentrations, estimates of ingestion determined using the gut fluorescence method were comparable to those determined from particle clearance rate data, and from the disappearance of chlorophyll *a*. As food concentrations declined, the gut fluorescence method yielded considerably lower estimates of ingestion than the other methods, with the highest discrepancies at the lowest food levels and following prolonged incubation. These results support the use of chloropigments as tracers of ingested phytoplankton when algal concentrations are high and grazers are actively feeding (i.e. spring bloom conditions). Alternate biomarkers should be considered for use during periods of low phytoplankton abundance (ie. summer).

Although the effect of temperature on the conservation of chloropigment products of grazing has not been investigated, it is probable that very low temperatures (e.g. 0°C) reduce the decay rates of faecal material and particles produced by the "sloppy feeding" which is characteristic of many copepods. Low temperatures may thus limit the *in situ* degradation of chlorophyll derivatives to colourless products. Chloropigment destruction, resulting from coprophagy, were probably minimal during the spring bloom in Conception Bay due to the relatively low biomass of grazers during this period of the year (Deibel et al., unpubl. data). Low microbial abundance and activity in the water column during the spring (Pomeroy et al. 1991), and slow decay rates of copepod faecal pellets incubated at 0°C (Urban 1992), offer additional support for the use of chloropigments as tracers of spring phytoplankton production in cold, coastal waters.

7.2. Development and Fate of the Spring Phytoplankton Bloom

The spring bloom in Conception Bay develops during mid- to late March in response to increasing light and decreasing wind stress. During the initiation of the bloom, the water column is nearly isothermal and $<0°C$, and throughout the bloom temperatures at and below the subsurface chlorophyll maximum are $\leq 0°C$. Small, chain-forming centric diatoms, in particular *Skeletonema costatum*, *Chaetoceros* spp., and *Thalassiosira* spp., generally dominate the phytoplankton bloom taxa. Changes in the composition of the dominant species, however, may occur as a result of storm-driven advection of offshore water and phytoplankton into the bay. Spring bloom diatoms are succeeded by a less diverse phytoplankton community, mainly small phytoflagellates, following thermal stratification and nutrient depletion of the surface waters.

The principal mesozooplankton grazers during the spring bloom are the calanoid copepods, *Calanus finmarchicus*, *Pseudocalanus minutus* and *Temora longicornis*, and the pelagic tunicate, *Oikopleura vanhoeffeni*. The chloropigment content in the gut tracts and faeces of copepod and oikopleurid grazers was generally a reflection of both body

size and phytoplankton concentration. The highest gut and faecal pellet concentrations occurred during the peak of bloom production. Variability within replicate samples of pooled copepods was often high (up to 7 fold) in both the *in situ* gut pigment study (Chapter 5) and in the laboratory grazing experiments (Chapter 3), and is indicative of intermittent copepod feeding.

Diel feeding rhythms were observed in the five most common species of calanoid copepods (*Calanus finmarchicus*, *C. glacialis*, *Metridia longa*, *Pseudocalanus minutus* and *Temora longicornis*). The feeding periodicity of the largest copepod, *M. longa*, was directly coupled to a strong vertical migration pattern, which was evidenced by the absence of adult females at depths above 100 m during daylight hours. *M. longa* was abundant in the upper mixed layer from dusk to dawn, and appeared to graze almost continuously throughout the night. *C. glacialis* also migrated to deeper waters during the day but tended to concentrate at depths immediately below the subsurface chlorophyll maximum. The depth range over which copepods vertically migrated tended to decrease with decreasing body size. With the exception of *M. longa*, all copepod taxa showed similar diel feeding rhythms during April, with low grazing in the late afternoon and an increase in ingestion at dusk. Peak feeding in these copepods, however, was generally observed within the SCM during midday, which may indicate daytime grazing in response to low abundance of visually feeding predators (i.e. fish). Grazing during both day and night may also enhance copepod spawning frequency (Runge 1985). A feeding rhythm was not observed in the pelagic tunicate, *Oikopleura vanhoeffeni*. The occurrence of faecal pellets in the trunk region of oikopleurids at all times suggests that these animals graze relatively continuously day and night.

Chloropigments are highly useful qualitative tracers of ingested phytoplankton. The primary fluorescent decay product observed in the gut tracts of *Calanus*, *Pseudocalanus* and *Temora*, collected throughout the bloom, was pyrophaeophorbide *a*. Although this derivative comprised up to 95% of the total chloropigments in *C.*

finmarchicus extracts, and appears to be a signature pigment of copepod grazers, it was not found exclusively in copepod gut tracts. Pyrophaeophorbide *a*, in addition to numerous other less-polar phaeophorbide *a*-type pigments, was also observed in the faeces of *O. vanhoeffeni* and the blue mussel *Mytilus edulis*. Oikopleurid pellets also showed much higher levels of undegraded pigments (chlorophylls *a*, *b* and *c*) than were found in the gut tracts of copepods, indicating the passage of partially digested and relatively intact algae through the gut. This is supported by electron microscopy observations of intact phytoplankton cells in *O. vanhoeffeni* faecal pellets collected during the spring (Deibel and Turner 1985, Urban et al. 1992). Oikopleurids occur in periodic blooms and have been estimated to have a grazing impact in Conception Bay equivalent to that of the copepod population (Knoechel and Steel-Flynn 1989). Faecal pellets of oikopleurids were abundant in sediment trap collections during May 1988, and confirm the role of oikopleurids as major grazers and contributors to the vertical flux of spring bloom production.

The chloropigments in oikopleurid faecal pellets and the faeces of blue mussels (*Mytilis edulis*) showed similar phaeopigment compositions, and greater diversity than those found in the gut tracts and faeces of copepods. When phytoplankton concentrations were high (early bloom), the gut tracts and faeces of all 3 grazer types showed higher percent levels of phaeophytin *a*-type pigments. As the bloom progressed, the fraction of phaeophorbide *a*-type pigments increased, and in blue mussel faeces, the percent of chloropigment represented by chlorophyll *a* decreased markedly, suggesting greater digestion and absorption efficiencies at low food concentrations.

The vertical flux of spring phytoplankton produced during 1988 occurred primarily in early May during a senescent phase of the bloom. Phytoplankton senescence, followed by mass sedimentation, was verified by the presence of high levels of chlorophyllide *a* and chlorophyll *a* in seston samples and in sediment trap collections. The sinking rate during the onset of mass sedimentation in early May was 20-23 m/d

throughout the water column. This flux probably represented the settling of small aggregates of interlocking chains of *Chaetoceros* spp. and *Skeletonema costatum*, which were abundant in the traps during this period. More than 75% of the flux of POM, POC and total chloropigments, in the form of intact diatom cells and chains and faecal material, occurred in May.

The proportion of phaeopigments comprising total chloropigment flux increased from 35% at 40 m to 67% at 240 m, and was associated with an increase in the relative abundance of copepod and oikopleurid faecal pellets with depth. Faecal pellet flux to deep traps was probably enhanced by the diel vertical migration of large calanoid copepods (i.e. *Calanus* spp., *Metridia longa*) from the upper mixed layer to deeper waters. Pyrophaeophorbide *a*, the dominant chlorophyll *a* derivative produced during zooplankton grazing, was the primary chlorophyll *a* decay product observed in both seston samples and sediment trap collections from depths below the euphotic zone.

The relatively constant carbon/chloropigment ratios at all depths throughout the bloom, and comparable losses of POC and pigment flux between near surface and near bottom traps, suggest that chloropigments are reasonably good tracers of phytoplankton biomass during spring. C/N ratios of the cumulative fluxes of POC and PON were similar at all trap depths and were low (7.0-8.8), indicating little decay of the bulk of sedimenting material. This resulted in a pulse of high quality particulate material (20% POM) reaching near bottom waters during the terminal phase of the bloom.

The chloropigment concentrations in the surface sediments and in the overlying flocculent layer reflected the seasonal production and flux of phytoplankton and faecal material. During March to mid-September, the top 10 cm of sediment were rich in both carbon (2.6-3.6%) and total chloropigments (>20 μg/g dry wt sediment). Although pigment concentration decreased with depth in the uppermost 5 cm, the composition, dominated by a series of phaeophorbide *a*-type pigments, remained uniform down to 10

cm, presumably due to the bioturbating activities of abundant deposit feeding polychaetes, and to pigment preservation processes following burial.

The fate of spring bloom production may differ from year-to-year due to various physical and biological controls. Highly variable meteorological conditions during April and May result in great interannual variation in the duration and magnitude of the spring bloom in Conception Bay (Deibel et al., unpubl. obs.). As a consequence, the extent of bloom utilization by herbivorous zooplankton, and the relative amounts of grazed and ungrazed phytoplankton in sedimenting material, may also be highly variable. Phytoplankton blooms which peak and terminate early in the spring (e.g. 1986 spring bloom) result in the massive sinking of intact diatoms, limited grazing impact and relatively low faecal pellet flux (Thompson et al., unpubl. data). The 1988 bloom, which was investigated in this study, was of lower peak magnitude and of longer duration, and showed a relatively higher degree of utilization by water column grazers, and a greater contribution of faecal pellets to the vertical flux of bloom production.

In conclusion, a large proportion of the spring phytoplankton bloom in Conception Bay settles to the benthos as intact cells and chains, and as faecal material of primarily copepod and oikopleurid grazers. Most of the particulate material sedimenting to deep waters and to the benthos occurs following nutrient depletion and subsequent cell senescence. This material is of high organic content, indicating a considerable degree of pelagic-benthic coupling during spring. Low water column temperatures, high phytoplankton biomass, and a delayed response of grazers to bloom production contribute to the effectiveness of chloropigments as quantitative and qualitative biomarkers of the fate and vertical flux of the spring phytoplankton bloom in coastal Newfoundland waters.

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Appendix A. Mean chloropigment concentrations (± SD) in the gut tracts of the dominant copepod taxa collected at 10, 25, 50 and 100 m during April 1989. Taxa include: *Calanus finmarchicus* (*C. fin.*), *Calanus glacialis* (*C. glacialis*), *Metridia longa* (*M. longa*), *Pseudocalanus minutus* (*P. minutus*) and *Temora longicornis* (*T. long.*). The data represent the means of 2-5 replicate samples of pooled copepods.

Species	Time hr	Date 1989	10 m (ng/cop)	25 m (ng/cop)	50 m (ng/cop)	100 m (ng/cop)
C. fin.	0015	04/20	1.4	2.6 (0.6)	4.6 (1.2)	-
	0030	04/19	1.8 (0.9)	3.9 (1.0)	6.2 (2.4)	-
	0400	04/20	0.2 (0.1)	4.7 (1.8)	6.1 (2.0)	-
	0420	04/19	1.0 (0.2)	4.3 (2.3)	9.7 (2.1)	-
	0805	04/20	-	1.7 (1.5)	4.7 (0.9)	9.1 (6.9)
	1000	04/19	-	4.0 (0.9)	7.4 (1.4)	5.8 (3.6)
	1150	04/19	-	14.5 (2.1)	11.2 (1.5)	7.4 (1.3)
	1200	04/20	-	5.1 (2.3)	8.0 (4.3)	11.0 (6.6)
	1600	04/18	-	1.5 (0.2)	2.5 (0.7)	1.5 (0.5)
	1830	04/22	-	3.2 (0.6)	3.4 (0.7)	-
	2000	04/19	-	4.0 (0.9)	9.9 (1.9)	7.5 (1.4)
	2020	04/18	-	3.8 (1.3)	5.3 (1.2)	1.2 (0.2)
	2200	04/22	-	-	6.6 (1.2)	-
C. glacialis	0015	04/20	-	17.3 (5.4)	22.0 (2.5)	-
	0030	04/19	6.8 (3.1)	13.6 (5.7)	32.1 (11.2)	-
	0400	04/20	2.3 (0.7)	20.5 (5.5)	23.3 (6.3)	-
	0420	04/19	-	12.7 (1.8)	34.1 (15.9)	-
	0805	04/20	-	-	-	16.8 (6.3)
	1000	04/19	-	-	-	15.0 (0.3)
	1150	04/19	-	-	25.5 (9.3)	15.4
	1200	04/20	-	-	15.3 (2.4)	21.9 (8.5)
	1600	04/18	-	-	6.3 (2.4)	-

Appendix A. continued.

Species	Time hr	Date 1989	10 m (ng/cop)	25 m (ng/cop)	50 m (ng/cop)	100 m (ng/cop)
C. glacialis	1830	04/22	-	5.9	14.4 (1.8)	-
	2000	04/19	-	13.1 (5.0)	24.7 (10.8)	23.4 (4.6)
	2020	04/18	-	16.3 (5.8)	19.7 (7.8)	4.0 (0.1)
	2200	04/22	-	-	17.2 (5.3)	-
M. longa	0015	04/20	7.8	10.4 (1.4)	10.3 (3.2)	-
	0030	04/19	8.5 (2.0)	9.1 (1.4)	14.2 (2.7)	-
	0400	04/20	-	12.7 (2.4)	12.8 (1.4)	-
	0420	04/19	6.6 (5.4)	17.0 (3.6)	11.2 (2.0)	-
	2000	04/19	-	-	-	7.7 (2.2)
	2020	04/18	-	14.1 (1.9)	7.8 (1.6)	8.1 (0.6)
	2200	04/22	-	10.2 (1.6)	9.9 (0.6)	-
P. minutus	0015	04/20	0.25 (0.08)	0.71 (0.12)	0.50 (0.14)	-
	0030	04/19	0.14 (0.03)	0.90 (0.42)	0.77 (0.13)	-
	0400	04/20	0.12 (0.03)	0.72 (0.26)	0.83 (0.19)	-
	0420	04/19	0.22 (0.02)	0.52 (0.05)	0.44 (0.06)	-
	0805	04/20	-	0.54 (0.34)	1.02 (0.27)	0.34 (0.10)
	1000	04/19	-	1.22 (0.16)	1.87 (0.38)	0.40 (0.08)
	1150	04/19	-	3.01 (0.72)	1.96 (0.43)	0.46 (0.26)
	1200	04/20	-	0.92 (0.54)	1.72 (0.28)	0.26 (0.27)
	1600	04/18	-	0.46 (0.05)	0.42 (0.04)	0.21 (0.10)
	1830	04/22	-	0.79 (0.09)	0.39 (0.03)	-
	2000	04/19	-	1.14 (0.03)	1.39 (0.26)	0.58 (0.13)
	2020	04/18	-	0.67 (0.13)	0.51 (0.06)	0.12 (0.01)
	2200	04/22	-	-	0.41 (0.03)	-

Appendix A. continued.

Species	Time hr	Date 1989	10 m (ng/cop)	25 m (ng/cop)	50 m (ng/cop)	100 m (ng/cop)
T. long.	0015	04/20	0.18 (0.06)	0.50 (0.05)	0.43 (0.27)	-
	0030	04/19	0.09 (0.01)	0.36 (0.15)	0.16	-
	0400	04/20	0.02 (0.03)	0.35 (0.06)	0.24 (0.06)	-
	0420	04/19	0.21 (0.06)	0.38 (0.03)	0.23 (0.05)	-
	0805	04/20	-	0.42 (0.23)	0.70 (0.23)	0.14 (0.04)
	1000	04/19	-	-	-	0.16
	1150	04/19	-	2.49 (0.75)	-	-
	1200	04/20	-	1.19 (0.38)	-	0.13
	1600	04/18	-	0.41	0.29 (0.05)	0.11
	1830	04/22	-	0.34 (0.09)	-	-
	2000	04/19	-	0.48	0.84	-
	2020	04/18	-	0.52 (0.03)	-	0.10 (0.04)
	2200	04/22	-	0.08 (0.02)	0.05 (0.01)	