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A Study of the Production and Measurement of Dissolved Organic Matter in Seawater

by

Jeffrey John Ridal

Submitted in partial fulfillment of the requirements

for the degree of Ph.D.

at

Dalhousie University

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To

Brenda and Dylan

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Abstract

Recent analytical developments in chemical oceanography have greatly emphasized the need for a better understanding of the quantity and physico-chemical nature of dissolved organic matter (DOM) in seawater. Measurements by a new high temperature combustion oxidation (HTCO) method indicate dissolved organic carbon (DOC) and nitrogen concentrations in seawater are several times higher than measured with traditional 'wet oxidation' methods, such as the persulphate and UV techniques. However, uncertainties in the accuracies of both HTCO and traditional methods limit the information that can be gained on the sources of oxidation-resistant DOC, and the pathways which alter its physico-chemical characteristics. Using ^{14}C labelling methods to circumvent the currently controversial bulk DOC methods, I investigated the extent to which DOC produced by marine algae is resistant to wet oxidation, the resistance of different molecular weight (MW) size fractions, and the effects of biotic and abiotic ageing on algal DOC to its resistance to wet oxidation procedures.

Batch culture experiments with a variety of marine phytoplankton showed that the amount of algal DOC resisting persulphate oxidation (DOC_{res}) ranged from 5-20% of the initial DOC in the samples. As much as 27% of algal DOC resisted UV oxidation by an autoanalyzer method; however, <5% resisted extended (6 hrs) exposure to UV irradiation. It was found that phytoplankton produced mainly low MW material, as on average ~80% was <10,000 nominal MW. High (>10,000 nominal MW) and low MW fractions generally showed similar reactivities to persulphate oxidation. Ageing of algal DOC solutions in the presence of bacteria for a period of about 1 year resulted in decreased proportions of DOC_{res} in the aged material, suggesting that bacterial alteration of algal DOC does not increase its resistance to persulphate oxidation over the long term. No significant changes to the resistance of DOC solutions were found from 250 hours exposure to sunlight.

A second aspect of the research into DOM resistant to wet oxidation methods involved a study of dissolved organic phosphorus (DOP) in seawater. I questioned whether the levels of DOP in seawater were underestimated by 'standard' methods, since organic and inorganic N:P ratios (the Redfield ratio) often show only a small variation in seawater. An oxidation/hydrolysis procedure that combined UV and persulphate methods was compared with the 'standard' UV and persulphate DOP techniques for a wide variety of NW Atlantic and NE Pacific seawater samples. The UV and persulphate techniques were 83±9% and 87±8% efficient for the Atlantic samples, and 90±6% and 71±9% efficient for the Pacific samples, respectively, relative to the reference DOP method. Size fractionation experiments indicated that the bulk (74±14%) of DOP in coastal and open ocean seawater was <10,000 nominal MW. The material most resistant to standard methods was found in the low MW fraction.

A mesocosm study examined DOP produced over the course of a mixed algal bloom. Very good agreement was found between persulphate and the reference method. Substantially higher 'DOP' values measured by the persulphate and reference methods than the UV method (up to 0.11 μM) were attributed to dissolved polyphosphates released into the medium from unhealthy phytoplankton cells. Most of the DOP recovered by ultrafiltration (>65%) was <10,000 MW, which is similar to the proportion of DOP with low MW in surface seawater.

These investigations of DOC and DOP do not support HTCO results which indicate the presence of a large pool of previously undetected marine DOM that is characterised by high molecular weight and high resistance to traditional wet oxidation methods.

List of Symbols and Abbreviations

AOU	apparent oxygen utilization
BAC	bacteria
C	carbon
Chl a	chlorophyll a
DOC	dissolved organic carbon
$DO^{14}C$	^{14}C-labelled dissolved organic carbon
$DO^{14}C_{res}$	^{14}C-labelled dissolved organic carbon that resists wet oxidation
DOM	dissolved organic matter
DON	dissolved organic nitrogen
DOP	dissolved organic phosphorus
dpm	disintegrations per minute
ESNW	phytoplankton culture enrichment medium
GC	gas chromatography
HNF	heterotrophic nanoflagellates
HTCO	high temperature combustion oxidation
LMW	low molecular weight (<10,000 molecular weight size fraction)
μCi	10^{-6} Curie (2.22×10^6 disintegrations per minute)
MW	molecular weight
N	nitrogen
NMR	nuclear magnetic resonance spectroscopy
NMW	nominal molecular weight
P	phosphorus
PDOC	dissolved organic carbon produced by phytoplankton

$PDOC_{res}$	phytoplankton dissolved organic carbon that resists wet oxidation
POC	particulate organic carbon
PP	particulate phosphorus
poly-P	polyphosphate
Ret	>10,000 molecular weight size fraction
Ret_{res}	>10,000 molecular weight size fraction that resists wet oxidation
SRP	soluble reactive phosphorus
TDOP	total dissolved organic phosphorus
TP	total phosphorus
UF	ultrafiltrate (<10,000 molecular weight size fraction)
UF_{res}	<10,000 molecular weight size fraction that resists wet oxidation
UV	ultra-violet

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Chapter 1

General Introduction

1.0 Preface

The characterization of dissolved organic matter (DOM) in seawater has been an important aspect of research in chemical oceanography since the Challenger expedition (Dittmar, 1884). However, marine DOM, which is presently defined as organic matter that passes through a submicron pore size filter, remains poorly quantified and characterized despite appreciable research efforts since that time. Williams and Druffel (1988) estimate that less than 20% of DOM in surface seawater can been identified as known biochemical substances, such as combined amino acids, carbohydrates, and lipids. Efforts to characterize marine DOM have been hindered by its low concentration in seawater, its resistance to chemical breakdown, the diversity of possible constituents, and the difficulties these features present to traditional and modern analytical methods.

Improved estimates of how much DOM is present in seawater, together with a better understanding of the roles that DOM play in oceanic cycles, are of critical interest to current global research programs attempting to pin down the forms, concentrations and fluxes of carbon in the global ocean. A more comprehensive knowledge of ocean carbon is in turn required for an understanding of the response of the global carbon system to increasing atmospheric CO_2 levels (Post et al., 1990).

In this introduction, I will first present the traditional view of DOM in seawater that has developed through research efforts in the first part of this century through to 1980. In the mid-80's, new empirical evidence supported a different understanding of the concentrations of DOM in the oceans, and new concepts on its lability and role in biogeochemical cycles. It was at this time that the research presented in this thesis was undertaken, and therefore the thesis research has been heavily influenced by these provocative new ideas.

Most recently, a modified picture of DOM in seawater has started to emerge, as results of intense research efforts over the past few years, including those presented in this thesis, have altered the ideas of oceanic DOM concentrations and concepts brought forth in the mid and late 1980's. This last perspective will be presented in the final Chapter. The dissertation is structured in this way to represent the flow of information about marine DOM that has occurred during the course of the thesis research. Of course, the present understanding of DOM in seawater is still very much in a state of change; much of the work undertaken in the past few years is only now being published, and each piece of new evidence changes our understanding of DOM in some way.

1.1 Traditional concepts of DOM in seawater

Dissolved organic matter in seawater is usually quantified as the concentration of dissolved organic carbon (DOC), although the concentrations of dissolved organic nitrogen (DON) and phosphorus (DOP) are also often of interest. Organically-combined dissolved nitrogen and phosphorus are particularly important in supporting primary production in ocean waters where inorganic forms exist in very low concentrations (Jackson and Williams, 1985; Smith et al., 1986). Modelling efforts toward assessing the contribution of DOM to the transport of organic matter into the deep ocean rely on DOC,N,P relationships (Toggweiler, 1989; Schlitzer, 1989; Sarmiento et al., 1990). Therefore, while most of the initial discussion will centre on marine DOC, reference will also be made to DON and DOP. DOP is discussed at length in Chapter 5.

There have always been discrepancies between estimates of DOC concentrations in seawater obtained from different analytical methods. Figure 1.1 shows levels of DOC measured by different methods for North Atlantic and North Pacific seawater. Lowest DOC values are found by 'wet oxidation' methods, in which a chemical oxidant (e.g. persulphate

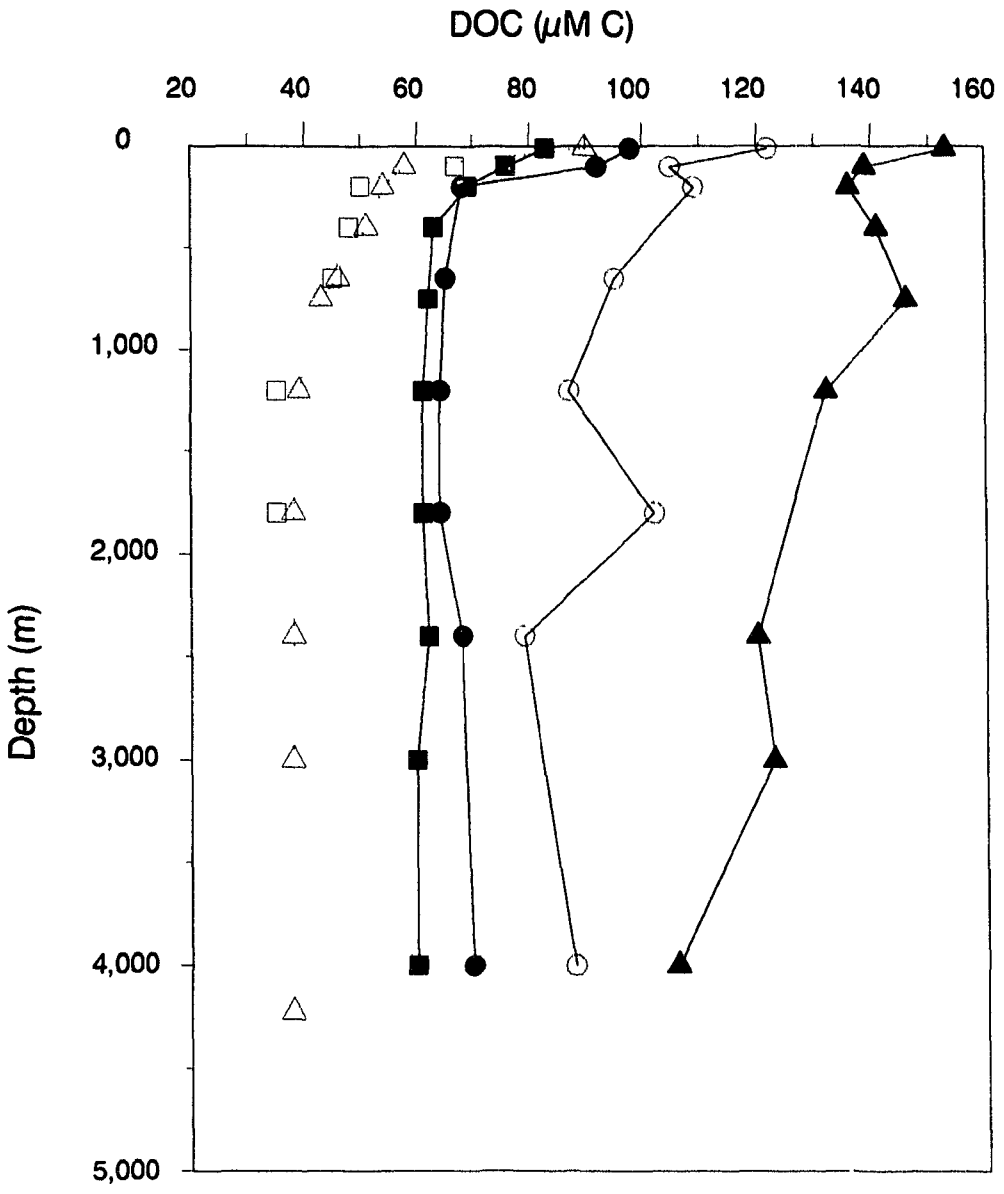


Figure 1.1. Vertical profiles of DOC concentrations in the North Atlantic (solid lines) and North Pacific (dotted line) measured with various analytical methods. A - DOC by UV oxidation in Central North Pacific (Druffel et al., 1989); open squares show results for NW Pacific by persulphate oxidation (Ogura, 1970). B-F - Typical profiles of DOC in the NW Atlantic by (B) dry combustion (MacKinnon, 1977), (C) persulphate and (D) wet combustion (Sharp, 1973), (E) dry combustion (Gordon and Sutcliffe, 1973), and (F) dry combustion (Skopintsev, 1966).

or perchloric acid) or high-intensity UV irradiation is applied to an aliquot of seawater. Highest DOC values are found by 'combustion' methods, in which samples are either dried and baked at high temperature or injected directly into a combustion tube, also maintained at high temperature (e.g. *ca.* 750°C; Sharp, 1973a). In these methods, the CO_2 produced by oxidation is typically measured by an infra-red detector, although alternative procedures such as gas chromatography and titrimetry have also been used (Wangersky, 1978). Mopper and Degens (1979) suggested that differences between wet oxidation and combustion methods result largely from incomplete oxidation by the wet oxidation procedures, and contamination of the dried sea salts by organic vapours in the case of dry combustion procedures. A carefully executed intercomparison study found that a dry combustion method gave values for Scotian Shelf seawater samples that were 5% higher than results from a UV method which, in turn, were 10% higher than persulphate method results (Gershey et al., 1979). These efficiencies were in good agreement with an earlier intercalibration between a persulphate method and a direct injection combustion method (Sharp, 1973a). It was found that persulphate oxidation of NW Atlantic (mainly Sargasso Sea) samples gave values that averaged 22% lower than high temperature combustion oxidation (HTCO), although surface (0-100 m) and deep samples (2000-5100 m) appeared to be oxidised more completely by persulphate (85%) than subsurface (100-600 m) and mid-depth (1000-1800 m) samples, which averaged 66% of HTCO values.

All DOC methods generated vertical profiles of similar shape, as shown in Figure 1.1. Values were highest in surface waters, and decreased gradually in subsurface waters. Wet oxidation results remained constant below 1000 m, while some structure was suggested in deep-sea profiles from combustion methods. The invariance of DOC concentrations in deep-water samples observed with wet oxidation methods, together with its apparent biological inertness (Barber, 1968) and old ^{14}C age (3500 years, Williams et al., 1969; 6000 years, Williams and Druffel, 1987), suggested that DOC was not an important contributor to oxygen consumption in the ocean's interior (Menzel, 1974; Broecker and Peng, 1982).

From this and other evidence, DOC was generally partitioned into two components: (i) labile and (ii) refractory to biological and chemical breakdown (Bada and Lee, 1977). In the traditional view of DOM, highest concentrations of DOC labile to chemical and biological breakdown are found in the surface layer of the ocean. Since radiotracer studies and analysis of specific 'tag' compounds, such as lignin, suggested that allochthonous inputs to the open oceans are relatively small, the major sources of labile compounds are thought to be exudation and cell lysis of phytoplankton and bacteria, solubilization of POC, and sloppy grazing and excretion on various trophic levels (Wangersky, 1978). Rapid uptake of labile DOC by bacteria in surface waters maintains very low concentrations of labile compounds in seawater transported to the deep-sea. Therefore, the bulk of DOC in deep seawater is assumed to be refractory to chemical and biological breakdown. Refractory DOC was suggested to be formed through alteration of DOC produced in surface waters by such mechanisms as biotic and abiotic oxidation processes, intermolecular condensation reactions, and metal complexation (Mopper and Degens, 1979).

Characterization of DOC by molecular weight (MW) size fractions indicated that most DOC was of low molecular weight, with 60-70% of DOC <1000 daltons (Maurer, 1976; Carlson et al., 1985). Using persulphate oxidation, Carlson et al. (1985) found only 1% of open ocean DOC with MW >100,000 daltons, while Sharp (1973b) observed that a colloidal fraction (0.003-0.8 μm) accounted for about 16% of total organic carbon in open seawater with his HTCO procedure. There was some evidence that the molecular weight of DOC increased with depth (Ogura, 1977), which was consistent with the traditional view that biotic and abiotic reactions produce inert, complex molecules (marine 'humus') which form the bulk of DOC in the ocean (Degens and Mopper, 1979; Skopintsev, 1981).

1.2 'Sugimura and Suzuki' ('S&S') DOM

A radically different picture of oceanic DOM was presented by Yoshimi Suzuki and coworkers, who found that a modified HTCO method gave DON (Suzuki et al., 1985) and DOC (Sugimura and Suzuki, 1988) values for NE Pacific surface seawater that were several times greater than wet oxidation values. Their vertical profiles of DOC showed previously undetected structure with depth and a very close association with AOU, as shown in Figure 1.2. They suggested that the high oxidation efficiency of the new HTCO method detected DOC and DON compounds not measured by other techniques. Comparison of their HTCO method with the persulphate method of Menzel and Vaccarro (1964) indicated that DOC and DON concentrations in NW Pacific surface seawater were respectively about 2.7 and 4 times greater than previously estimated with the wet oxidation technique. Better agreement was found for deep samples (HTCO/persulphate ≈ 1.4), and DOC values for oxygen minimum water were very similar to previous measurements in this region with the persulphate technique (Ogura, 1970). This surface to deep seawater gradient was analytically convincing, since differences between wet oxidation and the new HTCO method could not be easily accounted for by contamination or procedural blanks.

DOC results for central North Pacific samples, measured with the new HTCO instrument, were similar to the initial findings from the NE Pacific, and indicated that 'S&S' DOC was not a localized phenomenon (Druffel et al., 1989). It was observed that previously UV irradiated samples apparently released large amounts of CO_2 when analysed with the Suzuki HTCO method. The sum of UV and HTCO analyses from these samples were in excellent agreement with HTCO analyses of non-irradiated samples. As alluded to by Sharp (1991) and Wangersky (1992), further support for the 'S&S' DOC came from unpublished (and, therefore, not critically reviewed) reports of similarly high levels of DOC with other HTCO instruments.

This and other evidence presented by Sugimura and Suzuki (1988) pointed to three

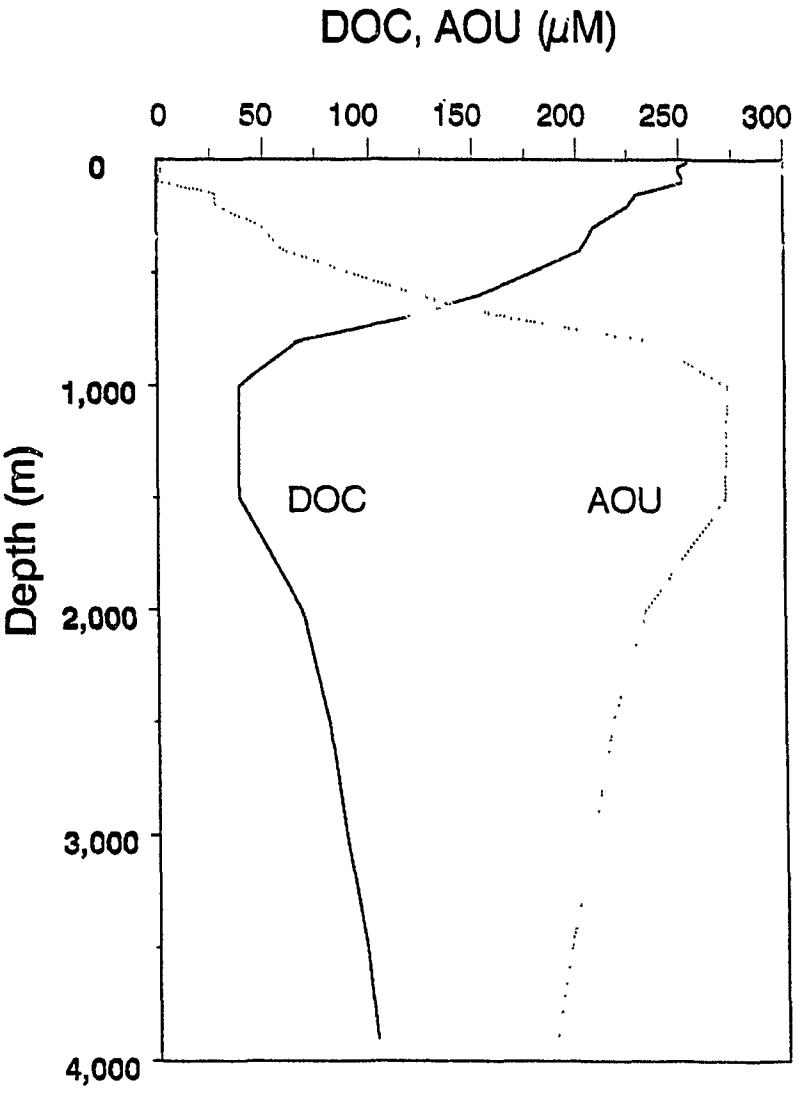


Figure 1.2. Typical profiles of DOC, measured with the HTCO method, and AOU in NW Pacific seawater by Suzuki and Sugimura (1988).

novel aspects of oceanic DOM. First, the very large gradient of the 'S&S' DOC from surface to the oxygen minimum, together with poor agreement with the persulphate method in surface and better agreement in deep waters, paradoxically suggested that the 'S&S' DOC could be broken down by micro-organisms, but was highly resistant to chemical oxidants. In contrast to the traditional view of DOC cycling as the turnover of a small pool of labile compounds, the 'S&S' DOC results suggested that much more of oceanic DOC is available on shorter timescales than previously imagined. Second, depth profiles of DON and DOC mirrored profiles of AOU. A plot of AOU versus DOC showed a high linear correlation with a slope of about -1 (Toggweiler, 1989). A similar close relationship with AOU was observed for DON. Sugimura and Suzuki (1988) therefore suggested that AOU could be explained by the *in situ* decomposition of DOC. Third, a gel filtration technique was used to isolate molecular weight size fractions of the DOM analysed by their HTCO instrument. As shown in Figure 1.3, about 75% of the DOC and DON analysed by the new HTCO method was >4000 daltons. Both DOC and DON high MW fractions were found to be resistant to persulphate oxidation, but as the concentrations of high MW fractions decreased rapidly with depth, their profiles suggested that these fractions were consumed by bacteria in subsurface waters.

The 'S&S' DOC has generated a lot of interest, especially in those cases where high DOC values provide a possible explanation for, or support, a number of recent developments in the field of oceanography. For example, inconsistencies between remineralization rates and new production estimated from sediment trap data could be explained by the export (or import) of DOM from the region of interest (Rintoul and Wunsch, 1990; Sarmiento et al., 1990). Differences between potentiometric and manometric alkalinity measurements might be explained by high concentrations of protonated organic compounds in seawater (Bradshaw and Brewer, 1988). As well, the advent of 'microbial loop' theory has emphasised the importance of the flow of carbon from bacteria to higher organisms. The understanding that most microbial activity is due to free-living bacteria, which are estimated to cycle 20-50% of

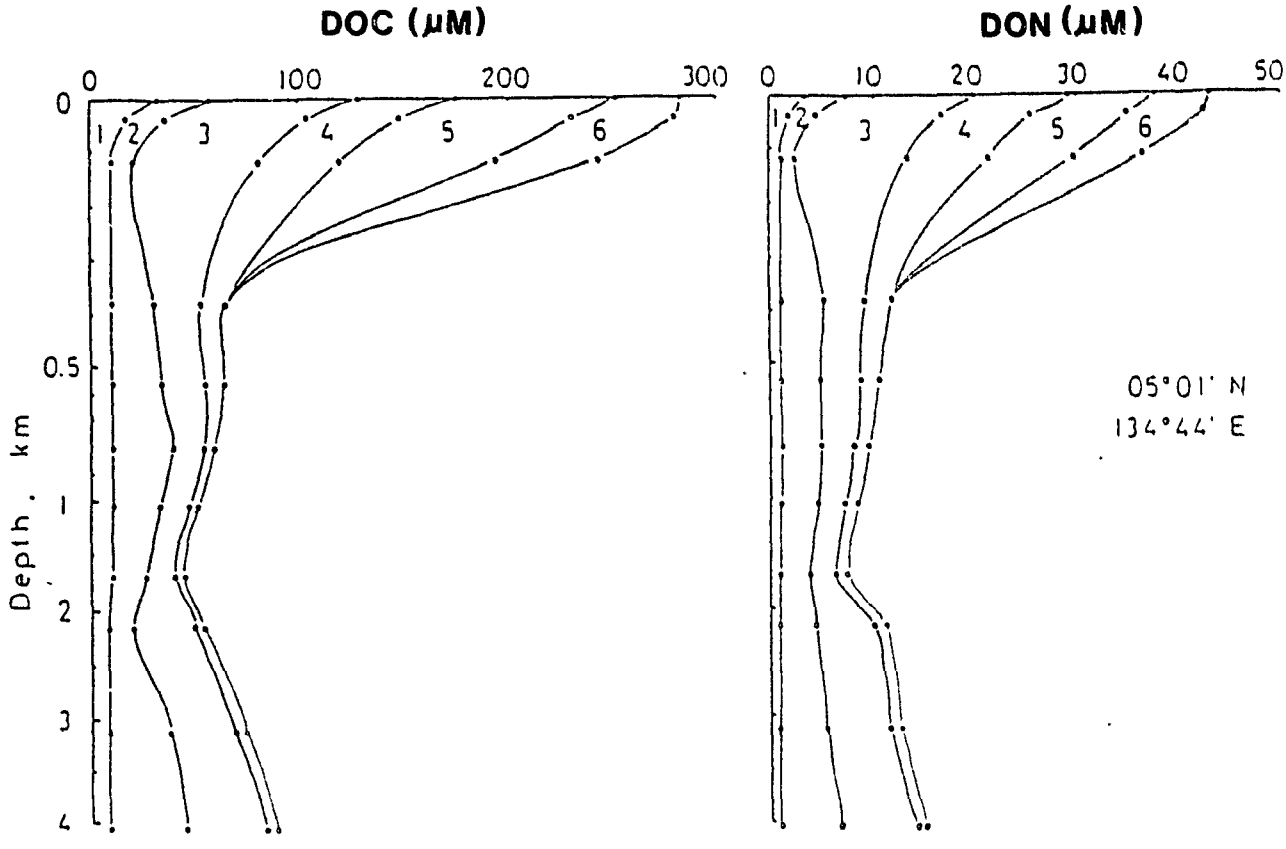


Figure 1.3. Vertical profiles of DOC and DON molecular weight size fractions by the HTCO methods of Suzuki et al. (1985) and Suzuki and Sugimura (1988). Molecular weight size fractions determined by size exclusion gel chromatography are as follows (in daltons): (1) <1,800; (2) 1,800-4,000; (3) 4,000-20,000; (4) 20,000-60,000; (5) 60,000-100,00; (6) >100,000. (Adapted from Suzuki and Sugimura, 1988).

primary production (Ducklow, 1991), fit well with higher estimates of microbially labile DOC in the oceans.

On the other hand, a number of problems developed in attempts to reproduce the 'S&S' DOM results. Although some experimenters were finding higher DOC values with commercial HTCO analysers than with wet oxidation procedures, no one was able to reproduce the very high 'S&S' DOC and DON values (Walsh, 1989), or their high inverse correlation with AOU. Problems with HTCO analysers and integrators at sea, a controversy concerning the role of catalysts in the combustion process, and a poor understanding of instrumental blanks stalled progress on the analytical front (Wangersky, 1992). In addition, no evidence had been presented to support higher oceanic DOP (or total dissolved phosphorus) values that might be expected from Redfield-type stoichiometry (Williams and Druffel, 1988; Jackson, 1988). If 'extra' dissolved P does not exist, this would indicate a previously unforseen uncoupling of the stoichiometric balance between N and P in the ocean.

In addition, Toggweiler (1989) pointed out that Sugimura and Suzuki's (1988) interpretation of the correlation between AOU and DOC was incorrect. An understanding of the relationship between DOC and AOU must have a knowledge of 'preformed' DOC concentrations associated with seawater at the point of isolation from the atmosphere, the role of mixing, and must account for the contribution of organic particles to oxygen consumption. For example, the role of organic particulate matter in oceanic O_2 consumption becomes vanishingly small, if one considers (*sensu* Sugimura and Suzuki) that downwelling seawater containing *ca.* 250 μM DOC, when reduced to 50 μM C by microbial oxidation in deep waters, would require 340 μM of O_2 to be consumed by respiration based on current understanding of O_2:C stoichiometry (170 moles O_2 for every 100 moles C; Takahashi et al., 1985). The oxygen demand by DOM would very nearly equal the observed dissolved O_2 concentrations in cold (0°C) surface seawater (350 μM; Broecker and Peng, 1982), suggesting that O_2 concentrations in the 50 μM DOC seawater measured by Sugimura and Suzuki (1988)

would be <10 μM, which is not observed.

While it seemed that DOC estimates of Suzuki and Sugimura (1988) were somewhat too high, or, at very least, not representative of global ocean DOC concentrations, the 'S&S' DOM results seriously questioned the credibility of wet oxidation values. It is not inconceivable that wet oxidation grossly underestimates seawater DOM, since, for example, humic organic matter from aquatic sediments is only 30-50% broken down by treatment with persulphate (Spiteller and Said-Jimenez, 1990). The high values of DOC by Sugimura and Suzuki (1988) were reported during a time of heightened interest in global carbon cycles. As shown in Figure 1.4, 'old' DOC values already predicted that oceanic DOC was a substantial pool of organic carbon. Scaling up the oceanic pool by the 'S&S' values increases the global ocean pool of organic carbon to more than twice its original value and greater than the combined estimates of atmospheric CO_2 and land plant biomass (Toggweiler, 1989). Since the flux of carbon between reservoirs is influenced by the current inventory of each reservoir and by their turnover rates, high concentrations of microbially labile DOC would play an important role in the exchange of CO_2 between the atmosphere and ocean (Post et al., 1990). The high inverse correlation between AOU and 'S&S' DOC suggested that previous concepts of oceanic organic carbon cycling would require revision (Toggweiler, 1988; Jackson, 1988; Legendre and Gosselin, 1989). Clearly, the potential influence of the high 'S&S' dissolved organic matter concentrations to our understanding of organic carbon, oxygen and nutrient cycling, and the global carbon budget, merited a considerable research effort from the chemical oceanographic community.

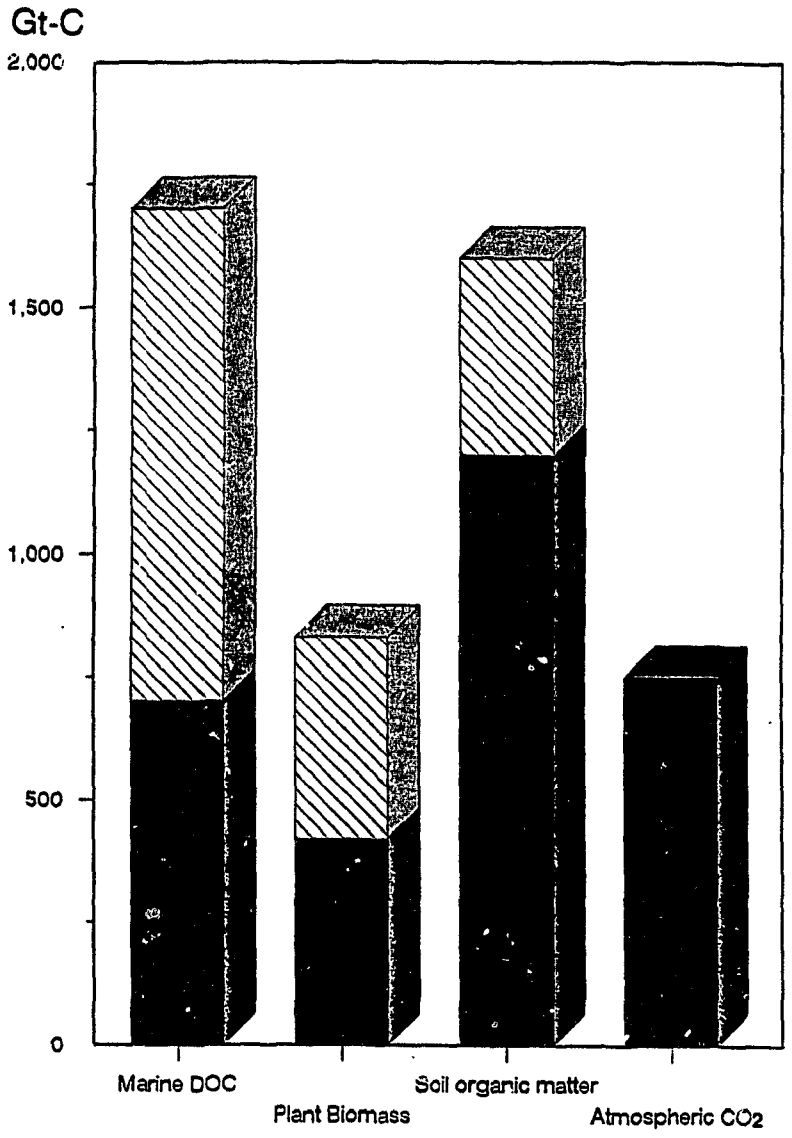


Figure 1.4. Comparison of oceanic and terrestrial organic carbon reservoirs in Gt-C (10^{15} g C) with the atmospheric CO_2 pool (Post et al., 1990). Cross-hatched areas show uncertainties in estimates. Uncertainty for marine DOC pool based on difference between wet oxidation and HTCO values (Toggweiler, 1992).

1.4 Objectives and Approaches

The challenge, as I saw it, was to investigate the DOM controversy with approaches that circumvent problems associated with the sophisticated, but poorly understood, HTCO equipment. The research could also be designed to address fundamental uncertainties regarding DOM with respect to its sources, size distribution, and lability to biological and chemical breakdown. It was decided that two aspects of marine DOM could be investigated that would meet the criteria outlined above. These two approaches toward a better understanding of marine DOM and the nature of its oxidation-resistant components were: (I) an investigation of DOC produced from marine phytoplankton; (II) an investigation of DOP in seawater.

The specific objectives of the thesis research were to:

(1) Investigate whether phytoplankton produce DOC resistant to wet oxidation methods.

(2) Investigate the effects of ageing processes on the stability of phytoplankton DOC to chemical breakdown.

(3) Investigate the measurement of TDP and DOP in seawater to assess whether a previously undetected P pool, implied by the DON and DOC measurements of Suzuki, could be observed.

(4) In the course of (1), (2) and (3), investigate DOC and DOP size fractions to to improve the characterization of DOM in seawater and to:

(i) compare the proportions of various MW size fractions with the amounts measured by Suzuki et al. (1985) and Sugimura and Suzuki (1988);

(ii) investigate the breakdown of each size fraction by wet oxidation methods.

In a comment on the new HTCO results and their implications, Williams and Druffel (1988) described the 'S&S' DOM in the following way:

> ...the 'extra', N-enriched, organic matter, derived from phytoplankton, is attractive to heterotrophic microorganisms, is probably polymeric, and has oxidation potentials and/or rates exceeding those of the standard compounds used in evaluating the efficiency of the wet, UV and earlier high temperature oxidation techniques.

This statement succinctly summarised what could be implied about 'S&S' DOC from the empirical data. Since recent evidence (Meyers-Schulte and Hedges, 1986) has strengthened the argument for relatively small inputs of land-derived DOC into the ocean, the main source of oceanic DOC in surface waters must be from photosynthetic plankton. A reasonable approach, therefore, is to attempt to isolate DOC refractory to chemical and photooxidative breakdown from cultures of phytoplankton. As described in Chapters 2 and 3, I used ^{14}C radioisotope methodology to provide estimates of the levels of chemically refractory DOC derived from marine phytoplankton. This approach replaces the currently controversial DOC techniques with a method than can provide highly precise measurements not compromised by analytical blank uncertainties. These Chapters also describe results from experiments with different size fractions of phytoplankton DOC, in an attempt to shed light on the sources and nature of high molecular weight DOC in seawater. High molecular weight DOM is of particular interest since it was found to account for the bulk of DOC analysed by Suzuki and Sugimura (1988).

The second part of the thesis research on marine DOM focuses on the measurement of dissolved organic phosphorus in seawater. As discussed in Chapter 5, while DOP concentrations in surface waters have been measured with numerous analytical methods, vertical distributions of DOP remain poorly characterised. As measurement of high DOC and DON concentrations bring into question whether the levels of dissolved phosphorus have been underestimated in seawater, re-examination of marine DOP and further study of its

distribution in seawater are pertinent at this time.

Detection of substantially higher DOP concentrations in seawater than commonly observed, through the application of a more rigorous DOP method than standard UV and persulphate methods, would provide additional support for the 'S&S' DOM values. In Chapter 6, I describe a new method for DOP analysis which combines the oxidation and hydrolytic capacities of the UV and persulphate methods. This combination method, as I call it in this thesis, was compared with UV and persulphate methods for analysis of NW Atlantic and NE Pacific seawater samples. The intercomparison study is one of very few to have examined DOM from both Atlantic and Pacific waters with the same analytical methods. Another unique aspect of this research was the characterization of DOP size fractions using a 'state of the art' crossflow filtration technique. Characterization of DOP size fractions in surface, subsurface, and deep seawater in both coastal and open ocean samples provides information concerning the sources and *in situ* diagenesis of marine DOP.

A mesocosm study is described in Chapter 7, which investigates the efficiencies of the three techniques used in Chapter 6 to detect total and dissolved phosphorus during a phytoplankton bloom. Characterization of DOP size fractions during the bloom identifies possible sources of similar size fractions observed in coastal and open ocean studies. Information gained in the course of the mesocosm study provided implications for speciation and cycling of phosphorus in coastal waters.

Chapter 2
Methods for Analysis of Dissolved Organic Carbon Produced from Marine Phytoplankton in Batch Culture

2.1 Introduction

This chapter details the ^{14}C analytical methods used to examine the efficiencies of persulphate and ultra-violet methods to oxidise dissolved organic carbon produced from cultures of marine phytoplankton.

2.1.1 Outline of analytical approach

The basic approach was to grow marine phytoplankton in defined media supplemented with ^{14}C-bicarbonate. The photosynthetically assimilated radiotracer entered the dissolved organic carbon pool through the processes of algal exudation and cell lysis. As the blooms progressed, samples were taken in time series, filtered, acidified and bubbled to remove remaining inorganic ^{14}C. The amount of fixed ^{14}C was counted by a liquid scintillation method, and values of the phytoplankton dissolved organic carbon (PDOC) were calculated from a knowledge of the initial specific activity of the culture medium. PDOC samples were oxidised by persulphate or UV techniques, and the amounts of phytoplankton dissolved organic carbon that resisted oxidation (PDOCres) again determined by liquid scintillation counting. A comparison of PDOC and PDOCres values indicated the resistance of the algal DOC to oxidation. In addition, size fractionation of PDOC samples into low molecular weight and high molecular weight (or colloidal) size fractions was accomplished by an ultrafiltration technique. Resistances of the different molecular weight fractions to persulphate oxidation were then analysed. As well, a number of experiments were carried out to examine whether

ageing of PDOC samples by abiotic, microbial and photochemical processes affected their reactivities to chemical oxidation.

2.2 Batch culture experiments

2.2.1 Phytoplankton cultures

Algae enrichment media (normally ESNW, Harrison et al. 1980) were prepared in 3-10-liter batches using dialysed (1000 dalton) or 0.2-μm filtered seawater taken from the Dalhousie Aquatron supply. The seawater base was autoclaved in the flasks prior to nutrient addition, and allowed to stand for two days to cool and equilibrate with the atmosphere. $NaHCO_3$ (200 μM) was added to the media prior to autoclaving to minimize salt precipitation during the sterilization process. The 10-L cultures were contained in 20-L polycarbonate carboys; other containers were borosilicate glass. All containers were prepared for use by a 24 hour soak with 0.05 M NaOH, followed by rinses with 0.1 M HCl, and distilled water. The containers were then filled with dialysed seawater and left to soak for 48 hours before use. A base soak step was particularly important for vessels that had been previously used for culturing experiments employing ^{14}C, since preliminary experiments showed that labelled surface active materials remained on containers despite cleaning with acid solutions. Some of the surface active material was found to be extracted, however, when the containers were soaked with seawater, and would have contributed to irregular experimental blanks had it not been removed.

The phytoplankton culture media were spiked with approximately 100 μCi L^{-1} of $NaH^{14}CO_3$ (54.6 mCi $mmole^{-1}$; New England Nuclear), and the solutions were thoroughly mixed. Unialgal inoculates of *Isochrysis galbana, Skeletonema costatum, Chaetoceros gracilis,* and *Phaeodactylum tricornutum* were added from stock cultures to obtain a cell density of about 10,000 ml^{-1}, after which the solutions were mixed and vessels sealed as shown in Figure

2.1. A summary of the experimental conditions employed in this work is listed in Table 2.1. Following removal of time-zero samples, the algal cultures were placed on a rotating table and grown at 20°C with 50-150 μEinst $m^{-2}s^{-1}$ of continuous illumination as measured with a 2π Licor photometer.

Replicate *Chaetoceros gracilis*, *Phaeodactylum tricornutum* and *Isochrysis galbana* cultures were prepared with the addition of 25 mg L^{-1} of the antibiotic streptomycin sulphate (SM). The antibiotic-containing cultures were used to assess whether bacteria rapidly respired PDOC or modified its reactivity, particularly during the early stages of bloom experiments. This broad-spectrum antibiotic was chosen since it was found to suppress 90% of bacterial activity over 7-day experiments (Jenson 1983), which were employed to examine PDOC from batch cultures of natural populations. Jensen also concluded from his work with this antibiotic that algal photoassimilation was not adversely affected by the application of this substance in cultures of marine phytoplankton.

In an additional experiment, an axenic *Synechococcus strain DC2* culture was grown in 2.5 L of autoclaved ESNW with 40 μEinst $m^{-2}s^{-1}$ of continuous light and shaken on a rotating table at 40 rpm. All manipulations with this culture were carried out in a laminar flow hood. Examinations with epifluorescence microscopy showed that the cultur remained axenic throughout the duration of the experiment.

2.2.2 Analysis of PDO^{14}C samples

The experimental protocol is illustrated in Figure 2.1. Samples were removed from the phytoplankton cultures by withdrawing culture material into sterile 60-mL plastic syringes, which were then fitted with 0.45-μm Millipore filters contained in plastic Swinnex filter holders. The samples were filtered into polypropylene containers using a constant pressure

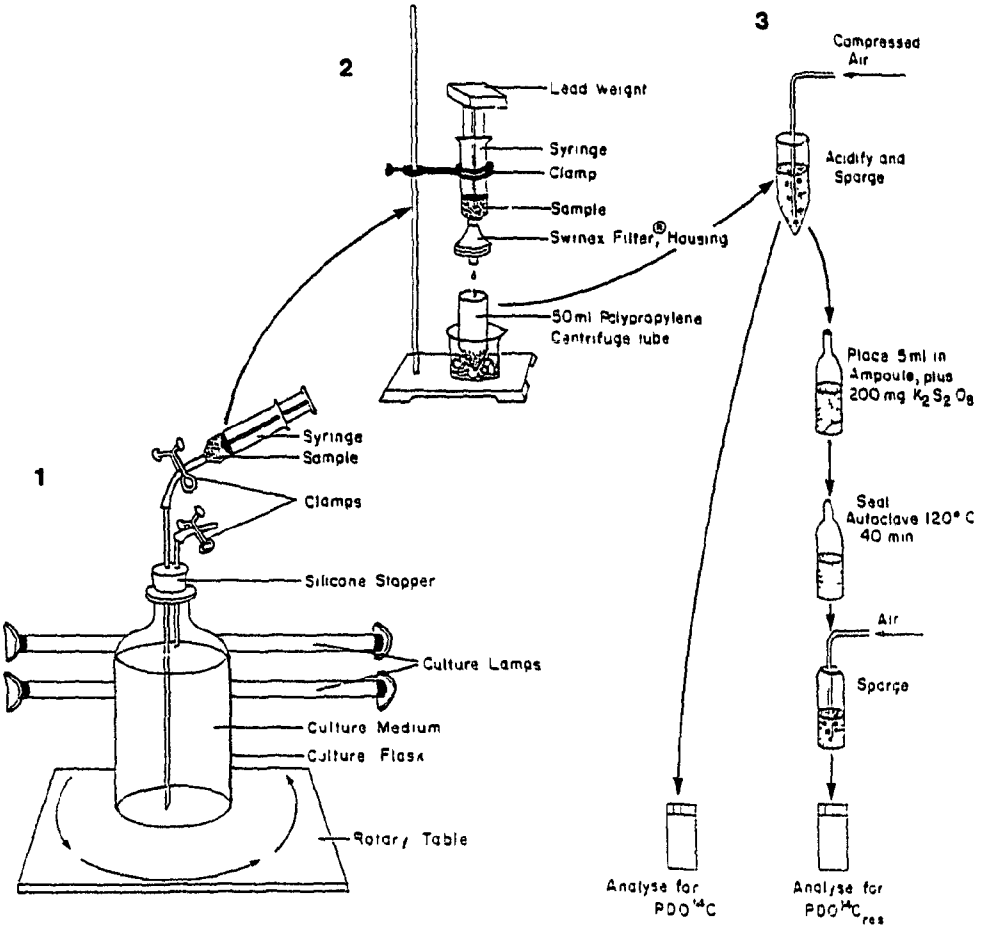


Figure 2.1 Experimental protocol for (1) sampling, (2) filtering and (3) analysing phytoplankton dissolved organic carbon

Table 2.1. Experimental conditions used to grow phytoplankton batch cultures in ^{14}C-bicarbonate supplemented ESNW culture medium.

Species	Culture volume (L)	Seawater base	Length of Culture (days)
Skeletonema costatum	10	f,d	42
Chaetoceros gracilis	3*	f	22
Phaeodactylum tricornutum	3*	f,a	22
	4*	f,a	7
Isochrysis galbana	10	f,d	42
	3*+	f,a	16
Synechococcus strain DC2	2.5	f,a	5

* Indicates that replicate cultures were grown with 25 mg L^{-1} Streptomycin added.

+ This culture was grown in seawater base supplemented with 5 μM of NaH_2PO_4 and 80 μM of $NaNO_3$ Base seawater from Dalhousie Aquatron Supply taken initially from 15 m depth in NW Arm Inlet, Halifax, Nova Scotia: f - 0.22-μm filtered; d - 1000-dalton dialysed; a - autoclaved.

(*ca.* 1 psi), applied by fixing a weight atop the plunger portion of the syringe. A low filter pressure was desired to minimize phytoplankton cell rupture. Approximately 40-mL sample were stripped of CO_2 by acidification to pH 2.2 with 3% H_3PO_4 and bubbling with compressed air at a flow rate of approximately 250 ml min^{-1} for 40 minutes. The air stream was previously saturated with H_2O by passing it through a frit immersed in distilled water. Since incomplete removal of $^{14}CO_2$ can be a problem, particularly for samples with low levels of fixed radiotracer (Sharp, 1977), it was verified that sparging to blank levels was obtained with this protocol, as shown in Table 2.2. Blank levels of approximately 36 and 40 dpm $sample^{-1}$ were obtained after *ca.* 30 minutes of sparging for both batches of stock ^{14}C-bicarbonate used in these experiments. These data also show that acceptably small amounts of organic ^{14}C were present in the ^{14}C-bicarbonate solutions. Contamination of stock ^{14}C solutions by labelled organic matter has been observed (Sharp, 1977; Smith and Horner, 1981). Following removal of inorganic ^{14}C, filtered 5-ml samples were pipetted in triplicate into plastic scintillation vials for PDO^{14}C analysis by the liquid scintillation counting method described below. Triplicate total organic carbon samples were taken by pipetting 5 ml of unfiltered material directly into scintillation vials. Particulate organic carbon (POC) estimates were made from the differences between total organic carbon and PDOC assays.

Assay of the ^{14}C radiotracer was accomplished by mixing 5-ml samples with 10 ml of New England Nuclear Formula 963 scintillation cocktail and counting for 10,000 counts (±1% coefficient of variation), unless otherwise specified, with a Beckman LS1801 liquid scintillation counter. This instrument employs a H# quench monitor calibrated against ^{14}C-toluene external quench standards (Amersham) to determine the counting efficiency of the samples. Detailed discussions of sample quench and H# quench monitoring are given by Niven (1990) and Horrocks (1978). In this work, the H# was derived from triplicate determinations by the quench monitoring system for each sample. The counting efficiency, which was

Table 2.2 Efficiency of inorganic ^{14}C removal from 40 mL ESNW seawater media acidified with 2 ml of 3% H_3PO_4 and bubbled with compressed air at approximately 250 ml min^{-1}.

time (minutes)	**$NaH^{14}CO_3$ solution #1 dpm (5 ml)$^{-1}$**	**$NaH^{14}CO_3$ solution #2 dpm (5 ml)$^{-1}$**
0	913, 423	106,773
15	139	84.1
30	39.2	36.7
45	41.7	35.7

Samples counted to ±1% coefficient of variation.

88 to 90% for the bulk of samples analysed in this work, was used to correct counts per minute to disintegrations per minute (dpm), which more accurately represents the activity of a sample.

Specific activities and calculation of photosynthetic carbon. Specific activities of the media were calculated from TCO_2 analyses using a coulometric method (Johnson et al., 1985) and ^{14}C activities of time-zero samples. Since the culture vessels were sealed from the atmosphere at all times other than during sample draw, the amount of dilution over the course of an experiment by $^{12}CO_2$ intake from sample draw can be readily estimated. In a 7-day experiment with a 3.5-L initial culture volume, approximately 1 L of sample will have been typically removed from the vessel. This represents an intake of about 15 μM CO_2 at room temperature, which is <1% of the CO_2 content of the seawater medium at the end of the experiment. Specific activity of inorganic pools were therefore assumed to be constant throughout the experiments. Total C calculations were not corrected for $^{12}C/^{14}C$ fractionation; a correction factor of 1.05 is commonly used (Strickland and Parsons, 1972).

Initial ^{14}C activity was measured by pipetting 1 ml of sample into 4-ml of pH 9.5 base solution. Failure to maintain alkaline conditions would result in loss of $^{14}CO_2$, as discussed by Kobayashi and Harris (1978). Samples were counted after allowing sufficient time for quenching of chemiluminescence generated from the basic conditions (Kobayashi and Harris, 1978).

PDO^{14}C results were converted to PDOC values in μMC based on the assumption that photosynthetic C pools were at isotopic equilibrium, i.e. equal to the specific activity of the inorganic pool. There is some disagreement in the literature on the rate at which photosynthetic C pools achieve isotopic equilibrium. Mague et al. (1980) and Smith and Geider (1985) examined the ^{14}C-labelling of intracellular and extracellular soluble organic pools from incubations of algae grown in culture. They concluded that isotopic saturation of these pools occurs in less than a day for fast growing species (*ca.* 1 doubling/day), such as those examined

here. However, the work of Storch and Saunders (1977) and Jensen et al. (1984) suggests that the extracellular organic pool of exponentially growing algae requires as long as 42 hours for isotopic equilibration with the inorganic pool. This disagreement may be associated with different specific growth rates of the phytoplankters studied, which were not discussed in detail by all authors in the aforementioned studies. There is agreement, however, that low molecular weight metabolites, such as amino acids, saturate within hours; while the high molecular weight fractions, such as polysaccharides, show an appreciable lag. This is attributable to the time required for the label to be incorporated into compounds labelled later in the metabolic sequence. From these observations, it can be stated that the values of PDOC, and particularly the high molecular weight fractions, were probably underestimated for the small number of samples taken early in log phase growth (<48 hours). Organic compounds present in the initial culture medium are not measured by the ^{14}C technique.

Labelling of the POC pool is a function of the number of doublings by the seed phytoplankton in the ^{14}C medium. The specific activity of POC, when determined as a fraction of the inorganic specific activity after (n) doublings, follows the expression $(1-0.5^n)$ (Welschmeyer and Lorenzen, 1984). I estimate between 3.5-7.5 doublings prior to stationary phase for these experimental cultures; isotopic POC equilibration was therefore >92% for the majority of samples.

2.2.3 Chemical oxidation of PDOC and analysis.

Persulphate oxidation analyses. Triplicate PDOC samples were pipetted into 10-ml Wheaton brand ampoules containing 200±5 mg of potassium persulphate. Careful measurement of K_2SO_8 was required to obtain precise experimental blanks, since ^{40}K ß-emissions contribute to the ^{14}C energy window. A blank value of 55 ± 2 dpm was obtained for persulphate-containing samples, compared with a value of 37 ± 0.8 for acidified seawater samples measured for 10,000 counts. Following addition of the PDOC samples, the ampoules

were sealed with a propane torch. During the sealing process, the ampoules were maintained under a positive pressure of oxygen to prevent contamination by propane or organic combustion products.[1] The samples were autoclaved for 40 minutes at 126-130°C (Strickland and Parsons, 1972) in a mini-autoclave located in a fume hood. Upon cooling, the samples were bubbled with compressed air at a rate of about 200 ml min^{-1} for 10 minutes to remove the CO_2 produced during the oxidation procedure. Further bubbling did not decrease the level of ^{14}C detected in the subsequent analysis. The samples were transferred *via* Pasteur pipet to plastic scintillation vials to assay the amount of PDOC that resisted oxidation ($PDOC_{res}$). As discussed in Chapter 3, it was found that very small amounts of residual $PDO^{14}C$ adhered to the ampoule surfaces; periodic counting of pH 12 NaOH rinses and entire crushed ampoules verified that these results were applicable throughout the experiments.

Ultra-violet oxidation analyses. Acidified (pH 2.5), O_2-sparged PDOC samples were also assayed for resistance to UV oxidation using both an autoanalyser instrument (Gershey et al., 1979; Chen and Wangersky, 1992a) and a batch mode instrument (Ridal and Moore, 1990), shown schematically in Figure 2.2. The autoanalyser system passes approximately 7 mL of sample through a quartz coil in very close proximity to a 550-W UV lamp (Conrad-Hanovia), which is positioned axially through the quartz coil. Due to the high flux of UV light subjected to the sample with this arrangement, the residence time of the sample in the coil is only of the order of 10 minutes. The batch mode system employs a 1200-W Hg lamp (Hanovia) and samples are contained in quartz tubes located 6 cm from the UV source. Quartz tubes were cleaned prior to use by irradiating acidified Super-Q for several hours. The tubes

[1]A stream of oxygen was directed into the interior of the ampoules *via* thin glass tubing positioned into the neck of the ampoule. As the neck thinned from heating, the tip of the glass rod was 'tacked' onto the upper inside wall of the ampoule neck and was used to pull seal the ampoule. This procedure provided a clean, reproducible seal, which can be elusive when a pull seal technique is not used. While the effects of contamination by combustion products are probably minimal in the radiotracer studies described here, this technique is particularly valuable for sealing samples to be analysed for DOC.

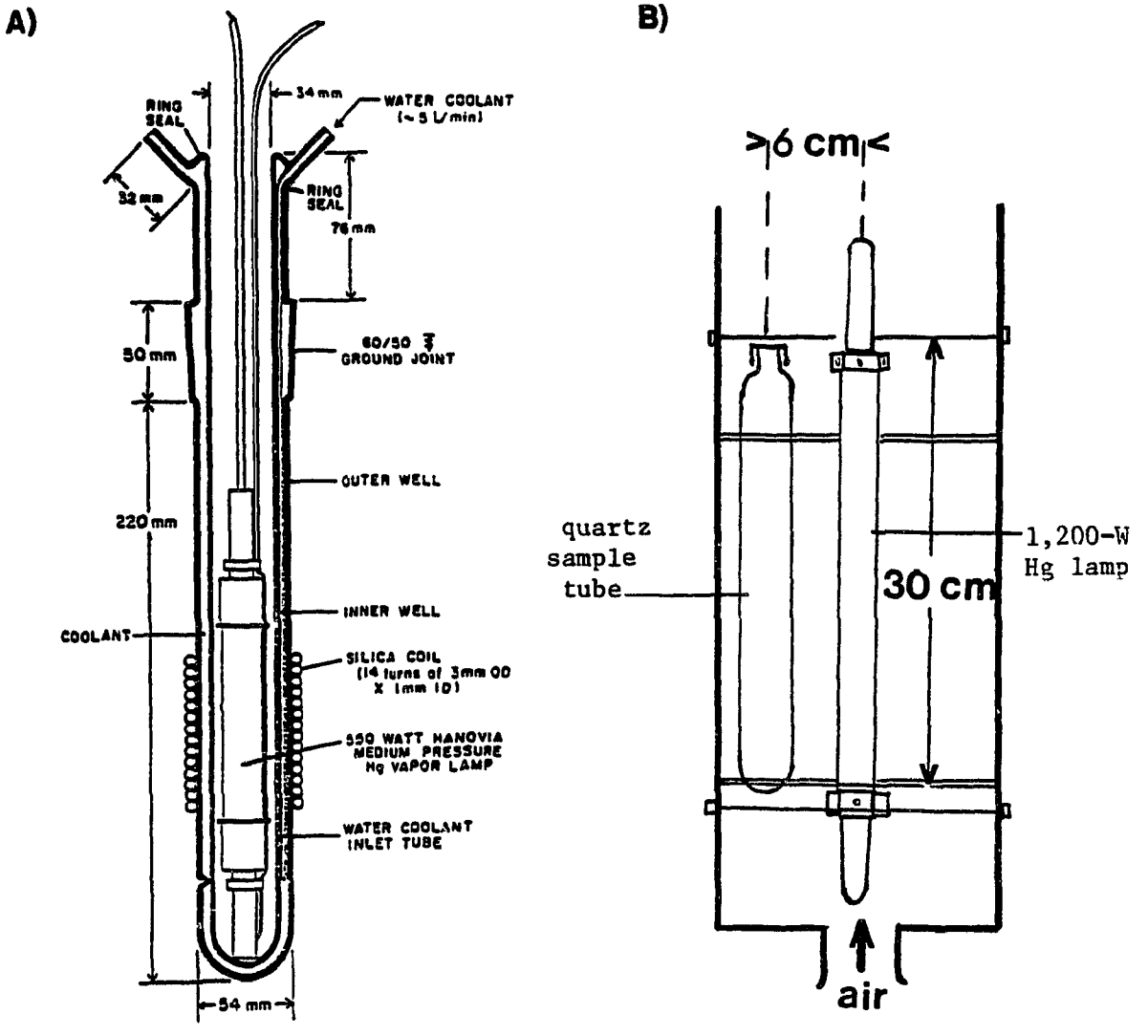


Figure 2.2. Schematic representations of UV autoanalyser (A) and UV batch oxidiser (B).

were handled with PVC gloves and wiped down with methanol prior to irradiation of samples. Maximum oxidation of DOC with this system requires several hours of photo-oxidation, and during this time sample temperatures are elevated to between 70-80°C. A variable timer controls the irradiation interval. Both UV systems were fitted with recently-purchased lamps, which were used for <100 hours prior to analysis. Lifetimes are estimated to be approximately 500 and 1000 hours for the respective 550 and 1200-W lamps.

Prior to UV analysis, PDOC samples were diluted with 3% NaCl Super-Q to total DOC values of less than 200 μMC, if necessary, since the autoanalyser system has shown reduced oxidation capacity for samples with concentrations greater than this level (W. Chen, pers. commun.). Residual ^{14}C activity in samples oxidised by UV autoanalyser was determined from assays of triplicate aliquots collected from the post UV irradiation stream. A small correction for dilution by the wash water was made to the subsequent ^{14}C assays.

To compare the autoanalyser and batch UV oxidation efficiencies, a duplicate series of samples were also treated by the batch UV system. Batch mode UV analyses were made by withdrawing 5-ml aliquots from 100-ml PDOC samples contained in quartz tubes. In a few cases, 30-ml samples were contained in 35-ml quartz tubes, and the entire tubes were removed after appropriate irradiation times. The stoppered tubes were tared prior to UV irradiation and the results corrected for typically small losses due to evaporation.

2.2.4 Ultrafiltration experiments

A TCF10 Thin Channel Amicon ultrafiltration unit, described in detail elsewhere (Niven, 1990), was fitted with YM10, and in a few instances YM100, series Diaflo membranes to provide respectively 10,000 and 100,000 nominal molecular weight (NMW) fractionations of PDOC samples. As shown in Figure 2.3, the molecular weight cut-offs of ultrafilters are not precise; compounds with molecular weights <10,000 are retained by the filter, while some

with molecular weights >10,000 pass the filter. Spherical molecules are more likely to be retained than irregularly-shaped molecules of the same molecular weight. As can be seen from the calibration data shown in Figure 2.3, the YM10 ultrafilter cut-off corresponds to 90% retention of 10,000 NMW spherical macromolecules. The material retained by the YM10 ultrafilter corresponds to a 1.5 nm-0.45 μm size fraction, and the YM100 corresponds to a 5 nm - 0.45 μm size fraction. Organic material in these size ranges has been traditionally referred to as colloidal (Sharp, 1973b)

Membranes were prepared by soaking the ultrafilters face down in Millipore Super-Q (changed frequently) for two days to remove the applied coatings. The filters were placed in the ultrafiltration unit and rinsed with 1 L of Super-Q. Additionally, the ultrafilters were conditioned by soaking overnight in dialysed seawater. Similar preparatory procedures have been found to result in minimal uptake of surface reactive materials (Niven, 1990).

Ultrafiltration of unacidified PDOC samples was carried out immediately after the filtration step. Following an initial 10-ml rinse, 60-ml samples were added to the cell. The cell was run at moderate peristaltic pumping speed and at 15 psi nitrogen pressure. Typically about 30 ml of sample was collected as ultrafiltrate. The retentate and ultrafiltrate fractions were collected in graduated cylinders and their volumes determined to ±0.2 ml. Upon completion of ultrafiltration procedures, 20 mL rinses of 0.005 M NaOH and then Super-Q were used to remove any strongly adsorbing PDOC from the unit and membranes. The membranes were stored in a dilute ethanol solution between uses, and rinsed with Super-Q and soaked for at least an hour in dialysed seawater prior to ultrafiltration of samples.

Analysis of ultrafiltered samples. After ultrafiltration of PDOC samples, samples were acidified and sparged. Aliquots of 5 ml were taken in triplicate of the ultrafiltrate and retentate fractions, pipetted into scintillation vials and assayed for ^{14}C activity, while three other 5-ml aliquots were treated by the persulphate oxidation procedure. Although only low molecular weight compounds pass the ultrafilter, both low and high MW compounds remain

in the retentate fraction. The activity due to the colloidal component was calculated by subtracting the activity of the ultrafiltrate from the activity of the retentate. The remaining activity was then divided by the concentration factor (typically 2 for these experiments) to give the activity due to colloidal compounds. Since these calculations usually involved the subtraction of two large values, ^{14}C precisions were maximized by counting these and the associated PDOC samples for 100,000 disintegrations, representing a statistical counting error of ± 0.5%.

2.2.5 Methods for Chlorophyll and microbial counts

Chlorophyll. Chlorophyll a samples were analysed following the method described by Strickland and Parsons (1972) with a few modifications. Initially, duplicate 25 or 50-ml samples were passed through 0.45-μm Millipore filters and the filters were immediately extracted in 90% acetone in the dark at 4°C for 20 hours. To minimize the time required for these analyses, filters from other batch cultures were placed in polypropylene centrifuge tubes, stored frozen, and then processed at the end of the experiments (all samples were measured within 10 days of sampling). Following solvent extraction, samples were centrifuged at 7000 RPM and 4°C for 10 minutes. Fluorescence measurements were made with a Turner fluorometer interfaced with an IBM personal computer. The contributions of phaeopigments to the fluoresence signal were assayed by measurements of the acidified samples. Calculations of Chl a and phaeopigments were generated from the calibration program of Shortle (1991).

Bacteria counts. Approximately 5-ml samples for bacterial counts were siphoned from the cultures with sterile syringes and placed in 20-ml glass scintillation vials. Samples were preserved by addition of formalin to a final concentration of 2% (vol/vol). Enumeration of bacteria was accomplished by epifluorescence microscopy using the acridine orange method of Hobbie et al. (1976), as modified by Schwinghammer (1988). Briefly, 1-5-ml samples were vacuum filtered (< 150 mm Hg.) through 0.2-μm Nuclepore filters (prestained or stained with

0.22-μm filtered Sudan-B black/ethanol solution (*ca* 0.1% wt/vol) for 24 hours) which lay atop a 0.22-μm Millipore filter on a glass sintered frit. Approximately 1 ml of 0.22-μm filtered acridine orange stain (0.02% wt/vol Super-Q) was then added to the filtration apparatus. The stain was left in contact with the black Nuclepore filter under low light for 2 minutes, after which it was filtered off under low vacuum and the filter and chimney were rinsed with 2-ml of 0.22-μm filtered Super-Q. The stained Nuclepore filters were then mounted onto a microscope slide covered with a very fine layer of Cargille Type A immersion oil to obtain uniform wetting of the filter onto the slide. A small drop of immersion oil was then added to the top side, the filter was covered with a cover slip, and placed in a small marked envelop for counting within 2 hours.

Counting of bacteria was accomplished with a Leitz Dialux EB 20 microscope fitted with an acridine orange filter and a Neofluar 100X objective (final magnification 1250x). This combination provided excellent visibility of the acridine orange preparations, and most orange and green fluorescing particles were readily identified as 0.2 to 1.2-μm-sized spherical, crescent, sigmoid and rod-shaped bacteria. A minimum of 20 random fields with a total of 400 bacteria were counted for each analysis.

2.3 Ageing experiments

I also investigated the changes of phytoplankton DOC reactivity to chemical oxidation methods brought upon by ageing of PDOC solutions in the presence of coastal marine microbes during experiments lasting several weeks to almost 1 year. The experimental methods are described in detail in Chapter 4, but are briefly outlined here. The PDOC substrates for these long-term experiments were taken from unialgal batch cultures of *Isochrysis galbana*, *Skeletonema costatum*, *Chaetoceros gracilis*, and *DC2 Synechococcus*. The cultures were allowed to grow for about two weeks before filtration, at which time all cultures had reached

stationary phases as determined by cell counts with a Coulter Counter. The filtered material was inoculated with small amounts of unfiltered NW Arm seawater, and replicates were spiked with sodium azide to determine the effects of abiotic reactions. Initial samples were extracted from the cultures and portions analysed by persulphate oxidation. Further sampling was done after weeks and months of ageing.

A short-term ageing experiment, examining the microbially mediated changes to PDOC over time periods of hours and days, was carried out with substrate from an axenic *Synechococcus* culture. Experiments were also undertaken to follow the ageing and possible microbial production of chemically refractory DOC from unfiltered *Phaeodactylum tricornutum* and *Isochrysis galbana* culture material. In a final study, PDOC samples were aged in quartz tubes in daylight to ascertain whether photochemical reactions produced appreciable changes to the resistance of samples to persulphate oxidation.

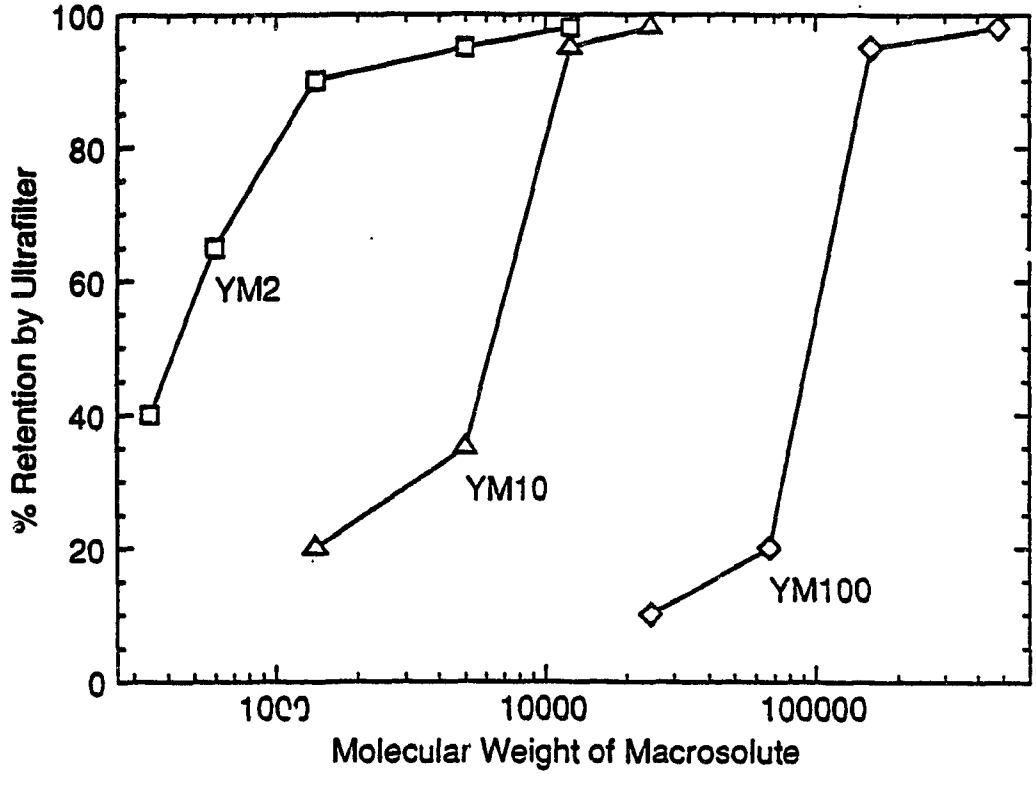


Figure 2.3. Percent retention of different molecular weight sperical macrosolutes by Amicon YM series Diaflo filters. YM10 and YM100 filters were used in this study. (Data from Amicon Corporation).

Chapter 3

Efficiencies of Persulphate and Ultra-violet Oxidation Methods for Analysis of Dissolved Organic Carbon Produced by Marine Phytoplankton

3.1 Introduction

As outlined in Chapter 1, the measurement of dissolved organic matter in seawater is at present a controversial issue in the field of chemical oceanography (Toggweiler, 1988; Williams and Druffel, 1988; Jackson, 1988). To review briefly, recent work using a high temperature combustion oxidation (HTCO) procedure has found values in surface seawater of dissolved organic carbon (DOC; Sugimura and Suzuki, 1988; Druffel et al. 1989; Suzuki et al., 1992) that are 2-4 times higher than measured by previous 'standard' methods, such as ultra-violet (UV; Armstrong et al., 1966; Collins and Williams, 1977) or persulphate oxidation (Menzel and Vaccaro, 1965; Sharp, 1973a) techniques. Sugimura and Suzuki claim that their HTCO method detects marine DOC that is resistant to oxidation by persulphate or UV, particularly that DOC with molecular weights greater than 4,000 daltons. It has been suggested that the low oxidation efficiency of standard chemical techniques to such colloidal DOC may be due to shielding of inner layers of folded, polymeric material from the chemical oxidizing agents (Druffel and Williams, 1988; Lee and Henrichs, 1992).

As well, the HTCO data presented by Sugimura and Suzuki (1988) exhibit strong inverse correlations with apparent oxygen utilization, a feature not previously noted for DOC profiles measured with the standard chemical techniques. Such correlations suggest that the extra DOC measured by the HTCO methods, while chemically resistant, is biologically utilizable. DOC may be transported from surface waters and decay gradually, as it is advected beneath the thermocline, a mechanism which links DOC to oceanic oxygen and nutrient cycles

(Toggweiler, 1988). The potential importance of high dissolved organic matter concentrations to oxygen and nutrient cycling, and to the global carbon budget, has challenged oceanographers to confirm, if possible, these new DOC results. However, attempts with various high temperature combustion oxidation methodologies to repeat the results of Sugimura and Suzuki (1988) have met with limited success (Williams, 1991), which may reflect oceanic variability of DOC or problems with the methods.

Very recent developments underscore this last point. Suzuki has now indicated that calibration errors resulted in erroneously high values reported in the 1988 paper (P. Wangersky, pers. commun.), although he apparently stands by his most recent DOC estimates for Pacific surface seawater of 200 μM C (Suzuki et al., 1992). However, Tanoue (1991) reports that he is unable to repeat even these lower values at the same oceanic stations with an *identical* HTCO instrument. As well, he found only very weak correlation between AOU and DOC in open ocean waters off Japan.

As discussed by Wangersky (1992), instrumental problems include difficulties associated with measuring trace quantities of organic matter against a 10-20 fold background of inorganic CO_2, variable and ill-defined instrument blanks, poor detector reliability (especially at sea), possible temperature dependent side products, variable catalyst efficiencies, and inadequate reference procedures to ensure that quantitative oxidation of marine DOC to CO_2 is accomplished with the technique of interest. These methodological problems therefore compromise attempts to analyze marine DOC, its modes of formation, microbial and chemical reactivities, as well as its spatial and temporal variations.

An alternative approach, one which circumvents many of the problems hindering DOC measurement, is to introduce ^{14}C radioisotope into the DOC pool via photosynthetic uptake and release by phytoplankton. The radiolabel can then be analysed with the technique of liquid scintillation counting to higher precision levels (e.g. ±1%) than normally obtained with bulk DOC instruments. Such an approach can be used to test the hypothesis that DOC resistant

to chemical oxidation methods is a product of phytoplankton growth and/or decay. This hypothesis is based on profiles which show maximum DOC concentrations in surface waters, rapidly decreasing concentrations with depth, and minimum values at the oxygen minimum (Sugimura and Suzuki, 1988; Druffel et al., 1989; Suzuki et al., 1992). Since evidence from lignin tracer (Meyers-Shultz and Hedges, 1986) and radiocarbon ageing studies (Druffel and Williams, 1987) indicates terrestrial DOC inputs into the open oceans are probably relatively small, the DOC profiles suggests photosynthetic production by marine algae at the surface and removal by respiration processes at depth.

In this study, the dissolved organic carbon produced from marine algae was investigated using a ^{14}C radiolabelling method described in Chapter 2, which is based on techniques developed to estimate the amount of extracellular release of organic compounds from phytoplankton (reviewed by Jones and Canon 1986). The radiotracer method can provide highly precise measurements of photosynthetic dissolved organic carbon (PDOC) with well-defined experimental blanks. It also enabled me to follow production of persulphate oxidation resistant PDOC through different physiological stages of phytoplankton growth, and to investigate the effects of microbial and chemical ageing on the susceptibility of PDOC to oxidation. Furthermore, because of the high precision of the ^{14}C method and decreased risk of contamination, the determination of PDOC size fractions is readily accomplished using ultrafiltration procedures. Individual size fractions can be then tested for their resistance to persulphate oxidation.

3.2 Methods

The general methods for these experiments are described in Chapter 2 and Figure 2.1. Time series measurements were made from cultures of *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Synechococcus* strain DC2. Replicate *Isochrysis galbana* and *Phaeodactylum*

tricornutum cultures were grown with an antibiotic added to limit bacterial growth, while the cyanobacterium culture was axenic. Experiments were also performed using gas chromatography to quantify the low molecular weight halocarbons produced from application of persulphate and UV oxidation methods to marine DOC.

3.2.1 Gas chromatographic analysis

Persulphate method samples. Filtered, acidified NW Arm seawater was bubbled with high purity N_2 gas to bring total halocarbons to very low levels. Aliquots of seawater were transferred by glass syringe into clean ampoules containing persulphate reagent, and the ampoules were sealed under a N_2 atmosphere with a propane torch. Samples were also sealed in ampoules without persulphate. Larger volumes than usual (10 ml instead of 5 ml) were used to minimize head-space volume. All ampoules were autoclaved and cooled to room temperature. Analysis of these samples for low molecular weight halocarbons by GC followed generally the methods described by Moore and Tokarcyzk (1992) with a few modifications. Ampoules were opened, 2-μl samples were quickly withdrawn and injected into the Tecmar purge and trap system, which already contained 5 ml of purged Super-Q water. Analysis then followed normal procedures.

UV method samples. Filtered, acidified seawater was purged for 30 minutes in a quartz container. A few drops of ultrapure H_2O_2 was added to the approximately 100 ml sample. A 25-ml blank was removed and the vessel then sealed with a Swagelok assembly. The assembly, which contained a Teflon fitting, was wrapped in Al foil to protect it from the high intensity light. The sample was irradiated in the 1,200-W Hg lamp apparatus for a period of 1 hour. A 25-ml sample was removed by a 50-ml syringe immediately after opening the Swagelok assembly, and duplicate 5-ml samples were analysed by GC.

3.3 Phytoplankton culture experiments results

3.3.1 Isochrysis galbana results

Results are presented in Figure 3.1 for *I. galbana* cultures with and without the antibiotic streptomycin (SM). Streptomycin was added to a replicate culture in an attempt to minimize bacterial growth and to ascertain whether $PDOC_{res}$ was rapidly respired by bacteria, particularly in the early stages of the experiments. The batch cultures with this flagellate showed typical phytoplankton bloom profiles with a maximum value of 65 μg Chl a L^{-1} measured in the SM culture, compared with 50 μg Chl a L^{-1} measured in the culture without SM. Higher Chl a levels in the SM culture may have been due, in part, to less nutrient uptake by fewer bacteria. Greater levels of PDOC also were measured in the SM culture, with a value of 310 μM C found in the SM culture versus 190 μM C for the culture not containing antibiotic. When normalized to maximum chlorophyll a values, these results indicate 20% more PDOC accumulated in the SM culture over the 16 day experiment. The fractions that resisted oxidation at the end of the experiments were 29.4 μM C in the culture with SM and 12.5 μM C in the culture without. Major accumulation of PDOC occurred during stationary and senescent phases of the algal blooms. As shown in Figure 3.1(c), microbial growth was observed in both cultures, although approximately 4 times fewer bacteria were observed in the SM culture. Presumably, bacteria grew in response to release of labile exudates by algae during stationary phase, as prior to this stage, bacterial numbers increased only marginally.

When $PDOC_{res}$ is taken as a percentage of PDOC, as shown in Figure 3.1(b), the values indicate the resistance of the PDOC during different physiological stages of algal growth. The $PDOC_{res}$ values range from 5-13% for both cultures and the most resistant PDOC appears to be associated with log growth phase. It is particularly interesting to note that despite the greater accumulation of PDOC in the antibiotic-treated culture, very similar percentages of PDOC were observed to resist oxidation for the SM and non-SM culture samples. If additional

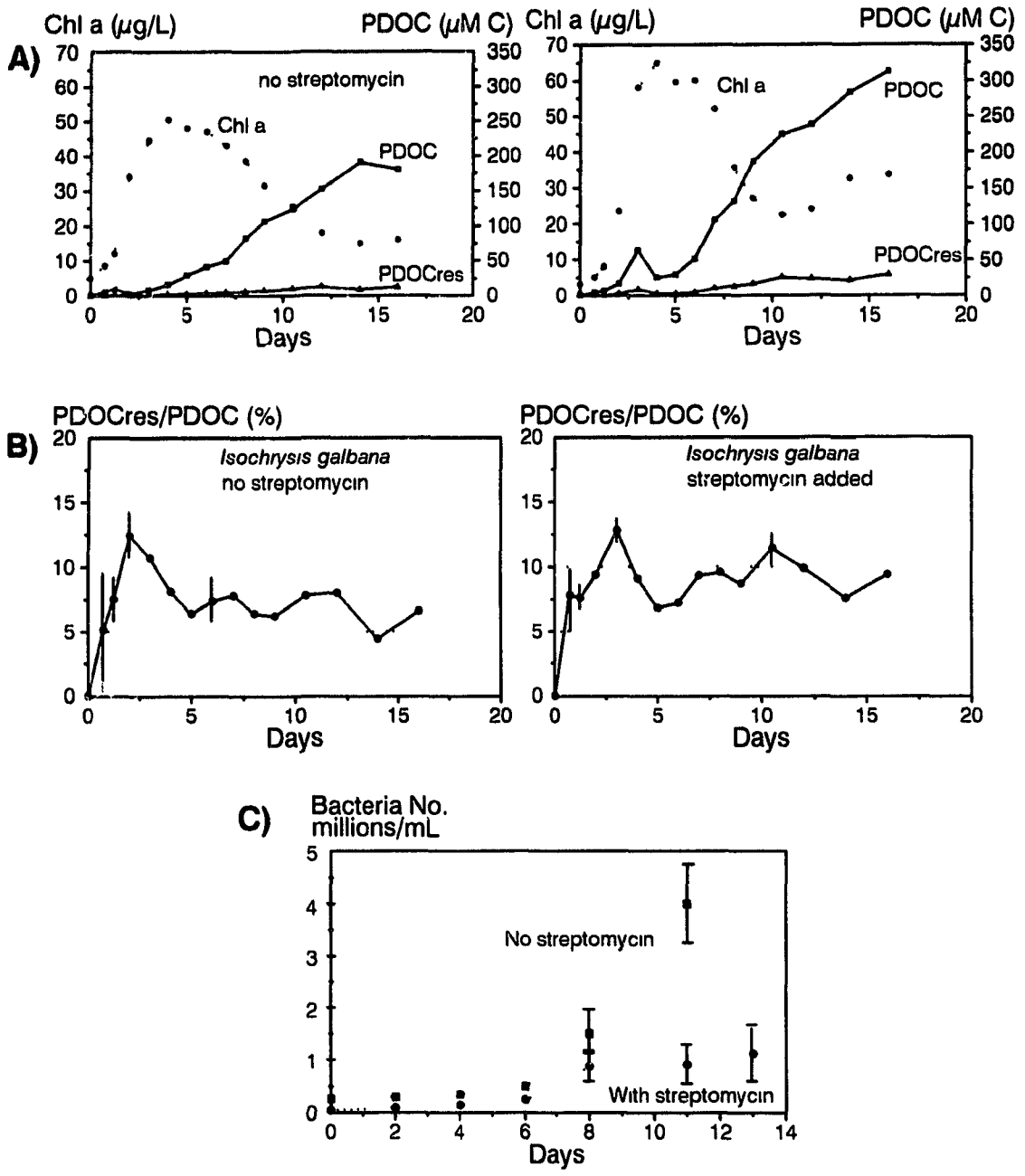


Figure 3 1. *Isochrysis galbana* batch culture results for cultures with no streptomycin added (SM) and with streptomycin added: (A) Progress of chlorophyll a (chl a), production of phytoplankton dissolved organic carbon (PDOC), and the percentage of PDOC resisting persulphate oxidation ($PDOC_{res}$); (B) Graph of $PDOC_{res}$/ PDOC; (C) Increase of bacteria in batch cultures

bacterial consumption and alteration of the PDOC occurred in the non-SM culture, as is suggested by the considerably greater numbers of bacteria, it did not significantly alter reactivity of PDOC to persulphate oxidation.

3.3.2 Phaeodactylum tricornutum results

The results from the *P. tricornutum* cultures are given in Figure 3.2. As observed for the *I. galbana* cultures, the bulk of the PDOC was produced during stationary and senescent phases of the blooms. Approximately 220 μM C of PDOC accumulated in the *P. tricornutum* culture without SM, 26.4 μM C of which resisted persulphate oxidation, whereas 250 and 30.5 μM C of PDOC and $PDOC_{res}$ respectively accumulated in the SM culture. Bacteria increased dramatically in both cultures from Day 3 to Day 4, though bacteria numbers levelled off or declined after this time, while Chl a levels increased to maximum values on Day 5. These observations suggest that bacterial regeneration of nutrients stimulated further algal growth.

Also shown in Figure 3.2 are the percentages of resistant PDOC measured for different stages of the diatom blooms. The $PDOC_{res}$ values were found to range from 7-15% of PDOC for Day 2-7 samples; $PDOC_{res}$ values were not measurable in samples taken earlier in the experiment. Following maximum values of about 15% on Day 2.5, $\%PDOC_{res}$ values decreased to 10% in both cultures on Day 3. Rapid bacterial growth in both cultures after this point suggest that the PDOC produced on Day 3 was also labile to bacteria.

3.3.3 Synechococcus results

The axenic *Synechococcus* culture results are given in Figure 3.3. Exponential growth was observed until about Day 2.5. A stationary phase followed, characterised by maximum Chl a values and a drop in the rate of ^{14}C uptake. Unlike the other cultures which showed a brief lag before PDOC could be observed, PDOC levels in the *Synechococcus* culture increased

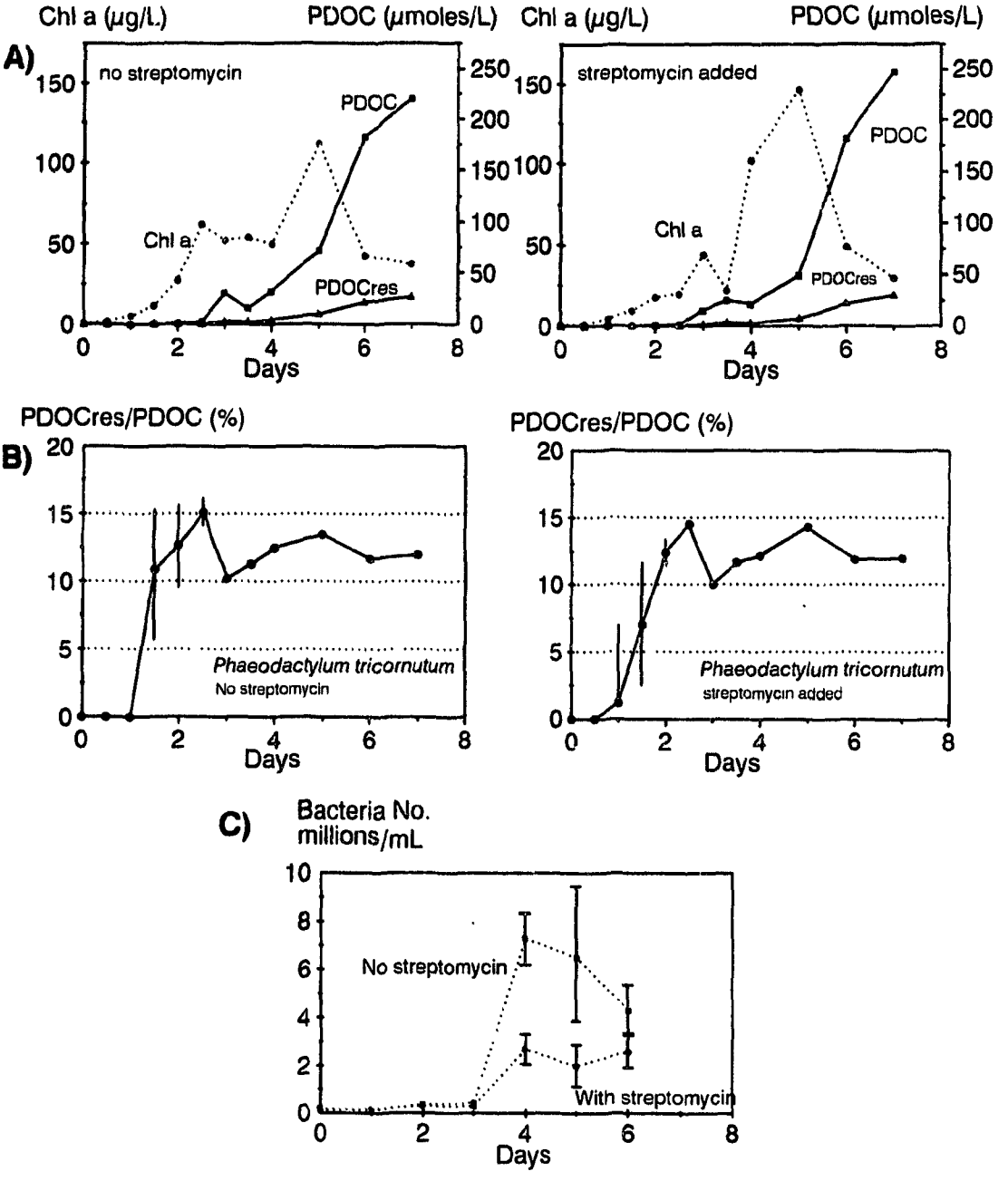


Figure 3.2. *Phaeodactylum tricornutum* batch culture results for cultures without streptomycin (SM) and with streptomycin added: (A) Progress of chlorophyll a, production of phytoplankton dissolved organic carbon (PDOC), and the fraction of PDOC resisting persulphate oxidation ($PDOC_{res}$); (B) Graph of percentage $PDOC_{res}$ of PDOC; (C) Increase of bacteria in batch cultures.

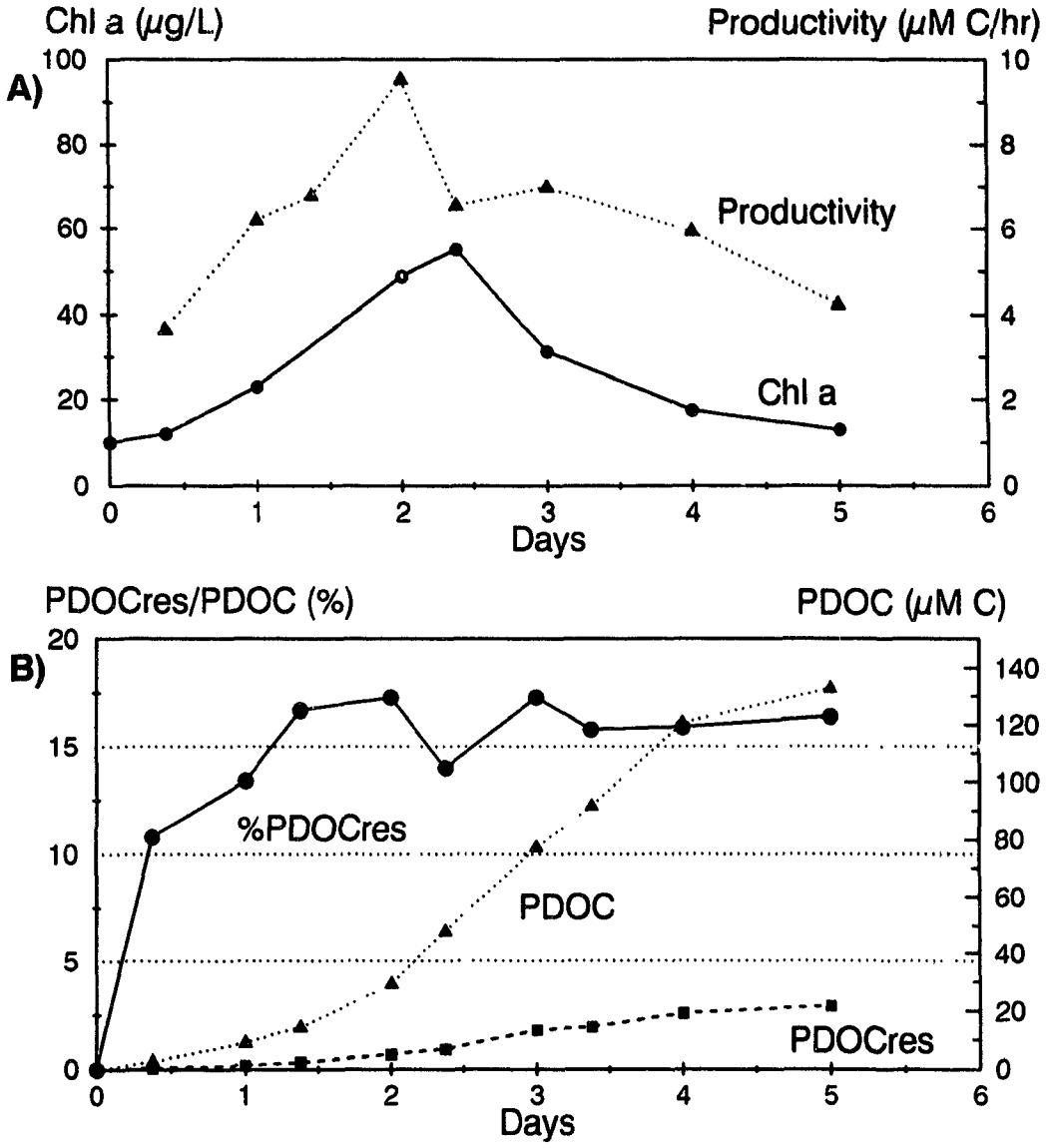


Figure 3.3. *Synechococcus* batch culture results: (A) Progress of chlorophyll a and productivity (^{14}C uptake); and (B) increase of PDOC, the fraction resisting persulphate oxidation ($PDOC_{res}$), and the percentage $PDOC_{res}$ of PDOC.

exponentially from the initial sampling day. PDOC made up between 8.0 and 9.1% of ^{14}C uptake for samples taken prior to Day 2,5, and following this point PDOC production increased to 12.5-20.0% of carbon uptake. By the end of the 5 day experiment, 133 μM C of PDOC and 21.9 μM C of $PDOC_{res}$ had accumulated in the culture.

As shown in Figure 3.3, *Synechococcus* $PDOC_{res}$ values ranged from 10.8-17.3% of the total PDOC. A rapid drop in productivity on Day 2.5 coincided with a relatively low $\%PDOC_{res}$ value, indicating 'release' of labile PDOC compared with that released on other days of the experiment. Since the measured reactivity of the PDOC sample from Day 2.5 also included the accumulated PDOC from log phase growth, corrected PDOC and PDOC*res* values were calculated by subtracting the values of the previous day. Values corrected in this way reflect PDOC and $PDOC_{res}$ values of only the most recently produced material. These results showed that during the 15 hour period of maximum productivity prior to Day 2, 18% of the PDOC produced resisted persulphate oxidation. However, in the 9 hour period following this productivity maximum only 8.9% of the PDOC produced was resistant to persulphate oxidation.

Similarly corrected $\%PDOC_{res}$ values cannot be calculated with confidence for the other algal cultures, since, unlike the axenic *Synechococcus* culture, these values would be compromised by bacterial uptake. However, it is observed for all cultures that $\%PDOC_{res}$ values decrease in early stationary phases when bacteria levels are low, consistent with inputs of chemically labile compounds, such as simple carbohydrates.

3.4 Ultrafiltration studies

3.4.1 Phaeodactylum tricornutum cultures

The size fractionation results for samples taken from the *P. tricornutum* cultures are shown in Table 3.1. Both *P. tricornutum* cultures produced similarly high levels (>68%) of low

molecular weight PDOC. Very significant proportions of PDOC were lost to the ultrafiltration apparatus for Day 1 samples; thereafter losses averaged about 4% of PDOC. Day 1 samples, of course, contained very low levels of PDOC; any uptake by the ultrafilter was proportionately large.

An interesting aspect of the ultrafiltration data is that the *P. tricornutum* colloidal fraction did not show greater resistance to persulphate oxidation, as would be suggested by the size fractionation results of Sugimura and Suzuki (1988). In this case, colloidal PDOC was generally more susceptible to oxidation than low molecular weight PDOC. Similar ultrafiltration results were found for the SM culture, suggesting that PDOC in the non-SM culture was not substantially altered by the higher numbers of bacteria present in this culture. Consumption or transformation of *P. tricornutum* PDOC by bacteria may have been expected to result in DOC with a greater proportion of high molecular weight compounds (Iturriaga and Zsolnay, 1983), due to preferential uptake of lower molecular weight substrate, and different reactivities to persulphate oxidation.

3.4.2 Synechococcus DC2 results

Synechococcus ultrafiltration results are presented in Table 3.2. The PDOC generated in this culture was >80% low molecular weight and 14-17% of this fraction resisted oxidation. In contrast with the results obtained for *P. tricornutum* cultures, colloidal PDOC exhibited generally greater resistance (15-22%) to persulphate oxidation than the low molecular weight fraction (14-17%). Further size fractionation with a 100,000 NMW ultrafilter revealed that very high molecular weight material was the most refractory to oxidation, as 24-28% of this material was not oxidised by persulphate.

Table 3.1(A). Ultrafiltration results for *Phaeodactylum tricornutum* cultures without streptomycin. Ultrafiltrate (UF): material <10,000 NMW; UF_{res} is the amount remaining of the UF fraction after persulphate oxidation; Retentate (Ret): material >10,000 NMW; Ret_{res} refers to the amount of Ret fraction remaining after persulphate oxidation. Loss values obtained from mass balance calculations.

Table 3.1(A)

Day	UF (μM C)	UF_{res} (%)	Ret (μM C)	Ret_{res} (%)	% UF	Loss (%)
0.00	0.00	0.0	0.00	0.0	0.0	0
1.00	0.06	0.0	0.00	0.0	56	44
2.00	0.69	15.7±1.3	0.20	1±5	72	6
3.00	23.5	12.7±0.2	5.01	6.2±1.5	76	7
4.00	29.0	12.5±0.5	3.45	10.3±5.0	91	0
5.25	53.2	14.9±1.7	13.7	9.7±1.5	74	7
6.25	147.0	11.4±0.3	32.1	4.2±1.5	80	2
7.25	162.0	13.3±0.3	43.6	8.7±2.1	74	6

Table 3.1(B). Ultrafiltration results for *Phaeodactylum tricornutum* cultures with streptomycin. Headings are as in Figure 3.1(A).

Day	UF (μM C)	UF_{res} (%)	Ret (μM C)	Ret_{res} (%)	% UF	Loss (%)
0.00	0.00	0.0	0.00	0.0	0.0	0
1.00	0.04	0.0	0.03	0.0	30	45
2.00	0.49	13.1±1.5	0.23	6±5	78	0
3.00	13.5	10.6±0.2	1.42	5±5	90	1
4.15	18.2	12.0±0.5	3.22	12.2±0.6	86	1
5.25	41.9	15.4±0.3	7.76	9.4±1.0	84	1
6.25	123.0	13.6±0.3	40.6	6.9±0.7	68	10
7.25	194.0	14.0±0.5	46.5	6.8±1.0	78	3

Table 3.2. Results from ultrafiltration of PDOC from *Synechococcus strain DC2*. Table captions are the same as those for Table 3.1.

Day	UF (μM C)	UF_{res} (%)	Ret (μM C)	Ret_{res} (%)	% UF	Loss (%)
0.00	0.00	0.0	0.00	0.0	0.0	0
1.00	7.25	14.1±0.4	0.77	16.1±5.2	81	11
2.00	26.84	14.7±0.4	1.09	15.3±4.9	92	5
3.00	67.84	16.4±1.2	5.84	21.9±3.8	87	6
4.00	100.0	13.7±0.5	13.4	20.7±1.1	83	6
5.00	110.9	16.8±0.5	11.9	17.1±6.5	83	9
YM100 ultrafilter results			>100,000 NMW (μM C)	Ret (%)		
4.00			9.83	24.0±4.4	83	6
5.00			2.19	28.5±2.5	83	9

3.5 Studies of the efficiency of the persulphate oxidation method

3.5.1 Substrate concentration

During the investigation of oxidation resistant PDOC, a number of experiments were done to test the sensitivity of the persulphate method to changes of some experimental parameters. Table 3.3 shows the efficiency of persulphate oxidation applied to *Synechococcus* PDOC of widely varying concentrations. The proportions of PDOC resisting oxidation agree within experimental error, indicating that yields of %PDOC are reasonably invariant to different concentrations of the same substrate. Comparable results were obtained with PDOC from other algae; significant differences between %PDOCres values therefore reflect qualitative changes of PDOC. A similar conclusion was reached by Williams (1969) who found near complete oxidation of ^{14}C-labelled glucose (>99%) and a mixture ^{14}C-labelled amino acids (*ca.* 96%) despite 100 fold changes of substrate concentrations.

Other parameters. Results also presented in Table 3.3 show that doubling the persulphate reagent concentration resulted in oxidation of an additional 4.8% of the labelled DOC. Increasing the autoclaving time from 40 minutes (Strickland and Parsons, 1972) to 3 hours resulted in 13.3% residual PDOC compared with the value of 16.6% obtained with the standard time. These results suggest that my standard procedures do not grossly misrepresent the oxidation capacity of the persulphate oxidation method.

Total organic carbon analyses. I also investigated the oxidation of total organic carbon samples with the persulphate technique, to determine if whole cells or cells lysed by sonication would show increased resistance to persulphate oxidation. Unfiltered, sonicated, and filtered samples of aged culture material were oxidised with persulphate. The results are presented in Table 3.4. It was found that unfiltered and filtered samples showed very similar resistance to oxidation. Sonication does not increase the amount of organic material resisting oxidation

Table 3.3. Efficiency of persulphate oxidation procedure with changes in Synechococcus DOC concentrations. Samples diluted with 3% NaCl Millipore Super-Q.

PDOC (μM C)	$PDOC_{res}$ (μM C)	$\%PDOC_{res}$
130.0	21.6	16.6±0.8
72.3	12	16.9±0.8
34.6	6.3	18.1±1.2
11.0	1.7	15.7±1.4
130.0	15.3[1]	11.8±0.5
130.0	17.3[2]	13.3±0.7

[1]Persulphate reagent concentration doubled to 400 mg sample^{-1}.
[2]Autoclaving time extended to 3 hours.

Table 3.4. Oxidation of unfiltered culture material by persulphate. Also shown are results obtained after replicate samples were sonicated at high settings for several minutes. All samples taken from senescent cultures.

Species	Sample	Total (dpm ml^{-1})	Resistant (dpm ml^{-1})	%Resistant (±0.5%)
DC2	unfiltered	45,085	5,209	11.5
	filtered	8,425	911	10.8
	sonicated + filtered	10,220	1,034	10.1
P. tricornutum	unfiltered	50,710	5,417	10.7
	filtered	22,406	2,279	10.2
	sonicated	50,700	5,376	10.7
I. galbana	unfiltered	10,948	685	6.2
	filtered	7,939	446	5.6
	sonicated	10,945	664	6.1

3.5.2 Volatile products other than CO_2

Trap experiments. I investigated whether significant proportions of PDOC samples were oxidised to volatile products other than CO_2, such as CO, low molecular organic acids, or transformed to low molecular weight halocarbons. Lee and Henrichs (1992) have postulated that such side products may be partially responsible for low DOC values measured by wet chemical oxidation methods, since these products would be missed by CO_2 detectors. The production of non-CO_2 volatiles from persulphate oxidation of PDOC was investigated by venting the gas sparging stream through a -50°C ($CaCl_2$/ice) cold trap and a series of two traps adjusted to pH 10.5 with NaOH. As shown in Table 3.5, 98.9±1.1% of the label was recovered by the dilute base traps and 0.8±0.6% was retained by the cold trap. Background values were measured when the traps contained weakly acidic solutions. A very small amount (<0.4%) of the ^{14}C label was sorbed onto the walls of the glass ampoules. A similar result was obtained by Williams (1969), who passed the oxidation products from the persulphate oxidation of a mixture of labelled amino acids through a dry ice trap and failed to find measurable accumulation of ^{14}C material. From this evidence, I concluded that my assays of the liquid sample following oxidation do not substantially underestimate the fraction of PDOC resisting persulphate oxidation to CO_2.

Gas chromatography experiments. The abundance of low molecular weight halocarbons produced by wet oxidation of marine DOC was investigated using gas chromatography. Analyses of coastal seawater samples treated with UV and persulphate DOC methods are given in Table 3.6. All of the major peaks were identified as low molecular weight halocarbons. Chloroform was the most abundant halocarbon produced. The total halocarbon concentration was very low after 1 hour UV irradiation, but accounted for approximately 3.1 μM C in the persulphate technique. Replicate samples analyzed by UV and high temperature analysis gave DOC values of about 100 μM C. This indicates that approximately 3±1.2% of the DOC sample was converted to low molecular weight halocarbons.

Table 3.5. Recovery of ^{14}C label following persulphate oxidation of aged PDOC from various algae.

	% Label Recovered				
Sample	NaOH traps	sorbed on ampoule[1]	$PDOC_{res}$	Total	cold trap
I. galbana	93.4	0.1	4.2	97.7±1.5	1.5±0.9
P.tricornutum	89.1	0.1	10.0	99.2±0.5	0.4±0.2
Synechococcus	78.7	0.4	20.6	99.7±1.2	0.4±0.1
			mean	98.9±1.1	0.8±0.6

[1]Values are averages of duplicate analyses. Amount of PDOC sorbed on ampoule measured from pH 12 NaOH extractions and/or by direct assay of crushed ampoule.

Table 3.6. Concentrations of low molecular weight halocarbons in coastal seawater samples after persulphate oxidation. Methyl chloride could not measured with the GC conditions employed. HTCO analysis indicated that the samples initially contained about 100 μM C

CH_2Cl_2 (μM)	$CHCl_3$ (μM)	CCl_4 (μM)	$CHCl_2Br$ (μM)	$CHClBr_2$ (μM)	$CHBr_3$ (μM)	CH_3I (μM)	Total (μM)
<0.01	0.99	<0.01	0.79	0.81	0.43	<0.01	3 03
							s_x ±1.06

Although it cannot be assumed that other carbon-containing volatiles, other than low molecular weight halocarbons, were not produced by persulphate oxidation of coastal seawater, the combined results of the GC and trap experiments suggest that the amounts of non-CO_2 volatiles produced by the persulphate method are small fractions of the total DOC

3.6 Comparison of UV and persulphate oxidation methods

Table 3.7 gives the results obtained from a variety of phytoplankton DOC samples in experiments comparing persulphate and autoanalyser UV oxidation efficiencies. All samples proved somewhat resistant to both persulphate (*ca.* 8-21%) and UV autoanalyser (*ca.* 15-27%) oxidation methods. The UV autoanalyser fared most poorly for PDOC produced from algae in log phase growth. Results from the batch mode UV oxidiser, shown in Figure 3.4, indicate that extended exposure to UV irradiation resulted in more complete oxidation of these samples. A 95% decomposition was observed for all samples after 6 hours exposure to high intensity irradiation from the 1200-W lamp. A small percentage (<1%) of PDOC was detectable for all samples even after 21 hours irradiation.

Table 3.8 shows time series results of the UV breakdown of a labelled DOC solution as determined by ^{14}C and high temperature combustion oxidation methods (Chen and Wangersky, 1992b). The HTCO method is expected to measure all carbon compounds present in the sample. Very similar decomposition rates were measured by the two techniques, indicating that the radiolabelled compounds are representative of the bulk reactivity of the samples, assuming complete oxidation of all carbon with the HTCO technique.

Table 3.7. Comparison of PDOC resisting persulphate and UV autoanalyser oxidation methods for a variety of samples.

Sample	$\%PDOC_{res}$ UV autoanalyser	$\%PDOC_{res}$ persulphate	Comments
I. galbana	21±1	10.0±1.1	exponential phase
	15±1.2	7.8±1.3	stationary phase
P. tricornutum	27±2	9.4±1.6	exponential phase
	15±5	12.8±1	aged
Synechococcus	17±2	20.5±0.9	aged

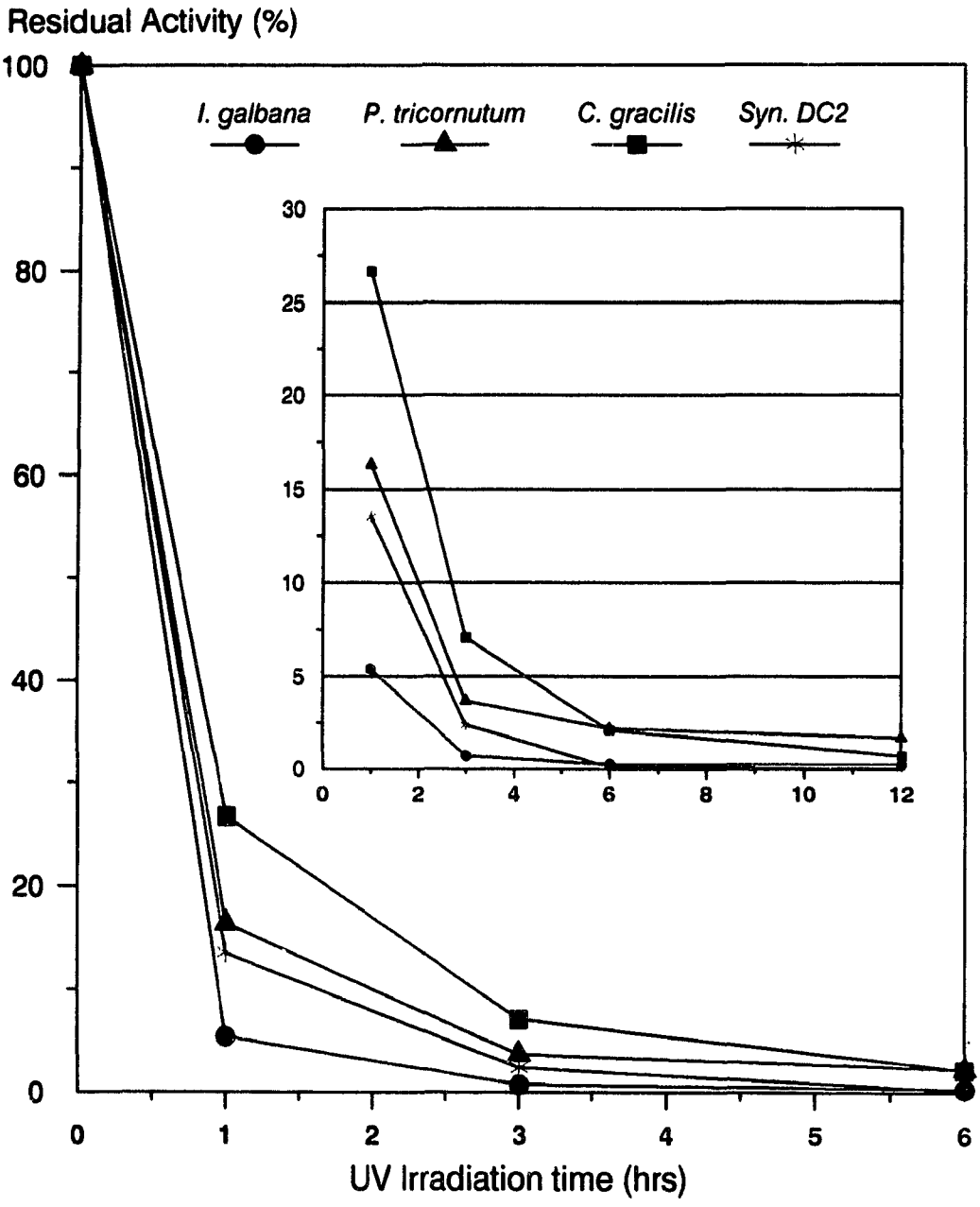


Figure 3.4. Oxidation of PDOC using batch mode UV irradiation procedures. PDOC samples are replicates of samples used for UV autoanalyser study. Insert shows longer time series measurements.

Table 3.8. UV decomposition of aged *Phaeodactylum tricornutum* culture material analysed by high temperature combustion oxidation and ^{14}C methods. Also shown are the values obtained following autoanalyser oxidation and after persulphate oxidation. b.d.l. (below detection limit).

Irradiation time (hours)	HTCO (μM C)	Remainder (%)	^{14}C (dpm ml^{-1})	Remainder (%)
0.0	192	100	2966	100
1.0	26	14	401	13.5
3.0	7	3.6	71	2.4
6.0	3	1.6	4.0	0.14
9.0	b.d.l.	-	2.6	0.01
UV autoanalyser	54	28	447±75	15±2.5
Persulphate	-	-	376±27	12.7±0.9

An interesting treatment of the time series data of the UV decomposition of *Skeletonema costatum* and *Chaetoceros gracilis* PDOC is shown in Figure 3.5. In this Figure, the data are transformed following the G model of Berner (1980). The G model was originally developed to model the decay of organic matter in sediments, but its use is informative here. This treatment shows that UV decomposition of PDOC can be partitioned into 2 or 3 groups of reactants, which can be characterised by apparent first order decay rates. Figure 3.5 indicates that while the bulk of PDOC is oxidised by UV at relatively fast rates (k_1 = 1-2 h^{-1}), some refractory fractions decay at much slower rates (k_2 = 0.2-0.4 h^{-1}; k_3 = 0.07 h^{-1}). In studies of the decomposition of marine DOC by high-intensity UV irradiation (Armstrong et al., 1966; Armstrong and Tibbets, 1968; Collins and Williams, 1977), single first order decay rates (k>0.4 h^{-1}) have been given as representative of DOC decay. Given the observations with labelled material, these rate constants may not characterize the reactivity of more refractory DOC components in marine samples.

3.7 Discussion

Phytoplankton DOC produced from axenic, non-axenic and antibiotic laced batch cultures was found to resist complete oxidation by the persulphate techniques. The proportions of PDOC that resisted oxidation ranged from 5 to 18%, and depended on algal species and growth stage. Interestingly, this range is comparable with differences observed between persulphate oxidation procedures and certain high temperature oxidation methods (Sharp, 1973; Gershey et al., 1979; Chen and Wangersky, 1992b). However, these values are substantially smaller than the proportions (50-65%) of DOC in surface seawater found to be oxidised by a recently developed high temperature method after application of persulphate (Sugimura and Suzuki, 1988) or UV oxidation (Druffel et al., 1989) methods.

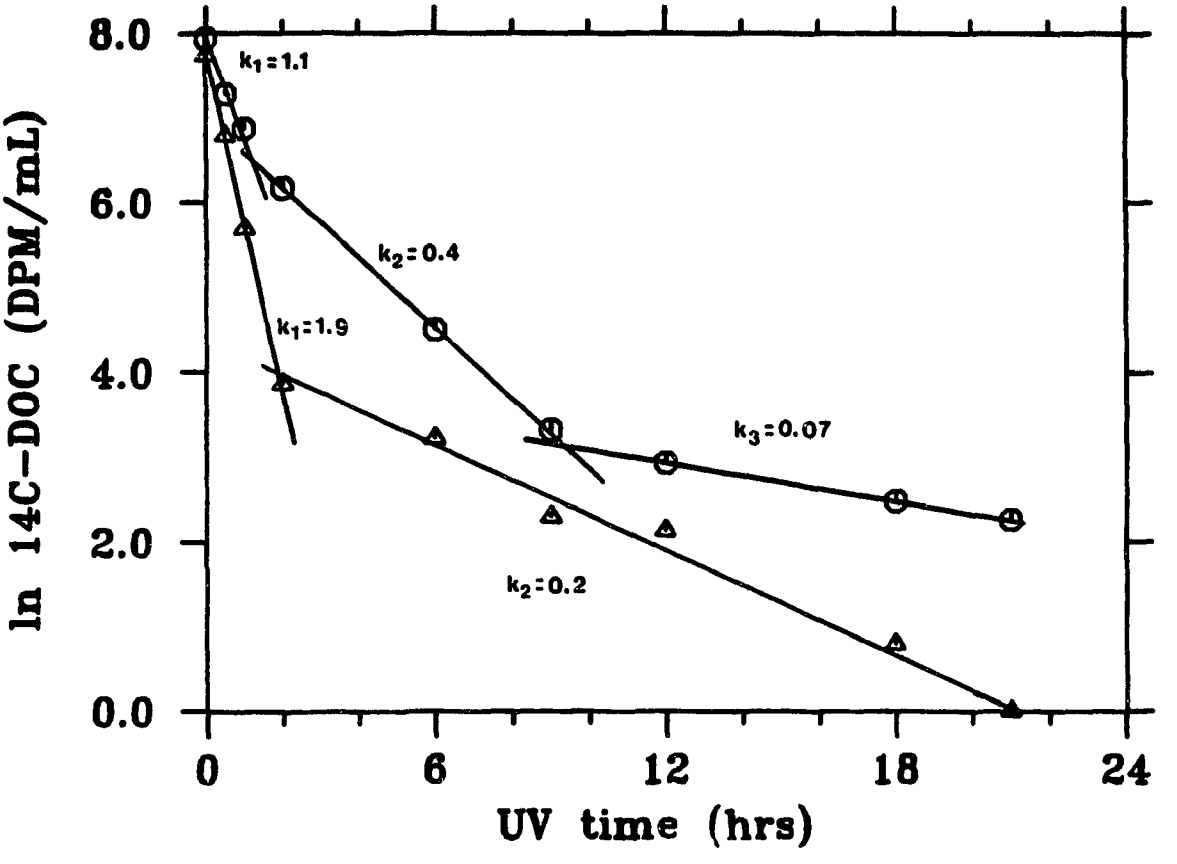


Figure 3.5. Rates of photochemical decomposition of PDOC from stationary phase (o) *Skeletonema costatum* and (Δ) *Chaetoceros gracilis* cultures. Individual rate constants are slopes obtained from linear portions of the data.

I observed that PDOC samples were not completely oxidised by the short-term UV autoanalyser method, but were quantitatively oxidised by extended exposure to UV irradiation. As discussed by several investigators (Collins and Williams, 1977; Gershey et al., 1979; Wangersky, 1992), many factors affect the efficiency of UV oxidation procedures, including the power and age of the UV lamp and concentration of substrate. As well, the intensities of UV wavelengths differ between lamps of different power and the opacity of quartzware to UV light increases with age through a process known as solarization (Hanovia Corp., pers. commun.). While efforts were made to ensure that both systems were operating maximally, some of these factors may have contributed to the lower efficiency of the autoanalyser for algal DOC. The batch UV system may have benefited from greater acid hydrolysis of refractory compounds due to higher sample temperature and longer reaction times than the autoanalyser method.

One problematic issue with PDOC determinations is the possibility of large inputs of dissolved materials from cell rupture during filtration. The possible extent of DOC input due to filtration was examined for *Isochrysis galbana* and *Phaeodactylum tricornutum* samples taken at maximum Chl a concentrations. Approximately 15-ml samples were filtered using only the weight of the plunger portion of the 50-ml glass syringe (*ca.* 0.05 psi) and compared with 50-ml samples filtered by applying liberal hand pressure. High pressures were required to pass this amount of culture through a single filter.[1] Analysis of the samples showed a 16 ± 5% increase of label (2884 *vs.* 2483 dpm) for the hand-filtered *I. galbana* sample compared with the 'gravity' filtered samples, and no appreciable difference between the two methods for *P. tricornutum* samples. Similar proportions of DOC were found after oxidation of the differently filtered samples.

[1]In routine procedures, two 25-ml samples were filtered using 1 psi pressure.

A study of the susceptibility of marine phytoplankton to cell breakage during filtration indicated that DOC inputs from cell lysis was most likely when filters were exposed to air prior to a filter rinse, presumably due to extreme osmotic shock (Goldman and Dennett, 1985). Such conditions were not employed in the present study. In addition, results of the Goldman and Dennett (1985) study indicated that *Isochrysis galbana* and *Phaeodactylum tricornutum* were relatively resistant to cell lysis even with application of the vacuum filtration technique. Therefore, while some cell rupture due to filtration is probably unavoidable in such studies, evidence suggests that the algae studied here were sufficiently robust to prevent very large inputs of DOC due to cell breakage during filtration.

Features common to all batch cultures were production of small levels of PDOC during active growth and production of large amounts of PDOC during stationary/senescent phases. Studies show that PDOC produced during log phase growth by healthy, non-stressed algae, constitute small fractions (<10%) of the total photosynthetically fixed carbon, while that produced from stressed algae constitutes larger amounts (10-40%) of production (Hellebust, 1965; Storch and Saunders, 1977; Sharp, 1977; Mague, 1980; Sellner, 1981; Sondergaard and Schierup, 1982; Jones and Canon, 1986 and references therein). My batch culture results are in line with published results, as I calculate that *P. tricornutum* and *Synechococcus* in log growth phase were found to release respectively 1-4% and 8-9% of ^{14}C uptake. Early stationary phase *P. tricornutum* released 29-34% and *Synechococcus* released 12-20% of ^{14}C uptake. As noted previously, the vast majority of PDOC was produced in the cultures following active growth phases. Therefore, as Sharp (1984) points out, contribution to oceanic DOC pools from dead or dying algae is probably much more important than amounts exuded during active growth.

Actively-growing algae may produce proportionately more resistant DOC than that produced during early stationary growth phases. Actively-growing algae are thought to release low molecular weight metabolites with higher proportions of free amino acids than

nutritionally stressed algae, which have been shown to release high amounts of carbohydrates in laboratory cultures (Guillard and Wangersky, 1958; Myklestad, 1974; Haug and Myklestad, 1976; Brokmann et al., 1979) and in coastal waters (Ittekkot et al., 1981; Ittekkot, 1982; Lancelot and Mathot, 1987). Greater resistance of PDOC from algae in log phase growth than in stationary phase may be related to the production of higher proportions of carbohydrates during stationary phase, since many carbohydrates (including polysaccharides) are known to be quantitatively oxidised by persulphate and UV methods (Williams, 1969; Gershey et al., 1979; Chen and Wangersky, 1992b). Some sulphur and nitrogen-containing organic compounds, however, are known to be resistant to chemical oxidation methods (Gershey et al., 1979; Chen and Wangersky, 1992b).

Size fractionation results for two different phytoplankton, *Phaeodactylum tricornutum* and *Synechococcus DC2*, identified the majority of PDOC as <10,000 NMW, in agreement with many studies of phytoplankton DOC (Wiebe and Smith, 1977; Mague et al., 1980; Iturriaga and Zsolnay, 1981; Sondergaard and Schierup, 1982; Jensen, 1983; Lignell, 1990). However, up to 50% of PDOC was composed of >10,000 NMW material in other studies (Chrost and Faust, 1983; Lancelot, 1984), indicating that molecular weight distribution of phytoplankton DOC may be affected by a number of factors. These factors include algal species, light intensity, physiological state (Sondergaard and Schierup, 1982; Wolter, 1982; Lancelot, 1984; Jones and Canon, 1986). In this study, maximum values of colloidal PDOC accumulated in the later phases of the batch cultures. These observations may have been due to increased inputs of macromolecules from algal cell lysis (Billen, 1983; Bronk and Gilbert, 1991), as well as to bacterially-mediated transformations of low molecular weight PDOC into higher molecular weight products (Iturriaga and Zsolnay, 1981, 1983; Jensen, 1983; Chrost and Faust, 1983; Lancelot, 1984).

Low molecular weight PDOC from *P. tricornutum* and *Synechococcus* showed similar reactivities to persulphate oxidation ($PDOC_{res}$ values ranged from 10.5-16.8%). However,

quite different colloidal material apparently was produced by these two species, based on the differences in reactivities. Colloidal DOC from *P. tricornutum* ranged from 6-12% refractory to persulphate, whereas 15-22% of colloidal DOC from *Synechococcus* resisted oxidation. It is apparent that physico-chemical properties other than molecular weight influence resistance to wet oxidation. The factors affecting the lability of organic compounds to oxidation have been recently discussed by Lee and Henrichs (1992). These properties include degree of N and S incorporation, amount of branching and cross-linkage, and degree of halogenation.

High molecular weight DOC produced by *P. tricornutum* would include extracellular carbohydrates and uronic acids (Ford and Percival, 1965). XAD resin extractable DOM produced in *P. tricornutum* cultures has been found to show similar NMR spectra as marine humics (Wilson et al., 1983). Interestingly, marine humics isolated by XAD resin have been found to be oxidised quantitatively by wet oxidation (Druffel et al., 1989), which is consistent with the relatively low levels of colloidal PDOC from *P. tricornutum* that resist persulphate oxidation.

Some of the refractory, very high molecular weight (>100,000 NMW) material detected in the latter stage of the *Synechococcus* culture may be derived from cell wall material sloughing off unhealthy algae. The outer sheath of cyanobacteria cell walls are gelatinous, hydrophilic colloids (Humm and Wicks, 1985), which are composed of highly cross-linked, lipopolysaccharidic polymers similar in molecular nature to the structures proposed for DOC resistant to wet oxidation (Williams and Druffel, 1988; Lee and Henrichs, 1992). While this colloidal material did show a high resistance to persulphate oxidation (24-28%), it made up a small fraction of the total PDOC (2-7%). However, since colloidal DOC may comprise a significant fraction of total marine DOC (Ogura, 1977; Suzuki and Sugimura, 1988), inputs to DOC by grazing may increase the proportion of such DOC in seawater (Caron *et. al.*, 1985; Taylor et al., 1985; Caron et al. 1991).

3.8 Summary and Conclusions

(1) Phytoplankton produce DOC that is resistant to persulphate oxidation, although the proportions are low (5-18%) compared with those values obtained using a high temperature combustion method on seawater samples (Suzuki and Sugimura 1988, 1992). The proportions of DOC resistant to persulphate that have been determined in this study are in good agreement with traditionally accepted differences between values obtained from persulphate and high temperature oxidation of marine DOC samples (Sharp, 1973; Gershey et al., 1979).

(2) Ultrafiltration studies revealed that colloidal PDOC (>10,000 NMW) showed variable reactivity depending on phytoplankton source. Colloidal PDOC was not necessarily more resistant to persulphate oxidation than low molecular weight PDOC, suggesting that physico-chemical factors other than molecular size are important in determining resistance to persulphate oxidation.

(3) Significant proportions of algal DOC are resistant (10-30%) to the UV flux in an autoanalyser method; however, long term exposure (6 hrs) to UV oxidation decomposes >95% of the PDOC for samples analysed.

There is ample evidence from these studies to suggest that analysis of low molecular weight, phytoplankton DOC may be underestimated significantly by wet oxidation procedures. This is noteworthy because many discussions of 'extra' DOC analysed by high temperature methods have assumed that it represents polymeric, high molecular weight DOC (Toggweiler, 1990; Kirchman et al., 1991). These assumptions may be misleading without direct characterization of the 'extra' DOC analysed by HTCO methods in each case.

Chapter 4

Effects of Ageing Processes on the Resistance of Phytoplankton DOC to Oxidation

4.1 Introduction

In the previous Chapter, studies of dissolved organic carbon produced in actively-growing and senescent phytoplankton cultures indicated that typically <20% of this material was resistant to persulphate oxidation. These values are much lower than the roughly 50% of surface seawater reported to resist this oxidative procedure (Suzuki et al., 1992). As discussed in Chapter 3, phytoplankton extracellular organic carbon is composed chiefly of low molecular weight metabolites, carbohydrates and proteins (Wangersky, 1978; Gagosian and Lee, 1981). However, marine DOC is very poorly characterized. Less than 20% of UV oxidizable surface water DOC has been identified as combined amino acids and carbohydrates, lipids or trace organic compounds (Williams and Druffel, 1988); although the relative proportions of such compounds may increase dramatically during bloom events (Ittekkot et al., 1982; Lancelot and Mathot, 1987). Significant fractions of marine DOC (*ca.* 20-50%) can be extracted by XAD resin; this macromolecular material has been found to be high in aliphatic carbon, but is otherwise not well-characterised (Harvey, 1983). As Hedges (1987) points out, "...lack of structural information makes it extremely difficult to identify biochemical precursors or to judge the probability of different chemical and physical transformations".

If the ultimate sources of marine DOC are relatively simple products of phytoplankton metabolism, what transformations to these precursors give bulk seawater DOC its complex nature and, if the HTCO data are correct, its resistance to wet chemical oxidation methods? Many chemical oceanographers have suggested that microbial and/or photochemical alteration of phytoplankton carbon are included in the processes which produce bulk seawater DOC

(Williams and Druffel, 1988; Toggweiler, 1989; Mopper and Zhou, 1990; Lee and Henrichs, 1992).

The advent of the microbial loop concept (Azam et al., 1983) has focused much attention on how bacteria affect DOC. Heterotrophic uptake and transformation of phytoplankton extracellular products by marine bacteria has been well-documented (e.g. Iturriaga and Zsolnay, 1981, 1983; Jensen, 1984; Lancelot, 1984). Such studies have found that 10-90% of phytoplankton DOC is taken up by bacteria in short-term (hours to days) experiments, with a literature mean of 45% (Baines and Pace, 1991). However, investigations into the uptake of marine DOC (Ogura, 1972, 1975) and phytoplankton DOC (Iturriaga and Hoppe, 1977; Chrost and Faust, 1983; Lancelot, 1984; Pett, 1989) have shown evidence for differences in the quality of DOC based on observed changes in rates of heterotrophic uptake over time. For example, about 60% of *Skeletonema costatum* DOC was consumed by bacteria in less than 32 hours at 10°C, but only an additional 10% was removed after 20 days (Pett, 1989). This study suggests that, although the bulk of the DOC produced by phytoplankton in the ocean is eventually consumed, a small percentage is refractory to consumers and may accumulate over time producing a significant fraction of bulk seawater DOC.

Bacteria are also producers of extracellular compounds in seawater (Rosso and Azam, 1987; Decho, 1990). Toggweiler (1989) and Lee and Henrichs (1992) postulate that alteration of PDOC via condensation reactions, particularly through reactions with nitrogenous bacterial enzymes, could produce colloidal DOC resistant to wet oxidation. As alluded to in Chapter 3, it has been postulated that highly cross-linked, polymeric material is resistant to wet oxidation procedures if reaction is limited to outer layers, leaving the inner core resistant to complete oxidation (Williams and Druffel, 1988). The oxidative radicals produced by the decomposition of peroxydisulphate react quickly with chloride ions producing free chlorine. Since chloride is in excess, it is conceivable that the majority of DOC does not undergo oxidation by the more oxidative persulphate generated radicals, but rather undergoes oxidative reaction with

other free radical moieties and free chlorine. Recent research into the free radical chemistry of persulphate in seawater supports these views (P.J. Wangersky, pers.commun.).

Seawater DOC also may be significantly transformed by abiotic processes. It has been thought that chemical reactions occur to produce polycondensates, or 'humic substances' (Gagosian and Lee, 1981; Skopintsev, 1981). Polyphenols and polyunsaturated lipids (Harvey et al., 1983) have been suggested as important precursors to marine humics. Association of relatively simple molecules with humic substances may increase their resistance to chemical and microbial oxidation. For example, Armador et al. (1989) found that high molecular weight humic acid-^{14}C glycine complexes were 50% more refractory to bacterial degradation than low molecular weight complexes. Carlson et al. (1985) and Brophy and Carlson (1989) have shown that labelled glucose monomers when incubated for periods of 3-6 months are incorporated into larger molecules. It was found that such condensates can be formed abiotically (Carlson et al., 1985); however, the 1989 study indicated that carbon transformation by biological processes was of greater importance.

Photochemical transformations of phytoplankton DOC and/or microbially-altered DOC have been suggested as potentially important routes by which oxidation resistant DOC may be produced (Williams and Druffel, 1988; Lee and Henrichs, 1992). Mopper and Zhou (1990) report that surface seawater DOC is less reactive to hydroxyl radicals than DOC from deep waters. They suggest that surface seawater DOC has been photochemically 'bleached', making it less reactive to hydroxyl radicals. It is notable that both UV and persulphate techniques are thought to oxidise DOM by free radical reactions (Lee and Henrichs, 1992).

DOC resistant to wet oxidation methods may come from other sources, such as from the egestates of protozoa and zooplankton (Lampert, 1978; Taylor et al., 1985; Jumars et al., 1989; Koike, 1990). The guts of zooplankters contain specialized enzymes and microflora; zooplankton egestates may provide inputs of DOC with unique physico-chemical properties. For example, proteins and polysaccharides were preferentially digested by *Calanus* copepods,

compared with cell wall material (Lee and Henrichs, 1990). Cell wall material of Gram-negative bacteria has been found to be indigestible to marine bacterivores (D. Bird, pers. commun.).

In Chapter 1, I noted that DOC characterised by traditional methods and by the HTCO method of 'S&S' differed markedly with respect to the terms 'labile' and 'refractory' DOM. I suggested that in the traditional view, the most 'chemically' and 'biologically' refractory DOC is found in deep ocean water, and is formed through 'ageing' processes (Bada and Lee, 1977). However, 'S&S' DOC results indicate that greatest concentrations of DOC refractory to wet oxidation methods is found in surface and subsurface seawater, and that DOC becomes less resistant to wet oxidation as it is aged by microbial processes (Sugimura and Suzuki, 1988; Druffel et al., 1989). Research described in this Chapter is directed toward a better understanding of the chemical reactivity of DOC as it is aged by biotic and abiotic processes.

4.2 Methods

4.2.1 Long-term ageing experiments

Unialgal *Isochrysis galbana* (Bigelow Culture Laboratory), *Skeletonema costatum*, *Chaetoceros gracilis*, and *DC2 Synechococcus* (Bigelow Culture Laboratory) cultures were grown from very small inoculates in culture media supplemented with approximately 1 mCi L^{-1} of ^{14}C bicarbonate. The cultures were grown for periods of about two weeks, by which time all cultures had reached stationary growth phases. The culture material was vacuum filtered (≤200 torr) through 0.4-μm Nuclepore filters, and the phytoplankton DOC solutions (0.5-5 L) were inoculated with 15-50 ml of NW Arm seawater taken directly from the Dalhousie Aquatron supply line. Initial samples were taken immediately and measured for their resistances to persu'phate or UV oxidation procedures. Starting concentrations of phytoplankton DOC ranged from 300-400 μM C from calculations based on the initial specific activities of the culture media.

The solutions were allowed to age in the dark at room temperature, and filtered samples were analysed after intervals of weeks and months. To prevent anoxia, solutions were bubbled very slowly with laboratory air, which passed first through a Super-Q bath. Graduations on the containers were used to record the levels of the solutions, and before sampling solutions were brought back to the mark with additions of small amounts of Super-Q. Additional inoculates of unfiltered coastal seawater were added at 1 month intervals. Since the unialgal cultures were not bacteria-free, some bacterial alteration of the phytoplankton DOC will have occurred prior to the initialization of these experiments. To investigate possible changes of reactivity due to abiotic chemical transformations, replicates containing sodium azide (0.2% wt/vol) were similarly aged in the dark at room temperature.

4.2.2 Short-term ageing experiment

An ageing experiment was done using DOC produced from the axenic *Synechococcus DC2* culture described in Chapter 3. The 0.45-μm filtered material was mixed 1:1 with coastal seawater, which had previously been filtered through 1.2-μm silver membrane filters to minimize rapid bacterial consumption by protist grazers. Though undetected, smaller heterotrophic nanoflagellates may nevertheless have been present (Cynar et al., 1985). In this experiment, the 1 L of seawater solution, initially containing approximately 60 μM C of phytoplankton DOC, was shaken at 30 RPM in the dark at room temperature for 20 days. Samples were taken at 12 or 24-hour intervals, filtered and analysed immediately to characterize short term changes of the $DO^{14}C$ as it was altered by microbial processes. To determine abiotic effects, a control solution was similarly prepared with 0.2-μm filtered seawater.

Size fractionation. Changes to $DO^{14}C$ size fractions during this study were determined by the ultrafiltration procedures described previously, with the following modifications to

improve precision for samples containing low amounts of colloidal material. Analytical precision was improved by increasing the ratio of retentate to ultrafiltrate by using a concentration factor of about 25 rather than 2. This was done by concentrating a 50-mL sample to 10 ml. A 50-ml aliquot of dialysed seawater was then added to the cell and the retentate again concentrated to approximately 10 ml. This amount was then diluted to 30 mL with dialysed seawater and analysed. Samples were counted for 100,000 disintegrations representing a 2 standard deviation counting error of 0.5%.

4.2.3 Ageing of unfiltered culture material

An experiment was also carried out with unfiltered *Isochrysis galbana* and *Phaeodactylum tricornutum* culture material to examine whether bacterial ageing of algal cells and associated debris would result in the production of DO^{14}C resistant to persulphate oxidation. Unfiltered material from senescent cultures was mixed 1:2 with 100-μm filtered NW Arm seawater. The flasks initially contained approximately 24 μM C POC and 13 μM C DOC of *P. tricornutum* culture material, and 92 μM C POC and 18 μMC DOC from an *I. galbana* culture. The flasks were stoppered with cotton batten, and stirred at 30 RPM on a rotary table in the dark at room temperature. Samples were extracted after 0, 1, 3, 7, and 28 days ageing. PO^{14}C values were estimated from the differences in ^{14}C activity between unfiltered and filtered samples. Filtered samples were analysed by the persulphate method. Samples for measurements of bacterial numbers were taken in 5 ml aliquots and sodium borate buffered formalin was added to the samples to a final concentration of 2% vol/vol.

4.2.4 Photochemical ageing study

Samples from *Synechoccoccus* and *Phaeodactylum tricornutum* cultures, which had been aged for many weeks in the presence of bacteria, were filtered to remove bacteria (0.2-

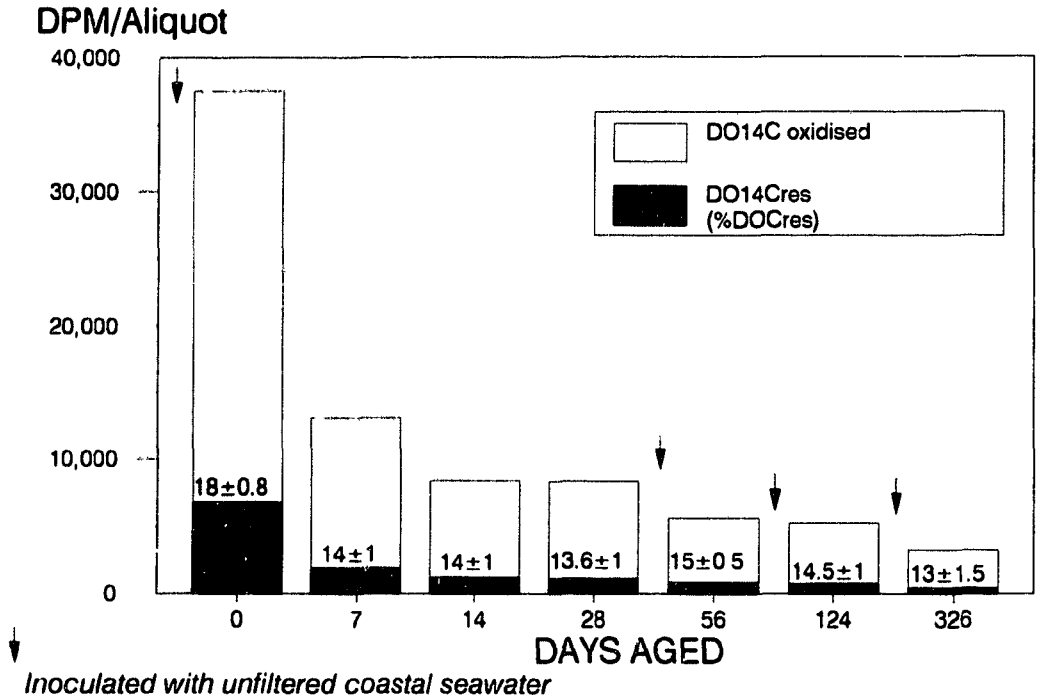
μm Nuclepore) and added to quartz tubes. The tops were covered, and the samples placed in sunlight during July. Replicates were covered with opaque paper. Duplicate samples were removed and analysed after 16, 48 and approximately 250 hours exposure to natural light.

4.3 Results

4.3.1 Long-term studies

Figure 4.1 shows the data for the ageing of *Synechococcus* DOC, plus the results obtained for a sodium azide control over a period of about 1 year. The labelled DOC decreased by 80% in the first two weeks of the experiment, while little change was observed in the control solution. These observations indicate that bacteria readily consumed the *Synechococcus* DOC. Further decreases of DOC were small after 2 weeks ageing, despite additional inoculations, suggesting that the remaining $DO^{14}C$ was refractory to further bacterial attack. The proportions of $DO^{14}C$ resisting oxidation decreased from an initial value of18 ±0.8% to an average value of 14 ±0.7% for all other solutions. Dilution of the control solutions with 3% NaCl Super-Q to the same activities as the aged samples indicated that more efficient oxidation of lower DOC concentrations was not the reason for the decreased values.[1] Samples from the control flask did not show any changes. Bacterial activity therefore appears to have preferentially removed, or altered, compounds resistant to persulphate oxidation within the first week of ageing. After this time, oxidation resistant compounds appear to have been consumed at similar rates as the rest of the DOC, since no significant changes were observed to the proportions of DOC resisting oxidation.

[1]See also Chapter 3, Section 3.5.1



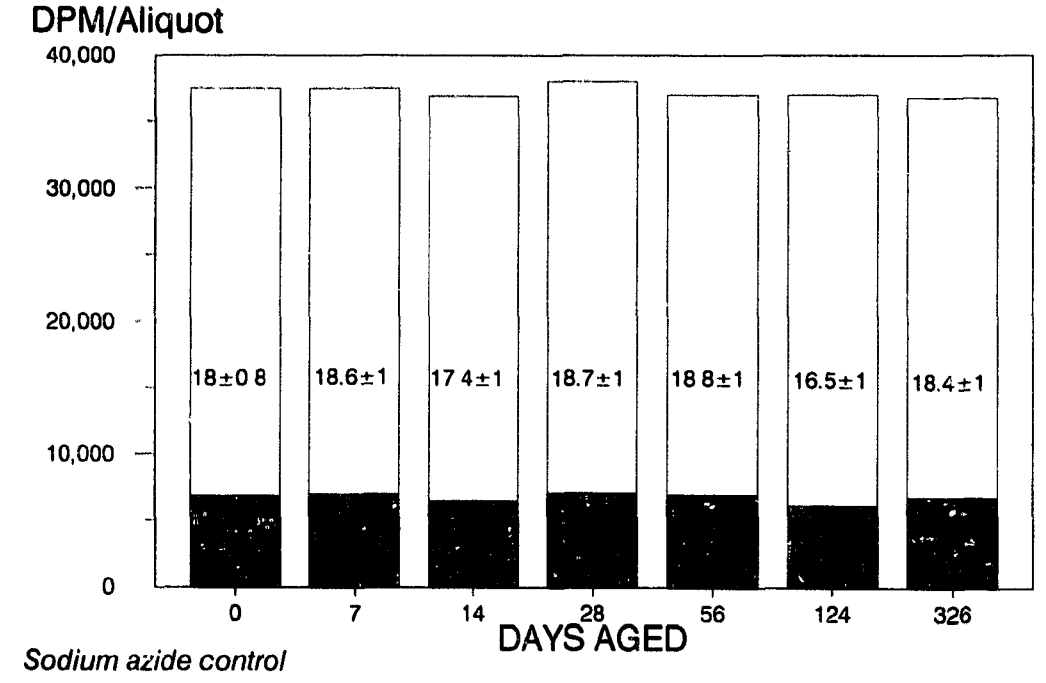


Figure 4.1 Results showing the long-term ageing of DO^{14}C taken from unialgal *Synechococcus* cultures. Culture was inoculated initially with 15 ml of unfiltered seawater. Additional 5-mL inoculations are also indicated. Also shown are results for a sodium azide control solution that was not inoculated with seawater.

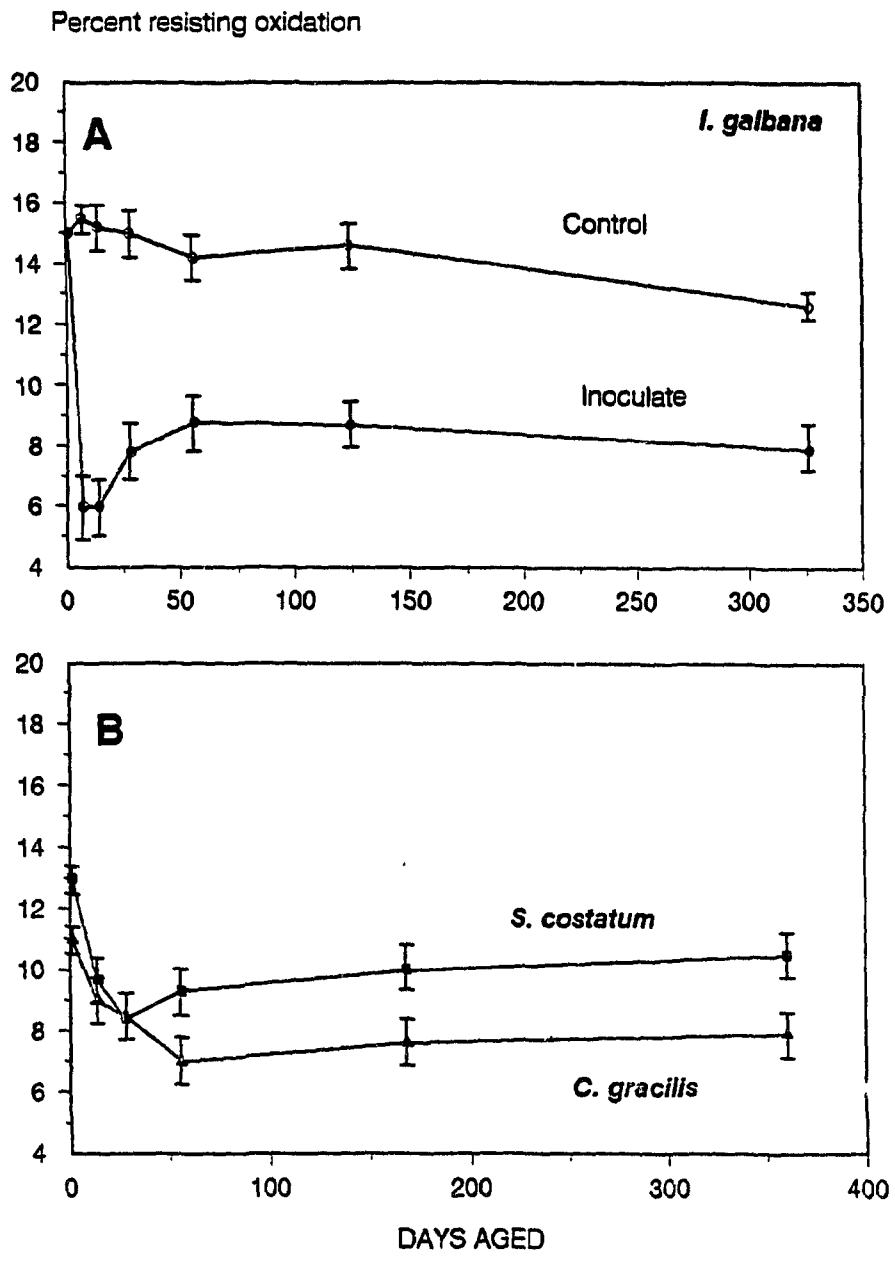


Figure 4.2. Summary of results from ageing of (A) *Isochrysis galbana*, and (B) *Chaetoceros gracilis* and *Skeletonema costatum* DOC solutions by additions of unfiltered seawater. Results of *I. galbana* sodium azide control are also given.

As shown in Figure 4.2, the same trends were observed for similarly aged *Isochrysis galbana*, *Chaetoceros gracilis* and *Skeletonema costatum* DOC solutions. After approximately 1 year of ageing, $\%DO^{14}C_{res}$ values were all somewhat lower than the initial values, and ranged from 5-12%. Sodium azide controls did not show any changes with respect to reactivity with time, indicating that dark, abiotic reactions (Carlson et al., 1985; Brophy and Carlson, 1988; Armadar et al., 1989) do not affect appreciably the reactivity of these solutions to persulphate oxidation over the time scales sampled. The relatively high temperature and concentrations of DOC (22°C and *ca.* 400 μM C) in comparison with natural conditions should have tended to enhance reaction rates.

4.3.2 Short-term studies

As the majority of changes to the phytoplankton DOC solutions occurred within the initial few weeks of the ageing studies, a more detailed examination of the short-term ageing of DOC from an axenic *Synechococcus* culture was undertaken. DOC produced from axenic culture was chosen as the substrate for this study, since no alteration of the phytoplankton DOC had occurred by heterotrophic bacteria prior to the commencement of the experiment. As well, a closer examination of the reactivity of DOC from cyanobacteria was merited, since these species are significant fractions of oceanic biomass, *ca.* 20% in Sargasso Sea waters (Li et al., 1991). *Synechococcus sp.* dominated phytoplankton biomass at several stations in the North Atlantic during the 1989 spring bloom (G. Harrison, pers. commun.).

The results of the experiment are given in Figure 4.3. After a short lag phase, a decrease of $DO^{14}C$ was observed in concert with an increase in numbers of small ($\leq$0.5 μm diameter), coccoid bacteria. As shown in Figure 4.3(b), $DO14C_{res}$ values also decreased over the course of the experiment. However, during the stage of rapid bacterial growth, $DO^{14}C_{res}$ material diminished at a slower rate than the non-resistant fraction, resulting in higher

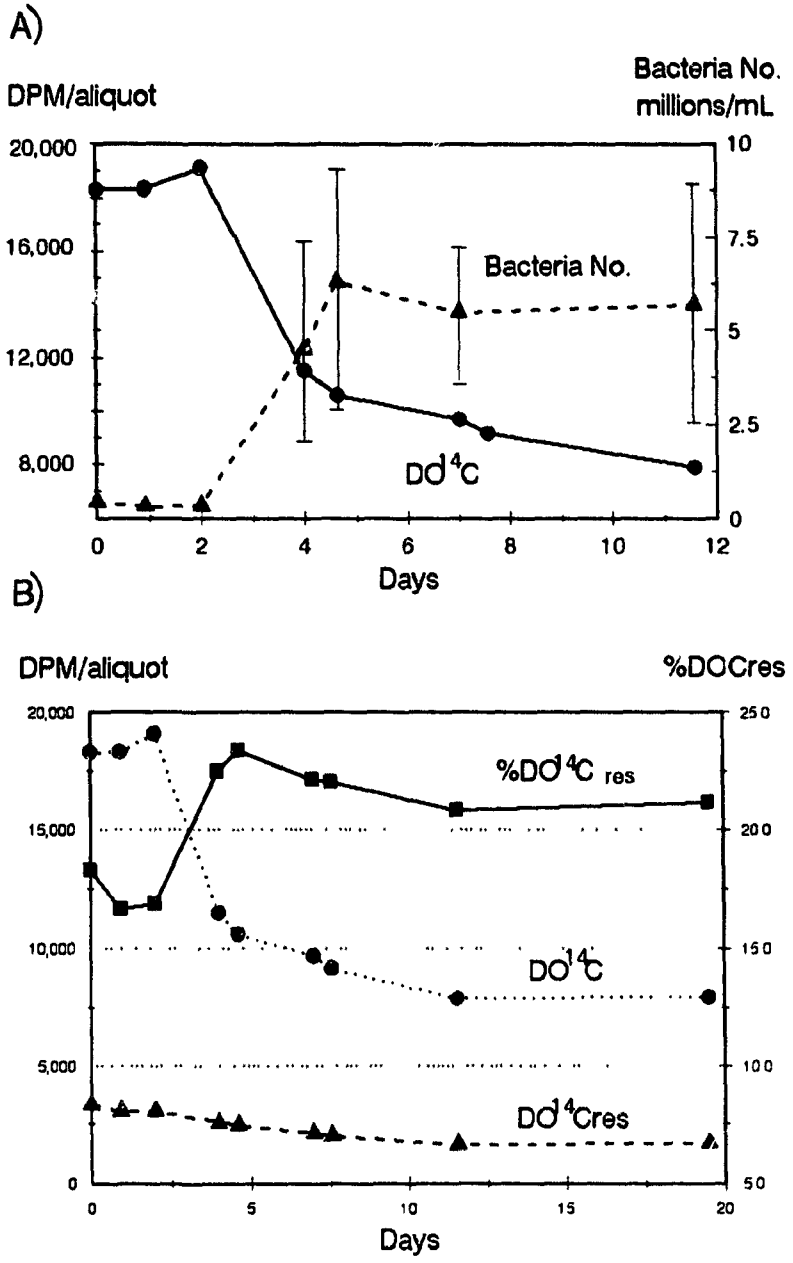


Figure 4.3. Short-term study of ageing of *Synechococcus* DOC taken from an axenic culture. Solution was inoculated with 1.2-μm filtered coastal seawater. In Figure 4.3 (A), bacterial numbers have error of approximately ±25% which is shown by error bars for high values.

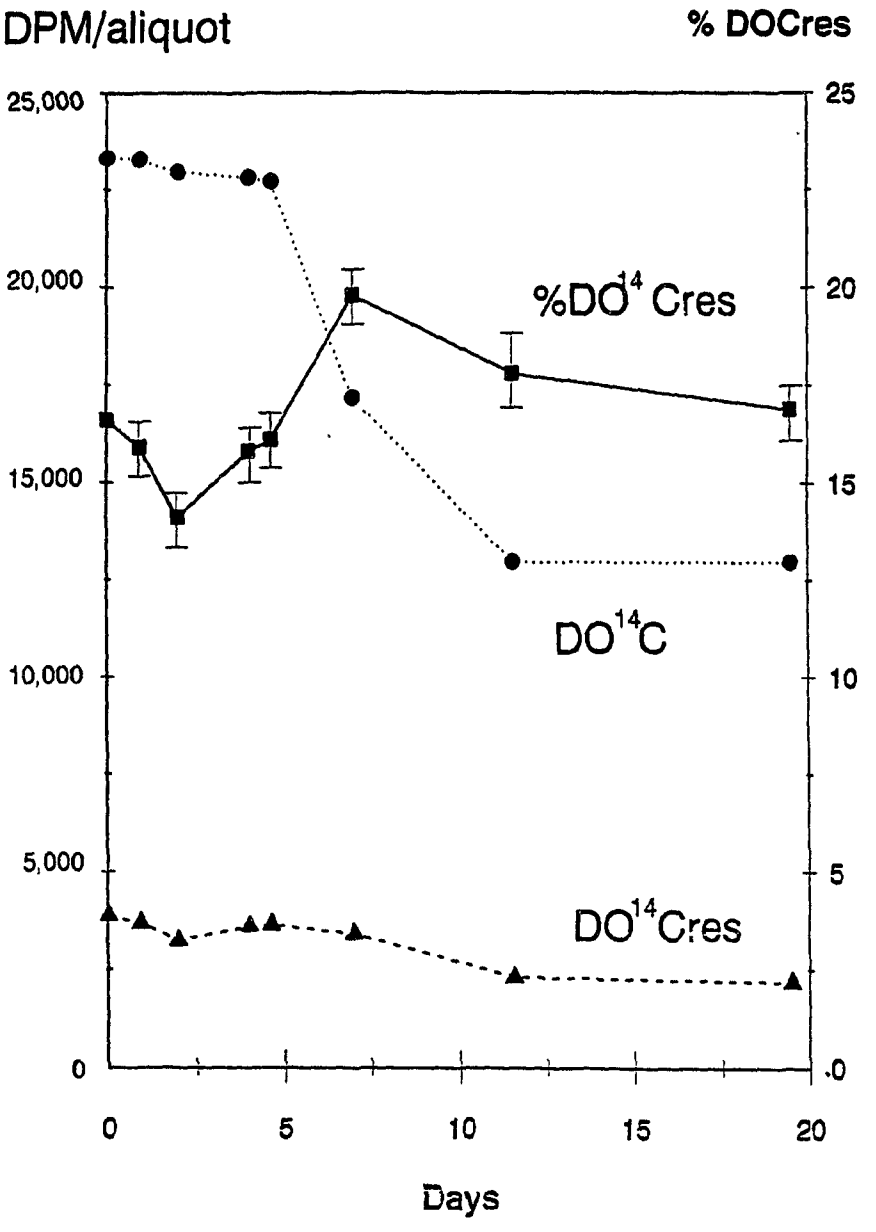


Figure 4.4. Results for *Synechococcus* "control" solution which became contaminated with bacteria, as is evidenced by the decrease in $DO^{14}C$ after day 6.

$\%DO^{14}C_{res}$ values. From day 2 to 4.6, 41% of the labelled DOC had been consumed. By day 11.6, a further 26% of the labelled DOC was consumed. During this later period, the $DO^{14}C_{res}$ fraction decreased at a rate similar to the rest of the labelled DOC, as $\%DO^{14}C_{res}$ values remained constant or decreased slightly. Somewhat similar trends were observed for the 'control' solution, shown in Figure 4.4, which became contaminated with large (*ca.* 1 x 0.4 μm), rod-like bacteria that bloomed after Day 6.

Ultrafiltration results. Ultrafiltration data given in Table 4.1 for the *Synechococcus* DOC solutions indicates that low molecular weight PDOC fuelled the majority of bacterial growth, as bacteria respired 56% of the low molecular weight fraction in the inoculated solution, and 53% in the 'control' solution. The low molecular weight fraction in the inoculated solution showed a trend toward increased resistance to persulphate oxidation as the material aged, though no discernable trend was observed for the 'control'(i.e. the solution not inoculated with microbes). Interestingly, the amount of colloidal material decreased in the inoculated solution, but increased substantially by Day 20 in the 'control' solution. It is suggested that the different microbial populations found in the two otherwise identical solutions may be responsible for the different size fractions observed.

4.3.3 Ageing of unfiltered culture material

The results from the ageing of unfiltered *I. galbana* and *P. tricornutum* culture material are given in Figure 4.5. While labelled $PO^{14}C$ pools decreased throughout the course of the experiment, short time scale increases and decreases of the labelled DOC and DOCres pools were observed over the first week of the experiment. These changes correlated with changes in the microbial communities, particularly for results from the *P. tricornutum* ageing solution. Increases of labelled DOC on Day 3 for both ageing solutions coincided with

Table 4.1. Data from ultrafiltration of PDOC from axenic *Synechococcus* cultures following inoculation with 1.2-μm filtered coastal seawater and control solution diluted with 0.2-μm filtered coastal seawater. UF: activity in the <10,000 nominal MW fraction; UF_{res}: activity in UF fraction remaining after persulphate oxidation; Ret: activity in the >10,000 nominal MW fraction; Ret_{res}: activity remaining in the Ret fraction after oxidation; %UF refers to the percentage of low molecular weight material in total sample. Losses to ultrafilter calculated by mass balance.

Inoculated solution

Day	UF	UF_{res}	Ret	Ret_{res}	%UF	Losses
	dpm ml^{-1}	(%)	dpm ml^{-1}	(%)		(%)
0.0	3524	16.8±0.5	99.6	17±5	96	1
4.6	2025	18.6±1.2	76.6	19±4	95	1
11.6	1490	18.2±0.3	81.4	15±2	81	14
19.5	1541	19.7±0.5	45.8	15±3	97	0.3

'Control' solution

Day	UF	UF_{res}	Ret	Ret_{res}	%UF	Losses
	dpm ml^{-1}	(%)	dpm ml^{-1}	(%)		(%)
4.6	4374	17.9±2.2	123.6	11.5±5	97	1
11.6	2083	17.1±1.3	158.4	16.2±1.5	80	14
19.5	2072	15.7±0.5	510.6	22.2±1.8	97	(-2)

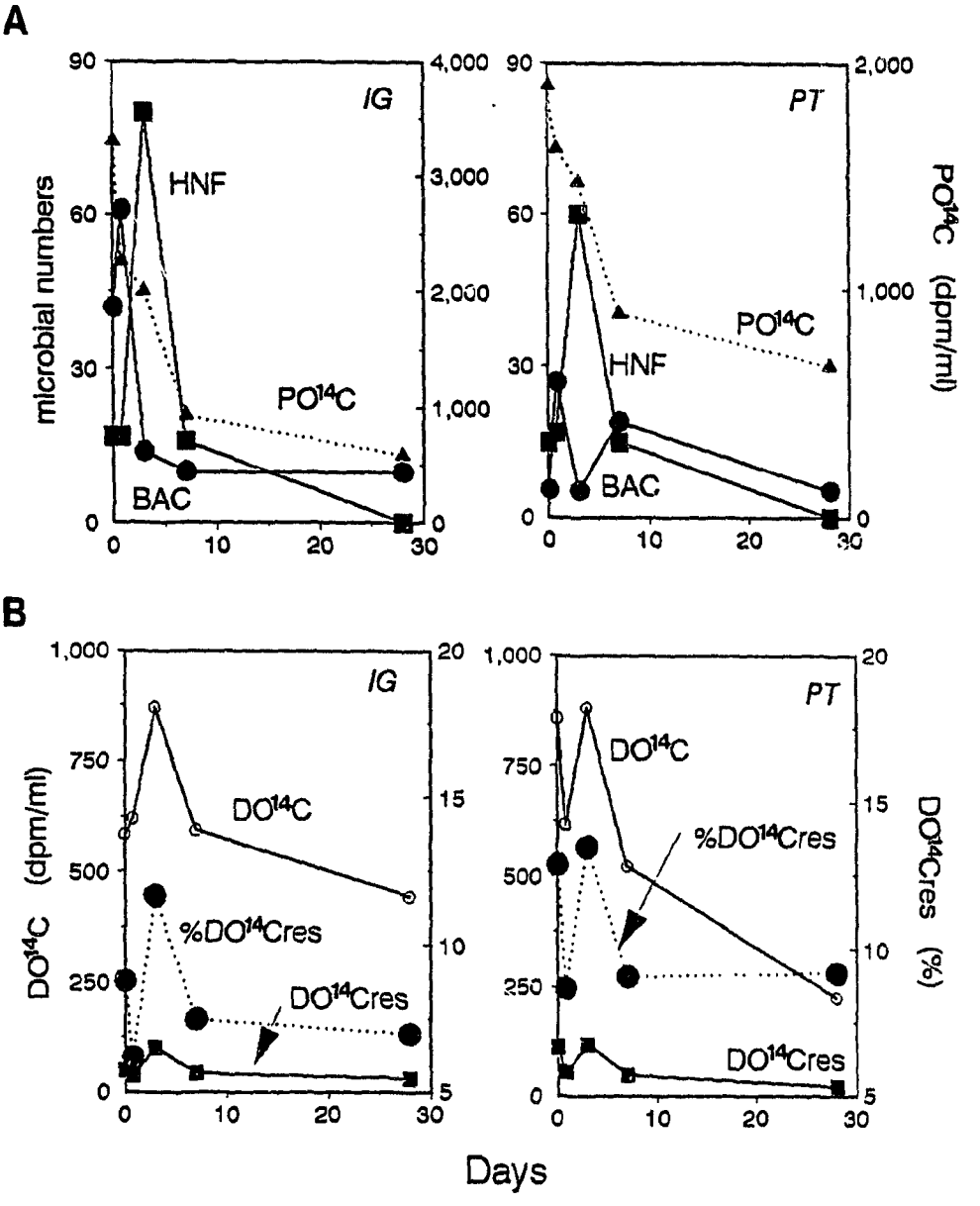


Figure 4.5. Results of the ageing experiment in which unfiltered *Isochrysis galbana* (IG) and *Phaeodactylum tricornutum* (PT) culture material was inoculated with unfiltered coastal seawater. In (A), graphs show changes to $PO^{14}C$, numbers of bacteria (BAC) and heterotrophic nanoflagellates (HNF) per ml. In (B), graphs show changes to ^{14}C-labelled DOC, $DO^{14}C$ recovered after persulphate oxidation, and the percentage of $DO^{14}C_{res}$. See text for discussion.

maximum observed concentrations of heterotrophic nanoflagellates, identified by their size (2-5 μm), distinct morphologies (monad-type, 5-15 μm long flagella) and the correlated rapid decrease of bacteria numbers. Nanoflagellates were also feeding on algal cell debris and intact algal cells, since heterotrophic flagellates have shown the ability to efficiently graze algae of similar size (Caron et al. 1985). Larger grazers may have also been present, although none were detected under microscope. I propose that increases of labelled DOC mainly reflect inputs from heterotrophic nanoflagellates, as well as other grazers that nmay have been present.

Solubilization of phytoplankton cells by bacteria does not appear to account for increases of $DO^{14}C$. Despite maximum numbers of particle-attached bacteria on Day 1 in both cultures (data not shown), $DO^{14}C$ declined or increased only marginally over the first 24 hours. This is consistent with tight coupling between solubilization and consumption of POC, as noted elsewhere (Schwinghamer and Kepkay, 1987; Pett, 1989). As well, the period of major $DO^{14}C$ increases occurred when bacteria numbers were low, due to consumption by bacterivores.

Net increases of 263 and 248 dpm/mL were observed for the respective *P. tricornutum* and *I. galbana* cultures between Days 1 and 3. $DO^{14}C_{res}$ values also increased by 64 dpm/ml. in both cultures. These observations suggest that approximately 25% of the labelled DOC produced in the two cultures was resistant to persulphate oxidation. However, by Day 7 most of this resistant DOC had been removed from solution. A possible removal process may have been through bacterial consumption, but if the DOC produced was in the colloidal size range, physico-chemical loss processes such as coagulation and surface sorption were probably more important in these stirred experimental microcosms (Johnson and Kepkay, 1992). By Day 28, $DO^{14}C$ from both solutions was less resistant than the starting material, consistent with the overall trends observed in the long-term ageing experiments, in which unfiltered seawater was also added.

4.3.4 Photochemical ageing

The results of the photochemical ageing studies are shown in Table 4.2. No significant differences were found between light and dark solutions, although trends toward higher $DO^{14}Cres$ values were found for all solutions after 48 hours. High outdoor temperatures (*ca.* 32°C) may have enhanced molecular reactions to increase the resistance of $DO^{14}C$ for both dark and light solutions, although the long-term ageing results of Section 4.2.1 suggest that such reactions are not important at room temperature. Ageing for an additional 200 hours did not produce significant differences between the light and dark *Synechococcus* DOC solutions. Some bacteria are likely to have grown in the *Phaeodactylum tricornutum* solutions, as the label decreased nearly 50% for both light and dark tubes. Nevertheless, these results are interesting, since Williams and Druffel (1988) have suggested that photochemical alteration of proteinaceous bacterial material could produce resistant DOC. However, any incorporation of label into resistant material (proteins or other compounds) formed by interaction with sunlight appears to have been small, as very little difference was again found between light and dark solutions.

4.4 Discussion

4.4.1 Ageing of filtered culture material

Ageing of phytoplankton DOC solutions produced variable results. Long-term bacterial alteration of phytoplankton DOC did not increase its resistance to oxidation. As shown in Figure 4.1, bacterial alteration of the initial $DO^{14}C$ resulted in a decrease in the total amount of resistant material, and as well, a decrease in the proportion of material resisting oxidation. All solutions aged in the presence of bacteria had lower $\%DO^{14}Cres$ values than those measured initially. Thus, although the $DO^{14}C$ remaining at the end of the experiment was refractory to further bacterial breakdown, it was slightly more labile to persulphate

Table 4.2. Changes to reactivity of microbially-aged DOC from *Synechococcus* and *Phaeodactylum tricornutum* cultures upon exposure to natural light in quartz vessels.

	Synechococcus solution			*Phaeodactylum tricornutum solution*		
Exposure hours	$DO^{14}C$ dpm ml^{-1}	$DO^{14}Cres$ dpm ml^{-1}	$DO^{14}C_{res}$ (%)	$DO^{14}C$ dpm ml^{-1}	$DO^{14}C_{res}$ dpm ml^{-1}	$DO^{14}C_{res}$ (%)
0	1498	317.8	21.2±1.8	16040	1595	9.9±0.1
16	1469	354.6	24.1±0.9	15811	1832	11.6±0.4
48	1426	338.6	23.7±0.9	15554	2182	14.0±0.5
Dark	1497	357.0	23.8±1.0	15267	2406	15.8±1.2
250	1409	314.0	22.3±0.1	8562	844.8	9.9±0.6
Dark	1439	314.6	21.7±0.4	8382	764.2	9.2±0.6

oxidation. This trend is consistent with observations of better agreement between HTCO and wet oxidation values for oxygen minimum seawater samples (Druffel et al., 1989). Barber (1968) observed that DOC values for deep ocean seawater samples did not change when inoculated with marine bacteria, while surface samples showed rapid decreases.

Contrary to the overall trends observed for ageing over long time periods, the short time scale experiment indicated that phytoplankton DOC can become slightly more resistant to persulphate oxidation, as $\%DO^{14}C_{res}$ values increased from 17 ± 1% to 23 ± 1%. The differences between these results and those from the early stages of the long-term ageing experiments may be best explained by preferential bacterial consumption of biologically and chemically labile compounds, present initially in the PDOC from the axenic culture, but which had been largely consumed by bacteria in non-axenic cultures prior to initialization of long-term ageing studies.

The short-term ageing experiment was designed to simulate maximal uptake of phytoplankton DOC by bacteria, since the axenic cultures should have contained relatively high concentrations of labile exudates. In addition, the inoculate was prefiltered to minimize bacterivores. In a similar experiment, Kirchman et al. (1991) followed the change in DOC measured by HTCO of 0.8 μm-filtered seawater taken from the NW Atlantic during the 1988 spring bloom. They found that 31 - 41 μM C was consumed in 1 to 3 day incubations with turnover rates ranging from 0.087 - 0.363 day^{-1}. A similar calculation with the *Synechococcus* data from Figure 4.3 gives a turnover rate of 0.210 day^{-1} for the 2.6 day time interval corresponding to rapid bacterial uptake of about 25 μM of *Synechococcus* DOC. These values should represent upper limits to the rate of DOC turnover in the ocean, since bacterivores and viruses may limit bacteria production in the open ocean when sufficient substrate exists (Ducklow, 1992). Nevertheless, such potentially high turnover rates would predict appreciable (25-40 μM C) short-term variability and very large DOC fluxes during the course of a phytoplankton bloom (Kirchman et al., 1991).

4.4.2 Ageing of unfiltered culture material

The ageing experiments involving unfiltered *P. tricornutum* and *I. galbana* culture material suggest that persulphate oxidation resistant DOC may also be produced from the grazing activities of heterotrophic nanoflagellates. Grazing of bacteria and algae (Goldman et al., 1985; Caron et al., 1985; Bratbak, 1987; Caron et al., 1991) cycles C back into the DOC pool, and thus forms an important flow of carbon in microbial loops (Azam et al., 1983). Recently, investigators have detected large numbers of sub-micron particles in upper ocean seawater (Koike et al., 1990; Wells and Goldberg, 1991; Longhurst et al., 1991). Experimental evidence suggests that sub-micron particles may be formed from the grazing of bacteria by nanoflagellates (Koike et al., 1990). These authors calculate that sub-micron particles comprise as much as 10% of surface marine DOC, since a substantial portion of sub-micron particles are not retained by 0.8 μm filters (Koike et al., 1990). Longhurst et al. (1991) have speculated that these particles may form part of the DOC resistant to wet oxidation DOC methods. In the present study, experiments with unfiltered culture material suggests that DOC produced through heterotrophic nanoflagellates is indeed relatively refractory, as *ca.* 25% of that produced resisted oxidation. However, these experimental results regarding the production of persulphate resistant DOC from the grazing activities of heterotrophic nanoflagellates should be considered as preliminary, with additional data needed at more appropriate time intervals (hours instead of days).

4.5 Summary and Conclusions

1) It was found that ageing of phytoplankton DOC in the presence of bacteria did not increase the total amount of material resisting oxidation.

2) Bacterial ageing was found to generally decrease the proportion of resistant DOC; however, in one experiment the proportion of resistant material increased. I propose that the increase was due to relatively high amounts of labile exudates present in the initial substrate taken from an axenic culture.

3) Increases in the amount of resistant $DO^{14}C$ in samples exposed to sunlight were small and similar to values found for dark controls.

4) Evidence suggests that DOC produced by heterotrophic nanoflagellates is relatively refractory (*ca.* 25%) to persulphate oxidation.

The results of Chapters 3 and 4 indicate that DOC produced or altered by various biological processes show different reactivities to persulphate oxidation. These observations suggest that DOC may be highly affected by the ecological history and present status of a given marine region. For example, the well-known phenomenon of phytoplankton species succession during different parts of the year in the North Atlantic ensures inputs of DOC with different reactivities to persulphate oxidation. As well, DOC from oceanic regions such as the equatorial and NE Pacific, where phytoplankton grow at near maximal rates, and where phytoplankton populations may be grazer controlled (Cullen et al., 1990), should differ in reactivity compared with areas which undergo seasonal bloom cycles.

Chapter 5

The Measurement of Dissolved Phosphorus in Seawater

5.1 Introduction

Phosphorus is an essential nutrient for marine organisms and productivity is strongly associated with its availability in the euphotic zones of the ocean on short (Smith, 1984; Jackson and Williams, 1985; Codispoti, 1989; Karl et al., 1992c) and long-term timescales (Redfield, 1958; Broecker and Peng, 1982). In the dissolved state, phosphorus exists in both inorganic and organic forms. Thorough reviews on the forms, measurement and cycling of phosphorus have been published, which include the works of Armstrong (1965), Hooper (1971), Fogg (1973), Burton (1973), Riley (1975), Froelich et al., (1982), and Cembella et al., (1984). To avoid unnecessary repetition, I will limit my background discussion to the most salient points of dissolved phosphorus speciation, sources and measurement in marine waters. Particular attention will be made to aspects of the measurement of dissolved P in seawater.

5.1 Speciation of phosphorus in seawater

5.1.1 Inorganic forms of dissolved P

Inorganic phosphorus exists mainly as HPO_4^{2-} and its metal complexes in seawater, which are illustrated in Figure 5.1. The predominance of HPO_4^{2-} is due to the pH of seawater which dictates phosphate ion equilibria, also shown in Figure 5.1. Thus, at seawater pH and typical seawater salinity, approximately 85% of orthophosphate is composed of HPO_4^{2-} (a generic name for all forms of $H_nPO_4^{n-3}$), of which 30% exists as the free anion and the remainder as alkali metal complexes. Other forms of dissolved inorganic phosphorus in seawater are pyropnosphate, metaphosphates and polyphosphates (hereafter all termed

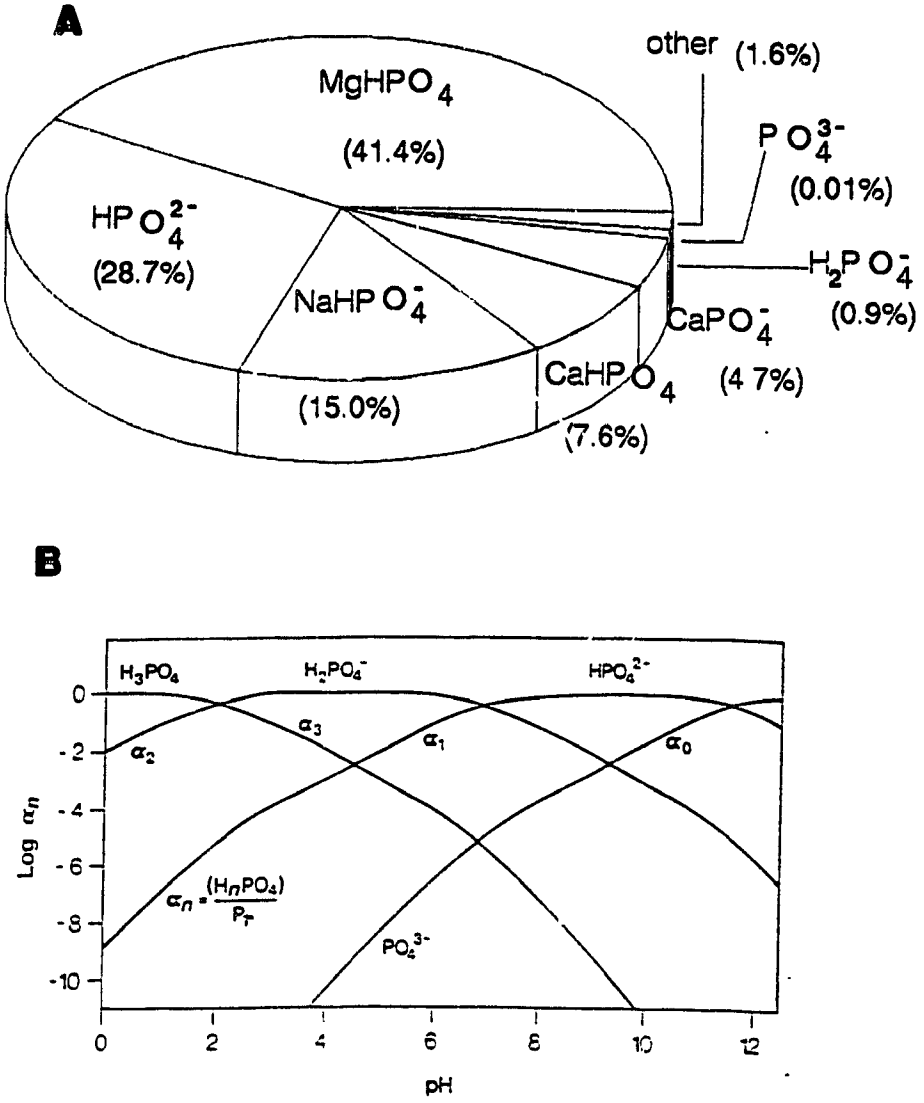


Figure 5.1. (A) Speciation of orthophosphate in seawater at 34.8 psu, 20°C and pH 8.0 (Data from Atlas et al., 1976). (B) Ionic phosphate equilibria as a function of pH.

polyphosphates). While polyphosphates are synthesized by marine bacteria and phytoplankton, and may compose significant proportions of cellular P (Aitchison and Butt, 1973), their concentrations in estuarine (Taft et al., 1975), coastal (Armstrong and Tibbets, 1968; and Solórzano, 1977) and open ocean waters (Strickland and Solórzano, 1968) appear to be relatively low. Consequently, polyphosphates have not been investigated in great detail, despite the possibility that transient compounds may be of significant importance to the cycling of phosphorus in seawater. Phosphite and other reduced P compounds appear to be of significance only in reducing sediments.

The sources and sinks of inorganic phosphorus have been reviewed by Froelich et al., (1982), and are briefly summarized here. The main sources of inorganic phosphorus into the ocean are fluvial inputs, dissolution of marine apatite and regeneration of organic matter from marine sediments. Hydrothermal and atmospheric inputs are believed to be less than 10% of the riverine flux. The major sinks of marine inorganic phosphorus are sedimentation and burial of organic matter and biogenic calcium carbonate (contains phosphorus at the ppm level), sorption and precipitation of phosphate onto clays and other particles.

5.1.2 Organic forms of dissolved P

The major groups of dissolved organic phosphorus (DOP) compounds in seawater are ester-P, lipid-P, and nucleotides including RNA and DNA (Karl et al., 1992c). These compounds reflect the components of the major forms of particulate organic phosphorus in seawater (marine bacteria, phytoplankton and zooplankton), from which DOP is ultimately derived. It has been suggested that phosphonates (organic phosphorus compounds that contain a C-P bond) may also contribute to DOP in seawater, since they are known to be synthesized by marine plankton (Kittredge et al., 1969).

The major pathways of DOP production in seawater have not been studied intensively,

but experimental results point to similar sources as for DOC (Pomeroy et al., 1962; Antia et al., 1963; Grill and Richards, 1964; Ketchum and Corwin, 1965). Major pathways for DOP production are expected to be cell lysis and decomposition, and grazing and excretion by zooplankton and protozoan grazers. Release of DOP from a broad range of marine phytoplankton (Kuenzler, 1970; Admiraal and Werner, 1983), and some bacteria (Sharpf, 1973) has been observed. DOP is released by actively growing algae, particularly during declining growth stages. However, Kuenzler (1970) noted that DOP levels decreased when orthophosphate supply was exhausted, indicating that phytoplankton are capable of reassimilating DOP. Bacteria and phytoplankton produce dissolved or surface-bound phosphatases to access extracellular organic phosphorus. Phosphomonoesters and diesters are particularly labile to enzymatic attack. In fact, some standard nutrient media contain organic phosphorus compounds, such as glycerophosphate, in lieu of orthophosphate. Rivkin and Swift (1979) noted that enzyme hydrolysable phosphorus concentrations were often greater than inorganic phosphate levels in the surface waters of the Caribbean and Sargasso Seas. From these and other observations, it is apparent that measurement of the organic forms of dissolved phosphorus is necessary to establish the full resources available to phytoplankton. Radiotracer estimates of biologically available phosphorus in oligotrophic regions were several times soluble reactive phosphorus (SRP) concentrations, and often equivalent to or greater than total dissolved phosphorus values measured by the standard persulphate procedure (Bossard and Karl, 1986; Orrett and Karl, 1987).

5.2 Characterisation of DOP compounds in natural waters

In a review of organic phosphorus compounds in natural waters, Hooper (1973) urged that more effort be applied to the classification and identification of organic P. This statement was reiterated some years later by Cembella et al. (1984), as very little work had been done in the interim on characterisation of specific organic P classes or size fractions in seawater.

I summarize below the very limited work that has been done on characterising DOP in seawater.

Strickland and Solórzano (1968) found only small amounts of open ocean DOP were hydrolysed by alkaline phosphatase, a monophosphoester-specific enzyme, and therefore concluded that DOP in seawater was mainly composed of nucleic acids. However, alkaline phosphatase was found to decompose 20-50% of DOP in Tokyo and Sagami Bay samples taken from the euphotic zone during a phytoplankton bloom (Kobori and Taga, 1979). Deeper samples contained no enzyme hydrolysable DOP, which led the authors to suggest that phosphate esters formed a major part of DOP during the phytoplankton bloom, and were recycled efficiently within the euphotic zone.

A gel chromatography investigation of the size fractions of DOP in Tokyo Bay samples taken during a phytoplankton bloom indicated the presence of two major P components: one with MW from 200-400 and the other with MW >5000 (Matsuda and Maruyama, 1985). Both fractions decreased with depth, and were therefore assumed to be labile to degradation. The authors suggested that the high MW compounds were mainly RNA, DNA and their fragments, based on evidence from the analysis of high molecular weight DOP collected from cultured algae (Minear, 1972). To my knowledge, no studies of the size fractions of DOP in open ocean seawaters have been published prior to the present study.

5.3 Measurement of P compounds

Unfortunately, standard techniques for measurement of phosphorus compounds in seawater are not able to differentiate finely between the inorganic and organic forms described in the preceding text. Techniques for measurement of dissolved phosphorus compounds may measure portions of the inorganic and organic pools; therefore, dissolved phosphorus fractions are often defined by the method of measurement.

5.3.1 Soluble reactive phosphorus (SRP)

Orthophosphate concentrations in seawater are typically estimated using the Deg:nes reaction, in which phosphate is reacted with molybdenum to form 12-molybdophosphoric acid, which is reduced to a phosphomolybdenum blue complex. The addition of antimony ions to the reactants increases the reaction rate and the stability of the complex (Murphy and Riley, 1962). The optical density of the solution is linear over a broad concentration range suitable for most analyses in seawater (up to 3.5 μM using a 10 cm cell). However, considerable evidence from freshwater studies indicate that SRP measurements include contributions of phosphate hydrolysed from bound sources, due to the acidic environment (pH 1) of the colorimetric method (Rigler, 1968). Bound sources might include labile phosphoesters, and phosphates complexed with colloidal matter (Stainton, 1980). As little as 30% of SRP may be free orthophosphate in oligotrophic lakes according to anion-exchange studies, whereas radiotracer studies indicate even a lower percentage of SRP is truly free orthophosphate (Chamberlin and Shapiro, 1973). This problem has not received as much interest in the analysis of phosphate in seawater; however, Cembella et al. (1986), have shown that approximately 10% of ATP and glucose-6-phosphate added to artificial seawater is hydrolysed to give molybdate reactive phosphorus. O-Phospho-DL-serine, phosphatidyl choline and several simple phosphonates do not react with the SRP reagents. The contribution of colloidal P to SRP measurements has been very recently investigated for samples from Tomales Bay using an ultrafiltration technique. Hollibaugh et al., (1991) found that colloidal DOP contributes less than 4% of SRP in these mesotrophic waters (SRP > 1 μM). However, organic contributions to SRP concentrations may be most substantial in oligotrophic surface waters, where phosphate levels are low and DOP levels are relatively high. Deep water SRP values should very closely estimate orthophosphate concentrations due to lower concentrations of labile organic matter.

5.3.2 Distribution of SRP in ocean waters

A summary of GEOSECS data for SRP concentrations in the western Atlantic and Pacific basins is shown in Figure 5.2. As can be seen from these diagrams, the classic nutrient profile shows low SRP concentrations in surface waters increasing to a mid-depth maximum and a slow decrease below this depth. Downwelling Antarctic seawater contains high SRP levels, and therefore a deep maximum is seen in high latitude South Atlantic and Pacific waters.The mid-depth maximum is usually assumed to reflect the release of phosphate by regeneration of vertically sinking organic particles (Broecker and Peng, 1982). Mid-depth SRP values are lowest in the deep water formation zones of the North Atlantic and increase as the water advects southward and into the Pacific basin. Therefore, high SRP levels are found in the NE subarctic Pacific, where mid-depth concentrations reach 3.5 μM.

5.3.3 Total and dissolved phosphorus (TP/TDP)

Traditional and present-day methods for the measurement of total and dissolved phosphorus are described in Table 5.1. TP and TDP values corrected for SRP values have been used for the estimation of organically-combined P. The tendency in method development has been to adopt procedures that have been shown to provide greater recovery from seawater samples, and show equivalent or better analytical precision than previous methods.

Early phosphorus methods (pre-1960) were time-consuming, imprecise and hampered by numerous interfering compounds and appreciable salt error. However, with the introduction of the Murphy and Riley (1962) method for orthophosphate determinations (and probably due to an improvement in the purity of chemical reagents and blank water), blank and precision problems improved. An early standard total phosphorus procedure was the perchloric acid method (Hansen and Robinson, 1953), which was recommended by Strickland and Parsons (1972). This method was nevertheless time-consuming and potentially dangerous,

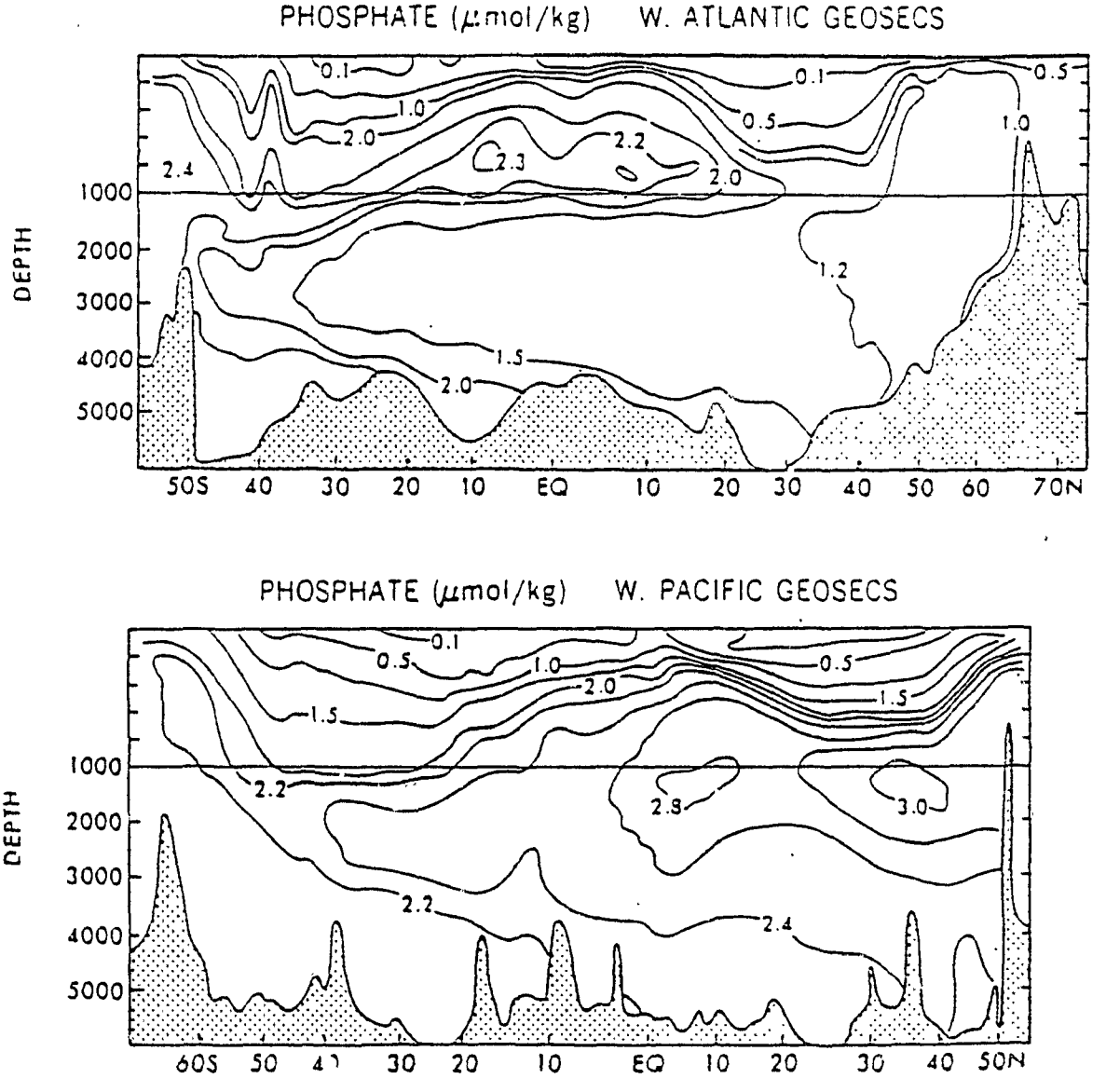


Figure 5.2. Summary of SRP distributions in Atlantic and Pacific Oceans measured during GEOSECS program (From Toggweiler, 1989. Permission to use copyright material pending).

since under certain conditions perchloric acid reacts explosively with organic matter.

The persulphate method was introduced by Menzel and Corwin (1965) and was shown to be rapid, precise and accurate relative to the Harvey (1948) and perchloric acid methods. The persulphate oxidant is more amenable for use at sea than perchloric acid, which facilitated shipboard analysis immediately after sampling to obtain quasi 'real-time' determinations.

The UV photo-oxidation method was the second important technique introduced during this time (Armstrong et al., 1966). The UV method was shown to give similar values for the oxidation of marine DOC as the persulphate method (Menzel and Vaccaro, 1964), but with regards to dissolved phosphorus it was found not to degrade polyphosphates, unlike all other methods. The UV method was viewed as a more accurate determination of organically-combined phosphorus in seawater, although it is recognized that compounds such as ATP contain polyphosphate linkages. When samples were brought to pH 2 by addition of sulphuric acid, the UV irradiation proved to be as effective for TDP determinations of freshwater samples as the persulphate method (Henriksen, 1970).

As can be seen from Table 5.1, other methodological developments for P analysis of marine samples have included dry-ashing and acid hydrolysis (Solórzano and Sharp, 1980), $Mg(NO_3)_2$ 'fusion' (Cembella et al., 1986), and a high temperature reduction method with GC analysis of the reduced phosphorus compounds (Hashimoto et al., 1987). No major discrepancies with the 'standard' persulphate method have been reported, although certain phosphonates have been shown to resist UV oxidation (Cembella et al., 1986).

5.3.4 Distributions of DOP in ocean waters.

Surface waters. Highest levels of DOP have been found in coastal and estuarine waters where levels of up to 0.8 μM have been measured (Strickland and Solórzano, 1968; Hollibaugh

et al., 1991), although the effects of sewage and river runoff cannot be neglected in these areas. DOP levels in open ocean seawater rarely exceed 0.5 μM. Jackson and Williams (1985) amassed a large data set of DOP concentrations for Pacific surface seawater samples ranging from the North Central Pacific to the Ross Sea. Of the 134 determinations made by the UV method, an upper value of 0.42 μM was obtained with a mean value of 0.20 μM. Similar DOP concentrations have been measured in the Pacific and Atlantic using perchloric acid, persulphate and UV methods.

Subsurface and deep seawaters. Very few depth profiles have been published which show DOP concentrations in subsurface waters. One of the earliest and most extensive determinations of DOP distributions in subsurface waters was published by Ketchum et al. (1955; using the Harvey (1948 method)) from profiles taken at numerous stations across the equatorial Atlantic. Due to relatively high uncertainties in the TDP and SRP determinations (a difference of ±10% was considered significant), these authors analyzed the data set statistically. About 95% of surface samples contained significant levels of DOP, with 50% of samples ranging from 0.25-0.50 μM. Significant differences between TDP and SRP were found for 67% of subsurface samples taken down to 1000 m, but only 5% of samples taken from below 1000 m showed significant differences.

On the other hand, DOP concentrations in subsurface and deep samples (300-2000 m) from the subarctic NE Pacific were reported to range from 0.65-2.1 μM (Strickland and Austin, 1960). Comparison with the Ketchum et al. (1955) data suggested variability between oceans, greater recovery of DOP with the perchloric acid method used for the Pacific study, or contamination problems.

A summary of DOP profiles determined using more reliable methodology is given in Figure 5.3. This very limited data set indicates that DOP is generally highest in surface waters and decreases as SRP increases with depth. DOP values are not detectable for Sargasso Sea samples below 500 m, and decrease to low but measurable values with depth in the North

Central Pacific off Hawaii. However, appreciable DOP levels are present in deep waters in the North Pacific gyre and in the Santa Monica Basin off California. Therefore, while surface DOP concentrations are similar, there is some evidence for higher DOP levels in deep Pacific seawater than in central Atlantic seawater.

There are two interesting features of the very deep profile in the North Pacific (Williams et al., 1980), shown in Figure 5.3. First, the DOP profile is somewhat similar to dissolved oxygen; both show a minimum at 1000 m. The similarity between DOP and O_2 profiles is reminiscent of the correlation found for DOC and AOU by Sugimura and Suzuki (1988). The persulphate-oxidisable DOC data do not show a similar correlation (Williams et al., 1980). Second, an increase is noted for the near bottom DOP sample. Williams et al (1980) suggested that near-bottom DOP levels may be elevated due to diffusion of refractory DOM from the sediments. The increase may have also been due to colloidal DOP derived from resuspended sediments.

5.3.5 Temporal variations of DOP in seawater

Temporal variations of DOP on monthly, weekly and hourly time scales are shown in Figure 5.4. The variation of DOP in the western English channel is plotted with SRP in Figure 5.4(A). A strong seasonal cycle is apparent with SRP decreasing in the spring and summer months, with a corresponding increase of DOP. Evidence for the production of DOP during a spring phytoplankton bloom in the Gulf of Maine is shown in Figure 5.4(B). Post-bloom measurements indicated lower SRP levels, higher particulate phosphorus levels, and increased DOP levels compared with pre-bloom conditions. DOP levels increased on a time scale of only hours (from early to mid-morning) in surface waters of the central North Pacific, as shown in Figure 5.4(C). The data are consistent with DOP production from increased photosynthesis during daylight hours, during phytoplankton blooms, and due to increased photosynthesis during spring and summer months.

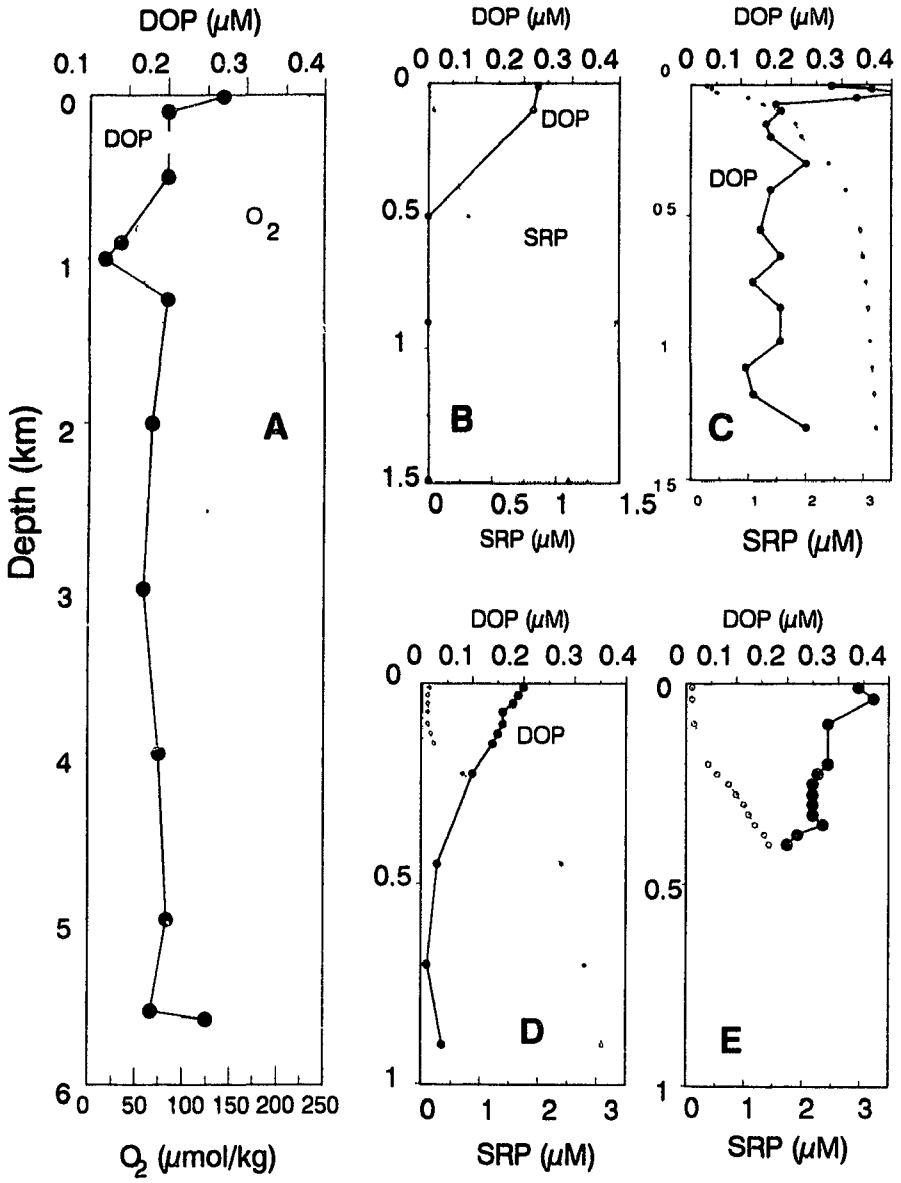


Figure 5.3. Summary of published DOP distributions. (A) Deep profile from the central North Pacific (28° N, 155° W) measured by UV oxidation (Williams et al., 1980); (B) Data reported for the Sargasso Sea (32° N, 64° W) as measured with the Harvey (1948) method by Kuenzler et al., 1963; (C) DOP measured off Southern California by UV oxidation (Holm-Hansen et al., 1966); (D) DOP measured in central Pacific near Hawaii by persulphate method (Smith et al., 1986); and (E) DOP measured in central Pacific near Hawaii by UV oxidation (Walsh, 1990).

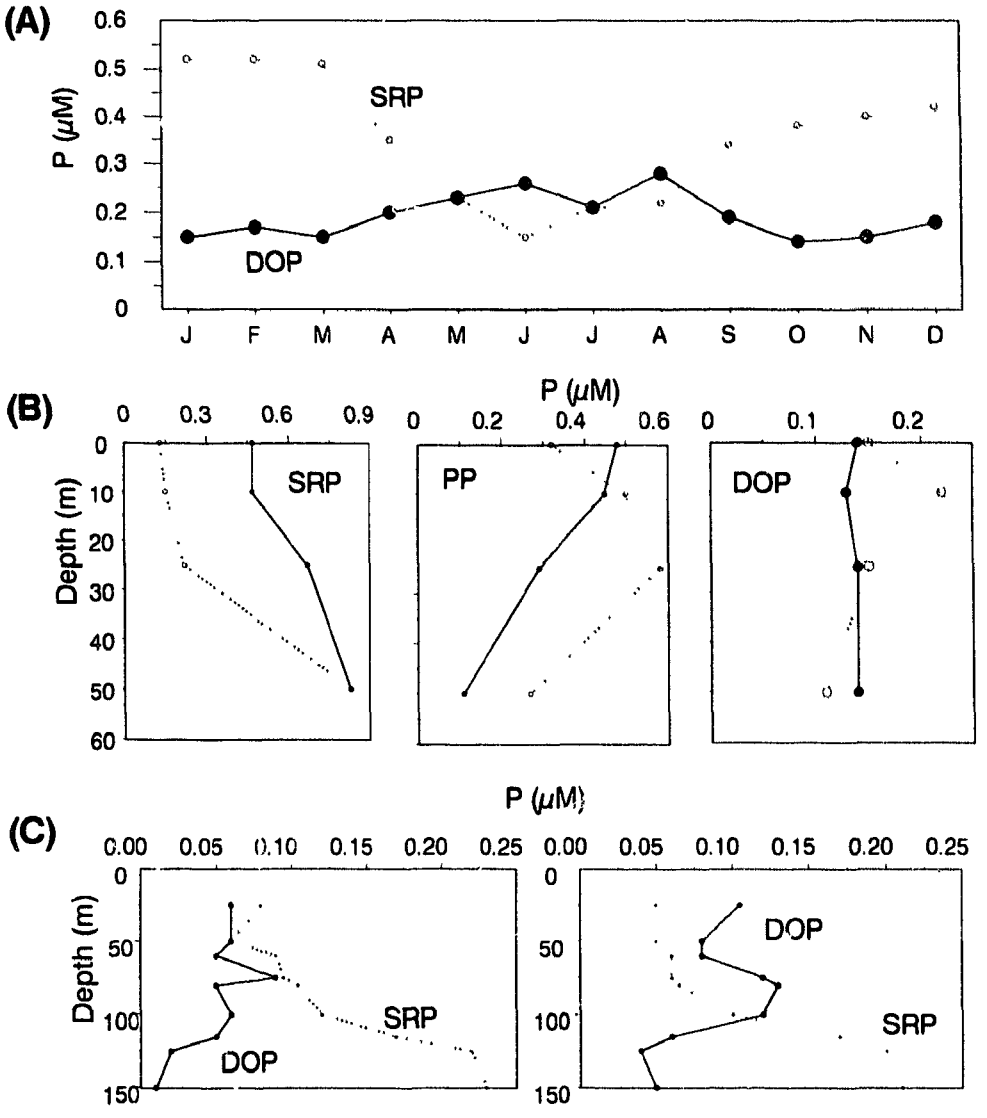


Figure 5.4. Temporal variations of DOP in seawater. (A) Annual changes in SRP and DOP concentrations in the English Channel. DOP measured by UV oxidation (Butler et al., 1979); (B) SRP, particulate phosphorus, and DOP concentrations before (closed circles) and after (open circles) a phytoplankton bloom in the Gulf of Maine. PP and DOP measured by persulphate method (Ketchum and Corwin, 1965). (C) Semi-diurnal variations of SRP and DOP in surface waters of the central North Pacific: (i) profile taken at 0500 hours, (ii) profile taken at 1000 hours. DOP measured by the persulphate method (Orrett and Karl, 1987).

5.4 Implications of high concentrations of UV and persulphate resistant DON,C to DOP

The reports of high concentrations of DON (Suzuki et al., 1985) and DOC (Sugimura and Suzuki, 1988) that resist conventional methods of analysis have led several authors to question whether similarly higher levels of DOP or TDP exist in seawater (Druffel and Williams, 1988; Jackson, 1988; Toggweiler, 1988), since dissolved inorganic and particulate C, N and P are often thought of as stoichiometrically linked in seawater in a ratio approximating 100-140:16:1, respectively (Redfield, 1958; Takahashi et al., 1985; Peng and Broecker, 1987). It was noted that nitrate and phosphate ratios vary in surface seawater where nutrients are present in low concentrations; however, Jackson and Williams (1985) found that a regression of TDN and TDP values for these seawaters resulted in a linear fit with a slope of 15. They therefore argued that DON and DOP were important links connecting organism N:P ratios and nitrate:phosphate ratios. If this relationship is applicable for the 'new' DON values measured by Suzuki et al. (1985), then a previously undetected P pool is required in surface seawater.

A modelling study provided further support for higher DOP values in surface waters than previously detected using conventional techniques. In an investigation into how the vertical flux of organic matter affects nutrient distributions in a 3-D ocean basin model, Toggweiler (1989) and Sarmiento et al. (1988) report unrealistic phosphate distributions were obtained when new production was solely represented by a vertical flux of particulate matter settling out below the photic zone. However, reasonable distributions were obtained when a long-lived (*ca.* 200 years) pool of DOM of an equivalent size to that reported by Suzuki and Sugimura (1988) for surface DOC was allowed to advect combined nutrients away from areas of high production. A C:P ratio of 100 was used to produce these results, requiring a TDP pool of about 2.5 μM in surface waters. Since traditional values are <0.4 μM for open ocean central gyres, a large pool of previously undetected dissolved phosphorus was implied.

These empirical and modelling results question the accuracy of traditional estimates

of TDP and DOP in surface waters. Detection of similarly high, or significantly higher, DOP values would provide additional support for high DON (Suzuki et al., 1985) and DOC values (Suzuki and Sugimura, 1988). It was also recognized that DOP is a poorly characterised pool of DOM. There is a paucity of deep profiles from major regions of the world ocean. It is unclear whether differences between DOP values in the Atlantic and Pacific deep water are due to different methodologies or indicative of true variations. Very little is known about the chemical composition and molecular size distribution of DOP in seawater. Further study of DOP would seem to be a useful endeavour during this time of heightened interest in the size and distribution of DOM in seawater.

Table 5.1. Some analytical methods for the analysis of total and dissolved phosphorus in seawater. Also included are method comparison studies.

Original Investigators	Method	Results/Comments
Redfield et al. (1937)	digestion with 50% peroxide, heating with NH_4OH, H_2SO_4.	-one of first total phosphorus methods for seawater samples.
Harvey (1948)	addition of 6 N H_2SO_4 and autoclaving for 5 h	-*ca.* 5% more P from seawater than Redfield method.
Hansen and Robinson (1953)	evaporation to salts, oxidation of residue with hot perchloric acid	-recovered an average of 0.2 μM more than Harvey method for surface seawater.
Ketchum et al. (1955)	oxidation/hydrolysis with H_2SO_4-H_2O_2.	-detected DOP in 95% of surface samples from equatorial Atlantic Ocean.
Menzel and Corwin (1965)	persulphate method	-recovery equivalent to Redfield method; 2-7% less than Harvey method for Indian Ocean samples. -full recovery from algal culture grown in synthetic medium.
Armstrong et al. (1966)	high-intensity UV method	-full recovery from selected organic phosphorus compounds. -did not release P from polyphosphates.
Strickland and Solórzano (1968)	UV irradiation followed by acid hydrolysis	-degraded polyphosphates

Table 5.1 (continued). Introduction of analytical methods for the analysis of total and dissolved phosphorus in seawater. Also included are method comparison studies.

Original Investigators	Method	Results/Comment
Jenkins (1968)	compared dry-ashing at 800°C, 30% H_2O_2 digestion, nitric and sulphuric acid digestion, perchloric acid oxidation and persulphate methods	-full recovery of coastal seawater with dry-ashing and persulphate techniques only.
Solórzano and Sharp (1980)	dry-ashing with $MgSO_4$ at 450-500°C, followed by acid hydrolysis	-100% recovery from selected P compounds
Valderrama (1980)	alkaline persulphate tested against acid persulphate method	-good agreement between methods for TP <2 μM; better recovery with alkaline persulphate at high TP values. -alkaline method has higher blank.
Cembella et al. (1986)	dry-ashing with $Mg(NO_3)_2$; tested against perchlorate oxidation, UV oxidation, and persulphate method.	-recovered *ca.* 96% of organophosphorus and polyphosphate compounds. -more efficient than other methods for phosphonates
Hashimoto et al. (1987)	high temperature reduction of P-compounds and GC analysis	-measured similar TDP levels in western Pacific seawater as persulphate method.

Chapter 6

Determination of Total and Organic Dissolved Phosphorus in Seawater

6.1 Introduction

As discussed in Chapter 5, DOP has been measured with a variety of analytical methods. Currently popular methods include acid persulphate (Menzel and Corwin, 1965), ultra-violet (UV) irradiation (Armstrong et al., 1966) and dry combustion (Solórzano and Sharp, 1980). Recently measurements of dissolved phosphorus levels in seawater also have been made with two novel techniques: the nitrate oxidation method of Cembella et al., (1986), which was developed to measure phosphonates, and a high temperature method in which phosphorus compounds are reduced to phosphine and detected by gas chromatography (Hashimoto et al., 1987). These procedures were found to measure the same levels of dissolved phosphorus in seawater as the standard UV and persulphate methods.

Despite this agreement, a re-examination of the dissolved organic phosphorus levels in oceanic waters was appropriate at the time of this study, as the findings of Suzuki et al. (1985) and Sugimura and Suzuki (1988) indicated that UV and persulphate oxidation do not completely oxidize marine dissolved organic matter. These results put into question whether significant amounts of organically-bound phosphorus have similarly escaped detection by standard methods. Underestimates of TDP may help explain estimates of biologically-available phosphorus that are 1.8-6.7 times greater than *in situ* total dissolved phosphorus determinations (Orrett and Karl, 1987). Toggweiler (1989) suggests that additional amounts of dissolved phosphorus are required to satisfy modelling constraints.

An inter-calibration workshop (US Global Flux Study workshop, Wood's Hole, 17-20 November, 1987) was held to compare the efficacies of standard DOC oxidation techniques with the high temperature combustion oxidation method of Sugimura and Suzuki. Although it was apparent that the high temperature combustion method yielded higher results than the

other DOC methods, it was found that the CO_2 released from a combination of UV irradiation and persulphate oxidation approached the levels measured by the combustion method (H. Livingston, pers. commun.). It was decided that such a combination would provide a demanding test with which to re-examine the dissolved organic phosphorus levels in oceanic waters.

This Chapter describes the development of the combination method (UV + persulphate) for total and organic dissolved phosphorus determinations. The standard UV and persulphate methods are also re-examined and improved. The efficiencies of the three techniques are compared for seawater samples taken from the NW Atlantic and NE Pacific. Seawater samples were also processed on board ship by ultrafiltration and the different DOP size fractions analysed by the three TDP methods. These size fractionation results are the first such data characterizing open ocean dissolved phosphorus compounds.

6.2 Materials and Methods

Methods for filtration and storage of seawater samples to be analysed for dissolved phosphorus generally followed Grasshoff (1983) and Koroleff (1983). Containers were soaked with 4.5 M sulphuric acid and rinsed repeatedly with deionised-distilled (Millipore Super-Q) water. All other materials contacting the samples were soaked or rinsed with 4.5 M sulphuric acid, and then rinsed many times with Super-Q. Samples were filtered through acid-cleaned 0.45-μm Millipore or 0.4-μm Nuclepore filters, after rinsing with approximately 200 ml of sample. Gelman Mini Capsule filters (0.45 μm), used to prefilter samples for ultrafiltration, were leached in 0.05 M Ultrex HCl, and rinsed with Super-Q. All materials were stored in plastic bags during field trips to avoid possible contamination sources.

6.2.1 Analysis of Soluble Reactive Phosphorus

Samples for SRP analysis followed the method of Murphy and Riley (1962) as modified by Koroleff (1983). In the Koroleff procedure, the single solution reagent used by Murphy and Riley is divided into two: an aliquot of a ascorbic acid solution is first added to the sample, followed by an aliquot of the second solution composed of sulphuric acid, ammonium molybdate and antimony ions. The final sample acidity is lower than that used with the Murphy and Riley method (0.1 M instead of 0.2). As noted in Chapter 5, it is likely that this method also measures labile DOP due to hydrolysis by the acid reagents (Rigler, 1968).

A 4-cm pathlength cell was used for the NW Atlantic study, whereas a 10-cm cell was used in all other work. The 4-cm cell required a volume of only 5-10 ml for analysis and was useful when sample volumes were limited; however, use of this cell required dilute acid rinses between samples and strict attention to blank values because the colloidal Mo complex tended to adhere to the walls of the cell. A 10-cm pathlength cell was preferred, as surface sorption proved less problematic and the longer pathlength cell provided more precise results. Spectrophotometric analysis was done at 885 nm with a LKB Spectronic 4000 spectrophotometer. Once samples had been removed for SRP analysis, the seawater remaining in each container was acidified to pH 2 with sulphuric acid and stored at 2 °C until later analysed for TDP.

6.2.2 Total Dissolved Phosphorus Analyses

Samples were analysed for TDP in three ways: with UV irradiation, with persulphate oxidation, and with the two oxidation methods applied sequentially to the same sample. In each case, the oxidized sample was measured using the Molybdenum Blue method, and dissolved organic phosphorus values were taken as the difference between TDP and SRP.

UV Irradiation. The 1200-W mercury arc lamp UV system described in Chapter 2 was used to photo-oxidize samples in quartz tubes. Ultrex H_2O_2 was added to the samples prior to photo-oxidation. Irradiation time was initially varied from 1 to 18 hours and was routinely 6 hours for most samples. Samples were weighed and diluted back to volume with Super-Q to correct for small amounts of evaporation. System and reagent blanks were negligible. KH_2PO_4 standards prepared in both Super-Q and aged seawater gave identical results.

Persulphate oxidation. The autoclave method of Menzel and Corwin (1965) with the improvements outlined by Koroleff (1983) was used. Autoclave heating time was initially varied from 30 minutes to 6 hours and was routinely 90 minutes thereafter. Autoclave temperature was 126 °C. Two concentrations of potassium persulphate were used:

(1) 4 mg ml^{-1}, the standard amount for TDP analysis (Menzel and Corwin, 1965).

(2) 40 mg ml^{-1}, as recommended by Strickland and Parsons (1972) for DOC analysis. In this case, solid potassium persulphate was added to individual containers, followed by the acidified sample, then gently heated in a oven to about 50 °C. The test tubes were swirled to dissolve all the reagent and immediately autoclaved. This method was used to test the efficiency of the persulphate method to liberate organically-bound phosphorus.

It was noted that colour development was seriously impeded in preliminary colorimetric analyses of the samples treated with the high concentration of persulphate. Slow colour development was attributed to the decomposition of the persulphate to sulphuric acid and a concomitant decrease in the sample pH. As noted by previous investigators (Koroleff, 1968; Riley, 1975; Solórzano and Sharp, 1980), colour development is sensitive to the acid:Mo ratio of the sample and is hampered at values greater than 5.0 (normality:percent). Analysis of samples treated with the high persulphate concentration was accomplished by preparing a Mo reagent containing double (5% wt/vol) the normal ammonium molybdate concentration. Separate standards and reagent blanks were analysed for each method. A bottle of ACS grade potassium persulphate reagent purchased from Fisher Scientific gave lowest blank values

(0.009 μM for the standard persulphate reagent concentration).

Combination method. With this method, samples were first UV irradiated and then treated by the persulphate techniques. Colorimetric analysis was carried out in the same way as for the standard methods.

Phosphorus measurements of unpolluted seawater by Molybdenum Blue colorimetry can be influenced by the presence of silicate and/or arsenate ions. However, at natural seawater concentrations, the contributions from these ions are negligible if absorbances are measured within 30 minutes after the addition of the reagents (Andreae, 1979; Koroleff, 1983). Tests done on open ocean surface seawater samples found no increases in absorbances for SRP and TDP methods once initial colour development had taken place (about 5 minutes). As care was taken to measure all samples within a narrow time interval (10-20 min) following the addition of the molybdate reagent, the slow development of colour due to non-P compounds in other samples (if present) should not affect the comparative nature of this study (Cembella et al., 1986).

It should also be noted that DOP values determined by the difference between TDP and SRP may correspond to upper limits for organically-bound P, since the difference between TDP and SRP may include contributions from inorganic phosphorus compounds (e.g. metaphosphates, polyphosphates), also broken down by the TDP methods. While oceanic concentrations of such compounds are not well-characterised, estimates of their levels in open ocean seawater (<0.05 μM; Armstrong and Tibbetts; Solórzano and Strickland, 1968) indicate they are generally at or near the detection limits of the DOP methods. Though short-term UV irradiation does not normally hydrolyse polyphosphate ions, the typical parameters used with the UV methods of these studies (sample pH 2 and heated to 70-75°C for 6 hours) should degrade most polyphosphate that may be present in any samples (Solórzano and Strickland, 1968).

6.2.3 Ultrafiltration method

Size fractionation for the phosphorus work was carried out using a Pellicon Millipore crossflow filtration apparatus as described in detail by Moran (1990). The crossflow filtration technique minimizes uptake of material onto the filter by creating a fluid flow that is tangential as well as perpendicular to the membrane. The tangential flow inhibits formation of a solute layer at the membrane surface, which improves filtration efficiency. Filtration rates of 15-50 L/hr enable large sample volumes to be processed rapidly and provide high concentration factors for the retentate material. This system is therefore particularly suited for oceanographic studies, where solutes of interest can be in very low concentrations (Moran and Moore, 1989; Whitehouse et al., 1989; Benner et al., 1992).

The crossflow system was fitted with a 10,000 nominal molecular weight 'PTCG' filter cartridge, which is constructed of polysulfone and polyethylene. As noted for Amicon ultrafilters in Chapter 2, size-cutoffs are not precise and retention of macromolecules will depend on both size and structure. The PTCG filter cartridge retains about 20% of 1000 MW and 90% of 10,000 MW spherical calibration macromolecules (Millipore Corp.). The cartridge and associated apparatus were initially cleaned by leaching with HCl and rinsing with Super-Q as described in detail by Moran (1990). Super-Q system blanks showed that the apparatus was non-contaminating for dissolved phosphorus.

The ultrafiltration procedure provided three fractions of dissolved phosphorus: TDP, which is the 0.4-μM-filtered sample; low molecular weight dissolved phosphorus (LMW, <10,000 NMW); and colloidal dissolved phosphorus (>10,000 NMW). Colloidal samples were typically concentrated by factors of 60-80 in the NW Atlantic and Tower Tank experiments (Chapter 6), while concentration factors of 10-15 were used in the NE Pacific study.

6.3 NW Atlantic Ocean study

6.3.1 Sampling and Analysis

North Atlantic seawater samples were collected in September, 1988 during a cruise to the three stations shown in Figure 6.1: the Scotian Shelf, Scotian Slope and Gulf Stream. Water samples, taken by a Niskin bottle rosette sampler, were immediately pressure-filtered (N_2, 5 psi) through 0.45-μm Millipore filters and stored in acid-cleaned Pyrex bottles at near freezing temperature. A 20-litre Gulf Stream surface seawater sample was also collected and pressure-filtered (0.45-μm, Gelman Versapor) directly into a clean polyethylene carboy. The sample was acidified to pH 2 with sulphuric acid, stored in the dark at ambient temperature, and analyzed ashore.

A number of 60-70-litre samples were collected by repeat casts of individual Go-Flo bottles at the Scotian Shelf and Gulf Stream stations, pressure-filtered directly from the Go-Flo bottles into a Teflon reservoir and processed by ultrafiltration aboard ship. The ultrafiltrate and retentate samples were stored in Pyrex bottles at near-freezing temperature. Replicate 10-mL samples were analysed for SRP within several days of sampling. After removing aliquots for SRP analysis, samples were acidified to pH 2 with sulphuric acid and stored at 2°C until later analysis for TDP (Grasshoff, 1983). UV analysis was carried out on 60-mL samples after addition of 150 μL of H_2O_2. Persulphate analyses were performed on 10-mL samples contained in Pyrex test-tubes and sealed with polypropylene screw caps.

Also presented are results for Sargasso Sea samples taken at Station NFLUX (38°8.6'N, 62°44.9'W) on April 11, 1991. Samples (approximately 450 ml) were frozen, unfiltered, in clean Teflon containers immediately after sample draw. Sargasso Sea samples were thawed in a refrigerator over 2 days. Solutions were therefore always maintained at <4°C prior to analysis to minimize microbial decomposition of labile compounds. Once thawed, samples were filtered through acid-leached and rinsed 0.4-μm Nuclepore filters. Triplicate 20-ml samples were removed for SRP analysis. Samples were then acidified to pH 2.5 with 150 μL

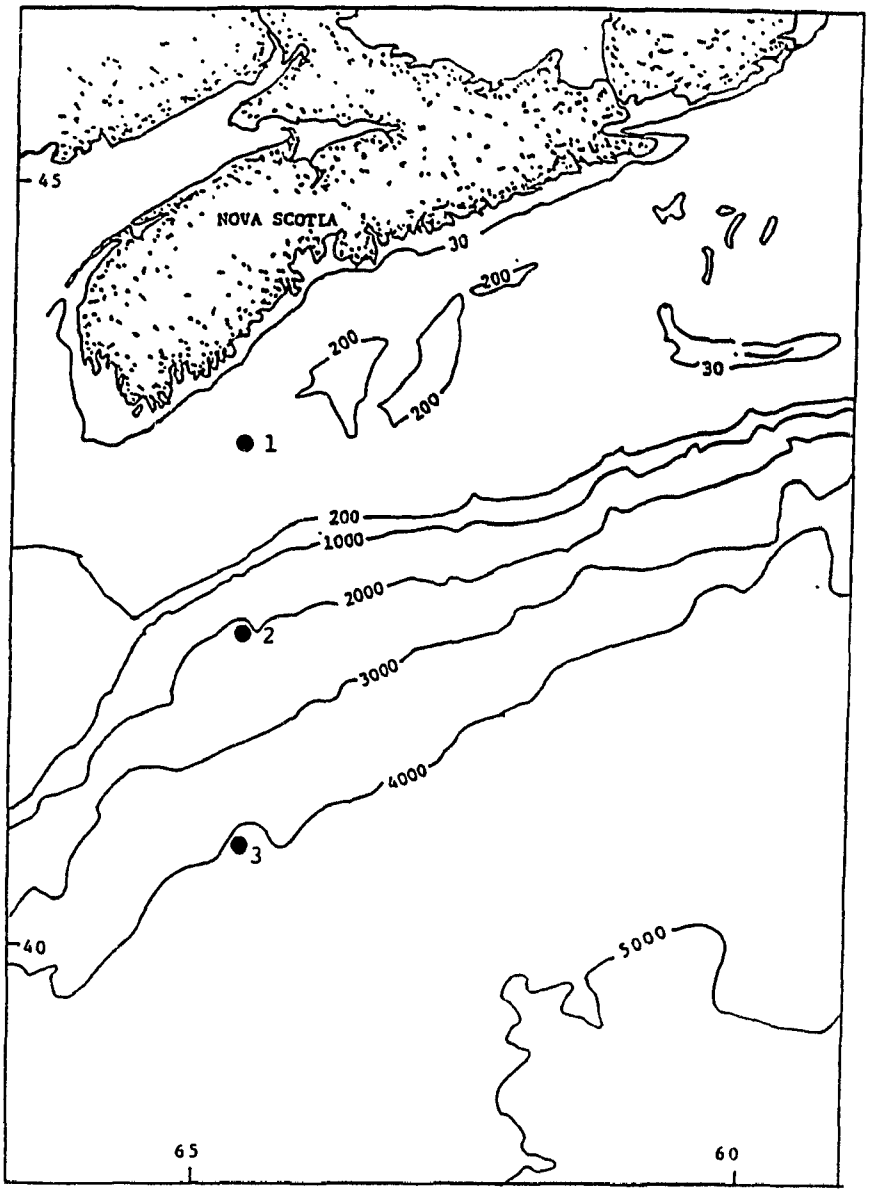


Figure 6.1. Sampling stations in the NW Atlantic collected from the *CSS Dawson*, September 6-11, 1988. Vertical profiles were taken on the Scotian Shelf (1), Slope (2) and Gulf Stream (3).

of 4.5 M H_2SO_4. A 20-ml sample was pipetted into a combusted ampoule for DOC analysis using a high temperature combustion oxidation method (Chen and Wangersky, 1992b). The remaining samples were treated by UV irradiation as described previously, except that 250 μL H_2O_2 was added to the 150-ml samples. After irradiation, samples were returned to the original containers, swirled to mix well, and aliquots were removed for analysis. Persulphate reagent was then added to the remaining samples. A 20-mL aliquot was pipetted into an ampoule for DOC analysis and sealed prior to autoclaving. Teflon containers were autoclaved with caps tightened and the tared vessels were returned to initial mass by addition of Super-Q after cooling with caps loosened under a fume hood. For the purposes of assessing DOC analysis, Wonder Water[1] (3% NaCl) samples were treated similarly, including the filtration step, except the small sample loss due to evaporation during UV irradiation was noted and the final results corrected accordingly.

6.3.2 Results and Discussion

Preliminary Results

The findings of Suzuki et al. (1985) and Sugimura and Suzuki (1987) indicate that the dissolved organic matter most resistant to chemical oxidation is contained in the surface layer of the open ocean. Hence, initial oxidation efficiency tests were performed on the 20-litre surface sample taken at the Gulf Stream station so that the optimum conditions for oxidative release of DOP could be determined. Figure 6.2 indicates that maximum release of phosphorus by UV irradiation requires at least 5 hours exposure, a much longer period than the 2 hours recommended by Armstrong et al. (1966). However, it should be recognized that the necessary exposure time will vary with the geometry of the apparatus, and the power and age of the

[1]Wonder Water is distilled water passed through a Millipore Super-Q system which is additionally purified by distillation through a 800°C catalyst-packed column. Water treated in this way is expected to contain extremely low levels of DOC (Chen and Wangersky, 1992b).

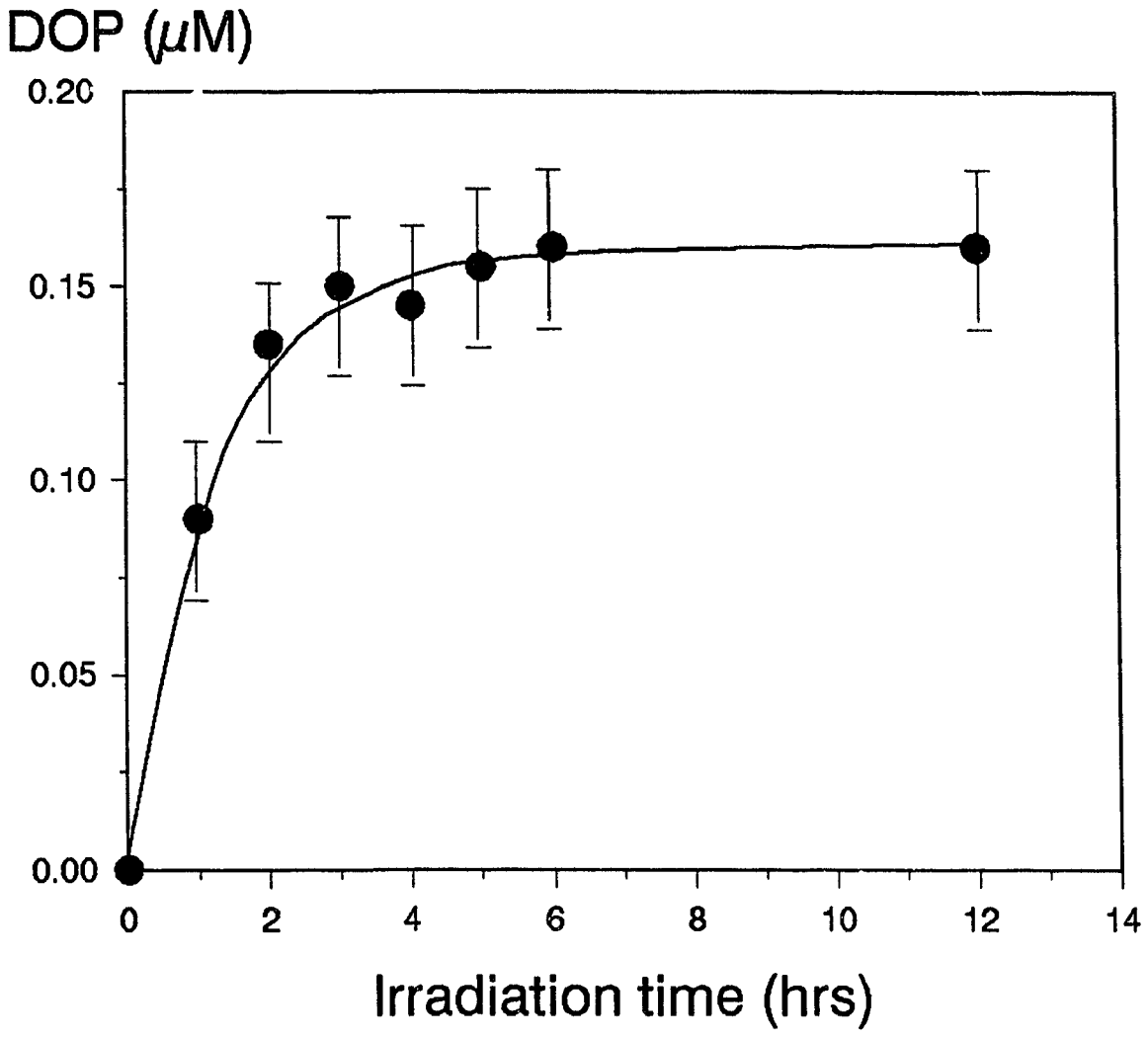


Figure 6.2. Observed DOP concentrations with UV oxidation method (1,200-W lamp) as a function of irradiation time.

lamp. All subsequent samples were irradiated for 6 hours.

It is shown in Figure 6.3 that the liberation of organically-bound P by persulphate oxidation was not complete in 30 minutes as commonly suggested (Menzel and Corwin, 1965; Koroleff, 1983). A similar observation was made by Sugimura and Suzuki (1988) for the persulphate oxidation of DOC where maximum values were found only after 4 hours of autoclaving. In the case of DOP, autoclaving samples with the standard amount of persulphate for as long as 6 hours did not release the same amount as that found with the combination methods. Persulphate oxidation alone resulted in these maximum values only when the higher concentration of persulphate was used.

Table 6.1 summarizes the results obtained using subsamples from the 20-litre Gulf Stream surface sample. Measurable increases in DOP were found with the combination method compared with the DOP concentrations measured by the standard techniques. Similar values were found with the combination method when both high and low amounts of the persulphate reagent was used. Since the low concentration method exhibited better precision and was employed with greater ease, the high concentration of persulphate was not routinely used to measure other samples in this study.

Results from Vertical Profiles

It was desired to compare the relative efficiencies of the UV, persulphate and combination methods for shelf, slope and open ocean seawater from a variety of depths. The TDP and DOP profiles measured with the combination method for the Scotian Shelf, Scotian Slope and Gulf Stream stations are displayed in Figure 6.4 together with salinity, temperature and phaeopigment (i.e. total fluorescence) data. Mixed layer depths ranged from 20-30 m at all stations and phaeopigments levels indicated that phytoplankton stocks were low in these waters in September, consistent with observed low nutrient levels (nitrate levels were below

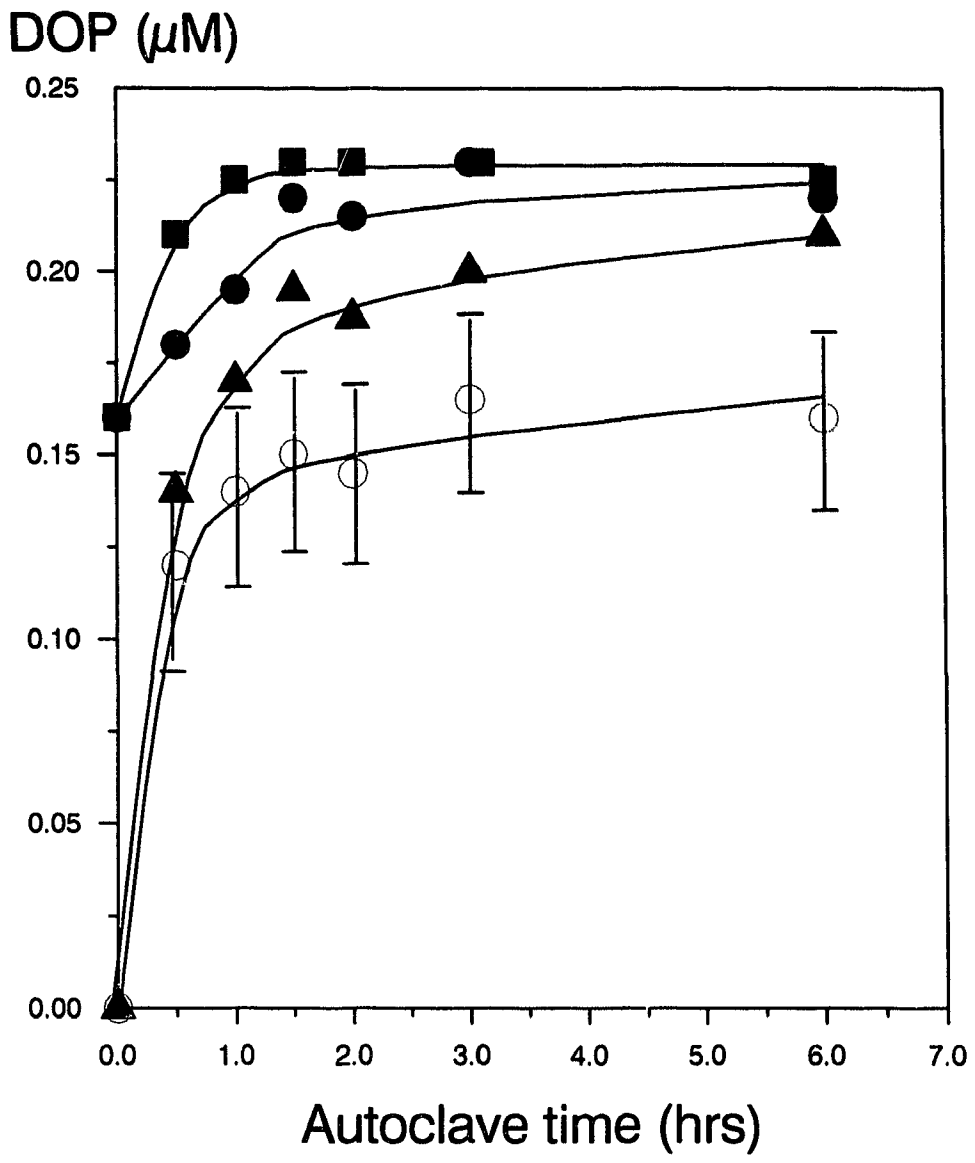


Figure 6.3. Observed DOP concentrations after application of the persulphate and combination oxidation methods as a function of autoclaving time. Samples analysed with 40 mg ml^{-1} persulphate: combination method (■), persulphate method (▲). Samples analysed with 4 mg ml^{-1} persulphate: combination method (●), persulphate method (o). Time-zero sample values for the combination method show DOP released by initial UV irradiation step. Curves fitted by eye to the data.

Table 6.1. Summary of DOP values obtained for Gulf Stream surface seawater analysed by UV oxidation, persulphate oxidation and the combination method.

	Oxidation method				
	UV	Persulphate reagent concentration		Combination reagent concentration	
		4 mg/ml	40 mg/ml	4 mg/ml	40 mg/ml
DOP (μM)	0.16 ±0.015	0.015 ±0.02	0.19 ±0.03	0.22 ±0.02	0.23 ±0.03

Values shown are for n=15±s.

detection in mixed layers at shelf and Gulf Stream stations). Higher chlorophyll levels were present in the Sargasso Sea station in April, where Chl a ranged from 1.3-1.6 μg/L in the upper 100 m (Kepkay and Wells, 1992). The corresponding DOP values are tabulated in Tables 6.2 and 6.3. DOP values measured by the combination technique were generally higher than the values found with the UV and persulphate methods individually; differences greater than experimental precisions are not observed for the great majority of samples.

A graphical method of assessing the relative oxidation efficiencies of the three methods for all samples measured is presented in Figure 6.5. The slopes of these graphs indicate that the UV technique liberates DOP with 90% efficiency of the combination method, whereas the persulphate technique is 87% as effective as the combination method. Recall that the UV and persulphate techniques have been 'optimized' for the purposes of this study; the experimental conditions more routinely used with these standard techniques would clearly have resulted in less favourable efficiencies.

Size Fractionation Study

I examined the partitioning of DOP between low molecular weight (LMW, <10,000 NMW) and colloidal (>10,000 NMW) size fractions for samples collected from the Scotian Shelf and Gulf Stream stations. The values obtained with the combination method are shown in Figure 6.6 and data from all methods are given in Tables 6.4 and 6.5. Recoveries of low molecular weight and colloidal DOP were in good agreement with the values for total DOP; therefore, any problems of contamination and sample processing loss were acceptably small. As DOP values in deep seawater samples (0.01-0.04 μM) are associated with relatively large errors (±0.02-0.03 μM), greater confidence is attributed to the colloidal values, which are much more precise (±0.002) due to high concentration factors. The results indicate 25±14%

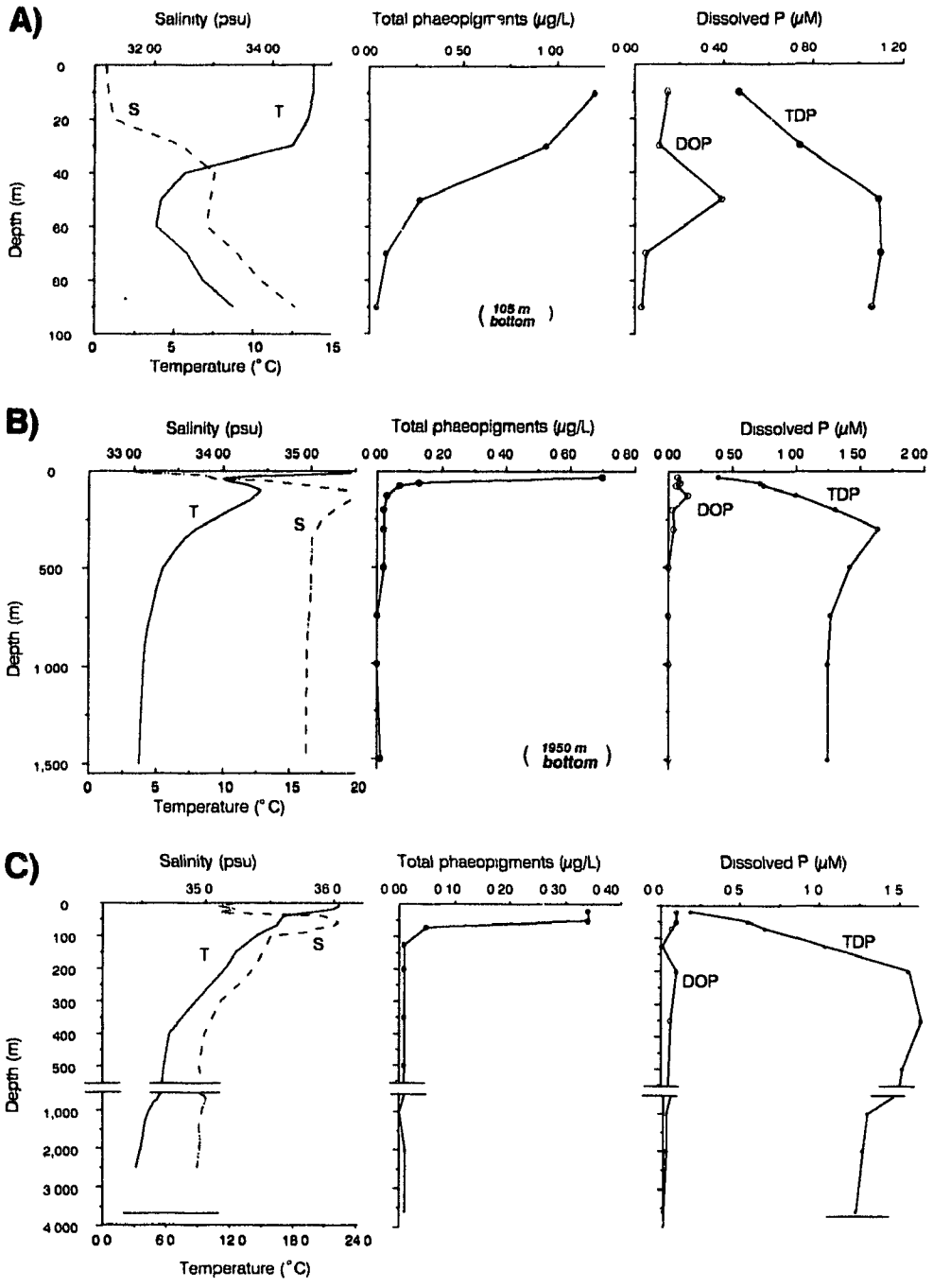


Figure 6.4. Salinity, temperature, and phaeopigment data from NW Atlantic samples taken with Niskin rosette: (A) Shelf Station, (B) Slope Station and (C) Gulf Stream Station. Also shown are total dissolved phosphorus values and corresponding DOP estimates obtained with the combination method.

Table 6.2. Observed DOP values for samples taken from Scotian Shelf and Slope Stations by Nisker rosette casts.

Depth (m)	Oxidation method		
Scotian Shelf	UV (±0.03 μM)	Persulphate (±0.04 μM)	Combination (±0.04 μM)
10	0.15	0.12	0.15
30	0.13	0.09	0.11
50	0.39	0.32	0.39
70	0.07	0.01	0.05
90	0.05	0.00	0.03
Scotian Slope Results			
30	0.06	0.04	0.07
60	0.12	0.05	0.10
75	0.06	0.02	0.06
125	0.15	0.13	0.15
200	0.05	0.00	0.03
300	0.00	0.00	0.04
500	0.00	0.00	0.00
750	0.01	0.02	0 00
1000	0.00	0.01	0.00
1500	0.00	0.00	0.00

Reported errors are the sums of respective TDP and SRP precisions (±s, n=3).

Table 6.3. Observed DOP values for samples taken at Gulf Stream Station by Niskin rosette casts. Also given are results for samples taken from Sargasso Sea.

Depth (m)	Oxidation method		
Gulf Stream	UV (±0.02 μM)	Persulphate (±0.03 μM)	Combination (±0.03 μM)
20	0.08	0.06	0.10
50	0.06	0.09	0.10
75	0.04	0.07	0.07
125	0.01	0.00	0.01
200	0.07	0.06	0.10
350	0.00	0.02	0.06
500	0.00	0.03	-
1000	0.00	0.00	0.02
2000	0.01	0.00	0.02
3600	0.00	0.00	0.03
Sargasso Sea Station NFLUX	SRP (±0.005)	UV-DOP (±0.010)	Combination-DOP (±0.015)
10	0.000	0.078	0.105
50	0.041	0.094	0.104
100	0.037	0.069	0.084

DOP values are TDP-SRP. Errors are sums of precisions for TDP and SRP determinations (Gulf Stream samples, n=4; Sargasso Sea samples, n=3). Sargasso Sea samples have lower associated errors due to use of 10 cm cell and more precise experimental blank determinations.

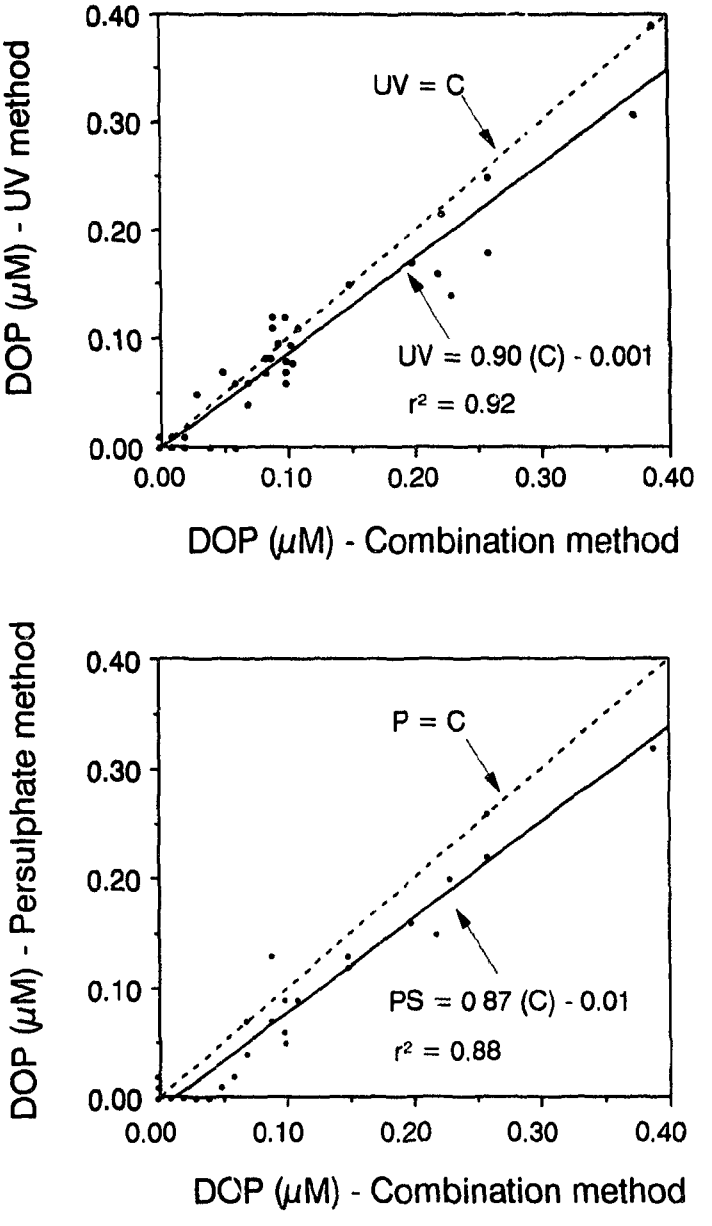


Figure 6.5. Linear regression fits (solid line) of DOP data obtained with UV method and persulphate method plotted against combination method results. Dotted line indicates ideal relationships. The slope values with 95% confidence limits are 0.90±0.06 and 0.87±0.08

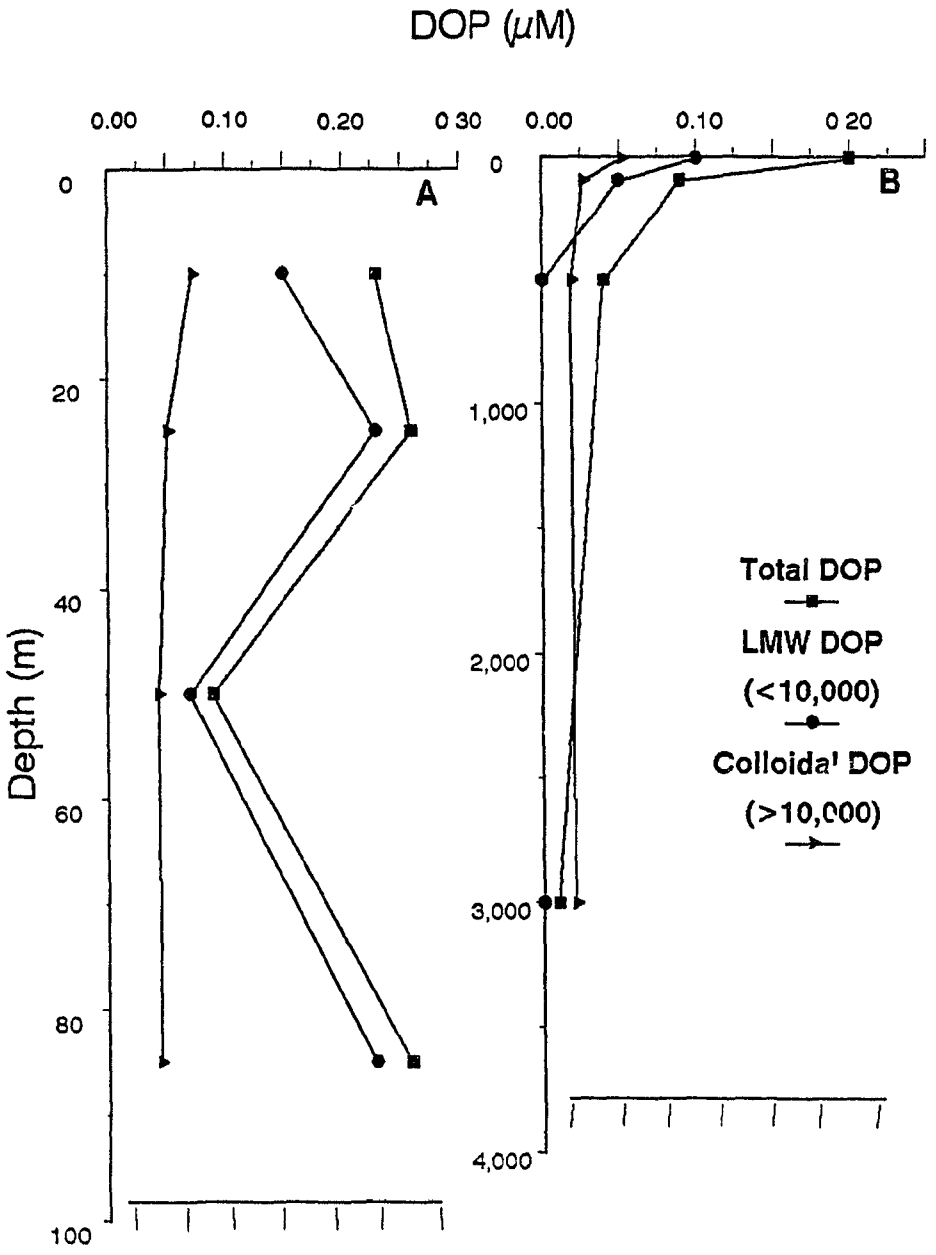


Figure 6.6. Depth profiles for ultrafiltered samples from (A) Shelf and (B) Gulf Stream Stations.

Table 6.4. Size fractionation results for Scotian Shelf samples.

	Oxidation Method		
Sample	UV	Persulphate	Combination
10 m - TDOP	0.14	0.20	0.23
LMW	0.07	0.12	0.15
Colloidal	0.069	0.071	0.072
25 m - TDOP	0.18	0.22	0.26
LMW	0.13	0.21	0 23
Colloidal	0.051	0.052	0 052
50 m - TDOP	0.11	0.13	0.09
LMW	0.06	0.10	0.07
Colloidal	0.040	0.039	0 044
85 m - TDOP	0.25	0.26	0 26
LMW	-	0.23	0.23
Colloidal	-	0.045	0.045

Errors in DOP estimates for TDOP and LMW determinations in μM: UV results - ±0.02; persulphate and combination methods - ±0.03. Colloidal results are better than ±0.002 due to concentration factors ranging from 18-86.

Table 6.5. Size fractionation results for Gulf Stream samples.

	Oxidation method		
Sample	UV	Persulphate	Combination
10 m - TDOP	0.17	0.16	0.20
LMW	0.10	0.07	0.10
Colloidal	0.052	0.049	0.052
100 m - TDOP	0.12	0.07	0.09
LMW	0.10	0.06	0.05
Colloidal	0.026	0.024	0.027
500 m - TDOP	0.01	0.01	0 04
LMW	0.00	0.00	0.00
Colloidal	0.015	0.018	0.019
3000 m - TDOP	0.00	0.00	0.01
LMW	0.01	0.00	0.00
Colloidal	0.019	0.018	0.021

Errors in DOP estimates for TDOP and LMW determinations in μM: UV results - ±0 02, persulphate and combination results - ±0.03. Colloidal results are better than ±0.001 due to concentration factors ranging from 68-75.

of DOP in coastal and open ocean samples from surface and subsurface depths was <10,000 NMW. Deep samples appear to be proportionately richer in colloidal DOP, as the colloidal fraction accounts for 50-210% of the total DOP values.

The Gulf Stream depth profile shown in Figure 6.6 indicates that low molecular weight DOP dominates in surface waters, but decreases rapidly with depth and is not detectable at depths greater than 500 m. Interestingly, although colloidal DOP levels also decrease with depth, measurable levels remain throughout the water column. While very few size fractionation data have been published, and none investigating open ocean DOP, these results are consistent with other studies in natural waters which also found that the low molecular weight DOP decreased rapidly with depth (Francko and Heath, 1979; Matsuda and Maruyuma, 1985); apparently due to high biological availability.

Results from the analysis of the crossflow filtered samples for dissolved organic carbon by the UV method of Gershey et al. (1979) found that <12% of UV-oxidisable DOC was colloidal, with highest values in surface waters (Moran and Moore, 1989). Carlson et al. (1985), using an Amicon ultrafiltration method, estimated that *ca.* 20% of persulphate-oxidizable DOC from surface seawater taken from Slope and Sargasso Sea stations was >10,000 NMW; slightly greater proportions of colloidal DOC were obtained for deep samples. These estimates are semi-quantitative given the current understanding that these wet oxidation methods underestimate DOC levels (Hedges et al., 1992)

Similar colloidal DOP values were obtained by all three methods These observations contrast with the results of DON (Suzuki et al., 1985) and DOC (Sugimura and Suzuki, 1987) size fractionation studies which indicate that the standard methods fail to fully oxidize colloidal DOM. The DOP results are in much better agreement with very recently reported size fractionation study of DOC in seawaters off Japan (Ogawa and Ogura, 1992), which

showed good agreement between values obtained by persulphate and high temperature combustion oxidation of DOC size fractions greater than 1000 and 10,000 nominal molecular weights. Further discussion of these and other (Benner et al., 1992) very recent studies of DOC size fractions is found in Section 6.4.4.

6.3.4 Summary

The sequential treatment of open ocean seawater with high intensity UV radiation and persulphate oxidation resulted in greater DOP levels for some surface samples than the values obtained with standard methods. Studies of shelf, slope and Gulf Stream seawater found that the individual UV and persulphate oxidation techniques were 90% and 87% as effective, respectively, as the combination method. These results indicate that careful measurement of DOP with standard techniques may be in error by a factor of about 1.25-1.5 for open ocean surface waters, but that the standard techniques adequately measure the DOP in other seawaters sampled. Analysis of crossflow ultrafiltered shelf and Gulf Stream seawater indicated that colloidal DOP comprised *ca.* 25% of the total DOP in surface waters. Results showing large changes of the low molecular weight fraction with depth at the shelf station, and a rapid decrease with depth at the Gulf Stream station are consistent with high biological availability of this fraction. Colloidal P forms the major portion of DOP in deep waters.

6.4 NE Pacific Ocean study

6.4.1 Introduction

Evidence has been presented which suggests that surface seawater DOC values may be 30-50% higher in the North Pacific than in the North Atlantic (Bauer et al., 1990; Suzuki et al., 1990: Kirchman et al., 1991). It was therefore questioned whether measurements of

DOP in Pacific seawaters made by the new two-step oxidation method would be higher than those measured by standard persulphate or UV oxidation procedures. I report here results of such a comparison study, using NE subarctic Pacific seawaters and including a profile taken at Ocean Weather Station "PAPA". Treatment of NE Pacific samples by crossflow ultrafiltration also provides a comparison of DOP size fractions from N. Atlantic and N. Pacific regions.

6.4.2 Materials, Methods and Sampling

Sampling was performed aboard the C.S.S. Parizeau (formerly of the Institute of Ocean Sciences, Patricia Bay) from 22 August to 7 September, 1990 during a Line P cruise (Figure 6.7). Water samples were taken with acid-cleaned 10-litre Go-Flo bottles. Samples for soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) were pressure-filtered (N_2, 0.35 kg cm^{-2}) through acid-washed 0.4-μm Nuclepore filters supported on polycarbonate filter holders. Samples were taken with thoroughly cleaned Teflon bottles after 250 ml of sample had passed through the filter. SRP values were determined immediately after sampling, using the Molybdenum Blue technique as described by Koroleff (1983) with a LKB Ultraspec 4050 spectrophotometer fitted with a 10-cm pathlength cell. Absorbances were determined at 885 nm and found to be linear over the sample concentration range. Analytical precision for shipboard SRP determinations was less than or equal to ± 0.01 μM, when taken as 1 SD of triplicate analyses.

Once subsamples had been extracted for SRP analysis, the remaining sample was acidified with sulphuric acid to pH 2 and refrigerated to stabilize the samples prior to TDP analysis. A replicate set of samples from Station PAPA were stored frozen in acid/persulphate cleaned 500-ml polypropylene bottles and analysed ashore to compare TDP recoveries obtained using IOS and Dalhousie UV systems.

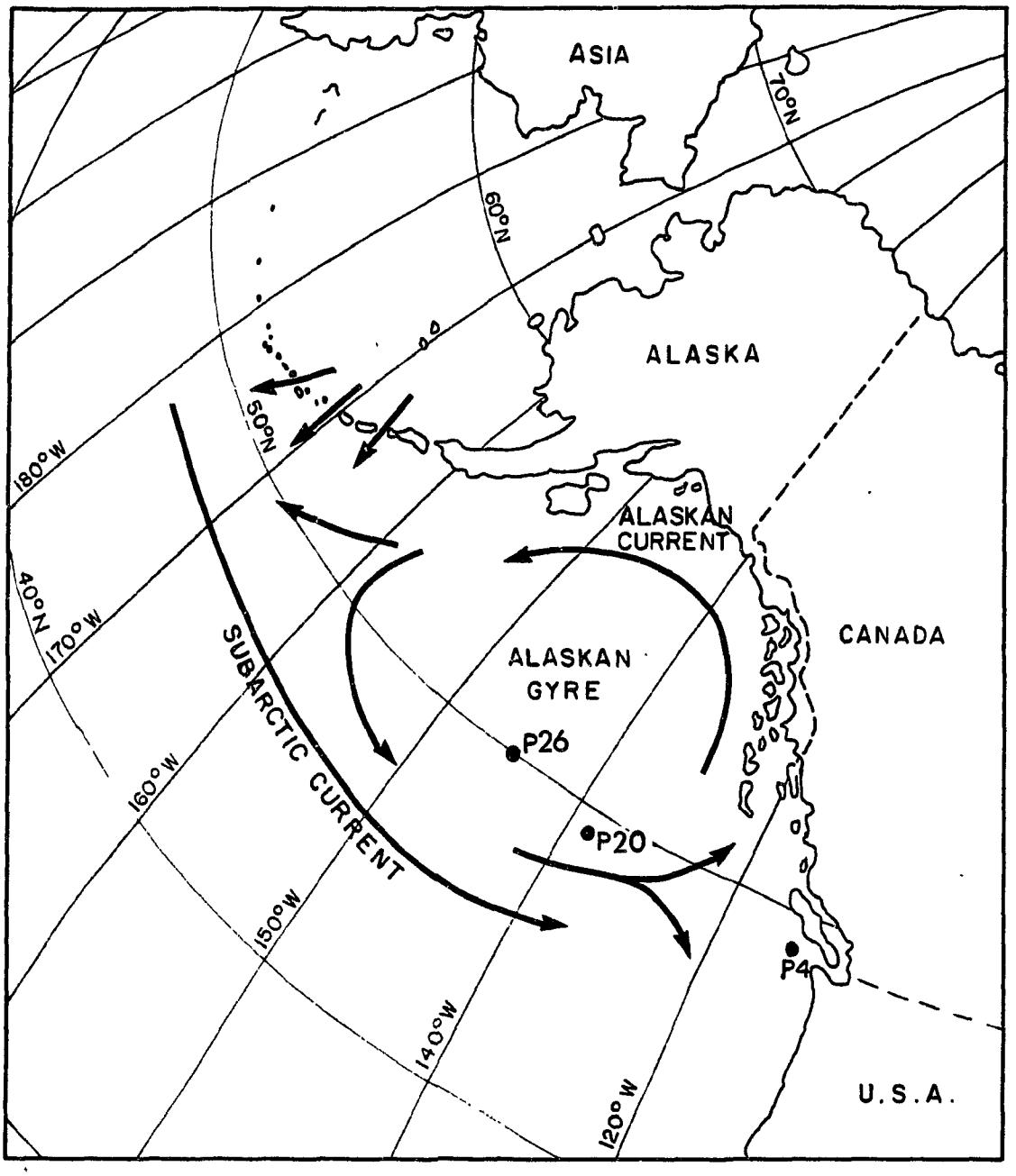


Figure 6.7. Stations where samples were collected in the NE Pacific Ocean from the *CSS Parizeau*, August 23 - September 2, 1992.

Samples for DOC analysis were taken at Station PAPA immediately after dissolved phosphorus sample draw. All-glass sample bottles were initially cleaned by soaking in 10% NaOH solution followed by rinses with double dialysed Milli-Q water (DMQ). The bottles were twice rinsed with small amounts of sample prior to collection of about 50 ml for analysis. Samples were acidified with 10% H_3PO_4 and stored in a refrigerator prior to analysis. Several subsamples were transferred with a previously baked glass syringe assembly to clean ampoules for DOC analysis by W. Chen at Dalhousie University. The ampoules were sealed by propane torch with the method described in Chapter 2 and stored on ice during transport to Halifax Shipboard DOC measurements were made with a Shimadzu TOC-500 instrument fitted with the Ionics pure platinum pillow catalyst. Integrated peak areas were corrected for a system blank by subtracting the values obtained from persulphate-oxidised DMQ and Wonder Water (21±2 μM C, see Appendix for a discussion of DOC blank).

Routine nutrient and chlorophyll samples were taken from 1.7-litre Niskin bottles and nutrients were processed on board ship using a Technicon AutoAnalyzer. Excellent agreement was found for SRP values determined manually and by AutoAnalyzer. Dissolved oxygen was determined on board ship using a semi-automated Winkler titration method, and temperature and salinity measurements were made from reversing thermometers and a salinometer

Several 6 litre samples were gravity filtered from Go-Flo bottles through 0.45-μm Gelman Mini Capsule Versapor polycarbonate filters into a 20-litre PVC carboy and ultrafiltered with the crossflow system described in Section 6.2.3, except a peristaltic pump was used in lieu of the more cumbersome air driven pump/compressor apparatus. This change had the added advantage of reducing the dead volume from 520 to 250 ml. The system was initially cleaned with sequential dilute base (Whitehouse et al., 1989), dilute acid and DMQ rinses. DOC and DOP analyses of processed DMQ water showed that the system was non-contaminating prior to sample processing. While at sea, the ultrafiltration system was rinsed

with 2 L of DMQ between sample runs, the entire system drained and rinsed with 500 ml of sample. The remaining sample was then processed until retentate volumes were approximately 500 ml. Concentration factors for the colloidal size range material ranged from 8 to 15. Ultrafiltered and retentate samples were collected in clean Teflon containers and 50 ml subsamples were removed by glass syringe for DOC analysis. Total DOC samples were taken midway through the 0.45-μM pre-filtration step.

6.4.3 TDP analyses

Total dissolved phosphorus determinations followed the methods outlined in Section 6.2.2 with the following changes. A shipboard UV oxidation apparatus was generously provided by Dr. J. Thompson of the Institute of Ocean Sciences. It was fitted with a recently purchased 550-W Hg lamp (Hanovia) that had been used for 25 h before experimental use. The lamp sat in a quartz cooling well through which a continuous flow of seawater (0.45-μm filtered) was maintained. Quartz tubes (15 cm X 1.80 cm I.D., 2.18 cm O.D.) contained 35-ml samples and the centres of the tubes were 4 cm from the walls of the UV lamp. Prior to sample analysis, all quartz tubes were checked for possible contaminating substances being released from the walls of the tubes during UV irradiation (including excessive leaching of silica) by irradiating distilled-deionised Millipore Super-Q water samples for 6 h. No detectable contamination was observed.

Surface coastal and open ocean seawater samples were used to determine the irradiation time necessary for maximum recovery. As shown in Figure 6.8, asymptotic values were achieved after about 6 h exposure. All samples were irradiated for 9 h to ensure maximum release of DOP by the UV method. Sample temperatures measured immediately following this UV irradiation period were 65 to 70°C. Due to the UV apparatus design, not all samples could be processed at the same time. Surface samples were processed immediately

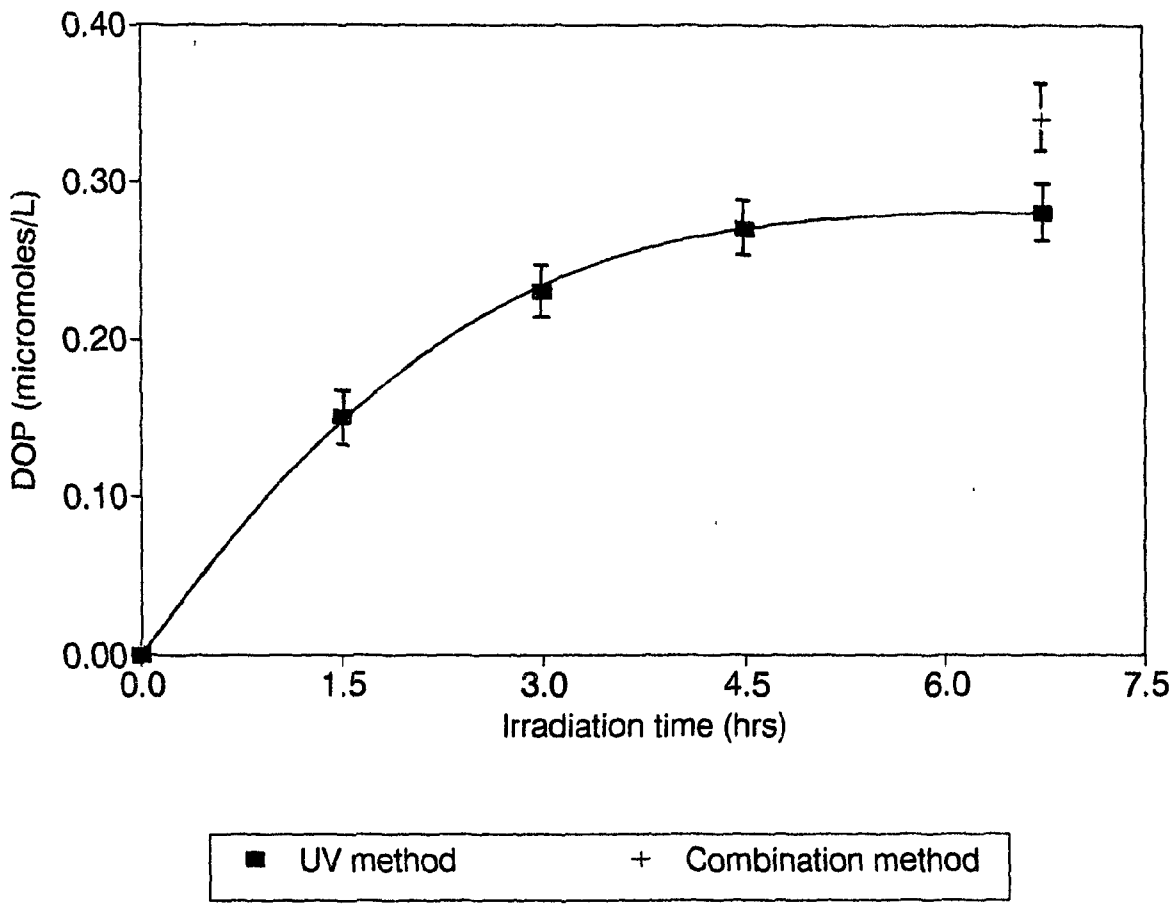


Figure 6.8. Observed DOP values from surface seawater samples as a function of UV irradiation time with the 550-W lamp method. Also shown is the value obtained from the combination method.

after collection, and all samples were processed within 32 h. Replicate samples from station P26 samples, stored frozen, were analyzed with the UV apparatus described previously (Section 6.2) which was equipped with a newly purchased 1,200-W Hg lamp (Hanovia). H_2O_2 (75 μl) was added to the samples prior to irradiation. All UV runs included a blank of Super-Q as a check against inadvertent contamination of the UV tubes, acid reagent or H_2O_2 solution. The analytical precision (1 SD) for triplicate TDP determinations by both UV methods was less than or equal to ±0.01 μM.

The acid persulphate method was modified for 25-ml sample volumes contained in Teflon reaction flasks. Teflon flasks (60 ml) were cleaned prior to analysis by leaching for several days with 4.5 M sulphuric acid, followed by applying the persulphate method with Super-Q water contained in the flasks. Analytical precision (1 SD) for shipboard TDP analyses by both the single step acid persulphate oxidation and the two-step, UV + persulphate (combination) method was ±0.015 μM.

6.4.4 Results and discussion

General characteristics of the region

The NE subarctic Pacific waters sampled are within the Alaskan gyre system which is bounded by the easterly flowing Subarctic Current to the south, and the westerly flowing Alaskan Current to the north (Figure 6.7). In this region, a 100-120-m layer of low salinity water caps the permanent halocline, and upwelling across the halocline is limited to a few tens of meters a year (Tabata, 1974). Chl a levels are uniformly low throughout the year and phytoplankton stocks are dominated by <10-μm sized plankton (Booth, 1988). High inorganic nutrient concentrations are observed throughout the year (surface NO_3 levels are always > 5 μM (Anderson et al., 1969)), which has been explained by grazer limitation of phytoplankton (Frost, 1987); Fe limitation (Martin et al., 1989); and grazer enhanced recycling and

preferential $[NH_4]^+$ uptake (Wheeler and Kokkinakis, 1990).

Oceanographic data shown in Figure 6.9 are measurements of samples taken at two open ocean stations (P20 and P26 "PAPA") in the NE subarctic Pacific. Stations P20 and P26 had mixed layer depths of about 30 m with the high nutrient concentrations typical of the subarctic Pacific waters (Anderson et al., 1969; Martin et al., 1989). Seasonal thermoclines and well-developed subsurface O_2 maxima also are observed in the upper 125 m at these stations. Chl a max values were 0.6 $\mu g\ l^{-1}$ for P20 and 0.3 $\mu g\ l^{-1}$ at P26, the latter being a typical value for phytoplankton standing stock at Station "PAPA" in August (Booth, 1988). At the coastal/slope station (P4, 1,300-m depth), the mixed layer was about 10 m, and in this layer nutrients were moderately depleted (0.45 μM PO_4; 0.8 μM NO_3; 3.0 μM Si), and Chl a levels were 0.8 $\mu g\ l^{-1}$ and uniform to 20 m.

Comparisons of TDP methods

DOP data are presented in Table 6.6 for the three NE Pacific stations as measured by UV, persulphate and combination methods. As found for NW Atlantic samples, the combination method measured more DOP than the other methods for the great majority of NE Pacific samples, with largest differences between methods generally found for samples taken from above 100 m. Samples taken from below this depth show better agreement between the methods. These trends suggest production of chemically resistant phosphorus compounds in upper layers by the biota, and degradation by microorganisms on time scales shorter than those of subsurface mixing processes.

Figure 6.10 presents regression analysis graphs derived from all NE Pacific samples analyzed by the three different methods. The slope of the graph is a measure of the relative efficiency of the UV or persulphate method when compared with the combination method. The slope of the line for the UV method was 0.71 ± 0.09 (95% C.I.) suggesting an approximate

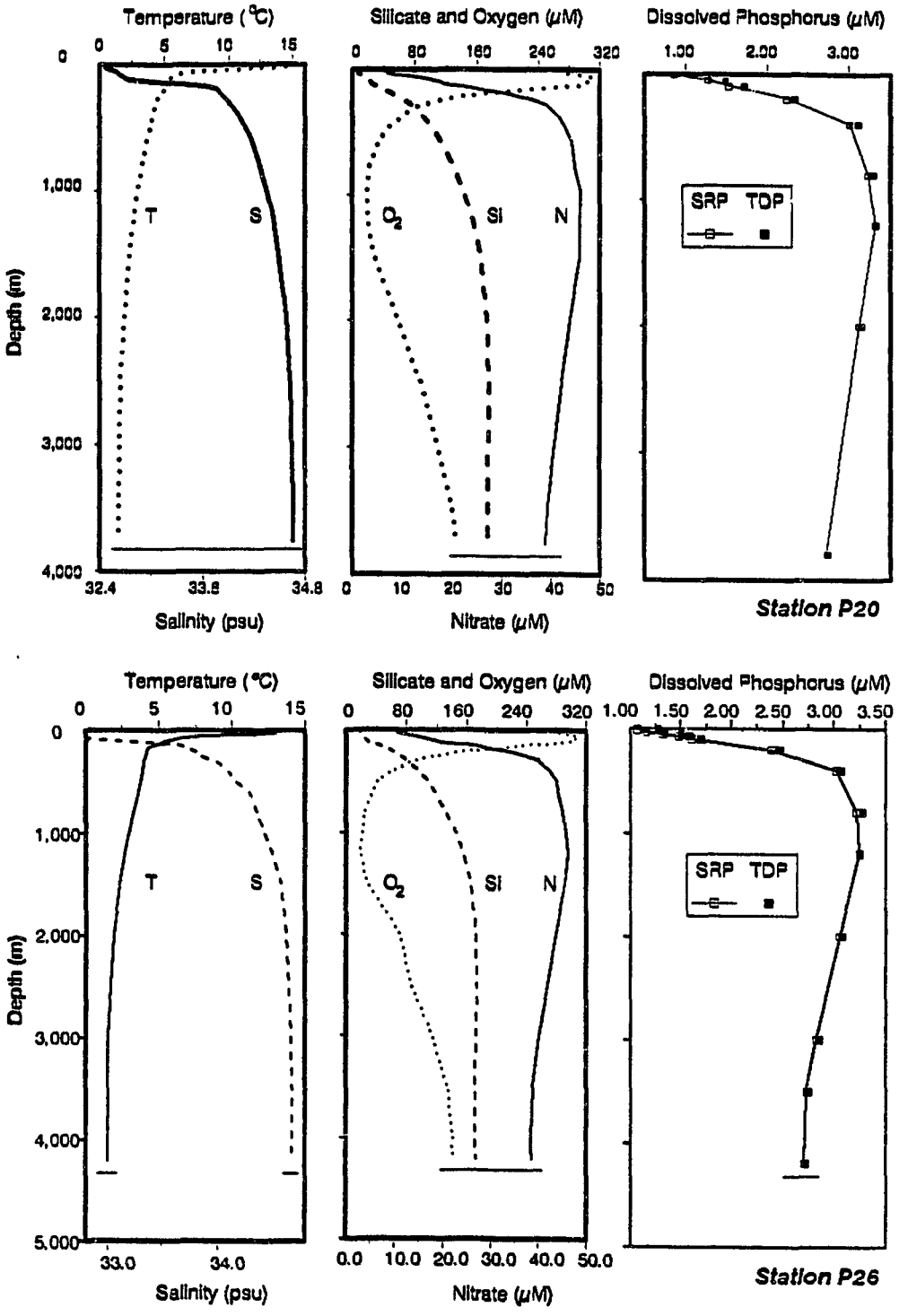


Figure 6.9. Hydrographic and nutrient data at Station P20 and P26. Also shown are TDP values measured by the combination method.

Table 6.6. Shipboard measurements of DOP from samples taken at slope (P4) and open ocean stations (P20 and P24) in the NE Pacific.

		DOP (μM)		
Station	Depth (m)	UV (±0.02)	Persulphate (±0.025)	Combination (±0.025)
P4	2	0.29	0.33	0.38
	10	0.24	0.33	0.31
P20	10	0.19	0.21	0.21
	50	0.17	0.21	0.21
	100	0.06	0.12	0.18
	200	0.08	0.06	0.10
	400	0.07	0.05	0.10
	800	0.07	0.08	0.06
	1200	0.00	0.02	0.00
	2000	0.00	0.05	0.03
	3800	0.01	0.03	0.01
P26	10	0.14	0.18	0.24
	50	0.13	0.14	0.17
	70	0.11	0.08	0.14
	100	0.11	0.11	0.12
	200	0.10	0.08	0.10
	400	0.06	0.04	0.07
	800	0.02	0.04	0.03
	1200	0.05	0.04	0.04
	2000	0.01	0.04	0.04

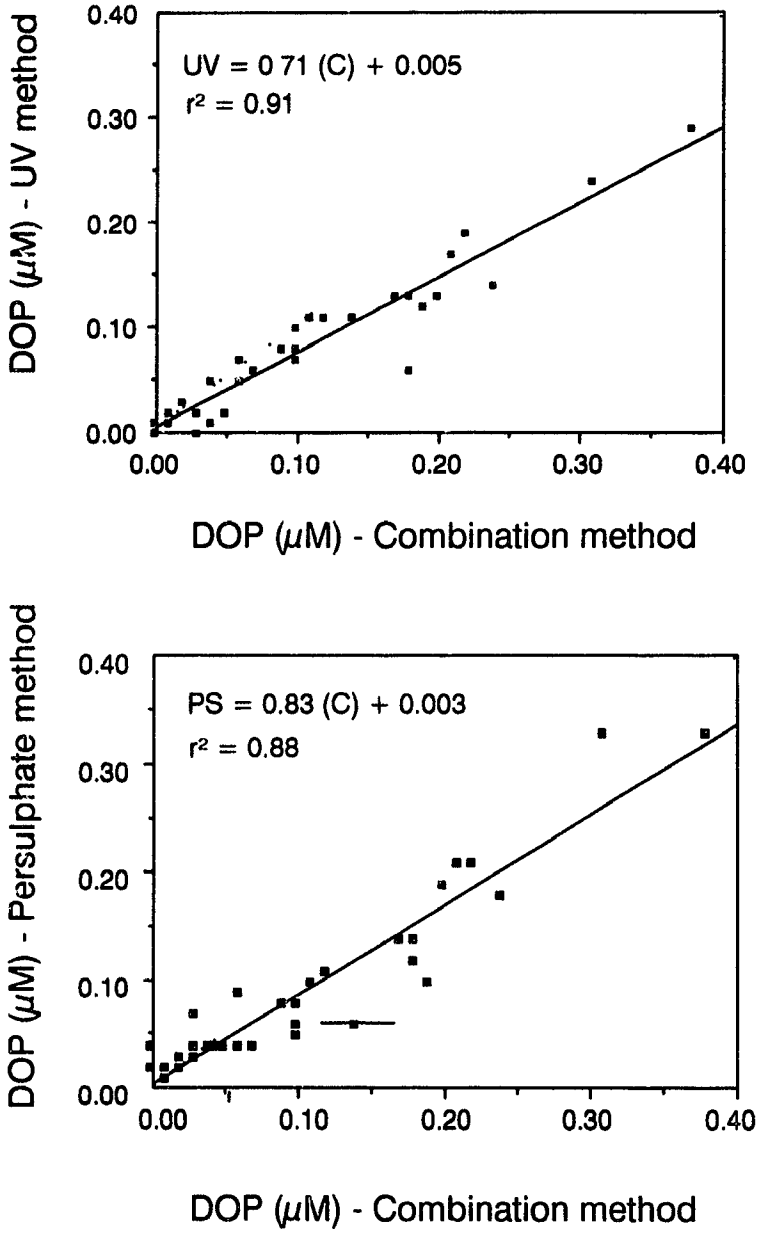


Figure 6.10. Linear regression fits of UV method and persulphate method data with data obtained from the combination method for NE Pacific samples. The regression slopes with 95% confidence limits are 0.71±0.09 and 0.83±0.09 for the respective UV and persulphate data

71% efficiency relative to the combination method, while the persulphate graph slope of 0.83 ± 0.09 suggests an 83% efficiency for DOP recovery by that method.

Since studies of DOP in marine environments are often limited to the upper water column (Jackson and Williams, 1985; Orrett and Karl, 1987), a statistical analysis of variance was performed for P20 and P26 data (n = 8) from the upper 100 m, pooled on the basis that these values were measured from similar oceanic environments. While the analysis resulted in a rejection ($p = 0.147$) at the 0.05 level of significance of the hypothesis that a difference between the combination and persulphate data exists, the hypothesis was accepted ($p = 0.0199$) at the 0.05 level of significance for an analysis comparing the UV and combination method results. However, the DOP levels measured by the combination method are still well within the range of DOP values (0.1-0.4 μM) previously reported for Pacific ocean surface samples measured with standard (Jackson and Williams, 1985; Orrett and Karl, 1987; Walsh, 1989) or specialized TDP procedures (Solorzano and Strickland, 1968; Cembella et al., 1986; Karl and Tien, 1992). A similar range of DOP values were measured with the combination method for surface seawater samples from the NW Atlantic.

When the relative efficiencies obtained by the correlation method were compared with those found from the earlier study with NW Atlantic seawaters (UV, 90±6%; persulphate, 87±8%), it appeared that the UV method used for this study performed poorly compared with the combination and persulphate methods. In order to test whether poor recovery was a function of UV apparatus, replicate samples from P26 were frozen and analysed at Dalhousie using the 1,200-W Hg lamp UV method. Regression analysis of these UV DOP values against the combination method values resulted in a slope of 0.63, in good agreement with the slope of 0.65 derived from a regression of the Station P26 values measured at sea. This comparison study suggests that the shipboard method was operating efficiently, therefore the relatively low DOP recovery obtained for subarctic Pacific Ocean samples may be evidence for different

reactivities to UV of DOP from differing oceanic regions. Other workers have noted that the UV method can result in variable recoveries of DOP for seawater taken from different marine environments (Solorzano and Strickland, 1968; Armstrong and Tibbets, 1968). As reviewed by Parsons and Lalli (1988), the NE Pacific and NW Atlantic represent disparate nutritive and ecological environments; it is perhaps surprising that even greater differences were not found between the DOP measurements from these two regions (see also the ultrafiltration results, Section 6.4.4).

The chemical nature of dissolved phosphorus compounds resisting UV oxidation (+ relatively mild acid hydrolysis) was investigated further by determining the amount of phosphate recovered by an additional, more rigorous, acid hydrolysis step. Additional acid hydrolysis should provide an upper estimate of any meta- and polyphosphates not recovered by the UV method, but measured by the combination method. As shown in Table 6.7, the concentrations of dissolved phosphorus compounds recovered by additional acid hydrolysis were small ($\leq$0.02 μM); therefore, the major portion of the additional phosphorus recovered by the combination method appears to be composed of organically-bound compounds that resist acid hydrolysis and UV oxidation.

Such chemically-resistant P compounds might include dissolved phosphonates. Phosphonates are known products of marine biota (Cembella, 1984), and have been identified in marine sediments by ^{31}P NMR (Ingall et al., 1990). Phosphonates resist perchlorate oxidation (Cembella et al., 1986) and acid hydrolysis (Cembella and Anitia, 1986). A common algal P metabolite, 2-aminoethylphosphonate, required a very long UV irradiation time (18 h) for quantitative release of the organically-bound phosphorus. Aminophosphonates are susceptible to breakdown by marine microbes, although degradation processes are inhibited by the presence of easily degraded orthophosphate-containing compounds, such as phosphate monoesters (Rosenberg and La Nauze, 1967). These compounds may therefore be degraded

Table 6.7. Some laboratory analyses of freeze-stored P26 samples, including results from acid hydrolysis of UV irradiated samples.

Depth (m)	DOP (μM)			
	Persulphate (±0.015)	UV (±0.01)	UV + H (±0.015)	Combination (±0.015)
30	0.14	0.13	0.15	0.18
50	-	0.12	0.14	0.19
70	0.10	0.11	0.11	0.11
200	0.03	0.05	0.04	0.06
400	0.04	0.02	0.02	0.05

Samples treated by acid hydrolysis were acidified to pH 1 with sulphuric acid and autoclaved in Teflon bottles for 1.5 hr, which are similar conditions to those recommended by Koroleff (1984) for analysis of dissolved polyphosphate.

slowly on biological time scales (days to weeks), but broken down on time scales shorter than those for subsurface mixing processes (tens of years), consistent with the observed trend for lower concentrations of chemically-resistant DOP in deeper waters (Table 6.7).

A relationship between DOP and AOU?

Figure 6.11 shows graphs of AOU vs. DOP, measured by the combination method, for the two open ocean stations. Both graphs show rapid decreases of DOP with depth to the O_2 minimum, with corresponding increases of AOU. DOP values below the O_2 minimum change marginally, while AOU decreases. These results are similar to one of the most provocative features of the Sugimura and Suzuki (1988) data, which was a high negative correlation of DOC with apparent oxygen utilization (AOU). However, such correlations were not found by Tanoue (1991), who re-examined DOC and AOU relationships at two stations in this region of the Pacific.

As discussed in Chapter 1, Sugimura and Suzuki (1988) found a high linear correlation between DOC and AOU, and suggested that consumption of O_2 in deep waters was driven by DOC regeneration. This approach was criticised by Toggweiler (1989), who pointed out that any discussion of the factors driving AOU for a given water mass must take into account 'pre-formed' DOM concentrations, the effects of mixing, and the contribution from regeneration of organic particulates. Unless DOP levels in surface waters in the areas of deep water formation are much higher than measured elsewhere, downwelling waters contain too little DOP to account for appreciable O_2 consumption, and therefore vertically settling organic particles are the main sources of organically-combined phosphorus regenerated in deep waters.

The apparent inverse relationship between DOP and AOU does, however, indicate a pool of slowly decomposing DOP in subsurface waters; very low DOP values are found in the

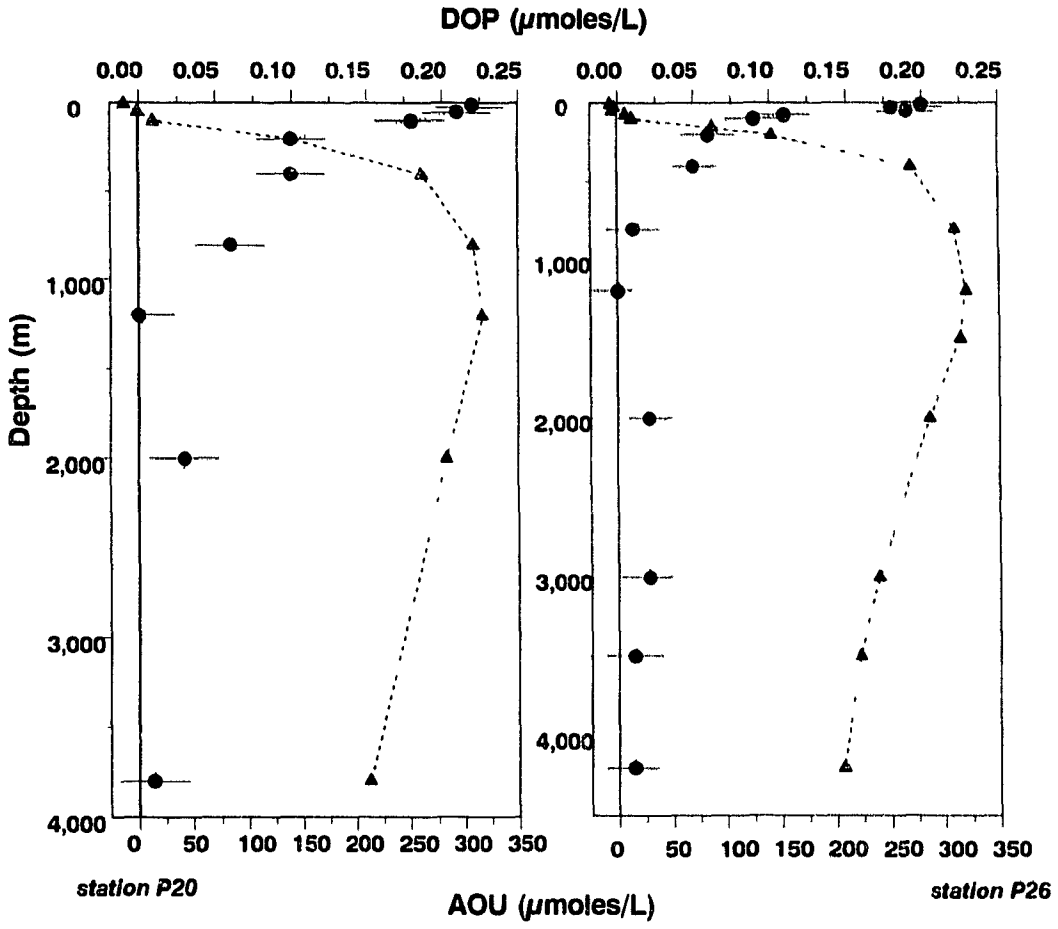


Figure 6.11. Vertical profiles of DOP (●) and AOU (▵) at the two open ocean stations

oldest waters. The observation that DOP values can fall to near detection limits in deep waters supports the idea that, within the timescales of oceanic mixing, all DOP measured by the combination method is available to the microbiological community. In the light of the measurement of a significant pool of very old DOC (Williams and Druffel, 1987), it would appear that this refractory DOM, having survived a number of oceanic mixing cycles, is depleted of P-containing compounds.

Size fractionation results

I employed crossflow ultrafiltration to investigate whether a similar molecular weight distribution would be found for samples from the NE subarctic Pacific as was found for NW Atlantic samples. Table 6.8 gives the size fractionation DOP and DOC data obtained using the crossflow technique. Total DOP analyses agree within experimental precision with the sum of low molecular weight and colloidal fractions, and the sum of DOC fractions averaged 102±20% of total DOC values. DOC and DOP analyses of 0.45-μm Gelman-filtered samples were in good agreement with 0.4-μm Nuclepore-filtered samples (Tables 6.8 and 6.9). These results suggest problems of contamination and processing losses were minimal.

DOP analyzed by ultrafiltration of surface samples was mainly <10,000 NMW material (75-85%), in agreement with the DOP fractionation results found for the NW Atlantic. While the portion of colloidal DOP was small (17-22%) in surface samples, subsurface samples became proportionately richer in colloidal P, with 28% of the 100 m DOP and 100-460% of the DOP in deep samples recovered in the colloidal fraction. A similar trend was observed for crossflow ultrafiltered samples from the NW Atlantic. It is also interesting to note that C:P ratios of the total DOP increase substantially with depth (from 300 to 9000), whereas the ratios in the colloidal fraction remain near constant (*ca.* 250). These observations

Table 6.8. Size fractionation data obtained by crossflow filtration of coastal (P4) and open ocean (P26) samples.

Sample		Size Fraction Total	LMW	Colloidal	%Colloidal
Station P4	2 m	0.29 (0.33)	0.20	0.063	22
	1000 m	0.01	0.01	0.010	100*
Station P26					
	10 m - DOP	0.20 (0.20)	0.16	0.032	16
	DOC^1	68	62	12.6	18
	DOC^2	64	78	9.7	15
	mean C:P	330	435	250	
	100 m - DOP	0.12 (0.09)	0.11	0.034	28
	DOC^1	75	54	10.1	17
	DOC^2	—	35	7.5	-
	mean C:P	625	370	215	
	1200 m - DOP	0.005	0.00	0.023	460*
	DOC^1	45	30	7.0	15
	DOC^2	36	30	5.1	14
	mean C:P	8100	-	263	

P26 DOP data are analyses of freeze-stored samples by the combination method (±0.015 μM, colloidal values ±0.002); Station P4 results were measured at sea (±0.025 μM, colloidal values ±0.005 μM). Results for Station P4 at 2 m obtained by the persulphate method. DOC^1 refers to shipboard analyses with the Shimadzu TOC-500 instrument. DOC^2 refers to analyses with Dalhousie instrument. Total refers to values after 0.45-μm Gelman prefiltered samples; LMW to <10,000 NMW fraction and colloidal to >10,000 NMW fraction. Values in parenthesis are TDOP values obtained for 0.4-μm Nuclepore filtered replicates. % colloidal values denoted with a * have high relative errors since TDOP values and LMW values are near the precision limits.

are consistent with preferential biological uptake of the low molecular weight DOP fraction, containing compounds such as phosphate monoesters which are labile to enzymatic hydrolysis, compared with the colloidal fraction, thought to contain components such as phosphoproteins, DNA and other polynucleotides which require extensive exoenzymatic breakdown prior to heterotrophic uptake (Cembella et al., 1984).

It was found that the majority of DOC is low molecular weight (83±25% by direct measure). The proportion of colloidal DOC recovered by ultrafiltration was relatively invariant with depth (15±2% by direct measure). These results are in good agreement with very recent high temperature oxidation studies of central Pacific seawater analysed by crossflow ultrafiltration (Benner et al., 1992; Ogawa and Ogura, 1992). Approximately 33% of open ocean surface DOC was found to have a nominal MW greater than 1000, compared with 22-25% in deep seawater samples (Benner et al., 1992). Similar proportions (30-37%) of DOC was >1000 NMW in coastal seawater off Japan, and only 5% of the DOC in these samples was >10,000 NMW (Ogawa and Ogura, 1992). Carbohydrates were found to comprise *ca.* 50% of open ocean surface DOC and *ca.* 25% of deep water DOC with molecular weight >1000 daltons (Benner et al., 1992). The levels of carbohydrate in the DOC isolated by ultrafiltration are consistent with the low concentrations of DOP in colloidal samples observed in the present work, and with the trend toward higher proportions of DOP in colloidal fractions of deep samples.

6.4.5 Conclusions

There is an increasing awareness that dissolved organic matter plays important roles in biological and chemical oceanic systems (Hedges, 1987). It is therefore important to examine critically the sizes of various organic compartments. Using a rigorous chemical

oxidation/hydrolysis procedure, I found that mixed layer DOP values ranged from 0.17 - 0.38 μM for NE Pacific seawaters. The standard persulphate method detected 83% and the UV method 71% of the DOP measured with the combination method, compared with respective values of 87 and 90% for North Atlantic seawaters. As discussed in the Appendix to this Chapter, DOC analyses using high temperature combustion oxidation techniques suggest that $\leq$0.07 μM P may not be accounted for by the combination method. Therefore, although small underestimates of the oceanic DOP may have been possible, these results suggest that historical oceanic analyses have not grossly underestimated DOP concentrations. A similar conclusion was reached by Karl et al. (1992b) based on the agreement between TDP results from UV and high temperature oxidation (Karl and Tien, 1992) analyses of central Pacific seawaters, and on their occurrence in the Redfield proportion with TDN values measured by high temperature combustion oxidation. DOP results from the NE Pacific fall to near the detection limits in oxygen minimum seawaters, suggesting that the bulk of measured DOP compounds are ultimately available to the marine microbiological community.

Appendix to Chapter 6

How much DOC resists oxidation by the combination method?

It may still be asked if the combination method does indeed quantitatively measure the DOP in seawater. We can only be comfortable that this goal has been accomplished if it can be shown that all the organic matter in the seawater sample has been destroyed. This goal can be obtained, in theory, by analysing samples for DOC before and after oxidation by the combination method. To this end, selected open ocean samples were analysed for DOC prior to and following application of the combination method by three high temperature combustion techniques: (1) the technique of Sugimura and Suzuki (1988); (2) the method of Chen and Wangersky (1991) and (3) a Shimadzu TOC-500 analyser containing the Ionics pure platinum catalyst.

The data from this exercise are presented in Table 6.9. Results from samples analysed with the technique of Sugimura and Suzuki (1988) were not conclusive. While the analysis indicated that roughly 55% of seawater DOC was resistant to the combination method, consistent with their NW Pacific results which showed 50-65% of DOC was resistant to persulphate oxidation, the results obtained for Wonder Water and oxidised Wonder Water are disconcerting. Both results are very similar to the DOC recovered from Gulf Stream seawater after it had been oxidised by the combination method. On the other hand, analyses by W. Chen show low values for replicate Wonder Water samples and a value of 150 μM C for the seawater sample. Unfortunately, persulphate-oxidised samples could not be analysed by his technique. Nevertheless, if contamination of Wonder Water samples occurred during sealing or at some other time, uniformly high results should have been obtained by both instruments. Results from the Sugimura and Suzuki analysis are more consistent with a large instrument blank. This speculation may be merited given current uncertainty regarding the accuracy of

values presented in the Sugimura and Suzuki (1988) publication (P. Wangersky, pers. commun.)

The results from analyses of Sargasso Sea and NE Pacific seawater samples are more consistent. Determination of instrument blanks remains problematic so upper and lower limits for the amounts of resistant DOC have been calculated. Upper limits were obtained assuming no instrument blanks exist. Lower limits were obtained when it was assumed that the values obtained from analysis of Wonder Water samples equalled the instrument blank. As shown in Table 6.9, the lower limits of DOC resisting oxidation ranged from 0-13 μM C, while upper limits ranged from 22-36 μM for seawater samples containing 58-108 μM C. While it is uncertain to what extent phosphorus-containing molecules form part of the resistant fraction, possible phosphorus contents were calculated assuming a C:P ratio of (500:1), an average value for dissolved organic carbon and phosphorus from surface samples oxidised by the combination method. Depending on the actual values of DOC blanks, possible P underestimates by the combination method range from 0.0-0.03 to 0.04-0.07 μM P. Since analytical precision for DOP methods typically range from $\pm$0.01-0.04 μM, the lower range of 'extra' DOP values would not significantly affect DOP estimations. The upper range values, if present, would be sufficiently large that comparison of the combination method with an absolute reference procedure should show significant differences. However, since standard persulphate and UV techniques generally show agreement with the combination method and rigorous dry-ashing techniques (e.g. baking at 450°C followed by weak acid hydrolysis, Solórzano and Sharp, 1980; with $Mg(OH)_2$ precipitation of all P compounds, centrifugation, and sequential treatment of the pellet to 8 M HCl hydrolysis, dry-ashing for 3 hr @450°C, and acid persulphate oxidation, Karl and Tien, 1992), it is difficult to imagine that significantly greater amounts of organically-bound P are present in seawater than the upper limit of 0.07 μM determined here.

Table 6.9. High temperature combustion analyses of seawater samples before and after oxidation by the combination method. DOP values obtained with the combination method are also shown.

SAMPLE	Suzuki HTCO (μM C)	W. Chen HTCO (μM C)	DOP (μM P)
10 m Gulf Stream	171	150	0.22±0.02
after oxidation	94	-	
Wonder Water	92	15	0.00
after oxidation	80	-	0.00
NE Pacific Seawater Station 'PAPA'	Shimadzu 500		
10 m	68-90	64-86	0.20±0.015
after oxidation	13-36	-	
100 m	61-83	-	0.12±0.015
after oxidation	6.7-29	-	
1200 m	45-64	36-58	0.00±0.015
after oxidation	8.3-31	-	
Wonder Water (3% NaCl)	0-21	0-22	-
after oxidation	1-22	-	
Sargasso Sea Seawater			
10 m	-	66-88	0.105±0.015
		4-26	
50 m	-	62-84	0.104±0.015
		—	
100 m	-	58-80	0.084±0.015
		6-28	
Dalhousie Aquatron	-	86-108	0.375±0.015
		0-22	
Wonder Water (3% NaCl)	-	0-22	0.00±0.005
		1-23	0.00±0.005

Gulf Stream sample stored acidified at 4°C for approximately 7 months prior to DOC analyses. NE Pacific results are for 0.4-μM samples analysed at sea.The Shimadzu TOC-500 instrument belonged to C.S. Wong and the combustion column was packed with the Ionics pure platinum catalyst. Treatment of Sargasso Sea samples is described in Section 6.2. Precision of the HTCO instruments (typically <±5%) is better than the accuracy due to uncertainties in the blanks.

Chapter 7

Testing Three Analytical Methods for Measuring Total and Dissolved Phosphorus in Seawater: a Mesocosm Bloom Experiment.

7.1 Introduction

Phytoplankton blooms are common events in coastal and open ocean waters (Glover et al., 1988; Karl et al., 1992a) and are known to result in uniquely high concentrations of dissolved organic compounds (Ittekkot et al., 1982). To pursue the applicability of methods for analysis of dissolved phosphorus in marine waters, and to investigate sources of DOP in seawater, a mesocosm bloom experiment was undertaken in January and February, 1990, using the Dalhousie University Aquatron Tower Tank facility. The bloom experiment also provided an excellent opportunity to extend the research to include a comparison of the UV, persulphate and combination methods for analysis of total phosphorus (TP) samples.

A bloom experiment is a unique approach to the comparison of total phosphorus and total dissolved phosphorus techniques, since it allows a comparison of the efficiencies of the methods on a wide range of organic material produced during the different algal growth phases. TP and TDP methods have generally been compared using selected chemicals, biological samples and/or seawater samples (see Chapter 5). The mesocosm approach enables a phytoplankton bloom to be initiated in a much larger water mass than typical batch culture experiments. 'Bottle effects' are minimized. Relatively large water samples (20-60 L) can be analysed by the crossflow ultrafiltration method, and concentration factors comparable to those for open ocean samples can be obtained. Most importantly, the mesocosm approach allows investigators to sample the biochemical changes produced by a phytoplankton bloom within a large vertical column of seawater maintained under controlled conditions. Bloom studies at sea can be confounded by the interplay of temporal and spatial variability, which often prevents assigning causal relationships between, for instance, primary production and

dissolved organic matter production (see, for example, Kirchman et al., 1991; Hollibaugh et al., 1991).

The principal objectives for this experiment were as follows:

(1) Determine the efficiency of UV and persulphate methods compared with the combination method for TDP analyses during a phytoplankton bloom, and test these approaches for TP determinations.

(2) Examine the dynamics of particulate and dissolved phosphorus fractions during the phytoplankton bloom.

(3) Characterize DOP by crossflow ultrafiltration procedures to shed light on sources of such size fractions in the open ocean.

7.2 Materials and Methods

7.2.1 Dalhousie Tower Tank

The Tower Tank facility is a 10.4 m high, 3.7 m diameter tank that contains a total volume of 117 m^3. An overhead array of mercury vapour and metal-halide lamps provided light levels near 40% of typical mid-day summer sunlight. Prior to filling the tank, the sides were rinsed for three days with sandbed filtered seawater and given a final thorough rinse with distilled water. The tank was filled with 1-μm filtered seawater on January 12 and 13, and a strong thermal gradient established at approximately 1 m. Stratification was accomplished by filling to the 1 m mark with ambient NW Arm seawater, then finishing the fill with seawater heated to 22°C. It was hoped that a thermal barrier would contain the bloom within the upper layer. However, the thermocline did degrade significantly over the course of the experiment as shown in Figure 7.1. Thermal degradation occurred relatively early in the experiment, and may have been caused mainly by additions and mixing in of seed material.

A plastic divider partitioned the tank into two halves: a dark side shielded by an

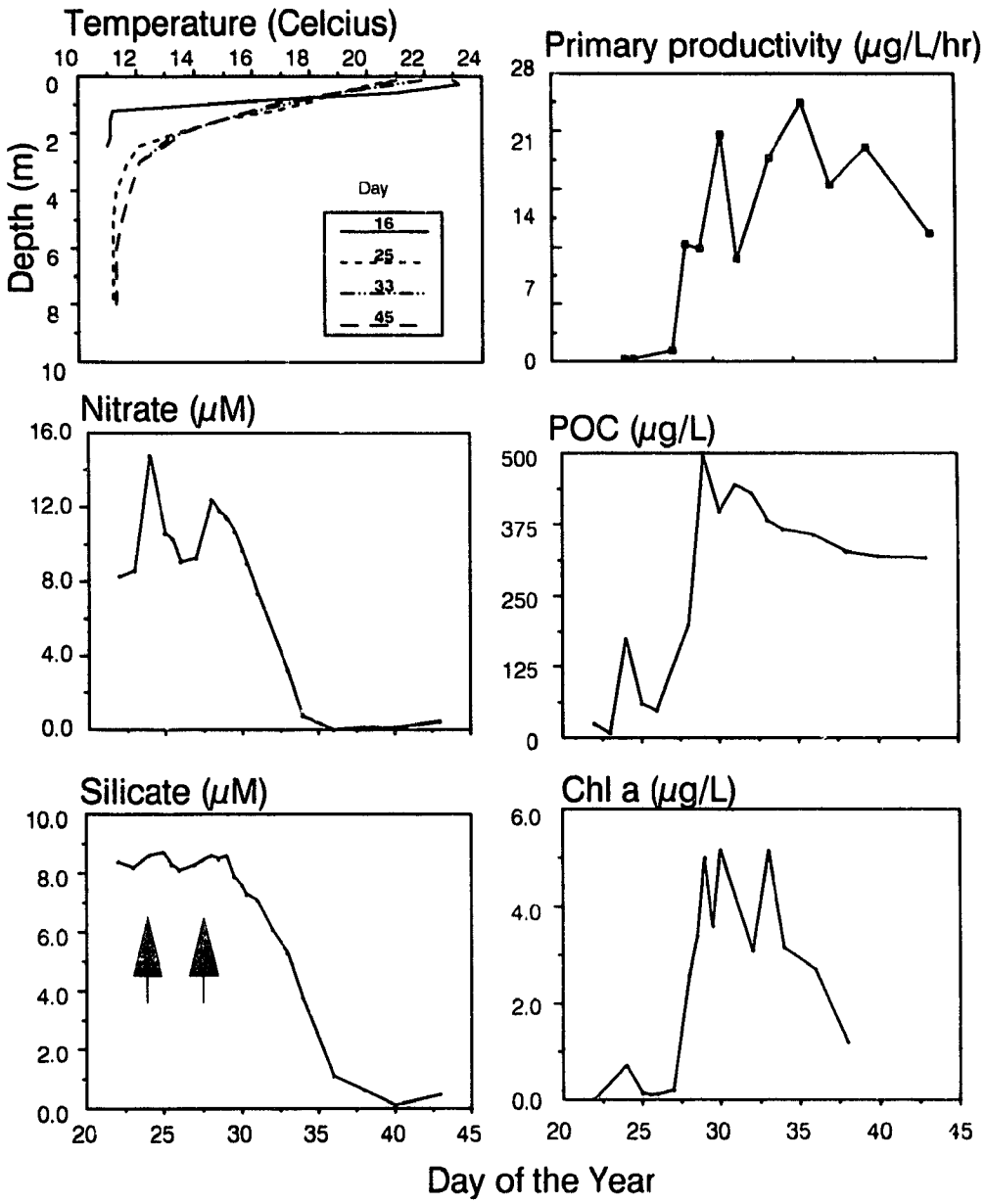


Figure 7.1. Characteristics of the phytoplankton bloom. Arrows indicate dates of additions of *Isochrysis galbana* cultures.

opaque plastic covering, and a light side that was protected from airborne debris by transparent plastic. Sampling hoses with dual entry ports about 0.25 m apart to reduce sampling patchiness were suspended by nylon line at depths of 0.3, 1, 4 and 8 m relative to the surface on the lighted side, and at 0.3 and 8 m on the dark side. Most sampling was done at 0.3 and 1 m depths. The hoses were connected to tap spigots imbedded into the tank walls. A conical sediment trap was deployed at 2 metres depth. The bottom of the trap was connected to a sampling hose, and the trap was sampled by slowly draining the volume of the hose (about 5 L) through pre-weighed filters.

Once filled, the tank was left unilluminated for 12 days to increase the amount of ^{234}Th in the surface waters to facilitate its measurement for another project. The tank was illuminated on January 22 (Day 22), and background values for SRP and DOP were taken. Approximately 15 L of *Isochrysis galbana* culture, grown in f/2, was added evenly to the lighted side on Day 24, producing a cell density of $5X10^3$ cells ml^{-1} at 0.3 m depth. Unfortunately, this cell density did not appear to initiate a bloom. A second 20 L carboy of dense culture was added on Saturday, January 26 to raise the cell density to 10^4 cells ml^{-1}, which proved sufficient to initiate a bloom. Nitrate and silicate were determined by standard Autoanalyser methods. Material retained by GFC filters were analysed for particulate organic carbon (POC) by the method of Wangersky (1976), and Chl a following Strickland and Parsons (1972). Primary productivity was measured by the method of Lewis et al. (1985). Bacteria were counted from stored samples using the methods described in Chapter 2. These measurements were taken with the assistance of S.B. Moran, S.E. Niven, R.J. Pett, and D.E. Slauenwhite. Due to the involvement of several researchers during this project, all samples were identified on a 'Day of the Year' basis to minimize confusion. This convention has been maintained in the present Chapter.

7.2.1 Phosphorus analyses

Samples for soluble reactive phosphorus (SRP), total dissolved phosphorus, and total phosphorus were generally collected at 0900 hrs each day, filtered if necessary (0.4-μm Nuclepore), and immediately transferred to reaction flasks. SRP measurements were then run immediately, while other samples were treated by UV oxidation or persulphate digestion. Analytical techniques for TDP and SRP analysis were generally the same as described in Chapter 6.2, with the exceptions noted below. Particulate phosphorus values were determined with the persulphate and combination methods by the difference between TP and TDP. The value of TDP-SRP has been termed "DOP", consistent with the terminology of Chapter 6; however, the term deserves particular qualification here since relatively high values of dissolved polyphosphate can be produced in batch culture compared with ambient open ocean levels (Strickland and Solórzano, 1968; Perry, 1976), as discussed below.

Estimates of polyphosphate levels. It was recognized that appreciable dissolved polyphosphate concentrations might be found during the phytoplankton bloom experiment, since marine algae can synthesize polyphosphate as cellular nutrient reserves when excess phosphate is available and growth is limited by some other trace nutrient (Solórzano and Strickland, 1968; Aitchison and Butt, 1972; Perry, 1976). Unused polyphosphate stores may be spilled into seawater upon cell death and lysis. Polyphosphates resist UV irradiation, but are rapidly depolymerized by hydrolysis at elevated temperature and sufficiently acidic conditions (Armstrong and Tibbets, 1968; Solórzano and Strickland, 1968; Hooper, 1973; Koroleff, 1984). Therefore, in order to detect the production of particulate and dissolved polyphosphates, samples were UV irradiated without acidification (*cf.* Chapter 6). Polyphosphate concentrations were estimated as the difference between the values obtained by UV irradiation, and by UV irradiation plus acid hydrolysis (Strickland and Solórzano, 1968), using the hydrolysis conditions described in Chapter 6.

Size fractionation study. The crossflow technique described in Chapter 6.2 was used

for this experiment. Prior to commencement of the bloom experiment, the ultrafiltration system was cleaned with overnight soaks of dilute acid and base solutions. Between sampling days, the ultrafilter was soaked overnight with dilute acid and rinsed by circulating approximately 20 L of Super-Q through the system. Samples were filtered with 0.2-μm Gelman Mini Capsule Versapor filters prior to ultrafiltration. Because of the sharp chemical gradients and inherent patchiness of a phytoplankton bloom (Wangersky, 1982), ultrafiltration samples were taken directly before samples for other analyses in order to minimize sample variability.

7.3 Results and Discussion

7.3.1 General features of the phytoplankton bloom

Figure 7.1 shows some of the biochemical characteristics of this bloom experiment. Additions of seed material can be easily identified as spikes in the nutrient profiles. Nutrients diminished rapidly after the second addition, including silicate, which was initially surprising since *Isochrysis galbana* should not require this mineral. However, microscopic examination revealed that *Chaetoceros sp.* also grew to significant numbers. This organism had grown in the Tower Tank in a prior experiment; perhaps our cleaning procedures had not removed all cells from the sides of the tank. While a mixed bloom was not intended, it suited the purposes of this study well, since it increased the variety of organic matter in the mesocosm. *Chaetoceros sp.*, such as *C. gracilis*, are often major contributors to phytoplankton blooms in Bedford Basin (Harrison, 1984), and other temperate coastal seawaters.

The productivity, POC, and Chl a data indicate that the phytoplankton bloom had multiple growth, stationary and senescent phases. As shown in Table 7.1, *Isochrysis galbana* dominated in early stages, giving way to the *Chaetoceros sp.* by Day 32. It is interesting to find that the relatively large diatom (10-15 μm) outgrew the smaller flagellate *Isochrysis galbana* (4-6 μm), given the large initial numbers of flagellates and relatively low nutrient

concentrations by Day 32 of the experiment. Surface area considerations would predict better nutrient uptake by the smaller flagellate.

7.3.2 Phosphorus results

Phosphorus results from samples taken at 0.3 and 1.0 m, and measured by the combination method, are given in Figure 7.2.

Particulate phosphorus results. The particulate phosphorus results clearly illustrate the various bloom phases of the experiment. A comparison of the particulate phosphorus data from 0.3 and 1.0 m indicates the dynamics of the two major phases of the experiment. The initial peak in particulate phosphorus occurred in the warmer waters of the surface layer (i.e. 22°C layer) on Day 29. A second phase (Day 32-40) appears to have initiated at 1 m depth. Temperatures at 1 m (16-18°C) were optimal for diatom growth, but not for *Isochrysis galbana*, a tropical flagellate.

Particulate phosphorus determinations by UV, persulphate and combination methods are shown in Figure 7.3. While good agreement was found between the results of the persulphate and combination methods, differences of up to 0.15 μM were obtained between the combination and UV techniques. As shown in Table 7.2, acid hydrolysis of TP samples recovered the difference between the UV and combination results. These results are consistent with the known chemistry of polyphosphates, and their production by marine algae (Solorzano and Strickland, 1968; Aitchison and Butt, 1972). Consequently, the bulk of the difference between UV and combination results is assumed to be cellular polyphosphate, which provides an estimate of their concentrations throughout the experiment. Polyphosphate estimated in this way comprised 15-40% of cellular P, in good agreement with 22-43% estimated for *Thalassiosira pseudonana* grown in N-limited culture, which was determined in a fashion similar to that used here (Perry, 1976).

Figure 7.4 shows the POC and particulate phosphorus values measured for 0.3 m

Table 7.1. Approximate numbers of *Isochrysis galbana* and *Chaetoceros sp.* observed by microscope.

Day	*Isochrysis galbana* (x 10^4 ml^{-1})	% of total	*Chaetoceros sp.* (x 10^4 ml^{-1})	% of total	Total # of cells (x 10^4 ml^{-1})
24	n.d.	-	n.d.	-	-
26	4	89	0.5	11	4.5
29	3	91	0.3	9	3.3
30	6	75	2	25	8
31	1.6	30	3.8	70	5.4
33	0.3	15	1.7	85	2
36	0.1	5	2	95	2.1

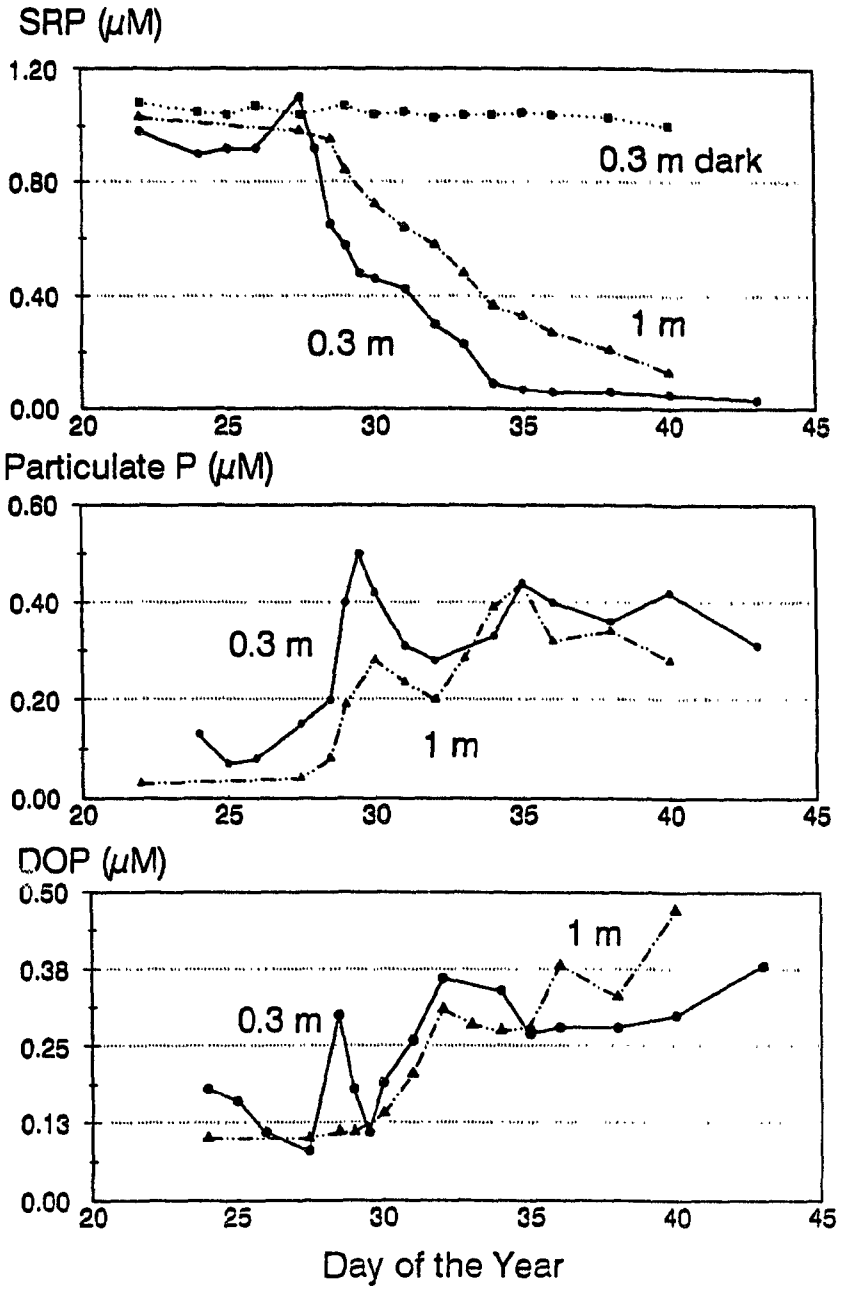


Figure 7.2. Changes in phosphorus concentrations during the bloom experiment as determined at 0.3 m and 1 m depths on the illuminated side of the tank. Also shown are SRP values for samples taken from the 0.3 m depth on the dark side.

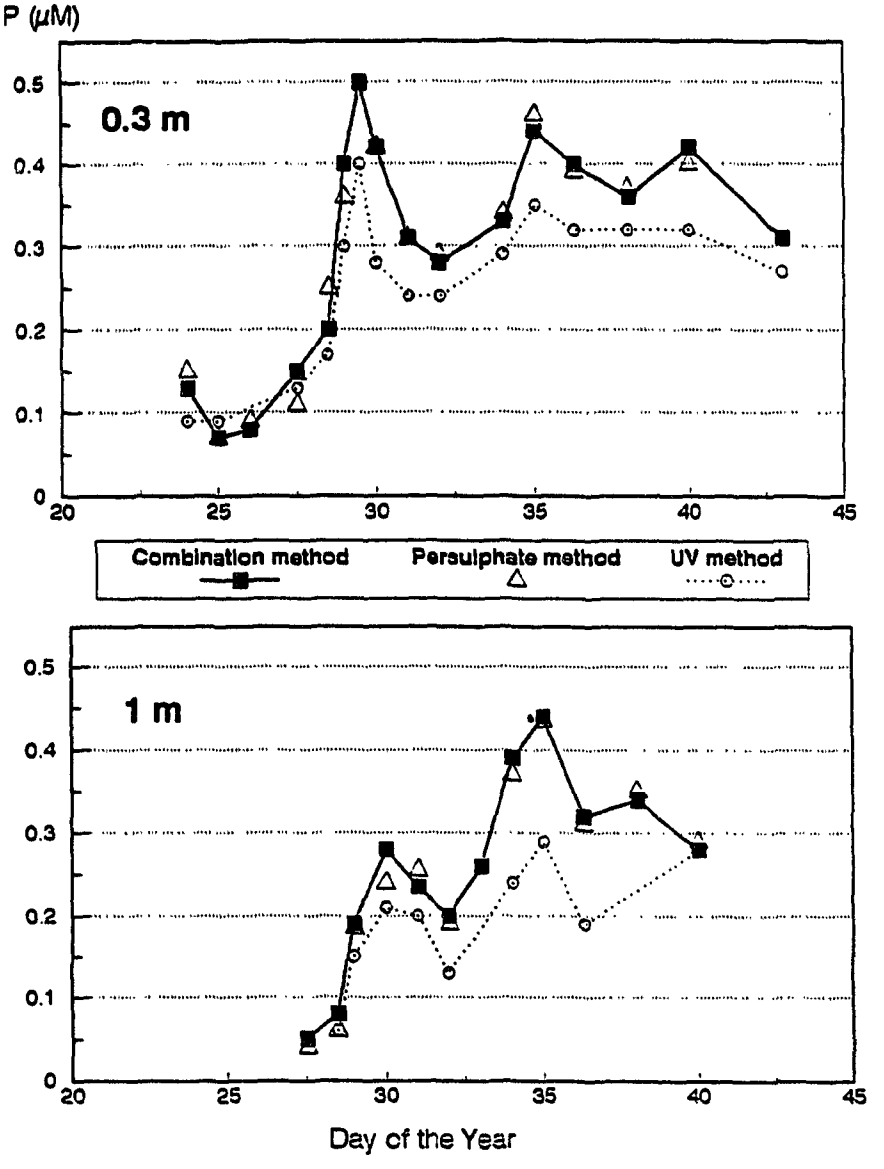


Figure 7.3. Comparison of particulate phosphorus data obtained by the combination, persulphate and UV methods for samples taken at 0.3 m and 1 m depths from the light side of the tank.

Table 7.2. Comparison of phosphorus recovered from filtered (TDP) and unfiltered (TP) samples after UV oxidation, UV oxidation and acid hydrolysis (UV + H), and UV oxidation followed by persulphate digestion (UV + P).

Day	sample	SRP (μM)	UV (μM)	UV + H (μM)	UV + P (μM)
34	0.3 m TDP	0.10	0.41	0.46	0.45
35	0.3 m TDP	0.13	0.33	0.39	0.40
	0.3 m TP	0.13	0.68	0.79	0.83
37	0.3 m TDP	0.06	0.26	0.32	0.33
40	0.3 m TDP	0.05	0.30	0.32	0.34
	0.3 m TP	0.05	0.62	0.80	0.77
	1 m TP	0.13	0.79	0.90	0.88
41	1 m TDP	0.13	0.42	0.49	0.49
	1 m TDP (dark side)	1.01	1.15	1.17	1.16
43	0.3 m TDP	0.03	0.31	0.40	0.41
	0.3 m TP	0.03	0.58	0.72	0.73

Typical precision of analyses: SRP ±0.005; UV ±0.015; UV + acid hydrolysis ±0.015; UV + persulphate ±0.02 μM.

samples. Linear correlation between the POC and PP data gives an r^2 value of 0.73 and a C:P ratio of 75. A significant amount of variability in the C:P ratios was observed over the course of the experiment, as evidenced by the scatter in the regression data. When C:P ratios were calculated from paired samples, a range of 50-127 was found with a mean of 88±23. These relatively low C:P values probably reflect the relatively high poly-P content of the particulate matter. An upper limit for C:P ratios of particulate organic matter can be obtained with the UV results. Correlation of particulate phosphorus values from UV analysis with POC data gives a C:P value of 90 (r^2 = 0.71). The mean of paired samples was 110±35. The UV results are closer to the 106:1 Redfield ratio normally associated with marine planktonic material. Particulate C:P ratios ranged from 63-106 for *Thallassiosira pseudonana* (Perry, 1976) and *Skeletonema costatum* (Perry and Eppley, 1981) in N-limited culture, but were 200-800 for P-limited cultures. C:P ratios of phytoplankton material are therefore indicative of macro-nutrient availability (Perry, 1976; Perry and Eppley, 1980). On the basis of C:P ratios, one would predict that the Tower Tank mesocosm was a N-limited system, which is consistent with nitrate levels that were very near detection limits (*ca.* 0.01 μM), while measurable reactive phosphate (0.1 μM) and silicate (1.1 μM) were present.

The observed C:P values can be used to calculate the export of particulate phosphorus out of the upper layer of the mesocosm. Sediment trap results indicate that a total of 1140 mg m^{-2} of particulate matter was collected over the upper 2 m layer during the 16 day phytoplankton 'bloom'. A comparison of suspended particulate (data not shown) and POC data suggest that the collected phytoplankton material was 50% organic matter by weight. Assuming that the bulk of the material collected in the sediment trap emanated from the upper 1 m (which is consistent with observations), then losses of about 48 mmol m^{-3} of POC and 0.48-0.64 mmol m^{-3} of particulate phosphorus are calculated. These losses should reflect similar decreases of total phosphorus at 0.3 and 1 m. Total phosphorus decreased by about 0.61 mmol m^{-3} at 0.3 m depth and 0.25 mmol m^{-3} at 1 m depth for an average of 0.43 mmol/m^3.

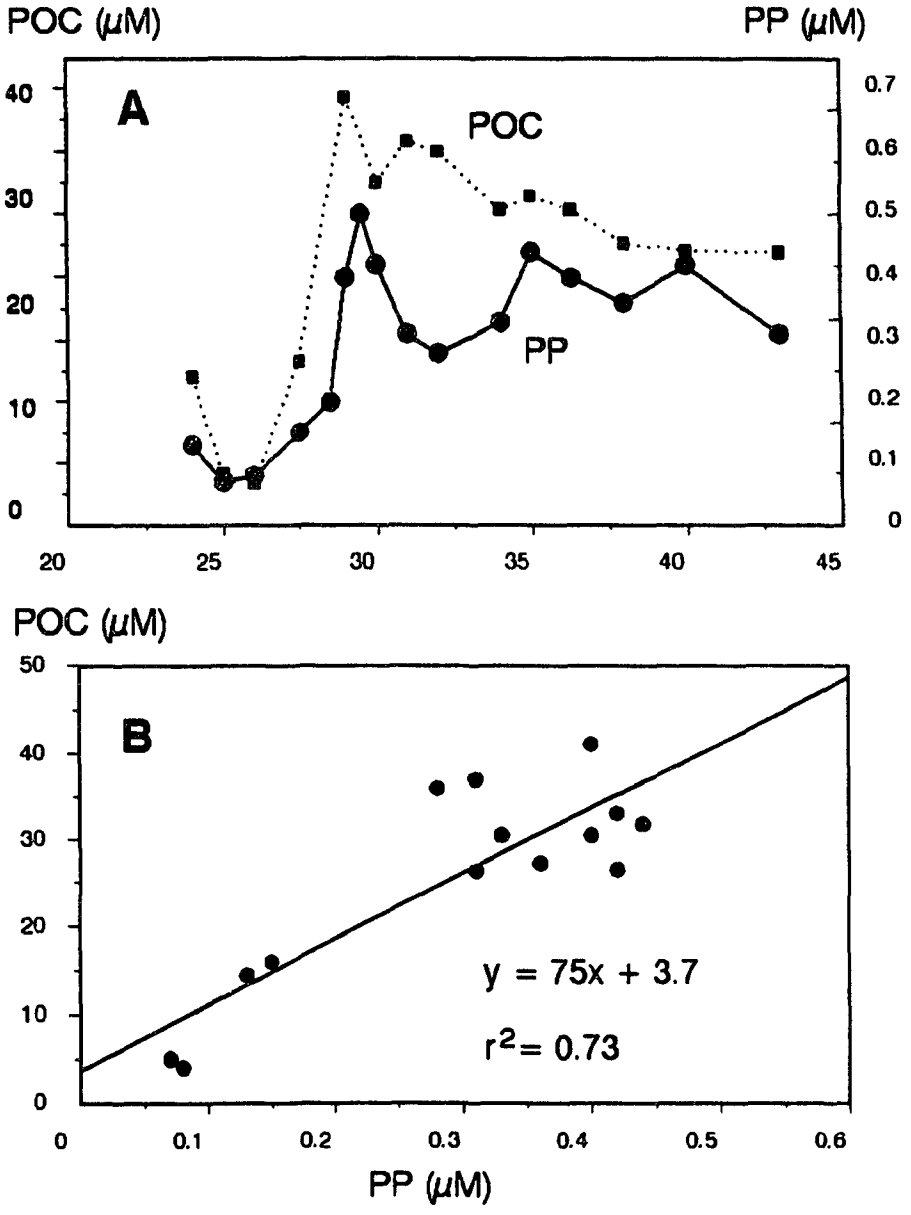


Figure 7.4. Comparison of POC and particulate phosphorus (PP) data: (A) time-course measurements of POC data (corrected for potential losses of bacterial carbon as in Table 7.3) and PP data obtained with the combination method; (B) linear regression of the data in (A). The slope of the line indicates changes of particulate carbon and phosphorus were related by an approximate value of 75.

Since sampling (1.5 m^3, equivalent to a ~0.14 m decrease of the surface layer) resulted in advective inputs of about 0.1 mmol m^{-3} of phosphorus into the upper 1 m layer, a corrected loss of 0.53 mmol P m^{-3} from the upper 1 m is obtained, in good agreement with sediment trap values. If polyphosphate constitutes *ca.* 25% of the sedimenting particulate P (which, unfortunately, was not confirmed experimentally, since the entire sample was required for another project), then about 0.13 mmol polyphosphate m^{-3} was exported out of the upper meter. Given that nutrient conditions were typical of coastal seawater levels, and the experiment included at least one coastal algal species, these results suggest that polyphosphates are an important aspect of particulate phosphorus cycling during phytoplankton blooms in temperate coastal systems. Karl et al. (1992a) also suggest that cellular polyphosphate production may be an important process in fueling *Trichodesmium* blooms in open ocean waters.

Contribution of bacteria to particulate phosphorus. Bacteria are significant contributors to particulate phosphorus. As shown in Table 7.3, bacteria accounted for as much as 0.08 μM, or 20% of particulate phosphorus. Microscope examination indicated that bacteria were mainly in the size range 0.7-1.2 μm, which suggests that most bacteria would have been removed by 0.4-μm Nuclepore filters used here. In addition, very good agreement was found between DOP results obtained for a sample filtered by 0.4 and 0.2-μm Nuclepore filters (see Table 7.4, Day 33), which further supports this claim.

On the other hand, bacteria may contribute to the 'dissolved' phosphorus fraction in studies which use filters with larger nominal pore sizes. For example, Hollibaugh et al. (1991) found that 75-100% of bacteria in Tomales Bay samples passed through GFC filters (1.2-μm nominal pore size). In this case, bacteria contributed *ca.* 27% to 'dissolved' organic phosphorus measurements. The relatively high phosphorus content of bacteria, and low DOP levels in seawater, therefore amplify the importance of filter pore size considerations for phosphorus studies. One of the better choices would appear to be 0.2-μM Nuclepore filters, since they

Table 7.3. Contribution of bacterial biomass to particulate carbon and phosphorus.

Day	Bacteria 10^9 L^{-1}	BC (μM)	% of POC	BP (μM)	% of PP
24	0.25	0.4	3	0.01	7
26	0.40	0.7	15	0.01	18
27.5	0.35	0.6	4	0.01	9
29	0.65	1	3	0.02	6
30	0.70	1	3	0.03	6
31	0.80	1	4	0.03	10
34	1.3	2	7	0.05	14
36	1.9	3	10	0.07	18
38	2.2	4	12	0.08	23
40	2.1	4	12	0.08	19
43	2.4	4	14	0.09	29

Bacterial carbon (BC) values calculated assuming 20 fg $cell^{-1}$ (Lee and Fuhrman, 1987). It was assumed that GFC filters retained only 25% of bacteria (Hollibaugh et al., 1991) and POC values were adjusted to include all bacterial carbon. Bacterial phosphorus (BP) values calculated based on C:P ratio of 45:1 (Goldman et al., 1987). It was assumed that 0.4-μm Nuclepore filters retained all bacteria for calculation of particulate phosphorus (PP) values.

should remove most bacteria while minimizing uptake of surface active material (Johnson and Wangersky, 1985; Section 7.3.3).

DOP results. The DOP data from 0.3 m and 1 m depths are given in Figure 7.5. An initial DOP spike from Day 26-29 at 0.3 m probably indicates input from dead or dying cells associated with the seed material. This phosphorus was evidently very labile as it was consumed at a rate similar to orthophosphate; decreases of DOP and SRP data over this period are accounted for by increases of particulate phosphorus. Decreases of DOP suggest appreciable activity of phosphorus-specific enzymes. Determinations of alkaline phosphatase levels would have been an interesting addition to the suite of measurements undertaken in this experiment.

A local maximum for DOP values of 0.36 μM was obtained at 0.3 m depth by Day 32 of the experiment, at which time particulate phosphorus levels were declining. Although experimental evidence indicates that DOP is actively released by phytoplankton (Kuenzler, 1970), a major source of the DOP measured on or after Day 31 in this experiment is likely to have been from death and lysis of *Isochrysis galbana* cells. Data in Table 7.1 indicates that cell numbers deceased rapidly at this time. Another possible source of DOP in marine waters is from the activities of zooplankton grazers (Pomeroy et al., 1965). While no large zooplankton were observed in microscopic examinations, cells with sizes and morphologies similar to heterotrophic nanoflagellates were observed in some samples stained with acridine orange.

A second phase of the experiment started about Day 33 when nitrate levels reached near detection limits and very low levels of SRP were maintained. Productivity remained relatively high during this phase; driven mainly by regeneration of organic nutrients, reflected in part by a decrease of DOP at 0.3 m depth. This decrease may indicate a stage at which enzymatic activity outstripped DOP production. Stable DOP levels from Day 35-40 could represent a 'steady state' between enzymatic regeneration and production. Phosphorus

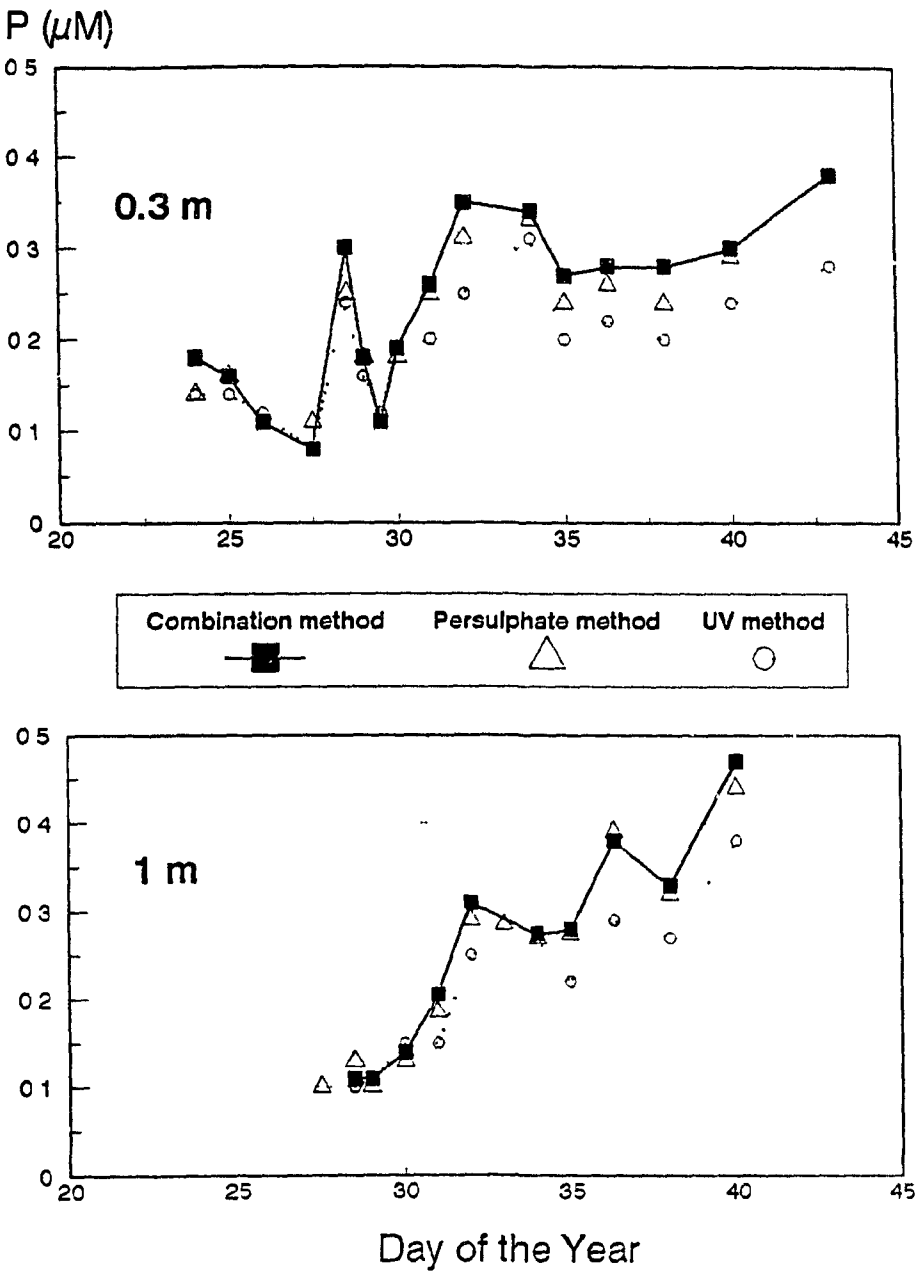


Figure 7.5. Comparison of dissolved phosphorus data (TDP-SRP) obtained by the combination, persulphate and UV methods for samples taken at 0 3 m and 1 m depths from the light side of the tank.

compounds most likely to be rapidly regenerated are phosphoesters and some phospholipids (Cembella et al., 1984). Levels of enzyme hydrolyzable phosphate were found to range from not detectable to 0.09 μM at different parts of the year in Cheasapeake Bay, with lowest levels recorded in springtime (Taft et al., 1977).

The regeneration rate of phosphorus compounds at 0.3 m depth can be estimated from SRP and the productivity data shown in Figure 7.6. The rates of SRP disappearance ('SRP-uptake') are compared with phosphorus uptake rates obtained from the productivity data, assuming a C:P uptake ratio of 100:1, and relatively small SRP uptake by bacteria (bacteria was $\leq$10% of particulate phosphorus during the period of rapid SRP uptake; see Table 7.3). The difference between the areas under each curve is a rough estimate of P-uptake from regeneration. A value of 0.3 μM L^{-1} day^{-1} for regenerated P-uptake is obtained in this manner, which is greater than the rate of production due to SRP uptake (*ca.* 0.2 μM L^{-1} day^{-1}; calculated to Day 33 when SRP levels reached constant low values (<0.10 μM)). Harrison (1984) measured SRP-uptake rates of 0.02-0.3 μM day^{-1} in Bedford Basin using radioisotope methodology. Simultaneous measurements of SRP regeneration in Bedford Basin samples ranged from 0.006-0.22 μM day^{-1} and were always less than SRP-uptake rates. However, these values were measured at relatively high SRP levels, where the bulk of P-regeneration probably takes place through microplanktonic grazers. When SRP is severely limited, as in the latter stages of this experiment, regeneration may occur directly between the external medium and phytoplankton internal pools, resulting in very short turnover times and high regenerated productivity (Harrison, 1984).

Efficiencies of DOP methods. A comparison of DOP results obtained with the UV, persulphate and combination methods is given in Figure 7.6. As found for measurements of particulate phosphorus, the persulphate and combination methods showed good agreement, while the UV technique measured appreciably lower levels of P in most cases. Linear regressions between the persulphate and combination results for filtered and unfiltered

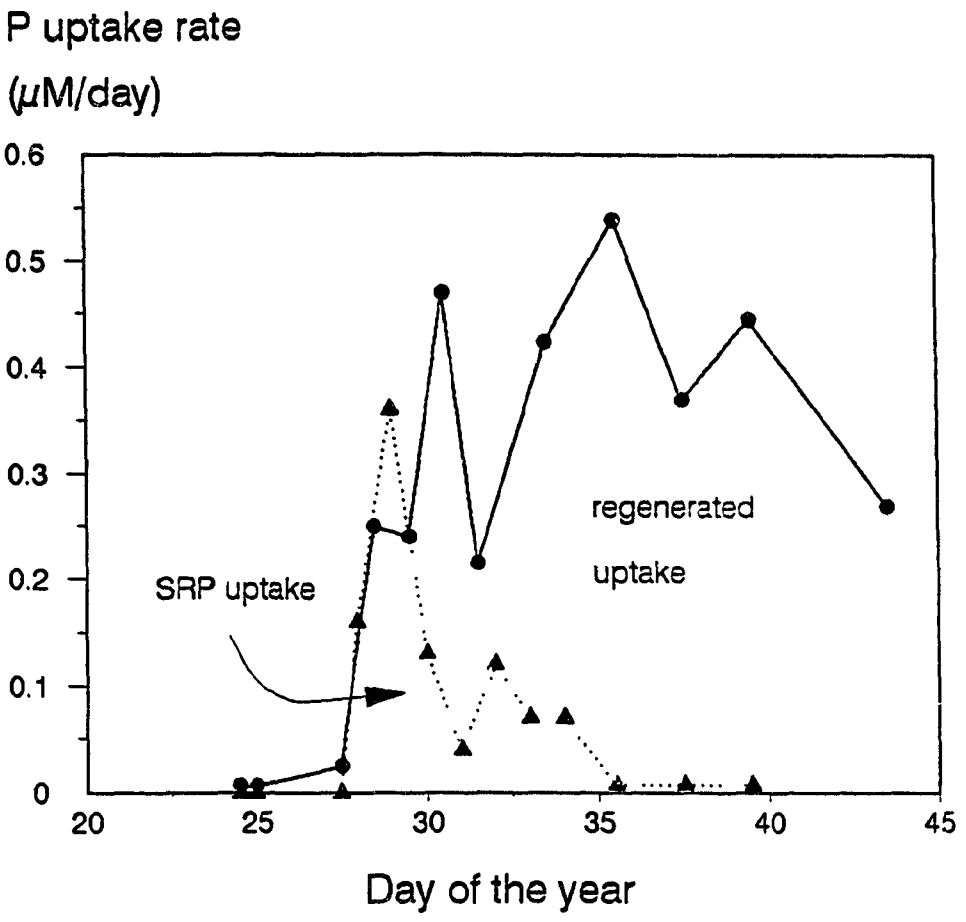


Figure 7.6. Line plots of phosphorus uptake rates. SRP uptake rates refer to rates of disappearance of SRP during the bloom phase of the experiment. SRP values did not change after Day 35. Solid line data are predicted P-uptake rates based on C-uptake data and an average C:P uptake ratio of 100, which would be consistent with observed C:P ratios of collected particulate organic matter. Regenerated uptake refers to P-uptake beyond that predicted by measured SRP levels, and its value is approximated as the difference between the areas under the gross P-uptake (solid lines) and SRP uptake (dotted) curves. Note that rate estimates are not corrected for changes in phytoplankton biomass.

samples are shown in Figure 7.7. The slopes of the best fit lines with 95% confidence limits indicate that the persulphate method is as efficient as the combination method for analysis of the SRP-unreactive components of total (97±3%) and dissolved (92±7%) phosphorus samples.

Differences between the measurements of 'DOP' by the UV and other two methods were not evident at the beginning of the experiment, but developed with the first declining phase of particulate phosphorus (about Day 30). Thereafter, the UV-resistant fraction remained a significant portion (10-30%) of the 'DOP'. Results of samples that were acidified prior to UV analysis, or from an additional acid hydrolysis step, are shown in Table 7.3. Most of the 'UV-resistant' fraction was degraded by acid hydrolysis. The "UV-resistant", acid-hydrolysable dissolved fraction is probably dissolved polyphosphates produced from cell lysis. Dissolved poly-P concentrations were 0.02-0.08 μM during most of the experiment. Comparable levels of dissolved poly-P were measured during bloom periods in two Scottish Sea Lochs (Solórzano, 1977) and at most times of the year in Chesapeake Bay (Taft et al., 1975). Therefore, while significant levels of dissolved polyphosphate can be produced during phytoplankton blooms initiated at ambient coastal nutrient concentrations, low polyphosphate levels in coastal and open ocean waters imply extracellular polyphosphate has a relatively short residence time and is readily taken up by planktonic organisms.

Polyphosphate levels determined in this work are rough estimates for at least two reasons. First, hydrolysis of polyphosphate can occur at the natural pH of seawater with the heating from UV irradiation. For example, Armstrong and Tibbets (1968) found 50% of polyphosphates present in English Channel seawater were hydrolysed after heating samples at 60°C for 15 hours. Since samples were heated to 70-75°C during the 6-hour UV irradiation period used in this study, some hydrolysis of polyphosphate compounds should have occurred. Second, as there is evidence to suggest that DOM is resistant to UV oxidation (Chapter 5; Chen and Wangersky, 1992b), acid hydrolysis may release organically-bound phosphorus that is resistant to UV. Short UV irradiation times recommended for accurate polyphosphate

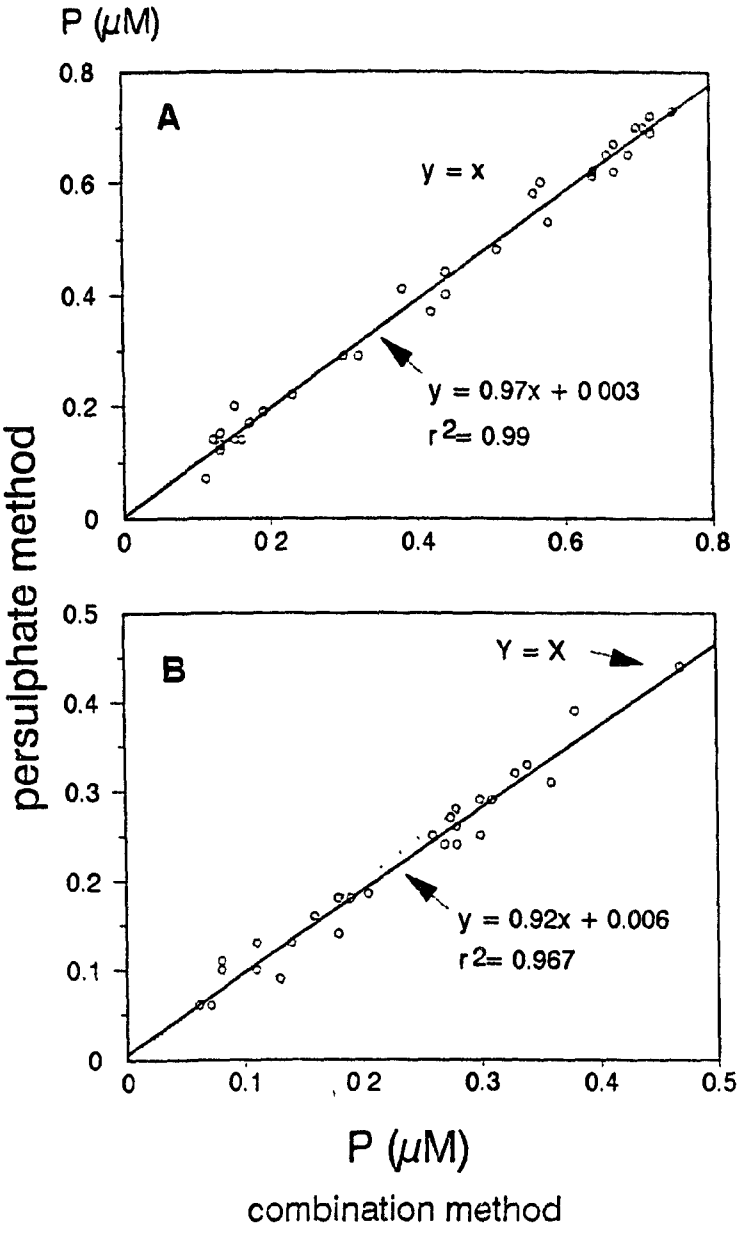


Figure 7.7. Linear regressions between persulphate and combination method results for the recovery of the 'unreactive' components of (A) total phosphorus and (B) dissolved phosphorus (normally referred to as DOP in text). The slopes of the lines with 95% confidence limits are 0.97 ± 0.03 for TP analyses, and 0.92 ± 0.07 for TDP analyses.

determinations with conventional high intensity lamps (<1 hour, Strickland and Solorzano, 1968) enhance the probability of incomplete oxidation. These authors do point out, however, that irradiation times could be extended if samples were kept well below 70°C. Further experimentation would appear to be merited before polyphosphate determinations can be considered accurate.

7.3.3 Size fractionation study

Effects of pre-filtration. The crossflow ultrafiltration technique is designed to process large volumes of water (10^1-10^2 L) to obtain high concentration factors for solutes of interest that often cannot normally be measured directly. Choice of pre-filter is limited if reasonable filtration times are to be obtained. In addition, pore size considerations are important for dissolved phosphorus determinations, since bacteria may comprise an appreciable fraction of total phosphorus, as discussed earlier. The pre-filter used here was the 0.2-μm Gelman Versapor membrane filter, which is composed of a pleated acrylic copolymer supported with a non-woven support material. This filter delivers high flow rates and is cleanable for trace metal purposes, which was a consideration for other projects associated with the Tower Tank study. Unfortunately, the high surface area (500 cm^2) of the filter also effectively removed a sizeable fraction of surface active material, as evidenced by the data reported in Table 7.4. It is obvious from these data that the true spectrum of colloidal material was probably not sampled, although it is possible that such highly surface active material would have been similarly sorbed by the ultrafilter during crossflow filtration.

Results. The ultrafiltration results determined with the combination method are presented in Figure 7.8. The low molecular weight fraction generally tracked total DOP (i.e. Gelman filtered values) and in most cases comprised the major portion of total DOP. Similarly good correspondence between TDOP and low molecular weight DOP was found for a depth profile taken at a Shelf station (Chapter 5, Figure 5.6). Colloidal DOP recovered by the

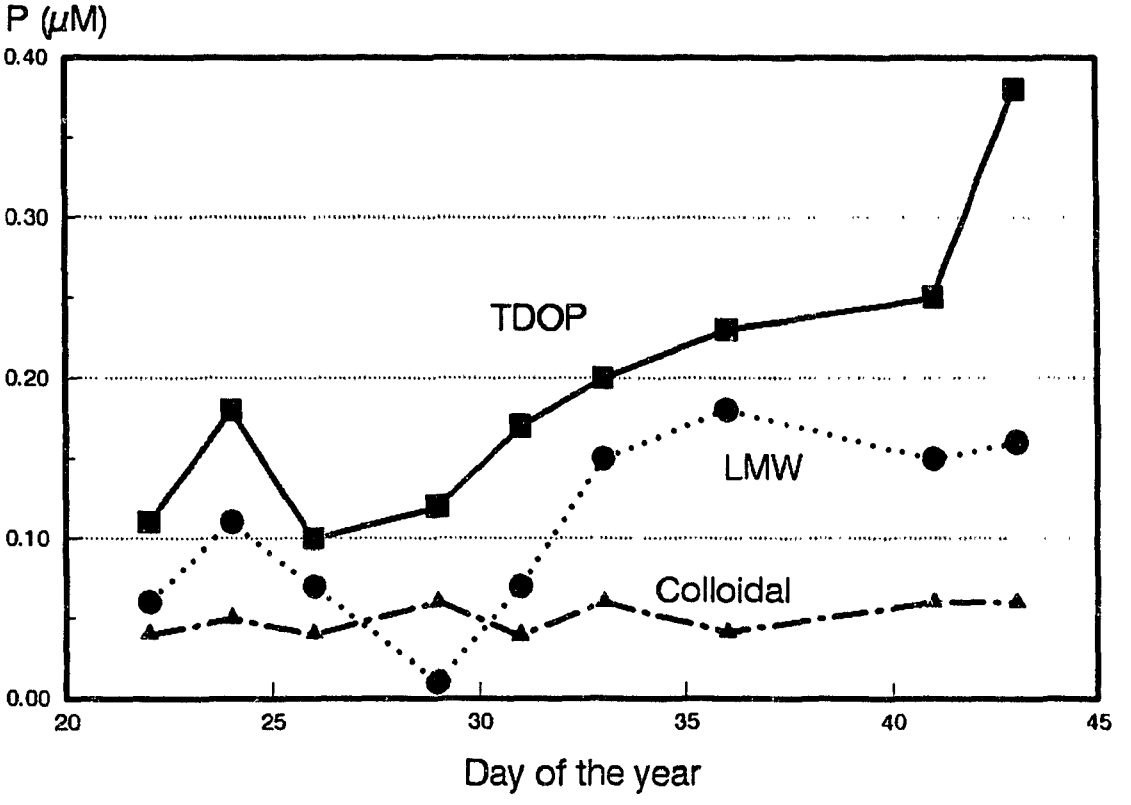


Figure 7.8. Crossflow filtration results for DOP over bloom showing variations of 0.2-μM Gelman filtered fraction (TDOP), low molecular weight fraction (LMW, <10,000) and colloidal fraction (>10,000 MW).

crossflow technique varied little over the course of the bloom, ranging from 0.04 to 0.06 μM by direct measure, which was 10-20% of the DOP fraction. Colloidal DOP was found to comprise similar proportions of TDOP in surface coastal and open ocean seawater (Chapter 5). As shown in Table 7.5, the UV oxidation and combination method results show very good agreement for analysis of colloidal DOP, in agreement with results for oceanic samples. The bulk of the disagreement between DOP results obtained with the UV and combination methods appears to be low molecular weight material. This result indicates that dissolved polyphosphates, which are assumed to compose most of the differences between DOP values measured with the two methods, are chiefly low molecular weight polymers with an upper limit of 125 subunits.

Mean recovery after crossflow ultrafiltration was 85±20% of total DOP; largest losses occurred on Days 29, 31, and 44 which correspond also to times of large losses onto the Gelman filter. The total amount of surface active material lost onto the pre-filter and ultrafilter relative to the DOP values obtained with Nuclepore filtration are reported in Table 7.4. Total losses ranged from 10-60% of the DOP values measured from Nuclepore filtered samples. The highest values of surface active material were observed at times of both relatively high particulate phosphorus and DOP values. While recognising that certain low molecular weight compounds (eg. phospholipids) are surface active in seawater, colloidal materials are known to be particularly susceptible to sorption and aggregation reactions (Johnson and Wangersky, 1985; Niven, 1990). One possibility, therefore, is that preferential removal of the colloidal fraction due to filtration may have dampened any response this fraction would have shown to the phytoplankton bloom, accounting for little observable change in the colloidal DOP during the course of the experiment.

Limited data suggest that the 0.45-μm Gelman pre-filter used for open ocean samples did not remove similarly large amounts of DOP. Comparison of NE Pacific samples taken at sea from the same Go-Flo bottles showed agreement within experimental errors between

Table 7.4. DOP recovered by combination method after different filtration processes.

Day	N-TDOP (μM)	G-TDOP (μM)	LMW (μM)	Colloidal (μM)	Losses (μM)	PP (μM)
22	0.11	-	0.06	0.04	0.01	-
24	0.18	_	0.11	0.05	0.03	0.07
26	0.10	-	0.07	0.04	-0.01	0.08
29	0.18	0.12	0.01	0.06	0.05 (0.11)	0.40
31	0.29	0.17	0.07	0.04	0.06 (0.18)	0.31
33	0.26 (0.27)*	0.20	0.15	0.06	-0.02 (0.04)	0.30
36	0.28	0.23	0.18	0.04	0.01 (0.06)	0.40
41	0.29	0.25	0.15	0.06	0.04 (0.08)	0.42
43	-	0.38	0.16	0.06	0.16	0.31

G-TDOP refers to 0.2-μm Gelman filtered samples, N-TDOP refers to 0.4-μm Nuclepore filtered replicates, * refers to a 0.2-μm Nuclepore filtered sample.
Bracketed loss values are total losses with respect to Nuclepore filtered samples.
Precision of combination method for total DOP (TDOP), low molecular weight (LMW; <10,000 daltons) and particulate phosphorus (PP) typically ±0.02 μM. Colloidal (>10,000 daltons) DOP values are better than ±0.004 μM.

Table 7.5. Comparison of UV and combination methods for analysis of crossflow ultrafiltered size fractions.

Day	TDOP (μM)		LMW (μM)		Colloidal (μM)	
	UV	Comb'n	UV	Comb'n	UV	Comb'n
22	0.13	0.11	0.06	0.06	0.037	0.036
24	0.15	0.18	0.09	0.11	0.047	0.045
26	0.12	0.10	0.07	0.07	0.036	0.044
29	0.10	0.12	0.01	0.01	0.051	0.059
31	0.14	0.17	0.06	0.07	0.039	0.043
33	-	0.20	-	0.15	0.060	0.055
36	-	0.23	-	0.18	0.035	0.039
41	0.20	0.25	0.12	0.15	0.050	0.056
43	0.29	0.38	0.12	0.16	0.055	0.059

TDOP samples pre-filtered through 0.2-μm Gelman filters.
Precision of combination method analyses as given in Table 7.4.
UV method results are typically ±0.015 μM.
Concentration factors for colloidal results range from 24-79. Analytical precision for colloidal analyses ranged from ±0.0010-0.0035 μM.

Gelman and Nuclepore filtered samples (see Chapter 5, Table 5.8). High concentrations of surface active materials may be a transient feature of open ocean chemistry occurring with phytoplankton blooms. Physical, physico-chemical and biological processes may act to reduce high concentrations of surface active material quickly. For example, while unusually high dissolved lipid concentrations were measured during the senescent phase of a spring bloom in Bedford Basin (Parrish, 1987), concentrations returned to normal levels within a short time (<5 days). The lipid fraction may therefore have been dispersed by advective processes, sorption onto particles or reassimilated by phytoplankton (Admiraal and Werner, 1983).

7.4 Summary

A phytoplankton bloom was used to investigate the efficiency of UV, persulphate and combination methodologies for determination of total and dissolved phosphorus in coastal seawater. The salient results are:

1) The persulphate method was as efficient as the combination method (97±3%) for total phosphorus determinations, and 92±7% as efficient as the combination method in recovering combined P from dissolved phosphorus samples.
2) The UV method, tested on samples irradiated at natural seawater pH, was generally not effective for analysis of TP and TDP samples. Poor performance of this method was due, in all probability, to its well-known inefficiency for depolymerizing polyphosphate linkages.
3) It was estimated that polyphosphates comprised 15-40% of particulate and 5-25% of dissolved 'unreactive' phosphorus after the onset of the phytoplankton bloom. These high concentrations suggest that polyphosphates should be of particular importance to P-cycling during phytoplankton bloom periods in coastal waters.

4) Bacteria can constitute an important phosphorus pool, as they were calculated to have composed as much as 20% of particulate phosphorus. Since bacterial sizes straddle conventional cutoffs for dissolved and particulate matter, filter pore size is an important consideration in phosphorus work.

5) Ultrafiltration results from 0.2-μm Gelman filtered samples showed similar proportions of colloidal and low molecular weight material as oceanic samples. However, uptake of surface active compounds by the Gelman pre-filter and the crossflow ultrafilter may have significantly altered the spectrum of P compounds recovered by ultrafiltration compared with that produced *in situ*. These results emphasize some of the difficulties associated with ultrafiltration methods. Particular care must be taken when these methods are used to analyse natural waters with high biological productivity. Attention to the surface active characteristics of the pre-filter must be given high priority if the colloidal fraction is to be accurately sampled.

Chapter 8

Principal Conclusions and Areas for Further Research

8.1 Introduction

The need for a better understanding of the quantity and physico-chemical nature of DOM in seawater has recently become greatly emphasized due to heightened interest in global carbon cycles, and the possibility that marine DOM concentrations are several times higher than measured with traditional methods of analysis. Present uncertainties in the accuracies of both HTCO and wet oxidation methods limit the information that can be gained on the sources, sinks, and lability of DOC in seawater. A ^{14}C approach was used as an alternative to the bulk DOC methods, since its use minimized problems of contamination, blank variability, low precision and unknown accuracy. Though the ^{14}C method employed here does not measure 'bulk' DOC concentrations in seawater, its use in laboratory experiments provides insights into the sources of marine DOC, and the pathways which alter its physico-chemical characteristics. The specific aims of the ^{14}C-DOC studies were to characterize DOC produced from cultured algae with respect to its resistance to wet oxidation, and investigate the reactivity of different size fractions of DOC. Research also included investigations into the effects of abiotic and biotic ageing processes on phytoplankton DOC.

Another facet of the thesis research on DOM in seawater was directed to the measurement of dissolved organic phosphorus. DOP is a component of marine DOM that had been largely ignored in the early stages of the controversy initiated by Suzuki and coworkers. However, the often observed correlation between N and P in seawater has raised questions of whether DOP levels were similarly underestimated in seawater. Inspection of literature also revealed that, prior to the work contained in this thesis, few vertical distributions of DOP in the deep ocean have been published, and no data existed on the size fractions of DOP in

seawater. As DOP is of interest in marine biological cycles, and has been used to represent DOM fluxes in modelling research (Toggweiler, 1989), further study of its concentrations, distributions and size fractions was merited.

8.2 Summary and Principal Conclusions

8.2.1 Results from experiments with DOC produced by phytoplankton cultures

It was found that phytoplankton do produce DOC that resists oxidation by the persulphate method and a UV autoanalyser method. Full breakdown of phytoplankton DOC (PDOC) was obtained, however, with a UV method that allowed extended exposure of samples to high-intensity irradiation. The amount of PDOC recovered after persulphate oxidation varied depending on species. DOC produced by *Isochrysis galbana* was generally less resistant (5-12% remaining after oxidation) than DOC produced by *Phaeodactylum tricornutum* (10-15% remaining) and *Synechococcus* (11-18% remaining). These values are similar to traditionally accepted differences between combustion and persulphate oxidation of surface seawater samples (~20% - Sharp, 1973; ~15% - Gershey et al., 1979).

Analysis of the volatile gases produced from persulphate oxidation of labelled PDOC indicated that ~1% of purged gases was not $^{14}CO_2$. GC analysis of seawater samples which had been oxidised by the persulphate method showed that 3 μM, or ~3% of the total DOC as analysed by HTCO, was low molecular weight halocarbons. Low molecular weight halocarbons were <<1% of DOC in samples treated by UV oxidation. While the levels of non-CO_2 volatiles other than low molecular weight halocarbons produced by persulphate and UV oxidation of the coastal sample were not determined, these results do not support suggestions that high levels of volatile products other than CO_2 are produced by traditional oxidation method (Lee and Henrichs, 1992).

Size fractionation results indicated that predominantly PDOC of <10,000 NMW was

produced in the *Phaeodactylum tricornutum* and *Synechococcus* cultures. The resistance of PDOC size fractions varied with algal source. The colloidal fraction of *P. tricornutum* DOC was less resistant (7.7±2.3% remaining after oxidation) to persulphate oxidation than the low molecular weight fraction (13.5±2% remaining). Colloidal DOC from *Synechococcus* showed generally similar resistance to oxidation (18.2±3%) as the low molecular weight fraction (15±1.5%).

The high proportions of low molecular weight DOC found in algal cultures are similar to recent size fractionation results from the central Pacific (Benner et al., 1992) and coastal NW Pacific (Oshawa and Ogura, 1992). DOC dominated by low molecular weight compounds is consistent with direct release by phytoplankton as a major source, while Bronk and Glibert (1991) suggest that predominance of high MW materials is evidence that grazing dominates DOC input.

Extrapolating the results observed with phytoplankton cultures to the ocean indicates that the character of DOC observed in seawater will vary according to the dominant phytoplankton species, growth phase, and mechanism of DOC production. Persulphate oxidation cannot be recommended for DOC analysis, as the efficiency of the method changes with these factors.

The results of the study of UV analysers are equivocal. As complete breakdown of all phytoplankton DOC was observed with one UV instrument, it was not established that phytoplankton DOC resists UV oxidation. What should be emphasized is that the oxidation efficiency of UV analysers must be rigorously checked and carefully monitored through any large series of measurements. There is at least one example in the literature in which the amount of DOC oxidized by UV varied considerably, depending on the age of the lamp (Williams and Druffel, 1987). On the basis of results obtained from this thesis research and elsewhere (Williams, 1969; Gershey et al., 1979), a UV system operating at peak efficiency should recover CO_2 from seawater samples oxidised with persulphate. Failure to do so would

indicate a need for further calibration and testing of the instrument.

8.2.2 Effects of Ageing of Phytoplankton DOC

Two major observations were made when phytoplankton DOC was aged in the presence of microbial populations. *Short-term* ageing (days) of PDOC from an axenic culture was found to increase the percentage of labelled DOC which resisted persulphate (from ~18 to ~23%). *Long-term* ageing (weeks to a year) of phytoplankton DOC solutions in the presence of bacteria decreased by 1-9% the percentage of DOC that resisted persulphate oxidation. Short term ageing was suggested to be due to preferential uptake of compounds labile to both bacteria and persulphate oxidation (e.g. low MW carbohydrates and amino acids), which were expected to be present in relatively high concentrations in the initially axenic solution. It was proposed therefore that bacterial uptake of labile compounds resulted in an increase in the proportion of oxidation resistant compounds in the short-term ageing solution. Long-term ageing results indicated that bacteria also eventually consume or alter the reactivity of oxidation resistant DOM, resulting in lower fractions of resistant compounds after ageing than in the initial solutions.

Limited evidence suggests that protist grazers produce relatively resistant DOC (~25% not oxidised). Further study of DOC produced by protist grazers is merited. No evidence was found to indicate that abiotic ageing reactions (chemical and photochemical) increased the resistance of DOC produced from phytoplankton.

These results support the idea that reactivity of DOC in surface waters to persulphate oxidation should be variable, depending on the mechanism of production and degree of ageing. Whether all these factors affect resistance to UV oxidation has not been thoroughly examined in this study. A general trend toward lower concentrations of resistant DOC with longer ageing is consistent with results which show better agreement between wet oxidation

and HTCO methods for deep-water samples (Druffel et al., 1989).

8.2.3 The measurement of DOP in seawater

Standard persulphate and UV procedures for the determination of DOP in seawater were compared with a method that combines the oxidation and hydrolytic capacities of these two methods. Results indicated that, although the combination method measured more DOP than the standard methods for many samples, dissolved phosphorus compounds have not been grossly undermeasured in seawater. The UV method was 90±6% and 71±9% efficient, and the persulphate method was 87±8 and 83±9% efficient relative to the combination method for NW Atlantic and NE Pacific samples, respectively. Differences between DOP methods were greatest in surface waters, where DOP levels are highest. Better agreement between the methods is observed for subsurface and deep-water samples, which is attributable to regeneration or removal of resistant P compounds in subsurface waters and, in general, to diminishing DOP concentrations with depth.

The range of DOP concentrations measured in surface waters was similar to traditionally reported values (Jackson and Williams, 1985). No evidence was found to support observations that North Pacific deep water contains ~0.2 μM DOP (Williams et al., 1980). Depth averaged subsurface values taken (0.07±0.04, n=8) from 100-400 m at different stations in the NW Atlantic were lower, but not significantly different, than subarctic NE Pacific values (0.11±0.03, n=7) for the same depths. Low DOP concentrations (≤0.05 μM) were found in oxygen minimum and deep-water samples from both regions.

Several of the NE Pacific samples were treated by the combination method and then analysed for DOC by two different HTCO analysers. Both DOC analysers were thought to be more reliable than the Sugimura and Suzuki (1988) method. The levels of residual organic carbon detected by HTCO analysis suggested that the amounts of organically-combined

phosphorus resisting oxidation by the combination method would not greatly increase our estimates of DOP in seawater.

Size fractionation experiments indicated that the bulk of DOP (74±14%) in coastal and open ocean surface waters at NW Atlantic and NE Pacific stations was <10,000 NMW. While DOP concentrations decreased with depth, a trend toward higher proportions of DOP with nominal MW >10,000 was observed in both the NW Atlantic and NE Pacific. These results suggest relatively slower decomposition of high molecular weight compounds. However, decreases of DOP levels to near detection limits in oxygen minimum waters suggest that all DOP is ultimately degraded by microorganisms within the timescale (~1000 yrs) of deep ocean mixing.

8.2.4 The measurement of total and dissolved phosphorus over a phytoplankton bloom

The persulphate method liberated combined phosphorus produced over a phytoplankton bloom with a similar efficiency (92±7% relative to the combination method), as found for oceanic samples (~85%), confirming that the persulphate method gives acceptable P recovery for a wide variety of samples. Total phosphorus values measured with the persulphate method were 97±3% of the combination values. Differences ranging from 0.02-0.15 μM between the UV method and other methods were attributed to the presence of polyphosphates, which are known to resist UV irradiation.

Predominantly low molecular weight DOP was measured during the phytoplankton bloom, although a significant amount of surface active DOP sorbed onto filters, and was therefore not characterized. Similar levels of low molecular weight and colloidal DOP were observed over the bloom as in coastal and open ocean surface seawater samples. The results are consistent with the possibility that direct release (exudation and cell lysis) from phytoplankton dominates DOP production in seawater.

8.3 A current perspective of DOM in seawater

In Chapter 1, I presented two views of DOM in seawater: one was the traditional view based mainly on evidence from wet oxidation methods, and the other was the view that quickly developed from the Suzuki et al. (1985) and Sugimura and Suzuki (1988) publications. After a small lag (that befits all biological systems), these publications initiated considerable research on DOM in seawater. A contribution to the large amount of recent research on DOM has been described in this dissertation.

A much needed intercomparison workshop was held in Seattle in July, 1991 (Williams, 1991). One of the most important aspects of this workshop was that it provided a forum to summarize the progress that has been made in DOC and DON research over the past few years. While most of the reports from this workshop are, as yet, not published, some of the most significant developments have come to my attention through personal communications from P.J. Wangersky, and W.H. Chen, who attended this conference. As well, several important articles have been published very recently, which, together with information presented at the intercomparison workshop, provide the groundwork for a nascent perspective of DOM in seawater, as discussed below.

8.3.1 DOP and DON

There is convincing published evidence to suggest that DOP levels are not significantly higher than previously measured with standard techniques (Ridal and Moore, 1990; Karl and Tien, 1992; Ridal and Moore, 1992; Karl et al., 1992b). In a soon to be published article, Suzuki states that he has been unable to repeat his very high DON values (~40 μM) in surface waters (P.J. Wangersky, pers. commun.). The vast majority of DON values measured in surface samples from the central North Pacific by high temperature oxidation procedures was less than 10 μM (Williams, 1991; Benner et al., 1992), and good agreement has been found

between wet oxidation and HTCO results (Walsh, 1990). Therefore, N and P stoichiometry for dissolved compounds in seawater does not appear to be very different from that of the Redfield relationship (Walsh, 1990; Karl et al., 1992b). From this evidence, it would appear that, while some N-containing compounds have been shown to resist UV oxidation (Gershey et al., 1979), compounds rich in N and P do not make a significant contribution to differences between wet oxidation and HTCO measurements.

8.3.2 DOC

There is little published evidence to support the very high 'Suzuki' DOC values (>250 μM) in surface seawater. All HTCO results published after the initial Sugimura and Suzuki (1988) communication have found values ≤240 μM, and most are <200 μM (Druffel et al., 1989; Cauwet et al., 1990; Bauer et al., 1991; Kirchman et al., 1991; Tanoue, 1991; Suzuki et al., 1992; Benner et al., 1992; Ogawa and Ogura, 1992; Martin and Fitzwater, 1992; Kepkay and Wells, 1992). Suzuki suggests that faulty calibration and calculation procedures contributed to his initial very high DOC values (P. Wangersky, pers. commun.). As discussed by Toggweiler (1992), however, researchers using HTCO instruments are still divided on the proper determination of the analytical blank. DOC values may be in error by as much as ±50 μM depending on the corrections applied to the data. The uncertainty is of the same order as DOC values in the deep ocean and is clearly unacceptable.

There is little evidence for a high inverse correlation between AOU and DOC. Tanoue (1991) re-examined the relationship between AOU and DOC at some of the same stations in the NW Pacific initially sampled by Sugimura and Suzuki (1988). Weak or statistically non-significant correlations were found between AOU and DOC. No apparent correlation was found between AOU and DOC for profiles taken from the North Atlantic, Drake Passage and Equatorial Pacific (Martin and Fitzwater, 1992).

Recent investigations into size fractions of marine DOM do not agree with the size exclusion chromatography results of Suzuki et al. (1985) and Sugimura and Suzuki (1988). As reported in this dissertation (Chapter 6), DOP samples from surface waters of the North Atlantic and NE Pacific were predominantly (74±14%) less than 10,000 NMW. HTCO results presented in Chapter 6 indicated that more than 90% of DOC in NE Pacific surface samples was <10,000 NMW. Ogawa and Ogura report that 50% of DOC in Japanese coastal waters is <1,000 NMW, and 95% is <10,000 NMW. Benner et al. (1992) found 67-77% of the DOC in surface samples from the Central North Pacific was <1000 NMW. These proportions of low molecular weight DOC are similar to the size fractions observed for DOC produced in algal cultures (Chapter 3). The most recent HTCO results are also similar to those obtained in older size fractionation studies of marine DOC (e.g. Sharp, 1973b; Ogura, 1977).

While the validity of the DON and DOC measurements of Sugimura and Suzuki (1988) have been recently seriously questioned, it is difficult to envisage how both sets of size fractionation results were adversely affected by faulty calibrations or other systematic errors. One way of reconciling the molecular weight results in the literature would be to suggest that Sugimura and Suzuki (1988) and Ogawa and Ogura (1992) sampled DOC emanating from different input mechanisms. However, this situation would require tremendous temporal and spatial variability for DOC size fractions in seawater; such oceanic variability would appear intuitively to be outside the ranges associated with dissolved organic constituents in seawater.

One of the contributions made by Sugimura and Suzuki (1988) was to bring greater attention to sampling and storage of DOC samples. There is now evidence to suggest that an appreciable fraction of DOC is microbially labile (hours -days) or semi-labile (days-weeks). Kirchman et al. (1991) found that DOC produced during the North Atlantic spring bloom can have rate constants as high as 20% day^{-1}. Similarly rapid rates of degradation have been found with DOC from algal cultures (Chapter 4; Chen and Wangersky, 1992a; Pett, 1989). Experimental evidence cited by Benner et al. (1992) indicated 49% of the >1,000 NMW

fraction in surface seawater was carbohydrate, but carbohydrates made up only 18-19% of DOC in deep water samples. The difference between carbohydrate concentrations with depth suggests that polysaccharides are important substrates supporting heterotrophic activity in the sea.

In summary, the emerging picture of DOM in seawater is more complex than the two views introduced in Chapter 1. DOC levels in seawater appear to be significantly lower than suggested by Sugimura and Suzuki (1988), but still higher than wet oxidation values. At present, analytical blank problems with HTCO instruments limit the accuracy of HTCO determinations. Contrary to the traditional view of low concentrations of labile compounds in seawater, as much as 50 μM of DOC in surface waters can be rapidly degraded by micro-organisms (Kirchman et al., 1991). Martin and Fitzwater (1992) report a 100 μM decrease in Pacific surface waters from 9° N to the equator. As Toggweiler (1992) points out, such gradients must be generated from high levels of DOC production and/or mineralization in surface waters.

8.4 Future research

8.4.1 Suggestions for future DOP research

There is still a dearth of deep profiles of DOP in regions of oceanic importance. Regions of particular interest are high latitude seas so that the concentrations of DOP in areas of deep water formation can be assessed. As discussed in Chapter 5, there is some suggestion that DOP levels are higher in Pacific deep waters than Atlantic. Evidence from this work is not conclusive. A comprehensive study of DOP levels in Atlantic and Pacific oceans is needed to resolve whether subsurface and deep water levels are significantly different. Reports of 0.5 0.8 μM DOP in 1,000 m water off Antarctica (Yanagi, Yasuda and Fukui, unpublished) may represent a source of high DOM to Pacific and Atlantic deep waters, but these

observations need confirmation as such high levels have not been previously reported (Jackson and Williams, 1985).

There are still relatively few size fractionation studies of DOP, DON and DOC in seawater. The temporal variability of DOM size fractions is not well known. If DOC inputs from grazing produce DOM with high molecular weights, as suggested by Bronk and Glibert (1991), different DOC input mechanisms will lead to DOM size fractions that are variable in space and time. Recently published evidence (Benner et al., 1992; Ogawa and Ogura, 1992) points to a dynamic pool of DOM with MW range from 1,000-10,000 daltons; further investigations of this size fraction are required to determine its modes of formation and lability to bacterial utilization.

Characterization of DOP compounds in seawater should be aided by two recent developments. Karl and Tien (1992) have demonstrated that their coprecipitation method removes most phosphorus compounds from seawater. Large amounts of phosphorus-containing compounds can therefore be obtained. A second technique is the ion retardation column method introduced for seawater analysis by Bronk and Glibert (1991) for separation of inorganic and organic forms of dissolved nitrogen. This method has been applied with success to HTCO analyses of DON (Chen and Wangersky, unpublished), and should be applicable to DOP analysis. These isolation techniques, together with ultrafiltration procedures, may facilitate the use of ^{31}P-NMR (Ingall et al., 1990) to characterize DOP in seawater.

8.4.2 Suggestions for future DOC research

Further research into identification and quantification of the sources and sinks of DOM are essential for an understanding of the exchanges between the aquatic biota and their environment, as well as the interaction of oceans and atmosphere in global carbon cycling.

One of the potential important sources of DOC in marine and freshwater systems is from grazing of bacteria and phytoplankton. This topic has been the focus of a numbers of studies (Lampert, 1978; Eppley et al., 1981; Linley et al., 1983; Taylor et al., 1985; Caron et al., 1985; Jumars et al., 1989; Caron et al., 1991; Weisse and Scheffel-Moser, 1991); however, with the exception of some measurements of dissolved free amino acids produced from grazing (Andersson et al., 1985; Roy et al., 1989) the nature of DOC produced from grazing has not been well characterized.

Bronk and Glibert (1991) have suggested that cell lysis, viral infection and sloppy grazing should produce high molecular weight DOM, presumably by spillage of algal macromolecular metabolites into the external medium. Although this process must certainly occur, I am not aware of studies that explicitly quantify the process. It is worth noting that I have not observed production of PDOC with large proportions of high molecular weight (>10,000 NMW) material during the senescent phases of algal cultures (Chapter 3). However, further investigation along these lines should use a lower molecular weight cut-off (>1,000), since open ocean studies indicate that the 1,000-10,000 NMW size fraction may be more dynamic.

Roy et al. (1989) produced conditions favorable to sloppy feeding by introducing large diatoms to copepods. A similar approach could be done with uniformly radiolabeled algae. Higher amounts of ^{14}C compounds in the dissolved phase compared with controls would indicate inputs from sloppy grazing (egestation would be small over the short term). Careful filtration followed by ultrafiltration would be used to determine inputs to the high molecular weight pool. Sonication of algal cells might prove to be a useful technique for experiments preliminary to grazing studies.

Another interesting aspect of grazing inputs to the DOC pool is the possible

particle counting methodology (Koike et al., 1990; Longhurst et al., 1992) and transmission electron microscopy (TEM; Wells and Goldberg, 1991; Kepkay and Wells, 1992), have identified the presence of high numbers of non-living organic particles in oceanic surface waters with sizes below 1 μm. Similar particles have been observed in freshwater systems (Leppard et al., 1990). A recent study has shown that marine tunicates filter, concentrate and ingest submicron material (Flood et al., 1992). Organic submicron particles contribute to the DOC reservoir since these particles are largely not retained by 0.8 μm filters, often used to provide an operational separation between dissolved and particulate carbon. In Chapter 3, I found that DOC >100,000 MW (0.01-0.4 μm size fraction) produced from *Synechococcus* was resistant to persulphate oxidation (28±4%). Therefore, if large numbers of organic submicron particles are to be found in marine surface waters, as evidence suggests, then this fraction of 'DOC' may contribute significantly to the difference between wet chemical oxidation and high temperature combustion measurements. A quantitative understanding of organic submicron particle characteristics and dynamics will provide better predictions of upper ocean carbon dynamics (Johnson and Kepkay, 1992). Colloidal particles have often been implicated as important components in the transport of surface active chemicals in the ocean (e.g. Moran and Moore, 1989; Niven, 1989).

There is some circumstantial evidence that most submicron particles are produced from the grazing of bacteria and cyanobacteria by heterotrophic nanoflagellates (Koike et al., 1990). I suggest that these particles may be remnants of grazer-indigestibile bacterial cell wall material. In Chapter 4, I found that chemically refractory DOC was produced from the grazing of POC by heterotrophic nanoflagellates. I suggest further study of the DOC produced from grazing heteroflagellates to determine the rate of production of submicron particles from heterotrophic flagellates, their resistance to wet oxidation, and the contribution of these

particles to DOC dynamics in nature.

One approach would be to produce and examine submicron particles in the laboratory. Cyano- and heterotrophic bacteria could be cultured in defined media and thoroughly labelled with ^{14}C-glucose or $NaH^{14}CO_3$. Labelled bacteria would be incubated in the presence of heterotrophic nanoflagellates, the population dynamics of all organisms followed by epifluoresence microscopy, and the production of submicron particles monitored by resistive particle counting (Longhurst et al., 1992). Submicron particles can be separated from bacteria and protist grazers by careful serial filtration. These particles can be collected by 0.05 μm Nuclepore gravity filtration (Koike et al., 1990) or by ultracentrifugation procedures (Wells and Goldberg, 1991). Filters would be radio-assayed to determine the rate of carbon transfer between size fractions. I would also attempt to show a direct link between grazing and the production of submicron particles using refined TEM procedures for counting submicron organic particles (Perret et al., 1991).

Laboratory production of labelled submicron particles facilitates physico-chemical characterisation of these small particles Samples containing known amounts of submicron particles can be assayed for their resistance to UV or persulphate oxidation procedures to determine to what extent these colloids may contribute to the discrepancy found between these standard procedures and the high temperature combustion method. The tendency of these colloids to coagulate or produce colloids of a different size spectrum under the influence of bubbling can also be examined, since this phenomenon is known to be of vital importance to DOC metabolism in oceans (Kepkay and Johnson, 1989; Johnson and Kepkay, 1992).

Another approach would be to examine production and dynamics of submicron particles in a lake or low-energy coastal embayment. The spring algal bloom provides an excellent opportunity to observe the *in situ* production of submicron particles. Successive

blooms of bacteria and heterotrophic flagellate grazers following algal blooms should provide a measurable pulse of submicron particles. The kinetics of particle appearance and disappearance could be determined by short time scale sampling, and may indicate removal processes. The information gained from this research would benefit those marine chemists interested in the role of organic submicron particles in the uptake of particle reactive elements and compounds in aquatic waters.

Bibliography

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

Aitchison, P.A. and Butt, V.S. 1973. The relation between the synthesis of inorganic polyphosphate and phosphate uptake by *Chlorella vulgaris*. *J. Exp. Bot.*, **24**: 497-510.

Anderson, G.C., Parsons, T.R. and Stephens, K. 1969. Nitrate distributions in the subarctic North Pacific Ocean. *Deep-Sea Res.*, **16**: 329-334.

Andersson, A., Lee, C., Azam, F. and Hagström, Å. 1985. Release of amino acids and inorganic nutrients by heterotrophic marine microflagellates. *Mar. Ecol. Prog. Ser.*, **23**: 99-106.

Andreae, M.O. 1978. Distribution and speciation of arsenic in natural waters and some marine algae. *Deep-Sea Res.*, **25**: 391-402.

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

Armador, J.A., Alexander, M. and Zika, G.G. 1989. Sequential photochemical and microbial degradation of organic molecules bound to humic acid. *Appl. Envir. Microbiol.*, **55**: 2843-2849.

Armstrong, F.A.J., Williams, P.M. and Strickland, J.D.H. 1966. Photo-oxidation of organic matter in seawater by ultra-violet radiation, analytical and other applications. *Nature*, **211**: 481-483.

Armstrong, F.A.J. 1965. Phosphorus. In: *Chemical Oceanography*, vol 1, J.P.Riley and G. Skirrow (eds.), Academic Press, London, Chapter 8.

Armstrong, F.A.J. and Tibbitts, S. 1968. Photo-chemical combustion of organic matter in

seawater for nitrogen, phosphorus and carbon determination. *J. Mar. biol. Ass. U.K.*, **48**: 143-152.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**: 257-263.

Bada, J.L. and Lee, C. 1977. Decomposition and alteration of organic compounds dissolved in seawater. *Mar. Chem.*, **5**: 523-534.

Baines, S.B. and Pace M.L. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. *Limnol. Oceanogr.*, **36**: 1078-1090.

Barber, R.T. 1968. Dissolved organic carbon from deep waters resists microbial oxidation. *Nature*, **220**, 274-275.

Bauer, J.E., Williams, P.M., Druffel, E.R.M. and Suzuki, Y. 1990. Deep profiles of dissolved organic carbon in the Sargasso Sea (abstract). *EOS*, **71**: 154.

Benner, R., Palukski, J.D., McCarthy, M., Hedges, J.I., and Hatcher, P.G. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science*, **255**: 1561-1564.

Berner, R.A. 1980. A rate model for organic matter decomposition during bacterial sulphate reduction in marine sediments. *Colloq. Int. CNRS*, **293**: 35-44.

Billen, G. 1984. Heterotrophic utilization and regeneration of nitrogen. In: *Heterotrophic activity in the Sea*, NATO conference series IV (15), J.E. Hobbie and P.J. LeB. Williams (eds.), Plenum Press, New York, pp. 313-356.

Booth, B.C. 1988. Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific in May and August, 1984. *Mar. Biol.*, 97: 275-286.

Bossard, P. and Karl, D.M. 1986. The direct measurement of ATP and adenine nucleotide pool turnover in microorganisms: a new method for environmental assessment of

metabolism, energy fux and phosphorus dynamics. *J. Plank. Res.*, **8**: 1-13.

Bradshaw, A.L. and Brewer, P.G. 1988. High precision measurements of alkalinity and total carbon dioxide in seawater by potentiometric titration. 1. Presence of unknown protolyte(s)? *Mar. Chem.*, **23**: 69-86.

Bratbak, G. 1987. Carbon flow in an experimental microbial ecosystem. *Mar. Ecol. Prog. Ser.*, **36**: 267-276.

Brockmann, U.H., Eberlein, K., Junge, H.D., Maier-Reimer, E. and Siebers, D. 1979. The development of a natural phytoplankton population in an outdoor tank with nutrient poor seawater. II. Changes in dissolved carbohydrates and amino acids. *Mar. Ecol. Prog. Ser.*, 1: 283-291.

Broecker, W. S. and Peng, T.-H. 1982. *Tracers in the Sea.* Eldigo Press, Columbia University, New York. pp. 275-314.

Bronk, D.A. and Glibert, P.M. 1991. A ^{15}N tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. *Mar. Ecol. Prog. Ser.*, **77**: 171-182.

Brophy, J.E. and Carlson, D.J. 1989. Production of biologically refractory dissolved organic carbon by natural seawater microbial populations. *Mar. Chem.*, **25**: 497-507.

Burton, J.D. 1973. Problems in the analysis of phosphorus compounds. *Wat. Res.*, 7: 291-307.

Burton, J.D. and Riley, J.P. 1956. Determination of soluble phosphate, and total phosphorus in sea-water and in marine muds. *Mickrochim. Acta*, **9**: 1350-1365.

Carlson, D.J., Brann, M.L., Mague, T.H. and Mayer, L.M. 1985. Molecular weight distribution of dissolved organic materials in seawater determined by ultrafiltration. *Mar. Chem.*, **16**: 155-171.

Carlson, D.J., Mayer, L.M. Brann, M.L. and Mague, T.H. 1985. Binding of monomeric organic compounds to macromolecular dissolved organic matter in seawater. *Mar. Chem.*, **16**: 141-153.

Carlucci, A.F., Shrimp, S.L. and Craven, D.B. 1986. Growth characteristics of low-nutrient bacteria from the northeast- and central Pacific Ocean. *FEMS Microbial Ecol.*, **38**: 1-10.

Caron, D.A., Goldman, J.C., Anderson, O.K. and Dennett, M.R. 1985. Nutrient cycling in a microflagellate food chain: II. Population dynamics and carbon cycling. *Mar. Ecol. Prog. Ser.*, **24**: 243-254.

Caron, D.A., Lim, E.L., Miceli, G., Waterbury, J.B. and Valois, F.W.. 1991. Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. *Mar. Ecol. Prog. Ser.*, **78**: 205-217.

Cauwet, G., Sempere, R. and Saliot, A. 1990. Carbone organique dissous dans l'eau de mer: confirmation de la sous-estimation antérieure. *C.R. Acad. Sci. Paris*, **311**: 1061-1066.

Cembella, A.D., and Antia, N.J. 1986. The determination of phosphonates in seawater by fractionation of the total phosphorus. *Mar. Chem.*, **13**: 205-210.

Cembella, A.D., Antia, N.J. and Harrison, P.J. 1984. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective. Part 1. *CRC Crit. Rev. Microbiol.*, **10**: 317- 391.

Cembella, A.D., Anitia, N.J. and Taylor, F.J.R. 1986. The determination of total phosphorus in seawater by nitrate oxidation of the organic component. *Wat. Res.*, **20**: 1197-1199.

Chamberlain, W. and Shapiro, J. 1969. On the biological significance of phopshate analysis: comparison of standard and new methods with a bioassay. *Limnol. Oceanogr.*, **14**: 921-927.

Chen, W. and Wangersky, P.J. 1992a. High temperature combustion analysis of dissolved organic carbon produced from phytoplankton cultures. *Mar. Chem.*, In press.

Chen, W. and Wangersky, P.J. 1992b. A high temperature catalytic oxidation method for the determination of marine dissolved organic carbon and its comparison with the photo-oxidation method. *Mar. Chem.*, submitted.

Chrost, R.H. and Faust, M.A. 1983. Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. *J. Plank. Res.*, **5**: 477-493.

Codispoti, L.A. 1989. Phosphorus vs. nitrogen limitation in new and export production. In: *Productivity of the Oceans: Present and Past*, W.H. Berger, V. Smetacek, and G. Wefer, (eds.), John Wiley and Sons, Chichester. pp 65-83.

Collins, K.J. and Williams, P.J. Le B. 1977. An automated photochemical method for the determination of dissolved organic carbon in sea and estuarine waters. *Mar Chem.*, **5**: 123-141.

Cullen, J.J., Lewis, M.R., Davis, C.O. and Barber, R.T. 1992. Photosynthetic characteristics and estimated growth rates indicate grazing is the proximate control of primary production in the equatorial Pacific. *J. Geophys. Res.*, **97**: 639-654.

Cynar, F.J, Estep, K.W. and Sieburth, J. McN. 1985. The detection and characterization of bacteria-sized protists in "protist-free" filtrates and their potential impact on experimental marine ecology. *Microb. Ecol.*, **11**: 281-288.

Decho, A.W. 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Ann. Rev.*, **28**: 73-153.

Dittmar, W. 1884. Report on researches into the composition of ocean-water collected by H.M.S. *Challenger* during the years 1873-1876. In: *Report on the Scientific Results of the Voyage of H.M.S. Challenger: Physics and Chemistry*. Vol I, pt. 1. John Murray, (ed.) H.M.S.O., London.

Druffel, E.M.R., Williams, P.M. and Suzuki, Y. 1989. Concentration and radiocarbon signatures of dissolved organic matter in the Pacific Ocean. *Geophys. Res. Lett.*, **16**: 991-994.

Ducklow, H.W. 1992. Oceanic bacterial production. In: *Advances in Microbial Ecology*, vol 12, K.C. Marshall (ed.). In press.

Emerson, S. 1987. Seasonal oxygen cycles and biological new production in surface waters of the subarctic Pacific ocean. *J. Geophys. Res.*, **92**: 6535-6544.

Flood, P.R., Deibel, D. and Morris, C.C. 1992. Filtration of colloidal melanin from sea water by planktonic tunicates. *Nature*, **355**: 630-632.

Fogg, G.E. 1973. Phosphorus in primary aquatic plants. *Wat. Res.*, 7: 77-91.

Fogg, G.E. 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Mar.*, **16**: 3-14.

Ford, C.W. and Percival, E.E. 1965. Carbohydrates of *Phaeodactylum tricornutum*. *J. Chem. Soc.*, 77: 7042-7046.

Francko, D.A. and Heath, R.T. 1979. Functionally distinct classes of complex phosphorus compounds in lake water. *Limnol. Oceanogr.*, **24**: 463-473.

Froelich, P.N., Bender, M.I. and Luedtke, N.A. 1982. The marine phosphorus cycle. *Am. J. Sci*, **282**: 474-511.

Frost, B.W. 1987. Grazing control of phytoplankton stock in the open subarctic Ocean: a model assessing the role of mesozooplankton, particularly the large calanoid copepods *Neocalanus* spp. *Mar. Ecol. Prog. Ser.*, **39**: 49-68.

Gagosian, R.B. and Lee, C. 1981. Processes controlling the distribution of biogenic organic compounds in seawater. In: *Marine Organic Chemistry*, Ch. 5, E.K. Duursma and R.Dawson (eds.), Elsevier, Amsterdam. pp. 91-118.

Gershey, R.M., MacKinnon, M.D., Williams, P.J.Le B., and Moore, R.M. 1979. Comparison of three oxidation methods used for the analysis of the dissolved organic carbon in seawater. *Mar. Chem.*, 7: 289-306.

Glover, H.E., Prezelin, B.B., Campbell, L., Wyman, M. and Garside, C. 1988. A nitrate-dependent *Synechococcus* bloom in surface Sargasso Sea water. *Nature*, **331**: 161-163.

Goldman, J.C., Caron, D.A., Anderson, O.K and Dennett, M.R. 1985. Nutrient cycling in a microflagellate food chain: I. Nitrogen dynamics. *Mar. Ecol. Prog. Ser.*, **24**: 231-242.

Goldman, J.C. and Dennett, M.R. 1985. Susceptibilty of some marine phytoplankton species

to cell breakage during filtration and post-filtration rinsing. *J. Exp. Mar. Biol. Ecol.*, **86**: 47-58.

Gordon, D.C., and Sutcliffe, W.H. 1973. A new dry combustion method for the simultaneous determination of total organic carbon and nitrogen in seawater. *Mar. Chem.*, **1**: 231-244.

Grasshoff, K. 1983. Filtration and storage. In: *Methods for seawater analysis*, 2nd edition. K. Grasshoff, M. Ehrhardt and K. Kremling (eds.), Verlag Chemie, Weinheim, pp.21-30.

Grill, E.V. and Richards, F.A. 1964. Nutrient regeneration from phytoplankton decomposing in seawater. *J. Mar. Res.*, **22**: 51-69.

Guillard, R.R.L. and Wangersky, P.J. 1958. The production of extracellular carbohydrates by some marine flagellates. *Limnol. Oceanogr.*, **3**: 449-454.

Hansen, A.L. and Robinson, R.J. 1953. The determination of organic phosphorus in seawater with perchloric acid oxidation. *J. Mar. Res.*, **12**: 31-42

Harrison, W.G. 1983. Uptake and recycling of soluble reactive phosphorus by marine microplankton. *Mar. Ecol. Prog. Ser.*, **10**: 127-135.

Harrison, P.J., Waters, R.E. and Taylor, F.J.R. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.*, **16**: 28-35.

Harvey, H.W. 1948. The estimation of phosphate and total phosphorus in seawater. *J. Mar. biol. Ass. U.K.*, **28**: 337-359.

Harvey, G.R., Boran, D.A., Chesal, L.A. and Takar, J.M. 1983. The structure of marine fulvic and humic acids. *Mar. Chem.*, **12**: 119-133.

Hashimoto, S., Fujiwura, K. and Fuwa, K. 1987. Determination of phosphorus in natural water using hydride generation and gas chromatography. *Limnol. Oceanogr.*, **32**: 729-735.

Haug, J. and Myklestad, S. 1976. Polysaccharides of marine diatoms with special reference to *Chaetoceros* species. *Mar. Biol.*, **34**: 217-222.

Hedges, J.I. 1987. Organic matter in sea water. *Nature*, **330**: 205-206.

Hellebust, J.A. 1965. Excretion of some organic compounds by marine phytoplankton. *Limnol. Oceanogr.*, **10**: 192-206.

Henriksen, A. 1970. Determination of total nitrogen, phosphorus and iron in fresh water by photo-oxidation with ultraviolet radiation. *Analyst*, **95**: 601-608.

Herbes, S.E., Allen, H.E. and Mancy, K.H.. 1975. Enzymatic characterization of soluble organic phosphorus in seawater. *Science*, **187**: 432-434.

Hobbie, J.E., Daley, R.J. and Jasper, E. 1977. Use of Nuclepore filters for counting bacteria by fluoresence microscopy. *Appl. environ. Microbiol.*, **33**: 1225-1228.

Hollibaugh, J.T., Buddemeier, R.W. and Smith, S.V. 1991. Contributions of colloidal and high molecular weight dissolved organic material to alkalinity and nutrient concentrations in shallow marine and estuarine systems. *Mar. Chem.*, **34**: 1-27.

Holm-Hansen, O., Strickland, J.D.H. and Williams, P.M. 1966. A detailed analysis of biologically important substances in a profile off Southern California. *Limnol. Oceanogr.*, **11**: 548-561.

Hooper, F.F. 1971. Origin and fate of organic phosphorus compounds in aquatic systems. In: *Environmental Phosphorus Handbook*, E.J. Griffin, A. Beeton, J.M. Spencer, and D.T. Mitchell (eds.), John Wiley and Sons, New York. pp.179-202.

Horrocks, D.L. 1978. A new method of quench monitoring in liquid scintillation counting: The H number concept. *Journal of Radioanalytical Chemistry*, **43**, 489-521.

Ingall, E.D., Schroeder, P.A. and Berner, R.A. 1990. The nature of organic phosphorus in marine sediments: New insights from ^{31}P NMR. *Geochim. Cosmochim. Acta*, **54**: 2617-2620.

Ittekkot, V. 1982. Variations of dissolved organic matter during a plankton bloom: Qualitative aspects, based on sugar and amino acid analyses. *Mar. Chem.*, **11**: 143-158.

Ittekkot, V., Brockmann, U., Michaels, W. and Degens, E.T. 1981. Dissolved free and combined carbohydrates during a phytoplankton bloom in the Northern North sea. *Mar. Ecol. Prog. Ser.*, **4**: 299-305.

Iturriaga, R. and Hoppe, H.-G. 1977. Observations of heterotrophic activity on photoassimilated organic matter. *Mar. Biol.*, **40**: 101-108.

Iturriaga, R. and Zsolnay, A. 1981. Transformation of some dissolved organic compounds by a natural heterotrophic population. *Mar. Biol.*, **62**: 125-129.

Iturriaga, R and Zsolnay, A. 1983. Heterotrophic uptake and transformation of phytoplankton extracellular products. *Botanica Mar.*, **16**: 375-381.

Jackson, G.A. 1988. Implications of high dissolved organic matter concentrations for oceanic properties and processes. *Oceanography*, **1**: 28-31.

Jackson, G.A. and Williams, P.M. 1985. Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. *Deep-Sea Res.*, **32A**: 223-235.

Jenkins, D. 1968. The differentiation, analysis and preservation of nitrogen and phosphorus forms in natural waters. In: *Trace inorganics in water*, R.A. Baker (ed.). Advan. in Chem Series, **73**: 265-280.

Jensen, L.M. 1983. Phytoplankton release of extracellular organic carbon, molecular weight composition, and bacterial assimilation. *Mar. Ecol. Prog. Ser.*, **11**: 39-48.

Jensen, L.M., Jorgensen, N.O.G. and Sondergaard, M. 1984. Specific activity. Significance in estimating release rates of extracellular dissolved organic carbon (EOC) by algae. *Proc. Int. Assoc. Theor. Limnol. Travail.* Congress in France (1983), **22**: 2893-2897.

Johnson, B.D. and Kepkay, P.E. 1992. Colloid transport and bacterial utilization of oceanic DOC. *Deep-Sea Res.*, **39**: 855-869.

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

Johnson, K.M., King, A.E. and Sieburth, J. McN. 1985. Coulometric TCO_2 analyses for marine studies: an introduction. *Mar. Chem.*, **16**: 61-82.

Jones, A.K. and Cannon, R.C. 1986. The release of micro-algal photosynthate and associated bacterial uptake and heterotrophic growth. *Br. phycol. J.*, **21**: 341-358.

Jumars, P.A., Penry, D.L., Baross, J., Perry, M.J. and Frost, B.W. 1989. Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. *Deep-Sea Res.*, **36**: 483-497.

Karl, D.M. and Tien, G. 1992. Magic: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. *Limnol. Oceanogr.*, in press.

Karl, D.M., Letelier, R., Hebel, D.V., Bird, D.F. and Winn, C.D. 1992a. *Trichodesmium* blooms and new nitrogen in the North Pacific gyre. In: *Biology and ecology of diazotrophic marine organisms: Trichodesmium and other species.* D.G. Capone and J. G. Rueter (eds.). Kluwar Academic Publishers.

Karl, D.M., Tien, G., Dore, J. and Winn, C. D. 1992b. Total dissolved nitrogen and phosphorus concentrations at US-JGOFS Station ALOHA: Redfield reconciliation. *Mar. Chem.*,in press.

Kepkay, P.E. and Johnson, B.D. 1989. Coagulation on bubbles allows microbial respiration of oceanic dissolved organic carbon. *Nature*, **338**: 63-65.

Kepkay, P.E. and Wells, M.L. 1992. Dissolved organic carbon in North Atlantic surface waters. *Mar. Ecol. Prog. Ser.*, **80**: 275-283.

Ketchum, B. H. and Corwin, N. 1965. The cycle of phosphorus in a plankton bloom in the Gulf of Maine. *Limnol. Oceanogr.*, **10** suppl. R: 148-161.

Ketchum, B.H., Corwin, N. and Keen, D.J. 1955. The significance of organic phosphorus determinations in ocean waters. *Deep-Sea Res.*, **2**: 172-181.

Kieber, D.J, McDaniel, J. and Mopper,K. 1989. Photochemical source of biological substrates in sea water: implications for carbon cycling. *Nature*, **341**: 637-639.

Kirchman, D.L., Suzuki, Y., Garside, C. and Ducklow, H.W. 1991. High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature*, **352**: 612-614.

Kittredge, J.S., Horiguchi, M. and Williams, P.M. 1969. Aminophosphonic acids: biosynthesis by marine phytoplankton. *Comp. Biochem. Physiol.*, **29**: 859-863.

Kobori, H. and Taga, N. 1979. Phosphatase activity and its role in the mineralization of organic phosphorus in coastal sea water. *J. exp. mar. Biol. Ecol.*, **56**: 23-39.

Koike, I., Shigemitso, H., Kazuki, T., and Kazuhiro, K. 1990. Role of sub-micrometre particles in the ocean. *Nature*, **345**: 242-244.

Koroleff, F. 1983. Determination of total phosphorus. In: *Methods for Seawater Analysis*, 2nd edition. K. Grasshoff, M. Ehrhardt, and K. Kremling (eds.), Verlag Chemie, Weinheim, pp. 167-173.

Kobayashi, Y. and Harris, W.G. 1978. LSC Applications Notes. New England Nuclear Applications Laboratory, New England Nuclear.

Kuenzler, E.J. 1970. Dissolved organic phosphorus excretion by marine phytoplankton. *J. Phycol.*, **6**: 7-15.

Kuenzler, E.J., Guillard, R.R.L., and Corwin, N. 1963. Phosphate-free sea water for reagent blanks in chemical analyses. *Deep-Sea Res.*, **10**: 749-755.

Lampert, W. 1978. Release of dissolved organic carbon by grazing zooplankton. *Limnol. Oceanogr.*, **23**: 831-834.

Lancelot, C. 1984. Extracellular release of small and large molecules by phytoplankton in the Southern Bight of the North Sea. *Est., Coast. Shelf Sci.*, **18**: 65-77.

Lancelot, C. and Mathot, G. 1987. Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. *Mar. Ecol. Prog. Ser.*, **37**: 239-248.

Lebo, M.E. 1990. Phosphate uptake along a coastal plain estuary. *Limnol. Oceanogr.*, **35**: 1279-1289.

Lee, C. and Henrichs, S. M. 1992. How the nature of dissolved organic matter might affect the analysis of dissolved organic carbon. *Mar. Chem.*, In press.

Legendre, L. and Gosselin, M. 1989. New production and export of organic matter to the deep ocean: consequences of some recent discoveries. *Limnol. Oceanogr.*, **34**: 1374-1380.

Leppard, G.G., Burnison, B.K. and Buffle, J. 1990. Transmission electron microscopy of the natural organic matter of surface waters. *Anal. Chim. Acta*, **232**: 107-121.

Lewis, M. R.,Warnock, R.E., Irwin, B. and Platt, T. 1985. Measuring photosynthetic action spectra of natural phytoplankton populations. *J. Phycol.*, **21**: 310-315.

Linley, E.A.S., Newell, R.C. and Lucas, M.I. 1983. Quantitative relationships between phytoplankton, bacteria and heterotrophic microflagellates in sheif waters. *Mar. Ecol. Prog. Ser.*, **12**: 77-89.

Li, W.K.W., Dickie, P.M., Irwin, B.D. and Wood, A.M. 1991. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. *Deep-Sea Res.*, **39**: 501-519.

Longhurst, A. R., Koike, I., Li, W.K.W., Rodriguez, J., Dickie, P., Kepkay, P., Partensky, F., Bautista, B., Ruiz, J., Wells, M. and Bird, D.F. 1992. Sub-micron particles in northwest Atlantic shelf water. *Deep-Sea Res.*, **39**: 1-7.

Mague, T.H., Friberg, E., Hughes, D.J. and Morris, I. 1980. Extracellular release of carbon by marine phytoplankton; a physiological approach. *Limnol. Oceanogr.*, **25**: 262-279.

Manny, B.A., Miller, M.C. and Wetzel, R.G. 1971. Ultraviolet combustion of dissolved organic nitrogen compounds in lake waters. *Limnol. Oceanogr.*, **16**: 71-85.

Martin, J.H. and Fitzwater, S.E. 1992. Dissolved organic carbon in the Atlantic, Southern and Pacific oceans. *Nature*, **356**: 699-700.

Martin, J.H., Gordon, R.M., Fitzwater, S. and Broenkow, W.W. 1989. Vertex: phytoplankton/iron studies in the Gulf of Alaska. *Deep-Sea Res.*, **36**: 649-680.

Matsuda, O. and Maruyama, A. 1985. Gel chromatographic characterization of dissolved organic phosphorus in eutrophic seawater during a phytoplankton bloom. *Bull. Plank. Soc. Jap.*, **32**: 91-99.

Maurer, L.G. 1976. Organic polymers in seawater: changes with depth in the Gulf of Mexico. *Deep-Sea Res.*, **23**: 1059-1064.

McKinnon, M. D. 1977. The analysis of the total organic carbon in seawater. Ph.D. thesis, Dalhousie Univ., 183 pp.

Menzel, D.W. 1974. Primary productivity, dissolved and particulate organic matter, and the sites of oxidation of organic matter. In: *The Sea*, vol V, E.D. Goldberg (ed.), Wiley-Interscience, New York. pp. 659-678.

Menzel, D.W. and Corwin, N. 1965. The measurement of total phosphorus in seawater based on the liberation of the organically bound fractions by persulphate oxidation. *Limnol. Oceanogr.*, **10**: 280-282.

Menzel, D.W. and Vacarro, R.F. 1964. The measurement of dissolved and particulate carbon in seawater. *Limnol. Oceanogr.*, **9**: 138-142.

Meyers-Schulte, K.J. and Hedges, J.I. 1986. Molecular evidence for a terrestrial component of organic matter dissolved in ocean water. *Nature*, **321**: 61-63.

Minear, R.A. 1972. Characterization of naturally occuring dissolved organophosphorus compounds. *Envir. Sci. Tech.*, **6**: 431-437.

Mopper, K. and Degens, E.T. 1979. Organic carbon in the ocean: nature and cycling. In: *The Global Carbon Cycle*, B. Bolin, E.T. Degens, S.Kempe and P.Ketner (eds.), John Wiley and Sons, Chichester, pp. 293-316.

Mopper, K. and Zhou, X. 1990. Hydroxyl radical photoproduction in the sea and its potential impact on marine processes. *Science*, **250**: 661-663.

Moore, R.M. and Tokarcyk, R.. 1992. Volatile biogenic halocarbons in the Northwwest Atlantic. *Global Biogeochem. Cycles*, submitted.

Moran, S.B. 1990. The marine geochemisrty of Aluminum. Ph. D. thesis, Dalhousie University. 258 pp.

Moran, S.B. and Moore, R.M. The distribution of colloidal aluminum and organic carbon in coastal and open ocean waters off Nova Scotia. *Geochim. Cosmochim. Acta*, **53**: 2519-2527.

Murphy, J. and Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. chim. Acta*, **27**: 31-36.

Myklestad, S. 1974. Production of carbohydrates by marine planktonic diatoms: I. Comparison of nine different species in culture. *J. Exp. Mar. Biol. Ecol.*, **15**: 261-274.

Niven, S. E. H. 1989. Solid-solution partitioning of Thorium in seawater. Ph.D. thesis, Dalhousie Univ. 175 pp.

Ogawa, H. and Ogura, N. 1992. Comparison of two methods for measuring dissolved organic carbon in sea water. *Nature*, **356**: 696-698.

Ogura, 1970. The relation between dissolved organic carbon and apparent oxygen utilization in the Western North Pacific. *Deep-Sea Res.*, **17**: 221-231.

Ogura, N. 1972. Rate and extent of decomposition of dissolved organic matter in surface seawater. *Mar. Biol.*, **13**: 89-93.

Ogura, N. 1975. Further studies on decomposition of dissolved organic matter in coastal seawater. *Mar. Chem.*, **5**: 535-549.

Ogura, N. 1977. High molecular weight organic matter in seawater. *Mar. Chem.*, **5**: 535-549.

Orrett, K. and Karl, D.M. 1987. Dissolved organic phosphorus production in surface seawaters. *Limnol. Oceanogr.*, **32**: 383-395.

Parrish, C.C. 1987. Time series of particulate and dissolved lipid classes during spring phytoplankton blooms in Bedford Basin, a marine inlet. *Mar. Ecol. Prog. Ser.*, **35**: 129-139.

Parsons, T.R. and Lalli, C.M. 1988. Comparative oceanic ecology of the plankton communities of the subarctic Atlantic and Pacific oceans. *Oceanogr. Mar. Biol. Annu. Rev.*, **26**: 317-359.

Peng, T.-H. and Broecker, W.S. 1987. C/P ratios in marine detritus. *Global biogeochemical cycles*, **1**: 155-161.

Peters, C.J., Luong, T.V., and Perry, R. 1982. Influence of bromide and ammonia on the formation of trihalomethanes under water-treatment conditions. *Environ. Sci. Technol.*, **16**: 473-482.

Perret, D., Leppard, G.G., Muller, M., Belzile, N., De Vitre, R. and Buffle, J. 1991. Electron microscopy of aquatic collloids: non-perturbing preparation of specimens in the field. *Wat. Res.*, **25**: 1333-1343.

Perry, M.J. 1972. Alkaline phosphatase activity in subtropical central North Pacific waters using a sensitive fluorometric method. *Mar. Biol.*, **15**: 113-119.

Perry, M.J. 1976. Phosphate utilization by an oceanic diatom in phosphate-limited chemostat culture and in the oligotrophic waters of the central North Pacific. *Limnol. Oceanogr.*, **21**: 88-107.

Perry, M.J. and Eppley, R.W. 1981. Phosphate uptake by phytoplankton in the central North Pacific Ocean. *Deep-Sea Res.*, **28**: 39-49.

Pett, R.J. 1989. Kinetics of microbial utilization of algae and their exudates in a coastal marine basin. Ph.D. thesis, Dalhousie Univ. 140 pp.

Pomeroy, L.R., Mathews, H.M. and Min, H.S. 1962. Excretion of phosphate and soluble organic phosphorus compounds by zooplankton. *Limnol. Oceanogr.*, **8**: 50-55.

Post, W.M., Peng, T.-H., Emanual, W.R., King, A.W., Dale, V.H. and DeAngelis, D.L. 1990.

The global carbon cycle. *Amer. Scientist*, **78**: 310-330.

Redfield, A.C., Smith, H.P. and Ketchum, B.H. 1937. The cycle of organic phosphorus in the Gulf of Maine. *Biol. Bull.*, **73**: 421-443.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. *Amer. Scientist*, **46**: 205-221.

Ridal. J.J. and Moore, R.M. 1990. A re-examination of the measurement of dissolved organic phosphorus in seawater. *Mar. Chem.*, **29**: 19-31.

Ridal, J.J. and Moore, R.M. 1992. The concentrations of dissolved organic phosphorus in the subarctic NE Pacific. *Limnol. Oceanogr.*, in press.

Rigler, F.H. 1968. Further observations inconsistent with the hypothesis that the molybdenum blue technique measures orthophosphate in seawater. *Limnol. Oceanogr.*, **13**: 7-13.

Riley, J.P. 1975. Analytical chemistry of sea water. In: *Chemical Oceanography*, (2nd ed.). Vol. 3, J.P. Riley and G. Skirrow (eds.), Academic Press, London. pp 421-424.

Rivkin, R.B. and Swift, E.. 1979. Diel and vertical patterns of alkaline phosphatase activity in the oceanic dinoflagellate. *Limnol. Oceanogr.*, **24**: 117-125.

Roy, S., Harris, R.P. and Poulet, S.A. 1989. Ineffiecient feeding by *Calanus helgolandicus* and *Temora longicornis* on *Coscinodiscus wailesii:* quantitative estimation using chlorophyll-type pigments and effects on dissolved free amino acids. *Mar. Ecol. Prog. Ser.*, **52**: 145-153.

Rosso, A. and Azam, F. 1987. Proteolytic activity in coastal oceanic waters: depth distribution and relationship to bacterial populations. *Mar. Ecol. Prog. Ser.*, **41**: 231-240.

Spiteller, M. and Said-Jimenez, C. 1990. A two step degradative procedure for structural studies of aquatic humic acids. *Org. Geochem.*, **15**: 449-455.

Sarmiento, J.L., Toggweiler, J.R. and Najjar, R. 1988. Ocean-carbon dynamics and atmospheric pCO_2. *Phil. Trans. R. Soc. London*, A **325**: 3-21.

Sarmiento, J.L., Theile, G., Key, R.M. and Moore, W.S. 1990. Oxygen and nitrate new production and remineralization in the North Atlantic subtropical gyre. *J. Geophys. Res.*, **95**: 18,303-18,315.

Schlitzer, R. 1989. Modelling the nutrient and carbon cycles of the North Atlantic 2. New production, particle fluxes, CO_2 gas exchange, and the role of organic nutrients. *J. Geophys. Res.*, **94**: 12,781-12,794.

Schwinghamer, P. 1988. Influence of pollution along a natural gradient and in a mesocosm experiment on sediment microbial numbers and biomass. *Mar. Ecol. Prog. Ser.*, **46**: 193-197.

Schwinghamer, P. and Kepkay P. 1987. Effects of experimental enrichment with *Spartina* detritus on sediment community biomass and metabolism. *Biol. Oceanogr.*, **4**: 289-322.

Sellner, K.G. 1981. Primary productivity and the flux of dissolved organic matter in several marine environments. *Mar. Biol.*, **65**: 101-112.

Sharp, J.H. 1973a. Total organic carbon in seawater - comparison of measurements using persulphate oxidation and high temperature combustion. *Mar. Chem.*, **1**: 211-229.

Sharp, J.H. 1973b. Size classes of organic carbon in seawater. *Limnol. Oceanogr.*, **18**, 441-447.

Sharp, J.H. 1977. Extracellular production of organic matter by marine algae. Do healthy cells do it? *Limnol. Oceanogr.*, **22**: 381-399.

Sharp, J.H. 1984. Inputs in microbial food chains. In: *Heterotrophic activity in the Sea*, J.E. Hobbie and P.J. LeB. Williams (eds.), Plenum Press. pp. 101-120.

Sharp, J.H. 1991. Review of carbon, nitrogen and phosphorus biogeochemistry. Reviews of geophysics, April supplement, 648-657.

Sharpf, L.G. 1973. Transformations of naturally occurring organophosphorus compounds in the environment. In: *Environmental Phosphorus Handbook*, E.J. Griffin, A. Beeton, J.M. Spencer, and D.T. Mitchell (eds.), John Wiley and Sons, New York. 393-413.

Skopintsev, B.A. 1966. Some considerations on the distributions and state of organic matter in sea water. *Oceanol. Acad. Sci. USSR*, **6**: 361-368.

Skopintsev, B.A. 1981. Decomposition of organic matter of plankton, humification and hydrolysis. In: *Marine Organic Chemistry*, Ch.6, E.K. Duursma and R. Dawson (eds.), Elsevier, Amsterdam. pp. 125-169.

Smith, R.E. and Geider, R.J. 1985. Kinetics of intracellular carbon allocation in a marine diatom. *J. Exp. Ecol. Biol.*, **93**: 191-210.

Smith, D.F. and Horner, S.M.J. 1981. Tracer kinetic analysis applied to problems in marine biology. *Bull. Fish. aquat. Sci. Can.*, **210**: 113-127.

Smith, S.V. 1984. Phosphorus versus nitrogen limitation in the marine environment. *Limnol. Oceanogr.*, **29**: 1149-1160.

Smith, S.V., Kimmerer, W.J. and Walsh, T.W. 1986. Vertical flux and biogeochemical turnover regulate nutrient limitation of net organic production in the North Pacific gyre. *Limnol. Oceanogr.*, **31**: 161-167.

Solórzano, L. 1978. Soluble fractions of phosphorus compounds and alkaline phosphatase activity in Loch Creran and Loch Etive, Scotland. *J. exp. mar. Biol. Ecol.*, **34**: 227-232.

Solórzano, L. and Sharp, J.H. 1980. Determination of total dissolved phosphorus in natural waters. *Limnol. Oceanogr.*, **25**: 754-758.

Sondergaard, M. and Schierup, H.-H. 1982. Release of extracellular oranic carbon during a diatom bloom in Lake Mosso: molecular weight fractionation. *Freshwater. Biol.*, **12**: 313-320.

Stainton, M.P. 1980. Errors in molybdenum blue methods for determining orthophosphate in freshwater. *Can. J. Fish. Aquat. Sci.*, **37**: 472-478.

Storch, T.A. and Saunders, G.W. 1978. Phytoplankton extracellular release and its relation to the seasonal cycle of dissolved organic carbon in a eutrophic lake. *Limnol. Oceanogr.*,

23: 112-119.

Stricklard, J.D.H. and Austin, K.H. 1960. On the forms, balance and cycle of phosphorus observed in the coastal and oceanic waters of the Northeast Pacific. *J. Fish Res. Bd. Can.*, **17**: 337-345.

Strickland, J.D.H. and Parsons, T.R. 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Board Can.*, 2nd Edn., No. 167: 311 pp.

Strickland, J.D.H. and Solórzano, L. 1968. Polyphosphate in seawater. *Limnol. Oceanogr.*, **13**: 515-518.

Stumm, W. and Morgan, J.J. 1970. *Aquatic Chemistry*. Wiley-Interscience, New York.

Sugimura, Y. and Suzuki, Y. 1988. A high temperature catalytic oxidation method of non-volatile dissolved organic carbon in seawater by direct injection of liquid samples. *Mar. Chem.*, **24**: 105-131.

Suzuki, Y., Peltzer, E.T. and Brewer, P. 1990. DOC analyses during the North Atlantic spring bloom (abstract). *EOS*, **71**: 81.

Suzuki, Y., Sugimura, Y. and Itoh, T. 1985. A catalytic oxidation method for the determination of total nitrogen dissolved in seawater. *Mar. Chem.*, **16**: 83-97.

Tabata, S. 1974. The general circulation of the Pacific ocean. *Atmosphere*, **14**: 1-168.

Taft, J.L, Taylor, W.R. and McCarthy, J.J. 1975. Uptake and release of phosphorus by phytoplankton in the Chesapeake Bay estuary, USA. *Mar. Biol.*, **33**: 21-32.

Taft, J.L., Loftus, M.E. and Taylor, W.R. 1977. Phosphate uptake from phosphomonoesters by phytoplankton in the Chesapeake Bay. *Limnol. Oceanogr.*, **22**: 1012-1021.

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

Tanoue, E. 1991. Vertical distribution of dissolved organic carbon in the North Pacific.

Annual Meeting of the Oceanographic Society of Japan, Tokoyo, Japan. Abstract translated by Taro Takahashi.

Taylor, G.T, Iturriaga, R. and Sullivan, C.W. 1985. Interactions of bactivorous grazers and heterotrophic bacteria with dissolved organic matter. *Mar. Ecol. Prog. Ser.*, **23**: 129-141.

Toggweiler, J.R. 1988. Deep sea carbon, a burning issue. *Nature*, **335**: 468.

Toggweiler, J.R. 1989. Is the downward dissolved organic matter (DOM) flux important in carbon transport? In *Productivity of the Oceans: Present and Past*, W.H. Berger, V. Smetacek, and G. Wefer, (eds.), John Wiley and Sons, Chichester. pp 65-83.

Toggweiler, J.R. 1990. Diving into the organic soup. *Nature*, **345**: 203-204.

Toggweiler, J.R. 1990. Bombs and ocean carbon cycles. *Nature*, **347**: 122-123.

Toggweiler, J.R. 1992. Catalytic conversions. *Nature*, **356**: 665-666.

Walsh, T.W. 1989. Total dissolved nitrogen in seawater: a new high temperature combustion method and a comparison with photo-oxidation. *Mar. Chem.*, **26**: 295-311.

Wangersky, 1976. Particulate organic carbon in the Atlantic and Pacific Oceans. *Deep-Sea Res.*, **23**: 457-465.

Wangersky, P.J. 1978. Production of dissolved organic matter. In: *Marine Ecology*, vol. 14, O. Kinne (ed.), Ch.4, John Wiley, London. pp. 114-220.

Wangersky, P.J. 1982. Model ecosystems: the limits of predictability. *Thalassia Jugoslavica*, **18**: 1-10.

Wangersky, P.J. 1992. Dissolved organic carbon methods: a critical review. *Mar. Chem.*, In press.

Wells, M.L. and Goldberg, E.D. 1991. Occurrence of small colloids in seawater. *Nature*, **353**: 342-344.

Welschmeyer, N.A. and Lorenzen, C.J. 1984. Carbon-14 labeling of phytoplankton carbon and chloropyll a carbon: Determination of specific growth rates. *Limnol. Oceanogr.*, **29**: 135-145.

Wiebe, W.J. and Smith, D.F. 1977. Direct measurement of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. *Mar. Biol.*, **42**: 213-223.

Wiesse, T. and Scheffel-Möser, U. 1991. Uncoupling the microbial loop: growth and grazing loss rates of bacteria and heterotrophic nanoflagellates in the North Atlantic. *Mar. Ecol. Prog. Ser.*, **71**: 195-205.

Williams, P.J. LeB. 1969. The wet oxidation of dissolved organic matter in seawater. *Limnol. Oceanogr.*, **14**: 292-297.

Williams, P.M. 1991. Scientists and industry reps attend workshop on measuring DOC and DON in natural waters. *U.S. JGOFS News*, 3 (1).

Williams, P.M., Oeschger, H. and Kinney, P. 1969. Natural radio-carbon activity of the dissolved organic carbon in the northeast Pacific Ocean. *Nature*, **224**: 256-258.

Williams, P.M., Carlucci, A.F., and Olsen, R. 1980. A deep profile of some biologically important properties in the central North Pacific gyre. *Oceanol. Acta*, **3**: 471-476.

Williams, P.M. and Druffel, E.R.M. 1987. Radiocarbon in dissolved organic carbon in the North Pacific Ocean. *Nature*, **330**: 246.

Williams, P.M. and Druffel, E.R.M. 1988. Dissolved organic matter in the ocean: comments on a controversy. *Oceanography*, **1**: 14-17.

Wilson, M.A., Gillam, A.H. and Collin, P.J. 1983. Analysis of the structure of dissolved marine humic substances and their phytoplankton precursors by ^{1}H and ^{13}C nuclear magnetic resonance. *Chem. Geol.*, **40**: 187-201.

Wheeler, P.A. and Kokkinakis, S.A. 1990. Ammonium recycling limits nitrate use in the oceanic subarctic Pacific. *Limnol. Oceanogr.*, **35**: 1267-1278.

Whitehouse, B.J. 1990. Crossflow filtration of colloids from aquatic environments. *Limnol. Oceanogr.*, **35**: 1368-1375.

Wolter, K. 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. *Mar. Ecol. Prog. Ser.*, **7**: 287-299.

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.*, **10**: 109-122.