The Measurement, Production and Degradation of Marine Dissolved Organic Matter

by

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Abstract

A significantly improved understanding of the amount of DOC and DON in seawater, their sources and sinks is of critical importance in understanding the global cycles of carbon and nitrogen. Recently, a major controversy about the measurement of DOC and DON and their chemical reactivity and biological lability has attracted much attention in the oceanographic community, mainly due to the high values found by Suzuki et al (1985) and Sugimura and Suzuki (1988) using a high temperature catalytic oxidation (HTCO) method.

Major modifications were made to the HTCO DOC system of Sharp (1973). The modified system was tested for accuracy, precision, and reliability. The total blank of this system was determined to be in the range of 10-20 μM carbon. Differentiating between the machine blank and the water blank was done by the use of "Wonder Water" (Millipore Super-Q water oxidized by HTCO), which was found to be essentially carbon-free. This result was confirmed by an experiment using ^{14}C.

The HTCO DOC method was compared directly to the UV photo-oxidation method: the former finds 5-60% more DOC in seawater, but not 2-4 times more.

Six methods of sample preservation were tested. Poisoning with $HgCl_2$ and acidification and storage under refrigeration were found to be both efficient and convenient. For the HTCO measurement, the latter was more practical, and thus preferable.

The HTCO system was also used to measure DON according to the method of Suzuki et al. (1985). This method was found to be efficient and precise. Results for seawater samples were in the range of those obtained previously by UV and persulfate oxidation methods. The very high DON values of Suzuki et al. (1985) could not be reproduced by the application of this method.

The production of DOC by phytoplankton was studied by culture experiments. The diatoms Chaetoceros gracilis and Phaeodactylum tricornutum, the flagellate Isochrysis galbana, and the natural algal assemblage in the Northwest Arm, Nova Scotia were found to release a significant amount of DOC in log phase growth. Maximum release took place during the stationary and decomposition stages. The dinoflagellate Alexandrium tamarense lived as both autotroph and heterotroph. The release of DOC by this species differed greatly from the other species, taking place only after the crash of the culture.

Long term studies on the use of algal exudates by bacteria in sea water demonstrated that DOC released by marine diatoms had high potential turnover rates. Microbial consumption of DOC followed first order reaction kinetics. A decay rate as high as 0.49 day^{-1} was obtained in this way for the DOC released by C. gracilis. The exudates of A. tamarense were found to be essentially bacteria resistant.

Two time-series observations in the Northwest Arm and Bedford Basin, Nova Scotia demonstrated significant increases in DOC during phytoplankton blooms. Similarities between the results from batch cultures and from spring bloom observations suggested that the bloom phytoplankton released significant amounts of DOC during and after the bloom.

List of Symbols and Abbreviations

Symbols

AOU	apparent oxygen utilization
°C	degree Celsius
^{14}C	radioactive ^{14}carbon isotope
DIN	dissolved inorganic nitrogen
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
DOP	dissolved organic phosphorus
dpm	nuclear disintegrations per minite
G_i	reactive fraction (i=01,02,03,04 for pools with decreasing lability) of the total quantity of organic matter undergoing decomposition
HTC	high temperature combustion
HTCO	high temperature catalytic oxidation
K_i	first order rate constant for G_i fraction
ln	natural logarithm
MW	molecular weight
NDIR	non-dispersive infrared
POC	particulate organic carbon
p	statistical probability
r	correlation coefficient
TDN	total organic nitrogen
TDP	total dissolved phosphorus
UV	ultraviolet
<, >	less than, greater than

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Chapter 1

General Introduction

1.1 Preface

While the study of the composition of organic matter in sea water dates back at least to the **Challenger** expedition, and is therefore almost as old as the science of oceanography itself, the lack of really sensitive methods for the determination of the total amount of organic carbon and for the multitude of individual organic compounds has obscured the importance of this field of research (Wangersky, 1984).

Until recently, dissolved organic matter (DOM) was considered not to be important to the global biogeochemical cycles of carbon or nitrogen. This conclusion was based on the following lines of reasoning. Various authors (Williams, 1971; Menzel, 1974; Williams, 1975), reviewing the ultraviolet (UV) photo-oxidation or wet chemical determination of dissolved organic carbon (DOC), argued for a universal DOC profile of surface water enrichment of 85-125 μM, sub-surface values between 35-75 μM, decreasing with depth, and then a uniformly low concentration of 35 μM to the bottom of the ocean. This "universal" DOC profile implied that any biological activity associated with DOC was restricted to the upper few hundred meters of the ocean

and below this depth the DOM was essentially inert.

Lack of a correlation between DOC and nutrients or oxygen added additional evidence to support this conclusion. While Ogura (1970) observed an inverse relationship between DOC and the apparent oxygen utilization (AOU) in the upper few hundred meters of the western North Pacific, the AOU was independent of the DOC concentrations below 500 meters. Only Craig (1971) claimed a relationship between DOC and oxygen consumption in the deep ocean, but this observation was indirect, based on data from the carbonate system, and criticized by others (Suess and Goldberg, 1971). The chance observation that food in a lunch-box from the sunken submersible ALVIN was well preserved after several months underwater demonstrated the apparent lack of bacterial activity in the deep sea (Jannasch et al., 1971). Finally, the apparent radiocarbon "age" for DOC from the deep ocean was shown to be very old, about 3400 years for the eastern North Pacific (Williams et al., 1969) and 6000 years in the central North Pacific (Druffel and Williams, 1990). Consequently, the DOC reservoir was thought to be geochemically inert, and thus not routinely considered in oceanic models of carbon cycling (Broecker and Peng, 1982).

The high DOC values, which were more variable with depth in deep water, obtained by high temperature

combustion methods (Skopintsev, 1960; Sharp, 1973; Gordon and Sutcliffe, 1973) were thought to be result of contamination and high system blank (Williams, 1975; Gershey et al., 1979), and hence were not generally accepted.

Recent measurements of DOC and dissolved organic nitrogen (DON) in the northwestern Pacific were several times higher than previous values determined by wet oxidation methods for oceanic surface and deep waters (Sugimura and Suzuki, 1988; Suzuki et al., 1985). By fractionating the DOC into molecular weight groups, Sugimura and Suzuki showed that more than 90% of the additional DOC uncovered by their technique was composed of compounds with relative molecular masses over 20,000. Previous work (Degens et al., 1970) had indicated that most of the DOC in seawater had relative masses of less than 5,000. The newly discovered class of large compounds made up most of the vertical gradient in DOC between surface and deep water. Moreover, a strong correlation between DOC and AOU from surface to deep waters was reported by these workers.

These dramatic new results have attracted widespread attention (Brewer et al., 1986; Toggweiler, 1988; Jackson, 1988; Williams and Druffel, 1988) and led to speculation

about a new DOM paradigm (Gribbin, 1988). Such speculation often asks the questions: What is the nature of the recently detected fraction of DOM, how is it produced, is it biologically labile on timescales important to the major biological nutrient cycles, and what fraction of the oceanic carbon pool does it represent? All these questions have a vital importance in understanding the oceanic distribution of DOM, its sources and sinks, the rates of its formation and bacterial mineralization, and hence are important to understanding the biogeochemical cycles of carbon and nitrogen (Jackson, 1988; Williams and Druffel, 1988).

The ocean is the largest reservoir of carbon in exchange with the atmosphere. Approximately 37,000 gigatons of carbon are stored there (Sundquist, 1985). Ninety-five percent of this reservoir is in the form of inorganic carbon, primarily bicarbonate. Most of the rest is in the form of DOC. Particulate organic carbon (POC), either living or dead, accounts for less than 1% of the total. Based upon recent high temperature catalytic oxidation (HTCO) measurements of DOC, the size of the DOC reservoir has been estimated to be 1630 gigatons (Peltzer et al., personal communication). This is about twice the atmospheric CO_2 pool and equivalent to all the living

biomass on land and in soils (Sundquist, 1985). Moreover, unlike the older determination of DOC, where most of the reservoir was thought to be inert, more than half of the reservoir determined by the HTCO method may be mineralized on time-scales much shorter than the ocean circulation. Real changes in the size of the DOM reservoir and the cycling of carbon through it can have a major impact on the global carbon budget and how the ocean affects the atmospheric concentration of carbon dioxide (Toggweiler, 1988; 1989).

There are many issues related to global oceanic flux studies where DOC and DON measurements can contribute to our understanding of the oceanic carbon and nutrient budgets and at the same time help us to discover important new information about the nature of DOM. DOM is approximately 50% carbon by weight; hence DOC is often used as a measure of this material. Likewise, DON provides information about the nature of the DOM since we can infer the bulk-C/N ratio of the DOM from the DOC/DON ratio. At the same time, process studies for measuring rates of primary and new production, DOC production, bacterial mineralization, etc., will provide additional insights into the nature of DOM. It is important to look at these issues individually.

1.2 Background

1.2.1 Measurement of DOC

The key to many of the problems associated with the production and utilization of dissolved organic matter is the ability to follow changes in concentration of these materials. Since the compounds involved are so diverse, and are usually present at such low concentrations, the measurement of all individual components is not feasible. Furthermore, for many purposes, and particularly for most biological investigations, the flux of carbon is the quantity sought, rather than the interconversion of compounds in the course of fixation and degradation of organic carbon. Therefore, measurement of dissolved organic carbon is preferred in most cases.

It would seem, at first glance, the measurement of dissolved organic carbon should be much simpler than the measurement of the concentration of any single compound. Unfortunately, this is not the case. Extremely sensitive methods - colorimetric, fluorimetric, chromatographic, etc. - exist for many compounds, or classes of compounds, even at the low concentrations found in natural seawater. However, no methods of comparable sensitivity exist as yet for DOC.

Despite a few attempts over decades, the measurement of dissolved organic carbon (DOC) in seawater has been a troublesome problem for marine chemists because the low DOC concentration and high salt content of seawater impose severe difficulties on all the methods employed. While there have been many ingenious approaches (Wangersky, 1965; 1975; 1978) to the measurement of dissolved organic carbon, the methods have largely fallen into four categories: 1. chemical oxidation; 2. ultraviolet oxidation; 3. dry combustion; and 4. direct injection high temperature catalytic oxidation (HTCO)(Wangersky, 1992).

There is a wide variety of chemical oxidation methods. The major difference between these methods has been the strength of oxidant used. The standard method for chemical oxidation has become a variant of the persulfate method (Menzel and Vaccaro, 1964), quite often with the modification proposed by Sharp (1973). While this method achieved some recognition as a "standard technique" (Strickland and Parsons, 1968) for DOC measurement, it has not found complete acceptance since the suspicion always exists that persulfate might not be strong enough to oxidize all the organics in seawater to carbon dioxide. Another major problem with chemical oxidation methods is that they are far from real-time methods. In fact, the

samples are usually stored for eventual processing ashore.

The UV photo-oxidation method came into wider use only after the advent of automated systems, allowing a greater density of sampling and promising close to real-time results (Collins and Williams, 1977; Cauwet, 1984). Although this method proved to be precise and easy to operate and it did measure somewhat higher amounts of DOC than the persulfate method (Gershey et al., 1979), it was not necessarily accurate: at least a few compounds are resistant to UV oxidation (Moore, 1977; Walsh, 1989). Another problem with this method is the degradation of the performance of the UV source with age; the output of the lamp decreases with age in a nonlinear fashion, and there is a large lamp-to-lamp variability. Furthermore, the UV oxidation for DOC is pH sensitive and it is difficult to keep standards and seawater samples in the same narrow range of acidity.

Although the dry combustion of sea salts has a high oxidation efficiency (Skopintsev, 1960; 1966; Gordon and Sutcliffe, 1973; MacKinnon, 1978), it suffers from being time-consuming and thus not a real-time shipboard method. It also has a high degree of susceptibility to contamination and a high system blank. During the drying

stage, most of the biolabile DOC and all of the volatile organics (MacKinnon, 1977) in seawater are lost before the combustion of the dry sea salts begins.

The ideal method for DOC analysis would give almost real-time results, to permit resampling in interesting regions, would permit enough replicates to allow statistical reliability , and would ensure complete reaction with all organic compounds present (Wangersky, 1990). The direct injection high temperature catalytic oxidation methods seem likely to fulfill these conditions.

Adapting the method of Van Hall et al., (1963), which was used for DOC in fresh water, Sharp (1973) developed a direct injection HTCO method for seawater. This method involved acid addition and sparging to remove carbonate, followed by injection into a catalytic oxidation chamber held at 950 °C in an atmosphere of carbon-free oxygen. The tip of the injecion needle was in a region of about 750 °C. Measurement of the CO_2 generated was by a non-dispersive infrared (NDIR) detector. Values of DOC found were much higher, in some cases twice as high, as those found by traditional wet chemical oxidation. While this technique raised serious questions about the validity of the wet oxidation results, the technology of the time did not allow the transformation of this equipment into a routine analytical tool.

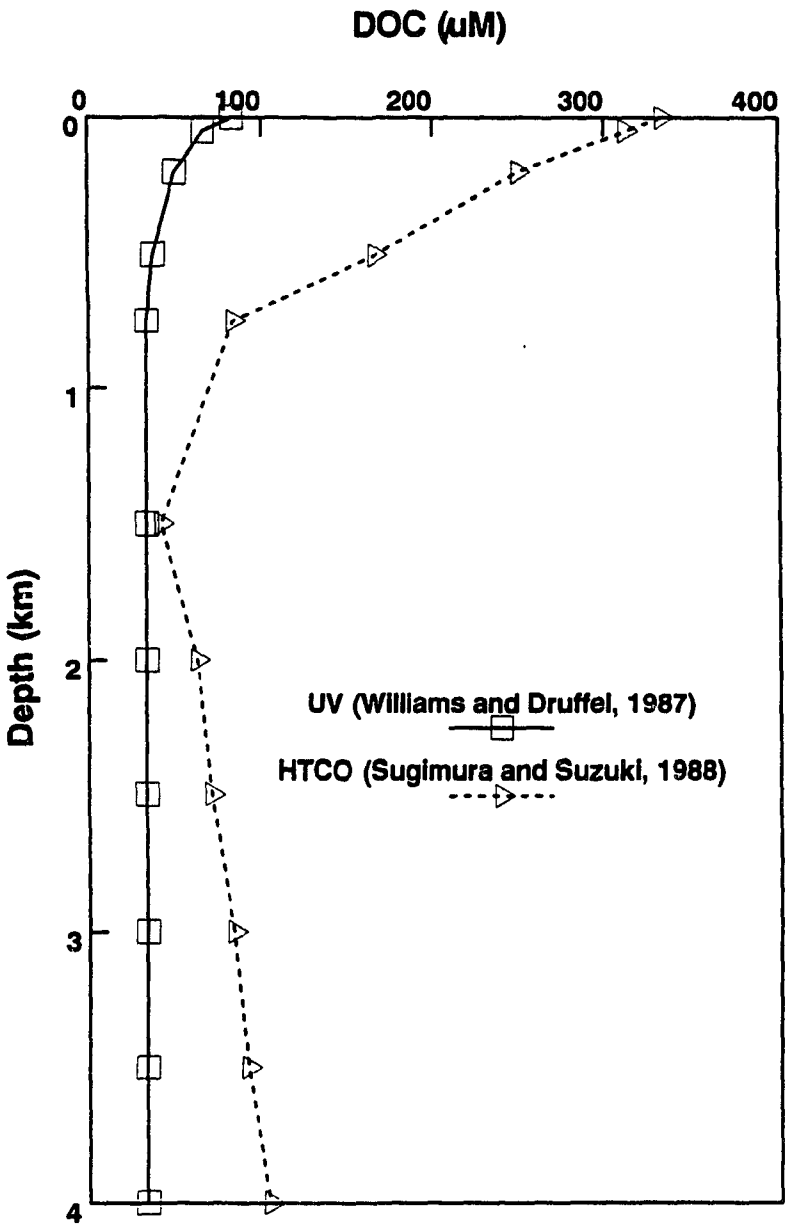
Sugimura and Suzuki (1988) have developed a modernized and improved direct injection HTCO system for DOC. Using this system, they have found DOC values between 3 and 5 times the accepted wet oxidation values for the northwest Pacific. Because of these high values, it was suggested that a major fraction of the marine organic carbon pool (Fig. 1.1) was missed by previous researchers. While the high numbers were surprising, the remarkable correlation between DOC and AOU captured the interest of the oceanographic community. This correlation suggests that a large portion of the DOC is resistant to chemical and photochemical oxidation, but biologically labile and rapidly recycled, in stark contrast to the prevailing wisdom. These results were found hard to reproduce by other workers and have sparked a controversy (Williams and Druffel, 1988).

In summarizing the current controversy, Williams and Druffel (1988) challenged the oceanographic community by setting as the first mandate the confirmation of the elevated DOC values of Sugimura and Suzuki (1988).

1.2.2 Production of Dissolved Organic Matter in Seawater

Although the nature, production and subsequent fate of the dissolved organic matter in seawater have been studied for decades, the picture that has emerged is full of

Fig. 1.1 DOC profiles in the Northwest Pacific



misconceptions and misinformation. Large discrepancies exist between the many methods employed and between the results obtained.

The rates of production and utilization of dissolved organic matter (DOM) are significant in a broad range of ecological interrelationships, such as food webs and carbon budgets. It is well established that phytoplankton is the major source of dissolved organic matter in the ocean (Anderson and Zeutschel, 1978; Thomas, 1971, 1977; Fogg, 1977, 1983; Sharp, 1977, 1980). There has been little argument concerning the importance of the contribution of organic matter from phytoplankton. The major disagreements usually concern the pathways by which these materials arrive in solution and the relative importance of the various pathways under natural conditions (Wangersky, 1978).

The primary source of dissolved and particulate organic matter in the ocean, in both the pelagic and coastal zones, is photosynthesis carried out by phytoplankton. Dissolved organic matter is derived from the phytoplankton in several ways: some organisms secrete a mucopolysaccharide sheath which continually degrades, adding polysaccharides to both dissolved and particulate pools (Ramus, 1972); those organisms which form blooms will

often produce more organic matter than can be utilized immediately by the bacteria and zooplankton present; sloppy feeding by zooplankton will add to the organic pool (Lampert, 1978); some organics will result from metabolic activities of zooplankton and higher organisms; and many species of phytoplankton, if not all, exude organic materials into solution as a normal part of their metabolism. There is considerable disagreement about the quantity of organic matter released to the ocean in the exudation of phytoplankton. Some workers hold that such exudation occurs primarily with unhealthy or senescent cells (Sharp, 1977, 1983), while others feel it to be a natural function of some species (Fogg, 1977; 1983). The proportion of the photosynthetically fixed carbon released in this manner varies greatly, from a few percent to half or more of the production of organic matter.

It seems superfluous at this point to list all of the organic compounds which have been found in cultures of marine phytoplankton, or in natural populations. In general, it could be said that any compound of biological interest which has been sought has been found. Those of low molecular weight, such as the members of the citric acid cycle and the compounds arising early in the photosynthetic process, have been found often, but there

are reports of materials of high molecular weight, even in the region of 100,000 (Hoyt and Soli, 1965; Iturriaga and Zsolnay, 1983). The number of compounds found will undoubtedly increase as better methods of concentration and identification are perfected.

Of the many classes of organic compounds recovered from algal culture media, by far the most ubiquitous are the carbohydrates. They range in molecular weight from what might really be called carbohydrate precursors or metabolites, including the small organic acids (Maksimova and Pimenkova, 1969), glycollate (Hellebust, 1965; Watt, 1969), and glycerol (Craigie and co-authors, 1966), through the simple sugars and sugar alcohols (Maruo and co-authors, 1965), to the large polysaccharides, including uronic acids and the sulfate esters of uronic acids (Lewin, 1956; Marker, 1965; Huntsman, 1972).

The mechanisms by which these carbohydrates and organic acids enter the medium have been matters of considerable dispute. It has been suggested that materials of low molecular weight, related to intracellular materials and possibly to storage compounds, may be excreted simply by diffusion through the cell walls (Watt, 1969), while the larger molecules may come from the breakdown of the materials making up either the polysaccharide sheaths or the cell walls themselves (Guillard and Wangersky, 1958).

In general, the rates of release of extracellular materials appear to be correlated with rates of photosynthesis (Eppley and Sloan, 1965; Huntsman, 1972). According to the results reported, it would seem that during periods of exponential growth most phytoplankton species release relatively minor amounts of the carbon they fix photosynthetically. When the population is subjected to stress, however, much larger amounts of material are released. Still greater amounts are released by senescent populations, probably as a result of death and decomposition. At this time we have no way of telling from which of these sources a given fraction of organic material has come, nor can we be sure of their relative importance.

Recent measurement of DON by Suzuki et al. (1985) and DOC by Sugimura and Suzuki (1988) in the Northwest Pacific are many times higher than the values found by previous wet oxidation measurements, with the greatest discrepancies to be found in the surface waters. While these high numbers have not yet been confirmed by other investigators, several workers (Fitzwater and Martin, 1992; Miller et al. 1992; Sharp et al, 1992; Williams et al, 1992; Chen and Wangersky, 1992a) have shown that HTCO techniques consistently show higher values for DOC than do the commonly used persulfate and UV oxidation methods. These

results suggest that there exists in the surface waters of oceans a hitherto undetected store of dissolved organic matter (DOM), in a form easily accessible to bacteria. It is likely that in the open oceans this extra DOM is produced autochthonously. How it is produced and what is its nature, however, are important questions which remain to be answered.

1.2.3 Microbial Degradation of Marine Dissolved Organic Matter

The breakdown of photosynthetically-fixed organic carbon in the oceans is one of the most important transformations in the global carbon cycle. Yet there is surprisingly little agreement on the processes involved and rates by which particulate and dissolved organic carbon (POC and DOC) are recycled to carbon dioxide and inorganic nutrients.

Bacteria are incapable of ingesting particulate matter; they have no mechanism of phagocytosis or pinocytosis and all organic matter must be soluble before it can be transported across the cell membrane. Of course, bacteria do utilize particulate organic matter, but these complex organic molecules must be acted upon by extracellular enzymes to produce soluble organic compounds. Dissolved organic matter can, therefore, be considered as

the preferred substrate for bacteria since it can be utilized without any investment of energy for the production of extracellular enzymes.

However, not all dissolved organic matter can be utilized by bacteria. In fact, most DOC in seawater is considered as biologically refractory and not available for microbial utilization (Jorgensen, 1976). The few estimates of the fraction that is biologically "labile" are typically in the range of 1 to 50% of the total DOC (Ammerman et al., 1984; Carlucci et al., 1987). Recent controversy over the actual quantity of DOC present in seawater suggests the percent labile fraction could be significantly underestimated, especially in surface waters. As observed in studies of POC mineralization (Westrich and Burner, 1984; Pett, 1989b), bulk seawater DOC is also mineralized in two or more stages (Ogura, 1972, 1975). The proportion of these fractions in the total DOC, however, is extremely variable in time and space, depending on activities of primary producers and their consumers. Consumption and transformation of DOC by bacteria rapidly eliminates much of the low molecular weight fraction of DOC, leaving more refractory materials to accumulate in seawater (Wolter, 1982; Chrost and Faust, 1983; Iturriaga and Zsolnay, 1983; Jensen, 1983; Bell, 1984; Brophy and Carlson, 1989).

Numerous determinations of uptake kinetics for bulk seawater DOC and algal exudates have yielded turnover times ranging from hours to years. Most of the determinations were based on either short-term, radiotracer investigations of simple organic compounds (Wright and Hobbie, 1966; Gocke, 1977; Hagstrom et al., 1984) and phytoplankton exudates and detritus (Iturriaga and Hoppe. 1977; Iturriaga and Zsolnay, 1983; Biddanda, 1988, Pett, 1989), or trace analyses of extremely labile exudates released by algae (Brockmann et al., 1979; Mopper and Lindroth, 1982; Lancelot and Billen, 1984; Bauerfeind, 1985). These studies have given some insight into the dynamics of bacterial uptake of simple labile components of dissolved organic matter in natural waters.

However, these determinations have their limitations. The single substrates added might be taken up in a manner different from naturally occurring solutes. At best, the technique of adding labelled organic compounds to samples of seawater measures a rate of utilization of a specific compound by some small portion of the population present; at worst, it selects conditions favourable only to bacterial utilization (Wangersky, 1978). For all ^{14}C uptake measurements the question remains as to whether ^{14}C labelled compounds are taken up in the same way as the ^{12}C

substrates already in the water sample (Bauerfeind, 1985). The uptake rates for the extremely labile compounds do not represent the bacterial uptake rates for bulk DOC in natural seawater. It is clear that the results of kinetic studies on the uptake of simple organic substrates are likely to apply to only the relatively small labile component of photoassimilated carbon released during the decomposition phase of a phytoplankton bloom.

To avoid the problems involved in the above methods, degradation of phytoplankton detritus was followed by POC and DOC analyses (Newell et al., 1981) or oxygen consumption (Bauerfeind, 1985.). Degradation of intact cells (Fukami et al., 1985) was followed by POC and DOC measurements. However, few rates of DOC degradation were obtained by these methods. All DOC analyses in these experiments were done by wet UV or chemical oxidation methods, which have been shown to underdetermine DOC content significantly (Wangersky, 1992; Chen and Wangersky, 1992a). No experiment has been done with dissolved phytoplankton exudates only and no DOC was measured by HTCO methods in the experiments reported.

Recently, evidence has accumulated showing that bacterial abundance in seawater had been underestimated by an order of magnitude and that bacterial production rate is

much higher than we believed in the past (see Azam and Cho, 1987). Thus the rate of bacterial utilization of organic matter in the sea might be much higher than previous estimates. High turnover rates of DOC of seawater in blooms (0.363 day^{-1}, Kirchman et al., 1991) and a diatom culture (0.49 day^{-1}, Chen and Wangersky, 1992b) have been reported. These results suggest the importance of sample preservation for DOC measurement: in a region of high primary productivity the time involved in sample taking and filtration may cause a considerable loss in DOC. Without some method of preservation which stops bacterial decomposition of the more biologically labile materials as soon as possible after sampling, we are really determining an undefined portion of the DOC, related in some unknown fashion to the in situ value (Wangersky, 1992). Without some method of preservation, DOC instruments can only be intercalibrated by being physically present in the same laboratory, not always a practical measure. Also, estuarine and coastal samples must often be taken to a shore lab for processing, some times several days later, again resulting in an unknown loss if the preservation is not applied properly.

1.2.4 Measurement of DON

The "Redfield-ratio" (Redfield et al., 1963), i.e. the ratio of C:N:P, is a empirical relationship among these major bioelements. It has provided a theoretical framework for studies of organic matter production, early diagenesis and nutrient regeneration in the marine environments.

DON concentrations and cycling must be investigated in conjunction with studies of DOC in order to understand the biogeochemical roles of dissolved organic matter in the ocean. In particular, productivity of the surface oceans may be largely controlled by the cycling of nitrogen through DON pools in surface and deep waters.

DON has been historically ignored in water column chemistry profiles for the same reason as for DOC: it was considered biologically inert, despite the fact that DON is by far the dominant form of fixed nitrogen in the oligotrophic surface waters and at least some of the DON pool must be labile. For the same reason as in the case of DOC, DON has recently attracted new interest in the oceanographic community.

Many DON compounds are known to exist in seawater, including urea, amino acids, nucleotides, nucleic acids, peptides, proteins, etc. The well defined compounds compose above 50% of the total
dissolved organic nitrogen (TON). Much of the remaining DON

has yet to be characterized (Williams, 1975). Because of the unknown nature, minute amounts and a wide spectrum of molecular sizes and structure, the determination of DON in seawater has been a difficult problem in marine analytical chemistry, comparable to that of DOC.

No method exists for a direct DON assessment independent of a dissolved inorganic nitrogen (DIN) measurement. The DON concentration is defined as the difference between total dissolved nitrogen (TDN) and the sum of the DIN concentrations of nitrate, nitrite, and ammonium that exist in the sample. TDN is determined by a digestion procedure to oxidize the organic nitrogen fraction into the inorganic forms.

The precision of the DON determination, however, is often hampered because DON is determined as the difference between two large numbers, TDN and DIN. As a result, even small relative errors in the TDN and DIN measurements can lead to large errors in the DON determination.

The accuracies and precisions of colorimetric DIN measurements using a spectrophotometer or an autoanalyzer are well established, and are generally higher than those for TDN (1-2% and 5-10%, respectively). Therefore, the key to accurate DON analyses depends solely on the accuracy and precision of TDN determination, which in term depend on the

completeness of the oxidation procedure applied. The TDN techniques most commonly discussed in the literature and now being used for seawater analyses are alkaline persulfate digestion (Koroleff,1969-1970; D'Elia et al, 1977; Nydahl,1978; Goulden and Anthony,1978; Solorzano and Sharp, 1980; Shepherd and Davies, 1981), photo-oxidation (Armstrong et al., 1966; Armstrong and Tibbits, 1968; Henriksen, 1970; Afghan et al., 1971; Manny et al., 1971; Lowry and Mancy, 1978), and thermal oxidation (Gordon and Sutcliffe, 1973; Suzuki et al., 1985; Walsh, 1989).

Although the persulfate and photo-oxidation TDN methods have generally been in reasonable agreement on the TDN concentrations of most natural seawaters, and have received some acceptance as standard methods for TDN measurements, their accuracy has always been questioned for the same reasons as for DOC analysis. The DON concentrations of oligotrophic surface waters, such as those in the North Pacific Gyre, have been measured to be about 4-8 μM by the wet oxidation methods (Jackson and Williams, 1985; Smith et al., 1986; Walsh, 1989). By contrast, concentrations above 40 μM DON have been reported for western North Pacific surface waters by Suzuki et al. (1985) using a high temperature catalytic oxidation method. The significant discrepancy between these results suggests

that there is a large and labile pool of organic nitrogen undetected by the wet oxidation methods.

Since this recent report of high DON values in seawater, efforts have been made to assess the accuracy of the HTCO method of Suzuki et al.. Walsh (1989) made a comparison between the more traditional UV photo-oxidation method and a high temperature combustion method, which combusted the seawater sample in a quartz furnace tube at 1100 °C without catalyst. He found good agreement between the two methods. While this study suggests that Suzuki's HTCO measurement over-estimated the concentration of DON, Walsh's HTC method is open to question, since no catalyst was used in his method. Pt catalyst was reported to be very important to the efficient oxidation of ammonia to nitric oxide (Connor, 1967a, b) and at equilibrium conditions, the products of the combustion of ammonia in oxygen are nitrogen and water (Connor, 1967a). The question is not whether Walsh's instrument failed to combust the DON at 1100 °C. It is hard to conceive of an organic molecule surviving such treatment. Rather the question is whether the nitrogen liberated from the DON was converted to nitric oxide or some other products, such as N_2.

Sometime later, Maita and Yanada (1990) made a direct comparison between the HTCO method and the persulfate

oxidation method for TDN measurement. They used the same type of HTCO system and the same detection method (i. e. spectrophotometry) as Suzuki et al. (1985). They found that the analytical values of TDN for several standard organic nitrogen compounds, humic acid prepared from marine and lake sediments, and natural seawater samples agreed very closely between the two methods.

More recently, a few studies (Williams et al., 1992; Hensell, 1992; Koike and Tupas, 1992) were carried out to examine the HTCO method for TDN measurement. An oxidation method similar to that of Suzuki et al. (1985) was used with a different detection method. A chemiluminescence detector was used instead of the colorimetric method. No DON values, either in surface or deep waters, were found to be much higher than those normally measured using persulfate or UV wet oxidation techniques or with Antek HT-TN analyzers. However, as pointed out by Williams et al (1992), the composition of the exit gases from the combustion tube are not known exactly. In addition to chemiluminescent-detectable NO, how much NO_2 or other NO_x species result from the high temperature combusion (HTC) of NO_3^- and DON or mixtures thereof? Is N_2 produced? Before these questions are answered the DON values obtained by chemiluminescence detection cannot be considered accurate.

More experiments are necessary to understand what is happening in the HTC of DON - the composition of the exit gases, the synergistic effect of DON with NO_3^-, the salt effect, etc.

On the other hand, since the high DON values reported by Suzuki et al. (1985) have not been repeated by other workers to date, and since their HTCO TDN method has only been used and tested once by other researchers, it is useful to duplicate their method and test its validity. Their method must be examined for possible interferences, and tested with culture medium in which we are able to maintain a nitrogen budget.

1.3 Objectives and Experimental Approaches of the Present Study

The new interest of HTCO methods for the determination of DOC (Sugimura and Suzuki, 1988) and DON (Suzuki et al., 1985) is bringing about a re-evaluation both of methods of preservation and analysis of DOC and DON samples and of the role of DOC and DON in the carbon and nitrogen cycles of the oceans. While the original studies of Sharp (1973) and Sugimura and Suzuki were field studies in coastal waters

and open oceans, there is much to be learned about methodology, sources, and sinks from laboratory studies.

Specific aims of this study are to:

i.) modify and test the HTCO method of Sharp (1973) to make it a reliable routine method for accurate and precise analysis of DOC;

ii.) determine the degree of discrepancy between the HTCO and a UV photooxidation method (Gershey et al, 1979) to investigate the nature of the DOC resistant to UV photo-oxidation, but available to some extent to biological degradation;

iii.) investigate methods of sample preservation for the labile DOC in order to find a reliable and convenient method for field use;

iv.) investigate the influence of physiological state of phytoplankton on the production of labile DOC. This study will give us some insight into the nature of the labile DOC and how it is produced;

v.) determine the rate of microbial decomposition of the labile DOC produced by unialgal cultures and cultures of natural populations. Information obtained will help us to understand the role of marine microbe in the global carbon cycle.

vi.) Duplicate the HTCO TDN method of Suzuki et al. (1985), examine it for possible interferences, test its validity, and then modify it if it is necessary.

vii.) Make a time-series study of DON and DOC in seawater during a spring phytoplankton bloom in Bedford Basin, Nova Scotia.

The modification of the HTCO method is described with details in Chapter 2. The oxidation efficiency of the method was examined by testing the effect of the combustion temperature used, the efficiency of the catalysts, the recovery of standard compounds and organics of marine origin, and comparison with a high temperature combustion method for POC measurement (Wangersky, 1976). Components of the system blank are determined by the use of deionized water treated by high temperature catalytic combustion, and the results checked by an experiment using ^{14}C.

A variety of methods for short term DOC sample preservation were tested using DOC samples containing highly labile organics produced by phytoplankton. Acidification with HCl and storage in the cold was also tested for long term preservation of DOC samples. The results of these experiments are also presented in this chapter.

DOC samples from various sources, including organic reference compounds, marine organic materials and seawater

from various parts of the world's oceans, were measured by both HTCO and UV methods. The results of these comparison measurements are presented in Chapter 3.

Chapter 4 presents the results of DOC production in cultures. Variations of DOC in cultures of the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum, the flagellate Isochrysis galbana, the dinoflagellate Alexandrium tamarense, and a natural algal assemblage from the Northwest Arm, Nova Scotia, Canada were followed by the HTCO and the UV photo-oxidation methods. Variations of molecular weight composition in two cultures, C.gracilis and I. galbana, were followed by membrane ultrafiltration and DOC analysis. All these variations were followed from the lag phase, through the log phase and into the stationary and senescent stages of the cultures. The shapes of the DOC curves obtained and the differences between the two methods were discussed with the results of molecular weight fractionation to get information about the composition and nature of the DOC released by the phytoplankton, and about the pathways by which these materials arrive in solution and the relative importance of the various pathways under natural conditions.

DOC decay was measured for substrates from cultures of the diatoms C. gracilis and P. tricornutum, the flagellate

I. galbana, the dinoflagellate A. tamarense and a natural algal assemblage from the Northwest Arm, by the HTCO method. Decay rate constants were determined using first-order reaction kinetics in the multi-G model (Berner, 1980). The results of these experiments are presented in Chapter 5.

The field work on DOC measurement is presented in Chapter 6. It consists of monitoring two spring phytoplankton blooms in Bedford Basin and the Northwest Arm of Halifax Harbor. The results of field work demonstrated temporal variations in the concentration and characteristics of organic matter in seawater. These variations were found to be correlated to the physical, chemical and especially the biological parameters in the sea.

The duplication and verification of TDN method presented by Suzuki et al. (1985) is described in detail in Chapter 7. Time-series samples were taken from a station in Bedford Basin from mid January to mid June, 1992. DOC and TDN were measured in our laboratory; DIN, temperature, salinity and chlorophyll data were provided by BIO. The preliminary processing and interpretation of these data is presented in Chapter 8. These data will be used to discuss the dynamics of carbon and nitrogen in Bedford Basin.

Chapter 2

Determination of Dissolved Organic Carbon

2.1 Introduction

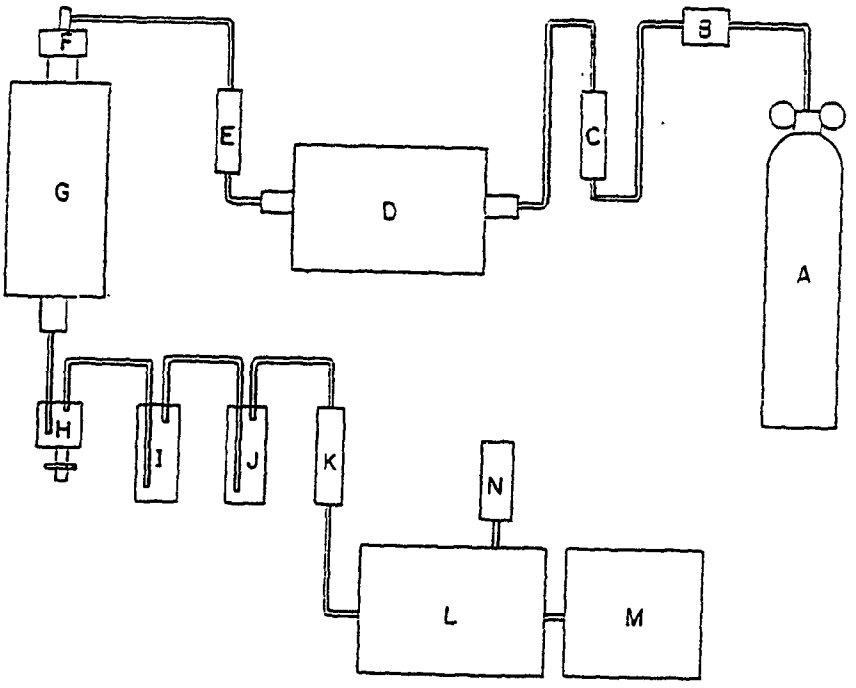
The determination of DOC in seawater is one of the more difficult problems in analytical chemistry, and one which has not yet been solved satisfactorily. This chapter presents a detailed description of a modified version of the HTCO DOC method of Sharp (1973). The oxidation efficiencies of this method and of the UV photo-oxidation method of Gershey et al. (1979) were evaluated. Components of the system blank were determined for both of the DOC units. The calibration of the two methods is also presented in this chapter. The method of DOC sample preservation is described in this chapter as well.

2.2 High Temperature Catalytic Oxidation Method

2.2.1 Instrumentation

In general, the HTCO device used follows the form of the Sharp unit, but with several modifications (Fig. 2.1). First, a vertically-oriented combustion furnace was used (Sugimura and Suzuki, 1988) rather than a horizontal one

Fig. 2.1 HTCO carbon analyzer



A. Oxygen bottle and regulator, B. Needle valve

C. Tank flowmeter, D. Pre-burner furnace

E. Drierite and Ascarite column, F. Sample injection port

G. Combustion tube and furnace, H. Water trap

I. Absorber, 12% ferrous chloride

J. Absorber, saturated silver sulfate

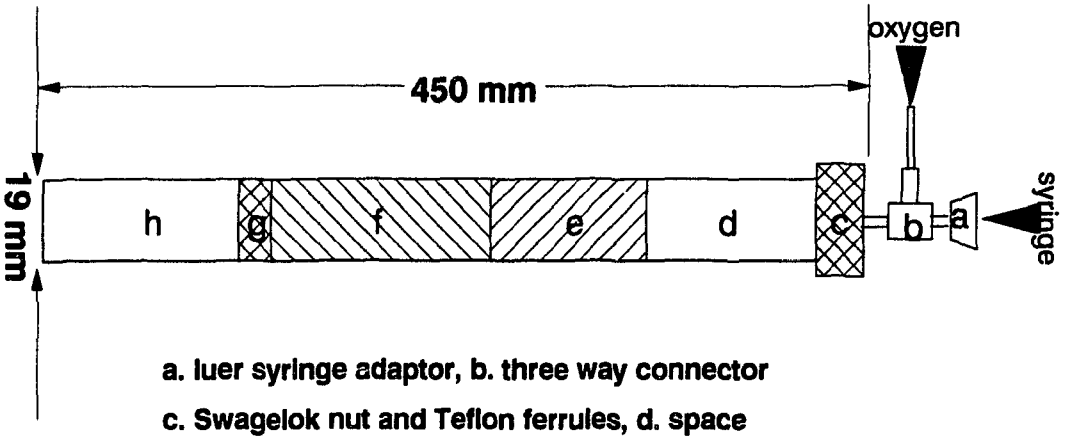
K. Magnesium perchlorate column, L. NDIR analyzer

M. Chart recorder, N. Analyzer flowmeter

(Sharp, 1973). This modification ensured that the salts were not allowed to collect in a cool zone at the very beginning of the furnace - a possible reason for incomplete combustion of the organic materials. A wider combustion tube was used, made of 19 mm ODx17 mm ID fused quartz tubing. In Sharp's unit, the combustion tube was made of 13 mm ODx11 mm ID fused quartz tubing. The larger size provided more room for gas expansion on evaporation and thus reduced pressure surges within the apparatus. Using the wider combustion tube stabilized the baseline of the non-dispersive infra-red (NDIR) CO_2 analyzer (type WFM, The Analytical Development Co. Ltd., England). Instead of 5% platinized asbestos, 5% Pt on Triton Kaowool (BDH Chemical Ltd.) and cobalt oxide on alumina beads were used as the catalysts. The former was in the upper section of the column, the latter (made by soaking alumina beads in a saturated cobalt nitrate solution, drying on a heating plate, and baking in a muffle oven at 700 °C) in the lower. The oxidation efficiency of this combined catalyst was compared to a pure Pt catalyst and a mixed catalyst. Fig. 2.2 shows the size, packings and connections of the combustion tube.

Another major modification was made to the sample injection system. Sharp (1973) used an in-line sample valve and a needle assembly. This type of assembly is prone

Fig. 2.2 The structure of the combustion tube and the injection port



a. luer syringe adaptor, b. three way connector

c. Swagelok nut and Teflon ferrules, d. space

e.5% Pt on Triton kaowool, f. cobalt oxide on alunina beads

g. quartz wool plug, h. space

to blockage of the needle by salt deposits. To clear this problem, the whole assembly had to be removed and cleaned or replaced. The whole apparatus then had to be reconditioned. This resulted in considerable down time, and often caused changes in machine response. The system required a great deal of experience to operate, and was basically a one-person machine (Wangersky, 1975).

The present unit used a microsyringe (Hamilton, Gas Tight) passing through and locking into a septumless port to make the sample injection. The syringe was removed from the injection port once the gas flow had stabilized (ca. 30 s) to avoid the problem of needle blockage caused by salt precipitation. A stainless steel Swagelok nut with teflon ferrules (Swagelok Canada Ltd.), instead of a silicone stopper, was used to seal the top end of the combustion tube. This sealing can withstand much higher temperatures and pressures without decomposing, and can thus reduce the machine blank considerably. A check valve was placed in-line before the injection port to reduce gas backflow caused by sample expansion. Scrubbers, consisting of a 12 % $FeCl_2$ solution and a saturated Ag_2SO_4 solution in series, were used to remove possible interfering chlorinated products of combustion (Skopintsev, 1960).

Peak height, rather than peak area, was measured. Peak height is less affected by the gas flow rate, which is

difficult to keep constant (Salonen, 1979). As long as the peaks have the same shape, peak height is an accurate measure of concentration. When occasional non-Gaussian peaks occurred, additional sample injections were made, until there were several replicates with normal peak shapes. Since each measurement took very little time (ca. 150 s), and since the shape of the resulting peak was instantly available, processing even two or three additional samples does not overburden the analyst.

Both peak height and peak area are affected by the inherent non-linearity of the NDIR response. This takes the form of a drop-off in proportional response at higher CO_2 levels. The concentration of CO_2 can be taken directly from a calibration curve when peak height is used as the measure; with peak area, however, the same carbon content can produce quite different peak areas, depending upon the relative distribution of quick- and slow-burning components. The best solutions to the calibration problem are either to use only the relatively short linear portion of the curve, or to linearize the response. I chose to use only the linear portion of the curve. Greater sensitivity and precision could have been achieved by computer-aided linearization, followed by integration; this option was not available to us. Without linearization, however, the use of

peak area, particularly in a computerized data handling system where the individual peak shapes are not routinely examined, can lead to precise but greatly inaccurate DOC values.

2.2.2 Procedure

A 10 ml filtered seawater sample was acidified with 1 M HCl (40 μl) to a pH of 2-3 and bubbled with oxygen (flow rate 150 ml min^{-1}) for 11 minutes to remove completely the dissolved CO_2. The bubbling was done automatically by the auto-sampler of the automated UV analyzer.

A 100 μl sample of the degassed seawater was injected directly onto the bed of the catalyst in the combustion tube by means of a Hamilton 250 μL gas-tight microsyringe with a Luer tip. The combustion tube was held at 710 °C. Newly packed combustion tubes had to be combusted at 500 °C in a muffle oven for 12 hours or longer before use. Usually 4-6 injections of Wonder Water, Millipore Super-Q water, standards or samples were required to clean the system and stabilize the analyzer at the beginning of the run. Samples were run with standards apportioned among them. One hundred μl was proven to be the optimum sample size for our system. The flow rate of the organic-free oxygen was 150 ml min^{-1}, which was also established as the optimum for our system.

The resulting gas stream left the combustion tube through a stainless steel tube and was cooled by the air; the water condensed was removed by a water trap. The gas stream was then bubbled through the scrubbers to remove possible chlorine species and passed through a $Mg(ClO_4)_2$ column to remove water vapor. The completeness of the removal of water vapor was checked using a second $Mg(ClO_4)_2$ column before the NDIR. No change in the NDIR output signal was found. For each run on the HTCO system, the scrubbers and the $Mg(ClO_4)_2$ in the drying column were changed. The final CO_2 concentration was detected by the NDIR CO_2 detector. The height of the peaks generated was recorded by a chart recorder (Model 232, Linear Instruments Corp.). The slope and intercept of the regression line were used to calculate the DOC values of seawater samples.

The operation of the system is rapid enough - 4 injections in about 10 minutes - for routine DOC analysis. The precision is normally 1-2% for seawater samples.

2.2.3 Oxidation Efficiency of the HTCO System

The efficiency of the HTCO system was examined by testing the effect of the combustion temperature used, the efficiency of the catalysts, the recovery of standard compounds and materials of marine origin, and comparison with POC measurements (Wangersky, 1976).

2.2.3.1 Effect of Temperature

When the temperature of combustion was increased from 710 °C to 900 °C, the DOC values were not increased (Table 2.1). However, the system blank was greatly increased, from 17 to 108 μmol C, and the baseline of the analyzer became unstable. The measurement became difficult to perform, and the precision decreased. For this reason the HTCO system was operated at 710 °C.

2.2.3.2 Efficiency of the Catalysts

About 10 g of pure Pt pillow catalyst, (IONICS Inc.), was added to the top of our catalyst column. The HTCO values for DOC (Table 2.2) with this combination are comparable to the values obtained using our catalyst alone. When the pure Pt catalyst was used alone in the combustion tube, the same or somewhat lower values were found (Table 2.2). This was probably due to the shorter contact time of the gas with the catalyst, because of much shorter length and looser packing of the Pt pillow catalyst column.

From the above comparisons we can see that our catalysts, consisting of 5% Pt on Triton Kaowool and cobalt oxide on alumina pellets, are at least as good as pure platinum catalyst for DOC measurements of seawater. A

Table 2.1

DOC values determined by HTCO method using different combustion temperatures.

Sample	DOC (μM)	
	700 °C	900 °C
Glucose	87.5	85.8
Antipyrine	120.8	122.5
Seawater from the North West Atlantic	102.5	100.0
Seawater from the Northwest Arm	113.3	115.8
Culture medium of a diatom	164.2	163.3

Table 2.2

Catalyst comparison HTCO measurements for Hawaiian Ocean Time Series (HOTS) station samples.

Catalyst	DOC (μM)*			
	Surface	O_2 minimum	Deep	River water
Pt	88.3	78.3	71.7	90.0
5% Pt + Co_2O_3	90.0	77.5	77.5	94.2
Pt + 5% Pt + Co_2O_3	94.2	77.5	77.5	96.7

* The experimental precision is typically ± 2%.

direct comparison still needs to be made with the 3% Pt catalyst used by Suzuki, et al., (1992).

2.2.3.3 Recovery of Standard Compounds and Marine Organic Materials

The results of the determination of the recoveries of reference materials dissolved in 3% NaCl solution are shown in Table 2.3. A high recovery was found for most of the compounds tested. The stable N-containing compound, antipyrine, has a recovery rate of 100%; and the very recalcitrant N, S-containing compound, sulfathiazole, has a 95% recovery rate (much higher than the 50% obtained by Sugimura and Suzuki, 1988). Our results are also in agreement with the dissolved organic nitrogen data of Walsh (1988).

2.2.3.4 Comparison with a High Temperature POC Method

Comparison of the carbon content of some marine organic materials as found by HTCO and high temperature POC measurements is shown in Table 2.4. In this series of comparisons, compounds were suspended in our low-carbon water (Wonder Water), and the suspension filtered through a precombusted 0.45 μm filter. Aliquots of the filtrate were

Table 2.3

Comparision of quantitative recovery of common cited reference and recalcitrant nitrogen &/or sulfur compounds dissolved in 3% NaCl solution using UV and HTCO methods.

Compounds	% Recovery				
	UV (This study)	UV (Walsh, 1989)	HTCO (This study)	HTCO (S&S* 1988)	HTCO (Walsh, 1989)
Glucose	102		98		
Urea	77	72.5	102		101
Thiourea	38		107	99	
Glycine	76	95.5	106		100
Caffeine	81		107	100	
Methyl Orange	85	48.2	91	95	95
Antipyrine	92	53	111	100	98
Sulfathiazole	84	83.5	97	53	102

* Sugimura and Suzuki

TABLE 2.4

Comparison of DOC contents of marine organic materials measured by HTCO and POC measurements.

Materials	DOC (μM)		HTCO/POC (x100)
	HTCO	POC	
Agar	142.5	140.0	102
D-Galacturonic Acid	143.3	146.7	97.5
Alginic Acid	130.0	128.3	101
Laminarin	113.3	118.3	96
Carragheenan	155.8	170.8	91

evaporated to dryness in precombusted quartz tubes, and carbon measured by the POC method of Wangersky (1976). The results were compared with HTCO determinations on the same filtrates. The differences between the two methods are within 5%. This determination serves as a more direct proof of the high oxidation efficiency of the HTCO method because the temperature of the POC measurement is much higher (1100 °C). This technique also allowed us to compare oxidation efficiency for compounds not easily put into solution, and of suspect purity.

2.2.4 Calibration of the System

The system was calibrated by use of an organic standard compound, usually potassium hydrogen phthalate or glucose. We prefer to use the former since it is sufficiently bacteriostatic to serve as a preservative and thus could be stored for long term use. A stock solution of 0.5 mg C/ml was used to make the working standards. Dalhousie Oceanography "Wonder Water" (Chen and Wangersky, 1992b) with 3% NaCl added was used to make the standard solutions. The water was prepared with a catalyst of 5% Pt on Triton Kaowool as packing in a high temperature (800 °C) furnace. A regression formula for standards from a specific run was used for calculation of carbon in samples from the

same run. With few exceptions, all regressions were linear with correlation coefficients of 0.995 or higher.

2.2.5 Blank Determination

The intercepts of the calibration lines were greater than zero and represent the instrumental blank of the system plus the carbon content of the water in which the standards were prepared, in this case, the Wonder Water. The total blank was usually around 15 ± 5 μmol C. Distilled or deionized water prepared in the usual fashion normally contains a small quantity of dissolved organic material, typically around 40-50 μmol C, about as much as the usual wet oxidation carbon values for deep-sea water. If the total blank as determined with the distilled water is subtracted from the seawater sample value, as is the usual practice in analytical chemistry, the resulting DOC value will be underestimated by an amount equal to the DOC content of the water used. It is necessary to determine the actual value of the two components of the total blank, the machine blank and the carbon content of the water, in order to calculate the proper DOC values for samples. This is not a simple problem; it could be solved by the use of "carbon-free" water, if such could be guaranteed to exist. The

blank could also be estimated to a given degree of accuracy by the use of a superclean water.

Although there is as yet no direct method for establishing the carbon content of the water used for the blank, it is possible to test the efficiency of the combined distillation and combustion system used to produce Wonder Water for the oxidation of those organic materials produced by phytoplankton. Two species of diatoms, Phaeodactylum tricornutum and Chaetoceros gracilis, and one picoplankter, Synechococcus sp., strain DC2, were grown in unialgal cultures, in the presence of ^{14}C bicarbonate. The culture media were filtered and the filtrates combined. The combined media were diluted with Super-Q water to a total ^{14}C count of 2500 dpm/ml. After passage through the high temperature distillation and combustion apparatus, the distillate count was reduced to 6.5 dpm/ml, or 0.26% of the initial value. This would represent a DOC value of 0.1 μmol for the water so distilled. For all practical purposes, this corresponds to a zero water blank.

The apparent completeness of the oxidation of all the organic materials contributed by the phytoplankton does not imply completeness of oxidation of all dissolved organic materials; compounds surviving the oxidation step may have

been present in either the water used for dilution or the seawater used as the base of the medium in which the organisms were grown. In either of these cases, such compounds would not have been labelled, and therefore their survival would not have been noted. Only an exhaustive survey of compounds released to solution by common species of phytoplankton, zooplankton, and planktonic bacteria could eliminate that possibility. However, we now have reasonable grounds for assuming that the contribution of our low-carbon water to the system blank is essentially zero, and the total blank of our system comes entirely from the instrumental blank, including contamination from the parts of the system, the catalysts, the carrier gas and the reagents used. This blank value must be subtracted from the sample peaks in our calculations of DOC values.

Suzuki et al. (1992) separated their water blank from their instrumental blank by collecting and re-injecting the water which had passed through their HTCO unit. This water was assumed to have no carbon detectable by their analytical system, so that the resulting blank should be completely due to the components of their analytical system. Their conclusion was that the greatest part of their total blank was due to the organic carbon in their original deionized water, and that the instrumental blank

was essentially zero. The blank, therefore, should not be substracted from the DOC value.

We repeated their experiment, using "Wonder Water" as our blank, and collecting the water after its passage through the analytical oxidation column. Re-injection of this water produced a peak essentially the same as, or possibly very slightly larger than, the original blank peak. Though this result does not determine the presence or absence of organic carbon in the 'Wonder Water", it does prove that the water does not contain any high temperature catalytic oxidizable organic carbon and demonstrates that the water does not make any contribution to the total system blank.

In conclusion, in our case, the major contribution to the blank was definitely from the analytical system, and not the carbon content of the original water. As long as these HTCO units are one-of-a-kind, and not purchased off a warehouse shelf, we can expect no general rule to apply for the calculation of blanks; the correct apportioning of the total blank will have to be determined in each case.

2.2.6 Sample Preservation

The reported high turnover rates of DOC (Kirchman et al., 1991; Chen and Wangersky, 1992b) emphasize the

importance of sample preservation for DOC and DON measurements. Without a reliable method of preservation, DOC values determined are not the real in situ values, and intercalibration between different laboratories is impossible.

Techniques that might be applicable to the post-filtration preservation of DOC/DON samples include: addition of acid, addition of a poison or preservative, and application of low temperatures or freezing.

The preservation methods for DOC analysis were tested using substrate from a culture of the diatom Phaeodactylum tricornutum in the log phase. Aliquots of GF/F filtrate of the culture were preserved by six different methods: (1) and (2) were both poisoned by a 2.5% mercuric chloride solution to a final concentration of 0.1% and acidified by a 1 M HCl to a final pH between 2 and 3, and stored at 2 and 20 °C respectively; (3) and (4) were both poisoned with mercuric chloride and stored at 2 and 20 °C respectively; (5) and (6) were both acidified with HCl but stored at 2 and 20 °C respectively. This test was conducted for a relatively short term (35 days).

Long term preservation by acidifying with HCl and storage under refrigeration was tested using samples from a dense culture of the natural phytoplankton population from

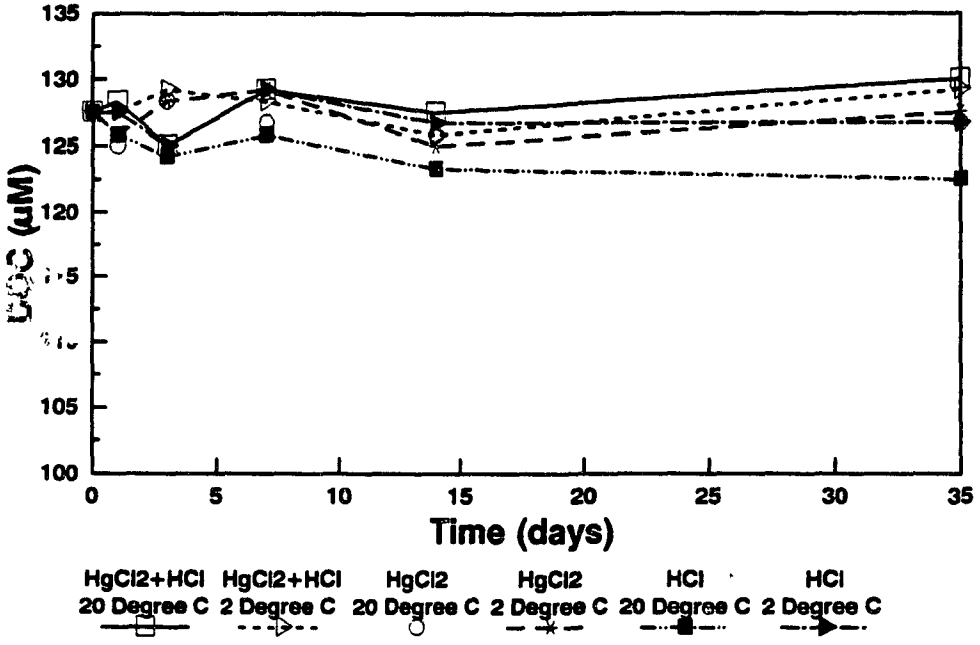
the Northwest Arm, and seawater from Bedford Basin, Nova Scotia, Canada. Samples (250 ml) were filtered (0.8 μm), acidified with HCl to a pH between 2 and 3 and stored at 2 °C in glass bottles (300 ml). The bottles had been combusted at 500 °C for 12 hours before use, and rinsed with the filtrates three times to reduce possible sorption by the glass surface (Sharp et al., 1992). The samples were sealed tightly by caps with Teflon linings. Samples for DOC measurements were taken with a 5 ml pipette to avoid any contact between the substrates and the caps. This experiment was conducted for 145 days.

In all the above experiments, samples for DOC analysis were taken at closely spaced time intervals at the beginning and at longer intervals later. DOC was measured by the HTCO method described above.

The results of the testing of short term sample preservation methods are shown in Fig. 2.3. With the exception of samples acidified with HCl and stored at room temperature, all the methods tested were satisfactory for DOC preservation for at least 35 days. Acidifying with HCl did not provide satisfactory preservation at room temperature; a consistent slow decrease in DOC was observed and about 7% of the DOC was lost after 35 days storage. Poisoning with mercuric chloride provided satisfactory

Fig. 2.3 DOC preservation

Culture of P. tricornutum in log phase



preservation for DOC at room temperature: no consistent decrease in DOC was observed. Poisoning with $HgCl_2$ had previously been found to be satisfactory for DOC preservation in the cold (2 °C) for as long as 87 days (Chen and Wangersky, 1992b). Poisoning with $HgCl_2$ thus seems to be the most convenient method for short term preservation of DOC, and poisoning and storage under refrigeration for long term preservation. However, since mercuric chloride is a highly toxic substance, it may cause problems in DOC measurement afterwards by producing a toxic gas in the exhaust of the DOC system, possibly harmful to the operator of the system and the Pt catalyst used in the HTCO system (Bauer et al., 1992). No poisoning effect was observed in our HTCO system when samples treated with $HgCl_2$ were analyzed, possibly due to the use of cobalt oxide in the catalyst column.

The results for long term preservation are shown in Table 2.5. Variations from the original values were within analytical error and exhibited no consistent trends for the 145 day storage. DOC samples preserved by this method are stable toward bacterial utilization, chemical decomposition and physical losses. Therefore, of the methods tested, acidification with HCl and storage in the cold appears to be the most convenient and practical method for DOC

Table 2.5

Preservation of dissolved organic carbon with HCl and storage at 2 C.

Day	DOC (μM)	
	Surface seawater	Culture filtrate
0	113.3	204.2
7	115.0	212.5
15	117.5	208.3
30	112.5	205.0
62	115.0	204.2
100	116.7	206.7
145	110.8	203.3

preservation for both short and long term sample preservation.

A pH of of 2.5 should be sufficient to stop bacterial activity, denature most enzymes, and preserve most organic matter (Sharp et al., 1992). There has long been a concern about the potential production of volatile products from hydrolysis of organic matter and precipitation of macromolecules from humic material in seawater. However, for the time tested, 145 days, we did not find such a loss. This kind of loss might be a much longer term process.

Sorption of DOM onto walls of storage bottles may occur. Since a large volume of sample was used, however, the loss from sorption must be negligible.

In order to test whether there is a portion of DOC in seawater which might disappear within minutes or hours (Fuhrman, 1987), a fast freezing of samples by immersion in a dry ice-acetone bath was tested as well. Samples were frozen within 2 minutes. No extremely labile DOC was detected in this experiment. The amount of this material must be too small to be detectable by the DOC method. In addition, this method was found to contaminate the sample easily. This was due to the differential contraction upon freezing of the storage bottle and its screwcap, loosening the seal and making the bottle permeable to acetone vapor of the cold bath.

2.3 Ultra-Violet Photo-Oxidation Method

2.3.1 General Description of the Method

The automated UV wet oxidation DOC analyzer is that described in Gershey et al. (1979), a slight variation on the original instrument of Collins and Williams (1977), and uses the same NDIR as the HTCO unit.

A block diagram and a schematic of the automated system are given in Figures 2.4 and 2.5 respectively.

A 6-10 ml of seawater sample is acidified by 40 μl 1 M HCl to a pH of about 2.5 and placed in the sample tray and bubbled for about 11 minutes with CO_2-free oxygen in order to remove inorganic carbon. It is then pumped into the system by means of a multichannel peristaltic pump. The sample is segmented with oxygen gas which is metered into the stream in the ratio of one part O_2 to three parts sample (by volume). The sample is then pumped through a silica coil which surrounds a water-jacketed medium-pressure mercury arc-lamp. After the sample leaves the irradiation coil, a solution of 1 M hydroxylamine hydrochloride in 1 M hydrochloric acid is added (0.04:1 by volume) in order to reduce any halogens produced during the irradiation and to insure that the pH is kept low enough

Fig. 2.4 Block diagram of photo-oxidation procedure for the determination of dissolved organic carbon in seawater (Adapted from Gershey, 1981)

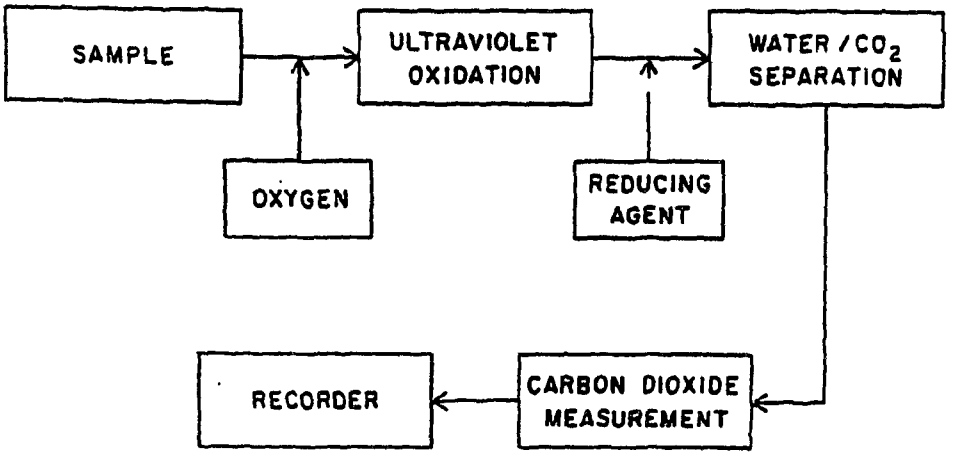
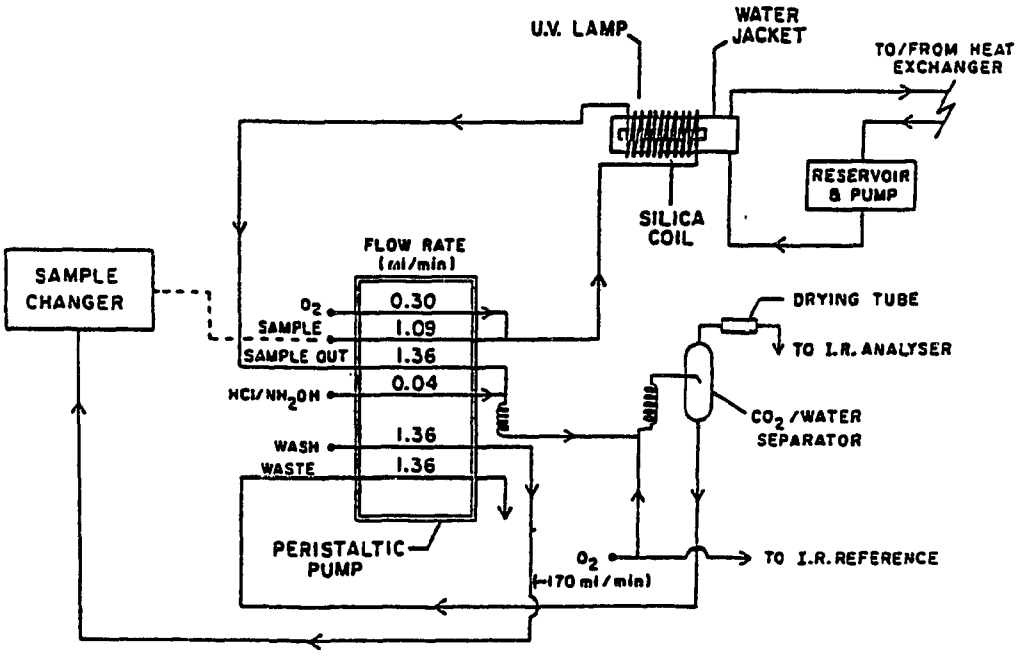


Fig. 2.5 Schematic diagram of automated photo-oxidation apparatus for the determination of dissolved organic carbon in seawater (adapted from Gershey, 1981)



for efficient removal of the CO_2 produced. The sample then passes into a scrubbing coil through which a stream of CO_2-free oxygen is flowing (ca. 170 mL/min). The CO_2 is transferred to the gas phase and is swept through a column of magnesium perchlorate in order to remove water vapor. The gas then passes through a non-dispersive infrared CO_2 analyzer.

After calibration with standard solutions, ten samples per hour can be analyzed by this system with a minimum of operator attention. The life of the UV lamp was followed. In this system it was a 550-watt medium pressure quartz mercury vapor type (Canrad Hanovia 673-0360). The lamp had a total radiated energy output of 202.7 watts with 46.8% of this being in the ultraviolet range of the spectrum (220-400 nm).

The change of the light intensity of the UV lamp was checked by the slope of the regression line of standards for every single run. When there was a evident decrease in the slope, the lamp was replaced by a new one. In order to guarantee the efficiency of the UV lamp for every run, the UV lamp was replaced more often than required by the manufacture's life time specification of the lamp.

2.3.2 Oxidation Efficiency of the UV Oxidation System

The measurement of oxidation efficiency for the UV oxidation system is not a straightforward analytical problem. Almost all of the compounds tested by various researchers, certainly all of the organic compounds expected to occur in biological systems and available as pure compounds, are essentially completely oxidized in these systems. Only a few nitrogen- or sulfur-containing compounds present analytical problems (Table 2.3).

We considered the possibility that the more complex marine polysaccharides, particularly those with uronic acid residues, might be difficult to oxidize in this manner. These compounds are difficult to obtain in pure form; we chose, instead, to prepare concentrated solutions of five such compounds, and to compare results from UV oxidation with HTCO values already compared to high temperature POC determinations, as described above (Table 2.4). The results of this experiment are shown in Table 3.1, and will be discussed in Chapter 3.

2.3.3 Calibration

Calibrating the response of a carbon analyzer is relatively simple; almost any organic carbon compound not

containing nitrogen or sulfur will serve as a standard, providing it is reasonably soluble in water and not too readily metabolized by bacteria. When the CO_2 detector is an NDIR, the response is usually set with a standard gas of known CO_2 content. The use of a standard solution will then ensure that the rest of the system is behaving properly. The analyzer is calibrated through use of an organic standard, usually potassium hydrogen phthalate. Stock solutions of approximately 0.5 mg C/L are used to make the working standards. Dalhousie Oceanography "Wonder Water" with 3% NaCl added was used to make the standard solutions. A regression formula for standards from a specific run was used for calculation of carbon in samples from the same run. With few exceptions, all regressions were linear with correlation coefficients of 0.995 or higher.

2.3.4 Blank Determination

The accuracy of the method is dependent on the blank value. The necessity for a zero water blank is easily seen. A total system blank consists of a contribution from the system itself, the machine blank, and a contribution from the water used to make the standards, the water blank. If we consider that the total system blank is all machine

blank, substracting this blank from our sample will produce a sample value too low by the amount of our water blank.

Using our Wonder Water the total blank of the system was determined to be equivalent to 10 ± 1 μM. Since the carbon content of Wonder Water is essentially zero (Section 2.2.5), this blank can be considered as the machine blank. The machine blank results from the carbon content of the reagents (organic and/or inorganic) and the carbon released by the silicone, Teflon, and Tygon tubing of the system. The inorganic carbon contribution to the blank (measured by switching off the UV lamp) was estimated at 7 μM. The organic carbon contribution to the blank was thus much smaller, ca. 3 μM being due to the organic impurities of the reagents and the leaching of the pump tubing.

2.4 Conclusion

The modified high temperature catalytic oxidation method for marine dissolved organic carbon measurement has a high oxidation efficiency and precision. It is robust and relatively easy to perform, and hence can be used for routine analyses. Components of the system blank can be determined satisfactorily by the use of deionized water treated by high temperature catalytic oxidation. The

calculation of the blank can be checked and confirmed by an experiment using ^{14}C.

Although most organic compounds tested by various researchers are fully recovered by the UV photo-oxidation, some nitrogen- and/or sulfur-containing compounds are resistant to UV oxidation. No clear indications of a connection between structure and ease of oxidation by UV has been found. In an intercalibration measurement our UV system produced results closely comparable to those of most of the laboratories using a UV method, including the UV/persulfate DOC analyzer of Collins and Williams (1977) at the University of Southampton, UK.

Chapter 3

A Comparison between the HTCO Method and the Ultraviolet Photo-Oxidation Method

3.1 Introduction

Since the advent of the high temperature combustion methods (Skopintsev, 1960; Sharp, 1973) there has long been a controversy over the real value of DOC in seawater (Wangersky, 1965; 1975). Until recently the discrepancy between the wet oxidation methods and the high temperature combustion methods, however, did not receive much attention from the oceanographic community. Recent measurements of DOC in the Northwest Pacific by Sugimura and Suzuki (1988) and Suzuki et al. (1985) have stimulated wide-spread interest because their DOC values are several times higher than those obtained previously with UV and persulfate wet oxidation methods and show a tight relationship with the apparent oxygen utilization (AOU). This work has now become critically important because of the increasing interest in the the fate of carbon, and particularly of CO_2, in the world ocean. The apparent presence of a large unsuspected and incompletely measured pool of organic carbon in both surface and deep ocean water must change our concept of the

roles of phytoplankton and marine bacteria in the oceanic carbon cycle (Wangersky, 1992). The hitherto undetected organic carbon reservoir in the surface waters could amount to a doubling of accepted estimates; the fate of this carbon, and in particular its turnover time, must be taken into account in any global modeling of the greenhouse effect (Toggweiler, 1988; Williams and Druffel, 1988; and Jackson, 1988).

In his recent critical review paper, Wangersky (1991) pointed out:"We are not aided in our understanding by the fact that much of the information in this field is still available largely as pre-prints of articles somewhere in the publication process, and probably not in their final form. What we can profitably do at this time is to examine carefully the methods we used for the determination of dissolved organic carbon, to discover, if we can, the reasons for the differences between methods, what these differences can tell us about the nature of the materials measured, and what improvements in methodology are needed".

The most widely used DOC method is intensive short-wave UV wet photo-oxidation. Although this method is quick, easy to operate and precise enough for most oceanographic purposes, it is not necessarily accurate; as with all wet combustion methods, the possibility is always present that

the use of still stronger oxidizing agents might lead to the discovery of still more organic carbon. In the absence of a generally accepted referee method, the only measure of completeness of oxidation has been the use of reference standards, pure or specially purified compounds of known composition. Since almost all such reference compounds have been shown to be converted to CO_2 by oxidants weaker than the UV technique, the use of such standards does little to increase the confidence of the seawater analyst.

A previous intercomparison (Gershey, et al., 1979) has shown that UV values of marine DOC are 5-10% lower than the dry combustion values of MacKinnon (1978) and 10% higher than persulfate oxidation values of Menzel and Vaccaro (1964) as modified by Sharp (1973). The HTCO values of Sharp (1973) are close to the dry combustion values of MacKinnon, but slightly higher, and are about 10% higher than UV values. A more recent comparison (Cauwet et al., 1990), while not finding the very high values of Sugimura and Suzuki (1988), finds clear but unsystematic differences between HTCO and UV-persulfate wet oxidation. An analysis of the differences between methods will be found in the Discussion section of this chapter.

The HTCO values of Sugimura and Suzuki (1988) are 50 to 400% higher than previous results found by UV and

persulfate methods (Williams and Druffel, 1988). Since no one has yet fully reproduced these results, the first priority of this study was to develop a similar HTCO system, to determine whether the elevated DOC values of Sugimura and Suzuki are in fact valid, and whose results can be compared with those of the older wet oxidation method, especially with the UV method used in our laboratory, to confirm the differences between HTCO and wet oxidation values.

In this chapter, an extensive comparison between our HTCO method and the UV photo-oxidation method of Gershey et al. (1979) is presented.

3.2 Methods

DOC samples from various sources, including organic reference compounds, marine organic materials, cultures of a variety of phytoplankton in their different growth stages and seawater from various parts of the world's oceans, were measured by both HTCO and UV methods (Chapter 2). Seawater samples were filtered (GF/F, precombusted) into glass bottles or tubes (Pyrex) with Teflon linings in the caps immediately after samples were taken by Niskin bottles, acidified by 1 M HCl to a pH of 2.5 (Gershey et al., 1979) and refrigerated until analyzed.

Two series of samples from the field were analyzed by the two methods. One group included samples taken from a variety of environments and at a variety of depths. These samples were preserved by a variety of methods; however, while the values found may not in some cases reflect the DOC content at the time the sample was taken, the differences between the two methods are a valid measure of what the methods saw at the time of analysis.

The second group of samples is a time series taken through a spring bloom in the Northwest Arm, an inlet of Halifax Harbour. The course of the bloom can be followed in the chlorophyll determinations run on the same samples. The samples were drawn from the pumping system of the Dalhousie Aquatron, and were run immediately. Preservation was not required.

3.3 Results and Discussion

3.3.1 Recoveries of Standard Compounds

Of the reference compounds measured (Table 2.3), the most resistant to UV oxidation is thiourea, whose recovery rate was only 38%. For the other recalcitrant N and/or S-containing compounds tested, 10-20% of the carbon content was missed by the UV method. Walsh (1989) found an average

recovery of 60% for these compounds by a UV oxidation method. The HTCO method, in contrast, showed a full recovery for almost all of the compounds tested, except for methyl orange, whose recovery rate by HTC was 91%.

3.3.2 Marine Organic Materials

Table 3.1 shows the results of the comparison of DOC contents of five marine organic materials in 3% NaCl solution as measured by both HTCO and UV methods. For agar, D-galacturonic acid and carragheenan, the UV method found as much DOC as the HTCO method did. However, the HTCO method found 10 and 17% more DOC for alginic acid and laminarin respectively than the UV method.

3.3.3 Bulk UV Oxidation Efficiency with Time

An experiment was performed to examine differences in the susceptibility of seawater DOC and common simple reference compounds to UV photo-oxidation. 0.45 μm filtered seawater, 83 μmol potassium hydrogen phthalate in Super-Q water, and 83 μmol glycine in Super-Q water were photo-oxidized in the bulk UV photo-oxidation unit (detailed description of this unit can be found in Ridal, 1992). Aliquots were taken at various time intervals and measured

Table 3.1

Comparison of DOC contents of marine organic materials in 3% NaCl solution measured by HTCO and UV methods.

Material	DOC (μM)*		HTCO/UV (x100)
	HTCO	UV	
Agar	71.7	73.3	98
D-Galacturonic Acid	189.2	192.5	98
Alginic Acid	184.2	166.7	110
Laminarin	140.8	120.8	117
Carragheenan	176.7	171.7	103

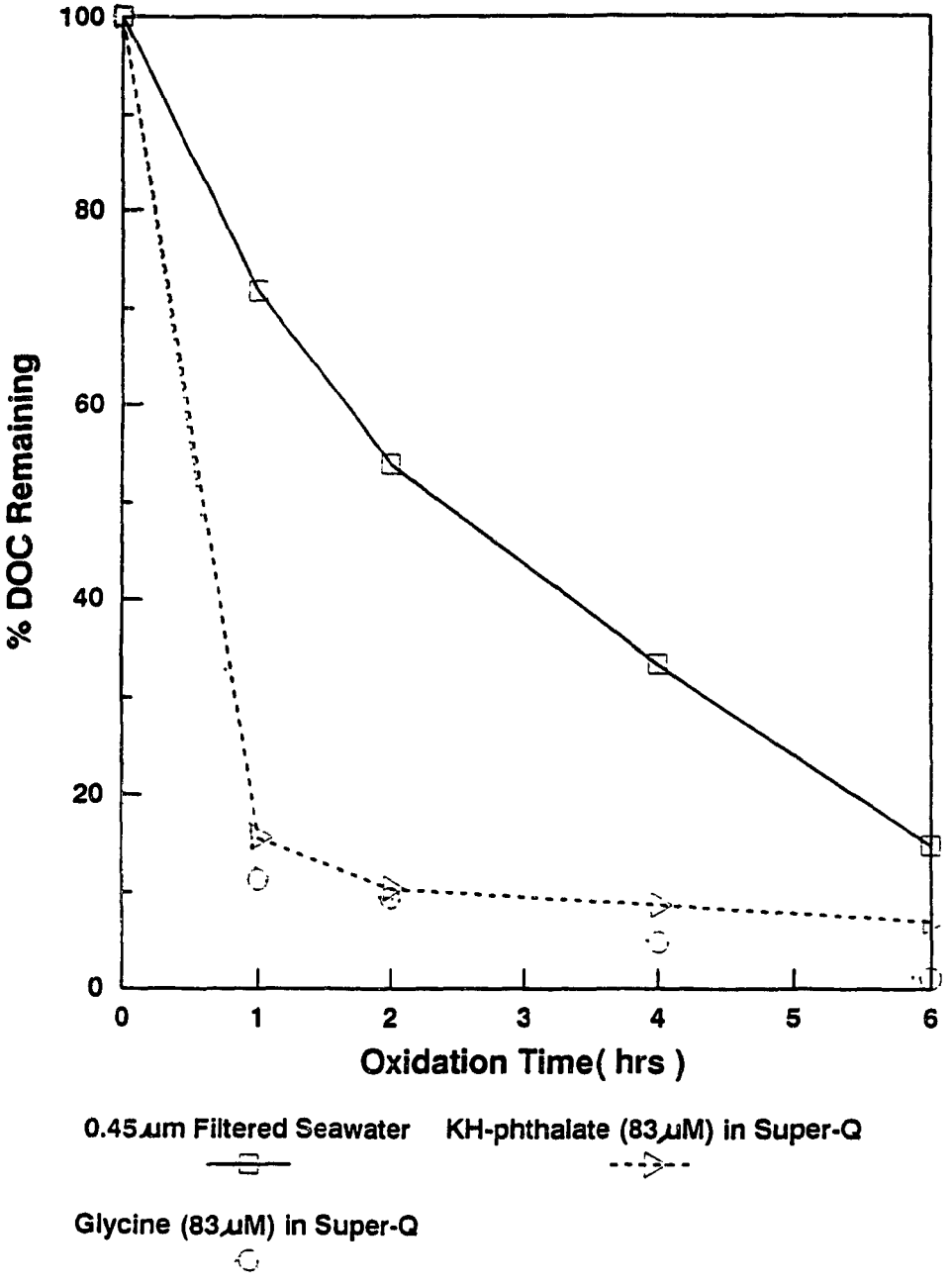
* Percentage standard deviation: ± 1-2%.

for DOC contents by the HTCO method. The results are shown in Fig. 3.1. Within an hour of photo-oxidation, most (ca. 90%) of the DOC in phthalate and glycine solutions had disappeared; however, only 30% of the DOC in the seawater was oxidized, and 50% after two hours. After 6 hours exposure almost all the DOC in the solutions of the two compounds had disappeared, in contrast, about 20% of DOC in the seawater remained. This result demonstrated that the bulk DOC of seawater is much more resistant to UV photo-oxidation than the common reference compounds.

3.3.4 Culture Samples

The results of analyses of culture media in which phytoplankton have been grown are discussed in Chen and Wangersky (1992) and will be discussed in greater detail in Chapter 4 of this thesis. The HTCO values were generally 10-100% higher than the UV values. The differences between the two methods are dependent on the background DOC of the seawater used, the algal species, the physiological state of the phytoplankton, and the activities of the bacteria in the media (Chen and Wangersky, 1992b; chapter 4).

Fig. 3.1 Bulk UV Oxidation Efficiency with Time



3.3.5 Field Samples

The comparison between DOC concentrations measured in seawater by the HTCO and UV methods is shown in Table 3.2. The HTCO values were consistently 5-60% higher than the UV values, with most results in the range of 15-40%. The largest differences in absolute amounts of DOC occurred in the surface water in most cases, except for the Arctic seawater, while the percentage differences varied in a more uncertain and complicated manner. The most important feature of these data was that the concentrations of the UV resistant organics in the surface seawater were less than the UV reactive organics. This is very different from the comparisons between persulfate and HTCO analyses reported by Sugimura and Suzuki (1988). When the UV DOC values were regressed against the HTCO values, the resultant linear relationship (r^2 = 0.90, n =13, $p < 0.0005$) had a slope of 0.88 (Fig. 3.2). The scatter around the regression line is to be expected, since the chemical composition of the various samples was not necessarily constant.

A similar comparison between the two methods was run on samples taken from the Northwest Arm (an inlet in Halifax Harbour), over the course of a spring phytoplankton bloom in 1991. DOC results of both methods are shown in Fig. 3.3. In this case the regression (r^2 = 0.71, n = 43, $p < 0.0005$) had a slope of 0.80 (Fig. 3.4). The difference

Table 3.2
Comparison of seawater DOC measured by the HTCO and UV methods.

Sample	DOC (μM)		HTCO/UV (x100)
	HTCO	UV	
North Pacific (Stn. P, 490 56′ 17" N, 144 59′ 87" W), 1-9-91. 0.4 μm filtered, acidified and kept at ~ 2 °C for 15 days.			
10 m	76.7	55.8	137
1200 m	62.5	45.0	139
The North Pacific (Hawaiian Ocean Time Series study site, 8-4-91. GF/F filtered, acidified and kept cool for 5 days.			
Surface mixed water	90.0	60.8	148
Oxygen minimum zone	45.8	29.2	157
Deep water	42.5	30.8	138
The North West Atlantic (Stn. 3, 41 10′ N, 64 33′ W), 9-9-88. 0.4 μm filtered, acidified and kept at 2 °C for 65 days.			
0 m	150.0	105.0	143
3616 m	91.7	65.0	141
Arctic (79 14.5′ N, 101 45.31′ W),4-9-90. 0.8 μm filtered, poisoned with $HgCl_2$ and kept unfrozen cold for 11 days.			
0 m	75.0	71.7	105
50 m	102.5	92.5	111
450 m	70.0	59.2	118
St. Andrews Bay, N.B. , Canada, 8-8-90. GF/F filtered, fast frozen by a mixture of dry ice and acetone and kept frozen for 4 days.			
2 m	120.8	110.8	109
10 m	112.5	101.7	111
The Northwest Arm, Halifax, Canada, 15-3-91 0.45 μm filtered, poisoned with $HgCl_2$ and kept at 2 °C for 14 days.			
12 m	101.7	92.5	110
The Bedford Basin, Nova Scotia, Canada, 13-1-92. GF/F filtered, acidified and kept in cold for 1 day.			
0 m	145.8	125.0	117
30 m	128.3	114.2	112

Fig. 3.2 Regression of UV oxidation values on HTCO values, collected stations

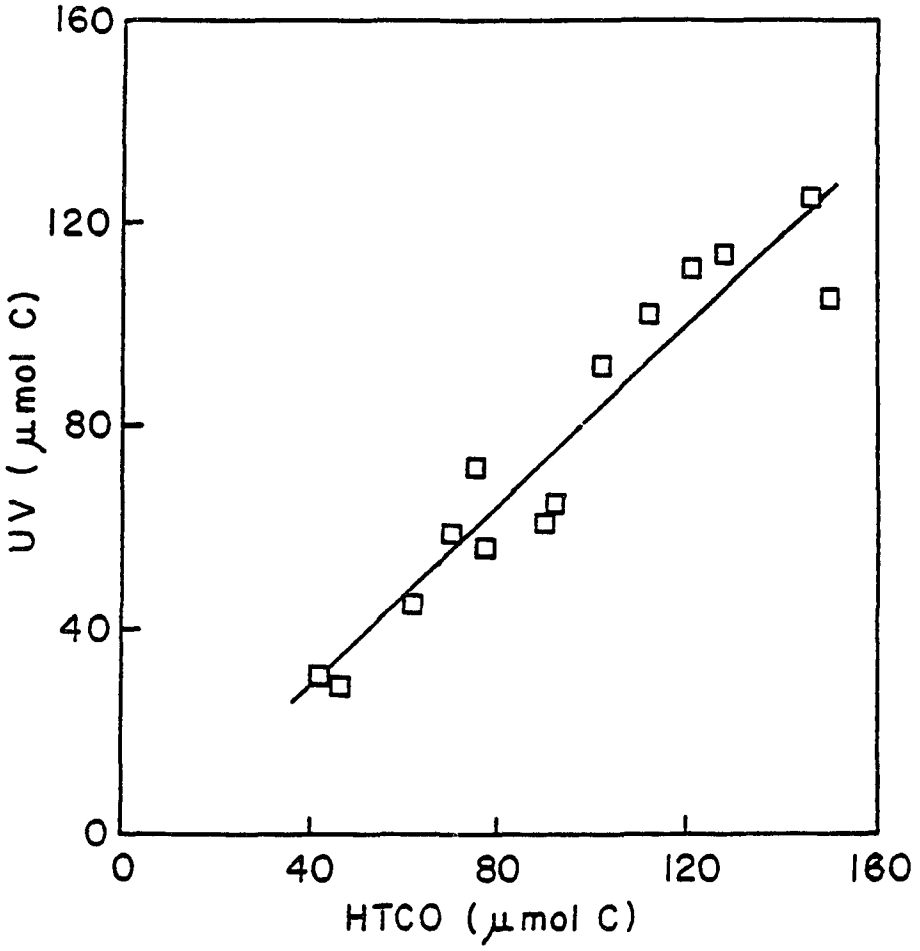


Fig. 3.3 DOC and Chlorophyll a in the Northwest Arm

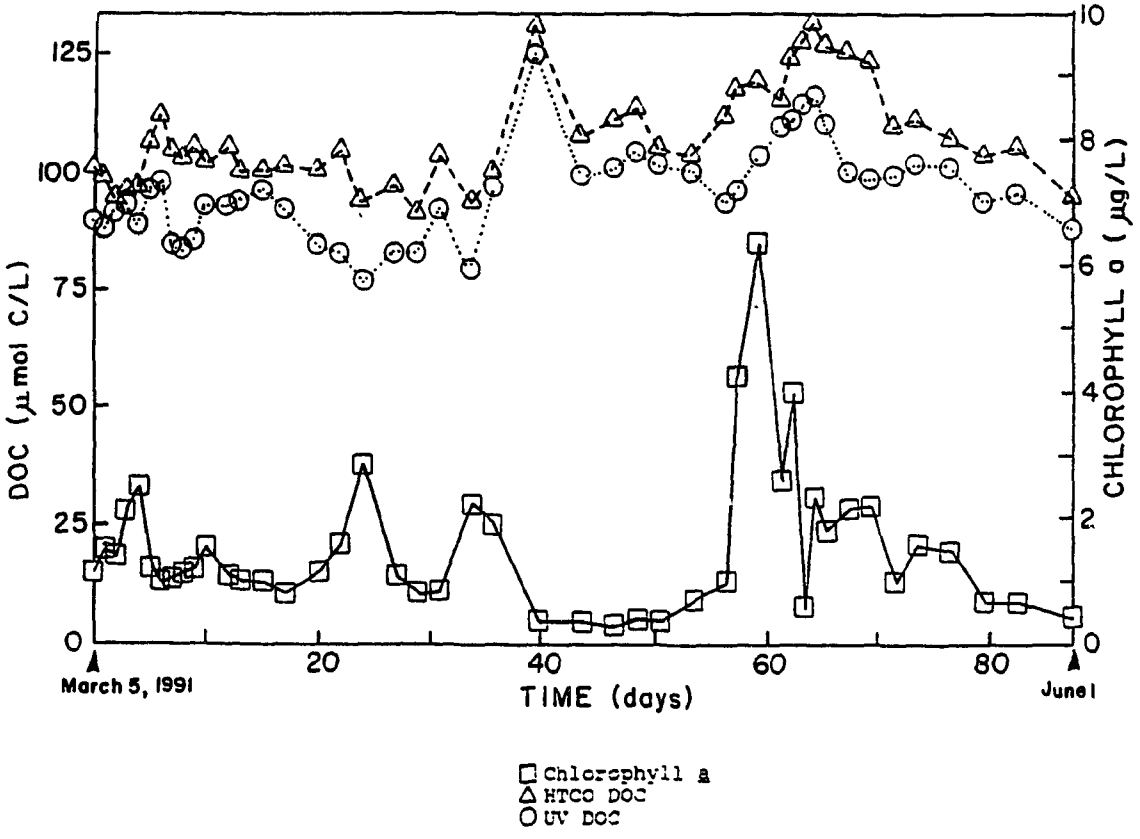
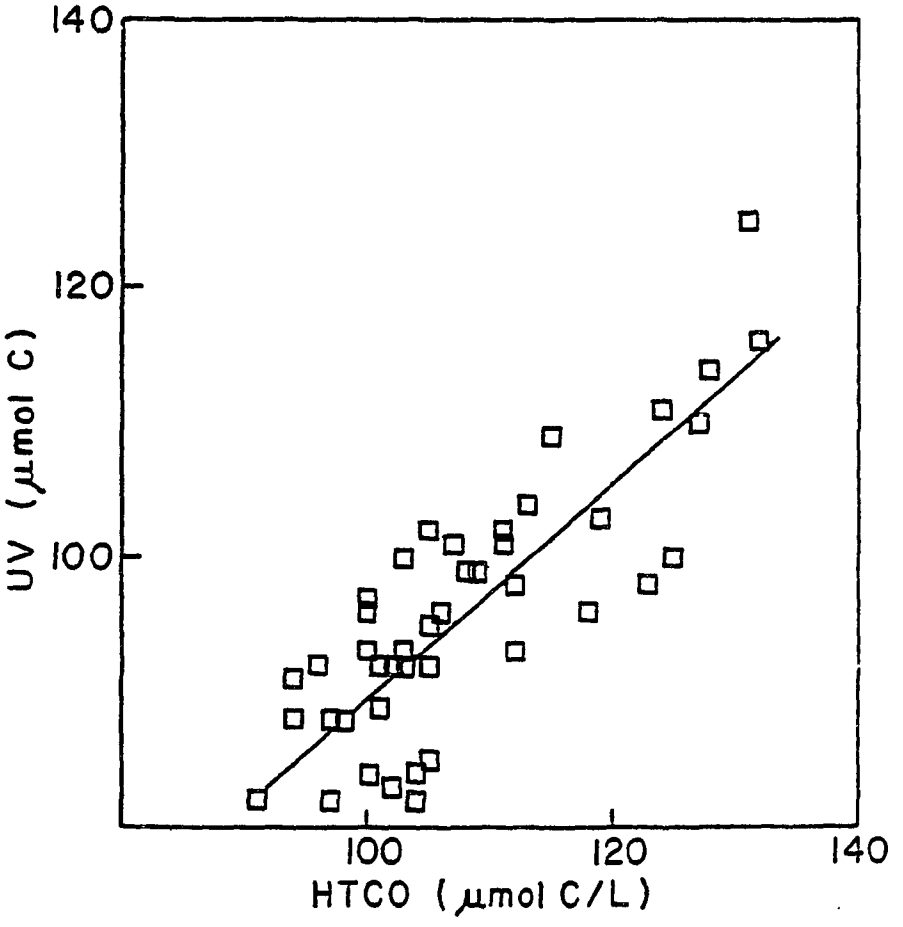


Fig. 3.4 Regression of UV oxidation values on HTCO values, Northwest Arm time series



in the slope, along with the increased scatter, probably resulted from a shorter interval between sampling and analysis, resulting in less loss of the "biologically labile" material, as well as from the inclusion of samples from the decay of the phytoplankton bloom. During that period, the differences between the two methods seemed to be accentuated (Fig. 3.4).

While the strength of the relationship between the two methods suggests that the older DOC results, found by UV wet oxidation, might be converted to their HTCO equivalents by the use of a simple factor, the distribution of the points around the regression lines, as well as the apparent seasonality of the larger differences, makes this a dangerous procedure, at least until many more such intercomparisons have been run. In the case of the two stations analyzed by Cauwet et al., (1990), no such relationship was found between UV-persulfate and HTCO determinations; in the deeper samples the wet oxidation values were often higher than those from HTCO. These results are difficult to explain.

3.3.6 Comparison with a UV System at BIO

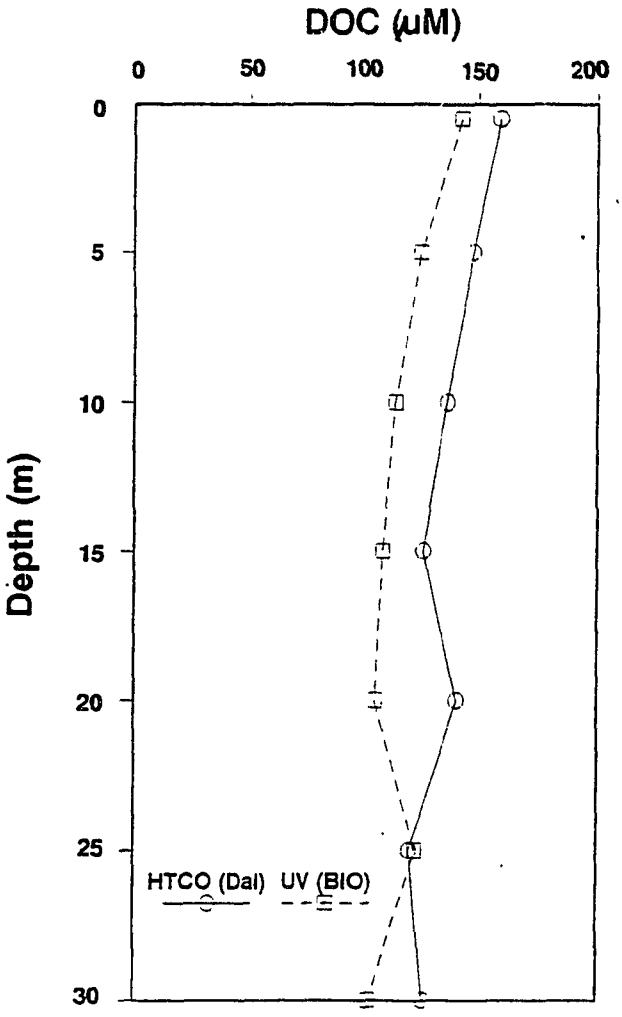
An intercomparison experiment was performed between our HTCO unit and the automated UV DOC analyzer (Cauwet,1984) at Bedford Institute of Oceanography (BIO),

Dartmouth, Nova Scotia. Seawater samples were taken from Bedford Basin on May 21, 1992. Results are shown in Fig. 3.5. The DOC profiles obtained by the two methods show a similar structure: a maximum in surface water, a minimum in the deep water (30 m) and a continuous decrease from the surface down to 15 M. The UV values were generally 10-20% lower than the HTCO values, except for one sample whose values for both methods were essentially identical. Although these samples were not run by our UV system, the difference between the BIO UV analyzer and our HTCO unit is similar to the difference between our UV analyzer and the HTCO system. This comparison again confirmed the difference between the two methods and provided additional evidence that our UV is as efficient as those being used in other laboratories.

3.3.7 Comparative Oxidation Efficiencies

Perhaps the best indication that the UV oxidation is missing some of the organic carbon measured by HTCO methods comes from determination of blanks for the two methods. Preparation of a distilled water which will produce a blank below the detection limit of the persulfate or UV oxidation methods requires care, but is not outside the capabilities of most analytical laboratories. However, even

Fig. 3.5 Comparison between the HTCO system and the UV ana lyzer at BIO



the most primitive of the HTCO methods (Wangersky, 1965, 1972) displayed a consistent organic carbon content of 50 μmol C in quartz-distilled water, whatever the original water source. This finding has now been confirmed by many laboratories.

While the nature of this material is not known, it can be shown that at least a portion of it is carried over into the distillate as droplets in the vapor stream. A fractionating column packed with glass saddles, or any other efficient trap, will lower the blank value appreciably. To bring the blank to zero, however, demands somewhat more heroic measures (Conway et al., 1973; Hickman et al., 1973; Petrick et al., 1981). Our solution, essentially the combustion of the organic carbon in the vapor stream in oxygen at elevated temperature and in the presence of a catalyst, removes all of the organic carbon visible to our analytical method. Proof that all of the organic carbon has been removed awaits the invention of still more rigorous methods of analysis.

From the comparisons we have run we can see that our catalysts, consisting of successive packings of 5% Pt on Triton Kaowool and cobalt oxide on alumina pellets, are at least as good as a pure platinum catalyst for DOC measurements of seawater. A direct comparison still needs

to be made with the 3% Pt on alumina catalyst used by Suzuki et al. (1992).

In our calibrations of both the HTCO and the UV methods for DOC, we are testing two quite separate functions of the instrumentation. The first - the response of the detector to a given amount of the final product, in this case CO_2 - can be checked with any compound available in sufficient purity and easily oxidized, or even with a series of standard gases. The second - the efficiency of the system in oxidizing all of the organic compounds present in seawater - is much more difficult, since we do not know the nature of these compounds. We do know that a few compounds of marine origin, the complex polysaccharides alginic acid and laminarin, while completely oxidized by HTCO, are oxidized with about 85% efficiency by our UV method. Others, including pure D-galacturonic acid, displayed no loss in oxidation efficiency. At least as yet we can see no clear indications of a connection between structure and ease of oxidation by UV; many more compounds may have to be investigated before correlations become evident.

In the original paper on the automated UV oxidation method, Collins and Williams (1975) tested the completeness of oxidation by analyzing medium in which Platymonas sp., a green flagellate, had been grown in the presence of ^{14}C

bicarbonate. More than 98% of the ^{14}C-labeled organic carbon in the medium was oxidized to CO_2 by their UV system. However, this finding cannot be extrapolated to all photosynthetically-produced organic carbon. In line with the then current thinking, their medium was held for a year, until the biologically labile material had been utilized by bacteria. It was considered that the remaining material would most closely resemble the older, biologically resistant material of the deep ocean, and that this material would be chemically resistant as well. Recent work has demonstrated, however, that at least some of the "excess" DOC resistant to UV and persulfate oxidation is biologically labile (Sugimura and Suzuki, 1988; Chen and Wangersky, 1992b). Also the rate of decay of the organic materials released by phytoplankton depends upon the plankton species present and their physiological state (Chen and Wangersky, 1992b; Chapter 5). With some species, the difference between the two analytical techniques can be seen even over long periods; with others, the HTCO and UV methods give similar values after a few weeks of storage. No single phytoplankton species can serve as a sufficient surrogate for the phytoplankton populations of the ocean, and biological resistance cannot be equated with chemical resistance.

At least at this time, there is no single compound that will fill both requirements for standardization of UV oxidizers. In the absence of a true referee method for DOC, and with the reported poor keeping qualities of the "excess" DOC found by HTCO methods, comparison of the oxidative efficiencies of different UV systems can really only be accomplished by intercomparisons, preferably done on adjoining workbenches with fresh material. In a somewhat less rigorous intercomparison, performed for the DOC/DON Workshop in Seattle, WA, our unit produced results comparable to those of most of the laboratories using UV methods (Hedges et al., 1992).

3.4 Conclusions

The extensive comparison between measurements using HTCO and UV methods shows that the HTCO method finds 5-60% more DOC in seawater than does the UV method. Most values in the areas and at the times sampled are in the range of 10-40% more, but not as high as 200% or more. No simple trends have been found that might explain the difference between the two methods. The differences might depend on many factors, such as the source of the sample, the time when the sample is taken, methods of sample handling and storage, and the time between sampling and analysis.

Chapter 4

Variations of Dissolved Organic Carbon in Phytoplankton Cultures as Measured by High Temperature Combustion and Ultraviolet Photo-oxidation Methods

4.1 Introduction

Recent measurements of dissolved organic nitrogen (DON) by Suzuki et al. (1985) and DOC by Sugimura and Suzuki (1988) in the Northwest Pacific are up to six times higher than the values found by previous wet oxidation measurements, with the greatest discrepancies to be found in the surface waters. These results suggest that there exists in the surface waters of the oceans a hitherto undetected store of DON and DOC, in a form easily accessible to bacteria. In the open ocean, this extra DOM is likely produced autochthonously. How it is produced and what is its nature, however, are questions which remain to be answered.

In this chapter, an attempt is made to reassess the influence of the physiological state of the phytoplankton on the nature and amount of DOC released, using a combination of phytoplankton culture and DOC analyses.

4.2 Methods

4.2.1 Culture Methods

The organisms used were the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum, the flagellate Isochrysis galbana and the dinoflagellate Alexandrium tamarense, as well as a natural algal assemblage taken from the Northwest Arm. They were grown in 20 or 2 liter batch modes at 20 °C, using a 16:8 hour light:dark cycle. The light intensity used was 100 $\mu E/m^2 sec$. The culture medium used was 0.2 μm filtered seawater from the Dalhousie Aquatron seawater system, except for the culture of the natural assemblage and a culture of a dinoflagellate. The former was grown in 75 μm filtered surface seawater from the Northwest Arm; the latter was grown in autoclaved dialyzed seawater. For the 2 L batch cultures inorganic nutrients of N (75 μM NO_3^-) and P (5 μM HPO_4^{2-}) were added. For the three 20 L batch cultures and the cultures of A. tamarense nutrients, vitamins, and trace metals were added to bring the medium to the Guillard and Ryther (1962) f/2 level and the Harrison medium (Harrison et al., 1980) respectively. For cultures of diatoms and the natural assemblage, Si (200 μM SiO_4^{4-}) was added. All cultures were bubbled with 0.45 μm filtered compressed air to keep cells suspended and the culture medium well

oxygenated throughout the experiment. Cell numbers were counted with a Coulter Counter (Model 1015 ZB) or under a microscope (for dinoflagellates only). For the culture of the natural assemblage, chlorophyll was measured instead of cell number.

4.2.2 Sampling and Filtration

Samples were taken at intervals for cell counting or chlorophyll and DOC analysis. Samples for DOC and chlorophyll analyses were filtered through Millipore 0.45 μm filters. Special precautions were taken in sample filtration to avoid contamination and cell breakage. DOC samples were acidified, and kept in the cold when storage was necessary.

Since filtration is necessary for the DOC measurement, ideally gravity filtration must be used. Unfortunately, gravity filtration is often too slow to be practical. Pressure or vacuum filtration is commonly applied. Generally, a pressure difference of less than 100 mm Hg was thought to be suitable for filtration of phytoplankton cells (Goldman and Dennett, 1985). The cell damage caused by the filtration is negligible.

A pressure filtration with a syringe was performed for the DOC samples. 0.45 μm Millipore filters were used.

Syringe and filter holder were rinsed thoroughly with Super-Q water before and after use. Filters were washed by filtration of 50 ml of Super-Q water. Contamination from the filtration kit and filter was found to be undetectable. Special precautions were taken in the filtration: it was done slowly and gently, with the pressure not exceeding 100 mm Hg.

An experiment was carried out to examine the effect of the filtration procedure, compared to gravity filtration. DOC samples taken from cultures in log phase and senescence were filtered by gravity and by our pressure filtration method. DOC was measured by the HTCO method (Chapter 2). The percentage differences between the two procedures are shown in Table 4.1. Samples filtered by pressure filtration gave higher DOC values in most cases. For cultures in log phase growth, the effect caused by pressure filtration was negligible or insignificant, except in the case of Isochrysis galbana. However, the filtration effects were significant for all cultures in senescence. Pressure filtration gave 2.5-33% higher DOC. The degree of difference was species specific and greatly dependent on the physiological state of the algae. In the sample of I. galbana more cells may pass through the 0.45 μm filter during pressure filtration than gravity filtration.

Table 4.1

DOC values for samples filtered by gravity and pressure filtration.

Species	Growth stage					
	Log phase			Senescence		
	DOC (μM)			DOC (μM)		
	PF*	GF**	PF/GF (x100)	PF	GF	PF/GF (x100)
C. gracilis	146.7	147.5	99.5	241.5	219.5	110.0
				383.7	288.3	133.1
P. tricornutum	167.5	165.8	101.0	225.0	219.2	102.5
I. galbana	165.3	150.0	110.2	326.7	293.3	111.4
A. tamarense	263.5	263.0	100.2	350.0	288.0	121.5
Natural assemblage	209.0	206.0	101.5	869.5	737.5	117.9

* Pressure filtration

** Gravity filtration

4.2.3 DOC and Chlorophyll Analyses

The DOC contents of all the cultures used was measured by both HTCO (Chapter 2) and UV photo-oxidation methods (Gershey, et al., 1979). Chlorophyll was measured by a standard oceanographic method (Parsons et al., 1984).

4.2.4 Molecular Weight Fractionation

Molecular weight fractionations of the DOC were obtained from a diatom culture and a flagellate culture using an Amicon high performance ultrafiltration system (TCF10, Amicon Co.).

Samples were filtered with 0.45 μM Millipore filters and then placed in the Amicon TCF10 thin channel ultrafiltration unit, and fractionated through ultrafilters under nitrogen gas pressure. The ultrafilters (Amicon Diaflo YM10 membranes, 10,000 MW cut-off) were cleaned by soaking successively 3 times in 1 l Super-Q water followed by soaking in 0.01 M aqueous NaOH for 24 hours prior to use. After use the ultrafilters were rinsed by Super-Q first, then soaked for 12 hours in 1 N NaOH, and stored in 10% ethanol until the next use.

The filtrates were then analyzed for DOC by both HTCO and UV methods.

4.3 Results

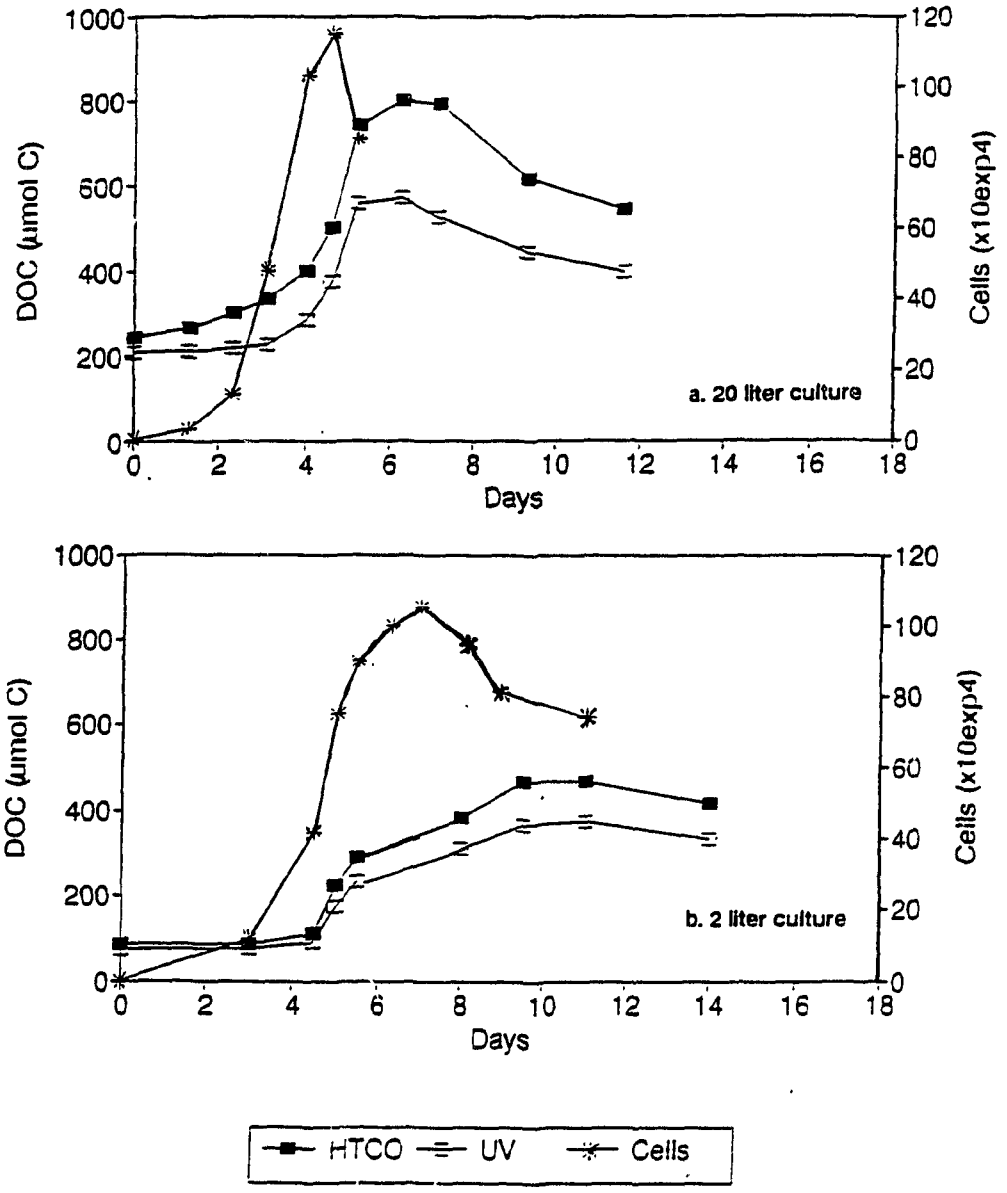
4.3.1 Chaetoceros gracilis

The changes in DOC of the culture media of a diatom, Chaetoceros gracilis, tracked by both HTCO and UV methods, are shown in Figs. 4.1a-b. Fig. 4.1a is for a 20 L batch culture. Fig. 4.1b is for a 2 L batch culture. During the lag stage of both cultures, little change in DOC was found by the HTCO method, while no change at all was detected by the UV method. During the log phase of growth, both HTCO and UV DOC increased rapidly as cell numbers increased. DOC continued to increase through the late log phase and until a few days after crashes of the cultures, then decreased. For the 20 L batch culture, the maximum increases were 560 and 360 μM for HTCO and UV DOC respectively, 375 and 300 μM for the 2 L culture.

The differences between the two methods remained small in lag and early log phases, then began to increase, and reached their maxima (265 and 100 μM for the 20 and the 2 L cultures respectively) at about 2 days after the crashes of the cultures. Afterwards the difference between the two methods decreased gradually.

The highest rate of change for the 20 L culture was found in the late log phase and within one day after the

Fig. 4.1 DOC in the cultures of the diatom Chaetoceros gracilis



crash, while the highest rate of change was found in the mid log phase for the 2 L batch culture for both methods.

The data shown in Fig. 4.1b are also expressed in Table 4.2, in which more data are included for the aged culture, 50 days old. The differences between the two methods were expressed as UV-resistant DOC, DOC(res). The percentages of the DOC(res) to total DOC are also listed in the table.

4.3.2 *Phaeodactylum tricornutum*

The DOC curves for the diatom *Phaeodactylum tricornutum* are given in Figs. 4.2a-b. They are similar to the *Chaetoceros gracilis* DOC curves. The greatest rates of production were found within 1-2 days after crashes of both the 2 and 20 L cultures. The differences between the two methods increased gradually from the early log phase to 2-3 days after the crashes, when they reached their maxima. Afterwards the differences decreased.

4.3.3 *Isochrysis galbana*

The curves obtained for the flagellate *Isochrysis galbana*, shown in Figs. 4.3a-b display significant differences from those of the diatoms. DOC increased

Table 4.2

UV resistant DOC in a culture of Chaetoceros gracilis.

Age of culture (days)	Cell # (x10E4)	DOC(HTCO) (μM)	DOC(UV) (μM)	DOC(res)* (μM)	DOC(HTCO) / DOC(UV) (x100)	DOC(res)* / DOC(HTCO) (x100)
0	0.2	88.3	75.0	13.3	118	15
3.0	12	86.7	78.3	8.4	111	10
4.5	42	112.5	91.7	20.8	123	19
5.0	75	225.0	175.0	50.0	129	22
5.5	90	291.7	233.3	58.4	125	20
6.3	100	-	-	-	-	-
7.0	105	-	-	-	-	-
8.0	93	385.0	312.5	72.5	123	19
9.5	78	468.3	366.7	101.6	128	21
11.0	70	466.7	375.0	91.7	124	20
14.0	-	416.7	333.3	83.4	125	20
50.0	-	238.3	163.3	75.0	146	32

* DOC(res)= DOC (HTCO) -DOC (UV)

Fig. 4.2 DOC in the cultures of the diatom Phaeodactylum tricornutum

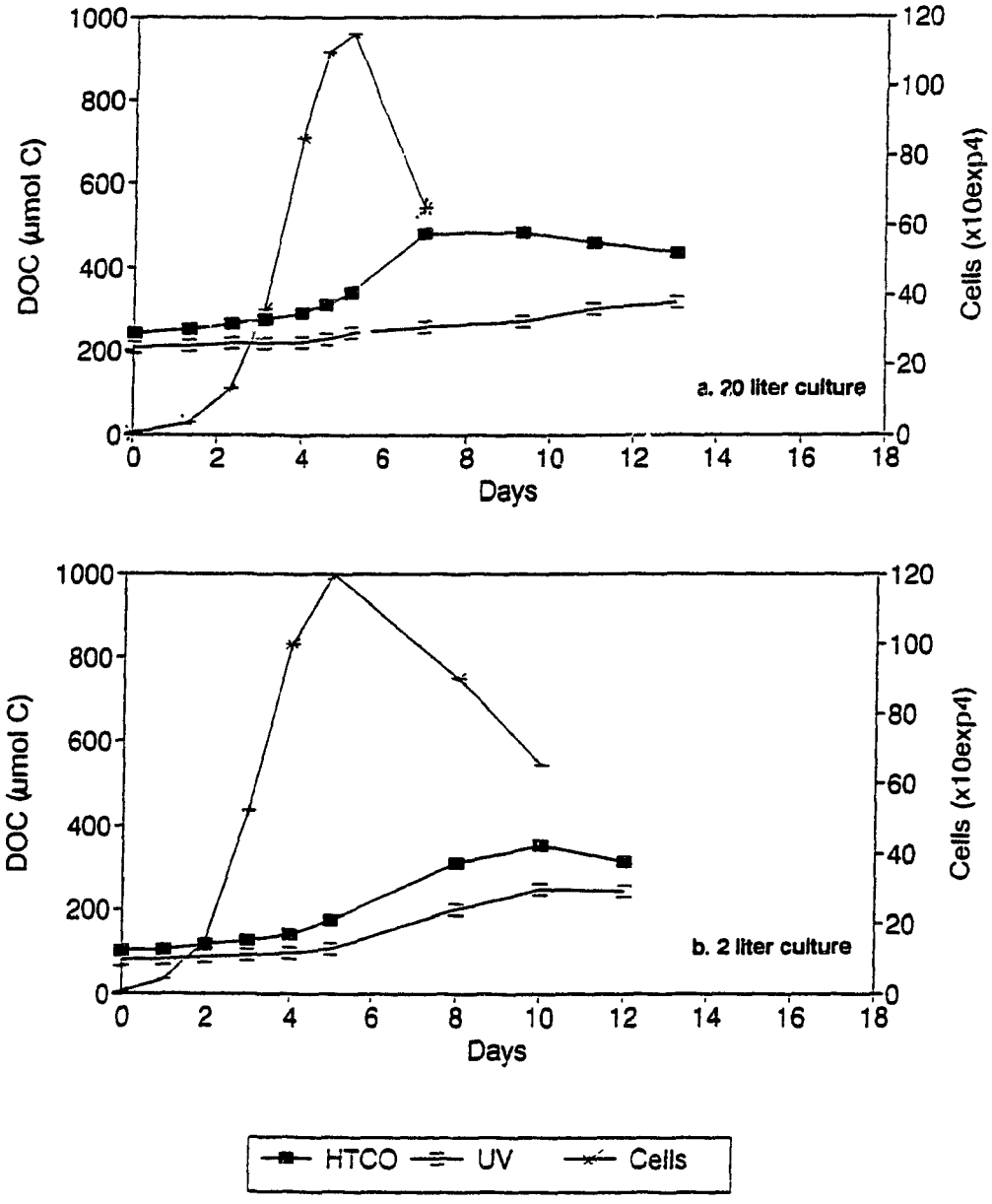
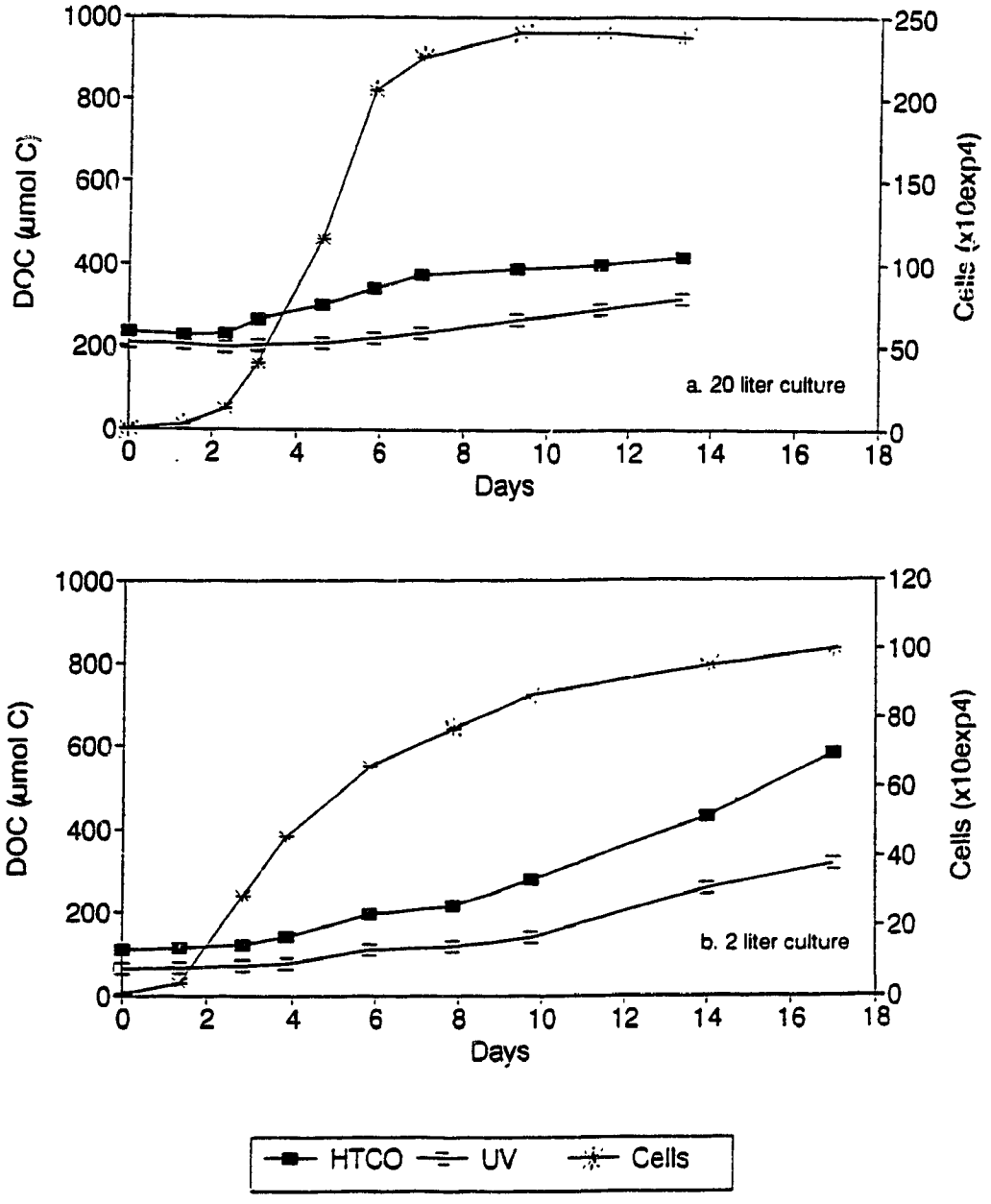


Fig. 4.3 DOC in the cultures of the flagellate Isochrysis galbana



continuously from the beginning to the end of the experiments. For the 20 L culture (Fig. 4.3a) the differences between the two methods increased from the early log phase to the late log phase and then remained relatively constant. The highest rate of production was found in the log phase. For the 2 L culture the difference between the two methods increased continuously from the beginning to the stationary stage (17 days old). The highest production rates were found in the stationary stage. The data shown in Fig. 4.3b is also expressed in Table 4.3, in which data for the aged culture (45th days old) are included. Both HTCO and UV DOC values, and the difference between them increased. The HTCO DOC increased from 580 to 965 μM; the UV DOC increased from 315 to 470 μM and the difference increased from 250 to 495 μM.

4.3.4 *Alexandrium tamarense*

The DOC curves obtained for *A. tamarense* are shown in Figs. 4.4a-b. They are very different from both the diatoms and the flagellate. The changes of DOC in the two dinoflagellate cultures followed a very similar pattern. Before the crashes (ca. 7 days) the UV DOC values decreased continuously and then increased rapidly to the end of the experiments (ca. 16 days). The HTCO DOC decreased at a

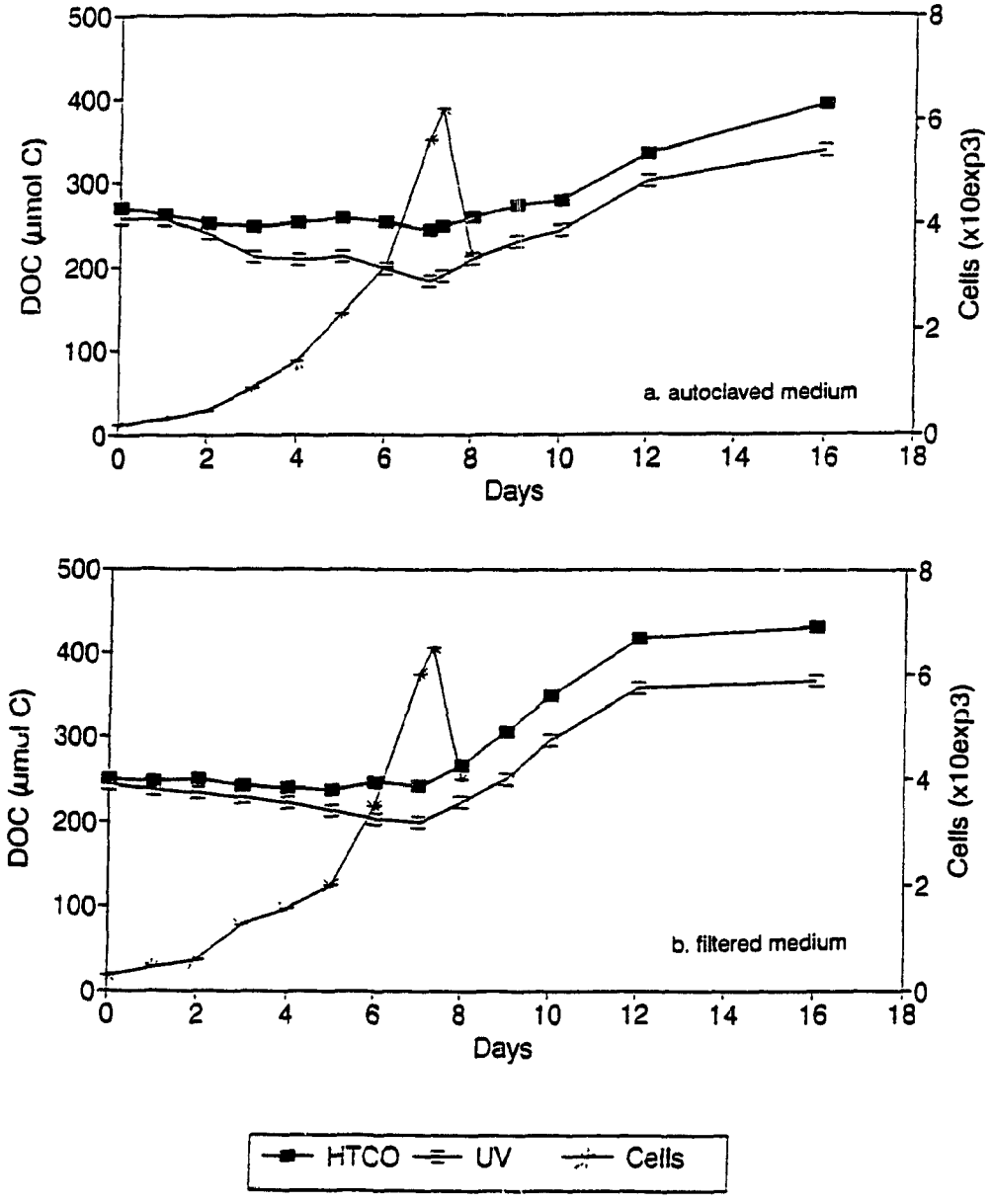
Table 4.3

UV resistant DOC in a culture of Isochrysis galbana.

Age of culture (days)	Cell # (x10E4)	DOC(HTCO) (μM)	DOC(UV) (μM)	DOC(res)* (μM)	DOC(HTCO) / DOC(UV) (x100)	DOC(res)* / DOC(HTCO) (x100)
0	0.55	110.0	65.0	45.0	169	40
1.35	3.5	115.0	66.7	48.3	172	39
2.85	29	121.7	73.3	48.4	166	40
3.85	46	141.7	76.7	65.0	185	46
5.85	66	195.8	110.0	85.8	178	44
7.85	77	215.0	118.3	96.7	182	48
9.85	87	279.2	140.0	139.2	199	50
14.0	-	429.2	256.7	172.5	167	40
17.0	-	580.0	316.7	263.3	183	45
45.0	-	966.7	470.0	496.7	206	51

* DOC(res)= DOC(HTCO) - DOC(UV)

Fig. 4.4 DOC in the cultures of the dinoflagellate Alexandrium tamarense



lower rate before the crashes and then increased immediately after. The differences between the two methods were small at the beginning, increased gradually until the crashes, then decreased for about five days and increased again.

4.3.5 Natural Assemblage

The results for the culture of the natural algal assemblage are shown in Fig. 4.5. Both the HTCO and UV DOC values decreased slightly before early log phase, then increased at a greater and greater rate until a few days after the crash, after which the increase was slower.

The differences between the two methods were small and constant until mid log phase, and then became greater towards the end of the experiment (ca.10 days). The most abundant organisms were found by microscopic examination to be the diatoms Leptocyliodros and Skeletonema.

4.3.6 Molecular Weight Fractionation

4.3.6.1 Chaetoceros gracilis

The results of molecular weight (MW) fractionation of DOC from the 2 L batch culture of the diatom C. gracilis are shown in Table 4.4. The low MW (<1000 MW) DOC in the

Fig. 4.5 DOC in the culture of a natural algal assemblage in the Northwest Arm

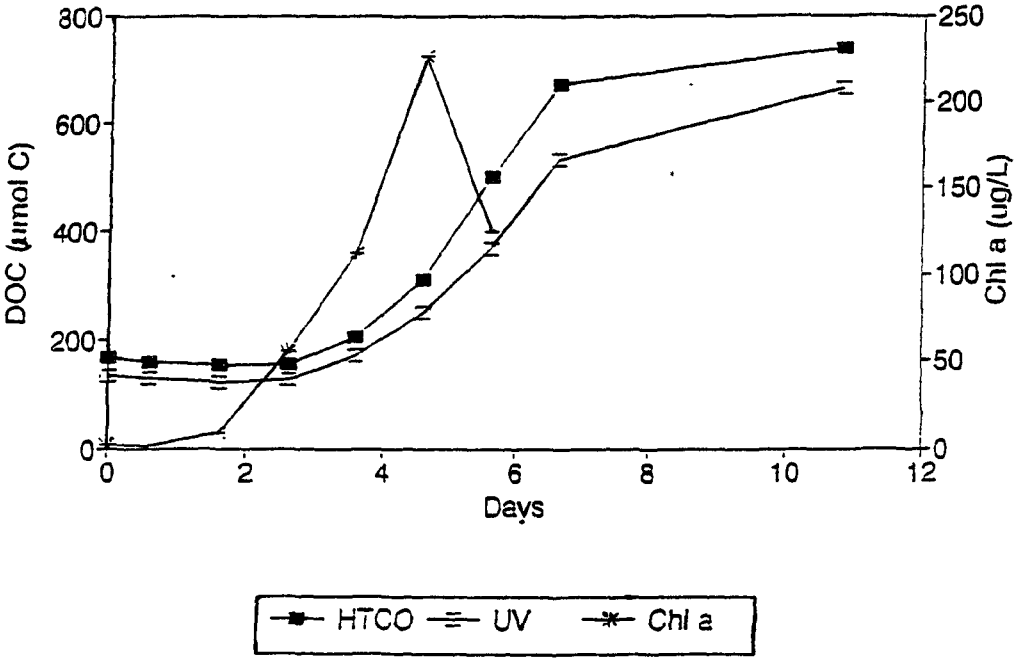


Table 4.4

Molecular weight fractionation of DOC in a culture of Chaetoceros gracilis.

Age of culture (days)	Cell # (x10E4)	TDOC* (μM)		DOC<10000 MW (μM)		DOC<10000 MW TDOC (x100)	
		HTCO	UV	HTCO	UV	HTCO	UV
0	0.2	88.3	75.0	80.0	69.2	91	92
3.0	12	86.7	78.3	75.0	69.2	86	88
4.5	42	112.5	91.7	92.5	77.5	82	85
5.0	75	225.0	175.0	180.0	143.5	80	84
5.5	90	291.7	233.3	239.2	195.5	82	84
6.3	100	-	-	-	-	-	-
7.0	105	-	-	-	-	-	-
8.0	93	385.0	312.5	330.0	274.0	86	88
9.5	78	468.3	366.7	393.3	315.4	84	86
11.0	70	466.7	375.0	355.0	287.5	76	77
14.0	-	416.7	333.3	300.0	243.3	72	73
50.0	-	238.3	163.3	133.3	98.3	56	60

* Total DOC.

culture medium decreased in the first three days, then increased until Day 9.5 and decreased afterwards. The percentage of the low MW decreased in the first 5 days, during the lag and the early log phase, and increased to Day 8, one day after crash, then decreased continuously to the end of the experiment, Day 50, a senescent culture. The highest percentage, 92%, was found at Day 0. The lowest, 56%, was found at the end of the experiment.

4.3.6.2 *Isochrysis galbana*

Table 4.5 shows the results of molecular weight fractionation for the 2 L batch culture of the flagellate *I. galbana*. While the total DOC and the low MW DOC increased continuously from the beginning to the end, Day 45, the percentage of low molecular weight decreased continuously, from 93% down to 27%, a very significant change. The percentage for an older culture would be even lower. A six months old culture was found almost not to pass a 0.45 μm filter at all. The culture medium became a viscous material.

4.3.6.3 Comparison of the HTCO and UV methods for MW fractions of DOC

From the data in Table 4.4 and 4.5, HTCO and UV values for the fraction of > 10000 MW can be calculated, allowing

Table 4.5

Molecular weight fractionation of DOC in a culture of Isochrysis galbana

Age of culture (days)	Cell # (x10E4)	TDOC* (μM) HTCO	TDOC* (μM) UV	DOC<10000 MW (μM) HTCO	DOC<10000 MW (μM) UV	DOC<1000 MW TDOC (x100) HTCO	DOC<1000 MW TDOC (x100) UV
0	0.55	110.0	65.0	102.5	60.8	93	94
1.35	3.50	115.0	66.7	103.3	60.8	90	91
2.85	29	121.7	73.3	103.3	64.2	85	88
3.85	46	141.7	76.7	115.0	64.2	81	84
5.85	66	195.8	110.0	131.7	75.0	67	68
7.85	77	215.0	118.3	138.3	79.2	64	67
9.75	87	279.2	140.0	150.8	88.3	54	63
14.0	-	429.2	256.7	159.2	113.3	37	44
17.0	-	580.0	316.7	205.0	133.3	35	42
45.0	-	966.7	470.0	260.8	180.8	27	38

* Total DOC

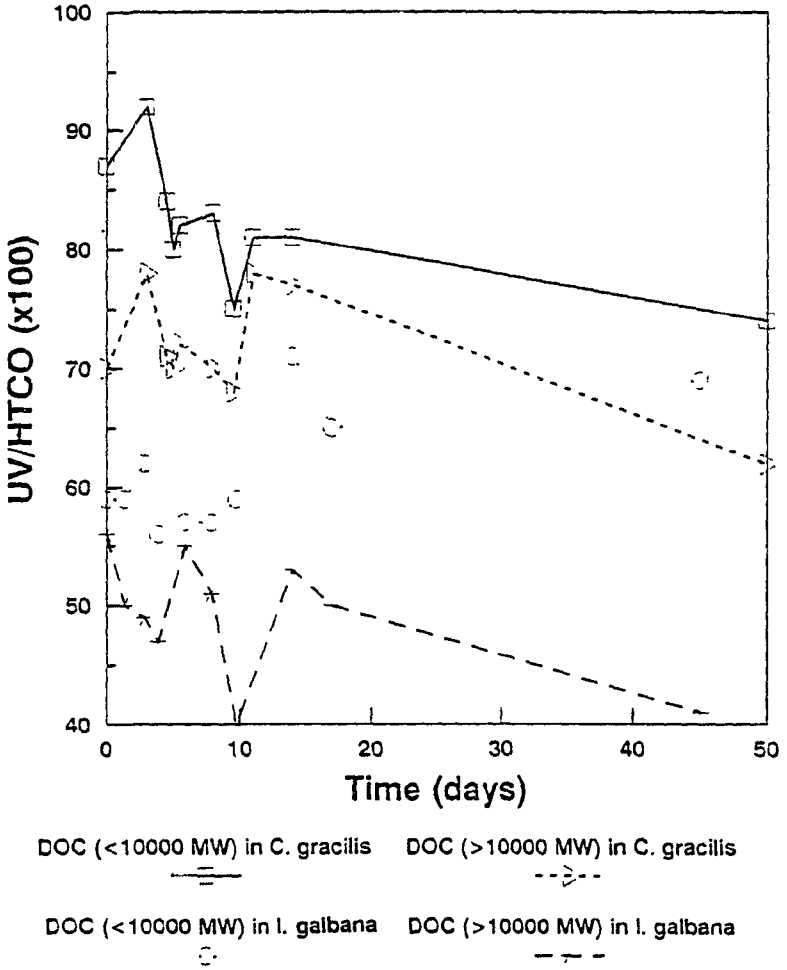
a comparison of the HTCO and UV methods for the two MW fractions of DOC in the two cultures. The results of this comparison are shown in Fig. 4.6. As seen from this figure, UV/HTCO ratios for the high MW fraction were generally lower than those for the low MW fraction in both cultures. The differences between the ratios of the two MW fractions were significant for most data points, and seem to be greater for the aged cultures.

These results strongly suggest that UV photo-oxidation is less efficient for high MW organic matter than for low MW organics in seawater from the Northwest Arm (the culture base) and in cultures of C. gracilis and I. galbana.

4.4 Discussion

In the DOC curves shown in Figs. 4.1-3 and 4.5, for cultures of the diatoms, flagellate and the natural algal assembly, there appear to be some generalities. During the lag and the early log phases the changes of DOC are small and hard to detect by either method, especially by the UV method. The UV method almost did not see any changes in DOC at these stages of growth. In the log phase, DOC increased rapidly as the phytoplankton bloomed. The DOC contents of the culture media thus appeared to parallel the photosynthetic activities as indicated by the cell numbers

Fig. 4.6 UV/HTCO ratios for two molecular weight fractions of DOC in cultures of C. gracilis and I. galbana.



or chlorophyll content. When the organisms were subjected to nutrient stress in the late log phase, DOC continued to increase, in most cases at even higher rates. The highest rates were registered, in most cases, a few days after the crashes of the cultures. This is in good agreement with the observation for carbohydrates (Guillard and Wangersky, 1958; Ittekkot et al., 1981; Ittekkot et al., 1983). A large amount of carbohydrate was released to the medium following stagnation of algal biomass development.

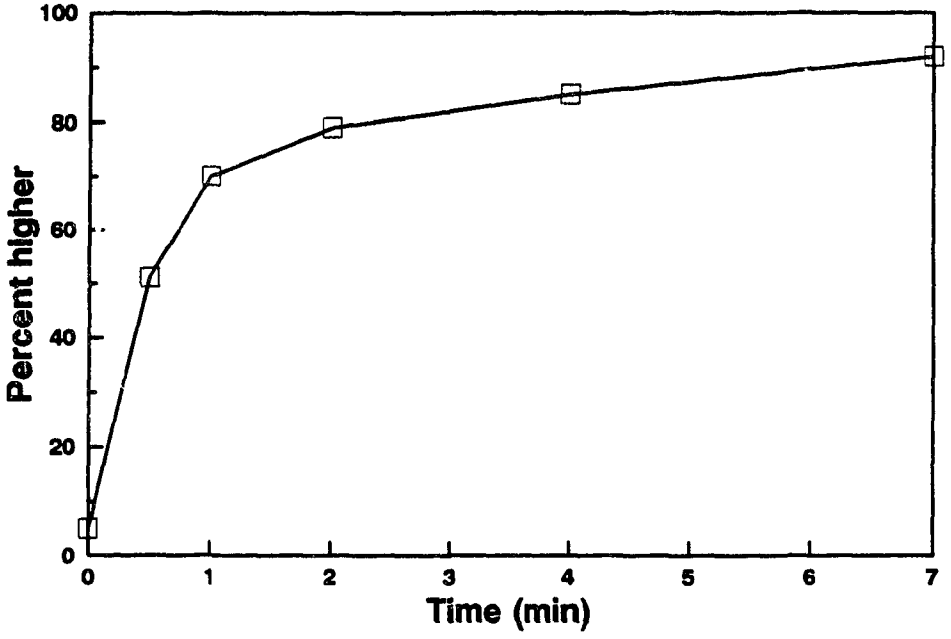
After crashes, DOC increased continuously for different lengths of time, from a few days to a few weeks, dependent on the species involved. DOC then began to decrease, probably from heterotrophic utilization by bacteria (Williams and Yentsch, 1976; Brockmann et al., 1979). Since the releases of DOC from the old cells and degradation of dead cell materials lasted for a much longer time, the greatest amounts of DOC were released by the senescent populations as a result of autolysis and degradation as suggested by Wangersky (1978).

The importance of cell lysis was demonstrated by the following experiment. In an attempt to determine whether there is a pool of extremely labile DOC, which might decay within minutes or seconds after sampling, we used a different method of sample processing. Instead of adding

$HgCl_2$ after filtration of the sample, samples taken from cultures were immediately put into a beaker contained $HgCl_2$, and then filtered within a minute. Much higher values of DOC were found by the new sample processing. For cultures of C. gracilis and P. tricornutum in log phase as high as 50% and 20% increases were found respectively. At first glance, this extra DOC was thought to be the extremely labile fraction which had previously been lost before and/or during the filtration procedure. However, it was also possible that this extra DOC might come from cell lysis due to cell damage and/or death caused by poisoning. A further experiment tested this and found it was due to more cell lysis caused by poisoning before filtration.

A sample from a Chaetoceros culture in log phase was poisoned with $HgCl_2$ to a final concentration of 0.1% right after being taken from the culture, and then filtered at 0, 0.5 1.0, 2.0, 4.0, and 7.0 minutes after poisoning. The results, presented as percent higher than the same sample poisoned after filtration, are shown in Fig. 4.7. The results show that the more damage the cells suffered, the more lysis of cells before and during filtration. These results clearly demonstrated the importance of cell lysis in DOC release and its dependence on the health of the cells.

Fig. 4.7 The poisoning effect of $HgCl_2$ on cell lysis of C. gracilis in log phase



As we can see from the results, the differences between HTCO and UV methods varied throughout the course of the cultures. There appears to be a general trend in the differences. During the early stages of growth, the differences were small or relatively small and relatively constant. They increased during the exponential growth, and reached their maxima some time after the crashes, after which they decreased gradually.

The DOC (res) shown in Table 4.2 and 4.3 and the low molecular weight DOC and its percentages of total DOC shown in Table 4.4 and 4.5 also changed with time. From these data we can conclude that the composition of organic matter released by the algae and the standing stock of DOC in the culture media vary with the different physiological stages of the cultures.

From the comparisons between the two methods we can see that HTCO always measures more DOC than the UV method, from 5 up to 100% more, with most values in the range of 15-50%. We can also see that the slopes of the HTCO DOC curves are generally greater than those of UV DOC curves. Thus we can conclude that the HTCO method is a more sensitive and better method for following the changes of DOC in culture media.

It was suggested by in-vitro experiments that the pattern of released compounds is to some extent species-specific (Allan et al. 1972; Haug and Myklestad, 1976). The different shapes of the DOC curves measured in this work, and the molecular weight composition of the diatom C. gracilis and flagellate I. galbana can serve as further evidences for this suggestion.

Perhaps the most startling manifestation of the release of organic materials by phytoplankton is, as suggested by Wangersky (1978), to be found in the dinoflagellates. This suggestion was confirmed by our experiments with dinoflagellate cultures. The changes of DOC in the culture media were much different from those of other cultures. The DOC continuously decreased before the crash. Since bacterial activities during this period were insignificant, as discussed later, the DOC reduced must be used by the healthy dinoflagellate cells and the uptake must exceed the extracellular release of photosynthetic products and the metabolites. Therefore, we suggest that the dinoflagellates live both as autotrophes and heterotrophes. Compared with other phytoplankton, DOC release by the healthy cells is much less significant in this species. The cell materials are released mainly after death.

Our phytoplankton cultures were not axenic through the experiments since we did not make efforts to prevent bacterial contamination. The number of bacteria and their activities were not measured. According to the work of Ridal (1992), however, the number of bacteria in the culture media (0.2 μm filtered seawater) at Time Zero would be zero or very small. The bacterial numbers in similar culture medium at time zero was found to be below the detection limit of the counting technique. However, two or three days later the bacterial number increased, possibly in part by introduction from the environment. This number remained small until the culture crashed. After the crash the bacteria bloomed. It is probable that before cultures approach their late log phase the production of DOC by phytoplankton is dominant and the utilization of DOC by bacteria is insignificant. After a culture crashes, however, the DOC contents of the culture media must be considered as the standing stock values, which are the net results of production by phytoplankton, mainly through autolysis and degradation, and utilization by bacteria. As we can see from the DOC curves for diatoms, DOC began to decrease some time after the crashes. At this point the utilization of DOC by bacteria exceeded the release of DOC from phytoplankton materials. For the other cultures, we

did not see the DOC decrease, as we did not keep them for longer times. The DOM released by different species of algae is different in nature; some types are more easily utilized by bacteria than others.

It has been suggested that heterotrophic bacteria turn over photosynthetic exudates at different rates, using first and to a higher extent the lower molecular weight products. Larger compounds will be removed at slower rates (Iturriaga and Zsolnay, 1983). Bacteria not only incorporate and respire the phytoplankton exudates but also transform them into relatively large molecular weight materials (Nalewajko and Lean, 1972; Iturriaga and Zsolnay, 1980; Carlson et al., 1985; Brophy and Carlson, 1989).

The results of molecular weight fractionation of DOC from Chaetoceros gracilis culture medium , Table 4.4, tell us that bacteria used mainly low molecular weight DOC and transformed some of it to high molecular weight materials, hence decreased the percentage of low molecular weight DOC in the total DOC. From Day 9.5 to Day 50 total DOC decreased 230 μM while DOC of low MW decreased 260 μM. This means 230 μM of low MW DOC was utilized and 30 μM of low MW DOC was transformed to high MW DOC by bacteria. The percentage of low MW DOC thus decreased from 84 to 56.

4.5 Conclusion

The amounts, composition and nature of the DOC released to the solution differ with algal species, physiological state and bacterial activity in cultures. In the cultures of diatoms (Chaetoceros gracilis, Phaeodactylum tricornutum and the natural algal assemblage in the Northwest Arm) DOC showed insignificant change in the lag phase, but increased significantly during the exponential phase. Maximum releases took place during stationary and decomposing phases. The flagellate Isochrysis galbana showed a similar pattern of DOC release; however, the composition and nature of the DOC differs greatly from that of the diatom C. gracilis. For an aged culture of this species, the high MW fraction was found to make up most of the difference between the HTCO and UV methods. The dinoflagellate A. tamarense showed a startling manifestation of DOC release. DOC in the cultures decreased continuously until the crash, and increased only after the crash. Since DOC released by this species is resistant to bacterial utilization (Chapter 5) and the bacterial activity was essentially negligible (the starting media were autoclaved or 0.2 μm filtered), the decrease of DOC before the crash strongly suggests heterotrophic activity in this species.

The HTCO DOC values were generally 10-100% higher than the UV values for cultures. The actual difference between the two methods for a specific culture is related to the background DOC of the seawater used, the algal species, the physiological state of phytoplankton, and the activities of the bacteria in the media, since the difference between the two methods is dependent on the nature and composition of the DOC being measured.

The UV method was found to be less efficient for high MW organic matter than low MW organics in seawater from the Northwest Arm and cultures of C. gracilis and I. galbana.

Chapter 5

Rates of Microbial Degradation of Dissolved Organic Carbon in Phytoplankton Cultures

5.1 Introduction

The rates of microbial decomposition of the labile DOC produced by phytoplankton are important in understanding the role of marine microbes in the global carbon cycle.

Numerous determinations of uptake rate of DOC by bacteria have been done; however, most of the rates reported were determined by short-term radiotracer methods, which have their limitations (Chapter 1). Few rates of DOC degradation were obtained by bulk DOC measurement.

Using the HTCO method developed in this study (Chapter 2), we followed the microbially-mediated decay of DOC from a phytoplankton culture in log and/or senescent phase of the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum, the flagellate Isochrysis galbana, the dinoflagellate Alexandrium tamarense and a natural algal assemblage from the Northwest Arm. DOC decay was also measured for a seawater sample from the Northwest Arm. Decay rate constants were determined using first-order reaction kinetics in the multi-G model (Berner, 1980; 1984).

5.2 Methods

The cultures used were grown using the method described in chapter 4. When the culture was brought to the desired growth stage, it was filtered through a 10 um Nuclepore filter and then through a 0.8 μm Nuclepore filter cartridge by gravity filtration. About 900 mL of filtrate was mixed with about 100 mL of 0.8 μm filtered seawater from the Northwest Arm. This was to ensure that natural bacterial populations in seawater existed in the decay medium. For two decay samples from the natural assemblage, filtered sediment leaching solution was added as well. About 20 g of fresh sediment taken from the Arm was mixed with 150 ml of seawater. After the sediment had settled, the solution was filtered through a GF/F filter. About 100 ml of the filtrate was added to the decay samples. This was to ensure the natural bacterial population on the surface of the sediment in the Arm existed in the samples as well, and to examine the effect of species, abundance and activity of bacteria in the turnover of DOC. Then the mixture was placed in the dark at 20 °C and bubbled with organic-free oxygen to prevent it from becoming anoxic. The bubbling was kept at its lowest rate (ca. 3 ml/min.) in order to reduce evaporation.

The effect of temperature on the decay rate of DOC was investigated using a culture medium of the diatom Chaetoceros gracilis in the exponential phase of growth. Two aliquots of 2 liters of 0.8 μm culture filtrate were kept at 10 and 20 °C respectively in the dark. The aliquots were bubbled with 0.22 μm filtered compressed air. The flow rate of the bubbling air was ca. 3 ml/min..

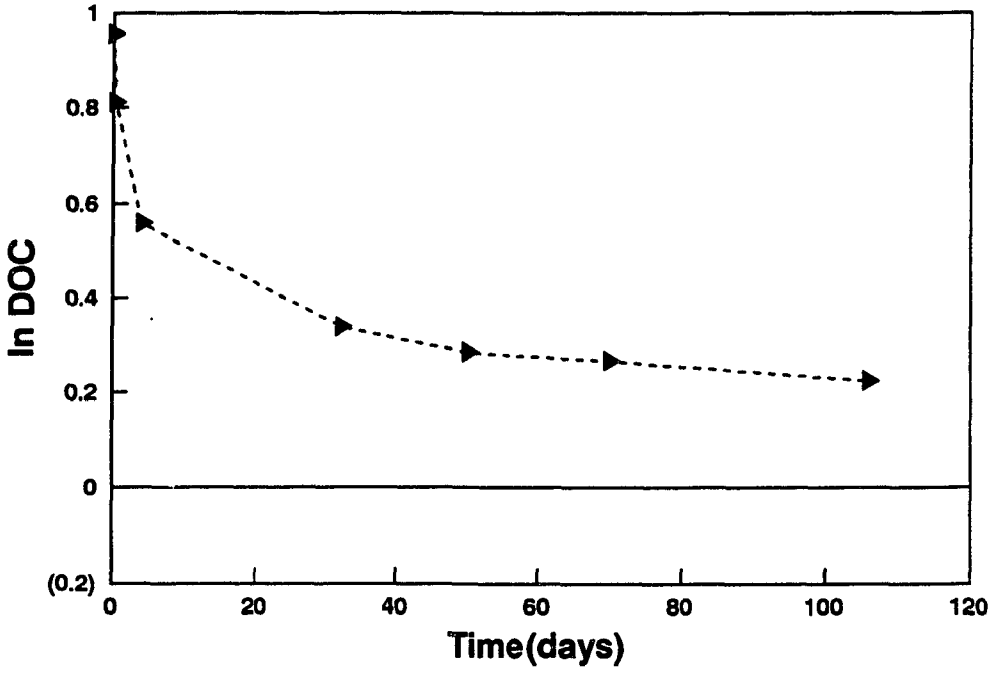
For all the above experiments, samples for DOC analysis were taken at time intervals, closely spaced at the beginning and farther apart later. DOC was measured by the HTCO method (Chapter 2).

5.3 Results and Discussion

The results of the decay experiments are shown in Figs. 5.1-8 and Table 5.1. The decay curves obtained can be described as sections of straight lines. For example, the decay curve in Fig. 5.5 is composed of 4 sections of straight lines: K_1, K_2, K_3, and K_4. The decay of the DOC followed the first-order reaction kinetics of Berner's (1980) multi-G model. The slope of each line is the decay rate for the corresponding DOC fraction.

The DOC in the cultures of the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum, and the natural assemblage decayed fastest in the first day, with the decay

Fig. 6.1 DOC Decay Curve
Sample from Chaetoceros Culture in Log Phase



Note: The unit of DOC used in Figs. 5.1-9 is mg/L.

Fig. 5.2 DOC decay curve

C. gracilis culture in senescent state

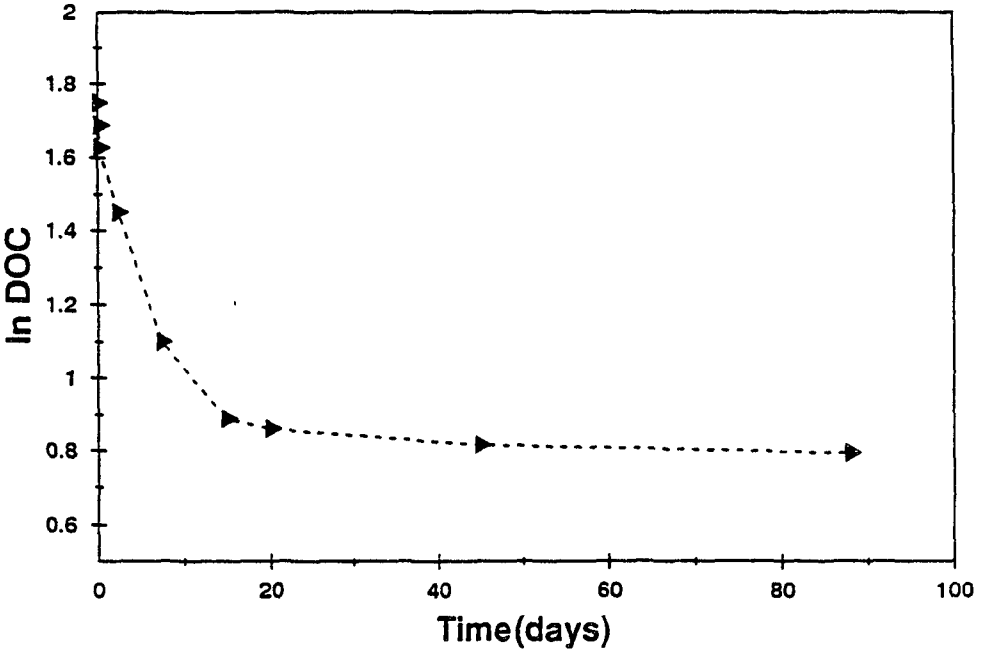
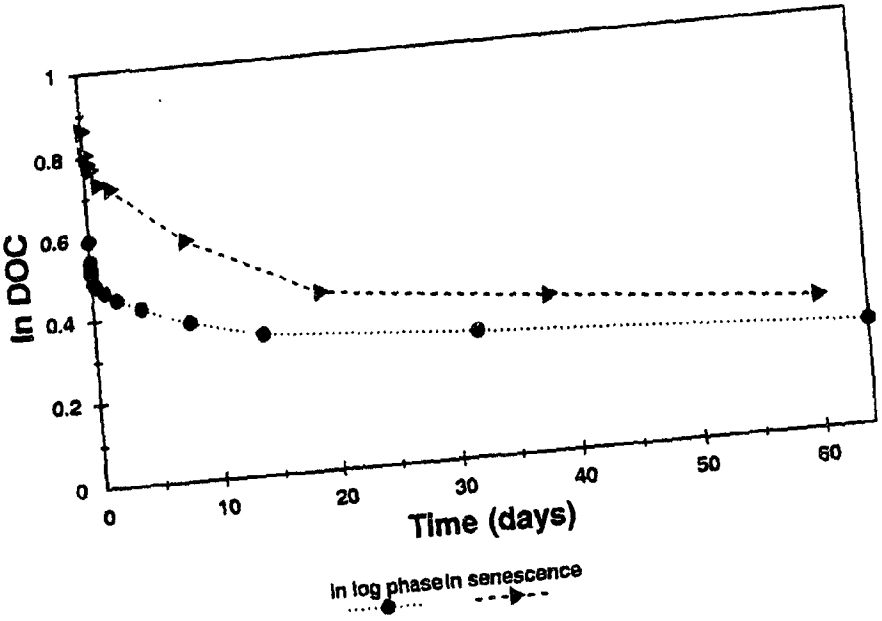


Fig. 5.3 DOC decay curves

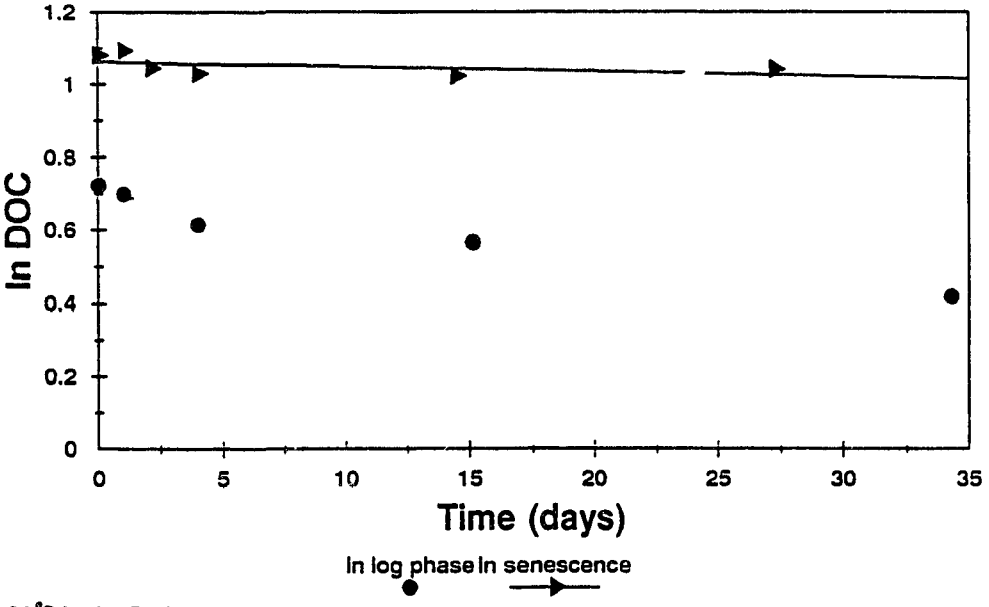
culture of P. tricornutum



0.8 um filtered, 20°C in dark
Bubbled with organic-free oxygen

Fig. 5.4 DOC decay curves

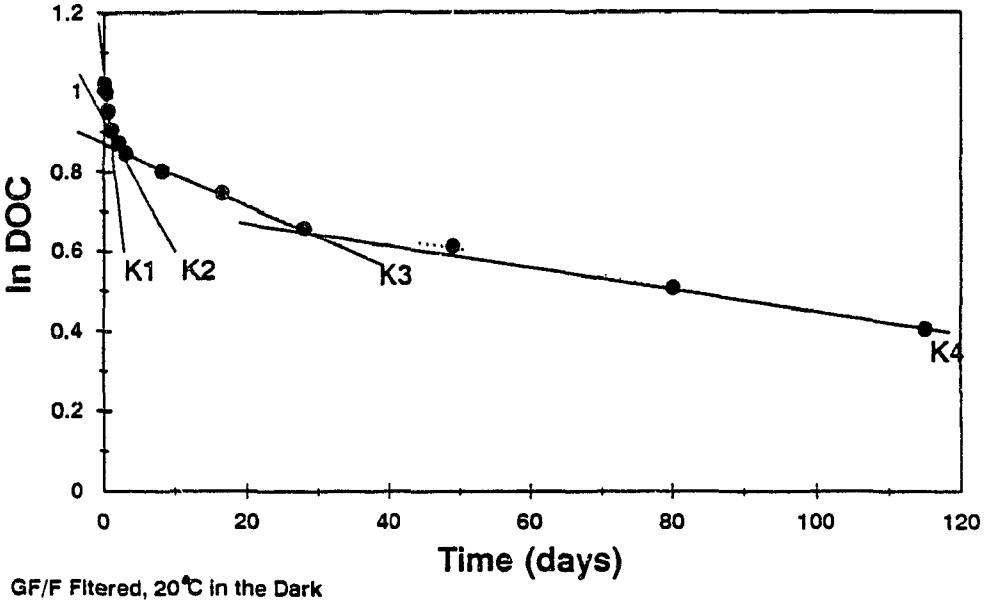
culture of I. galbana



20℃ in the Dark
Bubbled with Organic-free Oxygen

Fig. 5.5 DOC decay curve

culture of a natural algae assemblage

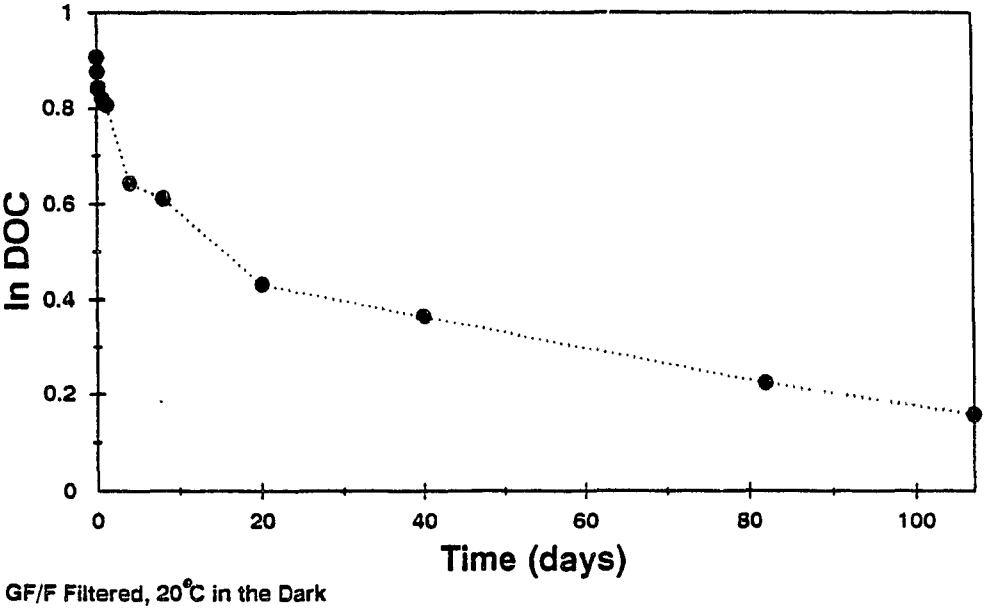


GF/F Filtered, 20°C in the Dark

Bubbled with Organic-free Oxygen

Fig. 5.6 DOC decay curve

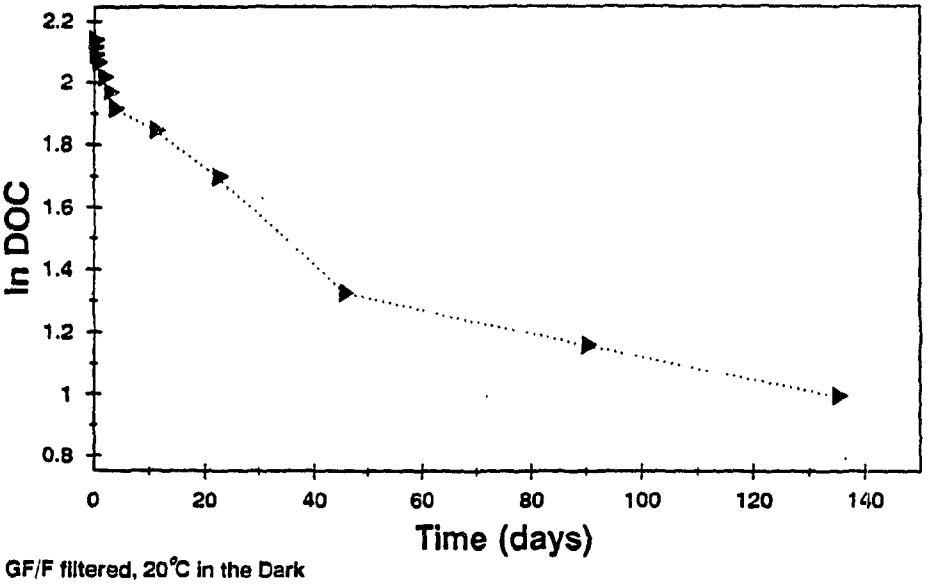
a natural algae assemblage in log phase



GF/F Filtered, 20°C in the Dark
Filtered Sediment Leaching Solution Added
Bubbled with Organic-free Oxygen

Fig. 5.7 DOC decay curve

a natural algae assemblage in senescence



GF/F filtered, 20°C in the Dark
Filtered Sediment Leaching Solution Added
Bubbled with Organic-free Oxygen

Fig. 5.8 DOC decay curve

0.2 μm filtered seawater

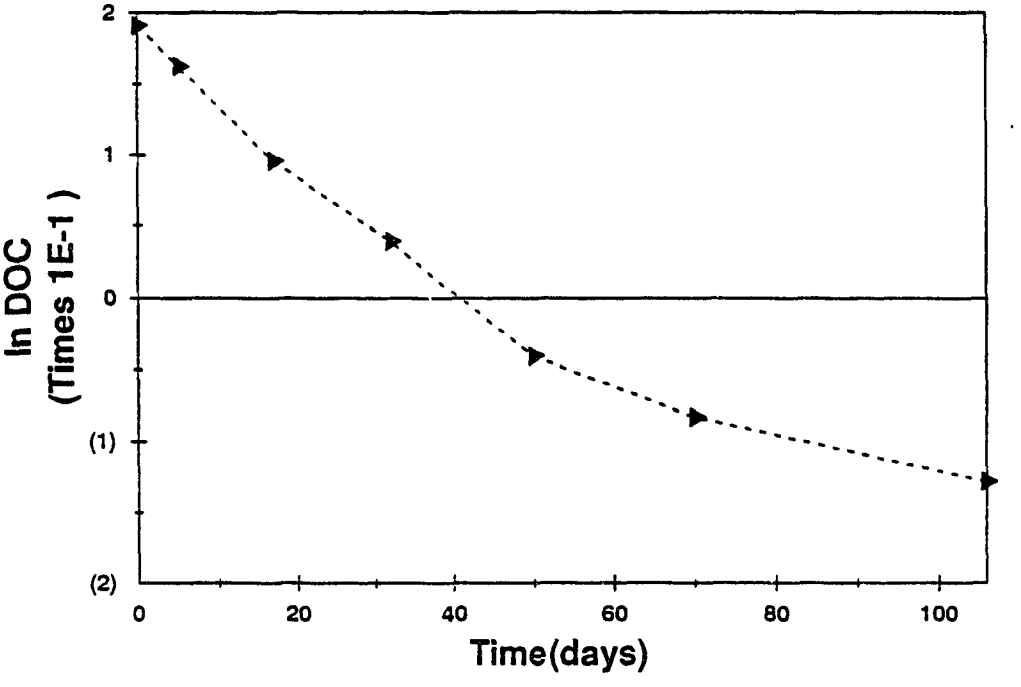


Table 5.1

Decay data of the filtrates from two dinoflagellate cultures in senescence.

Time (day)	DOC (μM) A	B
0	286.7	273.3
0.09	275.0	265.0
0.17	278.3	260.0
0.53	288.3	265.0
1.0	286.7	266.7
2.0	288.3	263.3
4.0	295.0	270.0
8.5	293.3	275.0
15.5	300.0	286.7
30.5	291.7	283.3
57.0	287.5	261.7

A: GF/F filtrate with 1/10 0.45 μm filtered seawater, 20 °C in dark, bubbled with organic-free oxygen. The beginning medium of the culture was autoclaved dialysed (2,000 MW retention) seawater with nutrients added to the Harrison medium (Harrison et al., 1980)

B: GF/F filtrate with 1/5 0.45 μm filtered seawater, 20 °C in dark, bubbled with organic-free oxygen. The beginning medium of the culture was 0.2 μm filtered seawater with nutrients added to the Harrison medium (Harrison et al., 1980).

rates ranging from 0.115 to 0.490 day^{-1} (see Table 5.2), and then slowed considerably. Most labile compounds were degraded within a few days to a few weeks. After that, the DOC decayed much more slowly. Beyond the curves there was no decay detectable by our HTCO method. The residual compounds were either bacteria resistant or degradable with a rate too slow to be detected by our method over a period of a few months.

No decay was observed for the DOC from the senescent culture of the dinoflagellate Alexandrium tamarense, see Table 5.1. This is possibly due to the toxic compounds released by the dying cells. These compounds may be bacteriostatic.

The decay rates and sizes of pools calculated by the multi-G model are shown in Table 5.2. The decay rates and pool sizes differed with species, physiological stage, and batches of the cultures. The rates obtained ranged from 0.115 to 0.49 day^{-1} for the G_{01} fraction, 0.022 to 0.075 day^{-1} for the G_{02} fraction, 7.6 to $27x10^{-3}$ for the G_{03} fraction, and $1.0x10^{-3}$ to $3.7x10^{-3}$ for the G_{04} fraction. The pool sizes varied from 5.0 to 13.5% for Pool G_{01}, 3.3 to 36.2% for Pool G_{02}, 7.2 to 35.6% for Pool G_{03} and 3.8 to 15.5% for Pool G_{04}.

As we can see from the decay curves of the diatoms (Figs. 5.1-3) and the natural assemblage in the Northwest

Table 5.2
DOC Decay Rates.

Substrate	Time (days)	DOC pool	% of TDOC**	K	Rate (day^{-1})
(1)					
C. gracilis	106	G_{01}	13.5	K_1	0.48
in log phase		G_{02}	19.2	K_2	0.068
(Chen & Wangersky,		G_{03}	13.5	K_3	8.0×10^{-3}
1992)		G_{04}	5.8	K_4	1.0×10^{-3}
(2)					
C. gracilis	88	G_{01}	11.5	K_1	0.49
in senescence		G_{02}	36.2	K_2	0.075
(Chen & Wangersky,		G_{03}	9.9	K_3	0.027
1992)		G_{04}	3.8	K_4	1.0×10^{-3}
(3)*					
P. tricornutum	64	G_{01}	10.5	K_1	0.336
in log phase		G_{02}	3.3	K_2	0.022
		G_{03}	7.2	K_3	0.011
		G_{04}	8.3	K_4	1.8×10^{-3}
(4)*					
P. tricornutum	60	G_{01}	8.9	K_1	0.194
in senescence		G_{02}	16.0	K_2	0.027
		G_{03}	10.5	K_3	0.013
		G_{04}	6.8	K_4	2.7×10^{-3}
(5)					
I. galbana	34	G_{01}	10.2	K_1	0.027
in log phase		G_{02}	16.0	K_2	6.5×10^{-3}
(6)					
I. galbana	27	G_{01}	3.7	K_1	1.3×10^{-3}
in staionary stage					
(7) Natural algal	115	G_{01}	10.8	K_1	0.115
assemblage in log		G_{02}	5.8	K_2	0.029
phase		G_{03}	13.7	K_3	7.6×10^{-3}
		G_{04}	15.5	K_4	2.9×10^{-3}
(8)*					
Natural assemblage	107	G_{01}	6.1	K_1	0.371
in log phase		G_{02}	17.0	K_2	0.052
		G_{03}	15.1	K_3	0.014
		G_{04}	14.4	K_4	3.1×10^{-3}
(9)*					
Natural assemblage	135	G_{01}	5.0	K_1	0.307
in senescence		G_{02}	15.1	K_2	0.049
		G_{03}	35.6	K_3	0.014
		G_{04}	12.5	K_4	3.7×10^{-3}
(10)					
Seawater	106	G_{01}	20.7	K_1	4.7×10^{-3}
(Chen & Wangersky,		G_{02}	6.7	K_2	1.6×10^{-3}
1992)					

* (3) and (4), (8) and (9) were from same batches of cultures.
** Total DOC

Arm (Figs. 5.5-7) and Table 5.2, the initial high turnover rates decreased abruptly as the DOC was consumed. In fact, the initial rates of decay decreased by an order of magnitude within hours to days. After a few weeks of decay the rates decreased to the order of 10^{-3}, two orders of magnitude slower.

Why does the bacterial oxidation rate decrease so rapidly? One possibility is that the nature of the DOM is changing (Wolter, 1982; Chrost and Faust, 1983; Iturriaga and Zsolnay, 1983; Jensen, 1983; Bell, 1984; Brophy and Carlson, 1989). The bacteria consume the most labile DOM first until only the most resistant or nutrient-poor fraction remains. An alternate possibility is that the bacteria require an additional nutrient to metabolize DOM and this coincidentally becomes limiting as the incubation experiment progresses (Parsons et al., 1980/1981; Kirchman et al., 1990; Kirchman et al., 1991). Either way, this simple decay experiment has given us insight into the possible nature of DOM and its cycling in the upper ocean.

The diatom C. gracilis, both in log phase and senescence, showed the highest decay rates and largest pool sizes for the fractions of G_{01} and G_{02}. This demonstrated that C. gracilis cultures have more highly labile DOC than

the other species species studied here. This result was consistent with the high percentages of low molecular weight DOC observed for this species in both log phase and early senescence, 82% and 84% respectively (Chapter 4, Table 4.3).

The DOC from the flagellate I. galbana decays much more slowly than that of the diatoms. Compared to the diatoms, in fact, no rate for the G_{01} pool was observed for substrate from the culture in mid log phase, and only a rate for G_{04} was obtained for the substrate from the culture in stationary stage. These results suggest that the DOC released by I. galbana is much less biolabile to bacterial degradation. This suggestion is supported by the result of molecular weight fractionation of the DOC released by this species, which was composed largely of high molecular weight material (Chapter 4, Table 4.5). The low values for the decay constants in seawater (see Table 5.2) suggest that these were in fact K_3 and K_4, the more labile DOC responsible for the K_1 and K_2 values having already disappeared before the experiment.

The substrates from the same batches of cultures, P. tricornutum and the natural algae assemblage, taken at different growth stages, decayed with significantly different rates for the G_{01} fraction, and with almost identical constants for all other fractions. This suggests

that the G_{01} fraction from a culture in log phase is more biolabile than that in the senescent stage. The substrates from senescent cultures of diatoms and the natural algal assemblage, whose major species were diatoms (Chapter 4), exhibited the high decay rates characteristic of biolabile DOC. This is reasonable because all of the cultures used were in their early senescent stages, within 3 days after the crash, and had high DOC values. In that time a large amount of DOC was released by the senescent cells as a result of autolysis and decomposition, while bacteria had not worked on this DOC very much (Chapter 4). There was therefore a considerable amount of biolabile DOC in these cultures. If substrates were taken from very old cultures, most of whose labile DOC had already been used by bacteria, they would display decay constants as low as those observed for seawater in Table 5.2.

The decay rates observed for the DOC from the two batches of the natural assemblage culture, both in log phase, differed in K_1, K_2, and K_3 significantly. The decay substrate with filtered sediment leaching solution added decayed with K_1 three times as high, and K_2 and K_3 twice as high as those for the substrate without the sediment leaching solution added. This result suggests that the species, abundance, and activity of bacteria are important factors in determining the turnover rate of the DOC.

The decay constants obtained demonstrated that biolabile DOC from phytoplankton cultures could be divided into four fractions which decay in hours to days, days to weeks, weeks to months, and months to years respectively. This is consistent with results obtained by ^{14}C tracer methods for DOC from detrital S. costatum cells (Pett, 1989). The amounts of material falling into each of the fractions were highly variable with species, ages of and bacterial activities in the cultures. The shapes of the decay curves were determined by both decay constants and sizes of each of the fractions.

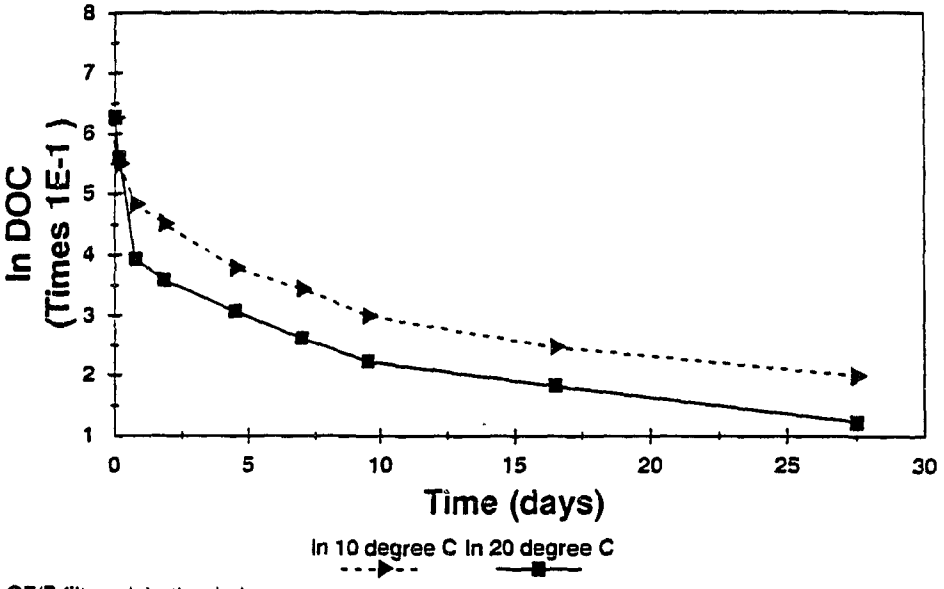
However, the decay rates observed in our experiments might be much higher than natural or in situ rates since most of the grazers of bacteria (e.g. microflagellates) were removed from the decay substrates by 0.8 μm filtration. Although the numbers of bacteria in the decay substrates were not measured, their abundances might be much higher than the natural levels in seawater and hence increase the decay rates of DOC by a large factor. It is obvious that the turnover rates obtained by these experiments are specific to the DOM and organisms existing in the substrates used, and cannot be generalized to natural seawater.

The results of the effect of temperature on decay of DOC are shown in Fig. 5.9. The bubbling air used in this experiment was not organic-free and hence produced some degree of contamination in the decay substrates. The contamination rate from the compressed air used was monitored using a controlled sample of Super Q water with salt in it: the DOC increased from 12.5 μmol at the beginning to 29.2 μmol at the 60th day. This rate of contamination was relatively minor compared to the decay rate of the DOC in the first 30 days. However, it was significant after 30 days and became higher than the decay rate of the DOC after about 45 days. In spite of the contamination from the bubbling air the results for the first 30 days, shown in Fig. 5.9, do demonstrate that the decay of DOC by bacteria and hence the growth of bacteria is temperature dependent: it was more rapid at 20 °C than at 10 °C. This is consistent with Takahashi and Ichimura (1971), who found a similar temperature limitation on glucose uptake in seawater.

Fig. 5.9 shows that the G_{01} fraction decayed much more slowly at 10 °C than at 20 °C. At 20 °C 35 μmol C disappeared within 0.75 day, giving a decay rate of 0.30 day^{-1}, while it took 4.5 days to decay the same amount of DOC at 10 °C. Moreover, at 10 °C it took two steps to decay

Fig. 5.9 Temperature effect on the decay of DOC

C. gracilis in log phase



GF/F filtered, in the dark
Bubbled with compressed air

this fraction of DOC, with decay rates of 0.15 and 0.036 day^{-1} respectively. Afterwards the decay took the same pathway at the two temperatures. This result suggests that the consumption of the highly labile DOC by bacteria is more sensitively affected by temperature change than are the later fractions. The high decay rates for the substrates from the diatoms and the natural algal assemblage demonstrated that phytoplankton can release highly labile DOC, which could be utilized by bacteria and disappear within hours to days. These results again confirmed the importance of sample processing and preservation for DOC measurement in seawater.

5.4 Conclusions

DOC released by phytoplankton differs greatly in its biolabiliy, according to the species and physiological stage of the algae. DOC released by the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum is highly labile and can be utilized by bacteria within days. That from the flagellate Isochrysis galbana has a much lower lability, and that from the dinoflagellate Alexandrium tamarense is essentially bacteria-resistant.

DOC released by diatoms decays rapidly at the beginning, slowing down within a few days to a few weeks,

and then decays at a much lower rate. The decay of DOC follows the first-order kinetics of the multi-G model, and is temperature dependent, with higher rates at room temperature and lower in he cold.

The high decay rate of the DOC released by phytoplankton confirms the importance of sample processing and preservation for DOC measurement in seawater.

Chapter 6

Temporal Variations of DOC during Spring Phytoplankton Blooms in the Coastal Waters of Nova Scotia, Canada

6.1 Introduction

In a spring bloom in temperate coastal waters, the production of phytoplankton increases very rapidly and then is terminated as a result of depletion of the major nutrients (i.e. nitrate, phosphate). It pulses over a period of a few days to weeks. The productivity and biomass of phytoplankton could be many times or even orders of magnitude higher during the spring bloom than in the other seasons. With such an intensive phytoplankton activity and dramatic changes in the physiological stages of the microalgae within a relatively short time, spring blooms permit study of the dynamics of organic production in real marine environments. Because the environment and the state of phytoplankton are changing during the course of the bloom, the amount and composition of both cellular and extracellular products are also changing with time.

Many laboratory culture experiments have demonstrated that marine phytoplankton are able to produce significant amounts of DOM during both active growth phase and stationary state (references in Chapter 1; results in

Chapter 4 of this study). The extracellular production of DOM by phytoplankton is a major source of DOC in seawater. The production depends on the activity of phytoplankton, their species and physiological state.

Until now, however, most of our knowledge about the dynamics of DOM production is from culture experiments. Few time series have been done in the field, mainly because of the complexity of the marine environment. In addition, the high cost of ship time makes it impossible to carry out a time-series measurement in the open ocean, while the relatively high background level and multiple sources of organic matter complicate the situation and make the the contribution of DOM from phytoplankton obscure in coastal waters.

In this study, two spring bloom time-series sampling projects were carried out, one in the Northwest Arm in 1991, the other in Bedford Basin, Nova Scotia in 1992.

6.2 Methods

6.2.1 Spring Bloom in the Northwest Arm, 1991

The sampling program was carried out from early March to early June. Seawater was taken about twice a week from the Dalhousie University Aquatron seawater supply system

before treatment. The seawater was drawn in at a depth of about 12 m in the Northwest Arm (Station A in Fig. 6.1) and pumped through an underground fibreglass pipeline to the Aquatron. The sampling time was usually around high tide (+ 1 hour).

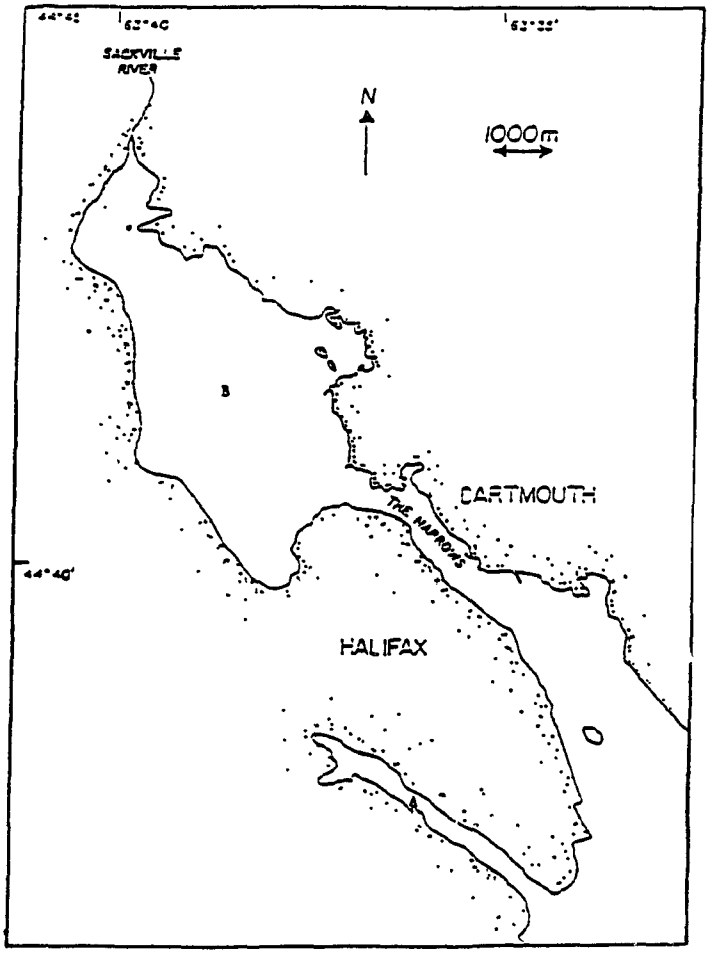
The seawater sample was filtered, immediately after being collected, through a precleaned 0.45 μm Millipore filter, using a 50 ml syringe. About 25 ml was collected in a glass test tube and analyzed for DOC immediately. When storage was necessary the filtered samples were poisoned by $HgCl_2$ to a final concentration of 0.1%, and kept in a refrigerator. The folded filter was stored frozen for chlorophyll measurement.

The DOC was measured by both HTCO (Chapter 2) and UV (Gershey et al., 1979) methods. Chlorophyll was measured by a standard oceanographic method (Parsons et al., 1984).

6.2.2 Spring Bloom in Bedford Basin, 1992

The time period of sampling was from mid January to mid June, 1992. Before the development of the spring bloom and after the bloom ended the sampling frequency was once a week or longer. During the bloom peak time the frequency was much higher: 4 times within a week. The sampling site was at the DREA Barge (Station B in Fig. 6.1) in the basin.

Fig. 6.1 Sampling sites in the Northwest Arm (A) and in Bedford Basin (B)



Temperature, salinity, depth, particle density, chlorophyll a, DOC, and nutrients, including phosphate, silicate, nitrate, nitrite, ammonium and TDN were measured. Except for DOC and TDN, all the other measurements were made by the Bedford Institute of Oceanography (BIO) using standard oceanographic methods.

Samples for DOC and TDN measurements were taken by a 12-l Niskin bottle from depths of 0.5, 5, 10, 15, 20, 25 and 30 m. Forty-ml samples were filtered through precombusted GF/F filters and acidified with HCl to a pH of about 2.5 and stored in a refrigerator. DOC and TDN measurements were run within a week of storage. DOC and TDN were measured by the HTCO methods described in Chapter 2 and 7 of this volume.

6.3 Results and Discussion

6.3.1 Spring Bloom in the Northwest Arm, 1991

The Northwest Arm is a small marine inlet lying along the southwestern boundary of Halifax Peninsula, with a length of about 5 km and a typical width of 300 m. The depth of the Arm is 13-19 m. The fresh water input into the Arm is very small. This water body is not restricted by a sill and has considerable circulation with offshore waters.

The tides in the Arm are mainly semidiurnal with a mean tidal range of 1.5 m. The general pattern of circulation is an intrusion of cold saline water at depth from the adjacent offshore region and a return flow removing the surface water from the Arm (Gregory, 1972). The effect of water displacement due to tidal movement was minimized by taking samples at high tides.

The results of the observations are shown in Fig. 3.3 for the comparison between the two DOC methods in Chapter 3. Here these time-series results are discussed for the production of DOC in seawater.

Fig. 3.3 shows a high peak of chlorophyll-a between Day 121 and 127, accompanied by a minor peak and then followed by a higher peak of DOC (both HTCO and UV values) between Day 127 and 134. The DOC peak at Day 104 is not confirmed since there is only one datum. Except for this point of datum, the variation of DOC in the Northwest Arm shows a good correlation with the chlorophyll, i.e. the phytoplankton biomass. This correlation suggests the phytoplankton-related source of DOC, in good agreement with the result and explanation of previous work on organic ligands in the Northwest Arm (Zhou, 1988; Zhou and Wangersky, 1989a).

In earlier field observations (Duursma, 1963; Morris and Foster. 1971; Wafar and Le Corre, 1984) an increase in

DOC was found several weeks after a phytoplankton bloom. Autolysis and degradation of the phytoplankton cells and debris were considered to be responsible for this increase. However, this time lag argued against the decay of phytoplankton itself being the main source of DOC. In contrast, in my data a DOC peak was found to accompany the Chl a peak and last with higher values until a few days after the Chl a decreased. This is very much in agreement with our culture results discussed in Chapter 4. Thus, the DOC peak observed resulted first from the phytoplankton bloom, then the phytoplankton under stress, and at last from the decomposing dead phytoplankton cells, from which the largest input of DOC came; and sloppy feeding from zooplankton grazing might also make a significant contribution to the DOC peak observed.

6.3.2 Spring Bloom in Bedford Basin, 1992

Bedford Basin is a small marine bay with an area of 17 km^2 and maximum depth of 90 m. The water exchange between the basin and the offshore waters occurs through a shallow inlet with an effective sill depth of 20 m and a minimum width of 375 m. The major fresh water input to the basin is from the Sackville River via Bedford Bay. During the spring, the increase of the fresh water discharge, mainly

from melting of snow, could significantly change the salinity of the surface water in the basin (Fig. 6.2). The terrestrial humic material input from the river could be another major source of dissolved organic matter in addition to *in situ* production of phytoplankton (Zhou, 1988).

The data collected from this spring bloom project are summarized in Table A.1 in the Appendix of this volume. DOC data discussed here were from the surface water samples (0.5 and 5.0 m depths) and for the time period from March 24 to June 11 1992 (three weeks before and 6 weeks after the bloom), since our major interests are in the aspects related to the phytoplankton bloom. DON results will be discussed in Chapter 8.

At 0.5 m (Fig. 6.3) on Day 94, well before the bloom started, there was a sharp DOC increase from the background value of about 115 μM to 136 μM, due to a pulse of fresh water. An extremely low salinity value coincided with this DOC increase. During chl-a peak time, from Day 112 to 118, DOC increased rapidly. After the bloom reached its peak, on day 121, chlorophyll concentration dropped rapidly to an extreme low value which coincided with a salinity low and a minor decrease of DOC. The drop of chlorophyll was due to both the crash of the bloom (sinking to deeper water) and dilution by fresh water. The increase of DOC from fresh

Fig. 6.2 Salinity in Bedford Basin during the 1992 spring bloom

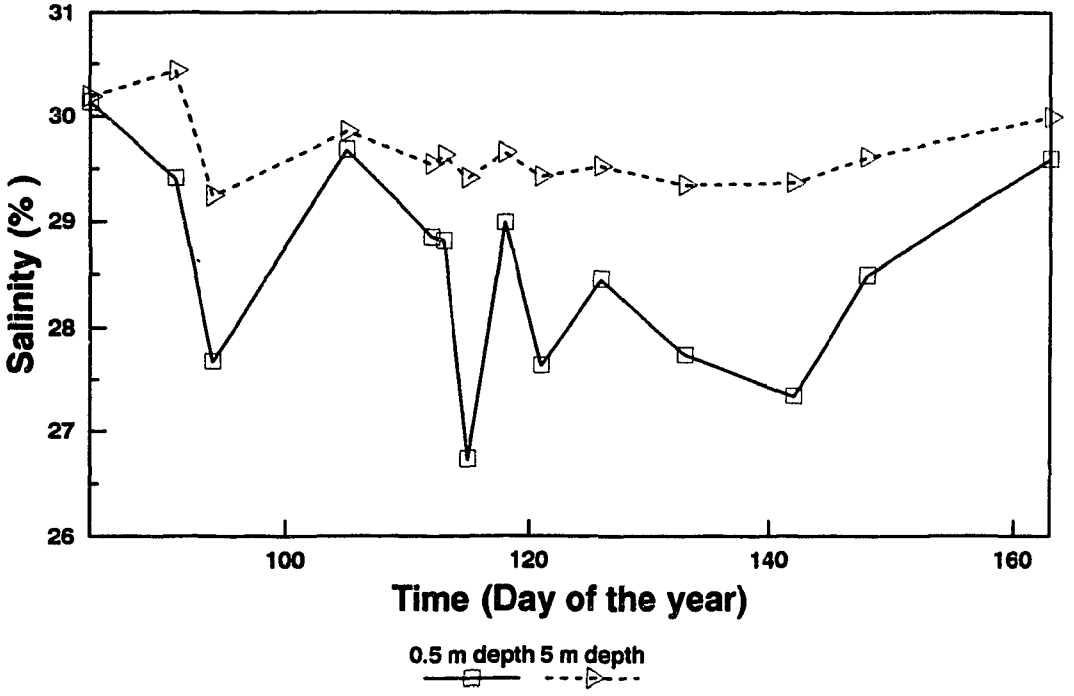
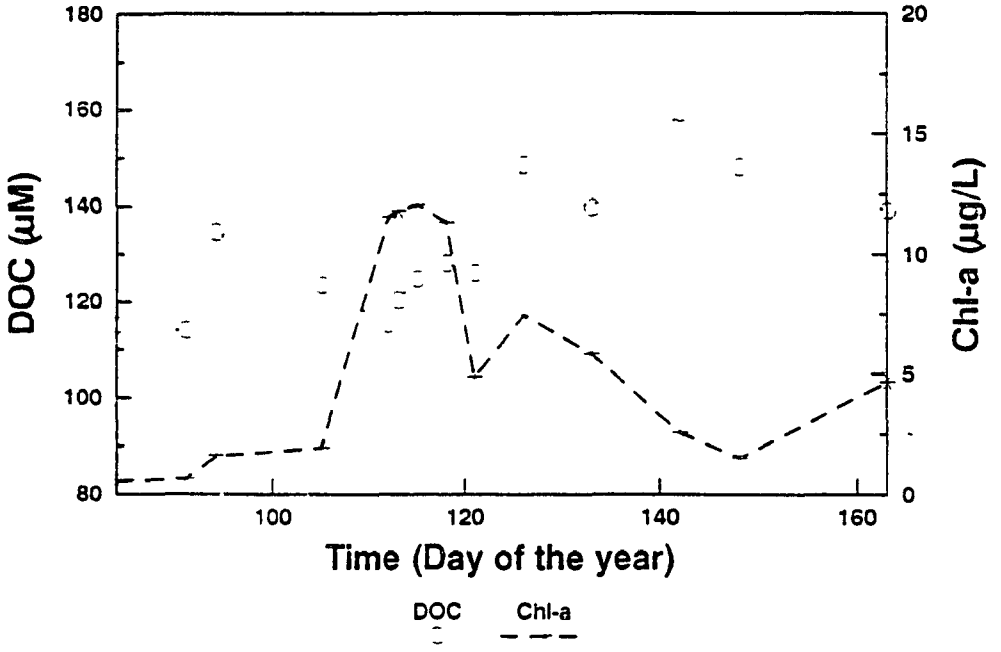


Fig. 6.3 DOC and Chl-a in Bedford Basin during the 1992 spring bloom



0 5 m depth

water input was less significant than the DOC decrease by cell dilution, resulting in the minor decrease in DOC concentration in the water. The release of DOC by stressed phytoplankton cells at this time was expected to be significant according to the results of culture experiments and the observations in the Northwest Arm. Following this minor decrease in DOC there was a sharp increase, reaching a peak value at Day 126. Since there was a co-occurring decrease of fresh water input (Fig. 6.2, an increase of salinity was observed at that time), DOC released by the decomposing cells at this time was responsible for this increase in DOC. After a minor decrease on Day 133, DOC reached its maximum (about 160 μM) on Day 142, which coincided with the minimum of salinity. The large input of fresh water at this time was responsible for this increase in DOC concentration, similar to the pre-bloom increase of DOC. DOC released from the cells at this time was much less important, since most of the cells had already decomposed or sunk to the deeper water. The chrolophyll a value at this time was rather low, close to the pre-bloom value. Due to the large input of fresh water after the bloom crash, DOC in the seawater increased continuously for a much longer time (about three weeks), and then began to decrease.

To examine the contribution of DOC from fresh water input, DOC in seawater before the phytoplankton bloom is plotted against salinity (Fig. 6.4). On Day 42, when there was little fresh water input, the correlation between these two parameters was poor (Fig. 6.4a, r^2=0.15). In contrast, however, on Day 49, 70 and 94 when there were significant fresh water inputs, the correlation between DOC and salinity was very close: DOC was negatively correlated with salinity, with a correlation coefficient of 0.91 (Fig. 6.4b). This close negative correlation between DOC and salinity and the high DOC concentration in the fresh water (371 μM, calculated from the intercept at 0 salinity in Fig. 6.4b) demonstrated that fresh water is another source of DOC in Bedford Basin.

At the depth of 5 m, as we can see from Fig. 6.2, salinity was much less affected by the fresh water input. There was only one significant drop in salinity on Day 94, accompanied by a DOC increase, while chlorophyll remained little changed (Fig. 6.5). Terrestrial organic material in the fresh water input was responsible for the increase of DOC in seawater at this time. Then DOC remained relatively constant until about 10 days after the phytoplankton peak. During the whole course of bloom development, peaking and crash, no significant changes in DOC were observed. This

Fig. 6.4 Correlation between DOC and salinity

in Bedford Basin during 1992 spring bloom

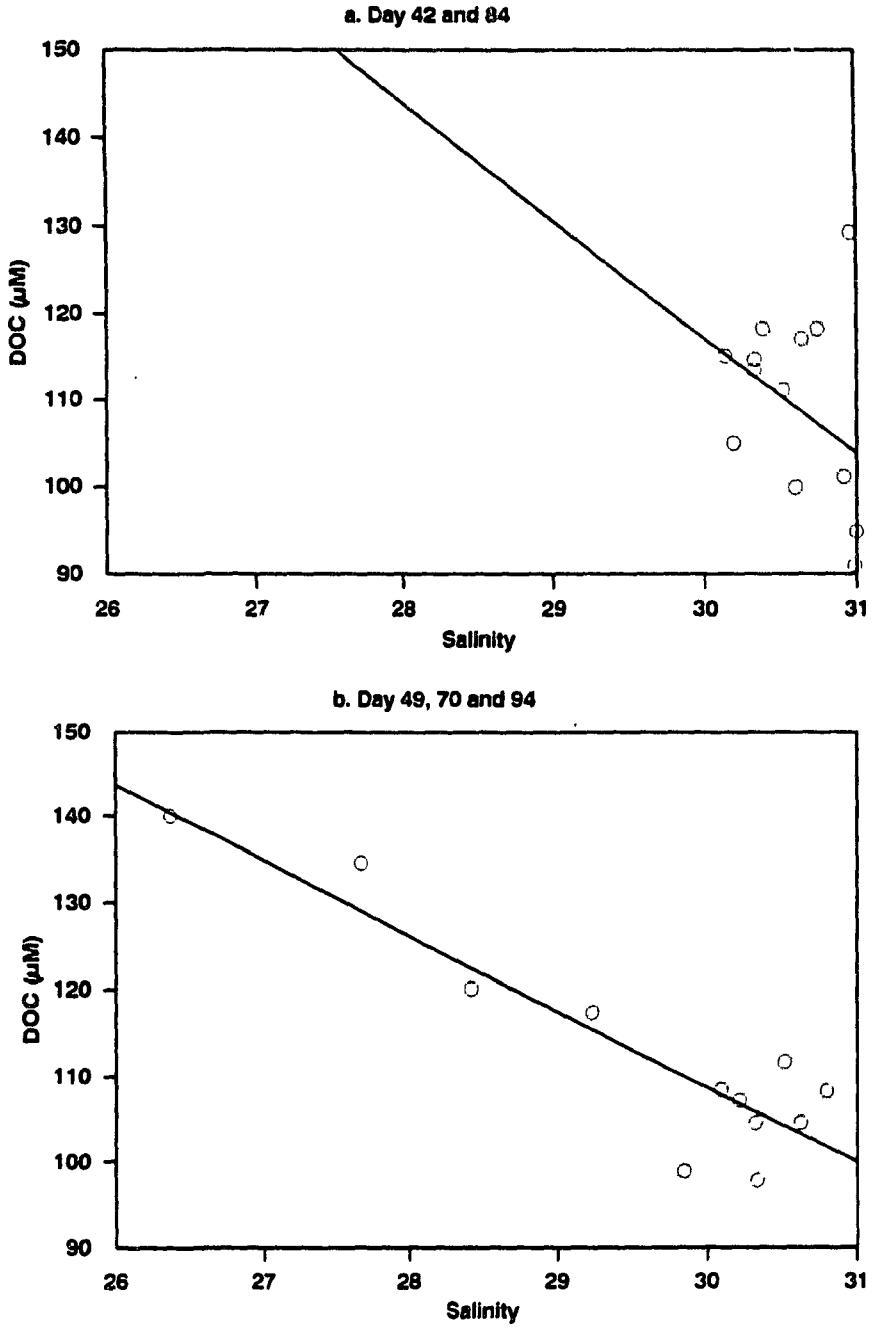
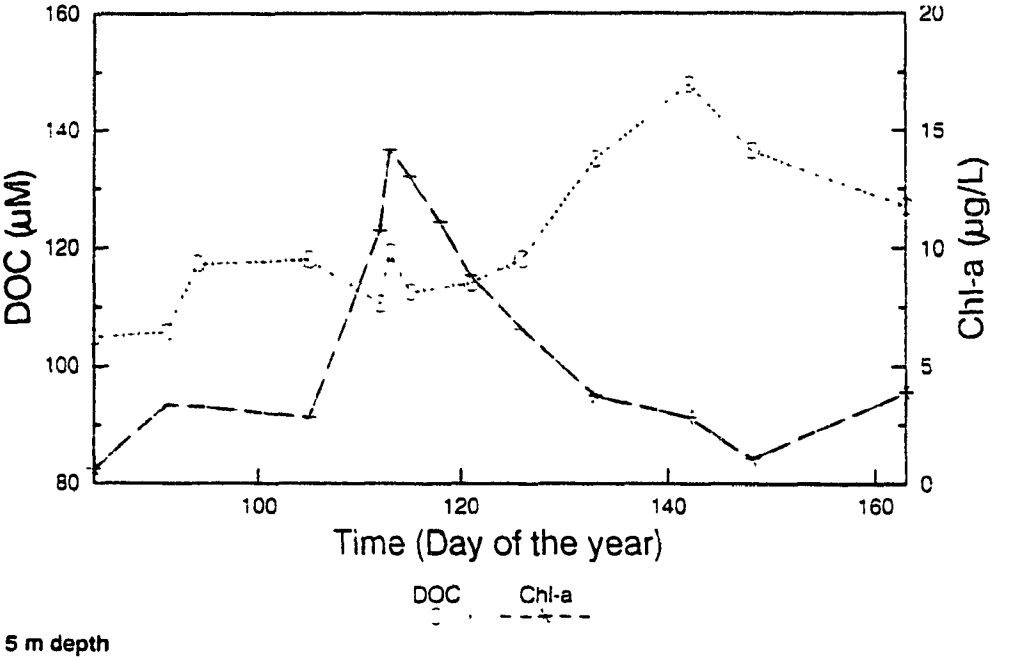


Fig. 6.5 DOC and Chl-a in Bedford Basin during the 1992 spring bloom



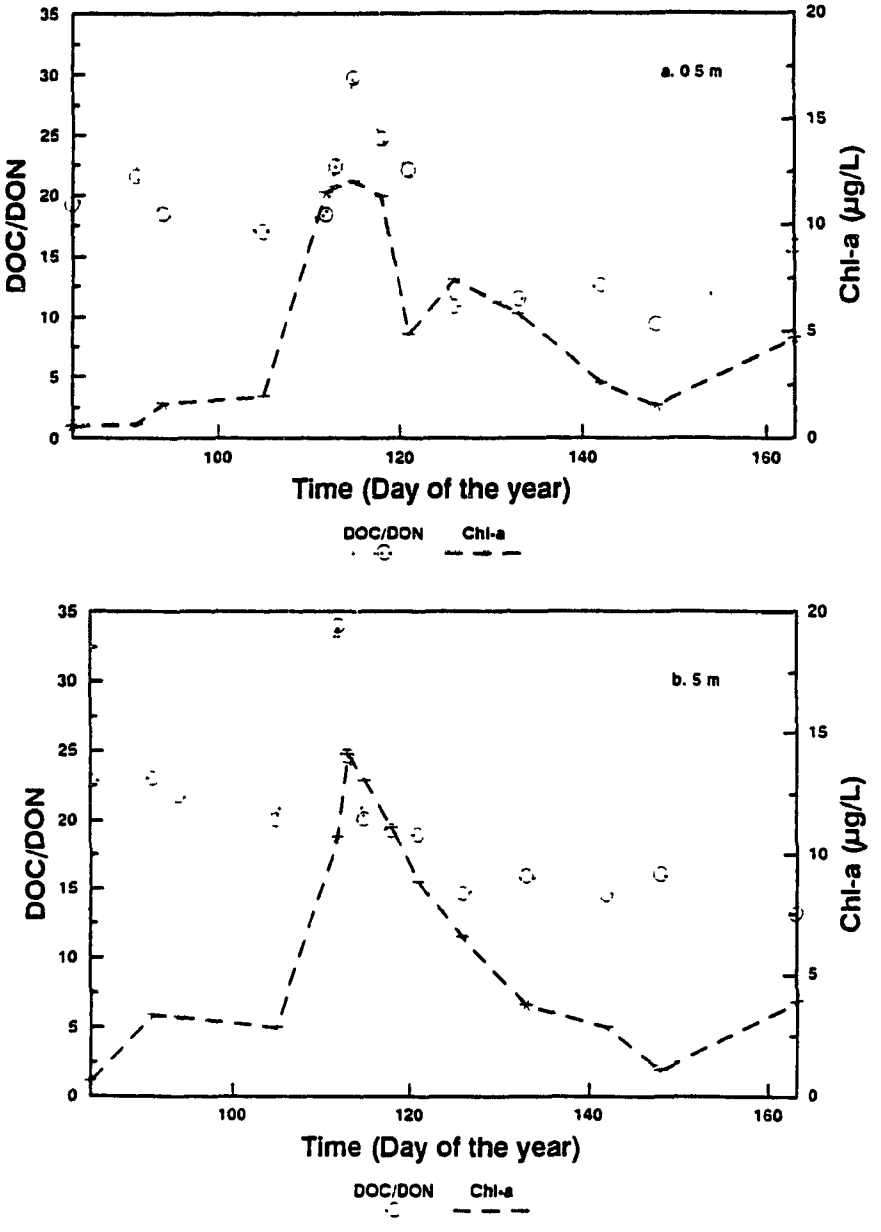
5 m depth

was hard to explain in terms of phytoplankton activity in the water, but may be due to physical processes such as tidal cycle and water mixing. Bacterial activity might also play a key role in this case. Since we do not have this information, the question remains unsolved. After Day 126 DOC increased rapidly and reached its maximum, 150 μM, on Day 142. Since there was not a significant fresh water input, the increase must have been from the release by the decomposing phytoplankton.

A significant increase in DOC concentration, about 45 μM at both depths, was observed after the spring bloom crashed. This was generally consistent with the observation in the Northwest Arm. However, the fresh water input from the Sackville River in Bedford Basin also made a significant contribution to the DOC pool. The relative contribution of the two sources, fresh water input and phytoplankton production, was dependent on factors such as the amount of the fresh water input, its DOC contents, the activity of the phytoplankton, and the mixing with deeper water and offshore water brought in by the tide, etc.

To examine the effect of the phytoplankton bloom on the composition of DOC, the curves of DOC/DON ratios are plotted together with chlorophyll a curves (fig. 6.6). The DOC/DON ratios at both depths followed a same pattern. It

Fig. 6.6 DOC/DON ratio in Bedford Basin during the 1992 spring bloom



is obvious that the phytoplankton bloom has a great influence on the DOC/DON ratio. During the peak, the ratio increased sharply, due to the uptake of DON, possibly by phytoplankton. After the bloom crash, the ratio decreased rapidly to values significantly lower than the pre-bloom values (about 6 lower at both depths), due to the release of DOM by the bloom phytoplankton. The DOM released by phytoplankton must have been much richer in nitrogen than the bulk DOM in seawater. This DOM would be more labile to bacterial utilization, i.e. a better food for bacteria.

6.4 Conclusion

The results of the two spring bloom time series studies demonstrated the dramatic temporal variations in the concentration of organic matter in seawater. These variations were found to be correlated to the physical, chemical and especially biological parameters in the sea.

The significant increase of DOC in seawater following the spring blooms, especially the bloom in the Northwest Arm, is consistent with the results from laboratory cultures (Chapter 4), demonstrating that extracellular DOC released from exudation, autolysis, decomposition, and zooplankton sloppy feeding of phytoplankton itself was an important source of DOC in seawater. DOM from this source

significantly changed the composition of DOM in seawater, resulting in an enrichment of organic nitrogen.

In Bedford Basin, however, the terrestrial organic material input from the Sackville River was also a important source of DOC in the surface water. The relative contributions from the two sources were variable with time and dependent on many environmental factors.

Chapter 7

An HTCO Method for the Determination of Marine DON

7.1 Introduction

Since the publication of the paper of Suzuki et al. (1985) no one has as yet confirmed their high DON values reported. Rather, a direct comparison between a novel high temperature combustion method (ANTEK, 1100 °C, no catalyst) with chemiluminescence detection, and the historical UV photo-oxidation method demonstrated a close agreement (Walsh, 1989). Also, a direct comparison between the HTCO method of Suzuki et al. (1985) and the persulfate oxidation method found no real difference (Maita and Yanada, 1990). The intercalibration exercise during the Seattle Workshop (Hopkinson et al., 1992) did not indicate that HTC or HTCO methods yield higher values than classical UV or persulfate techniques. Furthermore, no high dissolved organic phosphorus (DOP) values corresponding to the high DON were found by a new high temperature combustion technique (Karl and Tien, 1992), and the consistency of the TDN:TDP (total dissolved phosphorus) ratio in the oligotrophic Pacific Ocean was found to be in good agreement with conventional models of nutrient recycling

(Karl et al., 1992), supporting the validity of historical analyses of both TDN (and consequently DON) and TDP (and consequently DOP). Therefore, there appears to be little compelling evidence in support of the high (>30 μM) DON numbers reported by Suzuki et al (1985).

Since only one group of researchers (Maita and Yanada, 1990) has used and tested the same method as that of Suzuki et al., it is useful to duplicate their method and test its validity. Their method must be examined for possible interferences, and tested with culture medium in which we are able to maintain a nitrogen budget. Since few time-series measurements of both DOC and DON for natural seawaters have been made by HTCO methods at the same time and location, it is useful to make such measurements in order to study the organic cycle and nutrient regeneration in natural seawater.

In this chapter, a duplication and verification of the HTCO DON method described by Suzuki et al. (1985) is presented. The test of the HTCO DON method using culture medium with known nitrogen content has still to be done.

7.2 HTCO DON Methods

Since the DON concentration is defined as the difference between TDN and DIN, the determination of DON involves measurements of both TDN and DIN.

7.2.1 HTCO Measurement of TDN

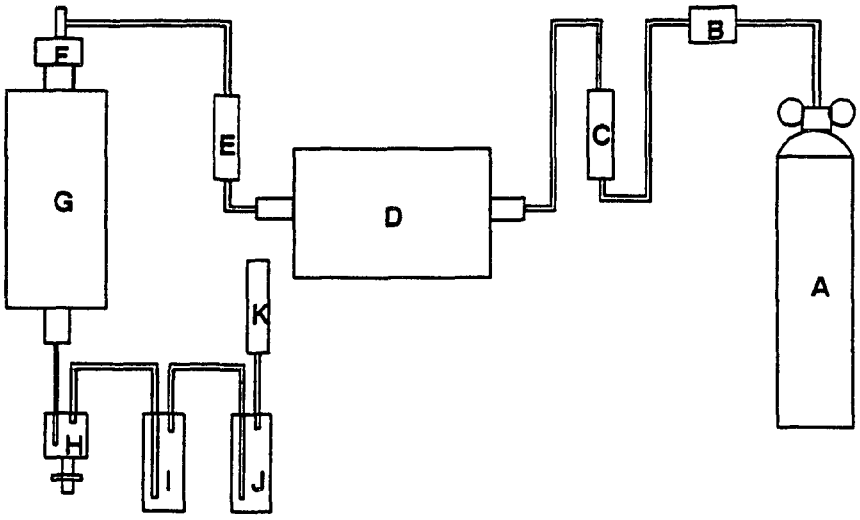
7.2.1.1 Instrumentation

In principle, the measurement of TDN was performed using the HTCO method described by Suzuki et al. (1985). The combustion apparatus of the HTCO DOC analyzer described in Chapter 2 of this thesis and a spectrophotometer (LKB Biochrom, Ultrospec 4050) with a micro-cell of 2-cm light path were used throughout this study. A schematic diagram of the instrument is shown in Fig. 7.1.

7.2.1.2 Reagents

All the chemicals used in this study are of reagent grade. The acid permanganate solution is prepared by mixing 5 ml each of 5% $KMnO_4$ in Super-Q water and 5% H_2SO_4 solution. The chromogenic reagent is prepared by dissolving 50 mg of N-(1-naphthyl)-ethylenediamine dihydrochloric acid, 4 g of sulfanilic acid and 20 g of sodium chloride in 700 ml Super-Q water, and mixing with 50 ml of acetic acid; the volume of the mixture is then adjusted to 1 L with Super-Q water.

Fig. 7.1 HTCO total dissolved nitrogen analyzer



A. Oxygen bottle and regulator, B. Needle valve, C. Tank flowmeter

D. Pre-burner furnace, E. Drierite and Ascarite column

F. Sample injection port, G. Combustion tube and furnace

H. Water trap, I. Absorber, permanganate solution

J. Absorber, chromogenic solution, K. flowmeter

7.2.1.3 Procedure

A 200-μl sample was injected by means of a micro-syringe (Hamilton, Gas-tight, 250 μl) into the oxidation column at 710 °C in an oxygen atmosphere. The flow rate of the organic-free oxygen is 200 ml/min. The generated gas is introduced by a gas dispersion tube (ACE 9435-08, pore size 25-50 μm) into an acid permanganate solution to transform the nitrogen oxides into nitrogen dioxide. The nitrogen dioxide is subsequently absorbed in 5 ml of the chromogenic reagent in a glass tube (25 mm diameter and 150 mm long) through another gas dispersion tube (the same type as above, Pyrex no. 9826). The determination of nitrogen dioxide is carried out by the measurement of the absorption of light at 545 nm in a 2-cm glass cell against that of the reagent blank as reference. The reaction time is 3 min per injection.

The system was also run exactly under Suzuki's operating conditions. The results obtained were compared with those obtained by the above operating conditions. The results of this comparison are shown in Table 7.1. The total blanks obtained and the system blanks determined by no-injection runs were both essentially identical, 3.0 and 2.8 μM, and 0 and 0.2 μM respectively. The TDN and DON values of seawater from the Bedford Basin determined by both conditions were comparable. They are all in the range

Table 7.1

Comparison between operating conditions of the TDN System.

Operating Conditions	This work	Suzuki et al. (1985)
Temperature (°C)	710	680
Sample volume (μl)	200	200
Oxygen flow rate (ml/m)	200	300
Reaction time (m)	3.0	4.0
Total blank (μM)	3.0	2.8
System blank (μM)	1.3	1.1
Water blank (μM)	1.7	1.7
System blank determined by no-injection run (μM)	0	0.2
TDN and DON value obtained (μM)		
Seawater from Bedford Basin		
#1	13.4 (10.0)*	13.4 (10.0)
#2	15.4 (12.5)	13.9 (11.0)
Scallop culture medium		
#1	37.5 (14.5)	32.5 (9.5)
#2	33.2 (14.9)	32.1 (13.8)

*Numbers in the ( ) are DON values.

of previously accepted values, although values obtained by using Suzuki's operating parameters were 0-13% lower than values obtained by using our operating parameters. These results suggest the importance of the oxygen flow rate. Too high a flow rate may reduce the efficiency of oxidation, transformation and absorption of the system.

7.2.1.4 Efficiency of the TDN Method

The oxidation efficiency of the combustion system for organic matter was tested in Chapter 2 this volume, and has proved to have a complete recovery for most organic compounds, including some refractory ones (see Table 2.2).

The transformation efficiency of the gaseous nitrogen compounds into nitrogen dioxide by the acid permanganate solution through the gas dispersion tube was tested by a second dispersion cell with acid permanganate solution. No extra nitrogen dioxide was found, indicating that the transformation was complete in one dispersion cell.

The absorption efficiency of the nitrogen dioxide by the chromogenic solution was checked using a second absorption tube. No absorption of the light was detected against that of the reagent blank as reference, demonstrating that the absorption was complete in one absorption tube.

The results of the determination of recoveries of the reference compounds are shown in Table 7.2. A 100% recovery was obtained for all the nitrogen compounds used, including the recalcitrant ones.

The accuracy of this method was tested by recovery of commonly cited reference and recalcitrant nitrogen compounds.

7.2.1.5 Calibration of the Method

Urea in 3 % NaCl solution (in Wonder Water) was used as the standard solution. The system was calibrated through use of this standard solution. A regression formula for standards from a specific run was used for calculation of nitrogen in samples from the same run. With few exceptions, all regressions for standards from 7.14 to 42.84 μM N were linear with correlation coefficients of 0.995 or higher.

7.2.1.6 Blank Determination

The total blank of the system was determined by the intercepts of the calibration lines. It was in the range of 3.0-7.0 μM. This total blank is composed of two parts: the instrumental blank and the water blank, in this case, the nitrogen content of the Wonder Water. The water blank, in

Table 7.2

Quantitative recovery of common cited reference and recalcitrant nitrogen compounds dissolved in 3% NaCl solution.

Compounds	Added (μM)	Found (μM)	Recovery (%)
Sodium nitrate	10	10.2	102.0
	40	40.2	100.5
Urea	10	10.1	101.0
	40	40.0	100.0
Thiourea	10	9.9	99.0
	40	40.4	101.0
Glycine	10	10.0	100.0
	40	40.2	100.5
Caffeine	10	10.2	102.0
	40	39.8	99.5
Antipyrine	10	9.9	99.0
	40	40.5	101.3
Sulfathiazole	10	10.1	101.0
	20	20.0	100.0
	40	39.4	98.5

turn, also consists of two components: DON and DIN. Since the DOC content of the Wonder Water is essentially zero, as demonstrated in Chapter 2, the DON content of the Wonder Water must be essentially zero, as well. The water blank of the Wonder Water thus must come entirely from its DIN content. The DIN content of the Wonder Water was determined indirectly. Wonder Water was passed through an ion retardation column to remove DIN (Bronk and Glibert, 1991). The eluate of the column was then run by the TDN system. The difference between the original Wonder Water and the eluate was thus used to determine the DIN concentration of the Wonder Water. Duplicate determinations showed that the DIN concentration of the Wonder Water is 1.7 μM. This is normally about one quarter to one half of the total blank and should not be subtracted from the sample values. The system blank, i.e. the machine blank is thus in the range of 1-5 μM.

The method of determining the system blank used by Suzuki et al (1985) was wrong. Their system blank was determined by using the same absorption reagents and analytical conditions, but without a sample injection. This is wrong because the system blank is very much sample volume dependent: the bigger the sample injected, the larger the system blank. The system blank of Suzuki et al

must have been underestimated, which in turn resulted in the overestimation of the sample values. The system blank of our TDN system as determined by Suzuki's method was essentially zero. The overestimation of the water blank by using a zero system blank will thus be about 2-5 μM. The overestimation of the TDN values for the samples will be 10-100%. Though this is a significant overestimate, it will not give values as high as those of Suzuki et al. (1985). The system blank of Suzuki's system might be much higher than our system blank (1-5 μM). If this were so, the overestimation of the TDN values for the samples must be much more significant. This might be a major reason for their very high TDN and DON numbers. We do not know what might have caused so high a system blank.

7.2.2 Measurement of DIN

Inorganic nitrogenous compounds, nitrate-, nitrite-, and ammonium-N, were determined by the standard oceanographic methods (Grasshoff, 1983).

7.2.3 Precision and accuracy of the DON method

The precision of the TDN method was determined to be better than 2% (Table 7.3). The analytical error for the

Table 7.3

Precision of the TDN method.

Samples	TDN (μM)
Thiourea	29.5
	28.5
	29.0
	29.0
average:	29 ± 0.4
Seawater from Bedford Basin	13.5
	12.1
	12.1
	13.0
	12.6
average:	12.7 ± 0.3

TDN measurement was estimated to be less than 2%, and because the DON is obtained by the difference between TDN and DIN, the analytical error for DON is estimated to be less than 5%, depending on the values of TDN and DIN and their relative size. For most of the DON values obtained the analytical error was about 5%.

7.3 Conclusions

The HTCO method for TDN measurement used by Suzuki et al (1985) was duplicated and shown to be accurate, precise, and reliable. The TDN and DON values obtained (Table 7.2 and Chapter 8) were consistent with those obtained by persulfate and UV oxidation methods and the HTCO methods used by others, except that used by Suzuki. Although we do not know exactly the reason for the overestimation, the underestimation of their system blank might be an important factor in causing the overestimation of their sample values.

Chapter 8

Results of DON Measurements

8.1 Introduction

Measurement of dissolved organic nitrogen is important in the examination of numerous oceanographic problems, including examinations of the role of DON in oceanic nitrogen cycling, the role of DON in surface water nitrogen dynamics, the importance of benthic fluxes of DON in sedimentary nitrogen cycles (i.e., denitrification vs. benthic fluxes), and the role of these fluxes as sources of DON to the oceans (Hopkinson et al., 1992).

The TDN and DON results measured by the HTCO method described in Chapter 7 are presented in this chapter. These results include a time-series study of DON in seawater during a spring phytoplankton bloom in Bedford Basin, Nova Scotia, a study of the excretion of dissolved nitrogen, both organic and inorganic, by sea scallops, and TDN measurements of seawater samples collected from a variety of environments.

8.2 Methods

8.2.1 Time-series Studies of DON in Seawater during a Spring Phytoplankton Bloom in Bedford Basin

The sampling method for this project is described in Section 6.2.2 in chapter 6. TDN was measured by the HTCO method described in Chapter 7. DON was calculated from the TDN data determined and the DIN data provided by BIO. The chlorophyll a data for the water sampled were also provided by BIO.

8.2.2 Excretion of DIN and DON by Sea Scallops

This experiment was carried out by Peter Cranford at BIO. Scallops (100 mm shell height) were placed in sealed 1 L containers and incubated for 20-60 min, depending on water temperature. Seawater in each chamber was mixed by a magnetic stirrer and temperature was regulated by a water bath. Filtered (5 μm) seawater containing 4 mg L^{-1} dried microalgae (Tetraselmis suecia) was used for all incubations. Four scallops were incubated at each temperature (6, 10, 12, 15, and 18 °C). Water samples were collected at the start and the end of each incubation. Samples for DON and DOC were filtered by the procedure described above. A control incubation, using empty scallop

shells and the same seawater, was conducted at 14 °C to account for any changes in nitrogen concentration not caused by the scallop. Nitrate plus nitrite was run by J. Anning and ammonia by P. Cranford at BIO.

8.2 3 Samples from Different Environments

Samples were collected by Drs. B. Hargrave and P. Keizer at BIO from Limekiln Bay at a salmon farm, Letang Estuary, and Bay of Fundy Shore on January 23, 1992. Two samples were also taken from the water tank and water incubated with sea scallops at the Bedford Institute of Oceanography. Samples were all filtered by precleaned GF/F filters and stored in a refrigerator for five days before analysis. DON and DOC were run by HTCO methods (Chapter 2 and 7). Ammonia was run by G. Phillips at BIO.

8.3 Results and Discussion

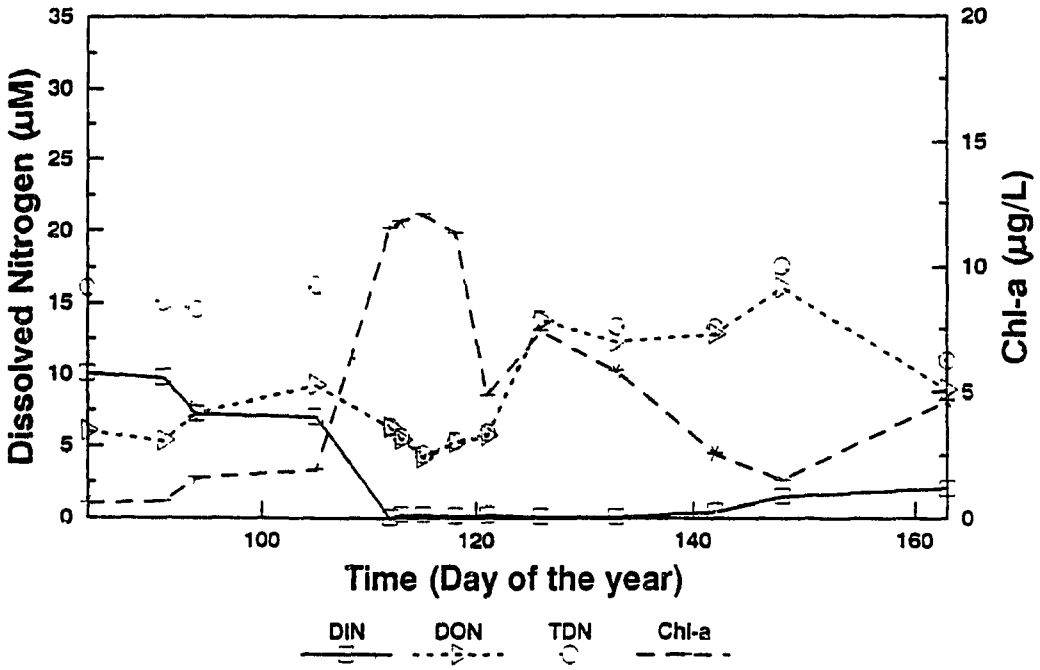
8.3.1 Time-series Studies of DON in Seawater during a Spring Phytoplankton Bloom in Bedford Basin

The data collected from this bloom project are summarized in Table A.1 in the Appendix of this volume. Data discussed here are from depths of 0.5 and 5.0 m during the time period from Day 84 to 163.

The data of chlorophyll and DIN shown in Figs. 8.1 and 8.2 indicate that the bloom had a single sharp peak and lasted for about two weeks, close to the classical concept of a spring bloom. The chlorophyll values started to increase rapidly at April 14 (Day 105), accompanied by a sharp drop in DIN. The peak values of chlorophyll coincided with the DIN minimum. After reaching the peak, the chlorophyll value dropped rapidly, possibly due to the processes of sinking and removal of the phytoplankton cells from the surface water, mixing of surface water with deeper water, and the fresh water input from the runoff of the river. After the bloom crashed, DIN remained essentially nil for about 30 days, and then began to recover slightly. At the end of this observation (Day 163, 50 days after peak), DIN values at both depths were still much lower than pre-bloom values. DIN from water mixing and regeneration of DON was taken up by phytoplankton during this time.

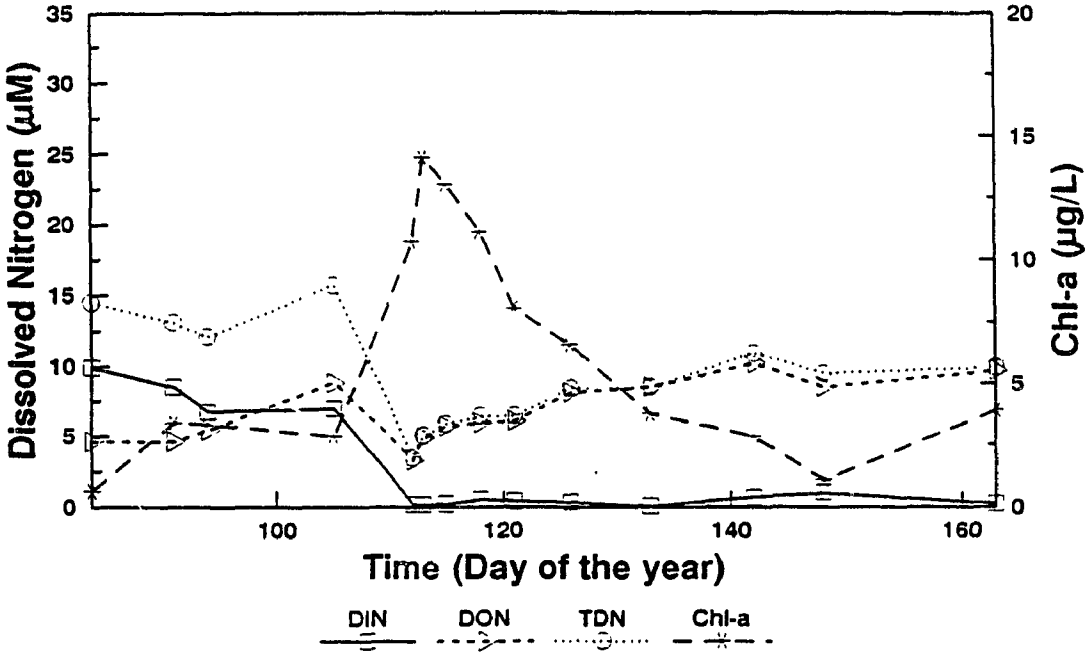
The TDN values remained fairly constant before the bloom started, dropped sharply due to the uptake of dissolved nitrogen, both DIN and DON, by phytoplankton and recovered to a value somewhat lower than prebloom values within 1 to 3 weeks after the bloom crashed. The composition of the TDN after the bloom differed greatly from that before the bloom. Before the bloom, DIN and DON

Fig. 8.1 Dissolved nitrogen and chl-a in Bedford Basin during the 1992 spring bloom



0 5 m depth

Fig. 8.2 Dissolved nitrigen and chl-a in Bedford Basin during the 1992 spring bloom



5 m depth

values were comparable; however, after the bloom DON values dominated over DIN values, with more than 90% of the TDN composed of organic species.

Before the crash of the bloom, DON variations at both 0.5 and 5 m showed a similar trend. The DON concentrations increased significantly before the bloom started, from 5.3 to 9.2 μM and 4.6 to 8.8 μM for samples for 0.5 and 5 m respectively. This was probably due to the input of organic matter from the runoff of the river since there was a corresponding sharp drop in salinity. During the phytoplankton peak period DON values dropped to the pre-bloom values, possibly due to the uptake of labile DON by phytoplankton in bloom, and recovered slightly during the peak time.

After the bloom crashed, DON concentrations increased continuously at both depths, but with different patterns. At 0.5 m after the crash (Between Day 121 to 126), there was a sharp increase in DON, ca. 8 μM in absolute value and 150% in relative value. This was consistent with DOC, which showed about a 45 μM increase in the DOC absolute value and about a 40% increase in relative value after the bloom crashed (Fig. 6.3 in Chapter 6). Since these DON and DOC increases did not coincide with a fresh water pulse (low in salinity), the DON and DOC were probably released

by the decomposing phytoplankton cells. After Day 126 DON remained relative constant, with a high value till day 148. This suggested that the release of DON from phytoplankton plus the DON input from river water during this time was balanced by the consumption of DON by bacteria and by dilution caused by mixing with deeper water and offshore water. Afterwards, DON began to decrease, suggesting that DON utilization by bacteria and dilution by water mixing exceeded DON supply from phytoplankton and fresh water.

At 5 m after the crash, DON continued to increase till Day 142 with a slower rate and reached a lower maximum value, then remained relatively constant until the end of this sampling program. If we had sampled for a longer time, we would have expected to see DON values decrease, because the release of DON from phytoplankton would be suceeded by microbial utilization some time later.

It is clear from Figs. 8.1 and 8.2 that during the phytoplankton peak there was not a corresponding increase of DON which would account for the amount of decrease of DIN. During this time DIN and a relatively smaller amount of DON were taken up by the algal bloom, turning into the particulate phase of phytoplankton biomass. After the bloom crashed, a significant amount of DON, close to that of the DIN taken up by phytoplankton during the peak time, was

released by the decomposing phytoplankton cells. DON concentration in Bedford Basin could be changed significantly by both river runoff and *in situ* phytoplankton production.

8.3.2 Excretion of DIN and DON by sea scallops

The results of this experiment are summarized in Table 8.1. As expected, there was no change in nitrate concentration during the incubation. Ammonia and DON were excreted by the scallops, with ammonia accounting for approximately 80% (s.d. = 16%) of the total nitrogen excreted. This is similar to published results for the mussel (*Mytilus edulis*, Bayne, 1976). Excretion of DON was correlated to water temperature (Fig. 8.3, r = 0.80) and scallop respiration rates (Fig. 8.4, r = 0.69).

These data will be useful for studies on scallop energy and nitrogen budgets (Cranford, personal communication, 1992).

8.3.3 Samples from Different Environments

The results of these analyses are shown in Table 8.2. Data for samples from Bedford Basin (Feb. 4, 1992) are included for comparison.

Table 8.1

Results of the scallop excretion experiment.

				Dissolved Nitrogen (µM)					Excretion (µM/h)		
temp (C)	length (mm)	time (h)	DOC	NO_3+NO_2	NH_4	Total	DON	DON Diff	DON	NH_4	NH_4 (%)
12	start		119.6	1.24	3.65	13.47	8.58				
	102	0.33	115.8	1.37	12.06	23.61	10.22	1.64	4.92	25.26	90.0
	100	0.40	140.8	1.27	6.82	21.27	13.19	4.60	11.51	7.93	45.7
	103	0.46	115.8	1.23	14.20	30.64	15.21	6.62	14.18	22.59	65.1
	103	0.51	346.7	1.21	13.27	28.69	14.21	5.63	10.88	18.61	67.9
6	start		115.8	1.24	2.32	12.69	9.13				
	103	0.66	237.5	1.54	5.97	15.03	7.52	-1.61	-2.41	5.47	100.
	102	0.73	249.2	1.05	5.54	17.76	11.17	2.04	2.78	4.39	86.9
	103	0.95	207.5	0.98	8.36	19.71	10.37	1.24	1.29	6.32	100.
	101	1.03	275.8	1.07	11.52	22.05	9.47	0.34	0.32	8.91	100.
10	start		178.3	1.11	2.79	13.08	9.18				
	102	0.41	105.0	1.17	12.53	24.00	10.30	1.12	2.69	23.36	97.6
	103	0.51	132.5	1.19	9.98	23.22	12.06	2.87	5.56	13.91	80.1
	102	0.60	112.5	1.16	11.27	23.22	10.79	1.61	2.68	14.13	96.1
	101	0.66	110.8	1.14	17.38	28.69	10.17	0.98	1.48	21.87	100.
15	start		112.8	1.17	3.31	12.28	7.80				
	102	0.30	118.3	1.21	8.22	19.65	10.22	2.42	8.07	16.37	73.3
	103	0.38	125.0	1.21	19.76	35.16	14.19	6.39	16.69	42.95	74.6
	102	0.46	113.8	1.33	13.99	26.24	10.92	3.13	6.69	22.87	83.3
	101	0.51	128.3	1.23	20.36	33.22	11.64	3.84	7.43	32.98	86.1
18	start		128.3	0.94	6.14	19.26	9.00				
	104	0.25	118.3	0.94	12.38	25.05	11.73	2.73	10.91	24.96	73.9
	103	0.30	126.7	1.06	13.71	31.67	16.90	7.90	26.33	25.23	51.0
	106	0.38	261.7	0.95	22.04	37.49	14.50	5.50	14.36	41.51	77.2
	102	0.43	385.8	0.99	17.32	33.22	14.91	5.91	13.65	25.82	69.1
14	start		143.3	0.90	2.60	13.44	9.95				
Control	104	0.50	182.9	0.94	1.96	15.38	12.48	2.54	5.08	-1.28	
(shells)	103	0.66	121.7	0.90	2.88	15.77	11.99	2.04	3.06	0.42	
	102	0.83	110.5	0.91	2.53	13.05	9.61	-0.33	-0.40	-0.08	
	102	1.00	113.8	0.99	2.56	14.22	10.67	0.73	0.73	-0.04	

Samples collected by P. Cranford. DOC and DN run by W. Chen (Dalhousie). Nitrate run by J. Anning and ammonia by P. Cranford.

Fig. 8.3 Correlation between excretion of DON and water temperature

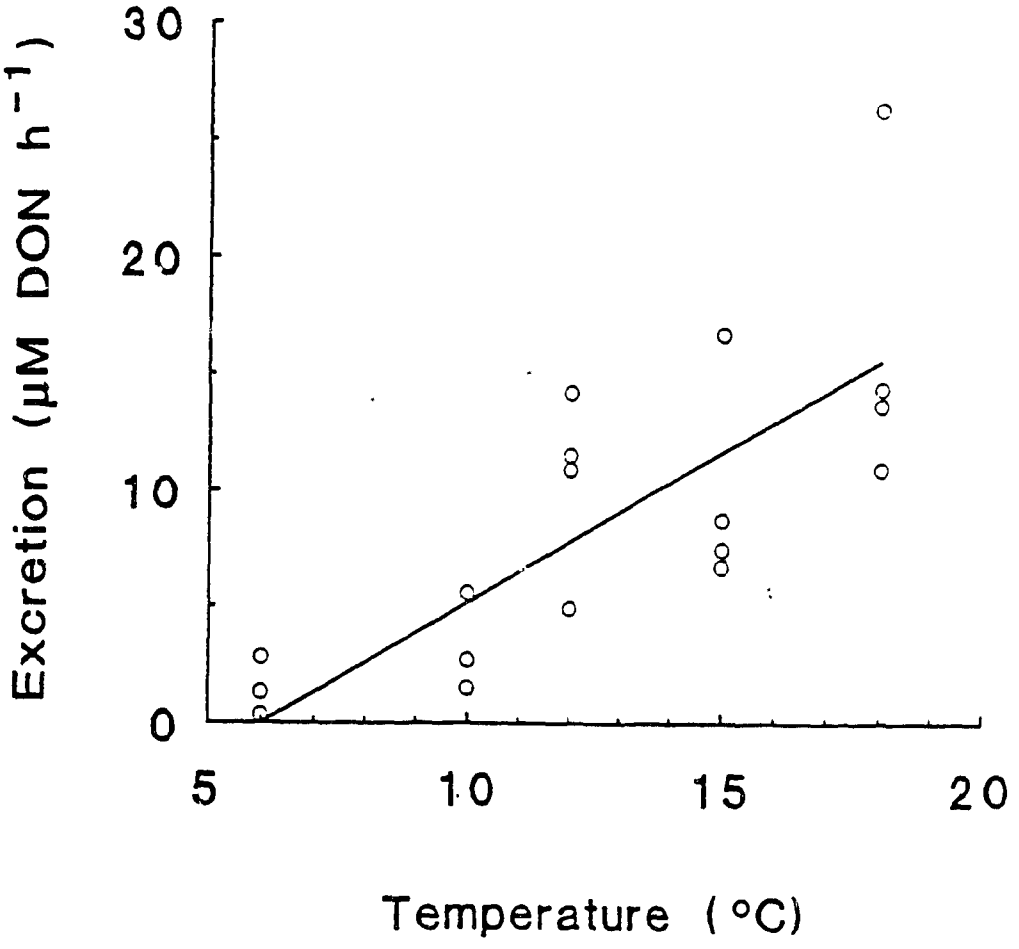


Fig. 8.4 Correlation between excretion of DON and scallop respiration rates

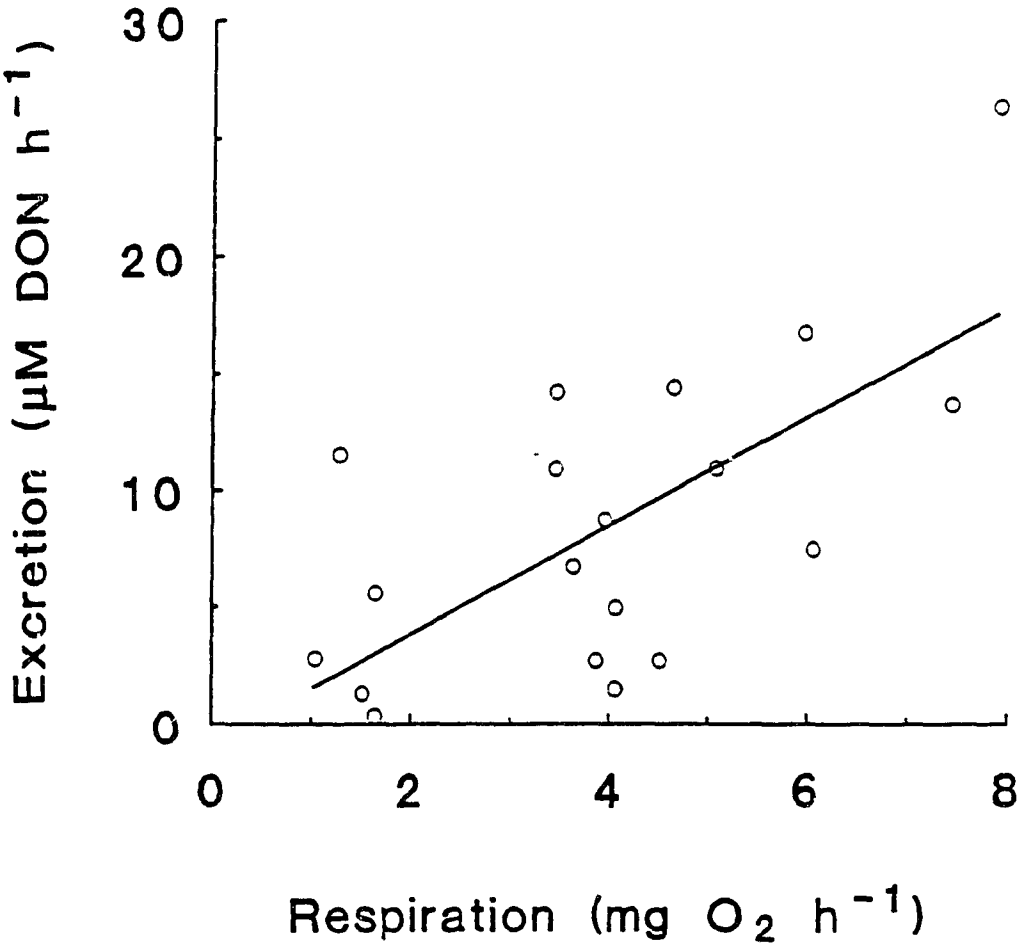


Table 8.2

Seawater ananlysis for DOC, TDN and ammonia.

Samples		DOC (μM)	TDN (μM)	ammonia (μM)
23-01-1992				
Limekiln Bay, New Brunswick				
Within salmon cage		119.9	16.2	2.12
At cage edge		109.1	16.2	1.82
50 m away from cage		101.8	24.7	0.70
23-01-1992				
Letang Estuary, N.B.				
Causeway		3800.0	98.8	4.07
Black harbor		587.5	115.6	9.95
23-01-1992				
Bay of Fundy				
Pocalogan Beach, N.B.		118.5	18.3	0.43
23-01-1992				
Bedford Institute of Oceanography				
Seawater (tank)		116.3	20.5	1.20
Scallop expt.		122.3	20.7	5.65
04-02-1992				
Bedford Basin, Nova Scotia				
DREA barge	0.5 m	120.2	21.1	1.43
	5 m	112.9	17.6	1.39
	10 m	99.3	21.3	1.53
	15 m	106.7	28.5	1.37
	20 m	96.8	17.6	1.19
	25 m	104.2	17.8	1.13
	30 m	107.9	20.5	1.47

The samples from the salmon cage site show the expected gradient for DOC and ammonia, though the size of the gradients are very different. Ammonia in the cage is three times as high as outside the cage, while DOC in the cage is only about 20% higher than outside. However, total dissolved nitrogen has a reverse gradient: the value in the cage is about 30% lower than value outside the cage. These results are difficult to explain since data for nitrate plus nitrite and other relative data are not available. The answer may be sought in the differences of bacterial activity and mixing of the waters.

For the samples from BIO, while ammonia concentration shows a great increase of 370%, DOC shows a minor increase of 5% and TDN 1%, which is within the analytical error of the method. As shown in the above section scallops excrete dissolved nitrogen, with ammonia accounting for 80% of the excretion. The total excretion of dissolved nitrogen would be about 5.5 μM. Where has this nitrogen gone? The suggestion again is the microbial activity in the sample: bacteria and/or algae used as food for scallops can take up dissolved nitrogen, both organic and inorganic.

The TDN values in Limekiln Bay (24.7 μM), Bay of Fundy at Pocaogan Beach (18.3 μM) and Bedford Basin (about 20 μM) are all similar. The results for the two samples from

L'etang Estuary are both very extraordinary because the environments are unusual. The water in the causeway contains the waste drained from a big paper mill, and Black Harbor is heavily contaminated by a large fish processing plant during the fishing season. Both samples show similar high TDN numbers (about 100 μM), but differ greatly in DOC numbers, 3800 and 587.5 μM for samples from the causeway and Black Harbor respectively. The C/N ratio for the two samples are 38 and 5 respectively. This difference in C/N ratio correctly reflects the difference in the composition of the contaminants from the two sources.

8.4 Conclusion

The TDN and DON values for normal seawater determined in this study are in the range of 3.4-34.3 and 2.1-12.8 μM respectively. All of these are well within the range of previous values determined by persulfate oxidation or UV photo-oxidation methods. It is obvious that we cannot reproduce the very high DON values found by Suzuki et al. (1985) in the North Pacific.

DIN was found to remain essentially depleted for 3 weeks after the bloom crashed and then recovered slightly. TDN values before and after bloom were comparable; however,

the composition was very different. After the bloom DON was the dominant species of dissolved nitrogen.

DON was found to be taken up by phytoplankton during the bloom development phase and then released by the senescent and decomposing cells after the bloom crashed. DON values were much higher after the bloom than before the bloom. Both fresh water input and *in situ* production from phytoplankton could be an important source of DON in Bedford basin.

The sea scallop excretes dissolved nitrogen, 80% as ammonia and 20% as DON. The excretion of DON is positively correlated to the seawater temperature and respiration rate of the scallop.

Chapter 9

Principle Conclusions and Areas for Further Research

9.1 Introduction

DOM in the oceans contains a total mass of carbon comparable to that in atmospheric CO_2 and accounts for most of the fixed nitrogen in surface ocean water. It has been shown that labile components of DOM support a "microbial loop" within which bacteria utilize a major portion of total carbon production at the sea surface. DOM has thus been increasingly recognized as a key and quantitively significant component in the global cycles of carbon and nitrogen.

Due to the structural complexity of DOM and great amount of sea salt in seawater, measurement of DOC and DON has long been difficult and full of controversy. No "referee method" was widely accepted. Although the HTCO methods showed higher oxidation efficiency and gave higher results than the more traditional wet chemical methods, they were more prone to contamination and suffered from a high and irregular blank.

In this study an intensive attempt was made to develop a HTCO method which is accurate, precise and reliable, for

both DOC and DON measurement. This method was compared with the more conventional UV photo-oxidation method which was reported to underestimate the DOC value in seawater significantly.

The second line of research in this study was directed to the study of the sources, sinks and lability of DOM in seawater. Both laboratory culture work and field observations were carried out.

9.2 Summary and Principle Conclusions

9.2.1 The HTCO DOC Method and its Comparison with the UV method

Major modifications were made to the HTCO system of Sharp (1973). A wider vertical combustion tube was used instead of a horizontal one. A combination of Pt and Co catalysts used in the combustion chamber ensure a high oxidation efficiency and less sensitivity to heavy metal poisoning. The replacement of the silicone stopper by the Swagelok nut and Teflon ferrules, the scrubbers used in line, and the lower furnace temperature used, 710 °C rather than 950 °C, all made contributions to lower the system blank significantly. The replacement of the in-line valve sampler by a micro-syringe needle injection and an in-line

check valve before the injection port made the system much easier to use and more reliable, and greatly increased its precision.

The modified HTCO method developed in this study for marine dissolved organic carbon measurement was shown to have a high oxidation efficiency and precision. Components of the system blank were determined satisfactorily by the use of deionized water treated by high temperature catalytic oxidation. The results were checked and confirmed by an experiment using ^{14}C.

Although most organic compounds tested by various researchers are fully recovered by the UV photo-oxidation, some nitrogen- and/or sulfur-containing compounds are resistant to UV oxidation. No clear indications of a connection between structure and ease of oxidation by UV has been found.

An extensive comparison between measurements using HTCO and UV methods was carried out. The results of this comparison showed that the HTCO method found 5-60% more DOC in seawater than did the UV method. Most values were in the range of 10-40% more, but not as high as several times more, as Sugimura and Suzuki (1988) found. No simple trends were found which might explain the difference between the two methods. This difference might depend on many factors

such as the source of the sample, the time when the sample was taken, methods of sample handling and storage, and the time between sampling and analysis.

9.2.2 HTCO DON Method and Results

The HTCO method for TDN measurement used by Suzuki et al (1985) was duplicated and found to be accurate and precise, and reliable to perform. The TDN (3.4-34.3 μM) and DON (2.1-12.8 μM) values obtained for seawater samples in this study were consistent with those obtained by persulfate and UV oxidation methods and the HTCO methods used by others. The very high DON values (>40 μM) found by Suzuki et al. (1985) in the North Pacific could not be reproduced. Although we do not know exactly the reason for the overestimation, the underestimation of their system blank might be an important factor in the overestimation of their sample values.

An excretion experiment with sea scallops was performed. DON and DIN were monitored. Sea scallops were found to excrete dissolved nitrogen, 80% in ammonium and 20% in DON. The excretion of DON is positively correlated to seawater temperature and respiration rate of scallops.

9.2.3 DOM Sample Preservation Method

Sample preservation is of critical importance because of the potential for both short- and long-term (hours to months) changes in DOC and DON following sample collection.

Six methods for DOC preservation were tested. Poisoning by $HgCl_2$ and acidifying with HCl and storage at cold temperature was demonstrated to be the most convenient and practical methods. However, for the analyses of DOC and DON by the HTCO method, the latter is preferred because of possible poisoning effects of Hg on the catalyst. Acidification and storage in the cold were found to be satisfactory for both short- and long-term preservation of DOC samples containing highly labile organics.

9.2.4 DOC Sources from Phytoplankton

Variations in dissolved organic carbon (DOC) in cultures of the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum, the flagellate Isochrysis galbana, the dinoflagellate Alexandrium tamarense, and a natural algal assemblage from the Northwest Arm, Nova Scotia Canada were followed using a high temperature catalytic oxidation method (HTCO) and an ultra-violet (UV) photo-oxidation method. Molecular weight fractionation was

performed for two cultures, C. gracilis and I. galbana. The shapes of the DOC curves obtained and the differences between the two methods were discussed along with the results of the molecular weight fractionation to elucidate the composition and nature of the DOC released by the cultures.

The amount, composition and nature of the DOC released to the solution were found to be different with algal species, physiological state and bacterial activity in cultures. In the cultures of diatoms (i. e. Chaetoceros gracilis, Phaeodactylum tricornutum and the natural algal assembly in the Northwest Arm in which diatoms were found to be the dominant species in the blooming phase) DOC displayed little or no change in the lag phase, and increased significantly during the exponential phase. Maximum releases took place during senescent and decomposing phases. The flagellate Isochrysis galbana followed a similar pattern of DOC release; however, the composition and nature of the DOC differed greatly from that of the diatom C. gracilis. The dinoflagellate A. tamarense showed a very different pattern of DOC release. DOC in the cultures decreased continuously until the crash, and increased only after. The decrease of DOC before the crash and the lack of bacterial activity in the cultures suggest heterotrophic activity in this species.

The HTCO DOC values were generally 10-100% higher than the UV values for cultures. The UV method was found generally less efficient for high MW organics than low MW compounds. The actual difference between the two methods for a specific culture is related to the background DOC in the culture base used, the species and physiological state of the phytoplankton, and the activity of the bacteria in the media, since the difference between the two methods is dependent on the nature and composition of the DOC being measured.

9.2.5 Lability of DOC Released by Phytoplankton

DOC decay was followed for samples from cultures of the diatoms Chaetoceros gracilis and P. tricornutum, the flagellate I. galbana, the dinoflagellate A. tamarense and a natural algal assemblage from the Northwest Arm, Nova Scotia, and from seawater of the Arm, using the HTCO method. Decay rate constants were determined using first-order reaction kinetics in the multi-G model. Decay rates as high as 0.49 day^{-1} were obtained, which demonstrated that DOC released by phytoplankton might be highly labile to bacterial utilization and degraded significantly within hours. The decay of DOC was found to be temperature dependent.

DOC release by phytoplankton differed greatly in biolability, according to the species and the physiological state of the algae. DOC released by the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum was very labile and decayed rapidly. DOC from the flagellate Isochrysis galbana showed much lower lability, and that from the dinoflagellate Alexandrium tamarense was essentially bacteria-resistant.

The high lability of the DOC released by the diatoms emphasizes the importance of sample processing and preservation for DOC measurement in seawater. Samples for DOM analysis must be processed and preserved as soon as possible after samples are taken.

9. 2. 6 Temporal Variations of DOM in Coastal Waters of Nova Scotia

The spring phytoplankton bloom is the most intense phytoplankton activity in the marine environment and usually lasts for only a few weeks. It is thus a special opportunity to study the dynamics of organic production by phytoplankton in the sea. Two time series studies were carried out in the Northwest Arm in 1991 and in Bedford Basin in 1992 (Chapter 6 and 8). In both observations, there were noticeable increases in the concentration of DOC

and DON during the course of the spring bloom development. This is in agreement with the results of batch culture experiments which showed that the production and accumulation of organic matter started in the exponential growth phase. As in the batch cultures, the maximum of DOC in Northwest Arm was reached about a week after the chlorophyll maximum. The significant correlations among DOC and chlorophyll demonstrate that the source of the increase in dissolved organic matter concentration is due to the in situ production by the bloom phytoplankton, rather than other sources such as fresh water runoff.

In Bedford Basin, however, there was an apparent correlation between the DOC peaks and the salinity lows. This correlation suggest that DOM input from the Sackville River is also an important source of DOC and DON in the surface water of the basin. The relative contribution of the two sources is variable with time and dependent on many environmental conditions.

9.2.7 Summary comments

In summary, my HTCO results do not support the very high DOC values reported by Sugimura and Suzuki (1988), but are still significantly higher than the UV oxidation results (Chapter 3). These results are in agreement with

recent published results (Cauwet et al., 1990; Ogawa and Ogura, 1992; Kepkay and Wells, 1992). My HTCO results (Chapter 7 and 8) also do not provide evidence to support the very high DON values of Suzuki et al. (1985). Recent comparisons made by Walsh (1989) and Maita and Yanada (1990) showed that HTCO and HTC methods found essentially the same values of DON in seawater as UV photo-oxidation and persulfate oxidation methods did. In addition, the DOP concentrations in seawater were found to be not significantly higher than previously measured with standard methods (Ridal and Moore, 1990; Karl et al., 1992).

Thus, the current situation in DOM measurements is that the HTCO methods find some more DOC, but essentially no more DON and DOP in seawater. From this situation, it would appear that the UV and/or persulfate oxidation resistant DOM found by HTCO methods contains little N and P; compounds containing N and P do not make a significant contribution to discrepancies between HTCO and wet oxidation measurements.

Polysaccharides have been shown to be more abundant and bio-reactive components of seawater DOM than has been recognized (Benner et al., 1992). Lipids low in N content were the major components of lipids released by phytoplankton in N-stressed stage (Parrish and Wangersky,

1990). Some marine polysaccharides are shown to escape UV photo-oxidation to some extent (Chapter 3). High MW compounds are shown to be more resistant to UV photo-oxidation (Chapter 4). These results suggest that high MW compounds such as polysaccharides, lipids and other unidentified macromolecules which are low in P and N contents could be important components of the DOM missed by wet oxidation methods.

The tight correlation between DOC production and phytoplankton activities and the high decay rates observed in this study strongly suggest a diurnal variation in DOC concentration in natural seawater, although direct observation had not been done in this study. Other indirect evidence supports this suggestion. For example, Mopper and Lindroth (1982) demonstrated pronounced diel variations in dissolved free amino acids in natural seawater. Burney et al., (1979) showed strong diel variations in carbohydrates. Zhou and Wangersky (1989b) observed diurnal fluctuations in the production rate of copper-complexing ligands in the culture of the diatom P. tricornutum. If such a cycle exists, then time of day and illumination become important parameters when sampling. We must know the diurnal variability before we can estimate the importance of day-to-day variability.

In addition to this daily cycle, there is a seasonal cycle in DOM as observed in the two time-series studies of DOM in seawater (Chapter 6 and 8). Temporal variations of DOM in seawater are important phenomena. Thus, the time of sampling for DOM must be taken into consideration if we are to understand temporal variations. For instance, comparisons of samples from one location in the winter versus those from another location in the summer must include the influences of seasonality. Likewise, time-series studies at a single station must be collected at similar time of day to reduce the influence of diel cycles.

Moreover, biological information must be taken simultaneously in order to properly interpret DOM data obtained, since the composition and concentration of DOM in seawater are influenced greatly by the activities of phytoplankton and bacteria in seawater.

9.3 Areas for Further Research

Since the structural characteristics of molecules making up DOM, the molecular composition of the combustion products and the mechanism of high temperature catalytic oxidation are all poorly understood at present time, a more powerful and straightforward "referee" method, however cumbersome or tedious, would be still necessary for

unambiguously establishing actual DOC and DON concentrations in seawater, as suggested at the Seattle DOC and DON workshop (Hedges and Farrington, 1992). This method has not been established. The sealed tube combustion followed by a mass spectral analysis of the combustion products is thought to be promising.

The accuracy and precision of the DON measurement suffered greatly from the presence of an order-of-magnitude larger total dissolved ir ganic nitrogen background, especially for samples from deep ocean waters. A new technique able to quantitively remove DIN from a sample prior to the TDN analysis would allow the present TDN method to be used to directly measure DON, greatly improving both the accuracy and precision of the DON measurement (Hopkinson et al., 1992).

The accuracy of the DON and DOP methods can be tested with culture medium in which we are able to establish an N and P budget. This will require the use of a completely artificial medium based on the Wonder Water. If the total N or P, including particulate, dissolved and volatile species, found by a method is equal to the budget, this method is likely to be accurate. By contrast, if it is not equal to the known budget, the method must not be accurate. By comparison with an accurate method we will be able to

determine in which forms these elements are missed or overestimated by the method tested.

Chemiluminescence NOx detectors (such as the Antek detector) are sensitive in determining nitrogen oxides (Walsh, 1989; Hansell, 1992). A chemiluminescence detector installed in line after the CO_2-NDIR analyzer on the DOC unit will allow a simultaneous determination of DOC and TDN, using the same furnace and catalyst column. This combined system will save both time and labour, and make the DOC and DON data more consistent.

In this study samples from cultures were all taken at about the same time of the day, and all in the light period to compensate the effects of diurnal fluctuations in DOC production by phytoplankton. If samples are taken at the ends of light and dark periods, daily cycles in DOC might be observed. Polysaccharides should have the same cycle as DOC, since they are important components of the DOC, both in cultures and seawater. Therefore, we should be able to observe a daily variation in the difference between the HTCO and wet oxidation measurements, as well.

In batch culture, both the physiological states of the organism and the environmental conditions are changing rapidly and constantly so that it is difficult to relate the production of the organic matter to a particular

environmental or physiological factor. The turbidostat cage culture system used in this laboratory for many other purposes (Parrish and Wangersky, 1987; Zhou and Wangersky, 1989b; Lombardi and Wangersky, 1991) provides a stable and controllable environment and cell density and thus makes it possible to study the effect of various stresses on the production of labile DOC and DON.

In our culture experiments zooplankton were excluded. However, zooplankton are an indirect source of DOM inputs from phytoplankton: spillage from phytoplankton cells during grazing, i.e. "sloppy feeding", and zooplankton excretion. These sources of DOM were shown to be important (Eppley et al., 1981). The qualitative significance of sloppy feeding as a mechanism for DOM production probably depends on the types of algae and herbivores involved (Azam and Cho, 1987). Systematic studies to determine this have not yet been done. Carefully controlled experiments are needed. DOC and or DON monitoring of continuous cultures with and without a zooplankton grazer should provide illuminating results.

"Sloppy feeding" can also produce small organic particles or colloidal organic materials which pass through traditional filters used for DOM sample processing. Through sloppy feeding, the colloidal organic load in natural

seawater could show both diurnal and seasonal variations. UV photo-oxidation and persulfate oxidation are known to be less efficient with particulate matter. Therefore, colloidal materials could make a significant contribution to the difference between the HTCO and wet oxidation measurements. Using filters of different pore sizes, such 0.02 μm and 0.8 μm respectively, we are able to get samples with and without colloidal organic materials in them. DOC and/or DON measurements of these samples by both the HTCO and wet oxidation methods could provide information about amount of colloidal organics and the discrepancy between the methods.

It would greatly benefit the interpretation of the results obtained from the culture and decay experiments done in this study, if the abundance and activity of bacteria in the culture media and decay substrate were measured. These measurements should be performed in further experiments on DOM production in cultures and its decay by bacteria.

Sea surface films play an important role in the sea-air exchange. The composition of the surface film is quite different from that of the subsurface water and is poorly characterized. What we do know is that surface reactive organic compounds, which are non-polar compounds and

hydrophobic classes, such as hydrocarbons and lipids, are enriched in the film. Protein and particulate organic matter were also reported to be enriched in the microfilm (Williams et al., 1986). Using HTCO techniques developed in this study we are able to measure total carbon, total nitrogen, POC, PON, DOC and DON in the surface film. These measurements will provide us with important information about the composition and nature of organic matter there.

Appendix

Table A1. Dissolved nitrogen and organic carbon in Bedford Basin during the 1992 spring bloom.

Date	Depth (m)	NO2 + NO (µM)	NH4 (µM)	TDN (µM)	DON (µM)	DOC-HTCO (µM)	DOC/DON
13/01/92	0	5.83	2.46	19.7	11.41		
13/01/92	5	5.62	2.12	21.2	13.46		
13/01/92	10	4.921	1.86	25	18.219		
13/01/92	15	4.557	1.9	34.3	27.843		
13/01/92	20	4.334	1.59	19.1	13.176		
13/01/92	25	7.284	1.4	19	10.316		
13/01/92	30	10.292	0.86	18.2	7.048		
28/01/92	30	13.494		16.2		113.9	
28/01/92	25	10.444		15.7		99.4	
28/01/92	20	11.197		15.5		97	
28/01/92	15	7.039		16.6		89.8	
28/01/92	10	9.617		17		98.8	
28/01/92	5	9.202		17.5		108.4	
28/01/92	1	9.087		22.5		108.4	
04/02/92	30		1.47	20.5		107.9	
04/02/92	25	9.989	1.13	17.8	6.681	104.2	15.5964
04/02/92	20	9.279	1.19	17.6	7.131	96.8	13.5745
04/02/92	15	9.334	1.37	28.5	17.796	106.7	5.99572
04/02/92	10	9.373	1.53	21.3	10.397	99.3	9.55083
04/02/92	5	9.251	1.39	17.6	6.959	112.9	16.2235
04/02/92	0.5	9.185	1.43	21.1	10.485	120.2	11.4639
11/02/92	30	9.111	1.41	32.7	22.179	129.1	5.82082
11/02/92	25	8.722	1.57	24.9	14.608	118.2	8.09145
11/02/92	20	8.636	1.98	23.8	13.184	117	8.87439
11/02/92	15	8.586	1.91	21.6	11.104	111	9.99639
11/02/92	10	8.148	2.36	20.5	9.992	118.2	11.8294
11/02/92	5	8.283	2.2	23.3	12.817	114.6	8.94124
11/02/92	0.5	8.556	2.23	19.4	8.614	113.4	13.1646
18/02/92	30	8.6	2.82	21	9.58	116.1	12.1189
18/02/92	25	8.444	2.89	20.5	9.166	118.7	12.9500
18/02/92	20	8.312	2.94	20.5	9.248	117.4	12.6946
18/02/92	15	8.148	3.06	18.6	7.392	104.5	14.1369
18/02/92	10	8.227	2.99	19.4	8.183	107.1	13.0881
18/02/92	5	8.715	3.6	20	7.685	108.4	14.1054
18/02/92	0.5	9.232	5.75	23.2	8.218	120	14.6020

25/02/92	30	8.252	1.99	15.6	5.358	117.9	22.0044
25/02/92	25	8.061	2.06	15.6	5.479	104.7	19.1093
25/02/92	20	7.748	1.97	17	7.282	106	14.5564
25/02/92	15	8.014	3.43	17.5	6.056	112.6	18.5931
25/02/92	10	7.994	2.65	18	7.356	108.7	14.7770
25/02/92	5	8.178	3.23	17	5.592	117.9	21.0836
25/02/92	1	8.746	5.74	26.1	11.614	181.2	15.6018
28/02/92	30	8.656	1.44	23.5	13.404	153.3	11.4368
28/02/92	25	7.969	2.32	19.2	8.911	117.9	13.2308
28/02/92	20	7.536	1.35	18.7	9.814	116.6	11.8809
28/02/92	15	7.93	1.59	18.3	8.78	115.3	13.1321
28/02/92	10	7.862	1.69	19.2	9.648	115.3	11.9506
28/02/92	5	7.753	2.07	22.1	12.277	126.8	10.3282
28/02/92	0.5	7.936	1.96	21.1	11.204	125.5	11.2013
03/03/92	30	8.308	2.39	19.2	8.502	121.6	14.3025
03/03/92	25	7.702	2.42	18.7	8.578	103.1	12.0540
03/03/92	20	7.424	3.77	19.7	8.506	103.4	12.1561
03/03/92	15	7.574	2.84	19.7	9.286	97.7	10.5212
03/03/92	10	7.319	2.4	18.3	8.581	100	11.6536
03/03/92	5	7.291	2.73	19.7	9.679	105.7	10.9205
03/03/92	0.5	7.564	3.54	21.6	10.496	107.9	10.2801
10/03/92	30	9.322	2.21	19.7	8.168	104.5	12.7938
10/03/92	25	9.817	2.02	22	10.163	101.5	9.98720
10/03/92	20	8.346	1.9	22.1	11.854	101.1	8.52876
10/03/92	15	7.889	1.93	20.2	10.381	104.5	10.0664
10/03/92	10	7.375	2.13	19.2	9.695	97.7	10.0773
10/03/92	5	7.267	2.72	19.2	9.213	98.8	10.7239
10/03/92	0.5	8.122	3.97	22.6	10.508	139.9	13.3136
17/03/92	30	7.904	2.02	16.3	6.376	121.5	19.0558
17/03/92	25	8.373	1.34	17.4	7.687	99.2	12.9049
17/03/92	20	8.9	1.49	16.9	6.51	91.7	14.0860
17/03/92	15	8.416	1.3	15.8	6.084	91.7	15.0723
17/03/92	10	8.243	1.45	16.3	6.607	97.9	14.8176
17/03/92	5	7.952	2	16.3	6.348	100.4	15.8160
17/03/92	0.5	8.142	2.32	23.3	12.838	137.6	10.7181
24/03/92	30	7.405	1.61	15.5	6.485	96	14.8033
24/03/92	25	7.346	1.51	13.5	4.644	94.7	20.3919
24/03/92	20	7.337	1.47	12.1	3.293	90.9	27.6040
24/03/92	15	7.446	1.38	12.1	3.274	101	30.8491
24/03/92	10	7.522	1.55	12.1	3.028	99.8	32.9590
24/03/92	5	7.53	2.39	14.5	4.58	104.8	22.8820
24/03/92	0.5	7.504	2.51	16	5.986	115	19.2114

31/03/92	30	7.412	1.41	13.5	4.678	107.1	22.8943
31/03/92	25	7.269	1.53	13.5	4.701	102.5	21.8038
31/03/92	20	7.075	1.55	13.5	4.875	100.2	20.5538
31/03/92	15	7.067	1.17	12.6	4.363	101.4	23.2408
31/03/92	10	7.025	0.93	14	6.045	111.6	18.4615
31/03/92	5	6.966	1.53	13.1	4.604	105.6	22.9365
31/03/92	0.5	7.427	2.32	15	5.253	113.9	21.6828
03/04/92	30	7.124		19.9	12.776	118.5	
03/04/92	25	6.203		12.6	6.397	107.1	
03/04/92	20	6.663		14.5	7.837	107.1	
03/04/92	15	6.906		13.5	6.594	108.2	
03/04/92	10	6.956		13.1	6.144	111.6	
03/04/92	5	6.73		12.1	5.37	117.3	
03/04/92	0.5	7.19		14.5	7.31	134.5	
14/04/92	30	6.846	1.65	18.6	10.104	121.8	12.0546
14/04/92	25	6.178	1.27	15.7	8.252	120.2	14.5661
14/04/92	20	5.81	1.19	14.7	7.7	111.6	14.4935
14/04/92	15	5.827	1.23	14.7	7.643	121.3	15.8707
14/04/92	10	5.827	1.15	16.6	9.623	117	12.1583
14/04/92	5	5.744	1.13	15.7	8.826	118	13.3695
14/04/92	0.5	5.768	1.19	16.2	9.242	123.5	13.3629
21/04/92	30	6.726	1.83	16.5	8	96.5	12.0625
21/04/92	25	6.157	1.37	13.5	6	100.7	16.7833
21/04/92	20	5.615	0.94	12.6	6.045	100.7	16.6583
21/04/92	15	5.503	0.86	11.2	4.837	98.9	20.4465
21/04/92	10	4.141	0.18	7.8	3.479	110.5	31.7620
21/04/92	5	0.109	0.04	3.4	3.251	110.5	33.9895
21/04/92	0.5	-0.01	0	6.3	6.31	115.2	18.2567
22/04/92	30	6.26	1.54	15.5	7.7	100	12.9870
22/04/92	25	5.401	1.07	16	9.529	95.7	10.0430
22/04/92	20	5.42	0.93	14.1	7.75	104.6	13.4967
22/04/92	15	4.598	0.65	11.2	5.952	91.2	15.3225
22/04/92	10	1.798	0.11	13.1	11.192	111.4	9.95353
22/04/92	5	0.078	0.07	5	4.852	119.2	24.5671
22/04/92	0.5	0.139	0.08	5.6	5.381	120.3	22.3564
24/04/92	30	5.589	1.5	13.6	6.511	93.4	14.3449
24/04/92	25	5.2	1.41	11.6	4.99	97.9	19.6192
24/04/92	20	5.151	1.23	11.2	4.819	90	18.6760
24/04/92	15	5.128	1.05	8.3	2.122	90	42.4128
24/04/92	10	3.777	0.46	9.2	4.963	94.5	19.0409
24/04/92	5	0.037	0.15	5.8	5.613	112.5	20.0427
24/04/92	0.5	0.024	0.19	4.4	4.186	124.8	29.8136

27/04/92	25	6.02	1.25	13	5.73	91.3	15.9336
27/04/92	20	5.39	1.17	10.8	4.24	94.3	22.2405
27/04/92	15	5.279	0.31	12.4	6.811	94.3	13.8452
27/04/92	10	3.8	0.11	10.8	6 '9	89.9	13.0478
27/04/92	5	0.487	0.04	6.4	5.873	98.7	16.8057
27/04/92	0.5	0.03	0.04	5.3	5.23	128.1	24.4933
30/04/92	30	5.574	1.63	10.8	3.6	83.9	23.3
30/04/92	25	3.708	1.25	8.9	2.9	79.9	27.55
30/04/92	20	3.078	1.17	10.8	6.5	87.8	13.51
30/04/92	15	3.293	0.31	8.4	4.8	83.9	17.48
30/04/92	10	0.826	0.11	10.8	9.9	87.8	8.87
30/04/92	5	0.388	0.04	6.4	6	114	19
30/04/92	0.5	0.143	0.04	5.9	5.7	126	22.11
05/05/92	30	4.274	1.56	12.3	6.4	99.7	15.58
05/05/92	25	3.925	1.44	13.3	7.9	94.4	11.95
05/05/92	20	3.751	1.13	15.3	10.3	101	9.81
05/05/92	15	2.882	0.93	10.8	7	102.3	14.61
05/05/92	10	1.377	0.61	8.9	6.9	110.2	15.97
05/05/92	5	0.149	0.17	8.4	8.1	118.1	14.58
05/05/92	0.5	0.02	0.03	13.8	13.7	148.4	10.83
12/05/92	30	5.195	1.99	17.9	10.7	138.1	12.91
12/05/92	25	3.749	1.49	13.3	8.1	109.0	13.46
12/05/92	20	3.023	1.01	15.6	11.6	125.8	10.84
12/05/92	15	1.837	0.53	6.4	4	109.0	27.25
12/05/92	10	0.017	0.05	6.4	6.3	112.0	17.78
12/05/92	5	0	0.02	8.5	8.5	135.0	15.88
12/05/92	0.5	0.003	0.05	13.3	13.2	139.6	10.58
20/05/92	30	4.162	2.06	18.2	11.9	126.0	10.59
20/05/92	25	3.193	1.58	13.9	9.1	120.0	13.19
20/05/92	20	1.336	0.78	11.9	9.8	140.5	14.34
20/05/92	15	0.141	0.42	11.3	10.8	126.0	11.67
20/05/92	10	0.035	0.46	7.6	7.1	136.0	19.21
20/05/92	5	0.042	0.63	10.9	10.2	148.0	14.51
20/05/92	0.5	0.02	0.4	13.2	12.8	159.2	12.44
27/05/92	30	1.411	1.26	19.8	17.1	111.5	6.52
27/05/92	25	1.867	1.21	10.9	7.8	107.3	13.76
27/05/92	20	1.655	1.06	17.0	14.3	115.6	8.08
27/05/92	15	1.264	0.66	10.4	8.5	107.3	12.62
27/05/92	10	0.138	0.48	10.3	9.7	121.9	12.57
27/05/92	5	0.215	0.78	9.5	8.5	136.4	16
27/05/92	0.5	0.293	1.18	17.5	16	140.4	9.25
11/06/92	30	3.853	2.02	15.1		103.0	
11/06/92	25	2.92	2.91	14.6		111.4	
11/06/92	20	1.458	1.43	9.9		106.7	
11/06/92	15	0.727	1.58	9.9		107.8	
11/06/92	10	0.257	0.06	5.7		117.4	
11/06/92	5	0.12	0.10	9.9		127.0	
11/06/92	0.5	0.097	1.93	10.9		139.0	

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