UNIVERSITY OF SOUTHAMPTON

Phosphorus in estuarine and oligotrophic ocean waters: Analytical and biogeochemical studies

by

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To my parents

Bolívar and Esperanza,

and sons

Alejandro, Isaac and Gabriel

A C K N O W L E G E D M E N T S

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

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Phosphorus in estuarine and oligotrophic ocean waters: Analytical and biogeochemical studies

by Franklin Isaac Ormaza-González

Analytical difficulties have precluded scientists from studying in a comprehensive manner in natural waters the main forms of P, i.e. dissolved inorganic (DIP) and organic (DOP), and particulate total (PTP) phosphorus, and their cycles. A long capillary cell (LCC) system for measuring DIP in natural waters at nanomolar concentrations in the range 1-500 nM was developed and proved easy to construct with relatively cheap and versatile components, compact and portable, and able to work on ship. Amongst 5 techniques for measuring DOP, that using $Mg(NO_3)_2$ as oxidant gave the highest DOP values, whilst the ultra-violet (UV) irradiation oxidation technique gave the lowest. The importance of dissolved poly-phosphates (PPs) was revealed in this work. The $Mg(NO_3)_2$ oxidation technique was modified for use in determining PTP. Surfactants widely used in automate systems proved to interfere in the colour formation of the phospho-molybdenum blue complex.

A study of DIP, DOP and PTP in four English estuaries was carried out. DIP varied in both concentration and behaviour. Concentration ranged from <0.05 μM (Beaulieu and Humber) to up to 55 μM (Itchen). Anthropogenic discharges were the dominant source of P in the Itchen and Thames estuaries. Non-conservative behaviour of DIP in the Beaulieu, and possibly also in the Humber, was detected with concentrations generally below 0.5 μM. In these estuaries the dominant fraction of P was PTP, which ranged from about 1 to 6 μM (average 3 μM), followed in importance by DOP. However in the Itchen and Thames DIP was the dominant fraction. The ratio of DOP/DTP varied greatly from < 1 to 80 %, with high values at the salinity end members. Previous studies of riverine PTP fluxes to the oceans appear low if the measurements reported are representative of world rivers.

The different forms of P were also comprehensively studied in the Sargasso Sea. The first nanomolar vertical profiles of DIP, and the processes affecting the well defined vertical structure observed are presented. It was found that 1) DIP is being utilized in biological processes at least down to concentrations of 1 nM. 2) DOP was constantly the dominant fraction (75 %) of the total P and proved to be an important reservoir of P for micro-organisms. DOP concentrations measured by the $Mg(NO_3)_2$ method were generally double those determined using the UV oxidation. The differences between these values were ascribed to the presence of dissolved PPs. 3) DIP was shown to be the limiting nutrient rather than nitrate. 4) Turnover rates within the euphotic layer for DIP were 0.3 $hours^{-1}$, and up to 0.5 $days^{-1}$ for DOP, whilst the overall residence time for P in mixed layer is about 2 years. 5) The application of a Fickian model to compute the diffusion of DIP to the euphotic layer from the nutricline was not adequate to balance export fluxes of particulate P from the this layer. 6) The use of the LCC technique for following the net removal rates of DIP *in situ et vitro* conditions was successfully demonstrated. Data from two profiles (0-4200m) show that PPs and DOP are present as well as the expected DIP in deep waters.

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*: Note that for Figures, Tables and Appendixes the first digit refers to the Chapter in which appear and the second digit is the sequential number of sections and sub-sections within the respective chapter.

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Abbreviations

A: Absorbance.
Å: Armstrong (10^{-8}m)*
Ac-c: acid oxidation under autoclave conditions method
AcPA: acid phosphatase activity
ADP: adenosine diphosphate
Al-c: alkaline oxidation under autoclave conditions method
APA: alkaline phosphatase activity
ATP: adenosine triphosphate
b: light pathlength
C: analite concentration
CA: cellulose acetate
CN: cellulose nitrate
CNA: cellulose nitrate-acetate mixture
Chl-*a*: Chlorophyll-*a*
CMP: cytidine monophosphate
d: difference between duplicates, Eq. 3.1
DE: decomposition efficiency
DDW: distilled deionized water
DIN: dissolved inorganic nitrate
DIP: dissolved inorganic phosphorus
DOC: dissolved organic carbon
DON: dissolved organic nitrogen
DOP: dissolved organic phosphorus
DOP_{change}: change of DOP in an interval
DOP_{prod}: DOP production in an interval
DOP_{reg}: regeneration of DOP into DIP
DTMAB: dodecyltrimethylammonium bromide
DTP: dissolved total phosphorus
EHP: enzymatically hydrolyzable phosphate
F: total flux of an analyte
F_A: advective flux
F_D: diffusive flux
FIA: flow injection analysis
GDP: Guanosine diphosphate
GF/F: borosilicate glass filter (Whatman, 0.7 μm nominal pore size)

GMP: guanosine monophosphate
GP: glycero-phosphate
G-1-P: Glucose-1-phosphate
G-6-P: Glucose-6-phosphate
High-t: high temperature oxidation method
ID: inner diameter
IRD: infrared emitting diode
k: molar absorption coefficient
K_m: half saturation constant
K_z: diffusion coefficient
LED: light emitting diode
m: meter
M: molar concentration
MP: metaphosphate
MR: mixture reagent
MW: molecular weight
n: number of samples analyzed
NADH: nicotinamide adenine dinucleotide phosphate
Nit-ox: nitrate oxidation method
NW: natural water
OD: outer diameter
ORS: oxidant reagent solution
P: phosphorus
Phaeo: phaeopigment
PIP: particulate inorganic phosphate
POC: particulate organic carbon
POM: particulate organic matter
PON: particulate organic nitrogen
POP: particulate organic phosphorus
PPs: poly-phosphates
PTP: particulate total phosphorus
pPn: phosphonate
PTFE: polytetrafluoroethene
r: regression coefficient
rFIA: reverse flow injection analysis
RSD: relative standard deviation
s: standard deviation in the Eq. 3.1
S: salinity
SD: standard deviation

SFA: segmented flow analysis
SRP: soluble reactive phosphate
T: temperature (^{0}C)
TP: total phosphate
UV: ultra-violet
Uv-ox: ultraviolet irradiation oxidation method
V: volts
V_{max}: maximum uptake
V_z: vertical water speed vector
X: average value.
Z: depth

Σ: total sum, Eq. 3.1
τ: residence time

: Note that the units (SI) agreed by the UNESCO Commission (see in references UNESCO 1985) have been used throughout the text of this thesis. However in some cases different unit systems had to be used because the data was not directly obtained by the author.

C H A P T E R

O N E

Literature review

1.1 Introduction

Despite the fact that phosphorus (P) is a vital element for all organisms living in natural waters, from bacteria to mammals, there are serious gaps in our knowledge about its role in biological processes and its global cycle. The gaps are mainly a product of a lack of information about organic P either in dissolved or particulate phases. Knowledge on dissolved inorganic phosphate (DIP) at nanomolar concentrations is also a problem, and there is a significant amount of papers in which DIP is simply reported as depleted because it is not detected by analytical techniques. According to the published literature (from 1970 to 1990) the study of dissolved organic phosphorus (DOP), particulate total phosphorus (PTP) and all their related chemical and biological processes (e.g. oxidation, regeneration, etc.) have not been studied as well as dissolved inorganic phosphorus (DIP).

Up until 1983, many reviews on phosphorus in marine, estuarine and fresh waters have been published (e.g. Redfield *et al.* 1937, Sverdrup *et al.* 1942, Graham and Morberg 1944, Redfield 1958, Redfield *et al.* 1963, Armstrong 1965, Riley and Chester 1971, Griffith *et al.* 1973, Spencer 1975, Pierrou 1976, Rigler 1978, Froelich *et al.* 1982, Wollast 1983, etc.). These works have mainly dealt with DIP, and have only touched on particulate total phosphorus (PTP), P in sediments, and input and removal of P from the oceans. Very little of this information relates to processes such as the desorption-adsorption behaviour of P with marine suspended material or sediments. There is almost nothing about the magnitude and cycling of DOP and PTP, with the only reviews found being those of Scharpf (1973), Hooper (1973), and Cembella *et al.* (1983). As regards the geochemistry of P, the reviews of Froelich *et al.* (1982), Romankevich (1984), and Mach *et al.*

(1987) contain significant amounts of conflicting information. Moreover, most of the information about DOP and POP (PTP in this work) refers to fresh (mainly lakes) and estuarine waters.

The global cycle of P has many unknown steps, such as, the atmospheric input of P to oceans and *vice versa*, quantification of the burial of P in oceanic, and to some extent in estuarine sediments, etc. Most of the reviewers have concluded that among the many gaps in the knowledge about P in marine environment, those concerned with the organic P fraction are particularly important.

The literature review carried out, as the first step of this research has given access to the following information.

1.2 Environmental phosphorus and its characterisation

Chemistry texts describe a very extensive variety of P compounds existing in the terrestrial environment. However here, only those compounds known or likely to occur in the aquatic environment, especially in marine systems, will be considered. These are: Orthophosphate, pyrophosphate, polyphosphate, and their respective organic esters. In addition, there are organo-phosphorus compounds with C-P bounds which have been found in algae (Baldwin and Braven 1968, and Kittredge and Roberts 1969), marine invertebrates (Quin 1967), and recently, these types of compounds have been determined in sea water in the dissolved phase (Cembella and Antia 1986). Reduced P compounds, such as phosphite have been rarely reported in the literature, because they have been overlooked in anaerobic environments where they may play a role in phosphorus oxidation-reduction cycles similar to those for sulphur and nitrogen. However, in keeping with this concept, both aerobic and anaerobic utilization of hypophosphite have been recorded in soil bacteria (Heinen and Lauwers 1974, and Foster *et al.* 1978). Recently Devai *et al.* (1988) have demonstrated , under laboratory conditions, that phosphine is released by bacterial reduction from a medium

containing inorganic phosphorus. From this observation, they suggested that the 25-50 % of the P deficit in open air sewage treatment plants can be explained in terms of the release of phosphine into the atmosphere. This new fact, despite being currently found only in open air sewage treatment plants, could give another perspective to phosphorus cycling in the hydrosphere. This process would make the P cycle comparable to those of major biogenic elements (e.g. carbon, oxygen, nitrogen, and sulphur) which contain gaseous substances (e.g. CO_2 , CH_4, N_2, NH_3, H_2S, and volatile sulphur compounds).

1.2.1 Chemical characterization of dissolved inorganic phosphorus

In sea water of salinity 34.8, and pH of 8.0 at 20^0C inorganic phosphates are principally found as HPO_4^{2-} (87 %), followed by PO_4^{3-} (12 %), and $H_2PO_4^-$ (1 %), (Kester and Pytkowics 1967). However, orthophosphate ions form metallo-phosphates complexes and chelates. Thus, overall, free orthophosphate represents 28.7 % of the total inorganic phosphate in sea water (Atlas *et al.* 1976). The rest is made up of $NaHPO_4^-$ (15 %), $CaHPO_4$ (4.7 %), $CaPO_4^-$ (7.6 %), $MgHPO_4$ (41.1 %), $MgH_2PO_4^+$ (0.1 %), $MgPO_4^-$ (1.5 %), and $H_2PO_4^-$ (0.9 %). These percentiles vary in accordance with not only pH but also concentrations and relative proportions of cations and competitive complexing anion ligands, temperature, and pressure (Cembella *et al.* 1983).

1.2.2 Chemical characterization of dissolved organic phosphorus

Few works have attempted to study the chemical structure and fractions of organic P and more importantly, even fewer of these works have gone toward linking the organic fractions with the metabolic functions of bacteria and plants, from which much (if not all) of the material must arise.

Different techniques have been employed to fractionate organic P (DOP). For example, Phillips (1964) separated the

DOP of sea water into six fractions using Thin Layer Chromatography. Three of them were identified as nucleotides or poly-nucleotides, the rest were believed to represent phosphorylated hydrocarbons. However, the identified species represented only 53-65 % of the total DOP.

Strickland and Solórzano (1966), Solórzano (1978) and Kobori and Taga (1977) determined sugar phosphates (generally phospho-mono-esters) using enzymatic approaches. They found that few samples from the open ocean failed to contain these compounds, but small amounts 0.03-0.45 μM were present in shallow coastal waters. They suggested that such material, if produced at sea, must be rapidly used or destroyed. Herbes *et al.* (1975) found that up to 50 % of the organic P fraction was hydrolysable by phytase, but Kobori and Taga (1977) observed that 1/3 of the organic phosphorus pool was hydrolysable by phosphatase enzyme. Enzymatic characterization may lead to conflicting information because of the unknown specificity of the action of enzymes on the organic P fraction.

Einsenrich and Armstrong (1977) reported an investigation on the presence of inositol phosphate esters in lake waters using gel permeation chromatography. They found that the total organic P present as inositol phosphate averaged up to 26 %. Minear (1972) using gel permeation on a Sephadex-25 column, found that about 80 % of recoverable organic P had a molecular weight (MW) of less than 50000 daltons. No more work regarding this technique has been found.

Only two works have reported organic P fractionation by ultrafiltration i.e. Hicks (1981), and Matsuda *et al.* (1985). Hicks reported that the bulk (i.e. about 80 %) of DOP has a MW below 10000 daltons followed in importance by high molecular weight > 50000 daltons (16 %) and only 4 % had a molecular weight between 10000 - 50000 daltons. He concluded that about 80 % of the DOP is readily available for phytoplankton, bacteria and other heterotrophic organisms. Matsuda *et al.* reached similar conclusions by observing that the MW fraction of < 500 daltons was predominant followed next

in importance by the highest MW fraction > 50000 daltons. Both fractions were rapidly converted to inorganic P by probably enzymatic action. They also made an availability test of each MW fraction (500, 10000, 100000 daltons) of the DOP to the red tide microorganism *Gymnodiniun sp.* This test revealed that every fraction was utilized as a source of P, but the 500-10000 daltons fractions showed the most successful stimulating effect on the growth. Using chromatography techniques, Matsuda and Marujama (1985) established that the high MW organic P fractions are the most stable compounds in the DOP pool in samples of sea water from the Western Pacific. They suggested that these compounds are associated with nucleotides in their chemical structure.

More recently, Admiraal and Veldhuis (1987) have reported the fractionation of DOP by using High Performance Liquid Chromatography; they claimed high sensitivity and achieved a separation of nucleosides and phosphorus containing nucleotides. Meck and Pietrzyk (1988) have employed liquid chromatography to separate, and a fluorescence technique to detect, phosphorus oxo-acids including diphosphonates.

Polyphosphates (PPs) either bound or not bound to organic compounds, are intracellular compounds which are believed to be for energy storage (Hoffman-Ostenhot 1962, Watanabe *et al.* 1989); they have been detected as a dissolved component in seawater by Solórzano and Strickland (1968), Armstrong and Tibbits (1968), and Solórzano (1978). Dissolved PPs levels can be as high as 50 % of the organic P pool. Lorenzen (1967) have suggested that PPs associated with high levels of DOP are an indication that this fraction is due to excretion by zooplankton and phytoplankton. Solórzano (1978) believes that significant levels of PPs can be found when a bloom of phytoplankton starts its declining life stage.

In summary, these few works carried out on sea water have shown the importance of DOP in biological processes, especially when DIP becomes exhausted (i.e. around the analytical zero) triggering the enzymatic systems which

together with bacterial activity mineralize the DOP. The DOP fraction, as it has been suggested by many authors (e.g. Chu 1946, Orret and Karl 1987, Sapozhnikov 1988), could be an important source of P, since it has been found that the dominant fraction of DOP in surface waters is mainly of labile chemical structure (Matsuda and Marujama 1985).

1.2.3 Fractionation of particulate and dissolved phosphorus

Phosphorus in the aquatic environment has been arbitrarily split into dissolved and particulate fractions. Dissolved forms and particulate fractions are those that respectively can and cannot pass through a filter of about 0.45 μm pore size. The definition given above must not be taken strictly, because of the mechanical problems associated with filters and filtration *per se*, i.e. clogging, pressure, and effective pore size, also type and shape of particles (Sheldon 1972). Fractionation can be done using either glass fibre or membrane filters. The first type have nominal pore diameters down to 0.7 μm (GF/F Whatman), and very high filtration rates, but the pore size is neither uniform nor evenly distributed on the filter surface. The second type could have a nominal pore size as small as 0.03 μm (e.g. Nuclepore, Millipore, Sartorius), the pore size is generally uniform and quite evenly distributed on the surface. Nonetheless, these membrane filters have a low filtration rate and also are prone to clog, and some of them such as the Millipore cellulose acetate release phosphate (Jenkins 1968), unless they are adequately treated. Thus type of water, filtration pressure, and kind of filters and their treatment must be specified when fractionation of P (or any other nutrient or trace metal) is made (Wagemann and Graham 1974, and Danielsson 1982, Morse *et al.* 1982).

1.3 Distribution of P in the oceans

Geographic, temporal, and horizontal and vertical distributions of DIP are quite well established. Two major

reviews on DIP (Armstrong 1965, Spencer 1975) have discussed its general distributions in coastal and open ocean waters. In general, horizontal distributions of DIP tend to decrease from the coastline towards open sea. Vertically, DIP is almost depleted, i.e. below or on the current detection limit of about 30 nM, in the photic zone during the season of high primary productivity; during the season of low primary production DIP is typically in the range 0.03-1 μM. Below or about the base of the photic layer levels of DIP start to increase, with a maximum at the minimum dissolved oxygen layer. From here downwards the levels of DIP are more or less constant, with occasional variability due to advective processes. The DIP well below the photic zone is regarded as conservative and some researchers have taken advantage of this to determine geostrophic circulation, especially in those zones, where the usual physical approaches fail or are not fully reliable, i.e. between 5^0N and 5^0S. (Tshuchiya 1985). Also, a correlation between conservative DIP, dissolved oxygen and carbon dioxide has been successfully employed to identify masses of water in Adriatic waters (Vukadin and Stojanoski 1983). Furthermore, Cabrera *et al.* (1974) have reported a good correlation of the so called conservative DIP and pH, achieving a regression coefficient r^2 of 0.989 in the North Pacific. The general oceanic distribution of DIP tends to remain constant, with some changes from season to season in the surface waters. To summarize, typical levels of DIP in the euphotic layer are around 0.7 μM, whilst below the euphotic layer, concentrations increase up to 2.0 to 3.0 μM in deep waters.

A summary of the oceanic content and distribution of DOP and POP (PTP) in the world oceans has been attempted by Romankevich (1984), whilst Sapozhnikov (1988) gave a more detailed study of the Pacific Ocean. Of the 23 worldwide oceanic areas studied and reviewed by the former author, only in 9 have measurements of DOP and POP been made (i.e. 39 % of all the areas), despite organic matter being the principal research target in all the areas. He found that reported DOP in ocean waters varies from the analytical detection limit (20

nM) to 1.68 μM. In the euphotic layer, typical values in the range of 0.06 - 0.97 μM are commonly observed, whereas in deep waters DOP varies from detection limit to 0.32 μM. Values as high as 0.97-1.94 μM of DOP have been reported below 500-1000m depth, at which depth the DOP content is generally lower than in the upper layer. The authors have also found irregular high concentrations in deep waters of the Equatorial Eastern Pacific Ocean. If correct, this observation is interesting, because it means that the DOP concentration can be more than 50 % of the DIP concentration, whilst in the euphotic layer the DOP levels can be higher than those of DIP. Sapozhnikov (1988) reported isolines of 0.4 μM in surface coastal and productive waters and 0.2 μM in the surface open and oligotrophic oceans. Below the euphotic zone isolines of < 0.2 μM were reported. It can be said that DOP is typically below 0.5 μM in the most productive waters. This type of concentration distribution would not be consistent with the new values of dissolved organic carbon (DOC) reported by Sugimura and Suzuki (1989), Suzuki and Peltzer (1990), Bauer *et al.* (1990), who are consistently finding a DOC concentration of 180-220 μM in the North Atlantic and 240-280 μM in the North Pacific. These DOC values imply concentrations of DOP of 1.7-2.6 μM if the Redfield ratio of 106:1 (C:P) is applied, which are several folds higher than the currently reported values. However, there should be pointed out that the Redfield strictly relates the average concentration of C, N, and P within the cell of the average marine phytoplanktonic population (see Redfield *et al.* 1963 and also Takahashi *et al.* 1985), therefore any deducted DOP values from DOC concentrations must be qualified and in this work these ratios are only employed to have baseline comparator.

Dissolved organic phosphorus in the surface waters of inland seas and coastal waters have on average higher concentrations than in the open oceans. In general, and depending on the biological activity, the DOP averages from only a few percent to more than 100 % of the soluble reactive phosphorus (SRP, i.e. DIP), Romankevich (1984), Smith *et al.* (1985), Smith *et al.* (1986), and Orret and Karl (1987),

Sapozhnikov (1988).

Three major reviews about the chemistry of the surface micro-layer (Liss 1975, Hunter and Liss 1981, and Hardy 1982) agreed in pointing out that both inorganic and organic P are

enriched in this layer. Surface roughness and wind speed in the presence of surface active molecules increase enrichment of phosphorus (Liss 1975, Graham *et al.* 1979, and Souza-Lima 1985). Enrichment factors vary greatly from place to place, from 1.5 to up 8. Williams (1967) has reported up to 2.6 μM PTP (or particulate organic phosphate, POP as the authors reported) in the surface micro-layer when the values of POP in the underlying waters were almost undetectable. The enrichment of organic as well as inorganic P in the surface microlayer seems to be a widespread feature, and has been observed in areas of high primary productivity (Peruvian Waters, Graham *et al.* 1979) and in waters of poor primary production (Sargasso Sea, Volastrykn 1979). No chemical characterization of the DOP in surface microlayers has been reported, but it could be expected to contain a good deal of phospholipids, because of this material's hydrophobic characteristics.

The study of the particulate phase of phosphorus (PTP or POP elsewhere) has been almost completely neglected. Romankevich (1984) reported that POP in the surface waters of the Pacific Ocean ranges from 0.11 to 0.82 % of the dry particulate matter. However the ratio C:P is up to 3.6 % (i.e. about 28:1); this ratio is more than three times higher than the Redfield ratio (106:1). The C:P ratio decreases with depth down to 0.6 % (i.e. 177:1). In general, according to the C:P ratios for particulate matter, the POP fraction of the dry organic matter is on average poorer in ocean floor areas, than in the areas of the continental slope and shelf. In the euphotic layer the particulate organic matter (POM) is richer in organic P than the dissolved organic matter especially before the end of the growth phase of phytoplankton.

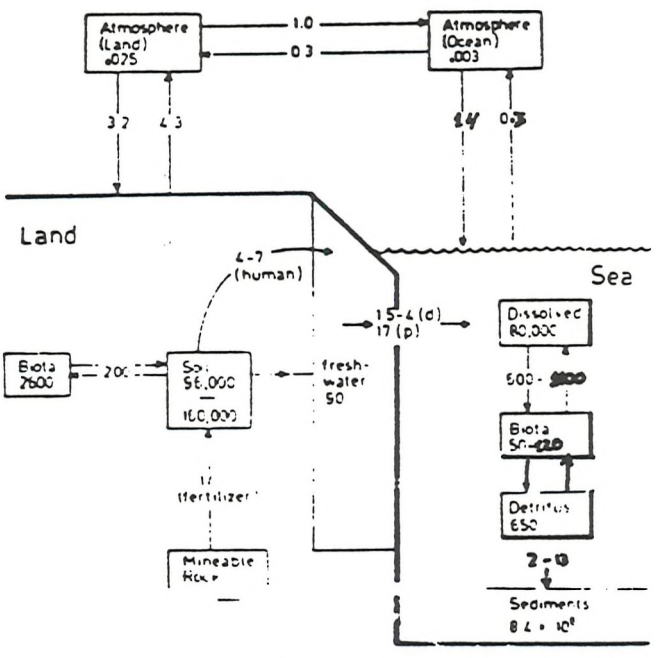


Fig. 1.1 Global cycle of P. Richeu (1983)
Note that units are Tg/year

1.4 Global cycle of phosphorus

Characterization of the global P cycle, i.e. sources, sinks, and pathways has been attempted by many authors (e.g. Pierrou 1976, Meybeck 1982, Richey 1983, Duce 1986, etc.). Figure 1.1 displays the main pathways and the annual inputs and outputs. The main source of P to the oceans is the world riverine system, which carries into the oceans about 560 $Gmol.yr^{-1}$ of particulate P, and 48-130 $Gmol.yr^{-1}$ as dissolved P (Pierrou 1976, Richey 1983, however for different estimates see, e.g. Lermant *et al.* 1975 and Meybeck 1982).

Estimates of atmospheric input, as dry deposition and rain together, varies from 32 (Graham and Duce 1979) and 45 $Gmol.yr^{-1}$ (Richey 1983), to 83-110 $Gmol.yr^{-1}$ (Pierrou 1976). This input is generally regarded as being 5-7 % of the riverine source. The atmospheric input of P could be important in certain local areas, especially those close to industrial zones or land with high agricultural use. Thus, for instance, the fallout of P to a lake (Wannia, Poland) has been determined to be as high as 50 % of the surface water inventory (Kowalczewski and Ryba 1981). This and other works (e.g. Sober and Bates 1979, and Sievering 1980) demonstrate that the atmosphere could be an important source of inorganic and organic phosphorus in local areas.

Another possible source of P to the oceans is from the ocean floor. According to Corliss *et al.* (1979), and Edmond *et al.* (1979), mid-ocean ridge hydrothermal processes could be a source of soluble P, if P were solubilised from sea floor basalt and carried into the ocean by P-enriched hydrothermal seawater. Phosphate deposits in the proximity of volcanic deposits support this idea (Froelich *et al.* 1977 and references cited therein). Nonetheless two points have been raised against this idea. 1) P analysis of model high-temperature sea water-basalt reactions at 400^0C yielded P concentrations of 13 μM, which ultimately will end up as metalliferous sediments rather than dissolved in sea water. 2) Data from the "cool" vents suggested that P is depleted in

the hydrothermal effluent rather than enriched (see Froelich *et al.* 1982 for details). Thus, it appears that hydrothermal circulation of sea water through the crust of the sea floor is not a source of P but a possible small sink.

Input from icebergs has not been reported, but in any case this should be a small source relative to those mentioned above.

The major sink for P is the oceanic sediments, which bury up to 420 $Gmol.yr^{-1}$ of the element (Richey 1983).

Another sink of P is the atmosphere. Richey (1983) based on few data, has proposed a sink of 10 $Gmol.yr^{-1}$. Graham *et al.* (1979) have found that the sea water surface micro-layer could be an important source of P to the atmosphere, particularly in those areas of extensive biological activity (e.g. Peruvian waters). Graham and Duce (1981), and Cheu *et al.* (1985) have considered that most of the P going to the atmosphere (in marine aerosol) is in the particulate phase.

Most of the information about net inputs and outputs of P in the marine environment are conflicting, mainly because of the poor data base. The cycle shown in Fig. 1.1. does not give a closed cycle, as has been previously reported by Riley and Chester (1975), and others. A point which most of the authors make, is that the P cycle is being affected by man's activities (e.g. Stumm 1973, Richey 1983, etc.).

Regarding the role of the organic P within the cycle, it can be said that this fraction has been generally overlooked, because of the belief that it is negligible. Very few pieces of work dealing with organic P suggest this material is significant in the P cycle. Thus, for example, Meybeck (1982) proposed a POP flux into the oceans of 260 $Gmol.yr^{-1}$. However, this estimate is poorly defined (Meybeck 1982, personal communication to Mach *et al.* 1987), because subsequently Mach *et al.* (1987) suggested a net flux of POP of less than 103 ± 30 $Gmol.yr^{-1}$. This latter value compares to the estimate of

Mach *et al.* for the discharge of DIP and DOP together of 30 $Gmol.yr^{-1}$. Thus if the total flux of P, which includes inorganic particulate fractions, of 550 $Gmol.yr^{-1}$ (Pierrou 1976 and Richey 1983) is assumed to be correct, POP can account for 25 ± 5 % of the flux of total P to the oceans.

Atmospheric input of organic P to the oceans has not been directly measured or studied. But, it has been observed that enriched organic P in the surface microlayer can be brought into an aerosol state by wind (Graham *et al.* 1979). The organic P in the marine aerosol, may be affected by, for instance, ultraviolet solar radiation (Cullen and MacIntyre 1990). The organic P fraction, though it has not been investigated, could be transported through the atmosphere to either land or other oceanic areas. Thus, the atmosphere would eventually be both a source and a sink of P in the cycle.

Graham and Duce (1981) measured atmospheric particulate P in the trade winds of the Hawaiian and Samoan islands. The concentration of P ranges from 0.3 to 0.8 $pmol.m^{-3}$ with an average 0.5 $pmol.m^{-3}$. Soluble reactive phosphorus was 20-25 % of the total particulate phosphorus (PTP) and appeared to be of crustal origin, whilst the rest was in the acid-soluble fraction (i.e. the organic fraction plus polyphosphates), and seemed to be of marine origin.

Estimation of the burial of organic P in sediments has again been neglected. Mach *et al.* (1987) carried out a statistical treatment of the available data on the POC and POP content of marine sediments, and found a close linear relationship (however, for a contrary opinion see Froelich *et al.* 1987). The relationship was:

$$[POP] = 5.3 \times 10^{-3} [POC] \qquad \text{(Eq. 1.1)}$$

where POC and POP are expressed in weight percent of the dry organic matter. Thus, if POC burial today is roughly 100 ± 25 $Gmol.yr^{-1}$ (Berner 1982), the rate of POP burial would be 53 $Gmol.yr^{-1}$. The POP would then be expected to be about 12 % of

the total P (430 Gmol.yr^{-1}) burial, and 10 % of the input (550 Gmol.yr^{-1}) when the data of Richey (1983) is used. However Froelich *et al.* (1987) found that burial of organic P in oceanic sediments is about 40 % of the total P input into the oceans. Like organic carbon, most of the material accumulates within the submarine margin of continents (Romankevich 1984).

1.5 The role of phosphorus in biological processes in the aquatic environment

Studies on the role of P in biological processes have generally assumed that DIP or the so called soluble reactive phosphate (SRP, i.e. free orthophosphate), which is measured by the phosphomolybdenum blue method (see below) is the only fraction which is biologically available. Nevertheless, many authors have reported that this is not necessarily true (e.g. Chamberlain and Shapiro 1973, Downes and Paerl 1978, Peters 1979, Bradford and Peters 1987 and references cited therein). Thus, Twinch and Breen (1982) found that SRP underestimates available P in the soluble fraction at low concentrations and overestimates available P in the soluble fraction at concentration higher than 0.65 μM. The problems with overestimation and underestimation of biologically available P could be caused by either analytical procedures, or by the fact that some organically bound P is readily available as well as the free orthophosphate (i.e. SRP or DIP). The problems with analytical procedures are discussed below (section 1.6.), whilst the role of inorganic and organic P in biological processes need to be briefly assessed separately.

1.5.1 The role of dissolved inorganic phosphorus in marine photosynthesis

Dissolved phosphorus as free orthophosphate (DIP or SRP) is among the inorganic micronutrients which are essential in photosynthetic processes. The role of DIP in photosynthesis is quite well established. DIP is a critical element in two or three steps in the photosynthetic pathways (Jagendorf

1973).

Briefly, photosynthesis is the synthesis of high energy organic compounds from low-energy inorganic compounds. This is done by phytoplankton absorbing and transforming either solar radiation or chemical energy (in this case chemosynthesis), derived from the reduction of inorganic compounds, into biological energy by mainly transforming the carbon dioxide and water into cellular carbon and oxygen. The process itself is complicated, thus for further details, the reader is referred to biochemistry textbooks (e.g. Calvin and Bossham 1962, Kuhl 1974). For less detailed information see for instance, Fogg (1973) Riley and Chester (1975), and Parsons *et al.* (1977).

The basic steps in photosynthesis are 1) capturing light energy (or chemo-energy) and transferring it into chemical forms, 2) changing inorganic chemical forms into suitable organic forms by bio-chemical reactions, and 3) fixing CO_2 using adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) produced in the earlier steps.

DIP is essential in at least two of these steps. The covalent ester bonds between two phosphorus atoms are at a higher "energy level" than covalent bonds between many other sorts of atoms. That is, it takes more energy for these to be synthesized, but conversely, they release more energy when they are either hydrolysed or participate in alternative reactions. The typical example of this sort of high energy bond is found in the enzymatic phosphorylation of adenosine diphosphate (ADP),

$$\text{ADP} + \text{DIP} \quad \Leftrightarrows \quad \text{ATP} + \text{water}$$

Utilization of the energy in the phosphate bonds of ATP occurs during other chemical reactions, e.g.

$$\text{ATP} + \text{Glucose} \quad \Leftrightarrows \quad \text{Glucose-1-P} + \text{ADP}$$

Thus formation of a phosphomonoester is an essential pre-requisite for many group-transfer reactions leading to new covalent bonds.

Another role of DIP is as a component part of some electron carriers, mediating biological oxidation-reduction reactions. Finally, DIP plays a very important role in most metabolic processes.

The DIP is taken up by phytoplankton from water by different mechanisms. These are trans-membrane electrochemical potential gradient, passive diffusion, facilitated diffusion and active transport (Cembella *et al.* 1983). The uptake is affected by many factors, including light, temperature, pH, relative concentration of DIP itself and other micronutrients. All these factors have been extensively reviewed by Cembella *et al.* (1983).

1.5.2 Dissolved organic phosphorus in biological processes

Generally, DIP is in short supply in oceanic surface waters, especially where there is high primary production processes or in oligotrophic open oceans. In these waters DIP can be exhausted or perhaps more precisely DIP is around the analytical detection limit of currently applied techniques. This occurs because *in situ* regenerative processes are not as fast as the uptake removal, and external supplies (i.e. DIP from rivers, atmosphere and advective or diffusive processes) are inadequate to balance the DIP removal. The shortage of DIP should eventually stop or slow down photosynthetic processes, but this does not seem to occur or has not been elucidated, but if so, that would mean DIP is a limiting nutrient. It is therefore logical to think that under these circumstances hetero- and auto-trophic micro-organisms utilize either intracellular excess inorganic phosphates or excreted DOP.

It has been reported that DIP entering the cell is transformed into condensed inorganic poly-phosphates (PPs), Harold (1966), Hooper (1973). Dissolved PPs are mainly linear chains containing pentavalent P. These compounds are believed to be used for energy storage within the cell (Hoffman-Ostenfof 1962, quoted by Hooper 1973). Harold (1966) provided evidence that PPs serve as a P source, reserve, and storage centre for P from which rapid biosynthesis of nucleic acids and phospholipids can take place. On the other hand, external PPs, i.e. dissolved PPs release by dying cells, could be used as a source of DIP. It has been demonstrated that even PPs coming from some commercially available detergents could serve as a source of DIP for the blue-green algae such as *Anabaena flos-aquae* (Stewart and Alexander 1971, Doemell and Brooks 1975, and Langowska 1982).

The PPs contents of living cells fluctuate within a wide range, from being undetectable when the cells are in the exponential growth phase, to being present at high levels in cells with nutritional deficiency, i.e. unfavourable environmental conditions.

It has been reported that when DIP is close to the analytical zero (i.e. 30 nM), phytoplankton are able to utilize DOP as an alternative source of P. Table 1.1 displays some reported cases in which DOP has been utilized (see Cembella *et al.* 1983 for more detailed information). Chu (1946) was one of the first researchers to report that under low DIP concentration, *Phaeocystis pouchetii, Skeletonema costatum*, and *Nitzchia closterium* were able to utilize phytin. He also reported that bacteria broke down glycero-6-phosphate (G-6-P) and lecithin (phospholipid) to orthophosphate. Since then many works on this topic have been published. Recently a series of studies carried out by a group of scientists in Holland (Veldhius *et al.* 1987, Velhius and Admiraal 1987, Veldhius *et al.* 1986, Veldhius *et al.* 1987) have shown how *Phaeocystis pouchetii* utilizes DOP when the surrounding water has a DIP concentration below 0.7 μM.

It has also been observed that bacteria can even utilize phosphonates (Cook *et al.* 1978) which are very resistant to chemical and thermal decomposition (Freeman and Doak 1957, Cembella and Antia 1986), and photolysis (Murai and Tomizawa 1976). This finding is very important because 1) it has been generally believed that only phospho-mono-esters and some short chain PPs are readily available forms of P, and 2) compounds with C-P bond were not believed to be biologically cleavable because of the strong C-P link. Thus, despite the fact that the bacterial strain used for the experiments of Cook *et al.* (1978) was isolated from soil and sewage, this observation would suggest that bacteria from other aquatic environment would be able to break down the refractory phosphonates compounds.

Table 1.1 shows that the utilization of DOP and PPs is commonplace among all genera of algae, and for some kinds of bacteria. The uptake of DOP or PPs by alga and bacteria have been reported in different types of natural waters (mainly lake waters), and also under laboratory conditions. Generally, this uptake has been found in association with the presence of phosphatase enzyme which hydrolyses phospho-mono-esters and some PPs.

Phosphatase is an enzyme, which is activated under acid or alkaline conditions. Chemically, it is a spherical molecule with a diameter of 60 Å and a MW of circa 80000 (Siuda 1984) or 89000 daltons (Kobayashi *et al.* 1983). The molecule is a dimer compound of two identical monomers, each containing two atoms of zinc (Plocket *et al.* 1962), and thus it is a metalloenzyme.

The Enzyme Commission of the International Biochemical Union has classified all the phosphatase enzymes (phospho-hydrolases) into five groups:

- Phosphoric mono-ester hydrolases E.C.3.1.3 (phospho-mono-esterases).
- Phosphoric diester hydrolases E.C.3.1.4

Algae	Compounds	Authors
-Bacillariophycea		
Cyclotlella cryptica	G-6-P	Kuensler (1965)
Fragilaria capucina	GP	Prosavoli (1968)
Skeletonema costatum	Phytin	Chu (1946)
-Chlorophycea		
Scenedesmus obliquus	GP, Lecithin	Laugowska (1982)
Chlorella pyrenoidosa	PPi, PP	Galloway/Kraus (1963)
-Chrysophycea		
Dinobryon sertularia	AMP, GP, UMP	Lehman (1976)
Synura sp.	GP	Prosavoli/Pintner(1960)
Ochramona danica	GP, G-1-P, G-6-P	Aaranson/Patni (1976)
-Cryptophycea		
Rhoclonuoma lens	G-6-P	Kuensler (1965)
-Dinophyceae		
Gymnodinium nelsoni	G-6-P	Kuensler (1965)
Gymnodinium uncatenum		" "
Pyrocystis noctiluca	AMP, GP, G-1-P, G-6-P Phytin, PPs, PPi	Rivkin/Swift (1980)
-Euglenophyceae		
Phacus pyrum	GP	Prosavoli/Pintner(1960)
-Haptophyceae		
Emiliania huxleyi	AMP, CMP, GMP, GP G-6-P	Kuensler (1965)
Ochrosphaera nepolitana	AMP, CMP, GMP, GP	Pintner/Prosavoli (1963)
Phaeocisthys pouchetii	Phytin	Chu (1946)
-Rhodophyceae		
Asterocystis ramosa	GP	Fries/Petterson (1968)
-Haptophyceae		
Paulova gyrans	AMP, CMP, GMP, GP GM	Pintner/Prosovoli (1963)
Hymenomonas sp.	AMP, CMP, GM, GP	" " "
Syracosphaera sp.	AMP, CMP, GMP, GP	" " "
-Xanthophyceae		
Vaucheria dichotoma	-GP, -GP	Aberg and Fries (1976)

Continuation Table 1.1.

Alga	Compounds	Authors
*****Bacteria		
Bacillus licheniformis		
" *mycoides*	Lecithin, -GP	Laugowska (1982)
" *Cuagulans*		
Azobacter agilis		
Pseudonomonas putida		
Bacillus cereus	pPn	Cook *et al.* (1978)
plus 14 unspecified strains		

Table 1.1 List of compounds, which have been reported as utilizable by algae species when DIP was deplected. Abbreviations.
AMP: Adenosine monophosphate; CMP: Cytidine monophosphate; GMP: guanosine monophosphate; GP: Glycerophosphate (mixture of α and β isomers where either isomer was tested individually it is indicated as αGP or β-GP); G-1-P: Glucose-1-phosphate; G-6-P: Glucose-6-phosphate; PPi: inorganic pyrophosphate; PPs: polyphosphates; pPn; phosphonate (i.e. 2-Aminoethylphosphonate). UMP; Uridine-(3' or 5')-monophosphate; AMP: Adenosine-(3' or 5')-monophosphate.

(phospho-di-esterases).

- Triphosphoric mono-esters hydrolases E.C.3.1.5
- Hydrolases splitting anhydride bonds in phosphoryl- containing anhydrides E.C.3.6.1
- Hydrolases splitting P-N bonds E.C.3.9

Hydrolases which split P-C bonds (phosphonatases) E.C.3.11.1. have also been reported (Cook *et al.* 1978).

Most of the studies on phospho-hydrolases have been concerned with phosphatase mainly because of the availability of methods (e.g. Strickland and Solórzano 1966, Perry 1972, Chrost and Krambeck 1986, Chrost *et al.* 1986, and Chrost *et al.* 1986) and it is thought that organic phosphorus is mainly composed of phospho-mono-esters (Brock 1966). However Kobori and Taga (1977), have found in the eutrophic waters of the Tokio Bay that these compounds only comprised up to 50 % of the total DOP.

Phosphatase (enzyme E.C.3.1.3) catalyses a reaction of the type

$$RO\text{-}H_2PO_3 + H_2O \Longleftrightarrow ROH + H_3PO_4$$

Specific phosphatases have been found, e.g. phosphoserine phosphatase, nucleotide hexase-mono-phospho-ester or phosphoglycerol phosphatase (Siuda 1984). Non-specific phosphatases are divided into alkaline and acid types according to the pH at which they exhibit maximal activity.

Phosphatase activity has been found to occur in cells of phytoplankton (e.g. Perry 1972, Veldhius *et al.* 1986, and Veldhius *et al.* 1987, etc.), zooplankton (Boavida and Heath 1984), and bacteria (Halamejko and Chrost 1984). In addition phosphatase activity (principally alkaline) has been reported as a dissolved form in natural waters e.g. Reichardt *et al.* 1967, and Francko 1983 (lake water), Wai *et al.* 1960, Strickland and Solórzano 1966, Mirkina 1974, Kobayashi and others 1983, Agatova *et al.* 1985, etc (sea water), Huber *et*

Place	Authors
Central North Pacific	Perry (1972)
Western Pacific (neritic and oceanic sediments)	Kobori and Taga (1979)
South West Pacific	Agatova *et al.*(1985)
Eastern North Pacific	Strickland and Solórzano (1966)
Eastern South Pacific	Dr. P Intriago (1985, per.comm.)
Japanese Coastal W.	Kobori and Taga (1979)b
Taiwan Sea Water	Wai *et al.* (1960)
English Channel	Davies and Smith (1988)
Southern North Sea	e.g. Veldhius *et al.* (1987)
North Atlantic (Sargasso Sea)	This work
North Western Indian Ocean	Mirkina (1979)
Venice lagoon (sed. and water)	Degobbis *et al.* (1986)
Australian estuary	Huber *et al.*(1985)
Loch Creeran and Etive (Scotland)	Solórzano (1978)
Subarctic lakes (Northen Sweden)	Jansson (1979)
Goardsjoem lake (Sweden)	Olsson (1983)
Commercial fish ponds (Israel)	Berman and Moses (1972)
Lake with fish aquaculture (Poland)	Chrost *et al.* (1984, and 1986)
Commercial fish ponds (Poland)	Matavolj and Flint (1987)

Table 1.2. Reported cases of APA or AcPA ocurrence in worldwide natural waters.

al. 1985 (estuarine water). Also phosphatase has been detected in oceanic, neritic (Kobori and Taga 1979), and estuarine sediments (Degobbis *et al*. 1986). Table 1.2 shows where it has been observed.

Alkaline phosphatase exhibits maximal activity in alkaline media of pH 7.6-10.0, whilst acid phosphatase has its maximal activity between pHs of 2.6-6.8. Alkaline phosphatase activity (APA) is more likely to be observed in a mass of water where aerobic conditions are present, whilst acid phosphatase activity (AcPA) can be found in stagnant, and interstitial waters and sediments.

Alkaline phosphatase is both an adaptive and constitutive enzyme. In the first case it is responsible for P assimilation from the environment, whilst in the second it is responsible for the intracellular formation of phospho-mono-esters (Kuenzler 1965). Constitutive phosphatases are regarded as constant in the cell, but the adaptive ones vary with the environmental DIP concentration. The adaptive phosphatases are classified as P repressible, whilst the constitutive are not. Nonetheless, it has been observed that some adaptive phosphatases are not P repressible (Forsberg and Chen 1980, and Alvarez *et al*. 1982). At present most, if not all, work related to APA in sea water has indicated that it is triggered during algal bloom conditions, at which time the surrounding water is DIP depleted. Thus the phosphatase enzyme in sea water is of the adaptive type. This enzyme is associated with the supply of DIP from DOP, and is related to the regenerative (mineralization) cycle of P from particulate matter (Olsson 1982). Utilization of DOP has also been associated with 5'-nucleotidase reported by Ammernam *et al*. (1984), Ammernam and Azam (1985). This enzyme is not of the adaptive or phosphate repressible type, i.e. DIP has not necessarily to be at nanomolar concentrations or depleted to be active. It has even been found in estuarine waters where DIP levels are well above those in open oceans (Ammernam and Angel 1990).

Concluding, 1) DOP plays an important role in primary

production processes in any water reservoir 2) two points need to be investigated: a) the effect of pH on APA, and b) the effect of APA on the chemical spectrum of DOP.

1.6 Methods for the analysis of DIP and DTP

There are many methods for the analysis of DIP, which are based on different techniques such as spectrophotometry, fluorometry, atomic absorption spectrometry, enzymatic analysis, etc. (see Kimerle and Rorie 1973, and Burton 1973). The spectrophotometric techniques are up to now the most popular, especially the phospho-molybdenum blue method reported by Murphy and Riley (1962).

The phospho-molybdenum blue technique has been slightly modified many times (e.g. Strickland and Parsons 1972, Johnson 1979, Koroleff 1983, etc.), and evaluated many other times (e.g. Kimerle and Rorie 1973, Burton 1973, Chamberlain and Shapiro 1973, etc.). In this method 12-molybdo-phosphoric acid is formed and is further reduced to phospho-molybdenum blue by using ascorbic acid. But about 6 other reductants have been reported in the literature (Burton 1973).

Between 1973 and 1983, no review about new approaches, and improvements made to previously reported techniques have been found. From the papers found, it could be concluded that the method of Murphy and Riley (1962) is still the most preferred procedure. Nevertheless, ingenious attempts to introduce new approaches employing other techniques rather than the spectrophotometric procedures have been published, for instance, using chemiluminescence (Matsumoto and Fuwa 1981), solvent extraction with cationic dyes (Motomizu *et al.* 1982, Koroleff 1983, Motomizu *et al.* 1984), fluorescence (Guyon and Shults 1969), X-ray fluorescence (Kham and Chow 1985), radio-isotopic dilution using ^{32}P (Parkhomenko 1984), enzymatic analysis (Pettersson 1979), chromatography (Hashimoto and Fujiwara 1987), atomic absorption spectrometry (Kubota *et al.* 1988). Perhaps, one of the most significant is that of Hashimoto and Fujiwara (1988) who proposed a method employing

hydride generation and gas chromatography. They claimed that only 100 mm^3 of sample is required per analysis. The detection limit was about 10 times that of Murphy and Riley (1962) with a relative standard deviation of 3 %. No interference from arsenic, silica and other elements that affects the spectrophotometric analysis was observed. However, this method seems to have three drawbacks, a) because it generates phosphine, which is a poisonous and inflammable gas, it is potentially unsafe, b) the whole system is quite bulky, and c) the method is not straightforward.

Attempts to improve the classic method have been made as well. Thus, from the performance point of view, 1) sensitivity: Fujiwara *et al.* (1982), and Lei *et al.* (1983) improved the sensitivity of it by using long capillary cells (up to 1000 mm long). 2) interference: Johnson (1979) overcame the arsenate and silicate interferences by adding sodium thiosulphate and hydrochloric acid. And 3) rapidity: Koroleff (1983), Fernandez *et al.* (1985), Eberlian and Kattner (1987), and Narusava (1988) have coupled the method to Segmented Flow Injection (SFA), while Johnson and Petty (1983), Johnson *et al.* (1985), and Worsfold and Clinch (1987) to Flow Injection Analysis (FIA). The most sensitive is that of Lei *et al.* (1985), whilst the most efficient in terms of analysis per hour is that of Johnson and Petty (1982) with 90 samples. A more detailed review about techniques for detecting DIP at low levels is given in Chapter 2.

Regarding the analysis of DTP and DOP, some improvements have been achieved on methods such as wet-oxidation, high temperature oxidation, and ultraviolet (UV) irradiation oxidation. In general, there are many methods for DTP or DOP, but the questions about which is the most adequate have remained unanswered. See Chapter 2 for a review of this subject. No automated system for DOP determination has been found, but semi-automated methods are described by, for example, Grashoff (1983), and Eberlian and Katner (1987).

As regards PTP or POP, one of the most used methods

(Solórzano and Sharp 1980) has not yet been compared against other methods such as that of Strickland and Parsons (1972). As PTP is even less considered in environmental studies than DOP, the respective methods for it are consequently less well known and applied.

1.7 Sampling and storage of samples for the analysis of phosphorus

In addition to a suitable and reliable analytical procedure, sampling and storage of samples for the analysis of DIP or SRP, DTP and PTP are vital steps, which have to be properly designed. Thus, the kind of sampling bottle, filter, storage container, type and concentration of any preservative, freezing, thawing time, and storage temperature have to be assessed prior to sampling and storage.

There is a good deal of literature on storage of samples for DIP analysis particularly, but none about storage of samples for DTP or POP. Most of the investigation work has been concentrated on coastal and estuarine water. There are however two examples for deep and open ocean waters (Morse *et al.* 1982 and Kremling and Wenck 1986). Published material about either sampling or the effect of different types of sampling bottles (i.e. Niskin, van Dorn, etc) has not been found. A very brief review of recent literature is given below.

As a general rule, any sample of water for the analysis of micronutrients should be filtered prior to storage. This is particularly required when sampling coastal waters having high primary productivity and suspended inorganic/organic material, but even those samples taken from oligotrophic waters should be filtered (Riley *et al.* 1975, Morse *et al.* 1982). Elution and adsorption problems for DIP when using filters have been reported (e.g. Schierup and Riewarance 1979). Glass fibre filters (e.g. Whatman) and membrane filters made from acetate, nitrate or nitrate-acetate mixture cellulose (e.g. Millipore,

Sartorius) and polycarbonate (Nuclepore) have been found to be the most suitable filter types, after being adequately cleaned by, for instance, boiling in deionized water or acid washed (Grasshoff 1983), and then rinsed with the sample. The detailed effects of pressure or vacuum during filtration seems to be a matter of supposition rather than of information. No specific work has been found dealing with this question, except Bloesch and Gavrielli (1983) who found that pressure is not a critical parameter, unless very high concentrations of certain algae species, such as dinoflagellate or colony forming species, were present. Despite this lack of information, one point is certain, i.e. if samples are not filtered either loss or contamination of DIP will occur (Thayer 1970).

Glass bottles are generally recommended for the storage of samples for DIP analysis (e.g. Koroleff 1983). Plastic containers have been shown to adsorb DIP onto their surface. Also adsorbed bacteria or phytoplankton on the bottle walls will adsorb DIP from the sample (Kremling and Weck 1986). Zhialiang *et al.* (1985) observed that polyethylene bottles halved the initial concentration of DIP in about 48 h at room temperature, whilst under freezing conditions ($-25^{0}C$) there were dramatic changes after 2 months of storage even in those sample with chloroform added. They also reported that the loss of DIP varies from bottle to bottle. Adsorption of DIP onto other plastic surfaces (polyvinyl chloride, polypropylene and polystyrene) has been showed earlier (Ryan 1966). However, Kremling and Wenck (1986) have reported that polypropylene bottles (Nalgene type 2105-0002) are effective containers for storage of phosphate and other nutrients. But they pointed out, as have Macdonald and McLaughlin (1982), that the longer the storage time the lower the precision of replicate analyses. They found a change of the relative standard deviation of up to 7 % in a time scale of 2 months, which was similar to that reported by MacDonald and McLanghling (1982) who employed glass carboys instead of polypropylene bottles. Thus, polypropylene containers would be suitable for storing samples, at least those waters samples chemically similar to

those of the North Atlantic.

Considering conditions of storage, all the research has indicated that quick freezing to 10-25 ^{0}C below zero, and rapid thawing are the best procedures. The use of preserving chemicals have also been investigated (e.g. Pichet *et al.* 1979). Chemicals such as thymol, potassium fluoride, sulphuric acid, tributyltin chloride, chloroform and mercury chloride, and their effect on DIP preservation have been studied. The use of chloroform has given conflicting results. Fitzgerald and Faust (1967) noted that this chemical disrupted some algal cells, so releasing DIP, whilst Thayer (1970) did not observe any significant change. Zhiliang *et al.* (1985) noticed that DIP increased during the first 11 days of storage and then started to steadily decrease when samples were kept at about 4 ^{0}C. Information about the use of mercury chloride is conflicting as well. Kremling and Wenck (1986) reported that in samples with and without this chemical under refrigeration DIP levels decreased by 50 %, but Dr. L Solórzano (pers. comm.) has found no significant changes of DIP in samples, with added mercury chloride, stored at room temperature (about 20 ^{0}C in darkness).

Concluding this section, "there is no single preservation method which may be recommended for all types of water, except that freezing should be of general applicability provided that it is done rapidly, and the solution is analyzed immediately after thawing" (Burton 1973). In addition to this, all samples must be filtered with pre-treated filters, stored in glass bottles without adding any chemical preservative. Analysis must be performed as soon as possible, preferably within two weeks, and cleaning and treatment of sampling and storage containers must be done carefully. Finally, the literature review has revealed that studies on sampling and storage of samples for the analysis of DOP and POP have been neglected.

1.8 Conclusions and research objectives

i) Much literature has been published concerning P in the aquatic environment, especially in fresh and estuarine waters. Most of this deals with DIP, and little relates to DOP and PTP.

ii) DOP levels have been reported to be higher than DIP or at least to represent an important percentage of the DTP in surface oceanic waters, and even in deep waters. In the surface micro-layer DOP is reported to be up to 8 times that in the underlying water.

iii) From the very few papers that have reported information on the chemical characterization of DOP in sea water, it can be observed that DOP in the surface has labile characteristics and therefore is potentially available to photosynthetic processes. There are many more papers in connection with fresh waters, especially lakes.

iv) The magnitudes of inputs and outputs in the P global cycle are not in balance. It has been shown that POP and DOP are important fractions of the total P riverine input. Atmospheric source or/and sink of P may be significant in local areas, where either high atmospheric disposal of industrial and agricultural waste inputs or high primary production processes are present.

v) Particulate organic phosphorus in terms of C:P ratios are generally lower (28:1) than the expected values (i.e. 106:1) in surface waters, whereas in deeper waters they go down to 177:1. In the surface micro-layer, concentrations of PTP are up to 8 times higher than in the adjacent layers.

vi) Dissolved inorganic phosphate is of crucial importance in photosynthetic processes, where a limited supply would slow down or even stop this processes. Thus DIP would eventually become a limiting nutrient.

vii) When DIP is depleted, or more precisely when its level is about the analytical detection limit, utilization of DOP by phytoplankton and bacteria becomes commonplace among many species of algae and some strains of bacteria.

viii) Assimilation of DOP is associated with APA and

sometimes with AcPA as well as with 5'-nucleotidase. Alkaline phosphatase activity has been reported either in dissolved or particulate phases in all natural water reservoirs. It has been generally detected when high primary productivity is present, and levels of DIP are low. Also, it has been observed in deep waters and surface waters with high concentrations of DIP.

ix) Work associated with the assimilation of DOP, and APA, has generally reported levels of DIP below the detection limit. Thus, it seems there is a need of a method for DIP determination with better sensitivity than that currently employed.

x) The worldwide presence of APA and utilization of DOP by phytoplankton and bacteria may justify the inclusion of DOP as a source of P for phytoplankton within the marine cycle.

xi) The phospho-molybdenum blue method has been the standard procedure for DIP determination, despite criticisms about over-estimation of DIP because of the hydrolysis of labile organic P compounds and PPs which are not an available source of P for algae. However, recent work has found there is not only an over-estimation of available P, but also under-estimation; these occur under low and high concentrations of DIP respectively. The cause of this is ascribed to the utilization of DOP by bacteria and phytoplankton.

xii) Many modifications to the Riley and Murphy (1960) method have been done in order to measure very low concentrations of DIP (i.e. < 30nM), but they are complicated or require expensive and cumbersome equipment, and generally have poor efficiencies, and have not been used extensively in environmental studies.

xiii) Others methods based on a different approach rather than the spectrophotometric techniques have been published, but few have accomplished a detection limit better than 30 nM, and moreover, generally these analytical procedures are not easy to perform.

xiv) Many methods for the determination of DTP or DOP have been published, and most of them use a final phosphomolybdenum blue determination step. However no extensive inter-comparison studies of these methods have been found.

1.9 Mains aims of the project

Obviously, this work can not pretend to bridge all the above mentioned gaps. Nonetheless attempts to bring about some solutions to the more conspicuous unknowns of the cycling of P in natural waters were made. Thus, the following research directions were followed.

a) To construct a system for the analysis of DIP at the nanomolar concentrations.

b) To make a rigorous inter-comparison of the principal techniques employed for DTP determination, especially those linked to colorimetric detection.

c) To modify or find a method for POP or PTP.

d) To investigate the behaviour and concentrations of different forms of P in some U.K. estuaries, especially in Southampton Water.

e) To study the role of the different P forms within the P cycle in an oligotrophic sea, i.e. Biological uptake and regeneration of DOP into DIP due to APA. Fluxes in and out of the euphotic layer, residence times etc.

f) To suggest a model of P cycling in oligotrophic oceans.

The following chapters describe the research carried out on the analytical problems and the application of the improved techniques in fresh, estuarine and open ocean waters.

C H A P T E R

T W O

Development of analytical procedures for the determination of different forms of P in natural waters

2.1. Analytical procedures for the determination of nanomolar concentrations of dissolved inorganic phosphate

The development of highly sensitive techniques (Garside 1982, Oudot and Montel 1988, Raimbult *et al.* 1990) have allowed the study of the fate of nitrate and nitrite at low nanomolar concentrations in seawater and have thus provided an improved understanding of the marine nitrogen cycle. In an analogous way, there is thus an obvious requirement for a simple and reliable technique for the determination of dissolved inorganic phosphate (DIP) at nanomolar concentrations. Information on DIP, or soluble reactive phosphorus (SRP) as it is sometimes termed, at very low concentrations is needed to help understand the cycling of phosphorus in fresh and surface marine waters where the detection limit of conventional methods (about 30 nM using 100 mm path-length cells, Strickland and Parsons 1972) is currently a limiting factor.

The determination of DIP at nanomolar levels is needed because among other reasons, it is generally reported (e.g. Perry 1972, Veldhuis and Admiraal 1987, Boni *et al.* 1989) that utilization of DOP and presence of active enzymes, e.g. alkaline phosphatase, in waters are connected to levels of DIP which are near or below the detection limit. It is also reported that DIP is depleted when it is at or below the detection limit level, and implied that hetero- and autotrophic micro-organisms are unable to utilized such minute amounts of DIP (see Cembella *et al.* 1983). However, recent

investigations indicate that P at nanomolar levels can be utilized by micro-organisms (e.g. Vadstein and Olsen 1989), thus challenging this earlier concept, and suggesting the need for information on kinetics of uptake, and actual concentrations and behaviour of DIP at this level. The research presented in this chapter, was aimed at developing a technique capable of detecting DIP at concentrations well below 30 nM. The technique had to be free of chemical interferences and give information comparable with previous methods, i.e. ideally the chemistry of the method should be similar to that of Murphy and Riley (1962).

There is a good deal of published work on methods for concentration of DIP at the nanomolar level, which employ diverse approaches (Kimerle and Rorie 1973). Fernandez *et al.* (1985, see also Motimuzu *et al.* 1984) manufactured an automated method whose chemistry was based on the phospho-molybdenum chemistry but they used malachite green instead of ascorbic acid as counter ions. This technique could detect down to 10 nM of DIP. Lei *et al.* (1983) reported an ingenious long capillary flow cell 0.5-1.0m long coupled to a spectrophotometric system and could detect 0.2 nM. Hashimoto and Fujiwara (1985 and 1987) described a gas chromatographic system which converted dissolved P into phosphine, which in turn was measured with an infrared gas analyzer thus providing a method 100 times more sensitive than the phospho-molybdenum blue colorimetry. Whilst this procedure may have future potential, specialized equipment, compressed gases etc. are required, making it less convenient than more standard colorimetric procedures, and its use for field studies has not been reported.

A high sensitivity method based on a spectrophotometric approach was considered preferable, because this analytical concept is generally easier to apply than other techniques. In this context a detailed consideration of theoretical aspects of spectrophotometric techniques for achieving very low detection is attempted.

The Lambert-Beer law states that the absorbance (A) of a monochromatic ray of light passing through a solution which has an analyte concentration (C), a molar absorption coefficient k, and a light path length b, is given by the equation:

$$A = kbC \qquad \text{(Eq. 2.1)}$$

The higher the value of A achieved the better the sensitivity is. If the concentration C, is kept constant the only way to improve sensitivity is by increasing either k or b; k can be improved chemically, and b by physically elongating the spectrophotometric light path-length.

For achieving higher values of k, different chromophores have been used. The typical method for DIP, based on the work of Murphy and Riley (1962), has a k of 22700 $mol^{-1}.l.cm^{-1}$, which means that the net absorbance A for a solution having 1.0 μM of phosphate is 0.227 using 100 mm path length cuvettes. Thus, if a good spectrophotometer (i.e. with low noise, ± 0.001A) is employed a theoretical detection limit of 10 nM can be achieved although a practical limit of 30 nM is given by Strickland and Parsons (1977).

Based on previous works (e.g. Itaya and Ui 1966, Hohenwalmer and Wimmer 1973, Hess and Derr 1985), Fernandez *et al.*(1985) constructed an automated Segmented Flow Analysis (SFA) manifold which used malachite green as the counter ion to the phospho-molybdenum complex. They claimed a k of 120000 $mol^{-1}.l.cm^{-1}$ for the modified method which is nearly six times higher than that reported by Murphy and Riley (1962). The detection limit of this method should be below 10 nM, but no value was reported.

Motomizu *et al.* (1983) reported a k of 78000 $mol^{-1}.l.cm^{-1}$ using malachite green in association with poly(vinyl-alcohol) to stabilized the colour. They reported a detection limit (0.3 nM) better than the method of Murphy and Riley (1962). Different values of k can be found when using malachite green,

Authors	Chromophore/ Reductant	Solvent Extrac.	k $M[P]^{-1}cm^{-1}$	Det. Lim. nM[P]	Prec. %
1	Ascorbic Ac.	no	22700	30	15
2	Malachite g.	yes_1	270000	?	?
3	Ethyl violet	no_2	78000	3	<10
4	Malachite g.	yes_3	250000	0.3	<1.1
5	" "	no	120000	<10	?
6	" "	no	80000	10	29
7	Ascorbic ac.	yes_4	125000	4	?
8	O-Hydroxyhydroquinone		250000	?	<5

Table 2.1. Different k reported for the phospho-molybdenum blue method. Authors: 1) Murphy and Riley (1962); 2, 3, and 4) Motomizu *et al.*(1982, 1983, and 1984). 5) Fernandez *et al.* (1985). 6) This work. 7) Koroleff (1983), and 8) Fuyita *et al.* (1985). Solvent extration carried out with 1) and 3) mixture of toluene and 4-methylpentan-2-one, 4) n-hexanol. Stabilizer 2) Poly(vinylalchol).

depending on the stabilizer employed. Thus, in this laboratory a k of 80000 $mol^{-1}.l.cm^{-1}$ was achieved using Triton X-100 (Sigma), whilst Fernadez *et al.* (1985), Lanzeta *et al.* (1979), and Hess and Derr (1975) determined a k of 120000, 80000 and 112000 $mol^{-1}.l.cm^{-1}$ using Flaminox (Fisher Co.), Sterox (Coleman Inst.), and Tween 20 (Sigma) surfactants respectively.

Another way of improving k is by concentrating the analyte by solvent extraction. Thus, Koroleff (1983) presented a modification of the method of Stephens (1963) in which the procedure and reagents are those of Murphy and Riley (1962), but isobutanol (used also by Stephens) or n-hexanol were used to concentrate and extract the phospho-molybdenum blue complex. Koroleff (1983) claimed that 4 nM can be detected with certainty. Motomizu *et al.* (1982, 1983, and 1984) have reported the use of many solvents (see Table 2.1) in association with malachite green and ethyl violet counter ions methods that gave k values ranging from 78000 to 270000 $mol^{-1}.l.cm^{-1}$ respectively, with a detection limit of 0.1 $ng.cm^{-3}$, and an RSD of less than 10 %. Nonetheless, the solvent extraction approach, from the performance point of view, is time consuming, and gives high blanks and poor precision due to excessive handling steps (personal communication of Prof. J.D. Burton and Dr. D. Purdie). Ultimately, these facts discourage scientists from employing this approach and only a few papers have reported the use of this method (e.g. Purdie 1983).

Concentration of the phospho-molybdenum blue complex onto the surface of a membrane filter (acetate or cellulose nitrate) instead of solvent extraction has been reported as another way of enhancing the concentration of the phosphate complex prior to colorimetric analysis (Taguchi *et al.* 1985). The authors claimed a concentration factor enrichment of up to 100 times, which gave a detection limit of 0.6 nM, with no interference from salts and moderate levels of silicate. However, the technique has the potential disadvantages of several handling steps with associated high blanks, and no

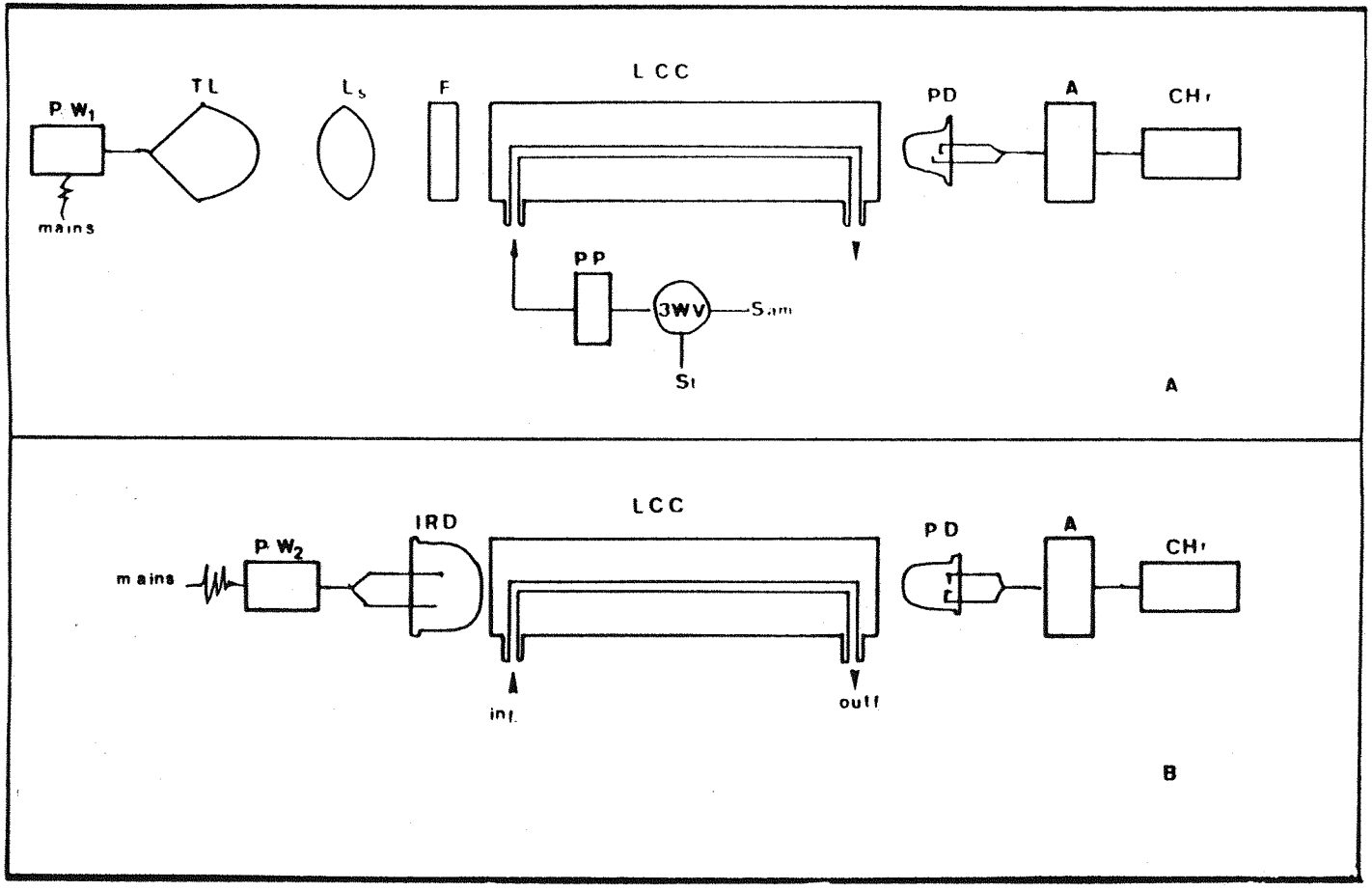


Fig. 2.1 Schematic arrangements of the LCC. PW1 and PW2 high and low intensity power supply; TL: tungsten lamp. Ls: convex lenses. F: optical filter. PD photodetector; IRD: infrared emitter diode;A: amplifier; CHr: chart recorder; 3WV: 3 ways valve; PP peristaltic pump; Sam: sample; Stan. Standard solution. inf. and outfl. inflow and outflow of sample.

environmental application of the technique has been reported.

Improving sensitivity by using longer path length cuvettes than those commonly used, has not normally been attempted because commercially available spectrophotometers cannot hold cuvettes greater than 100 mm in length, and thus for longer path length cells, special spectrophotometric systems have to be built.

Two papers have reported the use of long cells for liquid samples, i.e. Fujiwara *et al.* (1982), and Lei *et al.* (1983), which described the use of laser-induced thermal lens and long capillary cell (LCC) colorimetry, respectively. These two papers claimed detection limits of 0.2 nM for either sea or fresh waters.

Lei *et al.* (1983) used the LCC detection system with the classical phospho-molybdenum blue method. In the construction of the spectrophotometric system, they employed three types of light source, i.e. Xe discharge, W incandescent and He-Ne laser lamps. Figure 2.1A displays a diagram of the design. The light was focused by lenses, and the wavelength was selected with a low wavelength cut off filter (750 nm) or interference filters. A silicon photodiode detected the radiation passing through the LCC. The signal from the photodiode was amplified and then sent to a chart recorder or, alternatively to a personal microcomputer. Whilst the potential of the technique for detecting nanomolar concentrations of DIP was demonstrated, aspects of the design were cumbersome, difficult to implement and expensive.

Summing up, many methods has been reported for the determination of low concentrations of DIP. Most of them are based or connected to the phospho-molybdenum blue complex procedure with spectrophotometric detection. Whilst solvent extraction and phosphine generation techniques were not attractive methods, because of the handling, precision and blank problems, the methods using malachite green as counter ion and LCC spectrophotometry did appear attractive and worth

further investigation.

Initially the coupling of the malachite green method to a Flow Injection Analysis (FIA) or rFIA (reverse FIA) manifold was investigated, because this technique has proved to have a high sample throughput, is easy to construct, and is versatile and cheap. Briefly, the FIA systems associated with spectrophotometric procedures consist of a manifold through which reagents are pumped in a quasi-laminar flow to which a few mm^3 of sample are injected, whilst in a rFIA manifold the contrary is done. The formed coloured complex is detected by a spectrophotometer. The FIA systems substantially differ from the SFA manifolds because the latter used air bubbles to avoid diffusion inside the tubing, whilst the former rely on a quasi-laminar flow, and constant and reproducible residence time of the stream of sample, reagent and mixture of both inside the tubing. The FIA systems also have inherently a low contamination potential and are reproducible, sensitive, and portable (see, for instance Ruzicka and Hansen 1981, Ormaza-González 1987, for detailed reviews.

For the LCC spectrophotometry, it was initially intended to set up the same system as reported by Lei *et al.* (1981, Fig. 2.1A). However, different configuration of LCCs, and light source were investigated in order to tackle the drawbacks of lack of portability, and constructional features, and the use of expensive components.

2.2.1 Apparatus and methods

The apparatus employed for the construction of the system was essentially the same as that use by Ormaza-González (1987).

A 20 mm path-length cell (Fig 2.2) was constructed following the design of Betteridge *et al.* (1978). It was made from a block of Perspex into which was glued a LED diode and a photo-detector, which in turn, act as windows for the cell. The LED (see Gillesen and Shairer 1987 for a review) was a gallium phosphide red diode (Radio Spares; RS 588-291), which had a maximum emission wavelength of 635 nm, with a bandwidth of about 50 nm. The photo-transistor (RS 306-083) gave a maximum response at about 940 nm with an effective range of 300 to 1000 nm. The LED flow cell was enclosed in a light-proof box (made from Perspex) and was connected to the amplifier (Fig 2.3 shows the circuit based on that of Betteridge *et al.* (1979); slightly modified and constructed in the Department of Oceanography of the Southampton University (England). The amplified signal was sent to the chart recorder. A four ways switching valve (Rheodyne 50) was modified to allow injection of reagents for the rFIA system. The injection volume was varied by changing the length of the loop, which was made from Teflon tubing. To inject volumes below 100 mm^3, a rotary injection valve (Rheodyne 7010) was employed. In addition to the peristaltic pump a ChemLab auto-analyzer pump was sometimes used to pump solutions through the system.

Reagents were prepared as described by Fernandez *et al.* (1985), except that the normality of the sulphuric acid solution was changed from 2N to 8N (as suggested by the same authors in a personal communication because in the published paper there is a typographic mistake). The Flaminox surfactant which is used as a stabilizer for the mixed reagent (MR) and

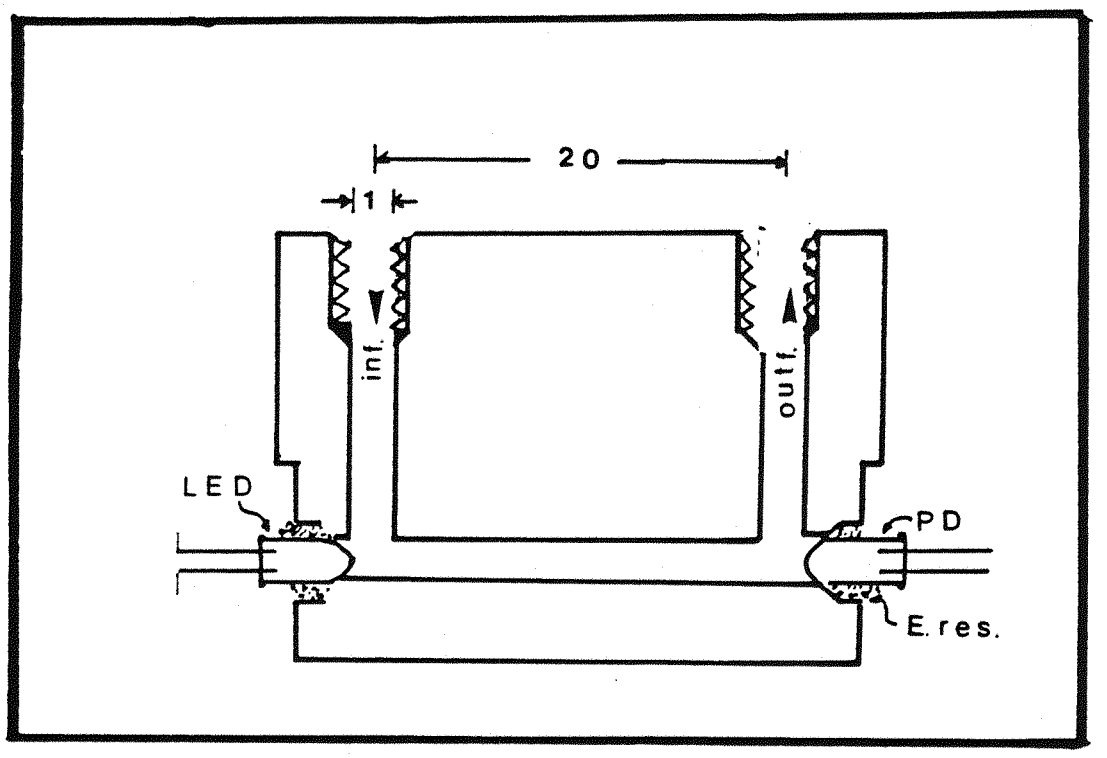


Fig. 2.2 LED flow cell made from Perspex. This a modified design of that of Betteridge *et al.* (1978). Dimensions in mm.

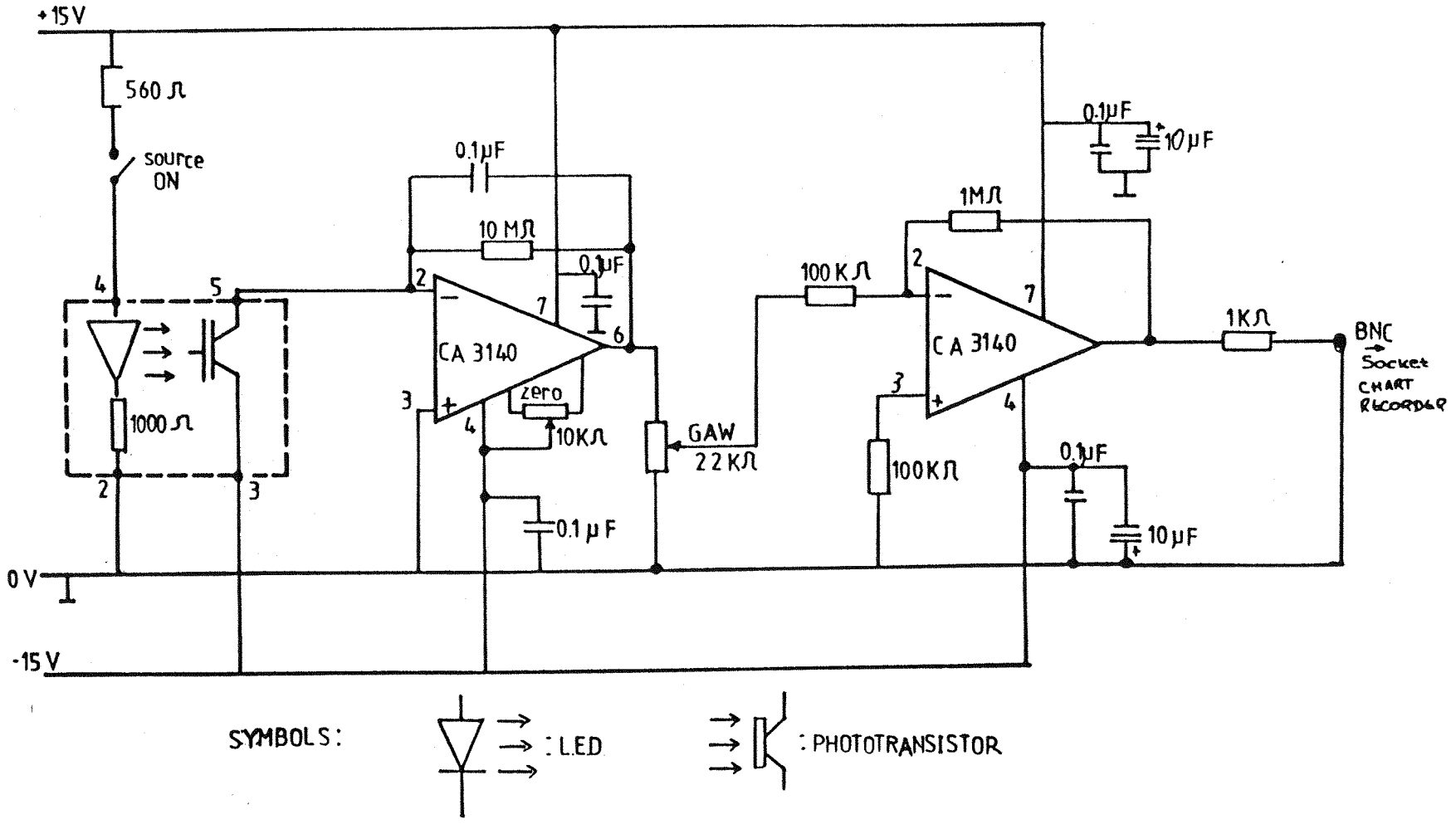


Fig. 2.3 FIA amplifier circuit diagram from Betteridge *et al.* (1978), with some minor modifications.

as a wetting agent, is no longer available from Fisher Co. It was replaced by Triton X-100 at similar concentrations to those reported for Flaminox.

Glassware and storage bottles were firstly washed with a warm (60^0C) 2 % (V/V) solution of phosphate-free Micro detergent. Secondly, they were thoroughly rinsed with tap and then distilled water. Thirdly, they were filled with or immersed in fresh 10 % (V/V) HCl solution and left for at least a week. Finally they were thoroughly rinsed with Distilled-Deionized Water (DDW). The HCl solution, glassware and storage bottles were kept just for the DIP work.

The DDW (18 Mega-ohm.cm; Milli-Q system) was used to prepare solutions and standards. Artificial sea water was prepared by dissolving 34 g of NaCl (Analytical grade) in a litre of DDW. Automatic pipettes (Gilson) fitted with previously cleaned tip were employed in the preparation of diluted standards.

For normal spectrophotometry a PYE UNICAM SP 500 MKII UV/Vis instrument was employed at a wavelength of 660 nm. The analytical protocol was the same as that given by Fernandez *et al.* (1985).

2.2.2 Optimization of the system parameters

Figure 2.4 shows the simplest manifold employed to test the malachite green method with a rFIA system. The effect of the following parameters on sensitivity, base-line drift and precision were investigated in turn: injection volume, length of the reaction tube, flow rate, concentration of the surfactant, detector, and temperature of the reaction mixture. The next experiments, unless otherwise specified, were performed using a spectrophotometer as detector, and a flow-through glass cell of 10 mm path.

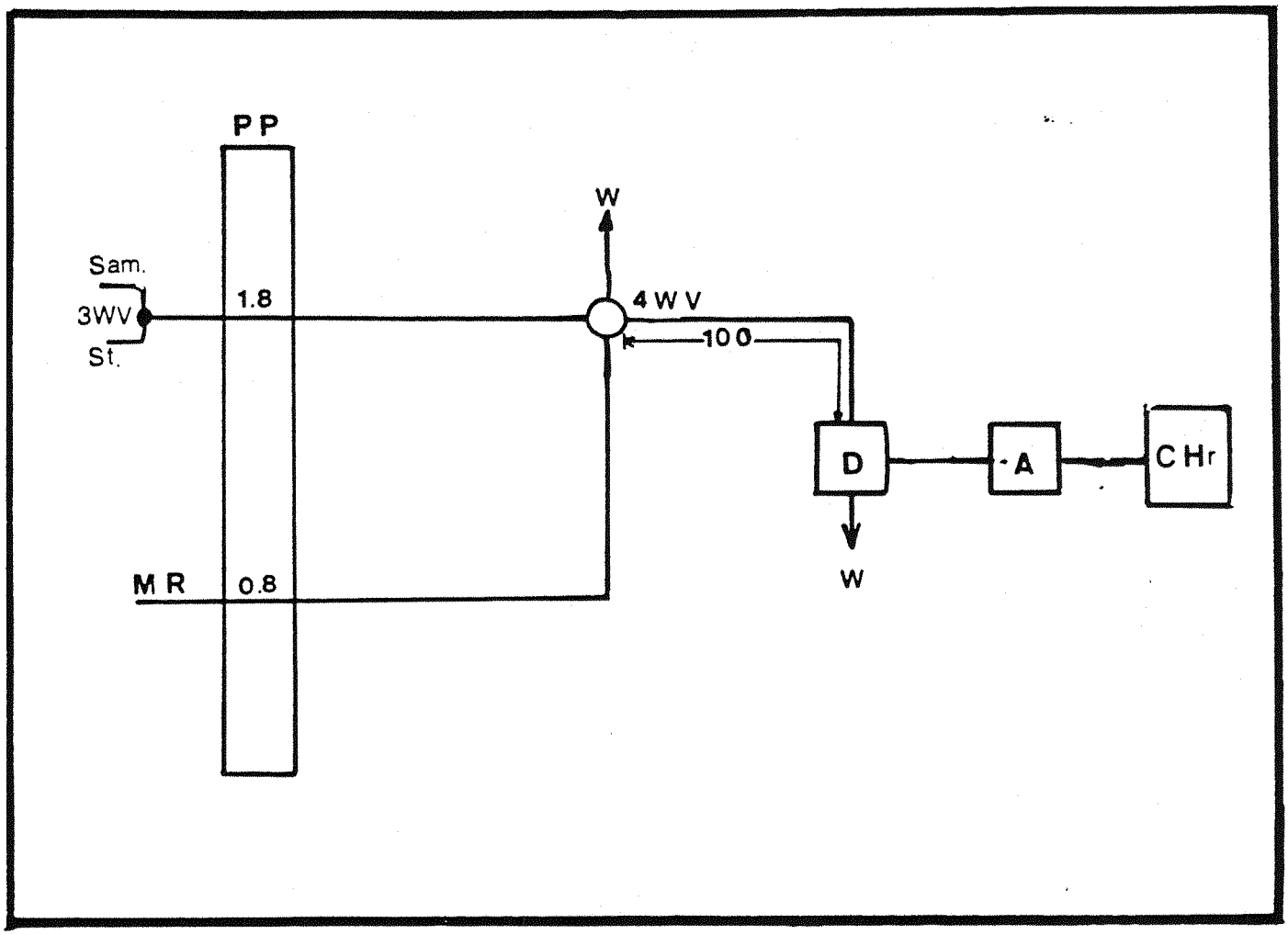


Fig. 2.4 The simplest manifold for a rFIA determination of DIP using malachite green as the chromophore. PP peristaltic pump with flow rate in $cm^3.min^{-1}$. W waste; 4WV 4 ways valve; MR mixture reagent solution; D detector (spectrophotometer or LED-detector); A amplifier, CHr. Chart recorder. See text for details of the system.

2.2.2.1 Injection volume

Ruzicka and Hansen (1979) suggested that for a FIA system with a reaction tube length of about 1000 mm, a flow rate of 1.8-2.0 $cm^3.min^{-1}$, and having mid-dispersion characteristics, injection volumes of about 100 mm^3 are optimum. Thus five different injection volumes were tested, i.e. 0.75, 0.50, 0.25, 0.125 and 0.020 cm^3. With the largest volume, the MR slug in the reaction coil was about 15 cm long and was unlikely to mix completely with the sample stream. From peaks obtained it was clear that the head of the slug of reagent was well mixed, whilst the middle did not seem to react. The tail of the slug was dispersed and mixed giving a small positive peak on the mean peak. The peak width was about 15 mm which was equivalent to 3 min. The baseline tended to drift by about 0.002 net absorbance units per minute.

When smaller volumes of MR were injected, although dispersion and drift of the baseline improved, refractive index problems affected peaks for concentrations below 1.0 μM.

Best results were obtained injecting 0.25 cm^3, when the sharpest peaks were attained. Drift of the baseline became significant at concentrations higher than 2.0 μM of DIP because of the memory effect (see below). Negative peaks for concentrations below 1 μM of DIP were observed and ascribed to a refractive index problem.

2.2.2.2 Length of the reaction coil

Reaction tube lengths of 1.0 and 0.50 m were tested. Using the latter length, higher peaks were obtained. This was probably due to 1) dispersion was not as high as in the 1.0 m length, and 2) less adsorption of the phospho-molybdenum-malachite green complex occurred on the tubing. However, further experiments showed that the MR in the 50 cm length coil was not fully mixed with sample, and thus the actual absorbance was caused by the MR itself, which according to Motomizu *et al.* (1982 and 1984) has a maximum absorbance at

630-620 nm. Kinetic experiments with the short coil showed no colour development because there was no mixing with the sample, whilst with the longer tube there was a colour development of only 50-60 % of the full value. It was therefore concluded, that the peaks given by the 0.5m tube were due to the MR itself rather to the phospho-molybdenum-malachite green complex, and thus, the 1.0 m long tube was chosen.

2.2.2.3 Flow rate

Using a reaction coil of 1.0m, the effect of the flow rate on height and shape of peaks was investigated. When the flow rate of the sample stream was below 0.5 or greater than 20 $cm^3.min^{-1}$, the height as well as the shape of the peak was negatively affected. A flow rate of about 1.8 $cm^3.min^{-1}$ appeared to give good results although negative peaks were still evident particularly at concentrations below 1.0 μM.

2.2.2.4 Concentration of Triton X-100

The surfactant, which acts as a stabilizer and wetting agent, has proved to be a source of problems (see Hess and Derr 1975, Lanzetta *et al.* 1979, and Fernandez *et al.* 1985), because it gives high blanks in the standard phospho-molybdenum blue method. The detergents previously reported by the above mentioned authors were Tween-20, Tergitol and Flaminox, but these are not currently available. It was thus decided to try Triton X-100 at similar concentrations to that reported by Fernandez *et al.* (1985). To investigate the effect of concentration, a series of blank solutions with different concentrations of Triton X-100 in the MR were processed. It was found that the addition of 4 cm^3 of 1 % (V/V) Triton X-100 to each 100 cm^3 of MR gave an adequate wetting effect as well as a low blank value. The stabilizing effect of the detergent on the malachite green reagent was not directly studied. However, it was observed that blank absorbances tended to increase drastically two or three days after the preparation of the MR. It thus was decided to prepare the MR daily.

2.2.2.5 Effect of heating the post injection tubing

Knowing that only 50-60 % of the full colour development was being achieved, in order to accelerate the reaction between sample and MR, the post injection tubing (about 0.40 m long) was immersed in warm water (about 50^0C). The residence time of the sample plus MR in the heating bath was roughly 4 seconds. However, no significant improvement in signal was found, because according to kinetic experiments run in stopped flow mode, the colour development with heating was only about 9 % higher than that obtained at room temperature. The use of a heating bath was thus discontinued because 1) heating the reaction tubing can produce air bubbles in the stream, which are undesirable for rFIA and FIA manifolds, 2) using a heating bath between the injection valve and detector is troublesome, specially for ship board conditions, 3) the refractive index problem was enhanced at higher temperature, and 4) there was only a small increase in sensitivity.

2.2.3 Limitations to the optimized procedure

Having chosen the optimum parameters a series of standards was run. A typical chart track and calibration are shown in Figs. 2.6 and 2.5 respectively. From the trace, two main problems are evident: 1) drift of the base line and negative peaks, with the former being more obvious at higher (2.0 μM) and the latter more remarkable at lower (< 0.2 μM) concentrations. The base line tended to drift about 0.17 % in transmission (T) per minute at low concentrations, while the negative peaks increased as concentrations of DIP decreased from 0.2 μM to analytical zero. Thus, for the blank, the negative peaks below the base line were 6.5 % T which is equivalent to about 0.2 μM of DIP. Peak widths were on average about 5 mm which is equivalent to 1 min.

The negative peaks and baseline drift could be ascribed to 1) adsorption/desorption of the phospho-molybdate complex onto the Teflon tubing, and colorimetric cell which has been called the "memory effect" by Eberlein and Kattner (1987), and 2) the

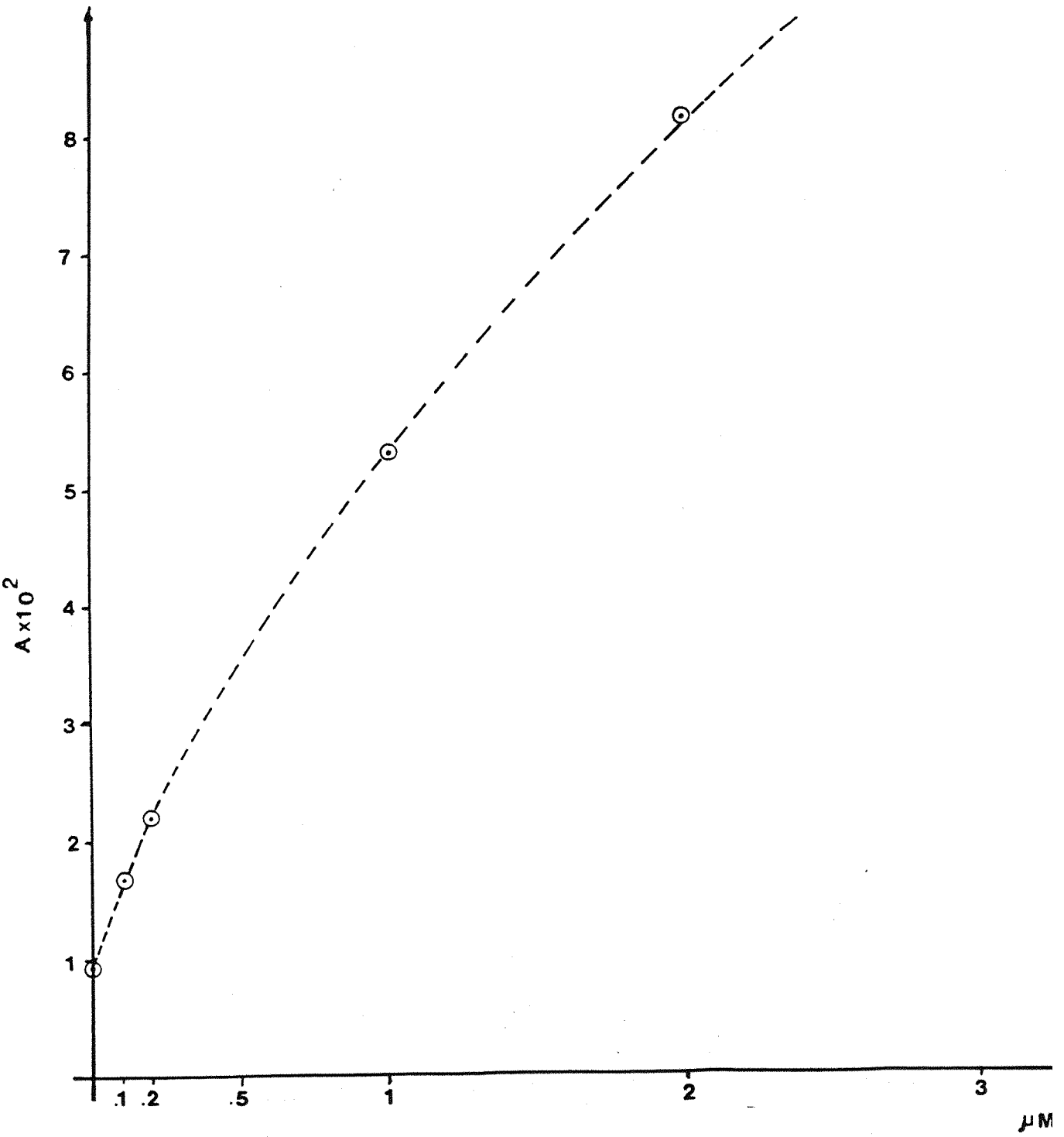


Fig. 2.5 Calibration curve for the malachite green/ rFIA system. The strictly linear range is 0-300 nM[P].

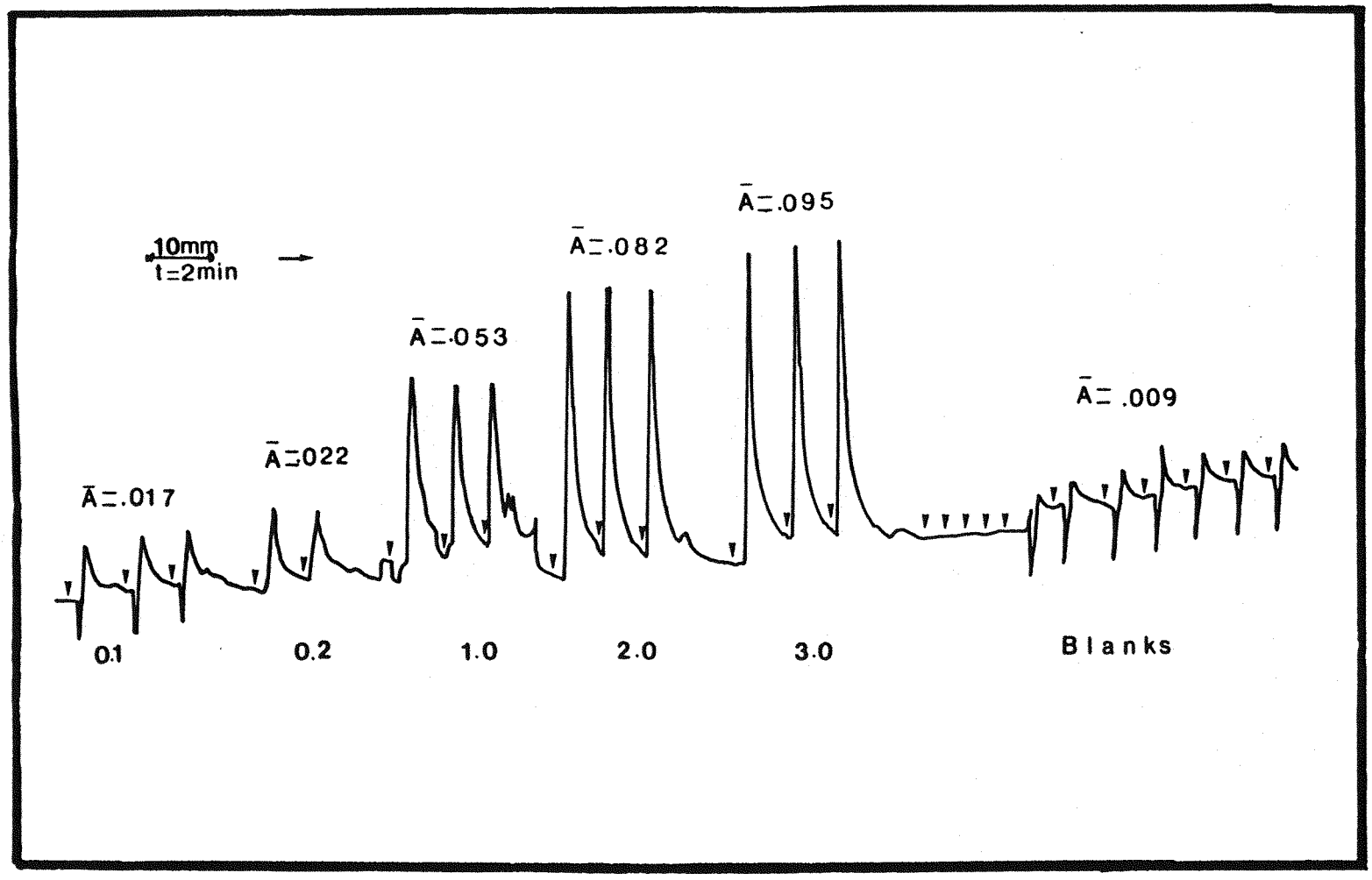


Fig. 2.6 Typical peaks obtained with/a/FIA/malachite green method. Arrows indicate individual baseline used for each measurement. Ā: average absorbance. DIP concentrations are in μM[P].

refractive index difference between the MR and the sample stream. The problems related to the refractive index and drift of the baseline were investigated as indicated below.

2.2.3.1 Baseline drift

The drift of the baseline appears to be almost completely due to adsorption/desorption of the phospho-molybdenum-malachite green complex on the polytetrafluoroethylene (PTFE) tubing. Both electrical noise and drift were insignificant. Thus, washing of the tubing wall was tried by injecting 0.250 cm^3 of 0.12 M HCl mixed with about 1 % Triton X-100 (5 cm^3 *per* 100 cm^3 of HCl solution), following the procedure of Fernandez *et al.* (1985). The injection of wash solution was made after the peak was recorded (see Fig 2.7A for manifold). The drift of the baseline was not improved but dispersion increased. A further approach to overcoming this problem may be to use glass instead of PTFE tubing as has been reported in SFA manifolds in association with the phospho-molybdenum blue method (Eberlein and Kattner 1987). These authors demonstrated that the memory effect was evident using PTFE tubing. Although, Johnson and Petty (1982) did not point out anything concerning this problem despite having used PTFE tubing for their FIA manifold for the determination of DIP. Fernandez *et al.* (1985) pointed out the necessity of using a glass sampler to avoid contamination and their manifold was made using plastic tubing.

2.2.3.2 The refractive index interference

This problem is likely to be quite difficult to overcome because of the significant difference in refractive index between the MR and samples of 0.0048. Nonetheless, attempts to tackle this problem were made. In the manifold B (Fig 2.7) the sample and MR were mixed at a T junction (in a 3:1 proportion) through 0.11 m of tubing, before injecting this mixture into the sample stream in an attempt to "matrix match" the reagent and sample refractive indices. However, negative

peaks were still almost the same size, whilst the higher dilution and dispersion of the MR gave smaller peak height for DIP standards.

2.2.3.3 The use of LEDs and Perspex flow-through cell

In a further experiment the physical configuration of the detector cell was changed and the first manifold was coupled to a Perspex flow-through cell (Fig. 2.2) with LED light source and a photo-transistor detector.

On the one hand, the negative peaks were no longer significant, but on the other hand the drift of the baseline was much more evident. The negative peaks presumably disappeared because of the construction and optics of the cell, which had a slightly longer diameter at the phototransistor window, so the parabolic refractive index gradients were flattened. The drift of the baseline was due to adsorption of the complex onto the Perspex cell itself, which became visibly contaminated by the malachite green or complex. The internal walls of the cell were a little rough, and thus the surface area for adsorption was increased relative to smoother surfaces. Furthermore, the LED output spectrum had its peak at 660 nm, whilst the MR had the maximum extinction at about 650 nm, and the sample plus MR solution had its maximum absorbance at about 630 nm. Thus a positive interference from the MR was observed and the use of this type of cell did not give satisfactory results under the experiment conditions.

2.2.4 Capabilities of the method

Useful measurements of peaks could be obtained at relatively high concentrations, despite the baseline drift and negative peaks problems. However, although it was possible to flush the post-injector tubing with plenty of sample without reagents to generate a baseline, the background refractive index signal made measurement of small peaks very difficult.

Table 2.2 displays values for absorbance, standard deviation (SD) and relative standard deviation (RSD) for different standards solutions for the method described. The detection limit (twice the SD of the blank) was 25 nM. The RSD ranged from 12 to 0.4 % for 0.1 and 3.0 μM DIP solutions respectively.

The method gave a k of 80000 $(mol.cm)^{-1}.1$ (calculated using the absorbance for a 0.1 μM DIP solution), which is about four times that reported by Murphy and Riley (1962) for the standard phospho-molybdenum blue method. It is however lower than the k reported by Fernandez *et al.* (1985, 120000 $(mol.cm^{-1}).1$). This dissimilarity could be ascribed to the different detergent, because both the k and the absorbance of blanks depends on the surfactant used. Fernandez *et al.* (1985) made use of Flaminox to achieve the value given above, but when they employed the alternative detergents NP-10 and 15-S15, both from Sigma (personal commun.), they found ks of only 60000-65000 $(mol.cm^{-1}).1$. Other authors have reported varying values of k dependent on the detergent employed, for instance, 112000 (Hess and Derr 1975, using Tween-20) and 80000 $(mol.cm^{-1}).1$ (Lanzeta *et al.* 1979, using Sterox).

2.2.5 Summary and conclusions of section 2.2

The technique has been shown to have a potentially good sensitivity, but its coupling to FIA or rFIA systems has been difficult. This is especially due to the refractive index and baseline drift problems. Also, the linear response of the method had only a very short range (0-100 nM). The "memory effect" drawback may be solved by replacing the PTFE tubing with glass. Manually, the technique worked as reported in the literature.

The malachite green analytical procedure coupled to a FIA or rFIA systems as developed was not adequate for the research intended, and therefore the use of a long capillary cell was investigated.

Concentration μM[P]	Blank	0.1	0.2	1.0	2.0	3.0
Absorbance. X	0.009	0.017	0.022	0.053	0.082	0.095
SD	0.001	0.002	0.002	0.004	0.005	0.005
RSD (%)	15	12	9.0	7.1	0.5	0.4
n	3	3	3	3	3	3

Tab. 2.2 The performance of the FIA system coupled to a malachite green method for the determination of DIP. X: average; n: number of samples and SD: standard deviation. Detection limit 25 nM (twice the SD of the blank absorbance readings). $k=80000$ $mol^{-1}.dm^3.cm^{-1}$, and linear range was 10 to 300 nM. See Fig. 2.5 for the calibration curve.

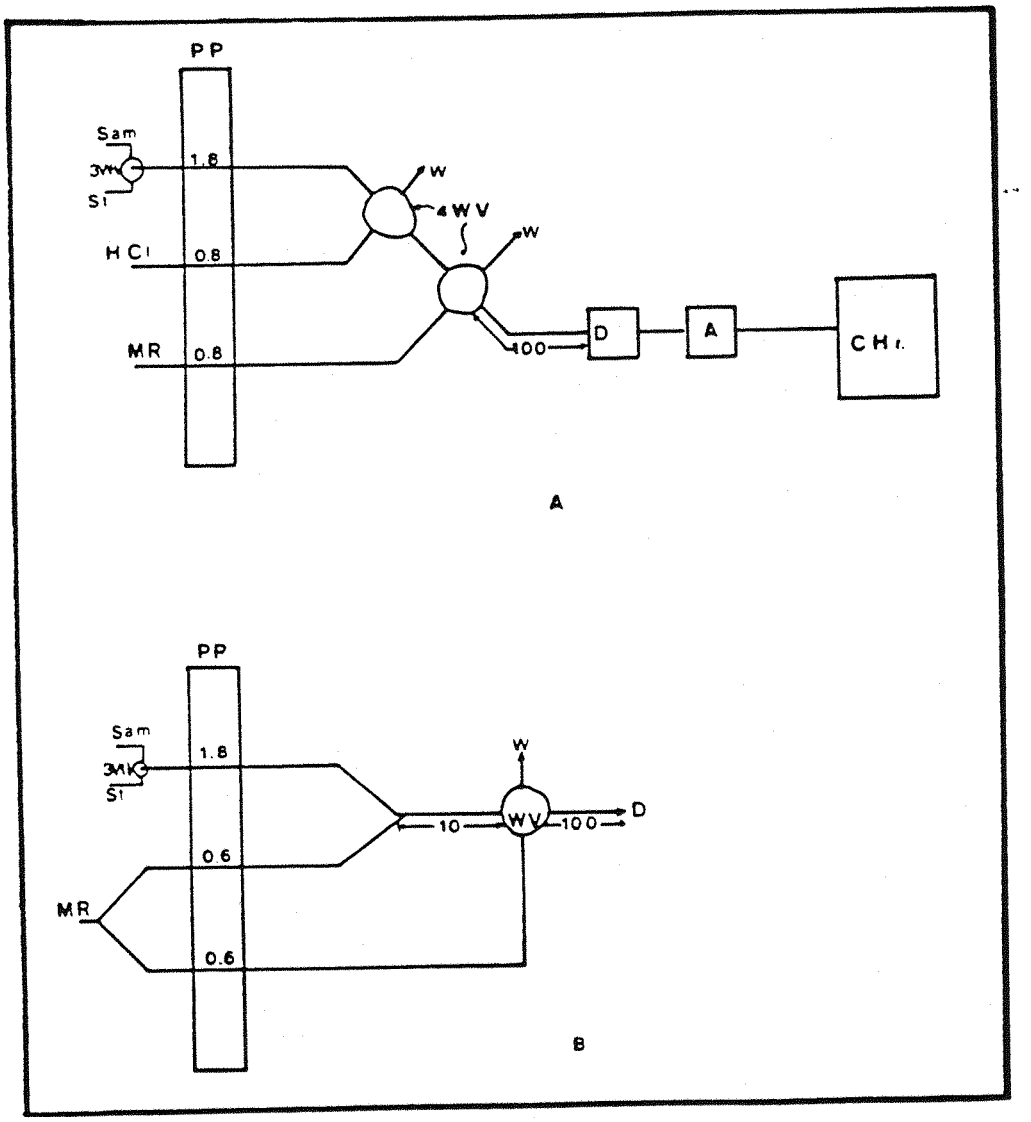


Fig. 2.7 Alternative manifolds developed to try to solve refractive index problems. In A an injection of 0.12 cm^3 of HCl is made. In B the MR solution is mixed with the sample (1:3 proportion). See Figs. 2.1 and 2.4 for abbreviations and text for details.

2.3 Development of a method for the determination of DIP in natural waters at nanomolar levels using a Long Capillary Cell

2.3.1 Materials and apparatus

Special attention was given to the treatment of the glassware used as indicated in the Section 2.2.1. When not kept in a 10 % (V/V) HCl bath, glassware was stored in dust-proof containers and was thoroughly rinsed before experiments in DDW. Glassware was only used for this particular analysis.

The components of the system are:

- Peristaltic pump: 8 rollers with four channels (REGLO-MS4-ISMATEC).
- Power Supply to give a current of up to 1.5 mA.
- Current stabilizer to supply a smooth current.
- Tungsten lamp and power source.
- Amplifier constructed in the Department workshop (see Diagram Fig. 2.3) based on the design of Betteridge *et al.* (1978)
- Infrared light emitting diodes (IRD) low (RS 306-077) and high (RS 635-296) power.
- Photo-transistors: RS 306-083 and RS 305-462.
- Chart Recorder: J.J. LLoyd Graphic 1002.
- Spectrophotometer: PYE UNICAM SP500 MKII UV/Vis.

- Tubing: Tygon 0.6 mm ID and teflon PTFE 0.8 mm ID. Silicon rubber joins.

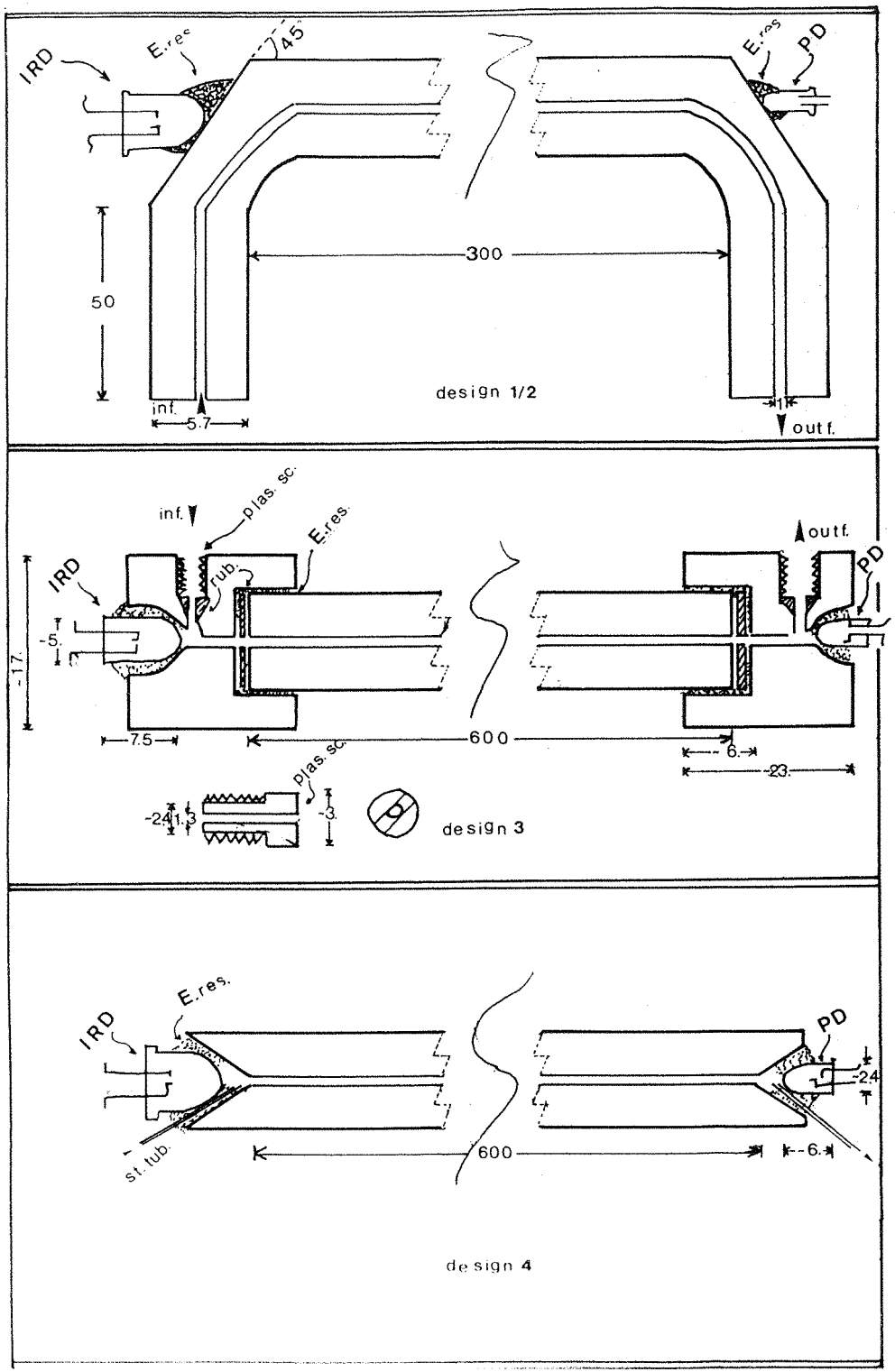
2.3.2 Development of the long capillary cell design

Figure 2.1 shows schematic diagrams of two approaches used. The system A is basically the same as that reported by Lei *et al.* (1983), whilst system B is the new approach developed.

The system A design of LCC is not shown in detail in the paper of Lei *et al.* (1983). Figure 2.8 displays different LCC designs investigated during development. The feasibility of design 1 was studied using two different sources of light; a Tungsten lamp and an infrared light emitting diode (LED). To obtain maximum internal reflection all the LCC designs were wrapped with aluminium foil, with brighter side facing the capillary tube. The ends of the LCC had to be protected from stray light using Al foil and light-proof tape. The Tungsten lamp had a controllable intensity. Convex silica glass lenses were used to focus the light with the LCC. An interference filter was employed to select a wavelength of 880 ± 10 nm. The selected wavelength was different to that reported by Lei *et al.* (1983), who employed a wavelength of 610 nm. The lamp was to mains powered through a stabilizer. The photodiode, with an 1 mm^2 sensitive area, was encased in a small Perspex block in order to protect it (not drawn in the diagram, Fig 2.8), and fixed, using fast setting epoxy resin. The signal from the photodetector was amplified by the amplifier and sent to a chart recorder. The lamp, lenses, filters, and LCC were on an optical bench, thus keeping the focal distance constant. The whole system was carefully covered by a black piece of thick plastic to exclude external light. To test the light-proof nature of the system, the photo-detector was switched on, and a torch light was directed onto it; no signal was seen.

With the light source switched off, the LCC filled with DDW, and the photo-transistor on, the 0 % T baseline was set up. Then the lamp was switched on to set up the 100 % T upper baseline. The chart recorder scale was set at 0.01 V, full scale deflection. The initial path length of the LCC was 300 mm, with ID and OD of about 0.8 and 7.0 mm respectively, i.e. similar to those reported by Lei *et al.* (1983).

Table 2.3 gives absorbance values for the system with different standard solutions. The absorbance of the LCC system was nearly five times higher than that of normal spectrophotometry. Although, the detection limit was about 5 nM, two drawbacks were found: low frequency noise (16 mHz),



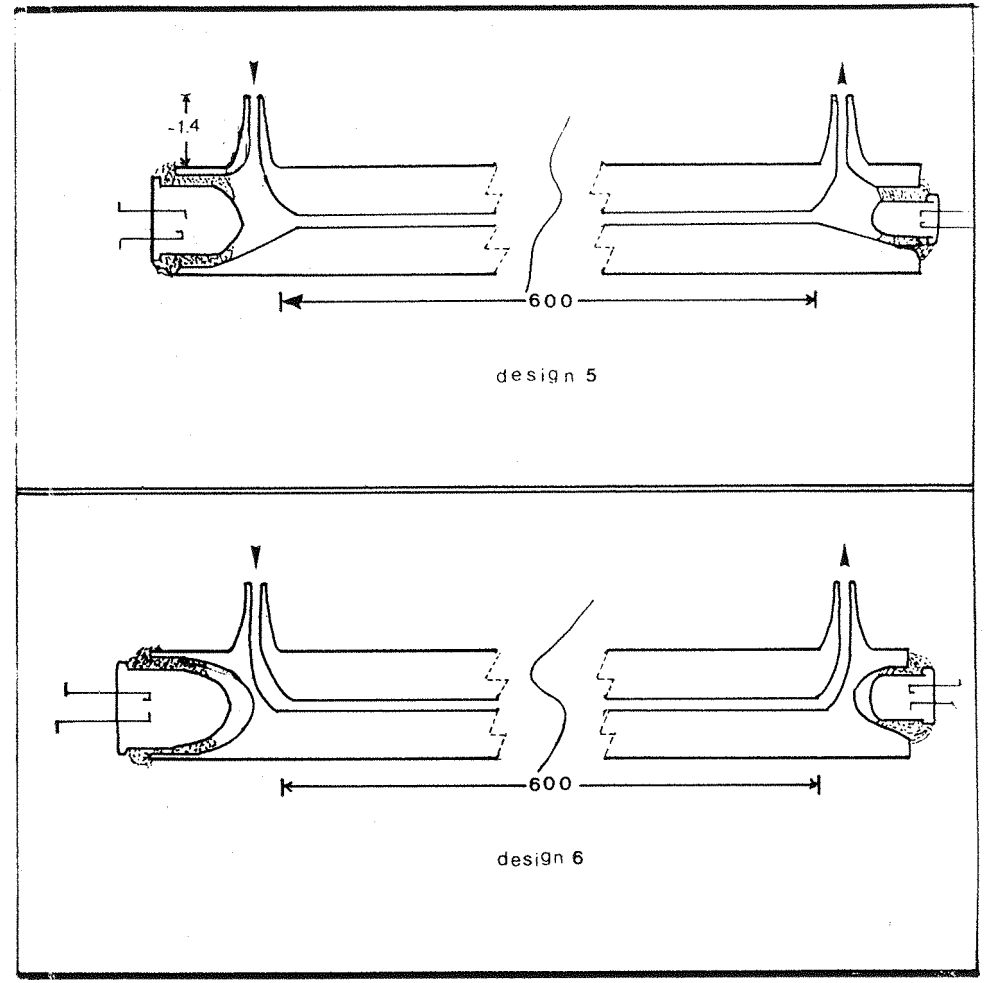


Fig 2.8. Cross sections of the different LCC designs. LCC design 1 is as 2, but with different source of light. OD 5.7-6.0, ID 1.0. IRD infrared emitting diode (low and high power); PD photodetector; plas. sc. plastic screw to hold the tubing into the LCC design 3; E. res. Epoxy resin; st. tub. stainless tubing 0.1 ID; rub silicon rubber. Units in mm. The diagrams are not necessarily at the same scale, for details refer to text.

and change of the upper baseline (100 % T) changed as a consequence of the variable output of the lamp. Increasing the intensity of the lamp did not solve this latter problem. It was decided to replace the Tungsten lamp with a more stable source from a system used in the spectrophotometric titration of dissolved oxygen.

Results using this source of light were also not satisfactory, because the absorbance value, for instance, for 100 nM droops from 0.100 to 0.047 A relative to the first design, and the electrical noise was about three times higher than with the previous lamp. Additional problems were that transmission increased or decreased with decreasing or increasing the flow rate (about 0.7 to 1.2 $cm^3.min^{-1}$) and the baseline drifted.

The first problem was solved by introducing the sample with a syringe rather than with a pump, but this produce an additional problem of increased possibilities of contamination. It was also later realized that there was a temperature effect on the photo-transistor because of the infrared radiation from the LED.

Lei *et al.* (1983) did not report problems with electrical noise, but nonetheless pointed out that it was most difficult to arrange the source of light. In subsequent designs, instead of a Tungsten lamp the use of an appropriate infra-red light emitting diode (LED) as the light source was investigated. As has been pointed out by Betteridge *et al.* (1978), Johnson and Petty (1982 and 1983), Johnson *et al.* (1985) and Ormaza-González (1987), LEDs are good substitutes for tungsten light sources. They have a stable light output, a relatively narrow spectral range, and a long life, and are small, robust, and inexpensive.

Two kind of infra-red LEDs (IRDs) are available, i.e. low and high power, which need a continuous maximum forward current of 40 and 150 mA respectively. Both have a maximum radiation output at 940-950 nm. The bandwidth between half

intensity points was 50 nm, and thus a beam of light generated by the IRD was nearly of ideal wavelength for the analysis of DIP, i.e. 885 nm. Also, the photo-transistor employed had a maximum response between 875 and 925 nm (RS Data Library, 1977).

The low power IRD and detector were cemented to the windows of the LCC design 2 (Fig. 2.8). Firstly the IRD was firmly glued and then the optimum position for the photo-transistor was found whilst power was supplied to both detector and emitter, by adjusting its position to give maximum transmission (T). The detector was held in this position until the epoxy resin hardened. Ambient light was excluded during this operation by using, for instance a black plastic bag.

The low power IRD gave only a very small output, i.e. the 100 % T signal without any DDW in the LCC was just 10 mV, and this was much lower with DDW in (5 mV). With this low level of signal, the response of the photo-detector was very noisy. A high power IRD was used in the same design with a variable power supply unit controlling its current between 0.075 to 0.15 amperes. With a forward current of 1.5 amperes this IRD had an output about 10 times higher than that of the lower power IRD. Thus the signal obtained for 100 nM DIP standard was 0.018 absorbance units, but the noise was still significant.

A major problem with the design 1/2 was that as much as 50 % of the energy was found to be going along the tube end near the IRD rather than along the other end near the photo-transistor. Additionally, the detector probably was less efficient because it was partially covered with the resin, and it was not possible to eliminate an air interface. Because of these problems LCC design 3 (Fig 2.8) was developed.

The end windows were made from Perspex because 1) its use has been reported in association with LEDs and photo-detector optical systems, 2) it is a transparent plastic, and 3) it is

easy to machine. Two lengths of LCC were investigated 300 and 600 mm, the latter because absorbances at least 6 times higher (applying the Lambert-Beer law) than that obtained in common spectrophotometry, were desired.

The IRD and sensor were glued into the new design, using the same procedure as indicated above. With a capillary of 600 mm long a net absorbance of 0..229 was obtained for a standard solution of 100 nM. This was about 5 times higher (see table 2.3) than using the 300 mm long cell (design 3) and more than 10 times higher than design 2 (see Table 2.3). The detection limit was below 10 nM, but the noise was still significant. Thus, it was demonstrated that 1) an IRD was a good source of light, and 2) the length of the LCC could be as long as 600 mm. However the detection limit was not low enough, and the noise was still a drawback. In addition, it was observed that the Perspex block ends and also the silicon rubber cement seals absorbed a significant amount of radiation.

In order to increase the intensity of radiation received by the photo-detector, and thus potentially reduce electrical noise found with amplification of small signals, a new design was made (Fig 2.8, design 4) in which the IRD and detector were in direct contact with the solutions. A much larger signal was achieved relative to the other designs. In this design stainless tubes of small ID (<0.5 mm) were used as out- and in-lets for samples. However this design was abandoned as encasing both diode and syringe together proved impracticable.

Design 5 was made to overcome the latter problem. Both emitter and sensor were encased in the LCC itself. With this design, the absorbance for 100 nM[DIP] was about 0.229, i.e. nearly 10 times higher than the absorbance obtained with the design 3. The noise was reduced to an acceptable level i.e. ± 6 mV, and a detection limit of about 3 nM was accomplished when the IRD had a forward current of 0.100 A. However, whilst design 5 give the best performance of those tested, problems of baseline drift still existed and experiments were undertaken to optimized this performance.

	Designs					
Conc. (μM)	1a	1b	2	5	5a	6
0.050	0.038	0.019	0.007	0.016	0.115	0.010
0.100	0.100	0.047	0.018	0.043	0.229	0.023
0.200	0.208	0.083	0.031	0.089	N. D.	0.042

Tab. 2.3 Absorbance values for the different LCC designs: 1a LCC with tungsten lamp. 1b: 1a but with different source of light. 2. low power IRD as light source. 5 and 5a high power IRD but different lengths; 300 and 600 mm respectively; 6 Normal spectrophotometry using 100 mm path length cuvettes.

2.3.3 Optimizing analytical conditions for design 5

2.3.3.1 Temperature effect

One of the potential problems using IRDs and photo-detectors, is the Johnson noise generated in the response of the latter (Dennis 1986). This is caused by an increase in temperature of the medium in contact with the photo-transistor by the infra-red radiation from the IRD. This is particularly so here where the sample volume is very small (<0.5 cm^3). Thus, it was observed that when the sample was left in the LCC transmission T decreased in time without any consistent trend. This effect was particularly observed after 10-15 min, and became greater if the forward current of the IRD was increased from 0.075 to 0.15 amperes. However when the sample was continuously flushed through the LCC the signal was stable. The sample can be introduced from either end, but it was preferable to inject it through the sensor end, in order to keep the sensor at constant sample and laboratory temperature. Also, it was observed in later experiments that a more stable response was obtained with a forward current of 75 mA on the IRD.

2.3.3.2 Flow rate effect

An effect of flow rate on transmission had been observed through all the experimental work, which was not correlated to the hydrodynamic characteristic of the cell. Different flow rates of 1.9, 0.54 and 0.22 $cm^3.min^{-1}$ of DDW were tested. For the second and third flow rates the effect on the T signal was evident. However with a flow rate of about 1.9 $cm^3.min^{-1}$ no changes in response were observed. Therefore, a flow rate of about 2 $cm^3.min^{-1}$ was employed thereafter. The exact reasons why the flow rate affects the signal were not directly investigated, but the most likely reason is that with the flow rate of 1.9 $cm^3.min^{-1}$ the sample inside was renewed quickly enough to avoid the increase of temperature on it (see above).

2.3.3.3 Drift of the baseline

The drift of the baseline appeared to be more evident with design 5 than with the previous designs. Electronic noise and adsorption of the phospho-molybdenum blue complex onto components of the LCC were regarded as likely causes of this problem.

Electronic drift was checked by registering the absorbance of DDW (i.e. 100 % T) which was pumped through the LCC for 1.5 hours. There was no drift of the baseline observed.

The adsorption hypothesis on two components was considered, i.e. 1) the delivery tubing: The PTFE and Tygon tubing could be the cause of the problem, because according to Eberlein and Katner (1987) a memory effect (i.e. adsorption/desorption) is evident for PTFE tubing. It is probably also a problem with Tygon, although Johnson and Petty (1982) did not report this effect in their FIA manifold for the determination of DIP. Thus a system was made in which the tubing manifold was entirely of glass and the sample was sucked through the LCC instead of being pumped from the IRD end. Almost the same drift in baseline was observed indicating the PTFE/Tygon tubing was not the major problem.

2) Adsorption onto the LCC walls, emitter and sensor, and epoxy resin: The design 6 (Fig 2.8) was especially constructed to investigate this problem. In this design the resin, emitter and sensor were not in contact with the sample stream which only contacted glass. The baseline drift was less but was still significant. It was thus concluded that some adsorption occurred onto the internal wall and components of the LCC.

At this stage, the next step was to investigate the use a wetting agent to 1) promote better flow characteristic and 2) avoid adsorption. The LCC design 5 was finally chosen because it gave the best results in terms of signal and low noise. The design 6 was abandoned due to its poor optics

design, i.e. the signal obtained from it was low and its construction posed more constraints than design 5. Thus, design 5 was used for the next experiments.

In section 2.4 it is explained why the samples for this LCC system must be spiked with Dodecyltrimethylammonium bromide (DTMAB) to give a final concentration of the detergent of 0.0001-0.0002 % . Due to the wider implications of the use of surfactants in DIP analysis, this work has been considered separately. At the recommended concentration this detergent proved to be the most appropriate in terms of low blanks, no chemical interference on the method; it gave a high wetting efficiency thus avoiding adsorption and improving flow characteristics.

2.3.4 Discussion of the LCC development work

A study of theoretical aspects of how the Lambert-Beer law applies to LCC systems has been briefly discussed by Lei *et al.* (1983). According to these authors the law behaved as predicted, but the relationship A=kbC (Eq. 2.1) cannot be strictly applied because the light beam travels by different paths through the cell (Fig. 2.13, page 76). Thus the actual path-length is longer than the overall direct length of the cell. This was corroborated by the results obtained. For instance with the 600 mm LCC, the net absorbance reading for 500 nM[DIP] standard solution was 1.011; this same standard gave an absorbance of 0.113 using normal spectrophotometry (100 mm path-length cell). Thus the LCC system gave an absorbance 8.9 times higher than the latter one, although the LCC was only 6.0 times longer.

The Lambert-Beer law also states that the Eq. 2.1 strictly applies to monochromatic light. The IRD has a bandwidth of ± 50 nm which is comparable with the bandwidth given by interference filters, for instance, those used in the first experiment (section 2.3.2) had a bandwidth of ± 20 nm.

In this case the IRD was suitable for the method, because

its maximum emission peak was in the range 900-1000 nm, with the maximum point at 950 nm. Thus, the maximum output wavelength was very close to the actual wavelength recommended for the analysis (895 nm). As the sensor was a photodiode with maximum response between 875 and 925 nm, the light source and emitter were closely matched. Furthermore, the response time of the sensor was 50 ns and reproducible and, the noise of the whole system was acceptable, i.e. 6 mV in a full scale of 1000 mV.

2.3.5 Analytical procedure

The final analytical system is shown schematically in Figure 2.9 and uses design 5 (Fig. 2.8) for the LCC. The phospho-molybdenum blue complex is initially developed in 25 cm^3 conical flasks using a sample size of 10 cm^3. The reagents used and the procedure are as given in Strickland and Parsons (1972). After a 10 min development period, 10 mm^3 of a 0.25 % (W/V) solution of DTMAB is added, the solution mixed, and after 30 min it is then pumped into the LCC. The peristaltic pump is connected to both inlet and outlet so that the sample is both pushed into and pulled out of the LCC. Any change in baseline is monitored by periodic introduction of a 0.0001-0.0002 % solution of DTMAB in DDW into the cell. The peristaltic pump is positioned up stream of the detector end of the LCC, and the sample and blank solutions are passed through at 2 $cm^3.min^{-1}$. The three way valve allows simple, bubble-free changes between solutions. Caution is required to exclude air bubbles from the pumped solution, if these are introduced into the LCC they can cause large and rapid changes in transmission. The output is monitored on a chart recorder (0-10 V range). The method is calibrated using standards in distilled water for both seawater and freshwater samples because of the difficulty in obtaining "phosphorus-free" seawater. The baseline against which blanks and standards are measured is obtained using DDW plus surfactant at the same concentration as in the standards. Alternatively for marine waters, low DIP seawater obtained by ageing in low density polyethylene at room temperature, can be spiked with DIP, and

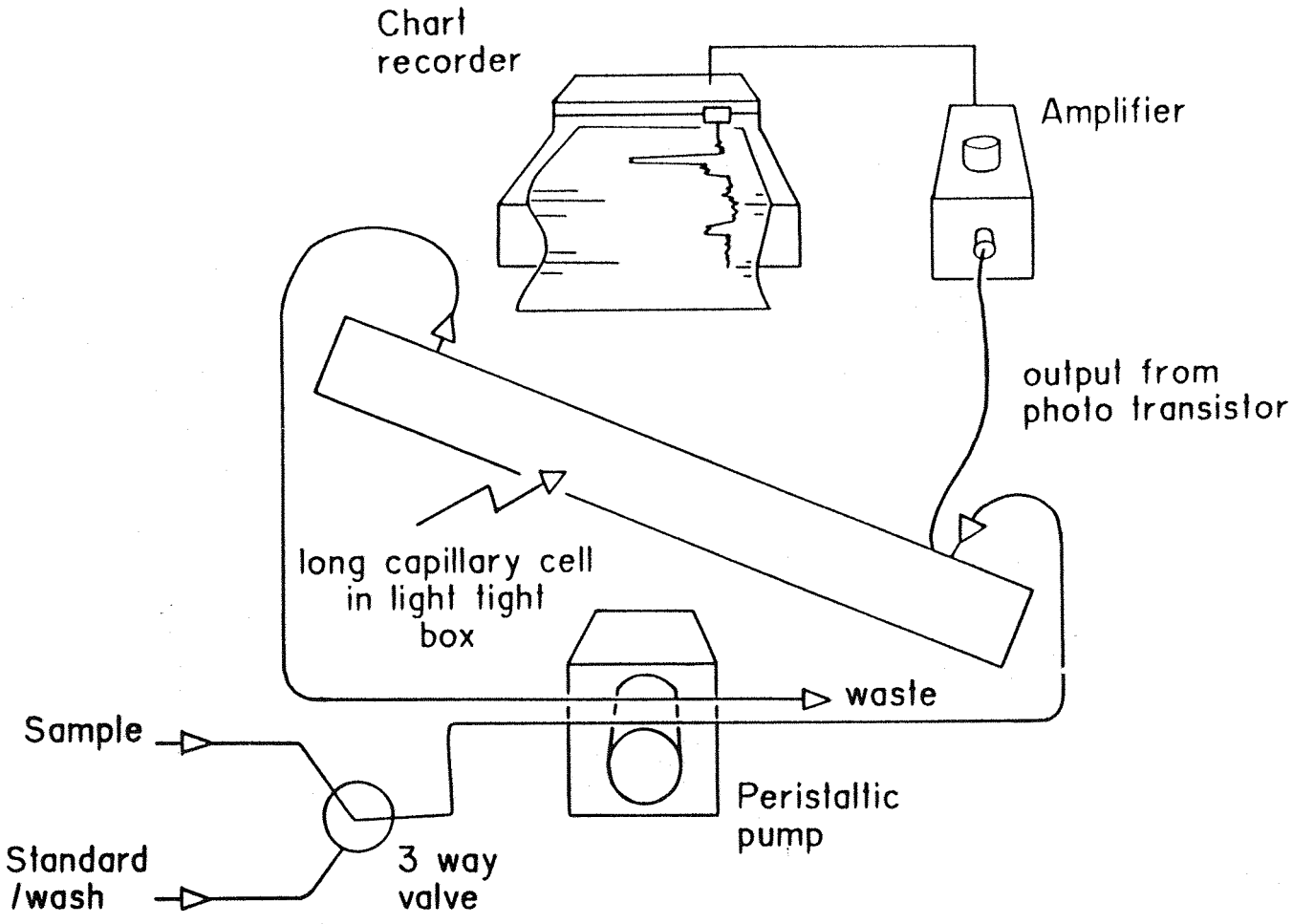


Fig 2.9 Schematic view of the LCC system constructed and its components.

the slope of the calibration curve obtained from these measurements. Whilst there is no significant difference in calibration slope between standards in DDW or seawater, there is a small decrease in transmission for seawater samples relative to DDW equivalent to about 13 nM for North Sea water, which is ascribed to differences in refractive index. Thus, for seawater samples the baseline was obtained using a seawater to which had been added the surfactant but no reagents; the analogous procedure is used for fresh waters.

2.3.6 Method performance

An example of typical chart recorder output is shown in Figure 2.10, and calibration curves are shown in Figure 2.11. Because of the non-monochromatic nature of the light source, some curvature of the absorbance-concentration relationship is to be expected at higher concentrations than the linear limit (500 nM).

About 30 individual samples per hour can be analyzed using this manual procedure, with a detection limit (2 times the standard deviation of the blank) of 1 nM DIP, but under ship board conditions electrical noise could increase the detection limit to 2 nM. Precision at the concentration of the blank (typically equivalent to 8 nM) is 6 %. A comparison of data obtained by the LCC system and conventional spectrophotometry is given in Table 2.4, and the increased sensitivity and precision is clear. The sample volume required (10 cm^3) is much less than other techniques such as solvent extraction (*circa* 350 cm^3).

2.3.7 Interference from arsenate

Interference from arsenate in the analytical procedure employed here was not directly tested. However, from the data on DIP obtained in the surface waters of the Sargasso Sea (chapter 4) and that reported in the literature, it can be deemed that there is no detectable interference from arsenate.

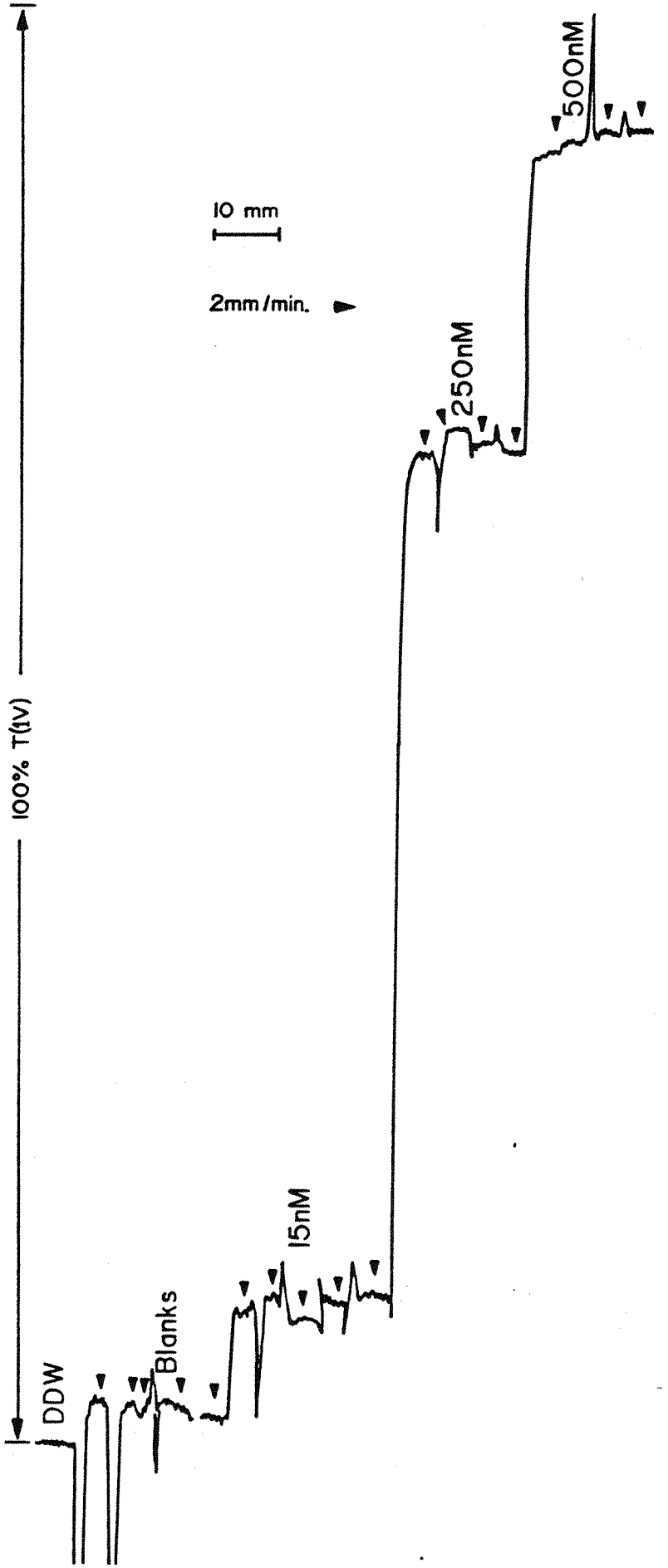


Fig 2.10 Typical chart recorder trace for the optimized LCC system. Arrows indicate plateau of individual samples.

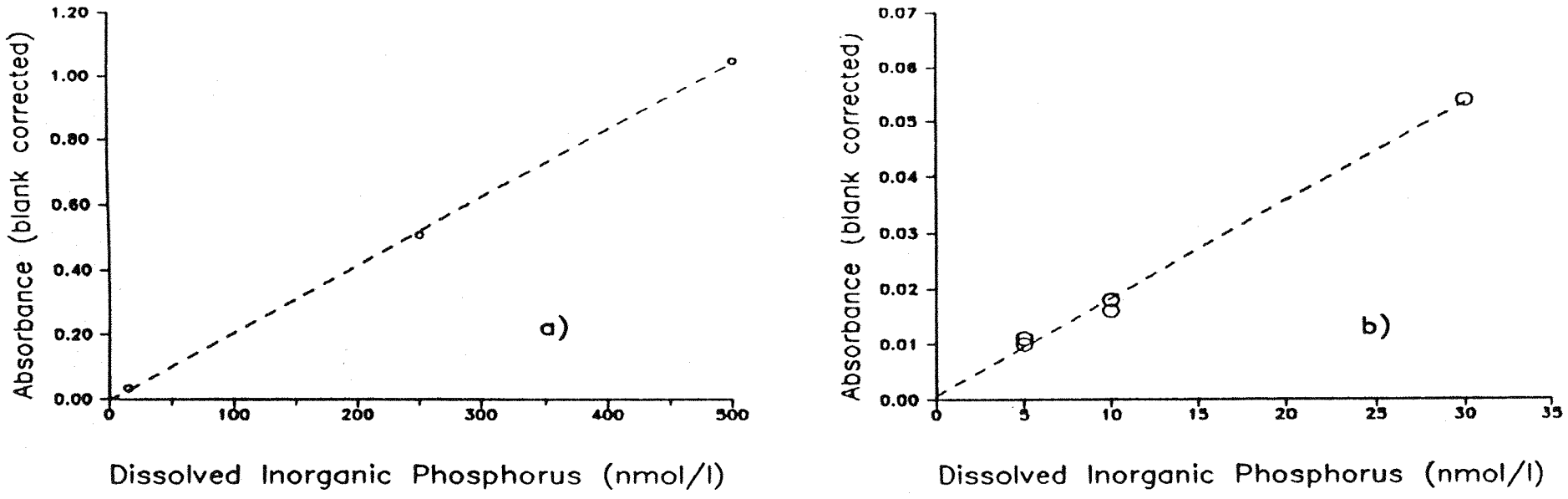


Fig 2.11. Calibration curve achieved with the optimized LCC system. a) 0-500 nM and b) 0-30 nM.

Samples (days from start of experiment)	Long Capillary Cell	Conventional spectrophotometry
0	21 ± 1	25 ± 12
2	156 ± 16	150 ± 10*
4	192 ± 6	179 ± 10*
8	55 ± 0	60 ± 5
10	16 ± 0	25 ± 12
12	11 ± 1	<
14	17 ± 1	<
16	43 ± 3	37 ± 12
18	22 ± 4	<

Tab. 2.4 Comparison data using LCC and conventional spectrphotometry (using 100 or 40 mm cell as indicated) for GF/F filtered solutions from a culture of *Phaeocystis pouchetii*. The time 0 sample is seawater without added culture; for the time=2 days onwards the data show the DIP added with culture being removed from solution with some recycling later, by the micro-organisms present. The data are the mean of three replicate analysis, and are given with ± one standard deviation. Data are in nM DIP. Samples below detection limit are indicated <. *) 40 mm cells, all other samples for conventional spectrophotometry used 100 mm cells.

In Sargasso Sea waters, concentrations of DIP at or below 2 nM have been observed whilst concentrations of dissolved arsenate are about 30 nM (Byrd 1990). Thus, even at molar ratios of about 15:1 to 1:1 for As:P, no significant interference was evident. A similar lack of interference from arsenic has previously been reported for high sensitivity methods (Koroleff 1983, Lei *et al.* 1983). These observations presumably reflect the relatively slow formation of the arseno-molybdate blue complex relative to the phosphorus equivalent (Chamberlain and Shapiro 1969).

2.3.8 Use of the system in environmental studies

The method has been applied to both marine and freshwater systems, and is thus applicable to natural waters in a range of environments where lack of sensitivity has previously precluded investigations. Figure 2.12 shows the DIP behaviour in *Phaeocystis pouchetii* cultures under short supply of DIP. The algae was cultured in two different flasks (dupli. 1 and 2 in the caption of Fig. 2.12) and samples were analyzed immediately by triplicate after subsampling and filtration. Table 2.5 gives data for nutrient depleted lake and stream waters in the Lake District of North-Western England; samples were filtered through Whatman GF/F filters, which had been rinsed with 70-100 cm^3 of sample to remove soluble P contaminants. Both data sets demonstrate the capabilities of the technique to precisely measure low concentrations of DIP. The second set of data also indicate the extremely low concentrations that can be found in natural waters, and it is therefore of great importance to maintain strict contamination control measures during sampling, storage, and analysis of these samples. There are clear analogies with developments in the field of dissolved trace metals in seawater over the last decade, and approaches used for metals may also be appropriate for low level P analysis.

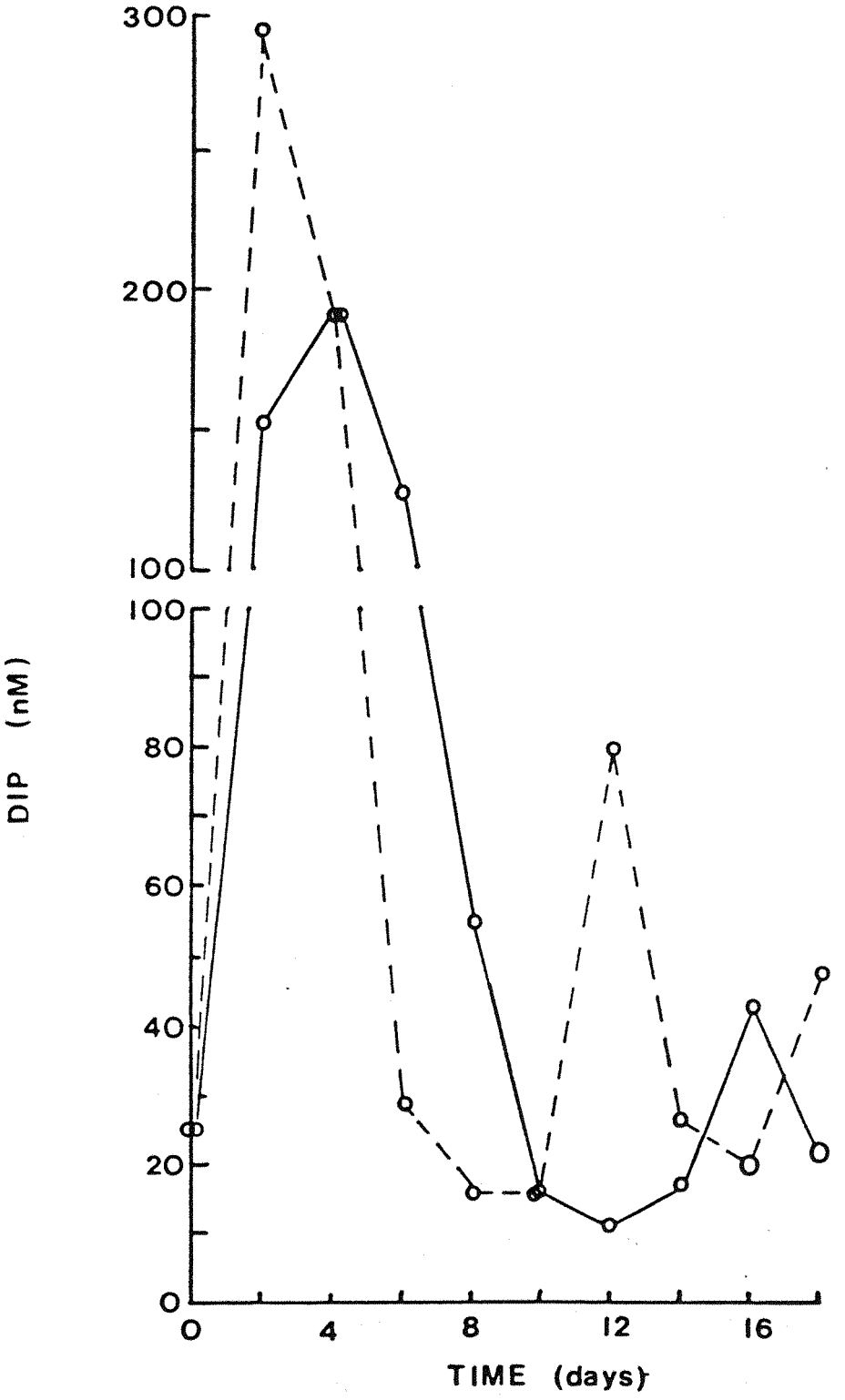


Fig 2.12. Mesurements of DIP by the LCC systems in cultures of *Phaeocystis pouchetii* in North Sea water. The initial peak corresponds to DIP added with the inoculum of the organism. The radius of the symbol is equivalent to one standard deviation. - - - -: dupli.1, ——:dupli.2

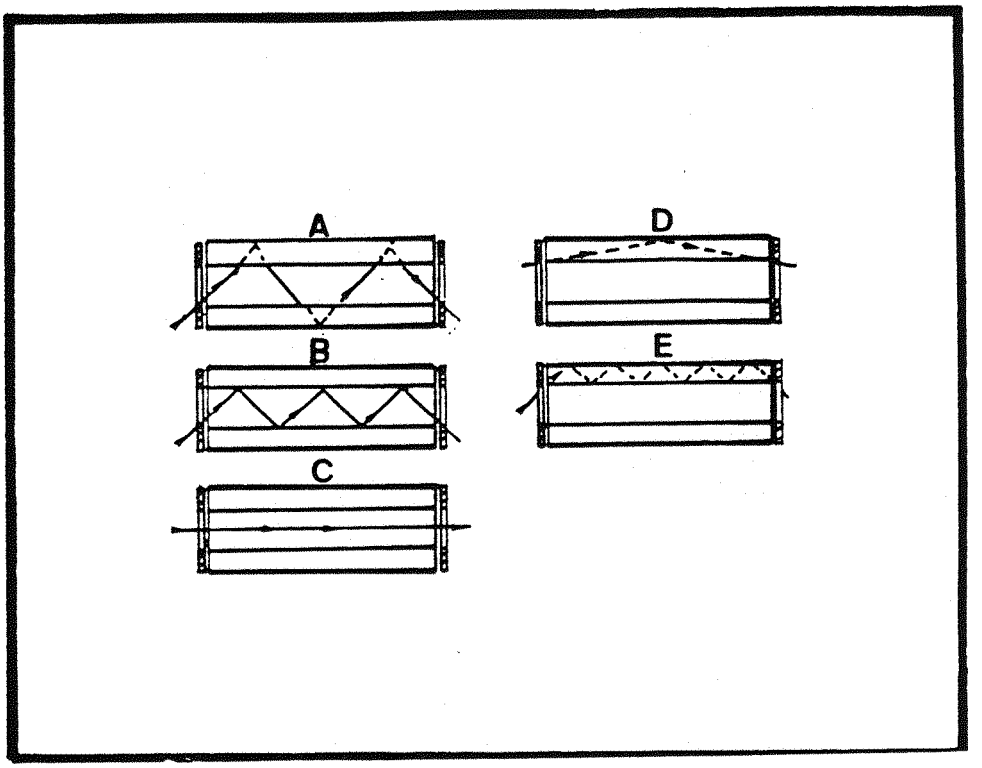


Fig 2.13 The different ways of a ray of light that travels along the LCC, whose external walls are wrapped with aluminium foil. After Lei *et al.* (1983).

Sampling site	DIP concentration [nM]	
	Replicate 1	Replicate 2
Eller Dubs	12 ± 2	* 22 ± 1
Mosedale Beck	7 ± 2	No sample
Doe House Gill	1 ± 0	<
Gaitscate Gill	5 ± 0	3 ± 0
Hard Not Gill	* 11 ± 5	2 ± 0
Wastewater	2 ± 1	3 ± 0
Ennerdele	8 ± 2	7 ± 2
Crummock	15 ± 1	12 ± 1
Buttermere	* 7 ± 0	<

Table. 2.5 Measurement of DIP in the Lake District (England). *: sample concentrations are dubious. < below 1 nM.

2.3.9 Conclusions for section 2.3

The LCC system described here provides a simple, rapid, and inexpensive technique for the determination of nanomolar concentrations of DIP in natural waters, which can be used in the laboratory or the field. The spectrophotometric system is reproducible, with low electrical noise. These characteristics together with selectivity, a good detection limit, linearity of the calibration curve and rapid and reproducible response comply with the *sine qua non* conditions of a flow-through cell established by Poppe (1980) and Ruzicka and Hansen (1981). The small refractive index effect noted should not be a problem in normal applications, as regions of significantly varying salinities such as estuaries have DIP concentrations generally higher than appropriate to this technique, and low DIP environments such as oligotrophic gyre waters and nutrient poor lakes, have relatively consistent major ion compositions. The LCC technique is compatible with conventional measurements at higher concentrations using the phospho-molybdenum blue method. As such, the potential limitations of the reactive DIP measurement, e.g. in not distinguishing between labile organic compounds and true DIP, must be considered during the interpretation of field data (Burton 1973, Cembella *et al.* 1983).

The use of an LED and photo-transistor combined with LCC system has proved to be a powerful technique, and it may eventually be applied to other analysis of others micronutrients where lack of a sensitive detector has previously limited their application. Drs. F. Lipschzultz (BBSR), P. Statham, and the author have already made initial work to measure nitrate-nitrite using this system and detected about 5 nM of nitrite. The application of the LCC technique to trace metal determination is also possible. The technique presented is a suitable, cheap, reliable, and versatile system which can advantageously replace former methods for the determination of nanomolar concentrations of DIP in natural waters.

2.4 The determination of dissolved total phosphorus in natural waters: An inter-comparison of five techniques

2.4.1 Introduction

The determination of dissolved organic phosphorus (DOP) in natural waters is important, because DOP plays a relevant role in biological processes, and has biogeochemical connotations (Smith *et al.* 1986). Since Chu (1942) reported that *Phaeocystis pouchetii, Skeletonema costatum* and *Nitzschia closterium* were able to obtain their inorganic phosphorus requirements from the organic compounds glycero-6-phosphate (G-6-P), phytin, and lecithin, many workers have reported that phytoplankton is capable of assimilating P from the organic fraction (e.g. Cembella *et al.* 1983, Veldhuis and Admiraal 1987, Veldhuis *et al.* 1987 etc). Also, DOP has been reported as a significant fraction of the dissolved total phosphorus (TDP, i.e. DOP plus dissolved inorganic phosphorus, DIP) in natural waters, particularly where there is biological activity. In surface marine waters, especially in oligotrophic waters, DOP levels generally surpass those of DIP (e.g. Smith *et al.* 1986, Orret and Karl 1987, Sapozhnikov 1988, Walsh 1989, Ormaza-González and Statham 1990d). Romankevich (1984) reported that DOP ranges from the detection limit (typically 0.030 μM) up to 1.68 μM in ocean waters. In the euphotic zone typical DOP values fluctuate between 0.06 and 0.97 μM in association with DIP levels near or below the detection limit. Below the euphotic layer DOP concentrations range from detection limit to 0.32 μM, although higher values have been reported (0.97-1.94 μM, Romankevich 1984). Regarding inland and coastal waters DOP is again usually at higher concentrations than DIP, and even in pristine rivers and estuaries DOP has been observed in some cases to exceed DIP concentrations (Ormaza-González and Statham 1990a).

In order to better understand the role of DOP in biological processes, and the P cycle in the aquatic environment, a precise and accurate means of measuring this fraction is essential.

Dissolved Organic Phosphorus is generally quantified as the difference between DTP and DIP fractions in a single sample. Most of the existing analytical approaches for DOP are based on the decomposition of the DOP to DIP with further phospho-molybdenum blue colorimetric analysis (Murphy and Riley 1962) or its modifications (e.g. Strickland and Parsons 1972, Fujiwara *et al.* 1982, Koroleff 1983, etc). However, non-spectrophotometric analytical approaches have also been developed, e.g. flame ionization photometry (Nakajima 1984), phosphine generation coupled to gas chromatography (Hashimoto *et al.* 1985 and 1987), electro-chemistry (Kolesar and Walser 1988a and 1988b), electro-thermal atomic absorption spectrometry (Kubota *et al.* 1988).

The phospho-molybdenum blue technique for DIP has received essentially universal acceptance as a standard procedure because, 1) it is simple, using only one mixed reagent, 2) the reaction is quick, 3) there are usually no interferences and 4) precision and detection limit are good. Nonetheless, the procedure for the determination of DOP has not yet been standardized. There are many quoted methods, and Table 2.6 provides a resume of most of these.

Each of these methods has its own advantages and disadvantages from the points of view of safety, reproducibility, detection limit, chemical interferences, operational range, ease of use, and the most important factor, decomposition efficiency (DE) i.e. the fraction of DOP present in the sample which is converted to DIP without losses during the analytical process.

According to the available literature no simultaneous intercomparison of more than two methods has yet been reported, see for instance Ridal and Moore (1990). Thus, it seemed worthwhile carrying out a concurrent inter-comparison of five of the most commonly used methods (see Section 2.4.2.2 below), which have potentially good DEs. Although the inter-inter-comparison was aimed mainly at assessing the DE, the

Author	Oxid. Agent	Range μM[P]	Precision RSD (%)	Comments
1	A and B	N.R.	N.R.	High/variable blanks
2	A	N.R.	N.R.	
3	C	0.50-2.90	N.R.	No autoclave
4	C	0.01-28.0	15.0	Autoclave.
5	C	0.25-7.00	8.0	Autoclave under alkaline conditions
6	D	0.12-18.0	1.0	High T (450^0C)
7	E	0.08-6.0	16	Possibility of violent reaction
8	N.O.	0.03-25.0	N.R.	UV oxidation, no hydrolysis.
9	F	0.050-N.R.	N.R.	Spattering salt problem
10	A @ C	N.R.-161.	2.0	Sample sealed in ampoules and autoclaved
11	C	0.03-N.R.	N.R.	Automate system eliminated handling

Table 2.6 Different methods reported for the analysis of DOP in natural waters: Authors 1) Redfield *et al.*(1937). 2) Harvey (1969). 3) Menzel and Corwin (1965). 4) Koroleff (1983). 5) Valderrama (1981). 6) Solorzano and Sharp (1980). 7) Strickland and Parsons (1972). 8) Armstrong *et al.* (1966). 9) Cembella *et al.* (1986). 10) Descas and Fangered (1981). 11) Jefries *et al.* (1979). Oxidant agents: A) H_2SO_4, B) H_2O_2, C) $K_2S_2O_8$, D) $MgSO_4$, E) ClO_4^-, F) $Mg(NO_3)_2$. Abbreviations. N.R.: Not reported; UV: ultraviolet; N.O.: no oxidant agent.

other factors important in the analysis as mentioned above, were also examined.

Two approaches to assessing the DEs of the methods were used. Firstly, the techniques were applied to a range of standard organo-P compounds having a diverse range of structure, and then secondly, natural water samples were analyzed. The samples were assumed to contain naturally occurring organic P compounds, which were representative of the spectrum of organic P materials in the aquatic environment.

2.4.2 Materials and methods

2.4.2.1 Cleaning procedures

All glassware was Distilled Water (DW) rinsed and then soaked overnight with sulphochromic solution (7 % w/v) potassium dichromat in 75 % (V/V) sulphuric acid, as suggested by Dr. L. Solórzano (pers. comm.) and finally rinsed with plenty of Distilled Deionized Water (DDW, MQ system 18 Mega ohm.cm). All glassware which could not be cleaned with sulphochromic solution was heated at 500^0C for 5 h, except Whatman GF/F filters which were heated for only 2 h. The glassware was only used for these experiments and intensively rinsed immediately before and after analysis, and thoroughly recleaned every 10-14 days. Reagents were prepared from analytical grade chemicals and stored as indicated below.

2.4.2.2 Methods employed and modifications

All the techniques tested rely on the conversion of DOP to DIP which is ultimately determined colorimetrically by the phospho-molybdenum blue method (Murphy and Riley 1962, as modified by Strickland and Parsons 1972). Their basic oxidation and hydrolysis principles are given below, together with the minor modifications used here.

1) Cembella *et al.* (1986): Heating a sample to dryness with $Mg(NO_3)_2$, then hydrolysis with HCl. This method is called hereafter Nit-ox.

Erlenmeyer flasks of 250 cm^3, glass boiling chips, and heavy glass reagent bottle stoppers were employed, instead of 120 cm^3 Erlenmeyer flasks, carborundum boiling chips and thick 2 mm glass stoppers. One half of the sample volume suggested was used, with a proportional reduction in the amount of added reagents.

2) Solórzano and Sharp (1980). Heating a sample to dryness with magnesium persulphate and baking the residue at 450-500^{0}C. Next hydrolysis with HCl. Hereafter the method is called High-t.

Erlemenyer flasks of 25 cm^3 were used instead of weighing bottles, in which 10 cm^3 of sample was evaporated to dryness. For the baking step, the flasks were capped with aluminium foil, which had been previously cleaned by heating at 400^{0}C for 2 hours. For the hydrolysis step, conical drop shape refluxing glass stoppers were employed to ensure efficient refluxing of the acid.

3) Valderrama (1981). Oxidation under alkaline conditions (pH starts at *circa* 9.7 and ends at 4-5) during the 30 min autoclaving step. The oxidizing agent was the same as in the Ac-c method. Hereafter, the method is called Al-c.

4) Koroleff (1983). Autoclaving acidified sample with potassium persulphate for half hour. Hereafter, the method is called Ac-c. Similar adaptations as for the Al-c method.

A sample volume of 10 cm^3, instead of 50 cm^3 was used and 0.8 cm^3 of oxidant reagent was added. Glass scintillation vials (25 cm^3) with screw caps of polypropylene were used.

5) Armstrong *et al.* (1966). Oxidation by ultra-violet (UV) irradiation. The method is thereafter called UV-ox.

Samples were irradiated for six hours using a 1 KW medium pressure Hg lamp. As the UV radiation output of a lamp will deteriorate with use, it is important to ensure that the lamp in use is operating effectively. On the basis of monitored lamp usage and comparison of decomposition efficiencies for photochemically labile (potassium oxalate) and more refractory organic (thiourea) the lamp during this work was considered as suitable.

Careful attention was given to making up volumes of samples to their original values, where evaporation (during the hydrolysis step or long UV irradiation period) was thought to be significant, i.e. when changes were greater than the precision of the method (> 7 %). In all cases evaporation losses were not more than 0.5 cm^3 (measured gravimetrically).

To carry out the spectrophotometric determination of the resulting DIP, 1 cm^3 of the mixed reagent (Strickland and Parsons 1972) was dispensed into 10 cm^3 of each of the treated and non-treated samples (i.e. for DTP and DIP respectively). However in the Ac-c method where reagents were added as recommended by Koroleff (1983), i.e. first, ascorbic acid was added to reduce the free chlorine formed during treatment, then the mixture containing the remaining reagents. For the spectrophotometric measurement a PYE UNICAM SP 500 MK II UV/Vis spectrophotometer was employed at a wavelength of 885 nm with 40 mm path-length cuvettes.

The colorimetric method for DIP was calibrated daily. For the AC-c and AL-c approaches, standard DIP solution were passed through the oxidation procedures. This was not done for the UV-ox, High-t, and Nit-ox methods, as previous workers (e.g. Strickland and Parsons 1972, Solórzano and Sharp 1983, Cembella *et al.* 1986) have demonstrated quantitative recovery of inorganic P standards which have passed these oxidation procedures.

2.4.2.3 Procedures for evaluating the methods

Two main sets of experiments were performed:

1) The DEs for 8 different compounds were individually investigated using each of the five methods. In the test of DEs of standard solutions organic-P compounds were added to both DDW and natural coastal water (NW; salinity *circa* 32, from Southampton Water, England, U.K.) to give concentrations of added DOP of 2.0 - 2.5 μM. The NW was vacuum filtered through Whatman GF/F filters. Stock solutions of standard compounds were contained in small pre-cleaned glass bottles and kept at -15 to -20^{0}C. For the preparation of daily working standards, one of these was gently thawed at time. Working standards were kept at about 0^{0}C and were discarded after 5 days of preparation. The organic-P compounds chosen are good representatives of the major classes of organic-P compounds occurring in natural waters (Hick 1983). Compounds chosen were:

i) phosphoric esters, mainly mono- and di- ester forms containing P-O-C bonds (e.g. Brock 1966, Siuda 1984, Solórzano and Strickland 1966, Kobori and Taga 1977), i.e. 4-nitrophenyl phosphate (npP), glycero-phosphate (G-P), glucose-6-phosphate (G-6-P).

ii) Poly- and meta-phosphates (PPs and MPs respectively, Solórzano and Strickland 1968), Trimeta-phosphates (MP), Tripoly-phosphates (PP).

iii) Nucleosides, which as well as the PPs are an important fraction in bloom events, (Admiraal and Veldhuis 1987), Adenosine-5-triphosphate (ATP), and Guanosine-5-diphosphate (GDP).

iv) Phosphonates (2-Aminoethylphosphonate, pPn) which despite being poorly studied and present at relatively low natural abundance (Quin 1967, Kittredge and Roberts 1969, Hilldebrand and Henderson 1983, Cembella and Antia 1986) are good compounds to test the efficiency of decomposition of the methods, as they contain strong P-C bonds and have been suggested as being relatively refractory (Cembella *et al.* 1986, Cembella and Antia 1986).

2) Four samples from the Itchen river, Southampton Water, and shelf waters of the North Sea (2 different samples) were also analyzed for their content of natural DOP, using all five methods.

2.4.3 Results and discussion

The first step was to assess the effectiveness of cleaning procedures. According to the blanks, which for all of the DTP techniques were consistent and close to the blank obtained for DIP, contamination problems were negligible. The DDW employed was assumed to be P-free, because the actual P levels found were between 5-15 nM (Ormaza-González and Statham 1990b, see also Gutschik 1985) which is insignificant relative to the DTP concentrations measured.

2.4.3.1 Decomposition efficiency of organic-P compounds added to DDW and NW

The results are shown in Figure 2.14 and their detailed data are in Appendixes 2.1.

Nit-ox:

The Nit-ox method had an averaged DE of 92 ± 7 % (range 84-101 %) and 93 ± 5 % (range 85-103 %) respectively for DDW and NW with added model compounds. Decomposition efficiency for all test compounds was > 90 %, except for npP with DEs of 80 ± 5% and 66 ± 10 % for DDW and NW solutions respectively. Loss of analyte due to spattering is potentially an inconvenience associated wit this method, but this was not the reason for the low DEs for 4-npP solution as experiments were done twice and spattering was successfully controlled by larger conical flasks with loosely fitting glass stoppers No difference was found between boiling vigorously (Cembella *et al.* 1986 suggested) and boiling gently.

High-T:

The High-t method had an average DE of 92 ± 5 % and 95 ± 6 % for DDW and NW solutions respectively. The lowest DE was

with GDP both in DDW and NW. No obvious explanation for this was found, but perhaps was due to high volatility of the compound under the procedure conditions.

There was no significant difference between the DE for DDW and NW matrices and the High-t method is free from any salt interference. This method gave good decomposition of compounds containing P-C bonds (i.e. 101 % and 89 % for DDW and NW respectively), contradicting the assumption of Cembella *et al.* (1986), that this technique would not decompose phosphonates.

A potential problem with the Hig-T method is losses of P during analysis, because the phosphorus liberated from the organic matter could form phosphorus pentoxide which will sublime above 350^{0}C (Andersen 1976) and be lost, unless alkaline salts are present to complex the oxidised phosphorus. Andersen (1976) and Solórzano and Sharp (1980) found poor and inconsistent recovery of organic P if, for instance, no calcium sulphate or magnesium sulphate were added to the sample prior to heating. Moreover, the presence of significant concentrations of humic material whose concentrations could be relevant in coastal and estuarine waters, could lead to potential inaccuracies (Andersen 1976) or possible interferences to the chemistry of the method.

Ac-c and Al-c:

The Al-c had similar DEs to the Ac-c technique. However, the Al-c method had a poor recovery for PPs and MPs, ATP, and GDP. The most likely reason is the final low acidity of the sample in this method, which starts as an alkaline solution, and the autoclaving time are not enough to complete the hydrolysis of organic and inorganic PPs to colorimetrically reactive forms.

Both methods had precision similar to the High-t method. The Ac-c procedure apparently had a salt interference, even though the analysis was performed as suggested by Koroleff (1983) in order to prevent formation of free chlorine, because

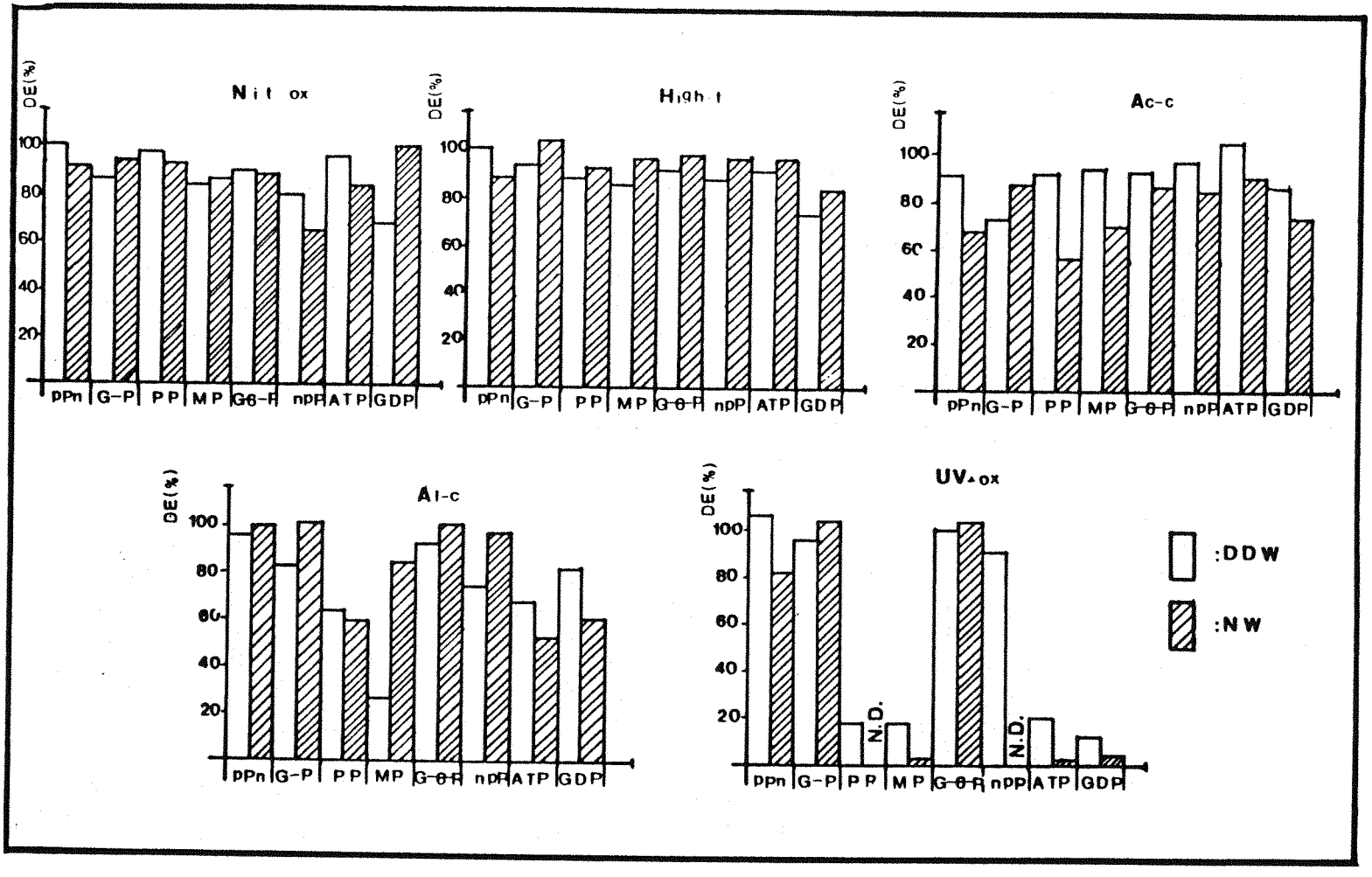


Fig 2.14 Decomposition efficiency (DE) of the five methods tested with eight different organic-P compounds. npP nitrophenylphosphate, GDP guanosine diphosphate, pPn phosphonate, see Table 1.1 for the rest of abbreviations. For detailed information see appendices 2.a, 2.b, 2.c, 2.d, 2.e. N.D. not determined.

the DEs for NW samples were consistently lower than for DDW, with the only exception of the G-6-P model compound. The lower values could have been probably due to a different pH conditions in the DDW and NW samples, as sulphuric acid is formed from the decomposition of potassium persulphate. Also the presence of humic acids in the NW samples could have been complexed the added model compounds. Complexation of organic-P by humic acids has been reported by Sholkovitz (1976) and these complexes are refractory.

UV-ox:

The UV-ox technique had excellent and reproducible average DEs of 93 % for P-mono-esters, and 101 % for phosphonates, which has been reported as being difficult to decompose (Cembella *et al.* 1986).

However, the UV-ox method gave only negligible breakdown of PP, MP, ATP and GDP, with DEs ranging from 3 to 20 % (Armstrong *et al.* 1966 reported this problem only for ATP). Henriksen (1970) and Koroleff (1983) have suggested that acidification prior to irradiation would lead to partial hydrolysis of PPs. However, when samples of the above compounds were acidified (1 cm^3 of concentrated HCl *per* 50 cm^3 of sample, *viz.* 0.25 M) and irradiation time was extended to 12 h, a DE of only 25 % was achieved.

Lennox (1979) has reported full decomposition of PPs when concentrated sulphuric acid and hydrogen peroxide were added to samples before irradiation and concluded that PPs were fully hydrolysed. However, he came to this conclusion when he compared his UV-ox data with a method based on acidic persulphate oxidation, which has been shown here to give incomplete breakdown of PPs.

If the DEs of PPs are excluded, the average DEs for the UV-ox method, for all the rest of the model compounds added to DDW and NW solutions, would be 98 and 95 % respectively. Therefore, this method appears well suited to breaking down P-monoesters, and some compounds containing P-C bonds such

as 2-Aminoethylphosphonate, 1-Aminoethylphosphonate and phosphonoformate.

2.4.3.2 Tests with natural water samples

A similar pattern of oxidation efficiency was found for the DOP in a range of natural water samples, in addition to the nearshore water used in the first set of experiments. The data are given in Table 2.7. Also Table 2.8. shows the DOP values found in the samples of NW that were spiked with the model organic-P compounds. The Nit-ox. method gave the highest value for DTP and thus DOP, whilst the UV-ox. method gave the lowest concentrations. This pattern was the same for all four samples, as well as the coastal water samples spiked with the organo-P compounds (Fig. 2.14). The Al-c method relative to the Ac-c method did not show a clear superiority as regards the relative efficiency of these methods. This contradicts that reported by Valderrama (1981).

The relatively poor performance of the UV-ox method is a reflection of its ability to only decompose partly some classes of organo-P compounds (Armstrong *et al.* 1967, Solórzano and Strickland 1968, Solórzano 1978, and Cembella *et al.* 1986). The mono-esters of orthophosphoric acid, known as enzymatically hydrolysable P (EHP), are the main type of organo-P compounds occurring in natural waters (Brock 1966, Siuda 1984). When the determination of EHP has been done using enzymatic methods (e.g. Strickland and Solórzano 1968, Chrost and Siuda 1986), the fraction of EHP relative to total DOP has been found to fluctuate between 18 and 50 % (e.g. Kobori and Taga 1977) in waters having high biological activity. Solorzano (1978) found only 19 % of the DOP was EHP in Loch Creeran (Scotland) waters. In the oceanic waters of the North Pacific the EHP as a percentage of the DOP has been reported as 10-20 % (Strickland and Solórzano 1966). These data suggest that the EHP fraction is normally a small percentage of the total DOP, potentially increasing up to 50 % under bloom conditions.

Dissolved PPs can represent a variable and significant part of the total DOP. Thus, for example, Armstrong and Tibbits (1967) found up to 0.05 μM of PPs where the DOP level was 0.16 μM. Solórzano (1978) found mean concentrations of PPs up to 0.08 μM where the DOP content averaged 0.13 μM, i.e. 62 % of the total DOP. Kobori and Taga (1977) reported PPs levels as high as EHPs concentrations in the Tokyo Bay.

It has been demonstrated that phosphomonoesters are not necessarily the main fraction of DOP in natural waters (e.g Strickland and Solórzano 1966, Kobori and Taga 1977), and that dissolved PPs may represent a significant fraction of total DOP. Therefore, the overall decomposition efficiency of the UV-ox method is likely to be poor, because of the partial decomposition of DOP. Its use has probably led to an underestimation of the organo-P compounds in natural waters. Also the Ac-c. and Al-c. methods may have similar problems to the UV-ox technique, because if a complete hydrolysis is not performed PPs would be under-estimated. Koroleff (1983) estimated that about 60 % of the PPs in samples would be hydrolysed but he commented that PPs were not significant in natural waters. Moreover, recently Ridal and Moore (1990) have reported a modified combination of the UV-ox and Ac-c techniques, which gave values of 1.25-1.50 times higher than those of the individual techniques. Although these authors did not report any reason, the higher values obtained by their modified technique are probably due to the treatment time which is up to three times longer than that recommended by the original authors for the composite techniques. Also, in the data reported by Hashimoto *et al.* (1987) concentrations of TDP and total phosphorus, obtained by their technique, were consistently higher than those of the persulphate autoclave method of Corwin and Menzel (1965, called here Ac-c). This data indirectly corroborates the findings in the present work that the Ac-c method can under-estimate DOP.

Method		Nit-ox	High-t	Ac-c	Al-c	UV-ox
Sample						
River Water	TDP	8.52±0.10	8.48±0.06	8.39±0.19	N. D.	8.20±0.05
	DIP	8.25				
	DOP	0.27	0.23	0.14	N. D.	0.00
Coast. Water	TDP	1.75±0.15	1.50±0.03	1.54±0.03	1.58±0.07	1.45±0.05
	DIP	1.06±0.06				
	DOP	0.69	0.44	0.48	0.52	0.39
Shelf Water 1	TDP	0.49±0.00	0.35±0.01	0.32±0.032	0.32±0.03	0.11±0.00
	DIP	0.07±0.02				
	DOP	0.42	0.28	0.25	0.25	0.04
Shelf Water 2	TDP	0.57±0.00	0.47±0.01	0.39±0.00	0.31±.01	0.22±0.05
	DIP	0.15±0.01				
	DOP	0.42	0.32	0.24	0.16	0.07

Tab. 2.7. TDP, DIP and DOP (μM) concentrations found for four different natural water samples by using the five methods. Shelf Water samples are from the North Sea, 1: bloom condition, 2: non bloom condition. Salinities for River, Coastal, and Shelf waters were 0.5, 32, and > 33 respectively.

		DOP methods				
Sample	DIP	Nit-ox	High-t	Ac-c	Al-c	UV-ox
1	1.79	0.54	0.48	0.0	0.13	N.D.
2	1.49	0.44	0.53	0.43	0.26	0.29
3	1.40	0.76	0.72	0.32	0.17	0.92
4	1.30	0.87	0.39	0.43	0.73	0.16
5	0.51	1.2	0.67	0.92	0.88	0.55
6	0.66	1.4	0.50	0.73	1.1	0.31
7	1.02	0.51	0.27	0.33	N.D.	0.13

Tab. 2.8 DOP levels (μM) detected in samples which were spiked with the organic P compounds. Numbers 1-7 refer to the different samples used per each model compound.

The High-t method has the disadvantage of heating the sample at 450^{0}C, which could produce phosphorus pentoxide that sublimes at about 300^{0}C if alkaline salts are not present. The production of phosphine from DTP under certain conditions (Hashimoto *et al.* 1987), could be another possibly way of losing P.

The Nit-ox method despite being an awkward procedure, gave acceptable recoveries of standard materials and the highest values of DOP for natural water samples. Its high efficiency is probably due to the use of a stronger oxidant (nitrogen dioxide) than in the others procedures, and also the use of a temperature not exceeding 300^{0}C which minimizes losses of volatile P compounds.

2.4.3.3 The determination of dissolved poly-phosphates

Because the UV-ox technique is not able to hydrolyze poly-phosphates (including meta-phosphates) whilst the Nit-ox method is, the difference in the DTP analyses of a sample separately treated by the two techniques would allow to measure the content of PPs. However, this fraction would mainly consist of PPs and also MPs, and perhaps organo-P refractory to UV irradiation which will also be included in the fraction. Until the chemical structure of this fraction is elucidated, in this work it will be simply called poly-phosphates.

2.4.3.4 Accuracy of the Nit-ox method

The tests above indicate the Nit-ox method is the best of the examined for the decomposition of natural and model organic P compounds. However, this inter-comparison does not necessarily demonstrate total decomposition of all natural organic P compounds occurring in natural waters. Recent work on measuring dissolved organic nitrogen (DON, Suzuki *et al.* 1985), and DOC (Sugimura and Suzuki 1988) in sea water suggests that earlier measurements were not accurate (although this is still a matter of contention, see Walsh 1989). The new

DOC values range from 180-220 μM, and 240-280 μM in the surface waters of the North Atlantic and Pacific respectively (Sugimura and Suzuki 1988, Suzuki and Peltzer 1990). If these values are true, the currently reported concentrations of DOP are far below what would be expected when applying the Redfield ratio C:P of 106:1, i.e. DOP should range from 1.7 to 2.6 μM !. Measurements made with the new techniques (Suzuki *et al.* 1895 and Sugimura and Suzuki 1988) appear to be oceanographically consistent, and the new values hint that a major reassessment of the cycling of dissolved organic matter (DOM) is needed.

In the absence of standard compounds for DOP in natural waters, the best strategy for assessing accuracy would appear to be the oceanographic consistency concept, but this would have to be tied to other concepts that are currently under review, like the apparent under-estimation of primary productivity in the oceans (Jackson 1988, Williams and Druffel 1988, Grande *et al.* 1989). Thus, in an attempt to independently assess the accuracy of the Nit-ox method, it was compared to a non-spectrophotometric approach, i.e. Inductively Coupled Plasma- Mass Spectrometry (ICP-MS). Two samples from natural water bodies and one sample from a sea water phytoplankton monoculture medium ($2X10^6$ cells.cm^{-3} of *Tetraselmuis succia*) were analyzed by both techniques (Table 2.9).

Because the ICP-MS technique is affected by sea water salts, the DOP has to be in fresh water. Thus, to obtain a DOP solution from the culture, 2 dm^3 of it were centrifuged and the particulate organic matter separated, washed and finally poured into 1 dm^3 of DDW and left to decompose. Within a week sub-samples were taken and filtered for analysis. The samples contained naturally occurring DOP in concentrations in the range 0.23-17 μM which represented 12-20 % of the DTP. Results from the ICP-MS and Nit-ox methods did not overall satisfactorily agree but the trend of concentrations was similar (Table 2.9). The main problem was that the ICP-MS

Sample	DTP		DOP	DOP/DTP
	ICP-MS	Nit-Ox	Nit-ox	%
A	<0.21	1.41±0.05	0.23	16
A + 5 μM	1.48±0.53	N.D.		
B	12.3±2.2	20.6±0.4	4.2	20
B + 5 μM	13.0±0.8	N.D.		
C	155±3	143±0.3	17	12
C + 15 μM	143±2	N.D.		

Tab. 2.9 Comparison of ICP-MS and Nit-ox methods for the determination of DTP in natural water samples. N.D.: Not determined. Samples A, B, and C are from the Beaulieu and Itchen R., and se water algal culture *Tetraselnuis succia* (green algae).The samples were spiked with DIP (5 μM)

technique showed high variability especially at low concentrations, which did not allow a meaningful comparison. Therefore, the question of accuracy still remains to be fully answered.

2.4.4 Conclusions for section 2.4

This exercise has shown there are major differences in the decomposition efficiencies of the tested methods, for a range of defined organo-P compounds and also for naturally occurring DOP in natural waters. The significant differences between the Nit-ox and the other methods indicate probable underestimation of DOP in earlier investigations where the Nit-ox method has been not used. The differences between Nit-ox and UV-ox methods can largely be ascribed to the presence of PPs, and perhaps UV refractory organo-P compounds. The combination of UV-ox and Nit-ox provides an empirical method for determining PPs.

The PPs have often been considered as negligible, whilst here it has been demonstrated that they can account for as much as 50 % of the total DOP in the natural water samples analyzed. On the basis of this intercomparison the UV-ox. technique is not recommended for the analysis of water samples where significant PPs may be present due to pollution or high biological activity. Further environmental studies using the Nit-ox technique in natural waters, especially ocean waters, will help to assess the accuracy of the technique in terms of oceanographic consistency. All values of total DOP will provide an important boundary condition for the detailed investigation of the organo-P fraction in natural waters including its chemical structure. This technique will provide a more reliable picture of the role of DOP in primary production processes.

The Nit-ox method seemed to be most suitable for any natural water and it can also be applied to the determination of PTP in natural waters. It was thus applied in all subsequent work.

2.5 Determination of total particulate phosphorus in estuarine and marine waters using magnesium nitrate as oxidant agent

2.5.1 Introduction

Particulate organic matter (POM) or in a broader sense particulate suspended matter (PSM) in both estuarine and marine environments plays an important role in the aquatic P cycle, as well as other chemical and biological processes, e.g. spatial oxygen distributions, pH, adsorption/desorption processes, supply of nutrients, etc. Major components of POM are compounds containing carbon, nitrogen, and phosphorus. Particulate organic carbon (POC) has been routinely studied, whilst the other two have been almost completely neglected. This is particularly true for Particulate Organic Phosphorus (POP) (Meybeck 1982). Perhaps, the main reason for the minimal information on POP is the lack of a reliable and straightforward technique.

The P in natural PSM is present not only as organically bound P but also as Pps, MPs and inorganic P. Therefore, the term PTP (particulate total phosphorus) more accurately defines the naturally occurring suspended phosphorus and thus will be used hereafter. Particulate total phosphorus can be estimated directly or indirectly. An indirect estimate can be given by measuring the total phosphorus (TP) concentration of an unfiltered, and a filtered sub-sample of water; the difference between these measurements is PTP. A direct measure is given by digesting the particulate matter which has been collected onto a filter, and then analyzing the P content of the resulting solution by a spectrophotometric technique based on the phospho-molybdenum blue method of Murphy and Riley (1962). The latter manner of determining PTP has two main advantages over the former. 1) Because the suspended particulate matter has been separated immediately from solution, interaction between suspended and dissolved phases

is diminished and 2) a low concentration of PTP is not an inconvenience as the PTP can be pre-concentrated by filtering large volumes of sample. Therefore, the technique to be developed has to measure PTP directly.

The analysis of TP and DTP has been carried out using different techniques (see section 2.4 for DTP) which have different ways of oxidizing the organically bound P. Recently, a new approach has been reported in which TP and DTP are determined by converting the TP or TDP to phosphine (Hashimoto *et al.* 1985 and 1987). However, the analysis of POP or PTP can be performed by techniques analogous to those for DTP analysis, such as, ignition at high temperature (Solórzano and Sharp 1980), digestion at 275^{0}C with sulphuric acid and hydrogen peroxide (Kattner and Bruckman 1980), autoclave oxidation (Jefries *et al.* 1979, perchloric oxidation (Strickland and Parsons 1972), and UV oxidation (Holm-Hasen, quoted by Perry 1976, and Demina and Belgayeva 1986). Apart from oxidizing the organically bound P, all of these methods must have a hydrolysis step to depolymerize condensed polyp-poly-phosphates (PPs) and metaphosphates (MP) to reactive P, as they are present inside phytoplankton cells (see for instance, Watanabe *et al.* 1989).

Perhaps the main problem with the above mentioned techniques, is that they are involved, requiring at least 2 hours of treatment and handling steps before colorimetric analysis. Additionally, the reports of these technique lack information about their efficiency of decomposing the PTP material, and the effects of experimental variables.

The idea of developing an improved analytical method for the direct determination of PTP, came after obtaining the result from the simultaneous inter-comparison of five technique for DOP. From this inter-comparison, the method of Cembella *et al.* (1986) gave the highest values of DOP in four natural water samples tested. Thus, the basic principle of this technique was investigated and optimized for the analysis of PTP separated from estuarine and marine waters onto filters.

2.5.2 Materials, apparatus and reagents

Glassware was the same as used in section 2.2. with the addition of 15 cm^3 centrifugation glass tubes. These were treated as specified in the same section, and kept for this purpose only.

Reagents: All reagents were analytical grade, unless otherwise specified.
- Oxidant Reagent Solution (ORS): 20 g of $Mg(NO_3)_2.6H_2O$ were dissolved in 95 % ethanol.
- 0.4 M [HCl]: 1 M [HCl] was prepared (89 cm^3 diluted in DDW to make 1.0 dm^3) and diluted accordingly.
- The mixed reagent (MR) for the colorimetric analysis was prepared as described by Strickland and Parsons (1972).

Different types of filters were investigated, i.e. glass fibre (GF/F, Whatman), cellulose nitrate (CN, Sartorius) and cellulose acetate (CA, Sartorius), cellulose nitrate-acetate mixture (CNA, Millipore), polycarbonate (Nuclepore), and polysulfone (Gelman) filters. Filters were treated as follows. GF/F filters were heated at 450^{0}C for two hours, whilst CN, CA, CNA were immersed in boiling DDW for 1 hour, and then thoroughly rinsed. Polysulfone filters underwent no treatment.

The method of Solórzano and Sharp (1980) was carried out as they described and it is based on the High-t method used in section 2.4. The technique presented here was compared to the method of Solórzano and Sharp. The determination of POC was carried out as Knap (1979) indicated and it is based on the decomposition of POC to CO_2 by high temperature combustion.

2.5.3 Development of the method

Initially, the technique of Cembella *et al.* (1986) as modified in section 2.2 was applied to the determination of PTP separated onto filters. In further experiments the evaporation time, volume of ORS, hydrolysis time and

temperature, strength of HCl, and the performance and P contamination from different classes of filter were investigated. For the method presented here, particulate suspended matter (PSM) was collected by filtering samples from monocultures of *Brachiomona sp, Gerodinium aureolum and Synechococcus sp*; then filters were stored at -20^{0}C until analysis, whilst for the method of Solórzano and Sharp (1980) acquisition of samples was carried out as they recommended, i,e after filtering the samples the filters were rinsed twice with 2 cm^3 of 17 mM[$MgSO_4$].

2.5.3.1 Optimization of the oxidation step

The evaporation time was controlled by the volume of DDW in the flasks with a constant amount of particulate P. Table 2.10 shows the results. A quite large range of PTP values was found for all three evaporation times and this can be explained in terms of reproducibility of filtration and sub-sampling. There was not a significant difference in PTP values when different volumes of DDW were used. This is expected because the oxidation of DOP only occurs during the nitrous oxide gas evolution step when all the water has been evaporated. A final medium volume of 25 cm^3 was used in subsequent work.

The effect of different volumes of ORS in 25 cm^3 of sample was investigated (Table 2.11). A volume of 0.4 cm^3 of ORS gave only 62 % of the PTP concentration given when 2.0 mm^3 was used. Volumes of 1.0, 3.0, and 5.0 cm^3 of ORS gave similar, but slightly lower results to 2.0 cm^3. Two cm^3 of ORS was shown to be adequate to oxidize the PTP, and was used in subsequent experiments.

2.5.3.2 Hydrolysis

Hydrolysis is a crucial step in the procedure, because in this process metaphosphates and polyphosphates (including ATP) are depolymerized to DIP. Polyphosphates are a major fraction of the PTP in the pure phytoplankton type sample, such as that

Evaporation time	Mean value	range	Volume of DDW
(min)	μM[PTP]		(cm^3)
40-45	19.4	17.9-20.8	15
50-55	20.6	19.6-21.6	25
75-80	17.5	16.8-18.8	50

Tab. 2.10 PTP values for different evaporation times. The evaporation time includes full NO_2 evolvement. Samples were analyzed in triplicate, and were taken from a monoculture of *Brachiomona sp*

ORS (cm^3)	Mean value	range
	μM[PTP]	
0.4	14.2	13.8-14.2
1.0	21.0	19.2-22.9
2.0	22.8	21.8-23.7
3.0	21.4	21.2-21.6
5.0	21.1	21.0-21.3

Tab. 2.11 PTP values obtained using different volumes of oxidant reagent solution (ORS).
Analysis was at least duplicate. Samples as described in Table 2.10.

used here. To test the effect of temperature on the hydrolysis step, sub-samples of oxidized culture media were treated with HCl as indicated above at 80 ^{0}C or room temperature. The value of PTP when hydrolysis was done at room temperature was only 1/3 that of the 80 ^{0}C hydrolysis. This indicated that 66 % of the PTP was present as PPs and/or MPs. This observation compares well with the experiment of Perry (1976).

Table 2.12 displays results for different hydrolysis times at 80^{0}C. This temperature was chosen, because 1) this or a very similar temperature has generally been reported as necessary for an efficient hydrolysis step (e.g. Solórzano and Strickland 1968, Strickland and Parsons 1972, Andersen 1976, Solórzano and Sharp 1980, Cembella *et al.* 1986), and 2) it gave an adequate heating without causing an excessive evaporation.

The results showed that the concentrations of PTP of the sample 1 which had no heating was 31 % of sample 3 which had been heated for 30 min. No further hydrolysis was evident even after 3 h heating and it was thus shown that hydrolysis is fully completed in 30 min.

The strength of the HCl solution also influences the hydrolysis. However, in order to investigate the effect of acid molarity on the depolymerization of PPs and MPs, it was firstly necessary to consider the effect of sample acidity on colour formation during the determination step. Thus, a series of DIP standards of 2 μM concentration were made in HCl solutions of 0.0, 0.2, 0.4, 0.6, and 1.0 M. These standards were subjected to the colorimetric analysis and the results are given in Table 2.13. When the final pH of the sample was in the range of 0.3 - 0.7 (pH) the colour formation was rapid as has previously been reported (e.g. Burton 1973). This final pH was obtained with samples having an acid molarity $\leq$ 0.2 M [HCl]. However, below a final pH of less than 0.30, the colour formation was almost completely suppressed. Therefore, the final strength of the treated sample before adding the MR must not exceed 0.2 M [HCl].

Hydrolysis of PTP polyphosphates with 0.1, 0.2, 0.4, 0.6, and 1.0 M [HCl] was investigated. Table 2.14 displays the effect of using different strengths of acid on the P content of samples hydrolysed. For the colorimetric analysis the samples were diluted when necessary to give a ≤ 0.2 M acid strength. The highest value of PTP was obtained in the sample with an initial HCl molarity of 0.4, whilst the lowest value, i.e. 23 % of the 0.4 M value, was found with the 0.1 M[HCl]. The concentrations of PTP obtained with higher acid molarity hydrolysis, were similar to the values achieved with 0.4 M hydrolysis. Thus 0.4 M was taken as the most appropriate acid strength for hydrolysis.

2.5.3.3 The effect of filter type on the analysis

The effect of different kinds of filters on the analysis of nutrients in natural waters has been reported by several authors (e.g. Wagemann and Graham 1974, Bickford and Willet 1981, Danielsson 1982). Recently, Bloesch and Gravieli (1984) have reported the influence of filters on the analysis of PTP. These authors used wet oxidation with $K_2S_2O_8$ (Szabo and Illi 1982), and with the addition of H_2O_2 and H_2SO_4 (Schmid and Ambuhl 1965). Bloesch and Gravieli (1984) found that the most appropriate filters were Sartorious and Millipore AC membrane filters, as these gave the lowest blanks close to their analytical zero. Sartorius filters were slightly better and also fully dissolved during analysis. GF/F filters gave blanks of up to 0.027 μmol/filter. As the analytical approach described here is different to that of Bloesch and Gavrieli (1984), it seemed important to investigate which type of filter was the most suitable.

Chemical compatibility with the method and the P content of the following filters was studied: borosilicate glass fibre GF/F (Whatman), CN, CA (Sartorius), CN, CNA (Millipore), polysulfone (Gelman), and polycarbonate (Nuclepore). The chemical compatibility was investigated by adding a known amount of organic phosphate (PPs or G-6-P) to a sample to

obtain final a concentration of 2 μM prior to the filtration and analysis treatment. Before analysis filters were treated as described in section 2.5.2.

GF/F filters, which were used in all the inter-comparison experiments gave no interferences with the colorimetric analysis. They did tear, thus centrifugation or re-filtration was required prior to adding the MR for colorimetric determination. Because the blanks when using these filters were high and variable (see Table 2.15), experiments to trace other possible sources of P contamination were carried out. The reagents, boiling chips and glassware were investigated. No significant blanks were found from these potential sources, as the absorbance readings were in the order of 0.001 in 1 cm path-length cuvettes. These readings were the same as those from DIP blanks readings. Therefore, the origin of blanks was ascribed to the GF/F filters themselves. This fact apparently contradicts information provided by Whatman Technical Services, who did not report any P present in the filters. The high blanks were consistent within batches but could show significant difference between batches (3 different batches were investigated). This variability was also reported by Bloesch and Gavrieli (1984).

The CA, CNA, CN, polysulfone and polycarbonate filters were shown to be unsuitable for the method, because they suppressed colour development. The GF/F filters were thus shown to be suitable for the method, but blank filters need to be run with each batch of samples, and the filters need cleaning before use.

2.5.4 Analytical procedure

The developed method is as follows: Filter the sample under low vacuum (0.2 bars) through Whatman GF/F filters of 25 or 45 mm diameter. Place the filter at the bottom of an Erlenmeyer flask which contains several glass boiling chips. Add 25 cm^3 of DDW and pipette in 2 cm^3 of ORS. Put the flasks on a hot plate inside a fume hood. Violent boiling will take

Samples	Hydrolysis time (min)	Mean value	Range	After 3 h
		µM[PTP]		
1)	0	5.09	5.02-5.17	16.8
2)	15	15.3	14.3-16.4	15.4
3)	30	16.5	16.4-16.7	16.8
4)	45	14.2	14.2-14.3	14.3

Tab. 2.12 Hydrolysis time for PTP analysis at 80°C. Samples are from the same culture as Tab. 2.10. Samples were taken when the culture growth phase was over. The strength of the HCl solution for the hydrolysis was 0.3 M.

HCl Molarity	Standard Sol.	Blank	pH
	Absorbance values		
0.0	0.036	0.001	0.80
0.2	0.038	0.000	0.58
0.4	0.008	0.000	0.36
0.6	0.003	0.000	0.22
1.0	0.003	0.000	0.16

Tab. 2.13 The effect of acid molarity on colour development of the method of Murphy and Riley (1962). Samples were analyzed in triplicate. pH was measured after addition of MR solution.

Molarity	Average	Range
	μM[PTP]	
0.1	4.16	3.62-4.70
0.2	16.4	16.4-16.5
0.4	18.3	18.7-17.9
0.5	17.5	17.9-17.1
0.6	16.6	16.4-16.8
1.0	15.8	15.6-16.0

Table 2.14. The effect of the acid molarity on hydrolysis. Hydrolysis time was 30 min. Temperature 80^{0}C. Samples were analyzed in duplicate.

	GF/F		Pc	Ps	CNA	CA	CN
	F1	F2					
Filter blank	0.006	0.084	N.R	N.R.	N.R.	0.001	N.R.
SD	<0.001	0.001	---	---	----	<0.001	-
Sample duplicate	12	12	4	4	4	4	4

Tab 2.15 Chemical compatibility and P content of different types of filters analyzed with the present method. F1 and F2 are different batches, Pc and Ps are polycarbonate and polysulfone filters, for other abbreviations and details see text. N.R; no data due to interference in the analysis step. Filter blank and SD are absorbance readings.

place when the volume is approaching 10 cm^3, and at this point diminish the heat and put heavy stoppers on the flasks but allow ventilation. After dryness is achieved increase the heat. When there is a dry residue nitrous gas will start to evolve; take the stoppers off until the brown gas is totally evolved, i.e. flask walls become transparent. Let the flasks cool, and then add 10 cm^3 of 0.4 M HCl, put on them refluxing stoppers and heat the solution at 80^0C for 30-35 min. When the flasks are cool, 10 cm^3 of DDW is added to give a final HCl acid solution of 0.2 M. Transfer 10 cm^3 of the diluted solution into glass centrifugation tubes, add 1 cm^3 of MR and centrifuge during 5 min at 5 gravity and then measure absorbance of the solution at 885 nm. Samples are analyzed in duplicate, and at least two blank filters are run with each set of samples. Calibration can be carried out as suggested by Strickland and Parsons (1972), although it is preferred to measure a set of standard solutions and prepare a calibration graph rather than multiple measurement of one standard. The whole oxidation and hydrolysis steps would take about 1.5 to 2 hours for a set of 26 samples including blanks and standards.

2.5.6 Inter-comparison of the developed method

The optimized method was tested with samples of cultured phytoplankton and particulate matter collected from natural waters. An inter-comparison with the method of Solórzano and Sharp (1980) was carried out. This technique was chosen, because methods such as this using ignition at high temperature are the most routinely used way of measuring POC, particulate organic nitrogen (PON), and PTP, in both marine waters (e.g. Knauer *et al.* 1979) and sediments (for PTP, e.g. Andersen 1976, Mehta *et al.* 1954; for POC and PON, e.g. Gibbs 1977). The analysis of POP in sediments using perchloric acid (a modification of the method for marine waters, see Strickland and Parsons 1972) has been compared with the ignition method, and both gave similar results (Andersen 1976). Therefore, although there are other methods (e.g. Schmid and Ambuhl 1965, Kattner and Brockmann 1980, Szabo and

Illi 1982), the technique of Solórzano and Sharp (1980) seemed the most suitable for an inter-comparison. The results are displayed in Table 2.16. A high decomposition efficiency of the developed method is thus inferred.

2.5.5.1 Accuracy of the method

Assessing the accuracy of the PTP method is difficult, because there is no particulate total phosphorus standard available. Therefore, the accuracy of the method had to be checked indirectly. Thus, POC analyses were also performed (Table 2.16) to enable a comparison of measured PTP and POC to the Redfield ratio; i.e. C:P of 106:1. If the POC:PTP ratio of the samples is close to 106 would support the idea of 100 % decomposition of PTP into DIP. The problems and limitations of this assumed C:P ratio are discussed in Chapter 4. Additionally, the decomposition efficiency of this method was expected to be acceptable, because of the report of Cembella *et al.* (1986) and this work results on the effectiveness of the technique to decompose many types of naturally occurring organic P compounds

Samples from the cultures gave ratios of C:P ranging from 85 to 206, whilst for natural waters 13 to 43. Values of PTP and POC reported in Chapters 3 and 4 gave similar C:P ratios to these. Moreover, Romankevich (1984) has reported that the C:P ratio in marine photic waters could be more than three times lower than the classical Redfield ratio.

The variation C:P observed could be ascribed to the chemical structure either of the PTP or of the natural chemical species of the waters, which would vary with pytoplaknton/bacteria species and location. The higher ratios found in a sample from the culture could be due to P-starved phytoplankton. The lower ratios in natural water samples may be explained in terms of DIP adsorption onto the surface of the particulate matter or/and accumulation of P inside the cells of micro-organisms.

It was also thought that the large differences in PTP values obtained between methods, such as that found for sample 3, could be due to problems in the ignition method, in which P can be lost as phosphorus pentoxide. The sublimation point of phosphorus pentoxide is 347^{0}C which is substantially lower than the ignition temperature (500^{0}C). The formation of phosphorus pentoxide during the treatment is prevented by the presence of alkaline minerals (Andersen 1976). This hypothesis is corroborated somewhat by Solórzano and Sharp (1980) themselves, who found poor recovery of P if there was no addition of $MgSO_4$ (Andersen added Na_2CO). Moreover, Andersen (1976) also recommended that the ignition method was not suitable for any class of particulate matter having a high content of humic material (see Section 2.4).

Another check on the accuracy of the methods was done by refiltering the residues of the filters left after analysis by the two methods. Thus, for a series of filter replicates, POC was measured before and after the PTP analysis. The initial sample had a content of 25 $\mu g.cm^{-3}$, whilst residues from the present, and Solórzano and Sharp (1980) methods had POC levels of 2.16 and 2.06 $\mu g.cm^{-3}$ respectively, i.e. the POC left on the treated filters is less than 10 % of the original content and within the analytical error of the POC technique. Thus implying that there was not significant difference in accuracy between the method presented here and that of Solórzano and Sharp (1980).

The two ways of testing the accuracy of the method could be criticized because 1) the accuracy of the POC method could be questioned because of the outcome of new data on organic carbon (see Chapter 4), and 2) the validity of the C:P Redfield ratios applied have to be qualified (see Takashashi *et al.* 1984). Therefore, the accuracy problem in the analysis of PTP needs further investigation.

Sample	Method	μM[PTP]	μM[POC-C]	POC/PTP	B/A
					%
1	A	0.35	10.3	29	138
	B	0.48		21	
2	A	0.77	33.8	44	102
	B	0.78		43	
3	A	1.96	53.2	27	201
	B	3.95		13	
4	A	32.0	7140	223	108
	B	34.6		206	
5	A	18.7	5360	114	134
	B	25.1		85	
6	A	13.9	N.D.		116
	B	16.1			
7	A	2.28	N.D.		116
	B	2.64			

Table 2.16 Intercomparison of methods A (Solorzano and Sharp 1980), and B (present method) carried out with different kinds of particulate matter. Samples:1 (non bloom), 2 (moderate bloom), and 3 (intensive bloom of *Mesodinimiun rubrum*) are from Southampton Water. Samples 4 and 7 are of *Brachiomona sp* sampled during decay and stationary stage respectively. Sample 5 is from a *Gerodinium aureolum* culture in a decaying stage, and sample 6 is from a *Synechococcus* strain WH7803(DC-2) bacteria culture. N.D. Not determined. All samples were analyzed in triplicate.

2.5.6 Capabilities of the method

The precision of the present method was 8.3 % RSD (it was measured as twice the SD of 6 replicate blanks), but at a level of 3.86 μM the RSD was 1.5 % (6 replicates), and for levels of about 36 μM the latter fell to 13 % . Precision could be affected when small volumes of sample containing high loading of phytoplankton are filtered. A precision of 3-4 % was observed when samples of 5 cm^3 of a culture with 2.1 X 10^6 $cell.cm^{-3}$ were filtered. The detection limit (measured as twice the standard deviation of 6 replicate blanks) is 0.11 μM, which is the minimum amount of DIP present in the final solution after full treatment. The amount of sample to be filtered depends on the POM load, thus for oligotrophic and estuarine waters a volume of about 1 and 0.020 dm^3 respectively will be sufficient. The method reported has a similar detection limit but a wider range than the method of Kattner and Brockman (1980) and Solórzano and Sharp (1980). Precision cannot be compared, because the above authors did not give this information. The blank of the reagent was close to zero absorbance i.e. ± 0.003-0.001, using a 10 mm path-path-length cell.

2.5.7 Conclusions of section 2.5

The developed method for PTP has been demonstrated to be more efficient, and easier to use than previously reported techniques. Finally, this technique is compatible to that of Cembella *et al.* (1986), which has been demonstrated to more efficiently convert DOP to DIP than other techniques (see section 2.4).

2.6 Surfactants: Wetting efficiency and their effect on the analytical procedure for DIP

2.6.1 Introduction

The use of surfactants has been reported in association with the automated analysis of DIP in order to improve flow and thus avoiding "tailing effects" (e.g. Hansen and Grasshoff 1983, Fernandez *et al.* 1985, Eberlain and Kattner 1987, etc.). The type of detergent used varies from author to author. For instance, Hansen and Grasshoff (1983) reported the use of Levor IV (manufacturer not reported), Fernandez *et al.* (1985) used Flaminox (Fisher Co.), and Eberlain and Kattner (1987) employed Aerosol 22 (GmbH, BadVilbel, FRG). The Sigma Co. Ltd. provides a kit of reagents for the determination of DIP using AutoAnalyzer systems which contains Brij 35 (Sigma). Each laboratory generally uses its own kind of surfactant. However, no study has been found which explains why the detergent and final concentrations were chosen, and whether or not the phospho-molybdenum blue method was affected.

It was thus considered worthwhile to carry out a series of experiments to determine the most appropriate surfactant for the LCC system described in section 2.3. The main strategy was:

i) To test the four kinds of detergents previously used.
ii) To work with similar final detergent concentrations to that reported before.
iii) To study how the type of surfactant and its concentration affects the chemistry of the method.
iv) To find a compromise between wetting capacity and any chemical interference.

2.6.2 Materials and methods

The investigated surfactants were:
a) Nonionic: 23-Laurylether (Brij 35), Sigma Co., and Polyxyethylene (Triton X-100) used in section 2.2.
b) Anionic: Lauryl sulphate.
c) Cationic: Dodecyltrimethylammonium bromide (DTMAB).

Solutions of 0.5 % either V/V or W/V depending on whether the surfactant was liquid or solid, were prepared in DDW. The final concentration of surfactants in samples plus reagents ranged from 0.0004 to 0.0013 % (V/V). The analytical method for DIP is that described by Strickland and Parsons (1972).

In general the experimental procedure for LCC spectrophotometry was as given in the Section 2.3, and the sample standard solutions of 100 and 500 nM of DIP, and blanks were spiked with surfactant once the colour was fully developed. The absorbances of the samples were measured periodically to check the kinetics of colour formation.

For samples where the DIP was determined by conventional spectrophotometry, sample absorbance were measured without adding detergents then again immediately after spiking with a detergent, and then periodically after this.

2.6.3 Results and discussion

A trial experiment using Brij 35 showed that immediately after spiking 55 cm^3 of 100 nM[DIP] solution with 100 mm^3 of this detergent, the absorbance reading in the LCC system dropped from 0.226 to 0.085. This absorbance then remained constant. The absorbance of a 500 nM[DIP] solution also decreased, in this case from 0.778 to 0.432, immediately after adding the detergent, but increased to 0.534 over 40 minutes.

A second experiment was done with sample solutions having different final concentrations of Brij 35 . Table 2.17. gives

the results of this experiment. No obvious pattern was found, but the following points are evident.

1) The samples without added Brij 35 gave higher absorbance than those with it.

2) The blank increased with increasing detergent concentration.

3) The highest absorbance reading for 100 nM[DIP] was given by the sample with the highest concentration of Brij 35.

4) The highest absorbance reading for 500 nM[DIP] was given by the sample with the lowest concentration of Brij 35.

5) Absorbance values for blanks and 100 nM[DIP] solutions with added Brij 35 did not significantly vary with time, except for the 500 nM solution.

Comparing this data with the initial reading (i.e. without detergent), for both the 100 and 500 nM standards the absorbance values dropped by about 50 and 40 % respectively. Further experiments using normal spectrophotometry showed how the addition of Brij 35 also reduced the absorbance value by about 45 % for both 100 and 500 nM DIP solutions. The interference appeared to stabilize after 2.5 - 3.0 hours (Fig. 2.15), by which time the absorbance had dropped by 24 and 21 % respectively in comparison to the initial absorbance values.

It has thus been shown that Brij 35 interferes with the chemistry of the method. A good wetting efficiency was achieved with Brij 35 concentrations higher than 0.0009 %, because when the LCC technique was used the flat plateau of the response signal was quickly obtained and the absorbance reading was the same after pumping the sample many times, in other words there was no adsorption of the phospho-molybdenum blue complex onto the wall of the capillary tube. However, the negative interference in the chemistry of the method was still evident.

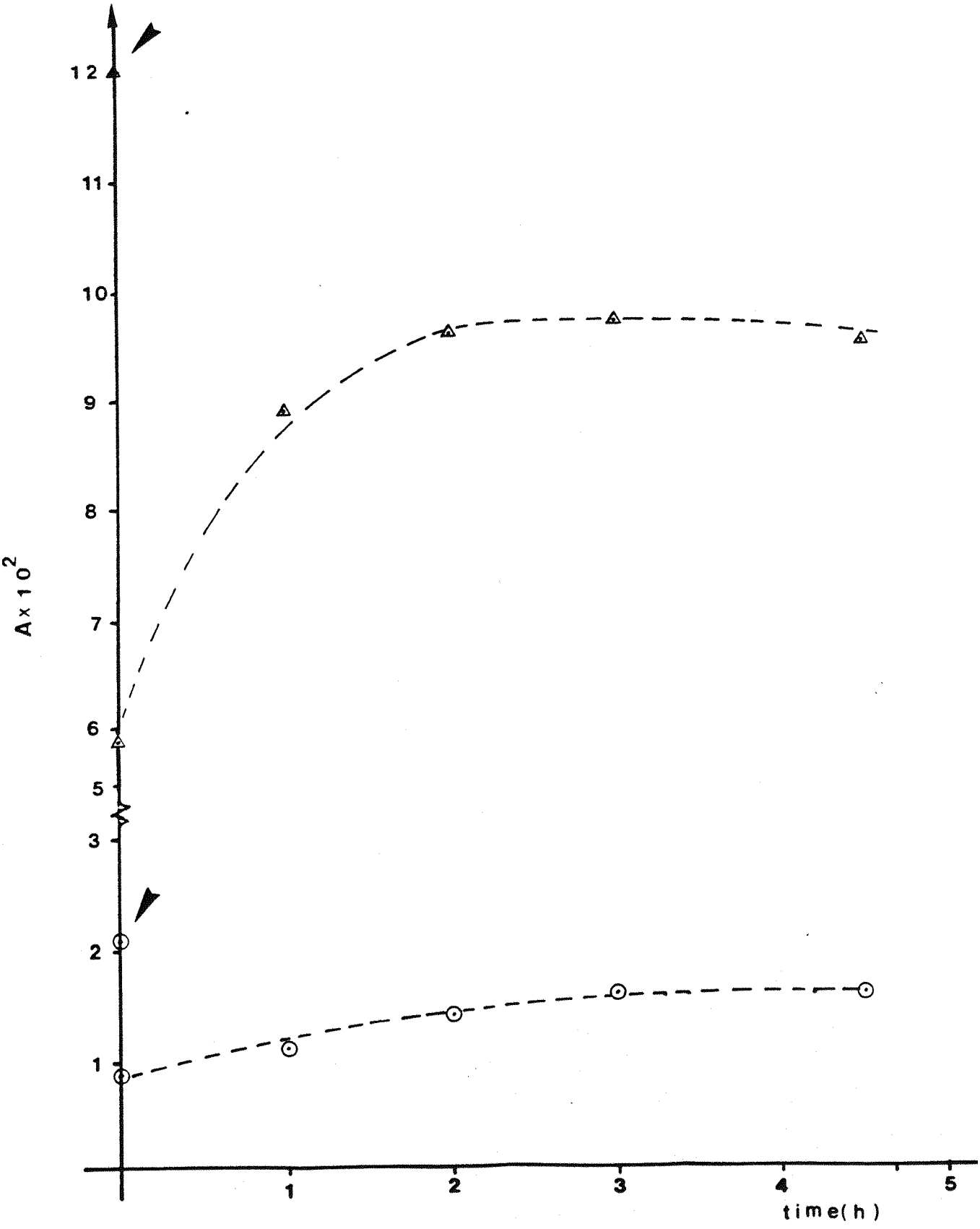


Fig. 2.15. The effect of the Brij-35 on colour formation of the phosphomolybdenum blue method. The detergent was added after full colour development was achieved. Arrows indicate the absorbance value of the sample prior to adding the detergent (0.001 % W/V). ⊙:100 nM, ◬: 500 nM standard solutions.

	Conct.	Final Detergent conct. (%)			
	(nM[P])	0.0013	0.00095	0.0004	0.00
	Blank	0.024	0.014	0.009	
t=0-0.5	100	0.132	0.111	0.109	0.226
	500	0.441	0.578	0.721	0.778
	Blank	0.024	0.013	0.011	
t=1.5-2.	100	0.127	0.107	0.103	0.226
	500	0.470	0.668	0.709	0.776
	Blank	0.023	0.015	0.008	
t=3.-3.5	100	0.128	N.D.	0.101	0.200
	500	0.545	0.706	N.D.	0.756

Table 2.17 Absorbance readings using the LCC system for standards with added DIP. Detergent used; Brij-35. N.D: Not determined. Time (t) in hours. Analyses are in triplicate.

A final concentration of about 0.001 % appeared to give good flushing of the LCC system, therefore a set of standard solutions were spiked with each of the of above mentioned detergents in turn at this concentration, and the absorbances were measured with both the LCC (Table 2.18). The detergent Triton X-100 gave similar results to those of Brij 35. Lauryl sulphate (cationic) gave the highest blanks and its wetting efficiency was poorer than the rest of the detergents, as indicated by the absorbance of the sample in the LCC tubing taking much longer to reach a flat plateau than the samples spiked with other types. The anionic detergents gave reasonable blanks and a good wetting efficiency, but still appeared to have some effect on the method chemistry. Although the absorbance of a 100 nM DIP solution was similar to the same standard without surfactant, the absorbance for the 500 nM solution was about 25 % higher than the same standard without surfactant. No further experiments were carried out with Triton X-100, Brij 35, and Lauryl sulphate.

Final DTMAB concentrations of 0.001, 0.0005 and 0.0001 % had a good wetting effect, but slight differences in absorbance readings were observed for standards. This was especially so for the 500 nM DIP solution. These differences between standards were regarded as due to adsorption at higher DIP concentration onto either the PTFE and Tygon tubing, or the inner wall of the LCC. An experiment was designed to examine this possibility and its magnitude.

Standard solutions containing 500 nM[DIP] and having the final DTMAB detergent concentrations mentioned above were treated as follows after colour development. The waste of the sample that passed through the LCC was poured into a flask, then the solution absorbance was analyzed again. The relative difference between the first and second readings were 5, 3, and 2 % for the samples containing 0.0001, 0.0005 and 0.001 % of detergent respectively. The second and third differences were below the precision of the system at that concentration, i.e. 3.9 %. It thus appears that samples containing these final DTMAB concentrations will pass through the LCC without

undergoing significant sorption processes.

Additional experiments carried out with normal spectrophotometry (Table 2.19), and a final concentration of the detergent DTMAB of 0.0005 and 0.001 % showed no interference on the chemistry of the method during the first hour after spiking. The absorbance readings made after one hour had a slight positive increase. This was more noticeable with the sample solution containing 0.001 % DTMAB. Further experiments demonstrated that excess molybdate in the sample solution slowly started to precipitate one hour after having added the DTMAB. This meant that there was no interference in the colour formation itself, but that the molybdate precipitation produced a turbidity blank during the spectrophotometric analysis.

Thus, dodecyltrimeehylammonium bromide at final concentrations of < 0.0005 % (V/V) proved to be a good wetting agent, had a low blank (< 5 nM) and gave no interference in the colour formation chemistry for at least one hour.

2.6.4 Conclusions for section 2.6

It has been shown that even minute concentrations of detergents can affect the chemistry of the traditional phosphate determination method. Although only a few of the available detergents have been tested, this exercise has shown that when wetting agents have to be used to improve flow characteristics in automated systems, their final concentrations have to be chosen with their blank, chemical interference and wetting efficiency effect in mind. From these experiments the DTMAB surfactant prove to be a good wetting agent for the LCC system described in section 2.3, whilst other detergents such as Brij 35 and Triton X-100 have been shown to interfere with the chemistry of the formation of phospho-molybdenum blue complex. Lauryl sulphate at the tested concentrations proved to have a poor wetting efficiency and high blanks

Conct. (nM[P])	Detergents a	b	c	
Blank	0.016	0.041	0.015	
100	0.193	0.225	0.108	t=0.0-0.5
500	0.920	0.894	0.482	
Blank	0.011	0.041	0.005	
100	0.221	0.226	0.097	t=1.0-1.5
500	0.993	0.918	0.617	
Blank	0.016	N.D.	0.003	
100	0.227	N.D.	0.102	t=2.0-2.5
500	1.00	N.D.	0.692	
Blank	0.016	N.D.	0.004	
100	0.230	N.D.	0.100	t=3.0-3.5
500	1.00	N.D.	0.711	

Table 2.18. The effect of different types of detergents on absorbance of the phospho-molybdenum blue method. The absorbance readings were made by using the LCC system. The final concentration of the detergents was 0.001 % W/V . Detergents are; a) Dodecyltrimethylammonium Bromide; b) Lauryl sul ate; c) Triton X-100. Time (t) in hours. N.D. Not determined. Analyses were in triplicate.

Conct.	DTMAB Detergent						
(nM[P])	N.Det.	t=0.0	t=0.5	t=1.0	t=1.5	t=2.0	
Blank	0.005	0.005	0.005	0.005	0.007	0.009	0.0005 %
100	0.030	0.029	0.030	0.033	0.040	0.040	
500	0.121	0.120	0.118	0.120	0.122	0.123	
Blank	0.004	0.005	0.004	0.003	0.003	0.005	0.001 %
100	0.023	0.024	0.025	0.020	0.020	0.018	
500	0.113	0.114	0.114	0.114	0.116	0.015	

Tab. 2.19 Absorbance readings (normal spectrophotometry) of standard solutions of 100 and 500 nM of DIP spiked with 0.0005 and 0.001 % (W/V) of DTMAB respectively. Time t in hours. N. Det.: Samples without detergent. Analyses in triplicate.

C H A P T E R
T H R E E

The behaviour of different forms of phosphorus in four English estuaries

3.1 Introduction

Generally, as has been previously noted in Chapter 1, the study of P in fresh and estuarine waters has been mainly restricted to the DIP fraction (see, for example, Burton and Liss 1976, Aston 1980, Froelich *et al.* 1985, de Jonge and Villerius 1989). Almost nothing has been reported on DOP or PTP in these environments (Meybeck 1982). One of the reasons for this is supposed to principally be the belief that DOP and PTP are not important fractions of P relative to DIP. However, two of the few studies regarding these P fractions in estuarine waters are those of Aston and Hewitt (1977) and Ormaza-González and Statham (1990a), whose results provided clear evidence for the presence of P forms other than DIP. The first paper studied the non-biological fate of P in sediments and suspended matter (as DIP, PTP and DTP) of a tidal semi-enclosed bay which was exposed to nearby sewage discharges (Walton, Essex, England). This work proved useful in assessing pollutant concentration, sources and distributions, and the role played in this particular bay by the net flux of PTP to the immediate coastal environment. The second paper dealt with dissolved and suspended particulate P behaviour and concentrations in four English estuaries and demonstrated that DOP and PTP could be important, and sometimes the main, fractions of P in some estuaries (e.g. Beaulieu and Humber). This paper was based on part of the data presented in this chapter.

Rivers are the main source of phosphorus to the oceans. There is a good estimate of riverine DIP inputs into the oceans but this is not so for DOP and PTP. The importance of

knowing the riverine flux of PTP into the oceans has been discussed by Froelich *et al.* (1982), Meybeck (1982) and Mach *et al.* (1987). Mach *et al.* (1987) argued that net removal by burial in sediments and input of DIP into the oceans are very different. They suggested that in order to balance these rates either a decrease of P burial or an increased input is needed.

Another factor that has precluded scientists from doing comprehensive P studies in estuaries is the lack of accurate techniques for the analysis of DOP and PTP, which has been due to the absence of a rigorous inter-comparison of the many techniques published rather than being no techniques *per se* (see section 2.4 and 2.5). Such an inter-comparison has been carried out and is described in section 2.2 and is summarized in Ormaza-González and Statham (1990c).

The data from Aston and Hewitt (1977) seem consistent, despite the fact that sample storage for the P analysis did not follow generally recommended procedures, i.e. unfiltered water samples were contained in polypropylene bottles and then stored frozen. The samples were thawed just before analysis and then filtered (see section 1.6 for details about sampling and storage of samples for P analysis). Nevertheless, this work demonstrated how useful information about not only DIP but also DOP and PTP could be.

Thus 1) appreciating that good information on DOP and PTP are very scarce and needed, and 2) having reliable techniques and storage-sampling procedures, it was decided to make a preliminary study of this element in the Itchen, Beaulieu, Thames and Humber estuaries (see Fig. 3.1 for locations).

There have been several studies of the chemistry of the Itchen estuary (e.g. Phillips 1980). However, only a few measurements of DOP (Collins 1976) and PTP (Phillips 1980) have been made in this area. The Beaulieu estuary which drains part of the New Forest (Hampshire, South England), is relatively pristine (Phillips 1980), providing a contrast to the other systems studied. No study of DOP and PTP has been

found in this estuary, although there is some work on DIP (see Coles 1977, Umnuay 1981). No data on DOP has been found for the Thames and Humber.

A detailed study of the different forms of P was made in the first two estuaries, whilst a less detailed investigation was carried out at tidal stations in the lower reaches of the Thames and Humber estuaries. The Thames and Humber provide the major river water input to the North Sea from England, and have average freshwater discharges of 154 and 246 m^3/sec respectively (Ormaza-González and Statham 1990a). These estuaries are subject to considerable inputs of a variety of pollutant materials, including treated sewage. The Itchen has much lower discharges of fresh water, which exponentially decrease from a winter high of about 12 to about 3 $m^3.sec^{-1}$ at the end of the summer. There are inputs of treated sewage to the river. The fresh water discharge of the Beaulieu is small, varying from about 1 in the winter to as low as 0.03 $m^3.sec^{-1}$ in the summer (Southern Water Authority).

The major objective of this work was to 1) assess the relative importance of DOP and PTP in the overall budget of P, 2) describe their distribution together with that of DIP during estuarine mixing processes, 3) observe any seasonal changes in the concentrations and behaviour of the different forms of P in the Itchen and Beaulieu estuaries, 4) estimate the fluxes of these forms of P into the neighbouring coastal waters, and 5) discuss the most probable processes during the P transport through this range of estuarine environments.

3.2 Sampling

The different dates during which the surveys were carried out in the above mentioned estuaries are shown in Table 3.1 (page 143), whilst Figure 3.2 gives a more detailed view of the Itchen estuary and Southampton Water. The sampling was planned in order to observe seasonal variability, thus initially a survey in the middle of each season and in during the transition between season. However logistical constrains

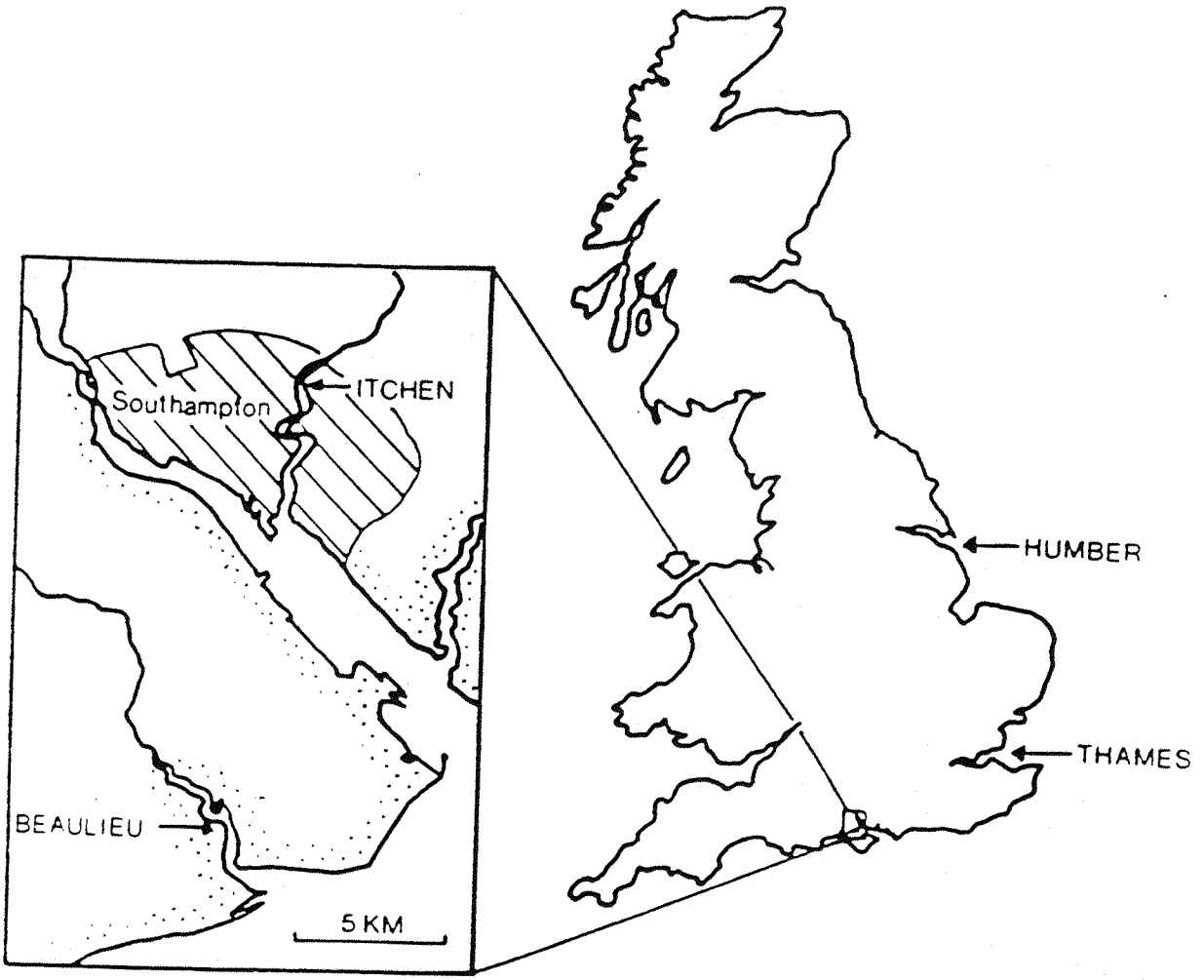


Fig. 3.1 Location of the Thames, Humber, Beaulieu and Itchen estuaries.

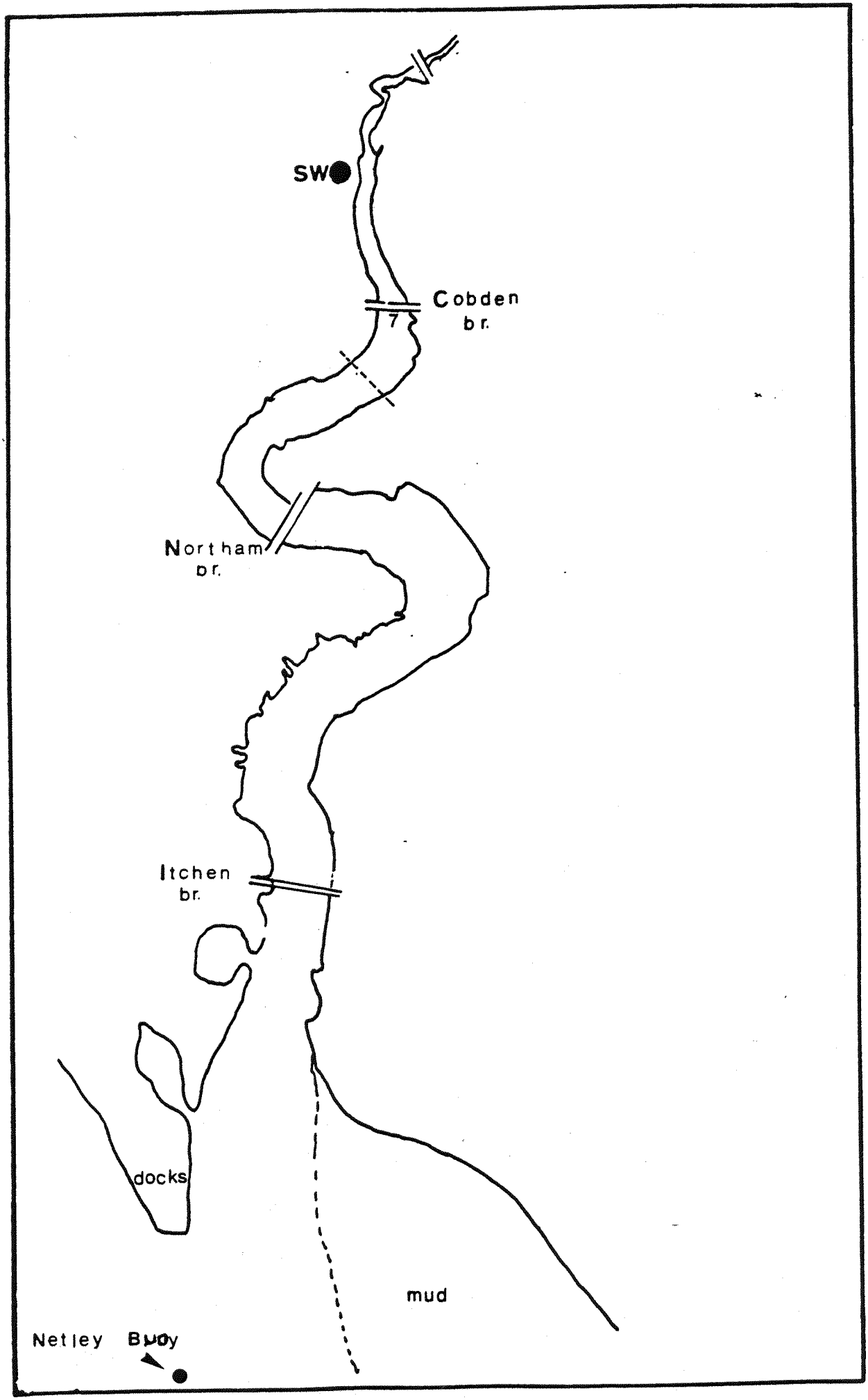


Fig 3.2 (Map) The river Itchen-Southampton water system. sewage works, SW :sewage treatment plant at Portswood.

greatly affected the initial strategy, and in the end half of the initially planned surveys were carried out.

For sampling in the Itchen and Beaulieu surveys, a non-contaminating pumping system was mounted on the 10m boat Labrax (from the Dep. of Oceanography, University of Southampton). Water was pumped from about 0.3-0.4m depth, and directed into a 1 dm^3 plastic beaker, in which a salinometer/temperature probe was immersed. Samples were taken from the overflow of the beaker every approximately 1 or 2 salinity units. Salinity generally ranged from *circa* 0 to 34. This covered the Itchen river from upstream of the Porstwood sewage treatment plant (Fig 3.2) to the Netley navigation Buoy, at which samples from a vertical profile were taken on three occasions. The vertical sampling was done by lowering a plastic tube, which had a heavy weight close to the opening in order to keep it at the desirable depth, and then pumping for 5-7 min at each depth to avoid carry over from other depths before collecting a sample. Samples for fresh water were taken at the head of the Itchen river by hand as the Labrax was unable to reach that point. Because the Beaulieu estuary is relatively shallow in its upper reaches, a small boat had to be used, or even sometimes samples were taken from shore. Samples from the Thames and Humber estuaries were taken during cruises CH42 (December 1988) and CH46 (February 1989) respectively of RRS Challenger, using a 10 dm^3 Go-Flo bottles deployed from a CTD-rosette.

3.3 Handling and storage of samples

All the glass containers, filtration system, Whatman GF/F filters, and aluminium foil were carefully cleaned in the laboratory, as indicated in section 2.2.1.

The main water sample was contained in a 0.25 dm^3 glass bottle (Duran) which had a polypropylene screw cap. The bottle was leak-proof. The GF/F filters were treated as indicated in section 2.5. Square pieces of aluminium foil (of about 60 X 60 mm) for storing GF/F filters were cut and heated at 450^{0}C

for two hours.

The GF/F filters (nominal pore size 0.7 μm) were used because they are easy to manipulate and have higher flow rates than other types of filters when dealing with the variable loads of suspended matter found, for example, in the Itchen (2 to 50 $mg.dm^{-3}$, Head 1969). Any colloidal (1-10 nm to 0.45 μm diameter, Moran and Moore 1989) or other micro-particulate forms passing the filter which were not available to the DIP technique would be measured as DOP.

Before taking the samples, the bottles (Duran) were rinsed at least twice with the sample. The tightly capped bottles were put into a light-proof insulated container, in which 2 freezer packs helped to keep the samples cool during transport to the laboratory, where they were immediately filtered under vacuum (not greater than 125 mm-Hg). The time between sampling and filtration was typically 3-4 hours. Samples for DIP, DTP and filters for POC, Chlorophyll *a* (Chl-*a*) and PTP (duplicates) were stored frozen below -10 ^{0}C. In the first survey a few samples for DOC analysis were also taken. It should be noted that no quick freezing of the water samples was possible, as has been recommended by some workers (e.g. Burton 1973, Morse *et al.* 1982).

In order to examine any blank from the Duran bottles and glass filtering system, in the first survey (16.Nov.88), a sample of DDW was passed through the filtration steps and stored in the same way as estuarine water samples. Four filters, two for POC and two for PTP were used to "filter" a DDW sample, and were then stored in the same way as the samples, in order to determine any blank from handling them.

3.4 Analysis

The analytical procedures, materials, glassware, equipment and reagents for the analysis of DIP, PTP and DTP were those described in section 2. For colorimetric analysis a 10 or 40 mm path-length cell was used. In general, a batch of fresh

reagents was prepared for each survey. Samples for DIP and DTP (DOP) were analyzed simultaneously. Triplicates of standards and blanks were performed daily. The decomposition efficiency (DE) for DTP analytical procedure was regularly checked, by using standard solutions of 2 μM P of phosphonate and polyphosphate, and the DEs ranged between 103 and 95 %. For measuring volumes of less than 10 cm^3, automatic (Gilson) pipettes were employed. For larger volumes, glass measuring cylinders were used.

The methods employed for POC and DOC analysis were those described by Knap (1979), and Statham and Williams (1983) respectively. The principle of these methods is based on oxidation of the organic carbon into CO_2 by high temperature and UV irradiation respectively, and then the measurement of the produced gas by means of an infra-red gas analyzer. Analysis of Chl-*a* was carried out as described by Strickland and Parsons (1972); acetone (90 % V/V) was employed as the pigment extractor and carrier, and the Chl-*a* was determined fluorometrically. The *in situ* measurements of salinity were performed using a portable salinometer (Electronic Switchgear) which had a combined temperature probe. In the laboratory salinity was re-measured using a Guildline Autosal salinometer to provide exact values. The average difference between salinity measured *in situ* and in laboratory for all analyzed samples was 2.5 % (a range of 1-4 %, in 98 % of samples); in the first and second surveys the difference was lower than 1 %. Valid comparisons at very low salinity (< about 3-4) were not possible as the conversion of the Guildline reading (conductivity) into salinity units becomes more problematic (i.e. less accurate) at low salinity.

3.4.1 Accuracy, precision, and blanks

Accuracy and precision can be assessed in terms of the self-consistency of the data and recovery efficiency for the DOP analysis, and reproducibility of the duplicates during analysis of samples respectively. In addition, measurements of blanks were carried out to check the effectiveness of

glassware and container cleaning treatment. The detection limits for DIP and PTP posed no inconvenience as concentrations were above them (see Section 2.1 and 2.5), but for DOP some samples had concentrations close to or below the detection limit (50 nM).

Consistency of the data is assessed in the section 3.5, and reproducibility, blanks, standards and DEs were checked as follows.

All the analyses were carried out in duplicate, with two spectrophotometric readings per each duplicate. The standard deviations in absorbance units of a batch of 10 samples and blanks, and standards analyzed in one run were 0.002, 0.006 and 0.013 for DIP, DTP and PTP respectively. These values were calculated by using the relationship

$$s = \sqrt{\Sigma d^2/(2n)}, \qquad \text{(Eq. 3.1)}$$

where s is the standard deviation, d is the difference between duplicates and n is the number of pairs of determinations (Ynaden 1951, quoted by Thompson and Howarth 1973). The relative standard deviations (RSD) achieved for the sample pair with the lowest absorbance reading (using 40 mm cells for DIP and DOP, and 10 mm cells for PTP) when the precision estimates above were used are 2.8, 7.3 and 8.7 % for DIP, DTP, and PTP respectively. However, it can be seen from the Appendixes (3) that on a few occasions the precision was poorer than these. It should be noted that the spectrophotometer (the same as used in section 2) readings alone gave a noise of ± 0.002-0.003 absorbance units. Therefore the standard deviation of low concentrations of DIP could be regarded as being due to just the spectrophotometer variability. For DTP and PTP the increase of s seemed to be proportional to the manipulation of the samples, i.e. the more manipulation the higher the s obtained; presumably because of increased random low level contamination.

The average absorbance readings of blanks, for DIP and DTP

in all the runs, using a 40 mm path-length cuvette, were 0.001 and 0.003 respectively, whilst for the PTP analysis with 10 mm cells, they averaged 0.085. The reason for this, explained in section 2.3, is the P content in the GF/F filters. This type of magnitude and variability of blanks from glass fibre filters has been previously reported by Bloesh and Gavrieli (1984). Blanks for POC filters averaged about 8 μmol[C]/filter.

The blank given by the Duran bottles, and by the sampling and filtration itself (<0.004 absorbance for both DIP and DTP analyses) indicated that the Duran bottles were not a source of contamination.

Attempts to investigate the effects on concentrations of the different forms of P by filtering water samples immediately after collection and doing so 2-4 hours later were hampered because of difficulties in using the glass filtration system on board. Because samples were kept cool inside a light-proof and insulated container and filtered after 2-4 hours, the potential removal of DIP by photosynthetic processes would have been stopped or slowed down considerably. Regeneration of DOP into DIP, by for instance APA, was unlikely as DIP was generally higher than 50 nM, except in a few samples from the Beaulieu, but activity of the 5'-nucleotidase enzyme cannot be disregarded (see Chapter 4). Adsorption of DIP either on suspended particles or glass bottles can not be assessed due to the lack of information, but in any case it should have been small. In general, it can be said that filtering the samples as done here is more satisfactory than doing so after many days or weeks as some scientists have done (e.g. Aston and Hewitt 1977).

Overall it can be said that sampling, handling, and analytical procedures were acceptable and therefore the data obtained would mainly be a real reflection of the situation in the investigated estuarine systems.

3.5 Results and discussion

3.5.1 The Itchen Estuary

3.5.1.1 Dissolved inorganic phosphate

Horizontal profiles show major changes along the estuary, with an overall non-linear mixing series between the salinity extremes for the 4 surveys (Fig. 3.3a and b, and 3.4a and b). The major features in the profiles of the first three surveys are: 1) The very high concentrations of DIP centred at about a salinity of 5. 2) The sharp increase in the second survey relative to the first (maximum concentrations for DIP of 55.0 μM and 20.1 μM respectively). 3) The conspicuous and sharp fall of DIP between 5 to 10 salinity, and 4) The linear decrease of DIP at salinity above 10 to the saline end members.

In the fourth survey DIP showed a slightly different behaviour and concentrations were lower over the whole salinity range. It was not possible to measure P at about salinity 5 because no sample was taken at this salinity as the salinometer broke down. However the maximum would be expected to have been centred about 2-5 salinity.

The concentrations of DIP in the Itchen River and Estuary reported here are significantly higher than those reported for other components of the Southampton Water system (Phillips 1980) and many other estuaries around the world (Meybeck 1982, however for even higher concentrations see Froelich *et al.* 1985). For example, Steffansson and Richards (1963) reported that the DIP levels in the Columbia river were almost invariant over the whole range of salinity, with an average of 1.19 μM. They concluded that both riverine and coastal waters had similar concentrations of DIP, and that there was a "buffering" effect which maintained the average phosphate concentration in the estuary relatively constant. Phosphate buffering by solution-mineral reactions has frequently been suggested as an important process in fresh and estuarine water

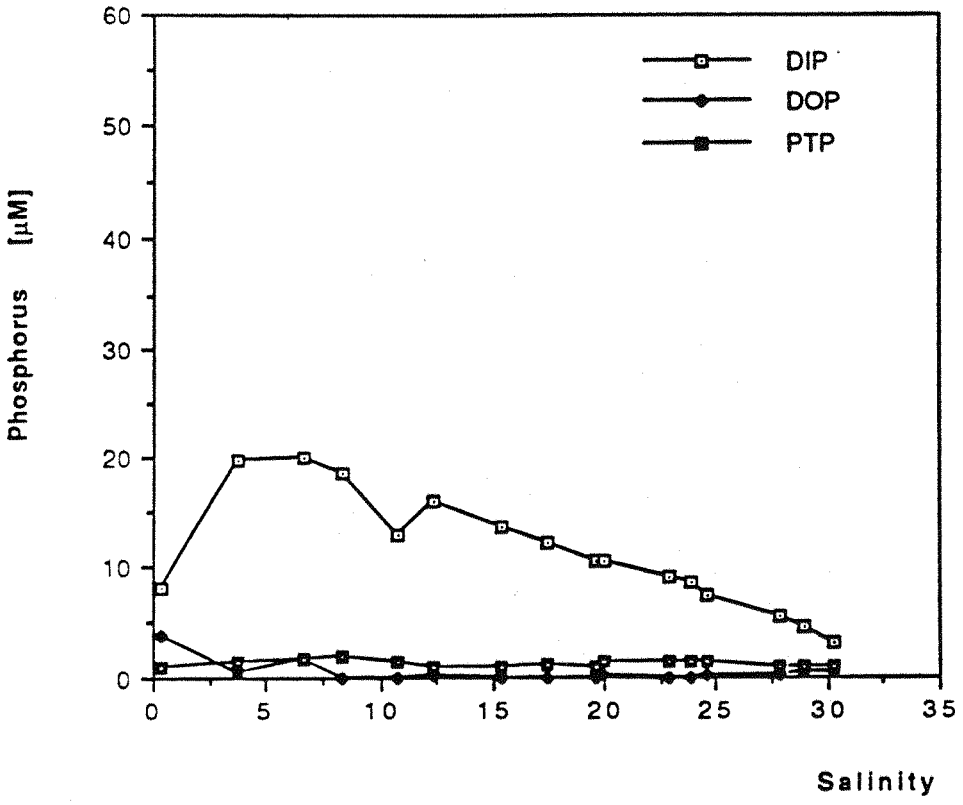


Fig. 3.3a Phosphorus phases in the Itchen and Southampton Water. Nov. 88

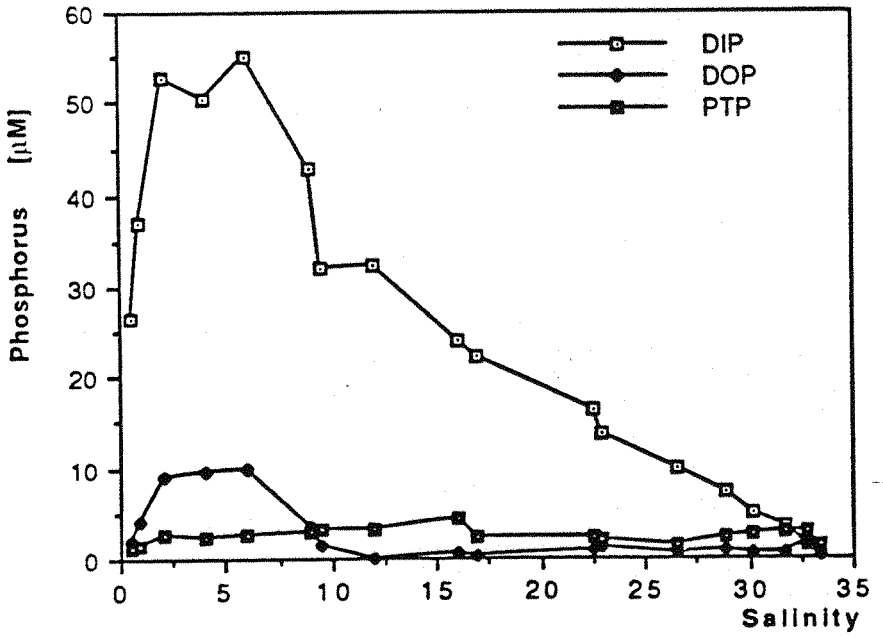


Fig. 3.3b Phosphorus phases in the Itchen R. and Southampton Water. Jul. 89.

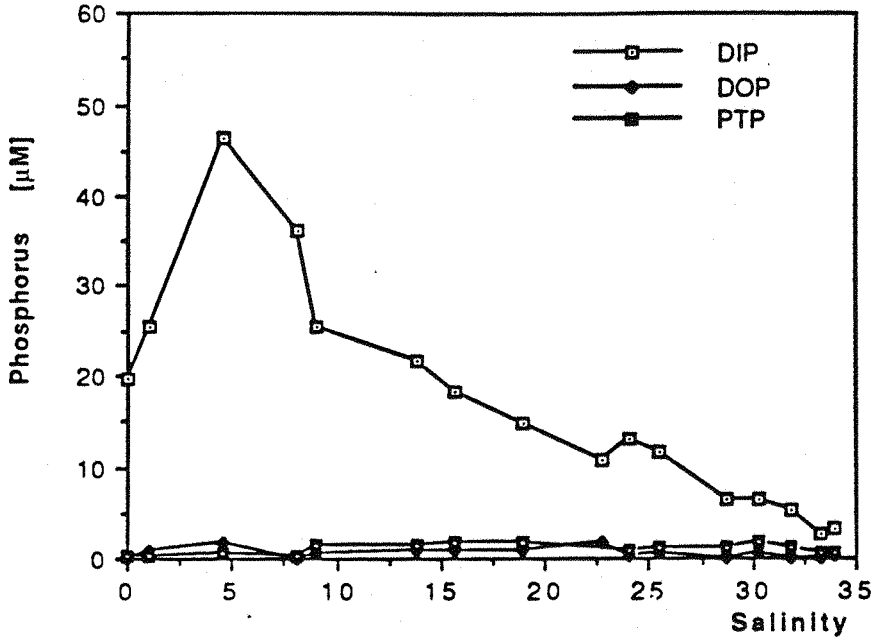


Fig. 3.4a Phosphorus phases in the Itchen R., and Southampton Water. Oct. 89.

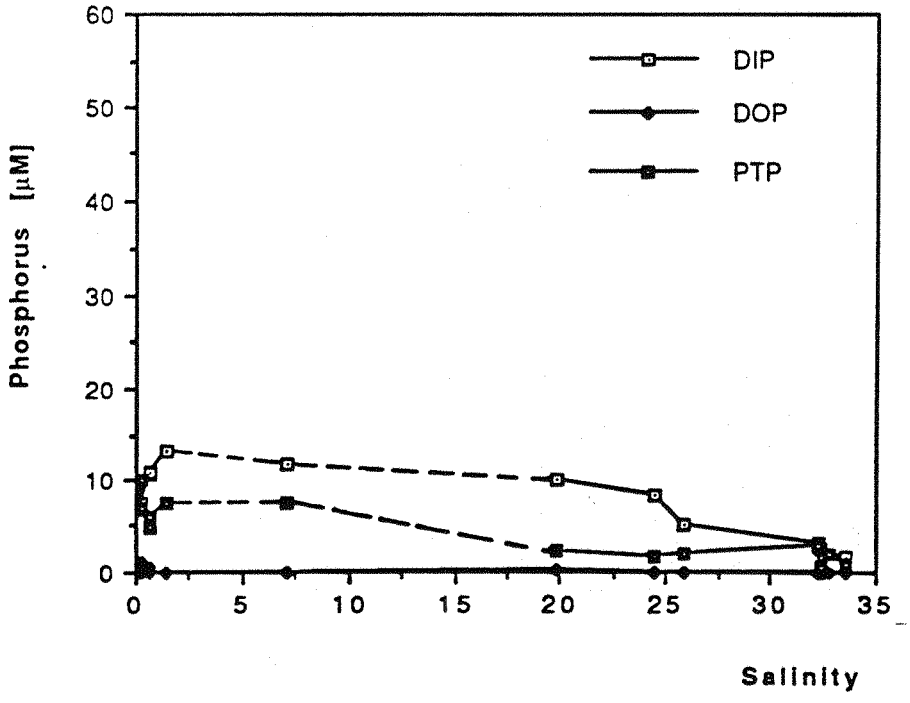


Fig. 3.4b Phosphorus phases in the Itchen River and Southampton Water. Dec.89.

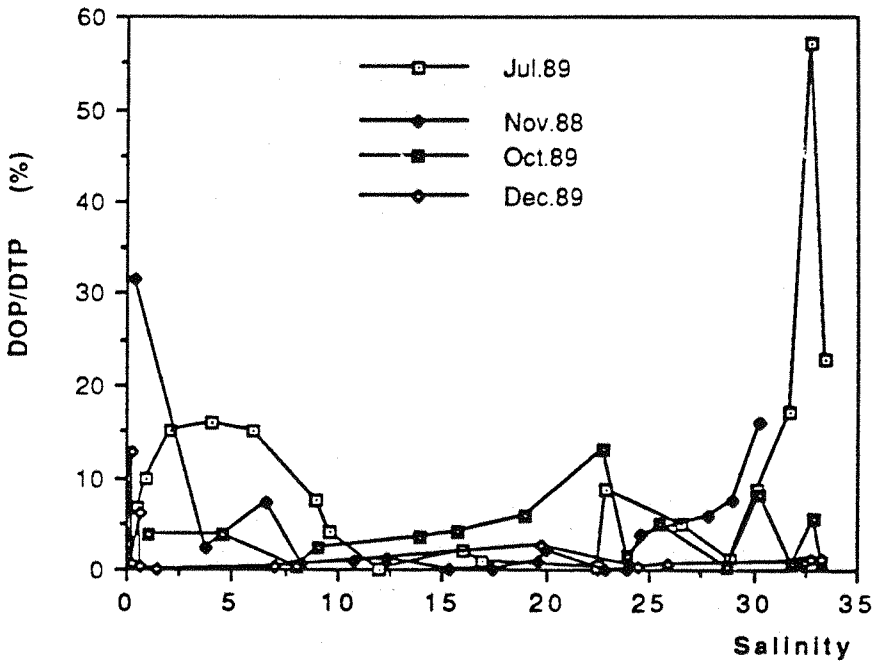


Fig. 3.4c DOP/DTP ratio in the Itchen R. and Southampton Water.

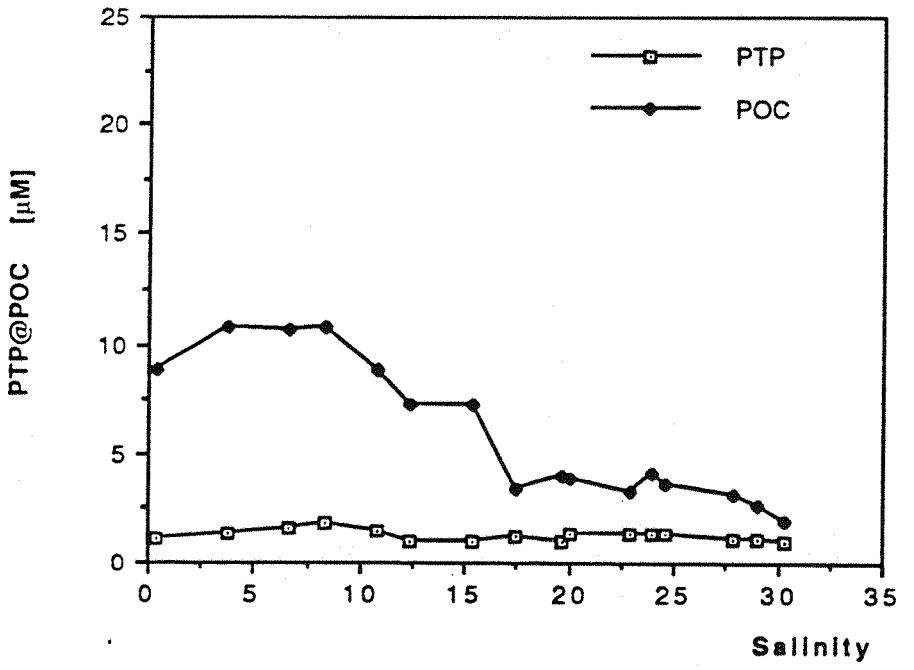


Fig. 3.5a. POC and PTP in the Itchen River and Southampton Water. Nov. 88. Note that POC is divided by 10.

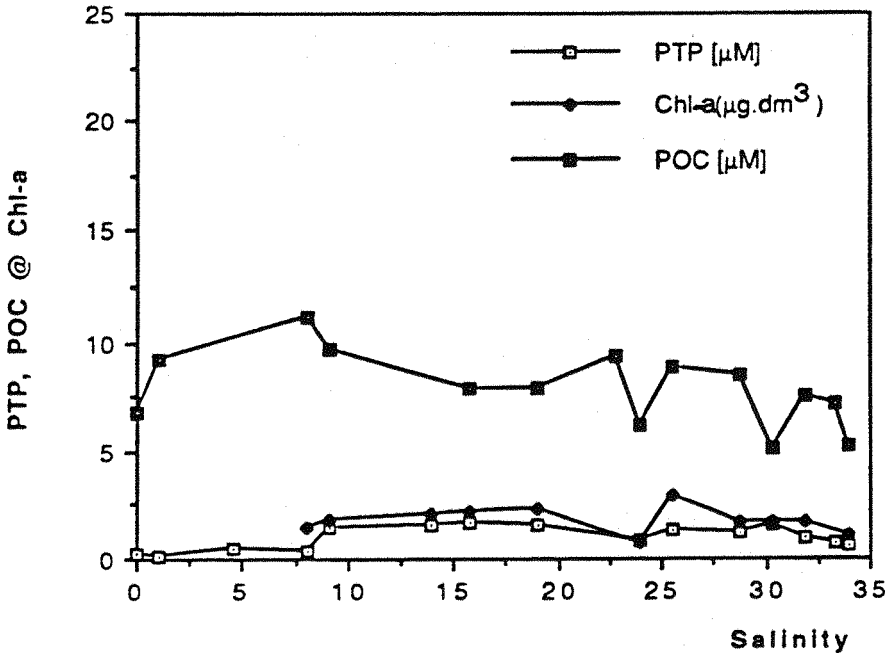


Fig. 3.6a. PTP , POC and Chl-a in the Itchen R. and Southampton Water. Oct. 89. Note POC is divided by 10.

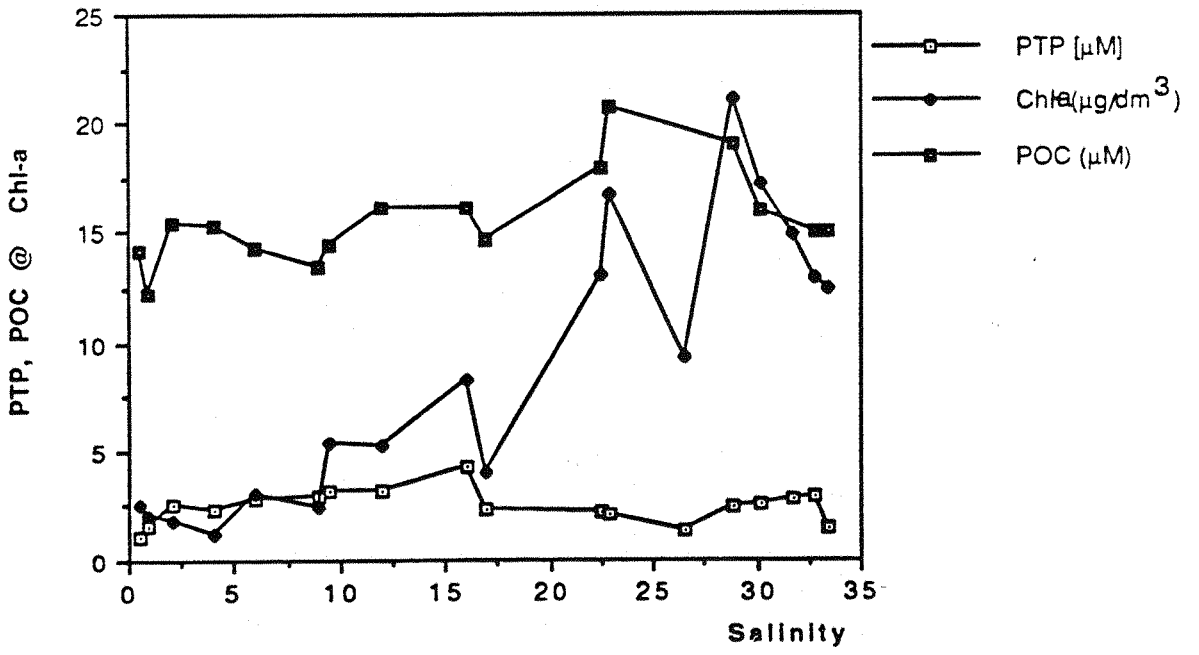


Fig. 3.5b PTP, POC and Chl-a in the Itchen R. and Southampton Water. Jul. 89. Note that POC is divided by 10.

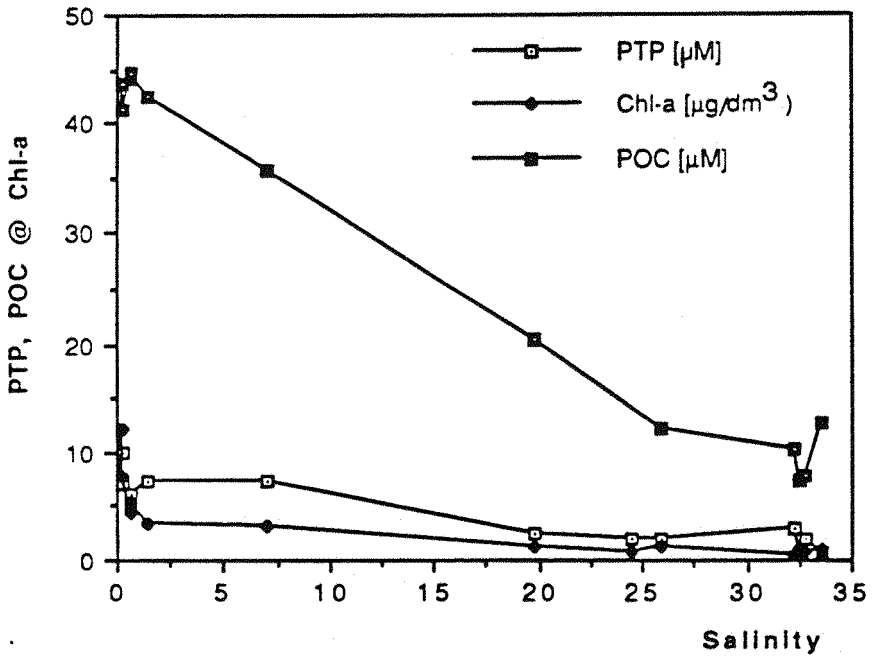


Fig. 3.6b. PTP, POC and Chl-a in the Itchen R. and Southampton Water. Dec. 89. Note the change of scale of the vertical axis.

(e.g. Mortimer 1971, Patrick and Khalid 1974, Stirling and Wormald 1977). The range of "equilibrium" concentrations varies from estuary to estuary; as examples, Pomeroy *et al.* (1965) reported a range of 0.71 - 0.91 μM and an average of 0.81 μM for Doboy Sound (Georgia, U.S.A.) whilst Butler and Tibbits (1972) and Morris *et al.* (1981), reported a range of 0.71 - ca 2 μM with an average of 0.97 μM for the Tamar estuary. It has thus generally been suggested that suspended matter and sediments in estuaries tend to maintain a DIP concentration of about 1 μM.

Perturbations to this general pattern have been observed where large urban or industrial inputs are present e.g. the Clyde (Mackay and Leatherland 1976). Lucotte and d'Anglejan (1988) also reported a lack of buffering reactions in the Lawrence Upper Estuary (Canada). However, in the Clyde study active removal of P was still occurring as inferred from the non-conservative behaviour of the DIP. Hobbie *et al.* (1975) have reported that about 60 % of the DIP entering the Pamlico River estuary (North Carolina, USA) was retained by estuarine sediments. Further insights into dissolved-particulate phase interactions for P have been made by de Jonge and Villerius (1989). These authors suggest that for the Ems estuary (Holland) calcite derived from the more saline reaches can act as a carrier phase for adsorbed P, which on transport upstream and reaching lower pH (<8.0) environments in fresher waters, can undergo dissolution and release of adsorbed P. This released P is again available to adsorb onto non-clay particles, thus giving the non-conservative behaviour of P in this estuary. A summary of DIP behaviour in worldwide estuaries is given by Carpenter and Smith (1984).

Iron hydroxyoxides coating suspended particles or in colloidal forms are reckoned to be the main scavengers of phosphate (e.g. Carpenter and Smith 1984, Smith and Longmore 1980) in estuarine waters. Adsorption onto hydro-oxides is favoured by: Low salinity and ionic strength, lower pH conditions (Morris *et al.* 1981, Lucotte and d'Anglejan 1988). Also the increase of temperature (Chen *et al.* 1973, Crosby

et al. 1984), organic matter (Sholkovitz 1978), redox potential (DeLuane *et al.* 1981), and coagulation processes (Bale and Morris 1981) contribute to the removal of DIP. However terrestrially originated humic acids reduce adsorption rates (Carpenter and Smith 1984).

In the data presented here, steep concentration gradients in DIP are evident above and below the input from the sewage treatment plant (2-7 salinity). These sudden increases reflect mixing fronts as the discharge disperses into the surrounding estuary. On the other hand the dramatic fall of DIP concentrations between 7-10 salinity range strongly suggests removal of DIP by the processes indicated above. There is good agreement in the scientific literature that the major removal of DIP occurs between salinities of 0 and 10, especially in the maximum turbidity zone, where there is an increased amount of suspended particles and appropriate factors assisting adsorption processes, mentioned in the previous paragraph. These conditions helped to reduce DIP concentrations by 25-50 % in the mentioned above salinity range. Hobbie *et al.* (1975) have reported 60 % removal of DIP, from the Pamlico estuary (N. Carolina, USA). Nitrate and nitrite concentrations of up to 250 and 20 μM respectively (summer months) respectively have been measured in front of the sewage treatment plant (Ormaza-González 1987) and these elements have shown to have non-conservative behaviour relative to both salinity and dissolved silicate in this river. If these concentrations were assumed to occur during the surveys, the dissolved inorganic nitrate ratios to DIP would be less than 5 which is near to the ratio of < 5 reported by Ward and Twilley (1986) in the Chesapeake Bay, but significantly lower than Head's (1985) proposed average ratio for estuaries of 10, thus suggesting that P content in the discharged sewage is higher than expected or the nitrogen is lower.

In the salinity range of 10-34, DIP showed a typical conservative dilution behaviour relative to salinity, although a slight increase of DIP can be observed at about 25 salinity.

If association of P with particles is important, and potentially reversible (Carpenter and Smith 1984), a rapid transport of these particles to the sediments must be occurring as the levels of particulate total P are not adequate to account for the removal of DIP inferred if there were conservative linear mixing between the discharge input and the estuary mouth. In the region below that influenced most directly by the treated sewage discharge (approximate salinity range 10-33), there appears to be linear conservative mixing between the high concentration below the sewage treatment works input and the low concentration seawater end-member, except in the fourth survey in which both distribution and concentration patterns were different to before.

Chen *et al.* (1973), and other workers, have examined the effect of temperature on phosphate adsorption, and found that the degree of removal is favoured by a rise of temperature. As there is a significant difference between the summer (about 20^0C) and winter (about 7^0C) river temperatures (data not shown), adsorption processes should be favoured during the former period. However, in the Itchen such removal processes do not appear significant relative to the inputs of DIP, as DIP concentrations are three times as high in the summer than in the winter.

A further reason for the high values of DIP in summer could be that adsorption sites were less abundant as the amount of suspended matter decreases due to a lower runoff and less mixing and resuspension than during winter.

In the fourth survey, although there is no DIP information between salinities of 10-20 (Fig. 4.4b), the following tentative observations can be made relative to the rest of the surveys: 1) The riverine input of DIP is higher than those of the first and fourth surveys, but lower than that of the second survey. 2) Between salinities of 2 and 30, DIP concentrations are lower than in previous surveys, and 3) The dilution slope in this salinity range of 10-33 is less steep.

The marked differences between the fourth and the rest of the surveys can be ascribed to 1) higher riverine water flow and 2) higher suspended particulate matter. During this survey the river flow increased by about 20 %, and the weather was unusually windy which led to an increased amount of suspended sediment. The re-suspended sediment would have desorbed DIP under anoxic conditions, but the contrary process occur under oxic conditions (DeLaune *et al.* 1981), which would explain the DIP behaviour.

Concentrations of DIP in the river water fluctuated from below 10 (winter) to about 20 μM (summer). This data combined with fresh water flow values (provided by The Southern Water Authority) allows the computation of the flux of DIP into the estuary (Table 3.2). The variability of the fluxes depend greatly on the combination of runoff, river water and sewage discharge.

At the other salinity extreme (salinity about 33.50) DIP concentrations were 0.68 (Jul.89), 2.75 (Oct.89), 1.65 (Dec.89), which are in the range reported by Tappin (1988); 0.08-0.96 μM, and Antai (1989); 0.60-2.5 μM, for the nearby English Channel and Netley Buoy waters respectively. These concentrations and distributions were highly affected by biological (uptake and probably regeneration), physical-chemical removal processes, and levels of DIP of the surrounding coastal waters. The flux (F) of DIP into the ocean can be calculated by:

$$F = Q(C-S(dC/dS)), \qquad \text{(Eq. 3.2)}$$

where Q is the fresh water discharge, C the concentration of the analyte (in this case, DIP) and S salinity at any section of the estuary, and dC/dS is the dilution slope. The latter value has been obtained statistically from data in the salinity range of 10-35. Table 3.2 shows that in the Itchen Estuary there was a net input of DIP into the estuary.

	Surveys			
Estuary	1	2	3	4
Itchen	16.Nov.88	19.Jul.89	2.Oct.89	14.Dec.89
Beaulieu	20.Jul.89	5.Oct.89	15.Dec.89	
Humber	Feb.89			
Thames	Dec.88			

Tab. 3.1 Surveys carried out in the Itchen, Beaulieu, Humber, and Thames estuaries.

Surveys	Itchen			Beaulieu		
	Estuary	River	Input	Estuary	River	Input
1	81.2	28.7	52.5	2.30	N.D.	N.D.
2	135	75.0	60.0	8.70	8.60	0.10
3	82.5	50.2	32.0	8.50	1.90	7.60
4	91.1	32.3	58.8	----	----	----

Tab. 3.2 Flux of DIP ($mol.sec^{-1}$) from the river into the estuary (River) and the estuary into the sea (Estuary). A positive input indicates flux of DIP from the estuary to the sea, whilst a negative input indicates a reverse case. The input for the Humber and Thames estuaries were 16219 and 78.7 $mol.sec^{-1}$ respectively.

3.5.1.2 Dissolved organic phosphorus

Dissolved organic phosphorus generally represented a small fraction of the total dissolved P in the Itchen estuary, although the DOP:DTP ratio ranged 58 - 0 % (Fig. 3.4c), with very low ratios (< 5 %) being found during winter (Nov. and Dec.). Concentrations fluctuated from below detection limit (0.05 μM, these points are plotted as 0.025 μM on figures) to 1.8 μM in winter and to 9.9 μM in summer (Fig. 3.3a and b, and 3.4a and b). The DOP:DTP ratio was most variable and often greatest at the salinity extremes. In the winter surveys the main source of DOP was the river with a range of 3.73-1.03 μM, whilst the treated sewage input was of secondary importance. In the summer the latter source dominated (see Fig. 3.3b). In the middle salinity range DOP concentrations decrease to very low or below detection limit levels reflecting either direct removal to particulate phases or oxidation to inorganic forms. The latter being most likely as levels of PTP at these locations did not increase. In all the surveys DOP tended to slightly increase from salinities greater than about 22. The increase was more notorious in the summer survey and this was associated with biological activity as can be deduced from the PTP and Chl-*a* data (Fig. 3.5a and b, and 3.6a and b), which confirmed the visual observation of patches of phytoplankton in sampling area. These blooms were of *Mesodinium rubrum*, which are obvious because of the reddish coloration of the water that makes them very conspicuous during summer months, especially in July (e.g. Antai 1990). The association of higher DOP concentrations with phytoplankton is also evident in the vertical profile (see below). Also it can be observed that DOP concentrations increase in the upper reaches (2-7 salinity), as a very likely consequence of sewage discharge. Thus, DOP behaved non-conservatively because of probable input in the low salinity range, removal in the middle salinity range and *in situ* biological production in the lower reaches of the estuary. The role of recycling of DOP via organisms is potentially important as has been demonstrated by Kobori and Taga (1977) and Taga and Kobori (1978), and Ammerman and Angel

(1990), who reported regeneration of DOP by nucleotidase and alkaline phosphatase enzymes in the Hudson and Tokyo Bay estuaries respectively. The first enzyme is particularly important because it is of bacterial origin and its activity is not regulated by DIP as is alkaline phosphatase. The relative importance of these processes cannot be elucidated from the data given here.

Generally, it has been shown that DOP in the upper reaches of the Itchen estuary has allochthonous and autochthonous origins. Thus the peaks at the low salinity end member can be explained in terms of 1) runoff of the fresh water, which drains agricultural land that is generally treated with fertilizer as well as herbicides, bactericides, etc that contains some organic P (Cook *et al.* 1978), and 2) the input from the sewage works. A fraction of the DOP is removed in the middle salinity range by unidentified processes. The allochthonous organic material is regarded as consisting of refractory material such as humic substances (Thurman and Malcolm 1983, Ertel *et al.* 1986) which comprised 40-80 % of the dissolved organic matter. Towards the salt water end-member, DOP concentrations start to increase in an irregular manner. In this region DOP could mainly be of autochthonous biological origin, particularly in summer. From the seasonal point of view DOP and DOP:DTP are lower in autumn and winter than in summer months when biological activity is reduced. In the winter survey there was a small but consistent increase in DOP concentration with increasing salinity, but at this time of year when biological activity is low the importance of this source is unclear. The data is suggesting that DOP is mainly of autochthonous origin in the estuary; especially in the mid and high salinity range.

With the samples of DOC it was not possible to determine the distribution of DOC along the whole the estuary (App. 3.2a) because most of the samples had extremely high concentration of DOC and were assumed as contaminated. The samples of DOC, which appeared uncontaminated because their levels were about the average of 2 $mg[C].dm^{-3}$ of DOC reported

by, for instance, Mayo (1970), showed that the concentrations at the low salinity end member was double that in the high salinity end member. The relationship DOC:DOP for the accurate measurements was below 200. This ratio at the last three stations near the high salinity end member increased from 100 to 208. These few data suggest that DOP becomes a more significant part of the organic matter at higher salinities. These ratios are well below those suggested by Meybeck (1982) which are about 1000. A more detailed discussion on processes (generation and regeneration) cannot be made, but it has been shown that DOP is an important fraction of the dissolved organic matter.

3.5.1.3 Particulate total phosphorus

The term particulate total phosphorus (PTP) was employed, because under estuarine conditions the particulate phase of P is not only organic but also inorganic due to the adsorption of DIP and other elements onto the suspended material. Thus the term PTP, in estuaries, is more precise than POP.

There was a marked difference in both concentration and behaviour of PTP in the four surveys. In winter (Oct. and Nov.), PTP averaged about 1 μM along the whole salinity range, except in the December survey in which the average for PTP was > 4 times higher. This was a clear reflection of re-suspended sediment present as a consequence of weather conditions. In summer PTP varied greatly from 1.09 to 4.71 μM, with an average of 2.44 ± 0.74 μM (Tab. 3.3, see also Fig. 3.5 and 3.6 a and b). In order to have a more comprehensive understanding of the biogeochemistry of the particulate suspended organic matter, POC and Chl-*a* were also measured. Particulate organic carbon fluctuated from 30 to 120 in the first and third surveys to 157 μM in summer months following similar pattern to PTP. But during the December survey POC increased by nearly two times (271 ± 159 μM) relative to the October survey, whilst Chl-*a* had a less defined pattern, although there was a clear decrease in concentration in winter months down to

Itchen Surveys	1	2	3	4
PTP	1.58 - 0.93	4.27 - 1.09	0.99 - 0.17	9.95 - 0.70
	1.24 ± 0.24	2.45 ± 0.74	1.73 ± 0.53	4.23 ± 2.89
POC	120 - 30	207 - 122	112 - 51	446 - 73
POC:PTP	47	64	80	64

Beaulieu Surveys	1	2	3
PTP	4.78 - 1.00	1.78 - 1.43	2.36 - 3.45
	1.82 ± 0.90	1.78 ± 0.59	1.90 ± 0.56
POC	256 - 49	208 - 32	292 - 54
POC:PTP	83	50	78

Tab. 3.3 PTP and POC concentration ranges and averages, and POC:PTP ratios in the Itchen and Bealieu estuaries. The POC:PTP ratios are average.

1-2 $\mu g.dm^{-3}$. Chlorophyll-*a* in summer steadily increase from 2 $\mu g.dm^{-3}$ to 21 $\mu g.dm^{-3}$ in high salinity waters (Fig. 3.5 and 3.6 a and b). Degraded Chl-*a*, i.e. phaeopigments, levels represented less than 30 % of the Chl-*a* during July, and then increased to above 50 % in the winter months (see App. 3). The concentration ranges for POC and Chl-*a* are similar to those reported before (e.g. Olausson 1980, Ward and Twilley 1986) but the POC is on average lower than the world rivers average of 417 μM (Meybeck 1982).

Information on PTP in other estuaries, as mentioned before, is scarce. The PTP concentrations presented in here are lower that those reported by Aston and Hewitt (1977), but no proper inter-comparison can be made due to the inadequate sampling and storage procedures followed by Aston and Hewitt. The concentrations of PTP reported here also seem to be larger than those given by Stevenson (1986), who proposed a C:P weight ratio of 100 (i.e. 258 mole ratio) for organic matter soil. Here the mole ratio C:P consistently varied from 80 to 47 (Tab. 3.3), which implies that suspended sediments in this estuary contain 3-5 times more PTP than the suggested average estuarine suspended sediment. The C:P mole ratio of the riverine suspended particulate matter was higher (83-283) approaching Stevenson's ratio; an exception is the December survey (44-92) in which there was a significant re-suspension of settled sediments. The December data would imply that re-suspended sediments are even richer in P than particles in the water column found under calm conditions. Another sign of the P enrichment in re-suspended sediments, whose loads decrease towards high salinity waters, is that PTP is quite uniformly distributed throughout most of salinity range (10-31), whilst POC tends to decrease with increasing salinity at values higher than 10, as reported by Ward and Twilley (1986). Particulate total phosphorus showed an increase in the region where DIP suddenly decreased (salinity range 5-10); this was particularly evident in the October and November surveys. In all the surveys, at salinities higher than 32, PTP slightly diminished in concentration and the C:P ratio increased to 177. The slight decrease could be ascribed to the lower

suspended particulate matter loads, but also release of DIP from suspended sediments could occur, as reported by de Jonge and Villerius (1989). Taking the lowest average PTP of 1 μM as a representative of other estuarine systems, the input of PTP into the nearby coastal waters will be 2-5 times higher than the predicted value (Mach *et al.* 1987) for this type of environment. In general, PTP appeared to be mainly of allochthonous origin as suggested by Hedges *et al.* (1986), with some anthropogenic contribution from the sewage treatment plant, plus *in situ* PTP production, especially at higher salinities.

3.5.1.4 Vertical distribution of phosphorus

The three vertical profiles (Fig. 3.7a,b,c) at the Netley Buoy were performed in order to observe the concentrations and behaviour of P with depth. In general the water column was well homogenous at the time of sampling, with salinity gradually increasing by 0.16-0.24 from the surface to 8m depth. Dissolved inorganic phosphate was constant with depth, except during October when there was a clear decrease below the surface and a minimum between 2-4m depth. Dissolved organic phosphate below the surface was relatively uniform with depth in the profiles, but there was a large decrease in concentration going from the summer into winter months from nearly 0.5 to $\leq$ 0.050 μM. Particulate total phosphorus concentration tended to increase with depth as a reflection of the higher suspended sediment load near the bottom, typically 8-10m at this location. The vertical distribution of PTP was also affected by biological processes. For example, the conspicuous peak at 2m depth in the July survey was positively correlated to a pulse of Chl-*a* (about 52.8 ug.dm^{-3}) and POC (283 μM). This unusual Chl-*a* peak was due to the presence of summer blooms of *Mesodinium rubrum*, which are sometimes located in subsurface waters at about 2m depth (Antai 1990). The presence of PTP near the bottom on the winter profiles suggests that PTP is not only composed of organic P but also of inorganic P. The POC:PTP ratio (see Appendices 3) greatly fluctuated with both depth and season.

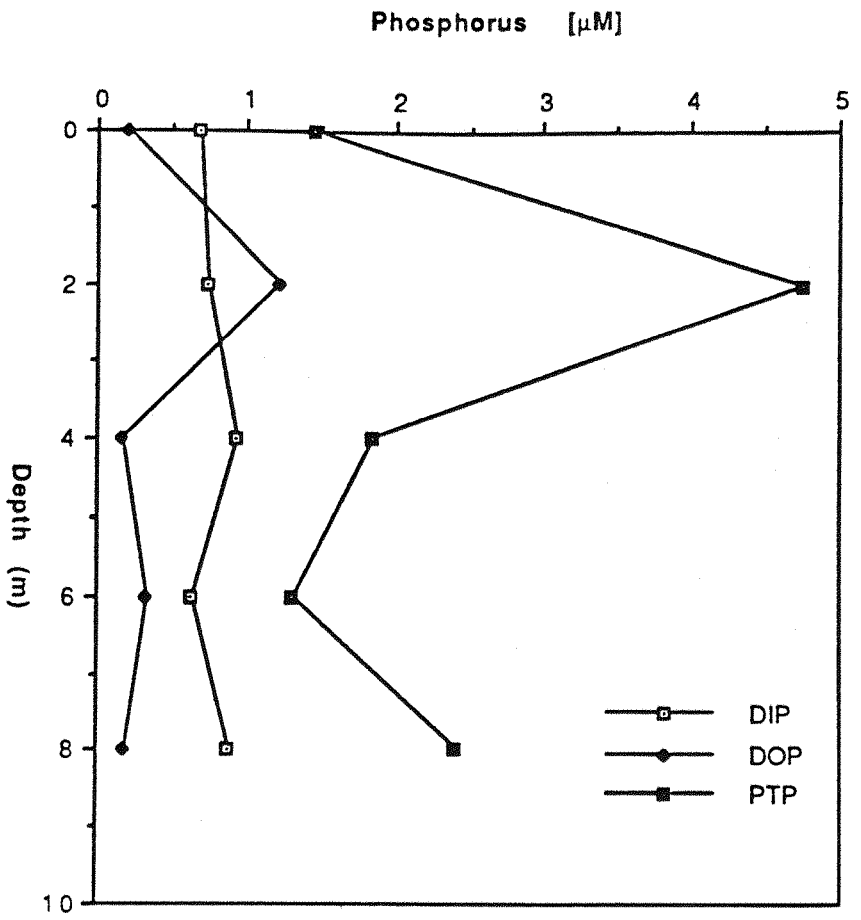


Fig. 3.7a Vertical profile of forms of P in the water column. Southampton Water (Netley Buoy). Jul.89.

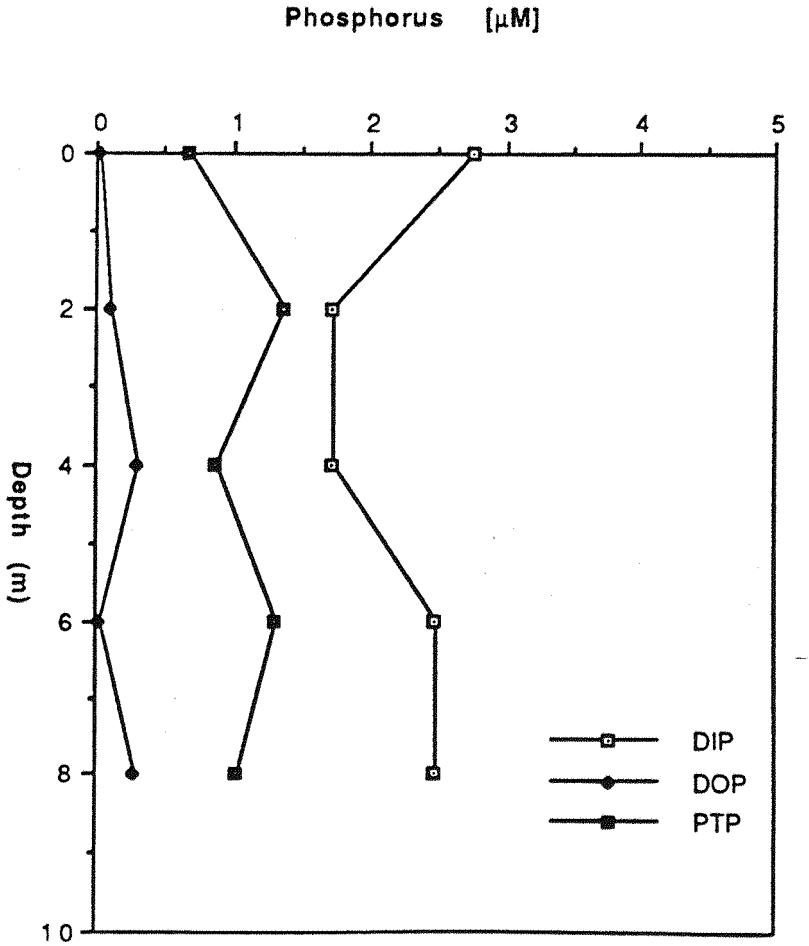


Fig. 3.7b Phosphorus phases in the water column of Southampton Water Netley Buoy. Oct. 89.

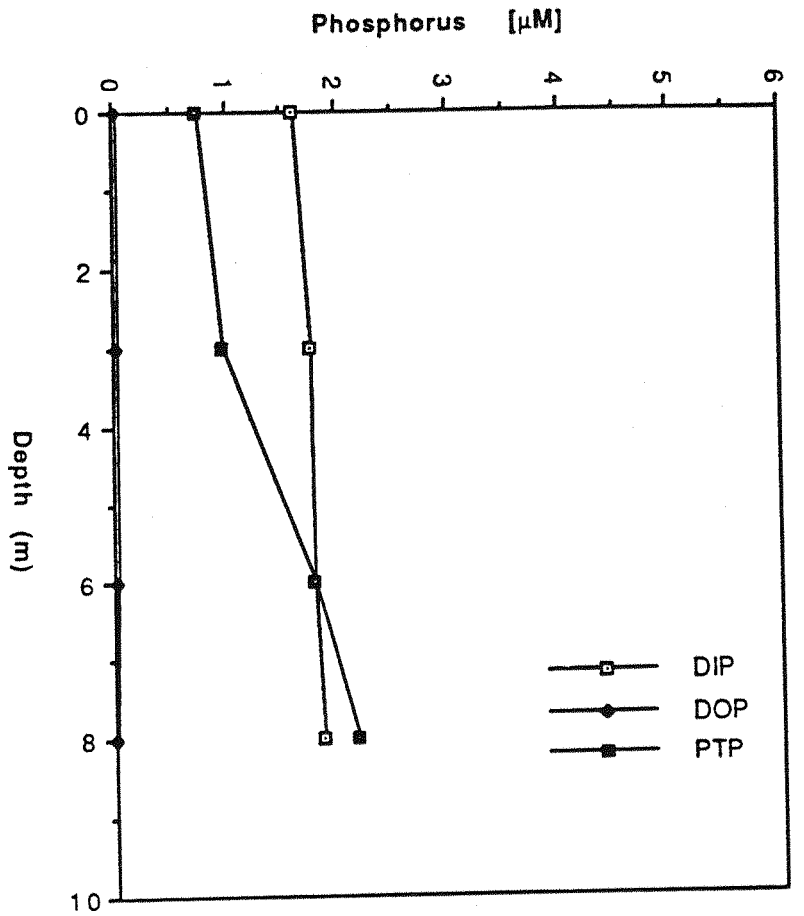


Fig. 3.7c Vertical profile of forms of P in Southampton Water. Netley Buoy. Dec. 89.

In summer POC:PTP ranged from 110 in the surface to 62 near the bottom, whilst in winter the equivalent values spanned from 167 to 62. This would imply that the POC:PTP ratio of the sediments would be about 62, i.e. nearly 5 times higher than that proposed by Stevenson (1986). Intense biological processes would also strongly affect the POC:PTP ratio; for instance in July (2m depth) POC:PTP was almost halved from 110 to 60 relative to the other surveys. The POC:PTP ratio is about the ratio of a healthy phytoplanktonic mixed population (Redfield *et al.* 1963). It is noteworthy that similar ratios were also found in oligotrophic waters (see Chapter 4).

The main lesson we can draw from these profiles is that DIP and DOP are essentially uniformly distributed, whilst the distribution and concentration of PTP are principally influenced by biological and sedimentary processes with the POC:PTP ratio of re-suspendible sediment being about 60.

3.5.2 The Beaulieu Estuary

The concentrations of the different forms of P in the estuary with respect to salinity for the three surveys (summer, autumn and winter) are shown in Figures 3.8 - 3.9. In July, concentrations of DIP were consistently low ranging from 0.060 to 0.26 μM at salinities of 16 and 34 respectively. By October DIP concentrations fluctuated from 0.28 to 0.87 μM at similar salinities, and in general showed the same pattern in the studied salinity range as the July survey. In December, at the same previous salinities, concentrations were in general higher than to those in October (0.87 and 0.85 μM respectively). There was thus a clear seasonal pattern, with concentrations differing by factors of 4-14. Similar concentrations have been reported before (Coles 1979, Umnuay 1981). The low DIP concentrations reflect a lack of major pollutant sources in this estuary (Phillips, 1980), and also biological and chemical removal processes. It appears that biological activity played an important role in the removal of about 70 % of the DIP during the summer time when DOP concentration (0.51 ± 0.06 μM) greatly surpassed DIP levels,

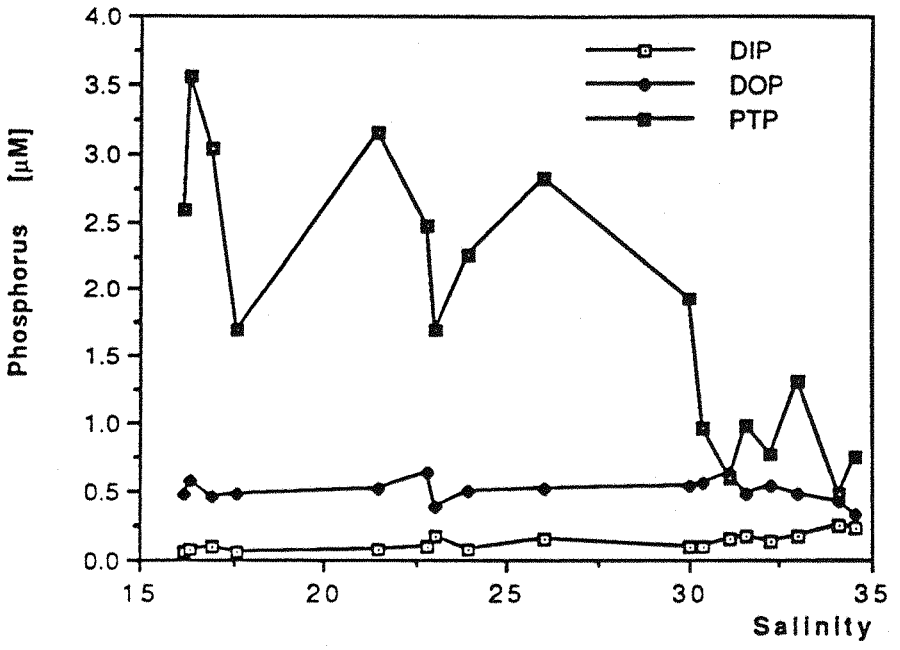


Fig. 3.8a Phosphorus phases in the Beaulieu Estuary. Jul. 89. Note the salinity offset.

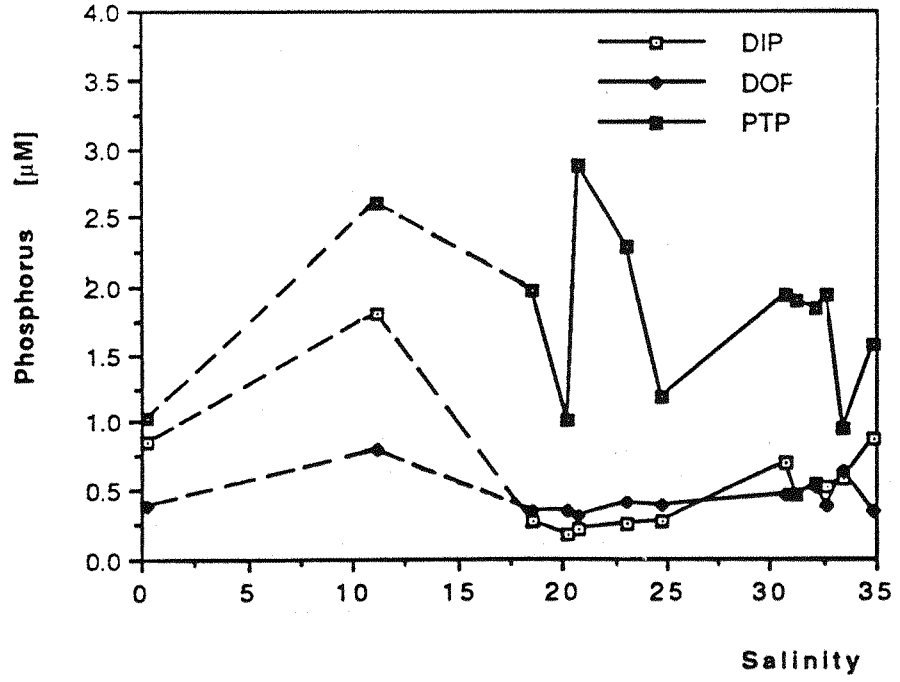


Fig. 3 .8b Phosphorus phases in the Beaulieu for Oct. 89.

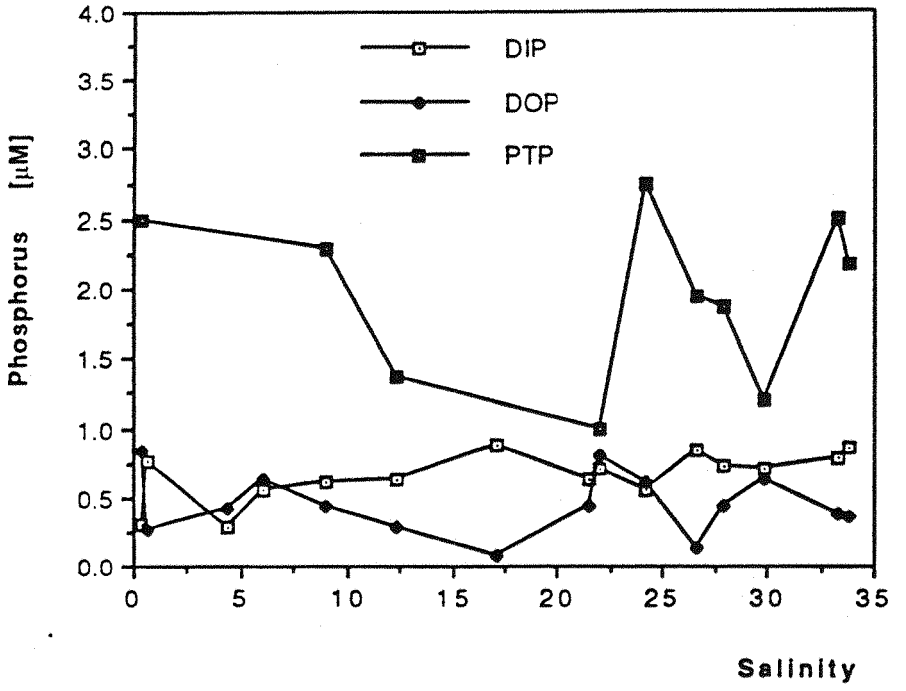


Fig. 3.8c Phosphorus phases in the Beaulieu River and Estuary. Dec. 89

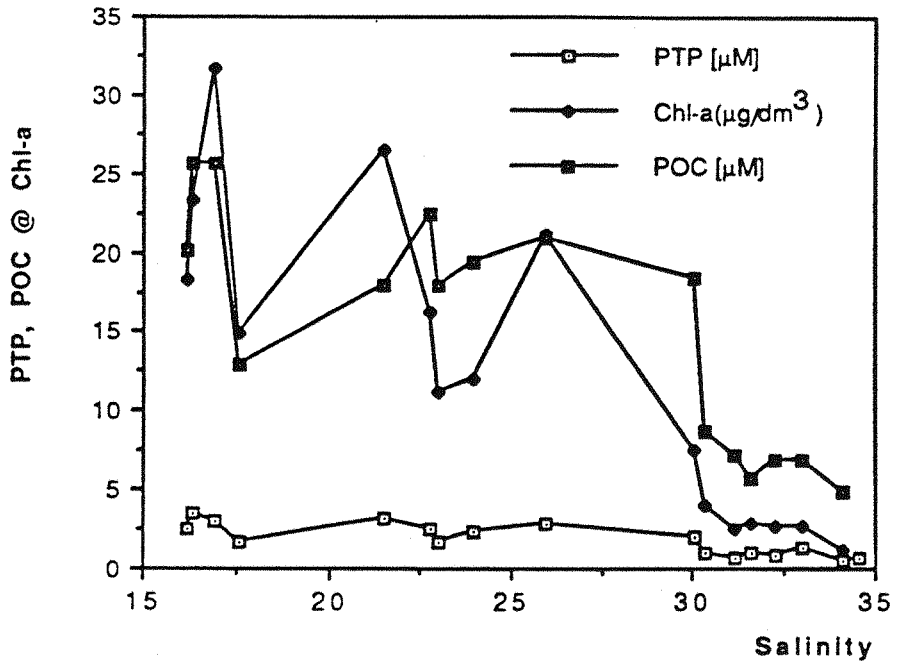


Fig. 3.9a. PTP, POC and Chl-a in the Beaulieu Estuary. Jul. 89. Note that POC is divided by 10 and the salinity offset.

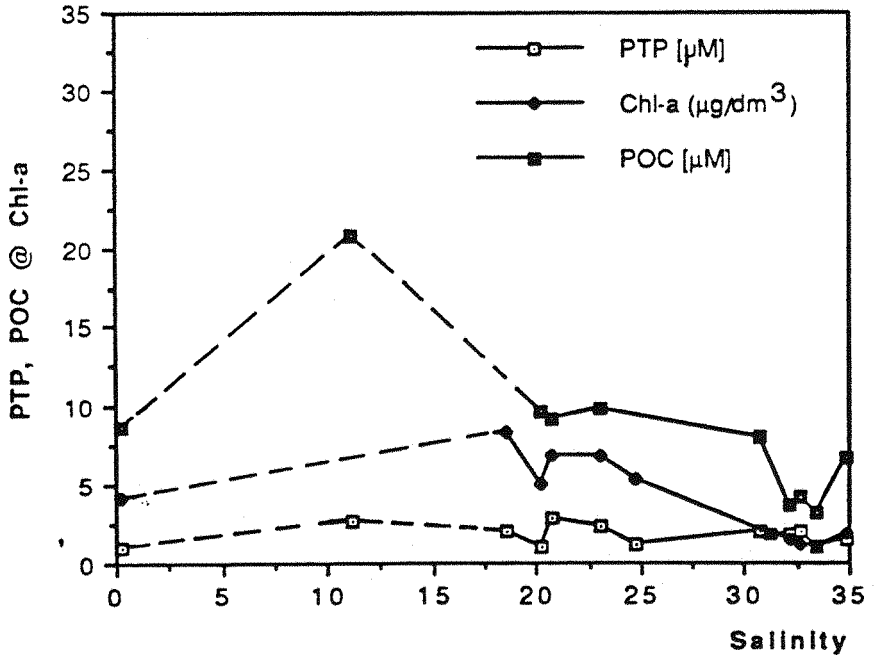


Fig. 3.9b. PTP, POC and Chl-a in the Beaulieu Estuary. Oct.89. Note that POC is divided by 10.

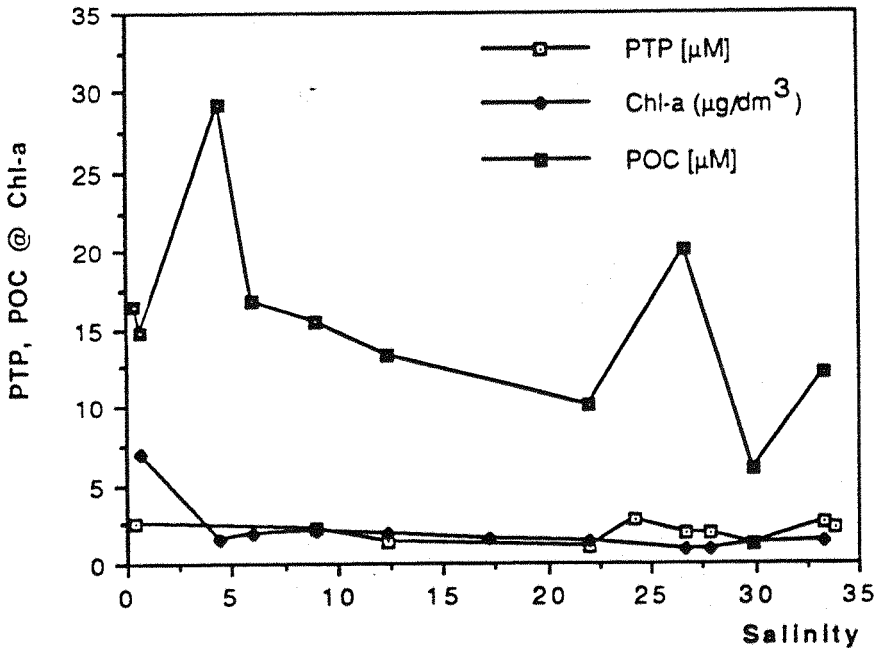


Fig. 3.9c PTP, POC and Chl-a in the Beaulieu Estuary. Dec. 90
Note that POC is divided by 10.

thus making DOP at 80 %, the main fraction of the DTP. After the summer months, DIP built up as a consequence of *in situ* regeneration of DOP into DIP and the tidal intrusion of coastal waters with higher DIP concentrations. Thus, DIP averaged 0.62 ± 0.20 μM in December with a maximum of 0.8 μM in the high to mid salinity zone, whilst DOP decreased by about 12 % to become 50 % of the DTP. Also, PTP slightly decreased by about 12 %. It is interesting to observe that the total P (DTP + PTP) remains almost constant throughout the seasons (i.e. 2.5 - 2.9 μM).

Overall, PTP proved to be the main fractions of P, with an average concentration of 1.8 ± 0.57 μM, i.e. double that found in the Itchen estuary. Particulate total P and POC appeared to principally be of riverine origin, because there was not a significant enhancement of PTP in summer relative to the riverine PTP background, assuming that riverine PTP concentrations were similar to those in winter. In summer POC varied from 256 μM in the middle salinity range to 49 μM towards the high salinity zone, whilst in winter values ranged from 100 to 30 μM for the same salinity range. The POC distribution is characterized by a sharp drop in concentration at salinities above 30. The POC:PTP ratios (Table 3.3) ranged from 50 to 83, with values of about 80 in the middle salinity zone and 30-40 in the lower reaches. This data indicates that the suspended matter is enriched in P during its passage through the estuary, as seen in the Itchen Estuary.

The intense biological activity in this estuary, particularly evident in the mid salinity zone, can be observed in the concentration pattern of Chl-*a* (Fig. 3.9 a and b). During summer Chl-*a* fluctuates between 15 and > 30 $\mu g.dm^{-3}$ and then drops to about 2/3 in the autumn, to further decrease in winter to concentrations below 3 $\mu g.dm^{-3}$. Degraded photosynthetic pigments followed an inverse behaviour. The lower reaches (> 30 salinity) had lower concentrations of phytoplanktonic biomass, because POC and Chl-*a* markedly dropped in this region.

The relatively constant concentration behaviour of DIP appears to be consistent with the "buffering" concept for this estuary, because of the almost uniform distribution of DIP along the salinity range. However, closer examination of the data indicates that sea water is transporting DIP into the estuary, as concentrations of DIP in the high salinity zone were generally higher than the middle zone (Figs. 3.8 a, b, and c). The data reported by Umnuay (1981) showed relatively high concentrations at the end salinity members with reduced values in the mid salinity range, thus indicating non-conservative behaviour.

The behaviour of DIP in the upper reaches is not clear as there is not enough information, but data from the December survey as well as from the literature (Umnuay 1981) suggest that removal of DIP is taking place in the salinity range of 0 - 5. Association of DIP with iron hydroxy-oxide phases is well known (e.g. Crosby *et al.* 1984, Lucotte and d'Anglejan 1988) and the formation of particulate iron phases which are documented in the Beaulieu (Moore *et al.*, 1979), can lead to removal of DIP from the water column. Moore *et al.* (1979) reported non-lattice particulate iron concentrations of about 3.6 and 0.83 μM at salinities below 5 and above 29 respectively. The high humic acid content and high water temperatures (24 ^{0}C) in the summer months would also favour adsorption and removal of DIP to particulate phases (Chen *et al.* 1973). Nevertheless, it seems the uptake of DIP by micro-organisms is the most significant process removing DIP agent in the salinity range studied. This is supported by the high phytoplankton numbers (and consequently Chl-*a*, see Fig. 3.9a), which were high at several locations (up to 240,000 cells cm^{-3} nanoplankton and 230 cells cm^{-3} larger dinoflagellates (>20 μm), D. Crawford, pers. comm.). The increase of DIP in autumn and winter months when biological activity is low, reflects both the increased importation of DIP from coastal waters and *in situ* regeneration of DIP from DOP within the estuary itself. As a result of the above processes PTP is the dominant component of the total P in this system, reflecting *in situ* biological activity and the presence of P adsorbing particles.

Dissolved organic phosphorus with concentrations *circa* 0.5 μM represented at least 50 % of the DTP in winter and up to 80 % in summer (Fig. 3.9d), thus it was the dominant fraction in the dissolved form of P. Due to the seasonal pattern it seems the source of DOP is mainly riverine, as it does not substantially change over the studied time. However, some *in situ* generation of DOP as well as regeneration of DOP into DIP must occur during the development and after collapse of the phytoplankton blooms.

3.5.3 Tidal cycle stations on the Thames and Humber Estuaries

3.5.3.1 The Thames Estuary

The concentrations of different forms of P in this estuary are shown in Figure 3.10a. The DIP concentrations were greater than at similar salinities in the Itchen, averaging 44 μM in the salinity range 19-22. Between salinities of 22 and 34, DIP decreased in a linear manner to about 2 μM. The dilution line obtained from the available data indicated that the fresh water end-member should have a concentration of 101 μM (r^2=0.919) and the slope dC/dS is -2.78. According to Meybeck (1982) the Thames river has the highest concentration of DIP in the world at about 80 μM, and the deduced value here (101 μM) is close to that of Meybeck. As in the Itchen Estuary, high concentrations would suggest inputs from sewage treatment plants or related anthropogenic sources. Using 101 μM DIP and assuming conservative mixing and a flow rate of 154 $m^3.sec^{-1}$ a flux of 15400 $mol.sec^{-1}$ (Tab. 3.2) into the Southern North Sea will be obtained.

Concentrations of DOP ranged from 0.8 μM to below detection limit, with the exception of a high value of 4.1 μM at a salinity of about 20 which coincided with a peak of PTP (6 μM). The peak of DOP at salinity of about 34 was regarded as dubious, because other samples at very close salinity had much lower values of DOP. The reasons of these DOP and PTP peaks could not be elucidated from the available information. The

forms of P in this estuary were dominated by DIP, the suspected source of which is the large sewage treatment plant upstream of the sampling site. Concentrations of DOP were similar to those in the Humber and Beaulieu but the DOP as a fraction of the total dissolved P (Fig. 4.10c) was the lowest found in this study, due to the high DIP concentrations (Fig. 4.10a).

The distribution of concentrations of PTP was similar to DIP with the highest values (up to 6.88 uM) in the 19 - 25 salinity range, and then a drop in concentration to low values (0.8 ± 0.4 μM) at high salinities.

3.5.3.2 The Humber Estuary

Dissolved inorganic phosphorus concentrations were low in the salinity range studied (28 - 34), ranging from 0.90 μM to 0.32 μM with an average of about 0.60 μM (Fig. 3.10b). Dissolved organic phosphorus was uniformly distributed through the salinity range at similar concentrations to DIP, with an average of 0.50 μM and a range of 0.10-0.86 μM. The DOP accounted for nearly 50% of the DTP for all the samples, and was thus a major fraction of the dissolved P (Fig. 4.10c). The PTP dominated the total P in the water column, varying from 1.02 to 6.03 μM. The Humber typically has very high loads of natural suspended particulate matter, and the potential for "buffering" processes leading to the association of DIP with particulate phases is thus high. There are significant inputs of iron-hydroxy-oxide materials to the Humber as a result of the titanium dioxide industry on the estuary. As previously mentioned, these iron phases could enhance removal of DIP to particulate phases. Inputs of P to the Humber are more dispersed than in the Thames, and the fate of such inputs at lower salinities is not clear from this study. An extrapolation for the P phases back to the riverine end member is not possible because of the relatively uniform distributions.

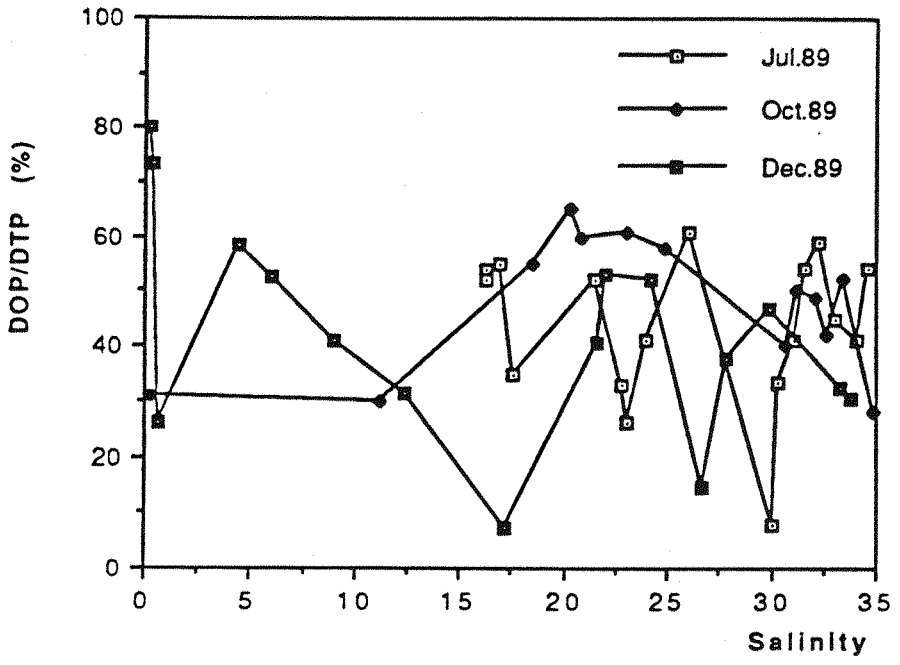


Fig. 3.9d DOP/DTP ratio in the Beaulieu Estuary.

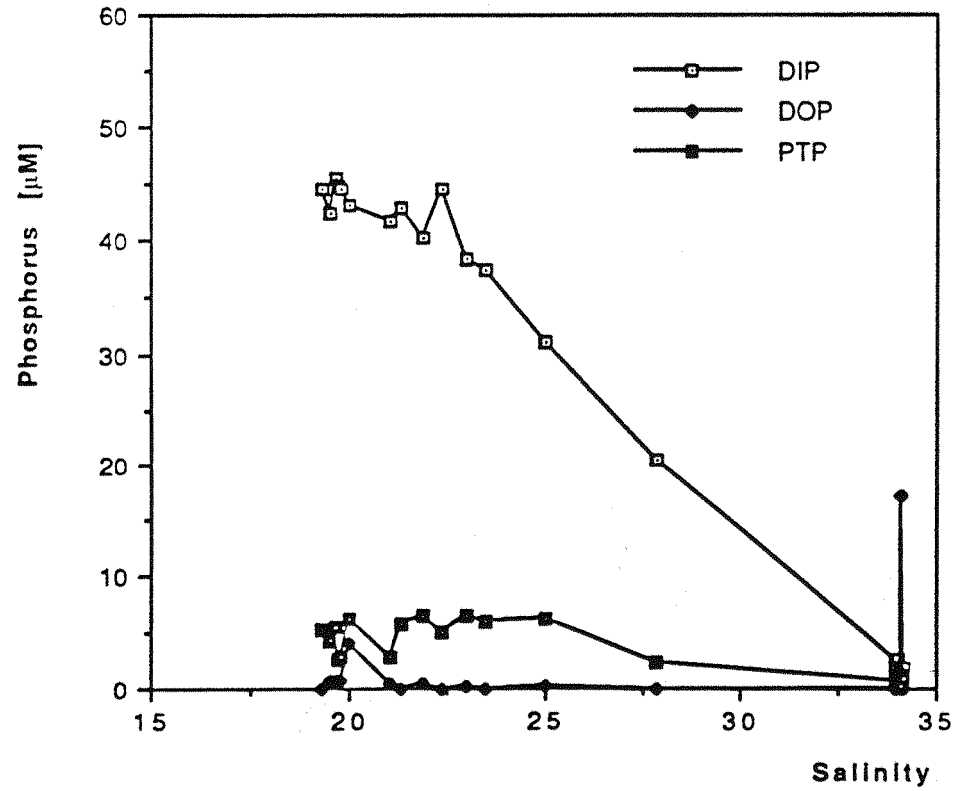


Fig. 3.10a Phosphorus phases in the Thames Estuary. Dec. 88. Note the salinity offset.

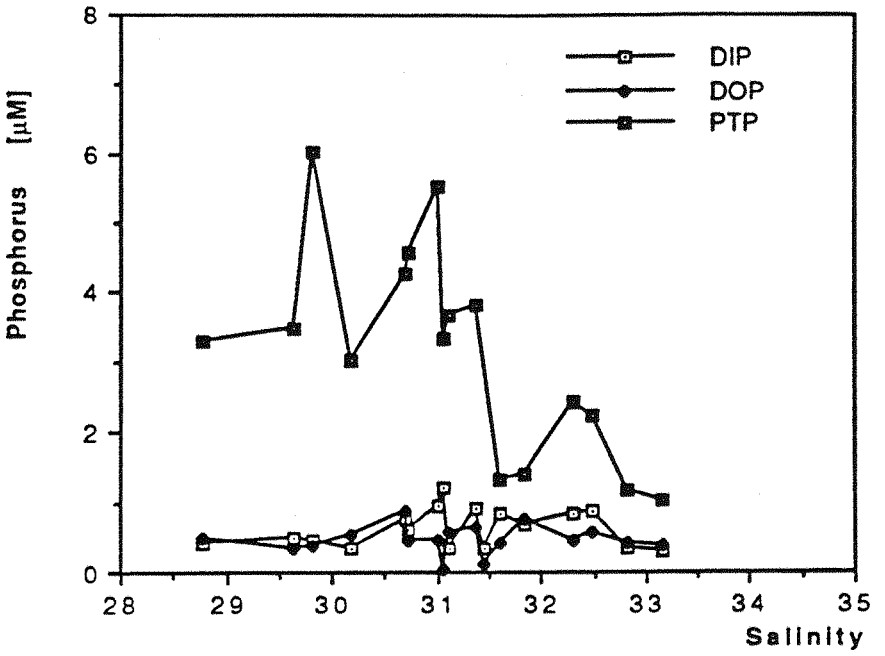


Fig. 3.10b. Phosphorus phases in the Humber Estuary. Feb. 89. Note the different salinity range.

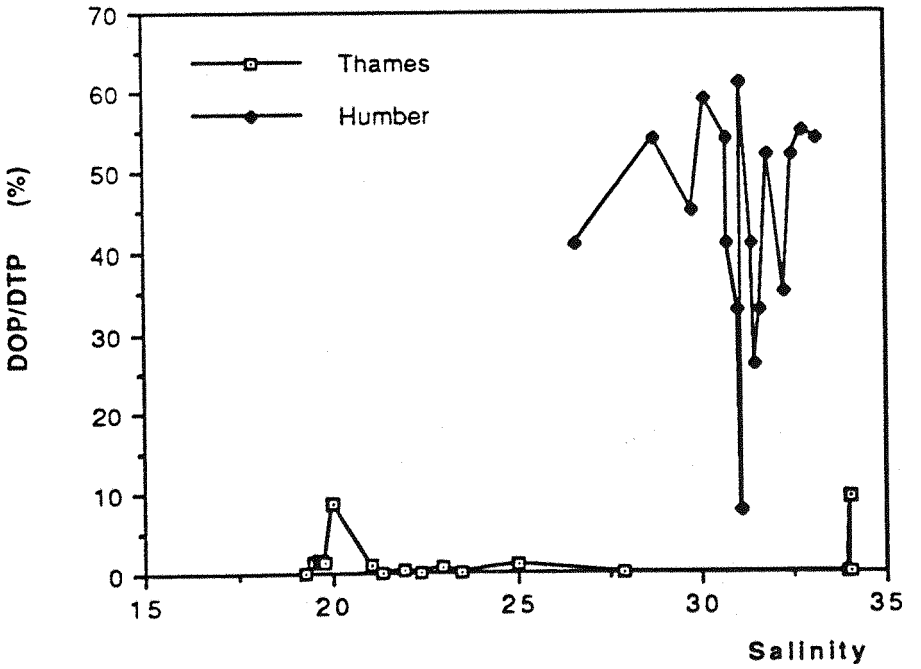


Fig.3.10c.DOP/DTP ratio in the Humber and Thames Estuaries.

3.6 Conclusions and summary

To make detailed conclusions about processes affecting P concentrations and behaviour in this type of environment from field data is difficult because of the highly variable and dynamic nature of the estuarine system (Morris *et al.* 1984). However important points about the relative importance of the fractions of P in the waters of estuarine environments can be made.

i) DIP concentrations varied greatly from estuary to estuary reflecting the importance of anthropogenic inputs ascribed to sewage treatment plants in the Thames and the Itchen, although other related anthropogenic sources, such as industrial activity could be important in some estuaries. Whilst adsorption of DIP onto particulate suspended matter through association with flocculated iron, and biological uptake, were regarded as the most likely removal processes, other factors such as transport and cycling in association with calcite may also be important. Non-conservative behaviour due to "buffering" of DIP concentrations was positively identified in the Beaulieu Estuary and can also be suspected to occur in the Humber Estuary.

ii) DOP concentrations were similar for the four estuaries, ranging from about 1 μM to below detection limit. However, the relative importance of DOP as a fraction of the DTP varied greatly. In the Beaulieu Estuary, it was the major fraction, and was also important in the high salinity range studied in the Humber. In the Itchen and Thames the DOP signal was obscured by large inputs of DIP ascribed to sewage treatment plants. Sources for DOP included allochthonous inputs, anthropogenic activities, and *in situ* biological production. Removal processes for the more refractory DOP of terrestrial origin were not clearly identified, but will include biological uptake (e.g. bacteria are reported as being able to assimilate phosphonate), and physico-chemical processes such as adsorption, and photo-chemical conversion to inorganic forms.

iii) Particulate P proved to be an important fraction of the total P, especially in the Beaulieu and Humber estuaries where it was the dominant fraction. For the four estuaries PTP ranged from about 6 to nearly 1 μM, overall averaging 3 μM. The ratio of POC:PTP for the Itchen and Beaulieu estuaries was 3-5 times higher than previously suggested values, and given the distribution of POC:PTP ratios in the whole salinity range and with depth, suspended sediments travelling through the estuaries investigated gradually became enriched with P. These facts challenge some other accepted concepts of the geochemical cycle of this element, suggesting that the riverine, i.e. estuarine, input of P into the ocean is underestimated.

iv) When major inputs of DIP were not present, as in the Beaulieu estuary, PTP was a major fraction of the total P. Clearly, when adsorption processes are significant in the estuarine environment, it is important to have information on PTP in geochemical studies of this element.

v) This study has demonstrated the potential importance of forms of P other than DIP in estuaries, and the need to consider these other forms if a better understanding of the biogeochemistry of P in the estuarine environment is to be attained.

CHAPTER

FOUR

Phosphorus cycling in the Sargasso Sea

4.1 Introduction

Oligotrophic waters are an important fraction of the world oceans, representing about 75 % of the total area (Lewis *et al.* 1987). They are located in the North and South gyres of the Pacific and Atlantic oceans, situated between 10-50 degrees latitude north or south. The Sargasso Sea in the North Atlantic and the North Pacific gyres are the most studied oligotrophic oceans, mainly because of the proximity of oceanographic laboratories based on the Hawaii and Bermuda islands, which allow scientists easy and quick access to these areas. The study described here is concerned with the Sargasso Sea (see Fig. 4.1).

Oligotrophic seas have relatively low primary production (e.g. Menzel and Ryther 1966, Platt *et al.* 1984), due to the low concentration of the dissolved inorganic nutrients phosphorus (DIP) and nitrogen (DIN). In the upper mixed layer, both DIN and DIP are generally reported to be below the detection limits (e.g. Perry 1972 and 1976, Jickells 1986, GOFS 1990) of the currently applied analytical procedures (see Chapter 2). Figure 4.2 shows a typical vertical profile for DIP. Similar patterns are also found for DIN.

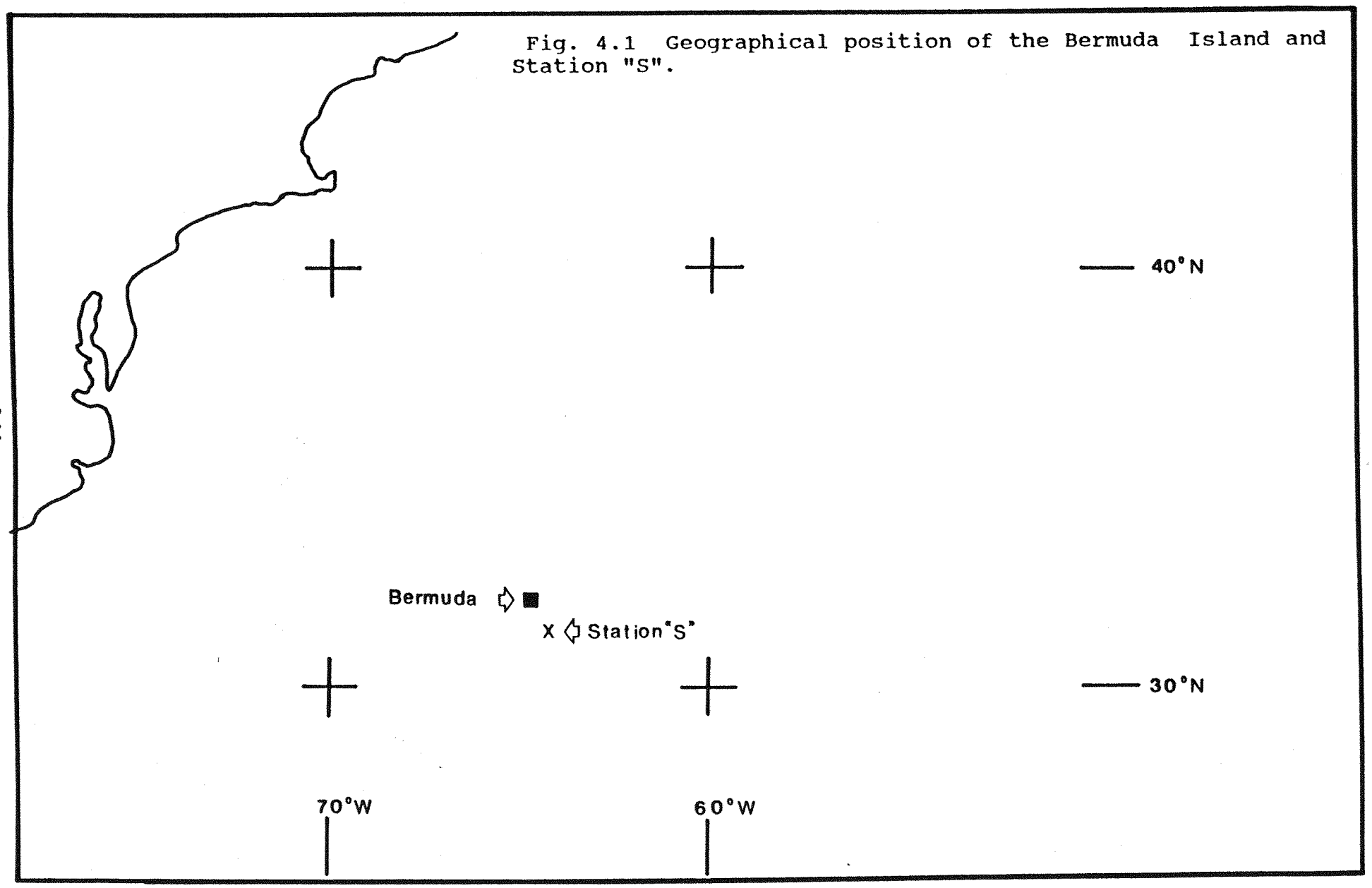


Fig. 4.1 Geographical position of the Bermuda Island and Station "S".

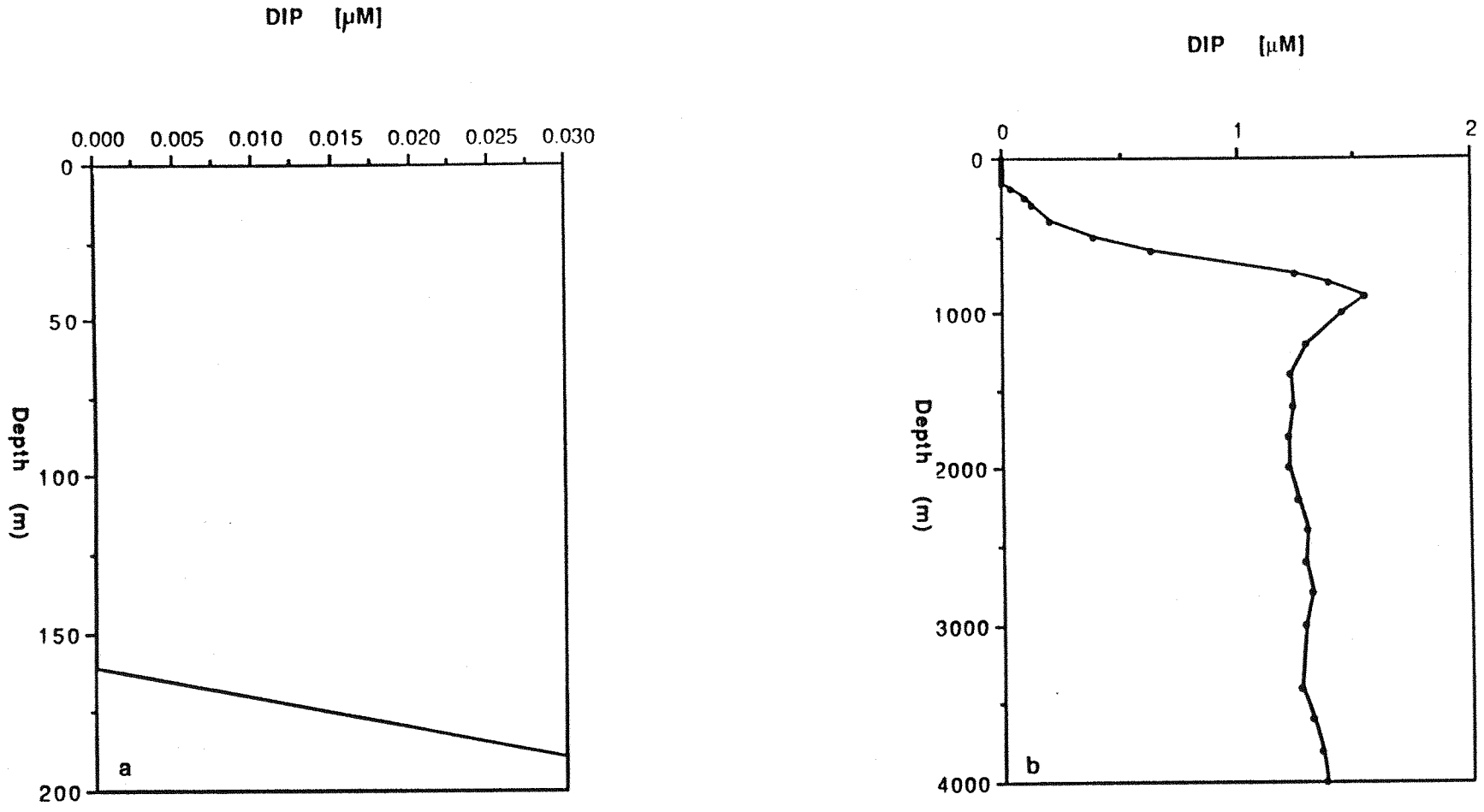


Fig. 4.2 Typical vertical distribution of DIP in the Sargasso Sea. Station "S". Jan.90. a) 0-200m and b) 0-4000m. Note that this profile was measured by scientists from the BBSR laboratory.

The reasons for the low levels of nutrients and other elements in upper mixed layers are 1) the general oceanic circulation, which keeps the oligotrophic waters isolated from nutrient rich coastal or/and upwelled waters, and 2) remoteness from the main land masses which restricts riverine and atmospheric inputs. Therefore, the input of nutrients via atmosphere and land is insignificant. These almost isolated water bodies do however have a net export of dissolved nutrients from the euphotic zone via sinking particulate organic matter (POM). If a steady state system is to be maintained there should be alternative sources; these are essentially two: 1) intrusion of cores or rings of nutrient-rich waters from near-coastal and northern oceans. These cores result from the general oceanic circulation. And 2) vertical diffusion and advection, whose intensity and magnitude of the depends greatly on meteorologic instabilities such as storms.

It is often inferred in the oceanographic literature that if inorganic nutrients fall below the limit of the techniques, it is an indication that the production of marine micro- and nano-plankton population is limited by these nutrients. Arguments about DIP *vs* DIN limitation have been and are widely discussed (for instance, Redfield 1958, Perry 1976, Smith 1894, Smith *et al.* 1986, Passhe and Erga 1989). Generally, marine geochemists and biologists argue opposite criteria. The former (e.g. Broecker and Peng 1982, Smith and Atkinson 1984, Smith *et al.* 1986) support the idea that P availability limits net organic production in the sea, whilst biologists (Ryther

and Dunstan 1971, Eppley *et al.* 1973, Perry and Eppley 1981) maintain it is N. Smith (1984) has concluded that P or N limitation depends on the balance of supply, removal and internal processes which adjust the ratio of ecosystem P:N availability. New developments in measuring DOC and DON (Suzuki *et al.* 1986, and Sugimura and Suzuki 1988) are implying that P is always a limiting nutrient, because the actual measured levels of DOP (including data of this thesis) are well below the expected values that comply with the average Redfield ratios of P:C:N of 1:106:16.

The analytical constrains on measuring DIP and DIN have hampered marine scientists in investigating the role of P and N at nanomolar levels in the Sargasso Sea. However, recently Garside (1985) and Glover *et al.* (1988) have reported the first data for DIN below 50 nM (detection limit of the colorimetric technique (Wood *et al.* 1967 as modified by Strickland and Parsons 1972) with concentrations ranging from detection limit (± 2 nM, Garside 1982) to above 100 nM in the upper surface layer (0-100 m). However according to the present literature, data for DIP below 30 nM have not yet been obtained, the concentration detection limit of the colorimetric method.

Very few DOP and PTP measurements have been made in oligotrophic oceans: e.g. in the North Pacific gyre; Perry (1972 and 1976), Perry and Eppley (1981), Orret and Karl (1987), whilst in the North Atlantic Gyre the only work reporting organic P in the surface micro-layer and the upper

few meters of the water column is that of Volastrykn (1979). Reported DOP values in the North Pacific range from 0.02 to 0.80 μM (Orret and Karl 1987), whilst PTP range from 11 nM (Perry and Eppley 1981) to 30 nM (Romankevich 1984). One single reported value of PTP in the Sargasso sea was 81 nM (Harrison and Harris 1986).

The microbiological uptake rates for P, using radioactive techniques fluctuates largely from 0.2 to 6 $nM.d^{-1}$ (Perry and Eppley 1981) in the North Pacific, and 5-10 $nM.h^{-1}$ (Harrison and Harris 1986) in the Sargasso Sea. The latter uptake rates are more than an order of magnitude greater than the former. Orret and Karl (1987) have also found similar values to those of Perry and Eppley (1981), but the former authors postulated than if effects of exudation of DOP from the cells, grazing or lysis processes were taken into account the actual incorporation rates should have been higher. This suggestion has been confirmed by results of Harrison and Harris (1986), who reported that previous work may have given a 1.5-2.0-fold under-estimation because of errors involved in the earlier techniques (see also Harrison 1983a, Glibert *et al.* 1982). Perry and Eppley (1981) reported uptake rates to be independent of time, but those from Harrison and Harris (1986) were time dependent at the beginning and stabilized at nearly 3 $nM.h^{-1}$ after about six hours. Harrison (1983b) reported similar rates for coastal waters.

Regeneration rates due to APA have been reported to vary between 3-5 $nM.h^{-1}$ (Perry 1972). Production rates of DOP in the North Pacific have been found to widely vary from 0.06 to 10 $nM.h^{-1}$ with an average of 0.3 $nM.h^{-1}$ (Orret and Karl 1987). Thus, regeneration of DOP into DIP are within the range of DOP production rates.

Residence times (τ) in the surface waters can be calculated by the expression $\tau=C/[dC/dt]$; where C and dC/dt are concentration and rates of removal of any analyte (DIP in this case). Residence times have been estimated to vary from 46 to 16 days with an average of 28 days in the North Pacific (Perry and Eppley 1981). But, Orret and Karl (1987) calculated 198-28 days, in the same oceanic area, whilst from the data of Harrison and Harris (1987), a τ of 0.5 days can be calculated in the Sargasso Sea.

As regards inputs, atmospheric sources have been considered insignificant (Duce 1986). For Nitrogen, a flux of 13-30 $\mu mol.m^{-2}.d^{-1}$ falling in the Sargasso Sea has been reported (Knap *et al.* 1986). In order to have a rough idea of the atmospheric P input into the Sargasso Sea, a value can be obtained by assuming that a Redfield ratio of 16:1 (N:P) can be applied; this value would be 1-2 $\mu mol.m^{-2}.d^{-1}$, and it is not far apart from that reported by Duce (1986); 0.033-0.80 $\mu mol.m^{-2}.d^{-1}$ which was based on direct measurements. Upward vertical diffusion rates for P have not yet directly been measured, but based on reported N diffusion of 190 $\mu mol.m^{-2}.d^{-1}$, suggested by Lewis *et al.* (1987), and again

applying similar N:P ratio, the P diffusion rate should be 11 μmol.m^{-2}.d^{-1}.

Removal of C, N and P from the upper water column (typically 120-150m depth) in open oceans is due principally to sinking of particulate matter. There is a substantial literature on this subject, for example, Knauer *et al.* (1979), Deuser and Ross (1980), Suess 1980, Deuser (1986), Pace *et al.* (1987), Knauer (1988), etc. Knauer *et al.* (1979) reported a value of 410 and 14 μmol.m^{-2}.d^{-1} for N and P respectively at 75m, whilst Knauer and Martin (1986) found 71 μmol.m^{-2}.d^{-1} of P. Generally, these removal fluxes are variable because they depend on primary production rates and depth (Suess 1980, Berger *et al.* 1988). However, if a steady state system is to operate, the magnitudes of inputs and outputs should match, but according to the above data they do not because the balancing diffusive fluxes are low (see Pace *et al.* 1987 and Knauer 1988). Thus, gains and losses of N and P into and from the surface Sargasso Sea waters range from *circa* 190 (Lewis *et al.* 1987) and 14 (Duce 1986) to 410 (Knauer *et al.* 1979) and 35 μmol.m^{-2}.d^{-1} (Knauer and Martin 1986) respectively. Such discrepancies are difficult to assess because 1) data was not taken at the same time, depth and place, 2) samples were taken over short periods, and 3) there is significant seasonal variation of fluxes even at depths of 3000m (Deuser and Ross 1980). From these data, it is obvious that Redfield ratios cannot rigorously be applied to these fluxes because the uptake and regeneration rates for P are higher than those for N and C (Smith *et al.* 1986), especially in water bodies of the

Sargasso Sea.

Furthermore, downward vertical diffusion of DOP and DON has recently been considered by Smith *et al.* 1986. They estimated that fluxes of DON and DOP of 6 and 4 % offset the vertical upward fluxes of N and P respectively.

The main objective of this chapter is to establish the magnitude of the DIP, DTP, DOP and PTP pools, study internal biogeochemical processes, and investigate possible sources and sinks of P in the euphotic layer of the Sargasso Sea; a model taking the various pools into account is proposed. This chapter also provides an insight of the deep water distribution of DOP and its relationship with DOC and DON, and also the genetic origin of P is estimated in the layer of 120-1000m depth.

4.2 Materials and methods

4.2.1 Cruises

Samples and data were collected during four cruises of the R/V Weatherbird II currently working in the long-term GOFS programm (Global Oceanographic Fluxes Study) run by scientists of the Bermuda Biological Station for Research (BBSR). The cruises were carried out on the (14-17)/Jan., (14-17)/Feb., (21-23)/Feb., and 27/Fe.-2/Mar. of 1990. They will be referred to as first, second, third and fourth cruises respectively.

There is a bi-weekly and monthly cruise to the Station "S" (31^0 50' N, 64^0 10' W, Fig. 4.1) which has been under constant scientific scrutiny for over 3 decades. There is no island effect due to its up-stream orientation to the average flow of water (Jickells 1986, Michaels 1990). A mainland effect is insignificant due to the distance from the American continent (Knap *et al.* 1986).

Samples were taken at or in the vicinity of station "S" using teflon coated Go-Flo or Niskin bottles, which were either placed in a CTD (Conductivity, Temperature, and Depth) probe or attached to a hydro-wire system. Samples for P analysis were taken after samples for analysis of gases were withdrawn. Sub-sampling, treatment and analysis of the samples were executed with the utmost care and trace-metal handling procedures were observed.

Sediment traps (Knauer *et al.* 1988) were employed to collect passively sinking particles at 150, 300, and 400m depth. This work was carried out by Dr. G.A. Knauer and M. Tuel (Minnesota University), who also determined *in situ* primary production using the C-14 technique. The sediment traps buoys were deployed before the first cast was done. Samples bottle casts were executed near the sediment trap buoys which acted as a drogue tracking essentially the same water body. Apart from this, scientists from the BBSR collected samples and data for inorganic nutrients, chlorophyll-*a* (Chl-*a*), dissolved oxygen, temperature (T), salinity (S), bacterial counts, and in two cruises ^{16}N and ^{3}H

uptake experiments were also made. For some of the information see Appendix 4.

4.2.2 Sampling and analysis of P pools

For DIP, DOP and PTP the storage, filtration and analytical determination procedures were carried out as outlined in Chapter 2. Samples were filtered immediately after sampling, using a glass filtration unit and poly-carbonate Nuclepore filters (0.45 μm nominal pore size), which had previously been treated as recommended by Grasshoff (1983), i.e. the filter was initially cleaned of surfactant used as a wetting agent (polyvynyl-pyrrolidone). No contamination or adsorption of P were detected with the Nuclepore filters by the LCC technique and similar results for samples were obtained to those using GF/F Whatman filters (see Appendix 4.1a). The effect of filtration pressure was also examined on board. A sample from about 10 m depth was filtered using 125, 250 or 375 mm-Hg vacuum (see Appendix 4.1b). No difference in DIP was found. However, generally filtration was performed at 250 mm-Hg vacuum. For PTP analysis GF/F filters were used. DIP and APA analysis were made on board. Dissolved PPs, DOP and PTP were analyzed in the shore laboratory a few days after the cruises. Analysis was performed as indicated in Chapter 2.

4.2.3 Cycling processes experiments

4.2.3.1 Enzymatic conversion of DOP into DIP

The analytical procedure for APA was that of Perry (1972). This consists in measuring the increase of fluorescence of the substrate 3-0-methyl-fluorescein-phosphate (MFP) when it is hydrolysed by the APA. To calibrate the analytical procedure a 3-0-methyl-fluorescein (MF, Sigma) solution was utilized at different concentrations of 1, 3, 5, 8, and 12 nM. The reagent blank was determined in both boiled and filtered sea surface water (as recommended by Perry 1972) and 0.05 N [NaOH] solution (Davies and Smith 1988). Blanks ranged from 100 to 50 $pM.h^{-1}$ similar to values reported by Perry (1972). The spectrofluoremeter (Perkin Elmer 650-10S) was calibrated before and after analysis, and no difference was found in the internal calibration.

Duplicates of unfiltered water samples (10 cm^3) were spiked with the substrate MFP (Sigma) to give a final solution concentration of 0.125 μM. This represents about half of the naturally occurring DOP concentrations. The samples were kept in light proof glass containers and incubated on deck in a container flushed with surface water at approximately the *in situ* surface water temperature (± 1^0C). Fluorescence was measured just after spiking the substrate (t=0) and then at t=30 and 60 minutes. Fluorescence readings were transformed to enzyme activity using a calibration curve (Fig. 4.3).

Suppression of the APA by DIP was investigated by adding DIP to unfiltered samples to give final concentrations of 5, 10, 20, 50, 100 and 200 nM. This experiment was repeated twice and carried out in triplicates each time. Fluorescence was measured at t=0, 30, 60, and 120 minutes. Fluorescence readings and time were linearly co-related.

4.2.3.2 Net uptake rates of DIP

The net disappearance of DIP with time was followed by a non-radioactive technique. Samples of surface water (10m depth) were incubated in thoroughly detergent and acid cleaned polycarbonate bottles (10^{-3} m^3). Seven sub-samples in total were spiked with 5, 10 (duplicate), 20, and 50 nM (duplicate) of DIP respectively. One of the incubation bottles spiked with 50 nM of DIP was also spiked with 750 nM of DIN (as nitrate). Apart from these spiked samples a sub-sample was kept as control, i.e without added DIP. Incubation was done on deck in the shade using un-screened bottles because phosphate uptake is independent of light (Perry and Eppley 1981), whilst samples were kept at surface water temperature by immersing in a vat flushed with fresh surface water. Analysis of DIP was immediately performed on filtered sub-samples at t=0, 1, 3, and 8 hours. Prior to this experiment, the potential sorption processes of DIP onto the wall of the polycarbonate bottles had been investigated, because 1) the effect of polycarbonate bottles on incubation experiments has not yet been determined and 2) DIP is a highly adsorptive analyte. Polycarbonate bottles, which had been carefully detergent and acid treated,

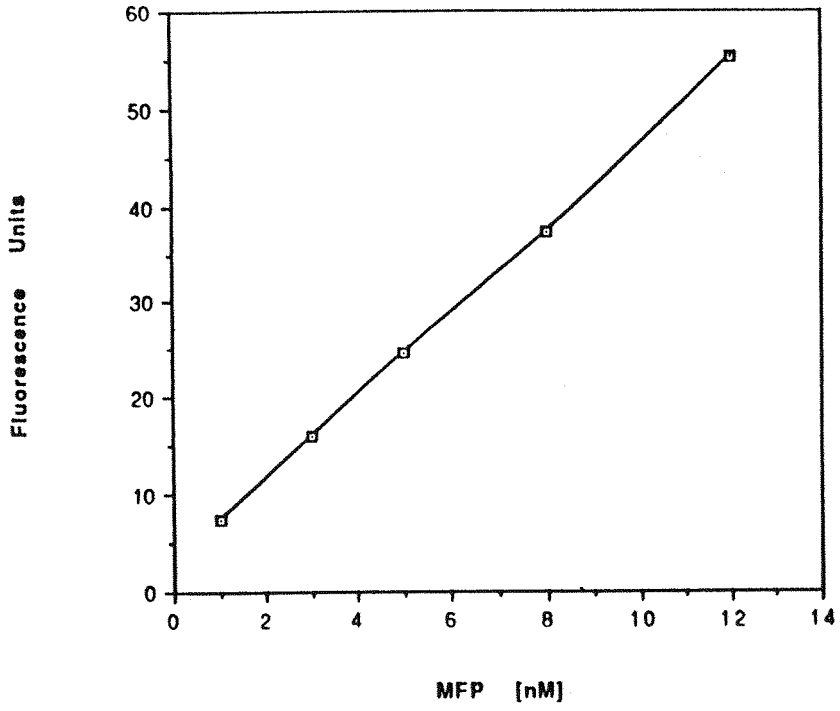


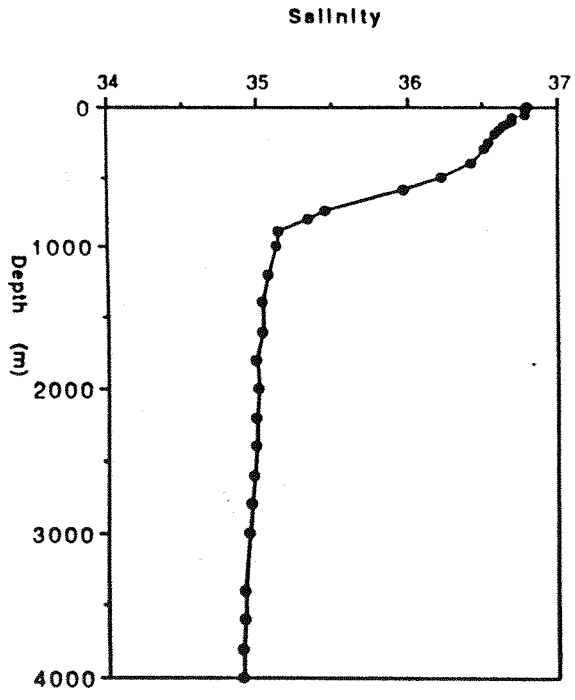
Fig. 4.3 Typical calibration curve obtained on board conditions for the APA assay. Refer to text for details.

were utilized to contain surface sea water filtered with treated Nuclepore 0.2 μm (duplicate bottles), and filtered and spiked (30 nM, duplicate bottles). The water samples were incubated as indicated above, and DIP analysis was performed at t=0, 1.5, 3, 6, and 10 hours.

4.3 Results

4.3.1 Hydrography and water masses system at the Station "g"

Figures 4.4, and 4.5 depict typical vertical profiles of salinity and temperature, whilst the T/S-depth relationship for the first cruise is shown in Fig. 4.6. This latter graph gives an indication of the presence of internal waves, which generally occur in the thermocline. Internal waves change the position of density horizons, that can ultimately affect the vertical distribution of nutrients, especially when there is a strong nutricline and low surface nutrients at nanomolar concentrations. However, some changes in the T/S-depth diagrams in the upper 200m layer could also be attributed to horizontal displacements or diffusion, because the particle traps generally drift between 15-20 nautical miles in about three days (M. Tuel, pers. comm.). The hydrographic data has the caveat of being preliminary information and was provided by the BBSR.



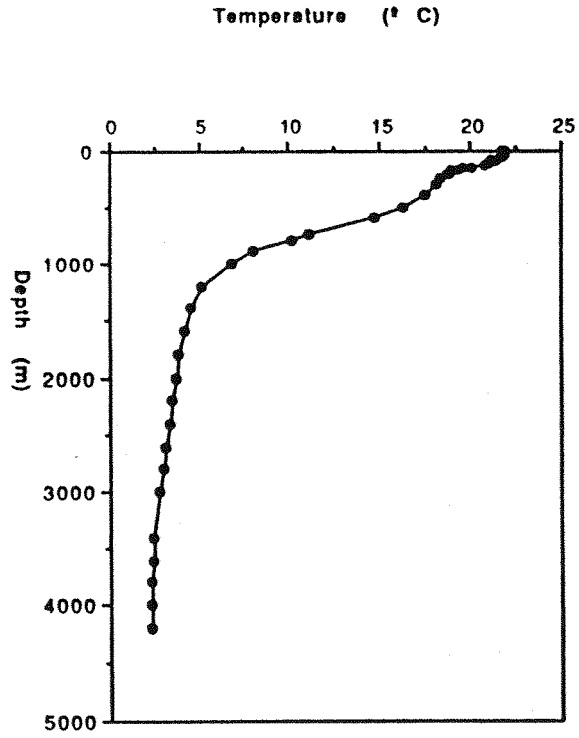


Fig. 4.4 and 4.5 Typical vertical distribution of salinity and temperature in the Sargasso Sea. Station "S".

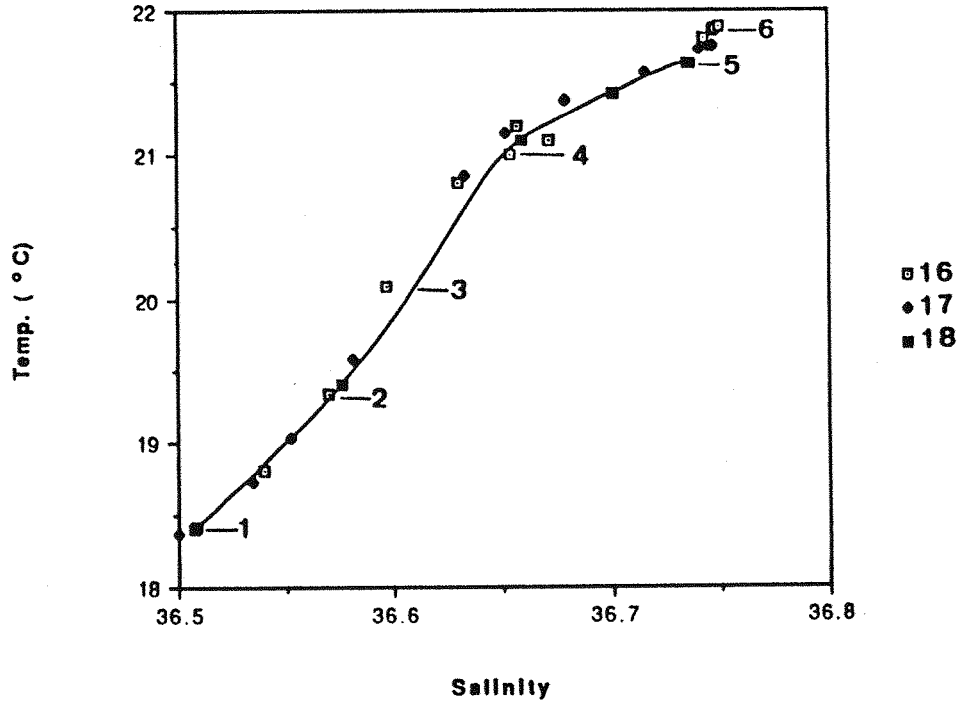


Fig. 4.6 T/S diagram for the upper surface layer (0-250m) in the Sargasso Sea. Station "S". The inserted numbers refer to sample depth (m), i.e. 1(250), 2(160), 3(140), 4(120), 5 (40), 6(2). For the symbols, numbers 16, 17 and 18 are the dates for Jan.90.

4.3.2 Phosphorus pools in the euphotic layer

4.3.2.1 Dissolved inorganic phosphate

The vertical distribution of DIP for the four cruises are displayed in Figs 4.7, 4.8, 4.9, and 4.10. For the first cruise DIP in the 120m upper water column ranged from detection limit (1-2 nM) to 16 nM. Characteristically, in the first cruise the profiles showed a maximum either in the surface (<10m depth) or at 40-50m depth. A minimum was typically observed between 50-120m, and below this the DIP dramatically increases at the top of the nutricline (phospho-cline thereafter).

In the first cruise there was a significant increase of DIP at 140m in the dusk profile (Fig. 4.7a) from 2 nM (dawn profile) to 19 nM (extrapolate from Fig. 4.7b). The rest of other profiles (4.7 c and d) also had similar concentrations at this depth (20 and 34 nM respectively). This dramatic change was found to be salinity dependent (Fig. 4.11.). In this Figure, it can be observed that at salinities between 36.60 and 36.75 DIP is quite scattered around 5 nM, but at salinity ranges of 36.60-36.50 there is a clear relationship, with the break or start point of the relationship just below 36.60 at which point DIP leaps to higher levels. These small changes of salinities (i.e. changes of position of the density horizons) at depths just at the bottom or below the mixed layer which generally coincided with the depth of the euphotic

layer, could lead to dramatic changes in the DIP levels in this layer (see below Discussion)

In the second cruise (14-17/Feb.), the DIP profiles had similar vertical structure and concentrations (Fig 4.8 a and b) to the first, but for the third cruise (21-23/Feb., Fig. 4.9), the DIP was below 2 nM throughout the 100m depth mixed layer. In the fourth cruise (28/Feb.-2/Mar.) the DIP profiles (Fig. 4.10 a to e) contrasted with the previous ones because they showed no clear pattern and higher concentrations of DIP. The dramatic increase of DIP or the start of the phosphocline for the fourth cruise was observed at about 160 m depth and not at 140m as in the three previous cruises. In general the series of profiles for the four cruises show that DIP could have a well defined vertical structure and high variability at nanomolar levels.

The mass of DIP integrated over the euphotic layer (0-120m) ranged between 417 and 947 $\mu mol.m^{-2}$ for the first and second cruises (Table 4.1). For the third cruise the mass of DIP was at the detection limit of the technique (i.e. DIP mass was between 0 and 100 $\mu mol.m^{-2}$) for most of the water column, whilst during the last cruise the DIP mass increased over 2-15 fold relative to the first three cruises.

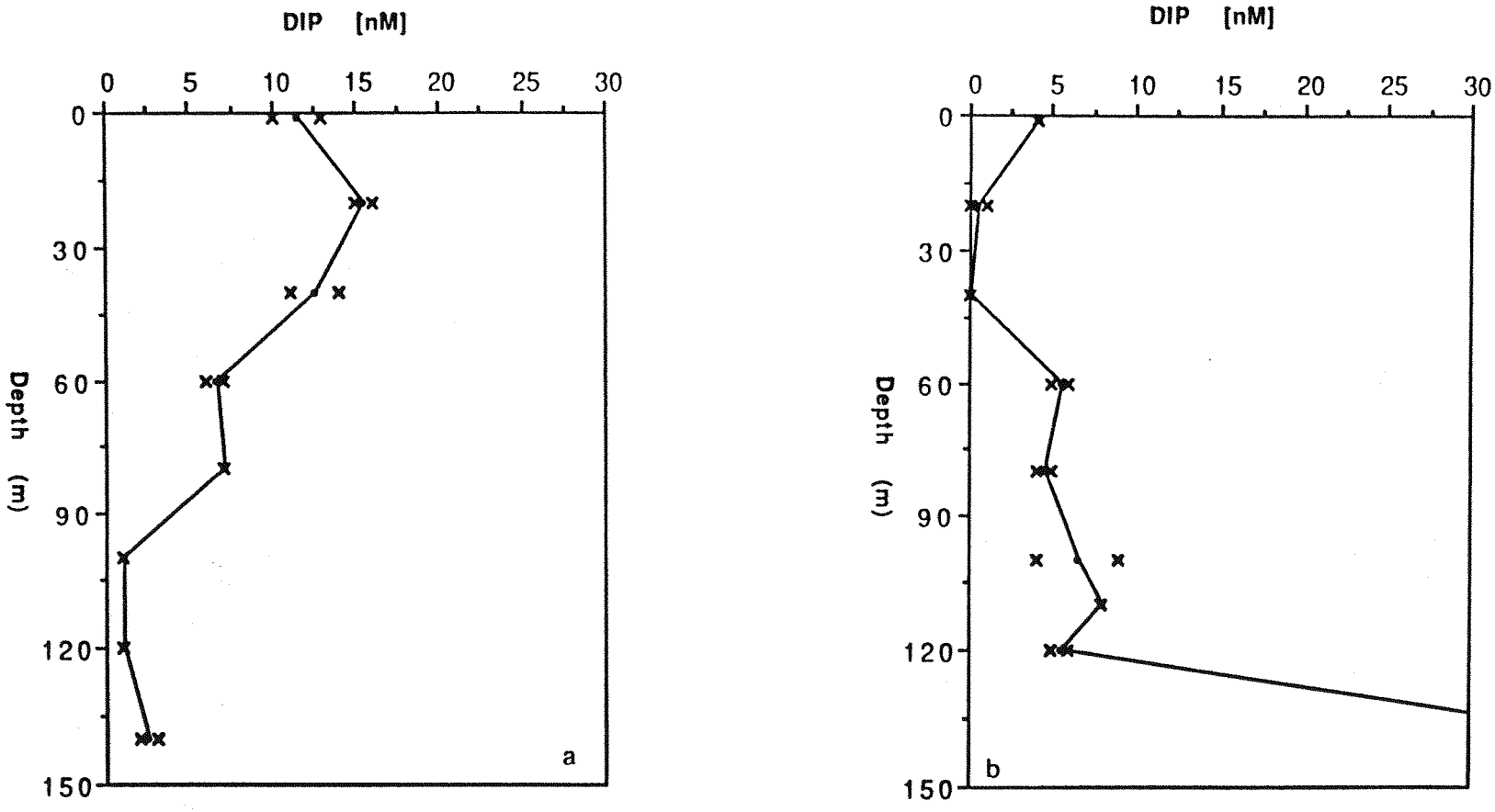


Fig. 4.7 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". a) 16.Jan (05:00), b) 16.Jan. (16:30).
Hereafter for all the Figures, analyses in duplicate are indicated with X, when duplicates are not plotted, see Appendices 4s for data.

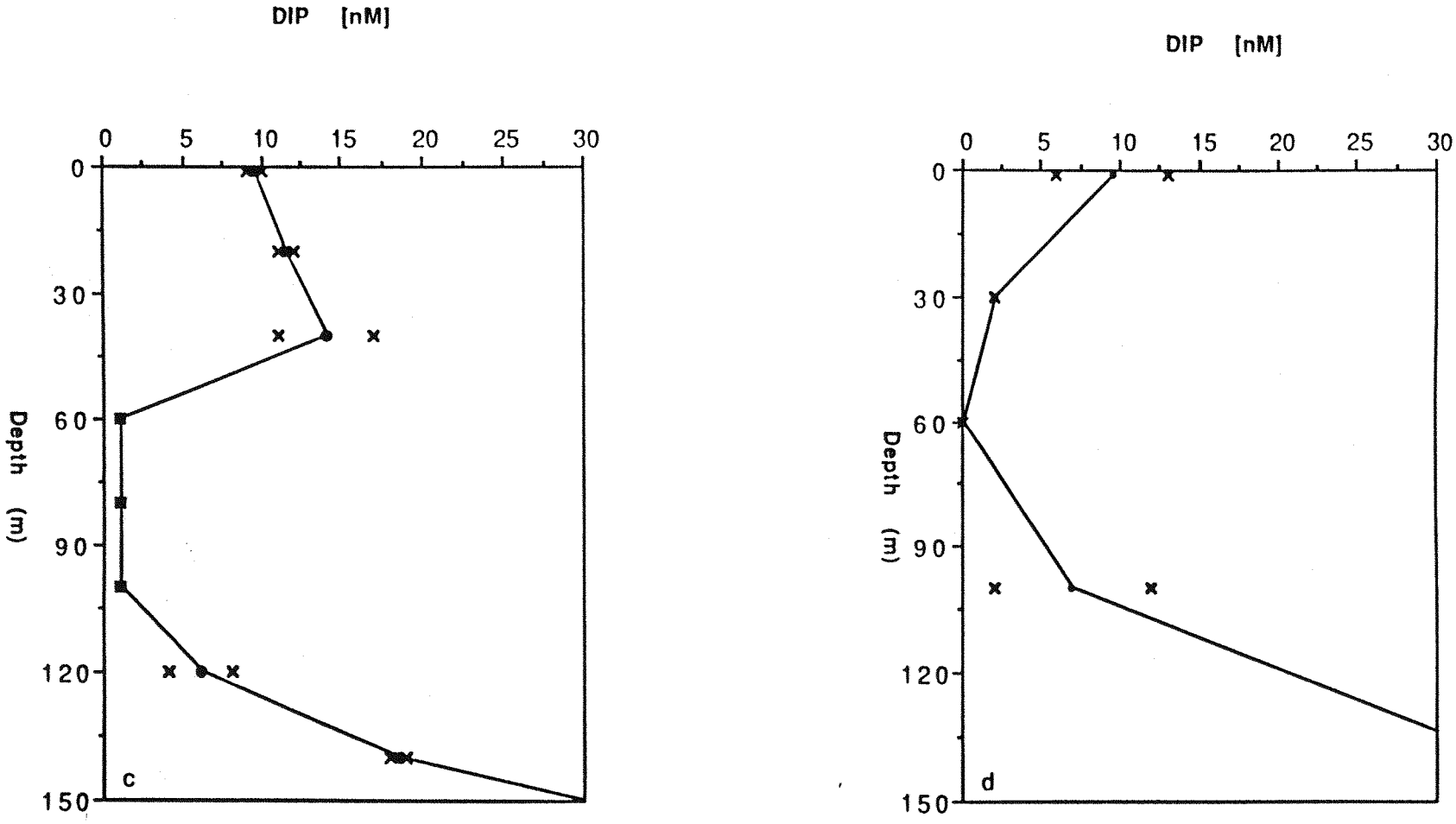


Fig. 4.7 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S".
c) 17.Jan. (13:00), and d) 18.Jam. (11:00).
Hereafter for all the Figures, analysis in duplicate are indicated with **X**, when duplicates are not plotted, see Appendices 4s for data.

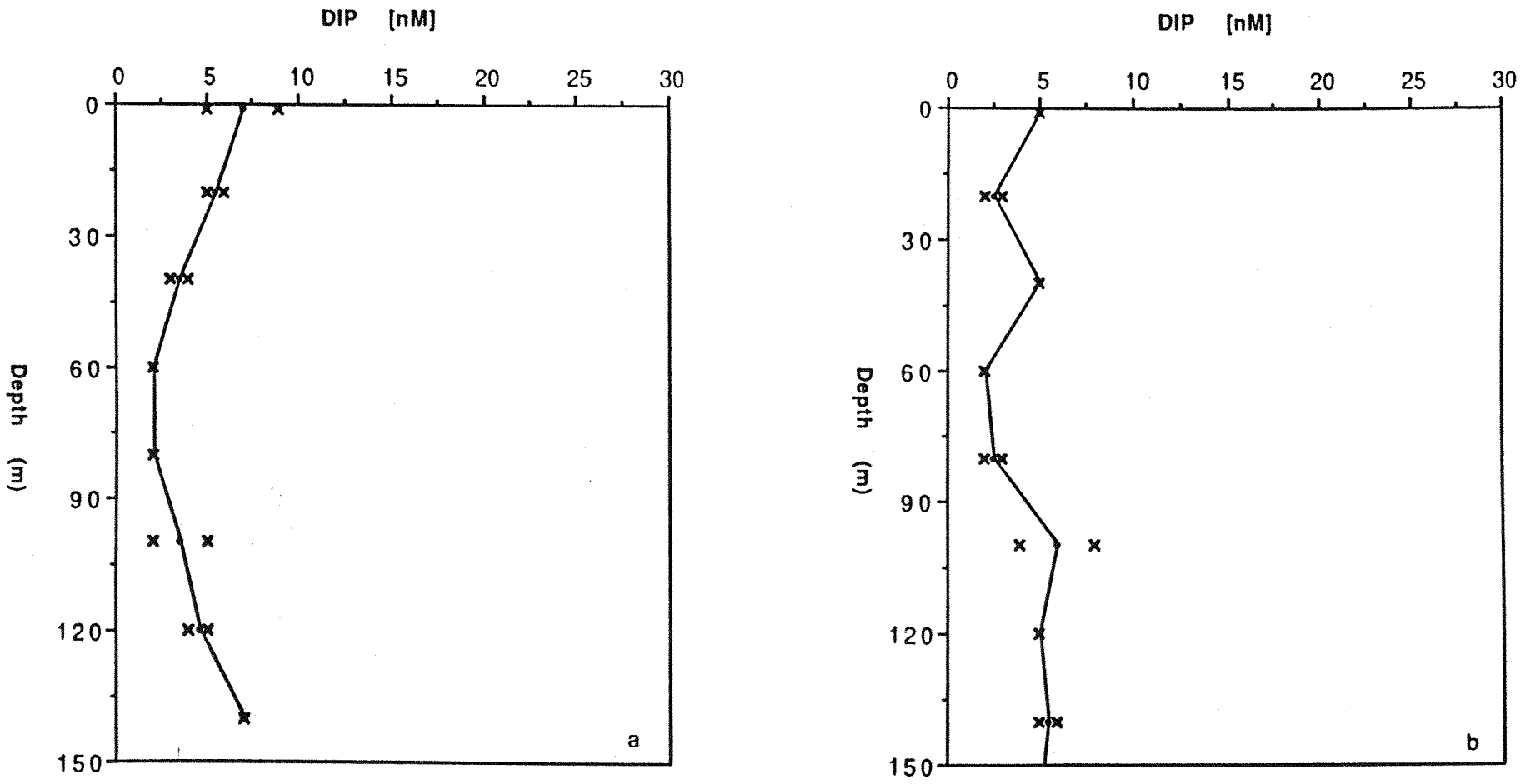


Fig. 4.8 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". a) 16.Feb. (04:00), b) 16.Feb. (11:00).

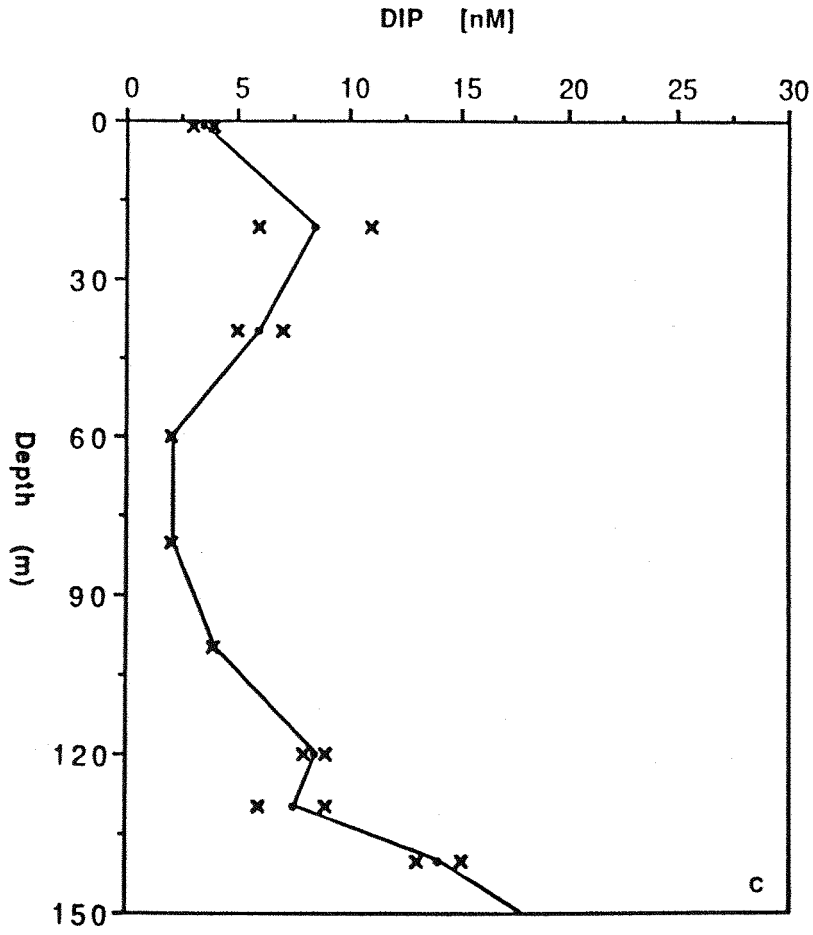


Fig. 4.8 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". a) 16.Feb. (04:00), b) 16.Feb. (11:00), and c) 16.Feb. (16:00).

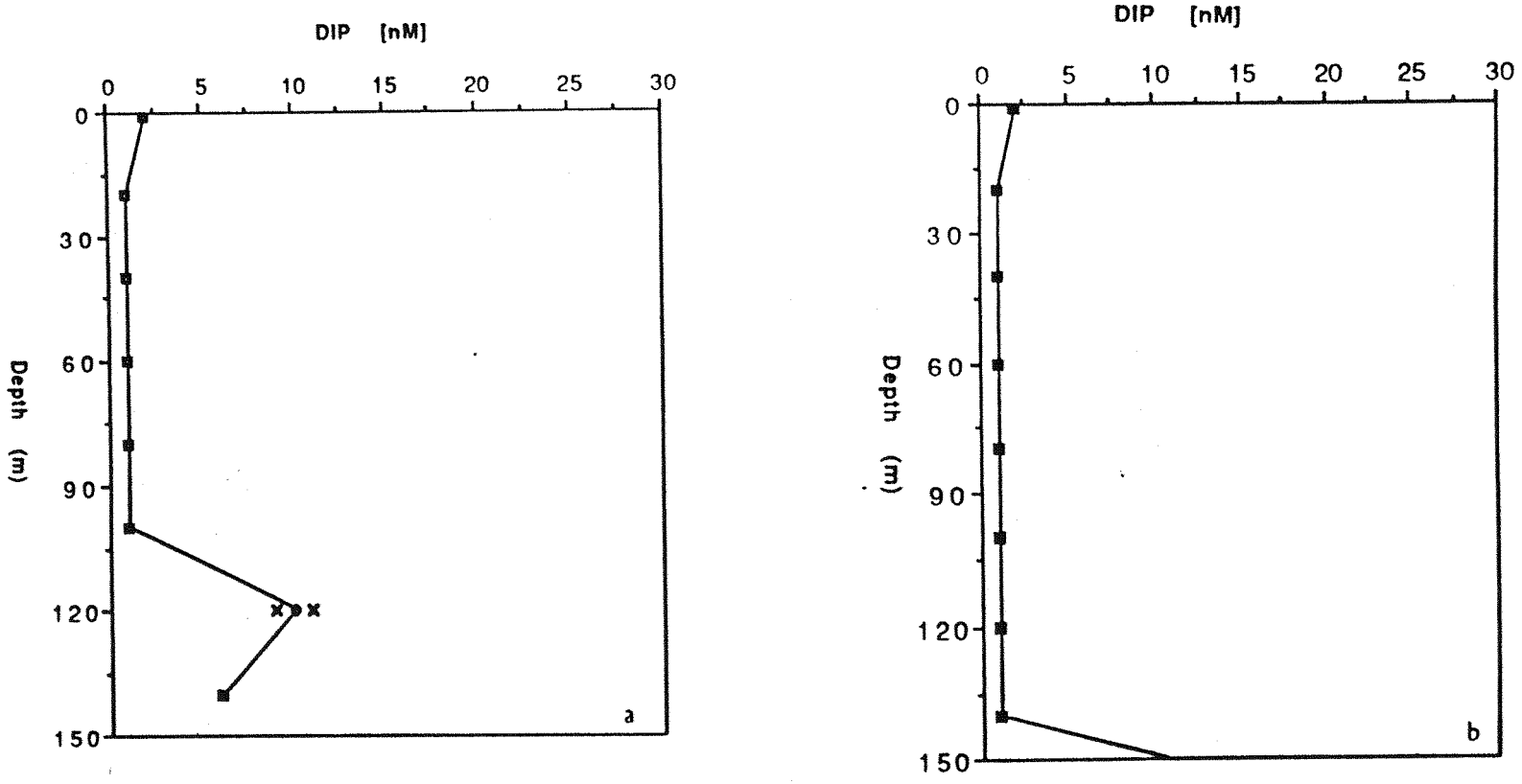


Fig. 4.9 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". a) 22.Feb. (05:00), and b) 22.Feb. (16:00).

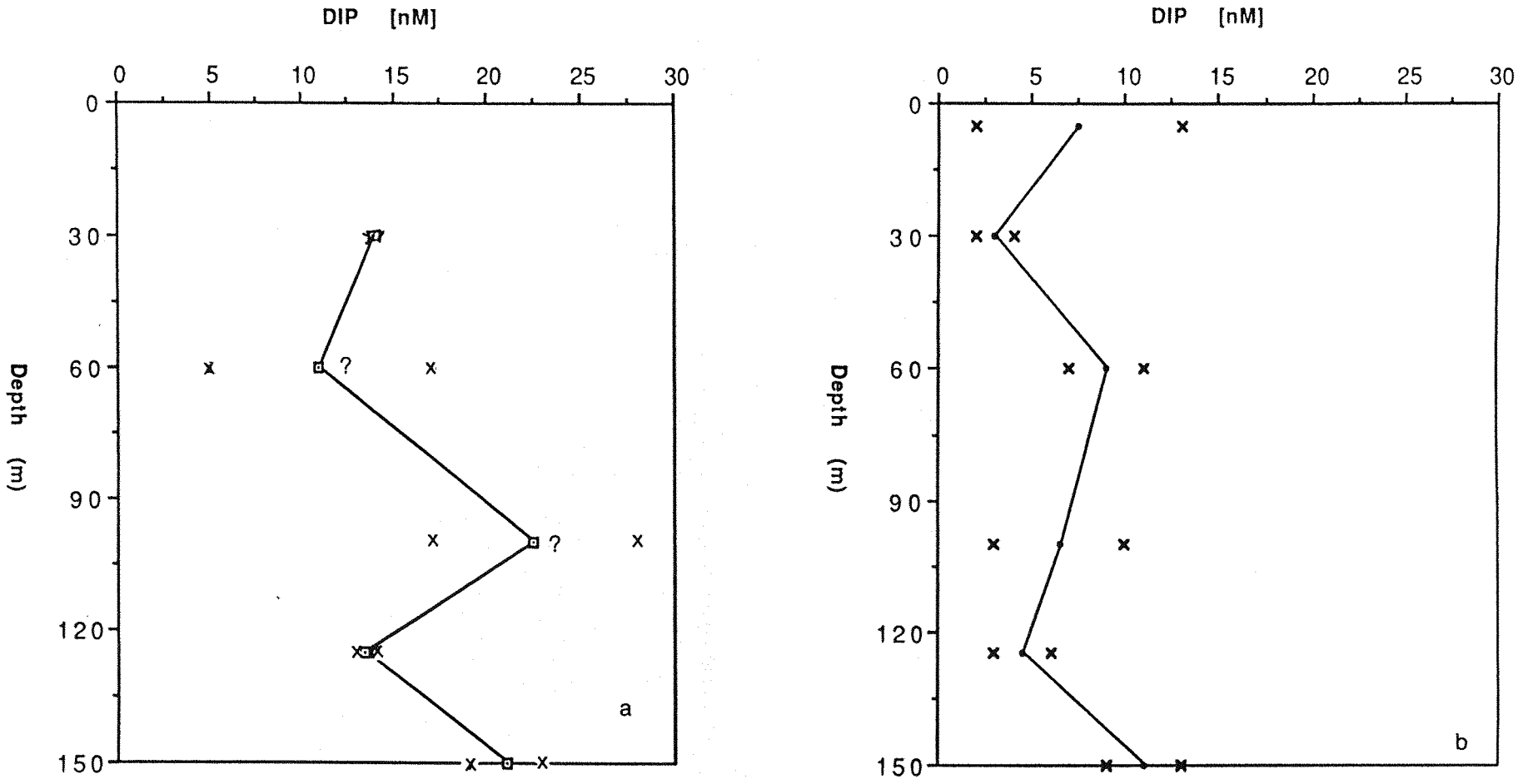
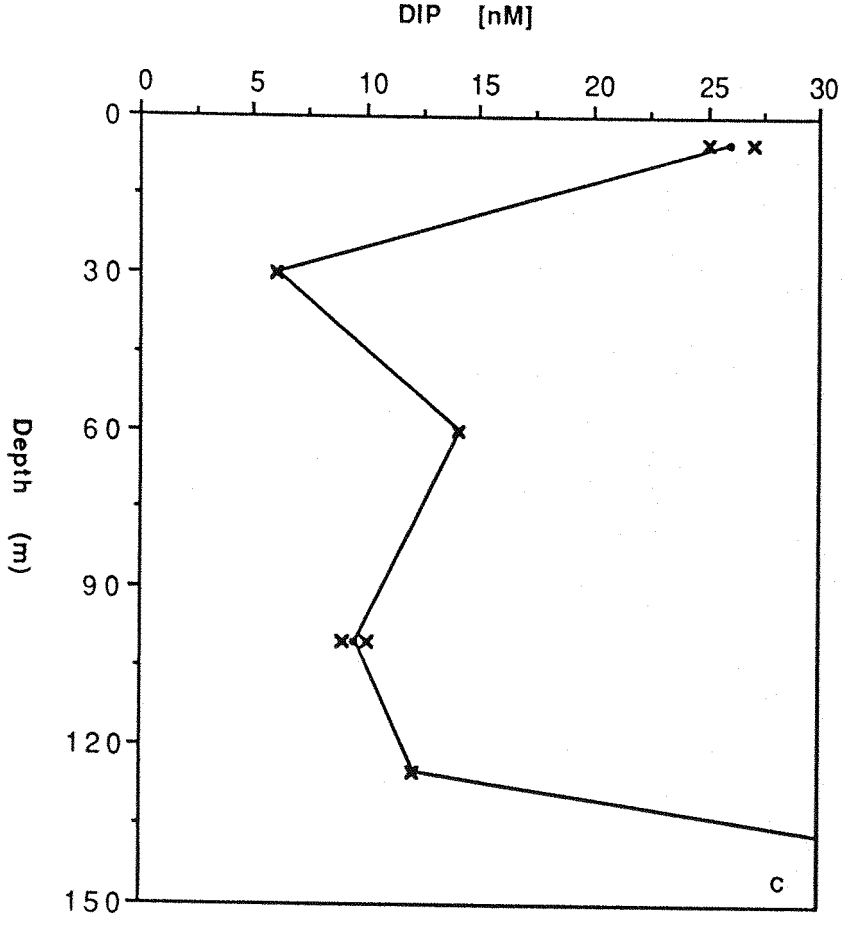


Fig. 4.10 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". a) 28.Feb. (10:45). b)28.Feb. (15:15). Note:The samples with a ?, means that the samples is regarded as dubious.



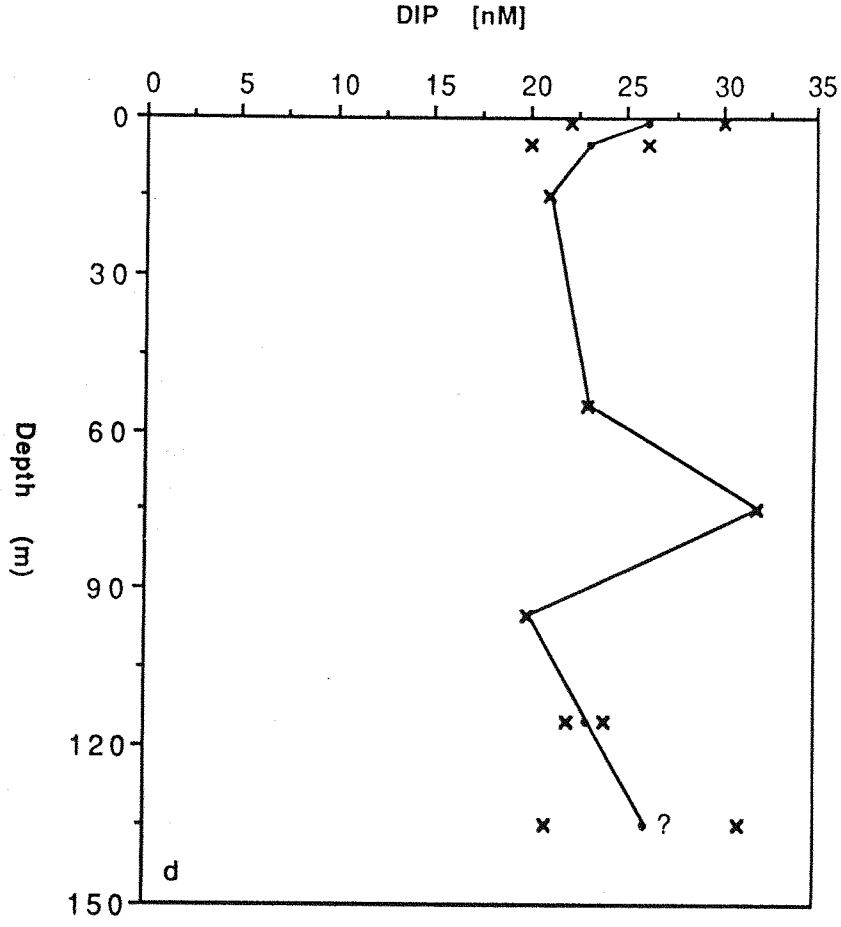


Fig. 4.10 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". c) 01.Mar. (02:30), and d) 01.Mar.(05:30). See 4.10 a and b for details.

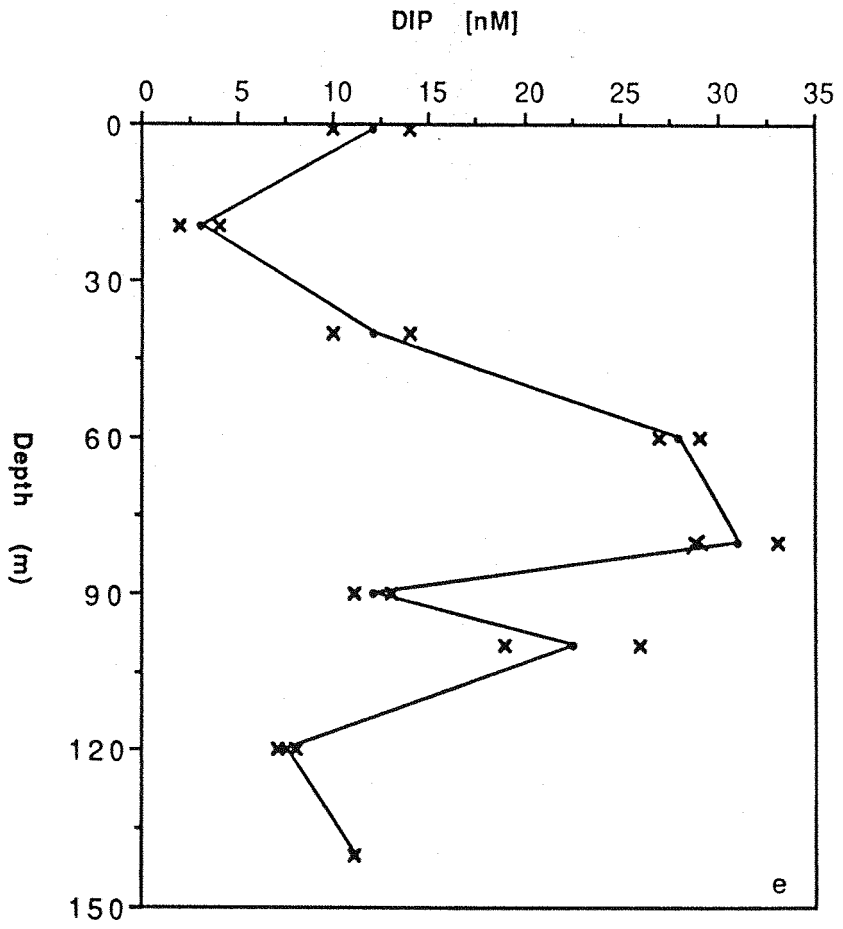


Fig. 4.10 Vertical distribution of DIP in the mixed layer of the Sargasso Sea. Station "S". e) 01.Mar.(10:00).

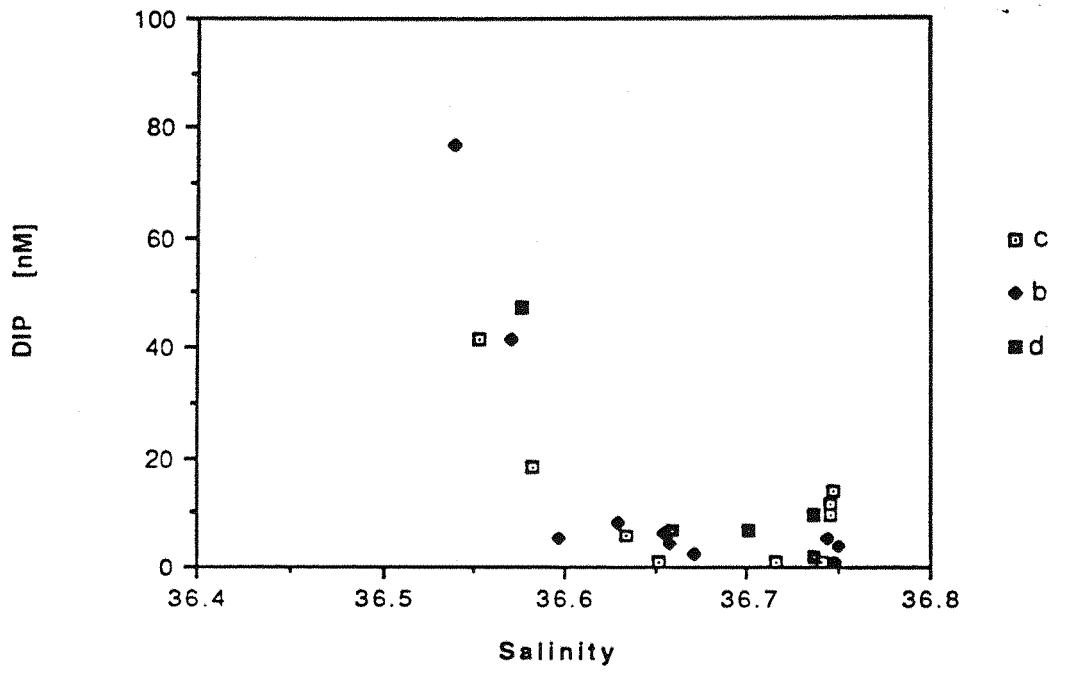


Fig. 4.11 Dissolved inorganic phosphate as function of salinity in the mixed layer of the Sargasso Sea. Station "S". The symbols denote b,c and d of Fig. 4.7.

Date(time)			
	16.Jan.(05:00)	16.Jan.(16:30)	
	947	463	
	17.Jan.(10:00)	18.Jan.(13:00)	
	873	647	
	16.Feb.(05:00)	16.Feb.(11:00)	16.Feb.(16:30)
	417	443	517
	22.Feb.(05:00)	23.Feb.(14:00)	
	103	<100	
	28.Feb.(10:45)	28.Feb.(15:15)	
	1203	573	
	01.Mar.(02:30)	01.Mar.(05:30)	01.Mar.(10:00)
	1513	2777	1440

Tab. 4.1 Total mass of DIP in the 120m upper mixed layer (photic) in the Sargasso Sea. Station "S". DIP pool is in $\mu mol.m^{-2}$.

4.3.2.2 Dissolved organic phosphorus

The vertical distribution of DOP for the first cruise is shown in the Figure 4.12. The profile taken at dawn shows the typical feature of dissolved organic P and C (e.g. Smith *et al.* 1986, Bauer 1990), *id est* a maximum near the surface, decreasing steadily down to 80-100m depth; below this depth there is no distinct increase. The dusk profile (Fig. 4.12b) showed a rather uniform distribution from 0 to 100m. This profile showed more scatter than the first one. Comparing both profiles, it can be observed that there was a removal of DOP between 0-50m depth, and in the depth range of 50-140m depth there was no a distinct change.

The mass of DOP in the euphotic layer for the first cruise is given in Table 4.2. and it is about 17200 and 16300 $\mu mol.m^{-2}$ for the dawn and dusk profiles. A net removal of 900 $\mu mol.m^{-2}$ can be observed.

For the rest of the cruises the vertical distribution of DOP is shown in Fig. 4.13 (a,b, and c respectively). The samples were analyzed by both methods described in the Section 4.2, i.e. Ultraviolet irradiation (DOP_{UV}) and Magnesium nitrate oxidation (DOP). The average values of DOP for the second cruise (200 nM) were higher than the previous cruise and there was not a clear vertical pattern. At 140m depth there was an anomalous concentration of DOP of about 1.0 μM which was regarded as suspect.

The DOP concentrations determined by UV oxidation technique were lower than those using the magnesium nitrate method, averaging 120 nM and showing a uniform distribution through the euphotic zone. The high concentration of DOP of 950 nM at 140m depth was also detected with the UV oxidation method. This single sample analyzed by both methods, had very likely been contaminated, because its DOP concentration was about 4 times greater than the DOP levels of adjacent depths and also there was observed no changes in other parameters such as Chl-*a*, nitrate, etc.

The third cruise (Fig 4.13b), a week after the second, showed a similar vertical pattern and concentrations to the second cruise. No high irregular value was observed, thus corroborating the idea mentioned in the above paragraph, i.e. the sample was contaminated. For the fourth cruise (Fig 4.13c) there was a clear decrease of DOP suggesting regeneration of DOP into DIP have occurred. The average DOP was 144 ± 37 nM over 0-140m, and there was a clear increase between 100-140m, then below this depth DOP values started to decrease (see below the deep vertical profile Fig. 4.19 and 4.20).

The total mass of DOP in the euphotic layer for the occupied stations (Table 4.2.) range from 16300 to 23200 $\mu mol.m^{-2}$, and it was the main fraction of total P in the water column (see below).

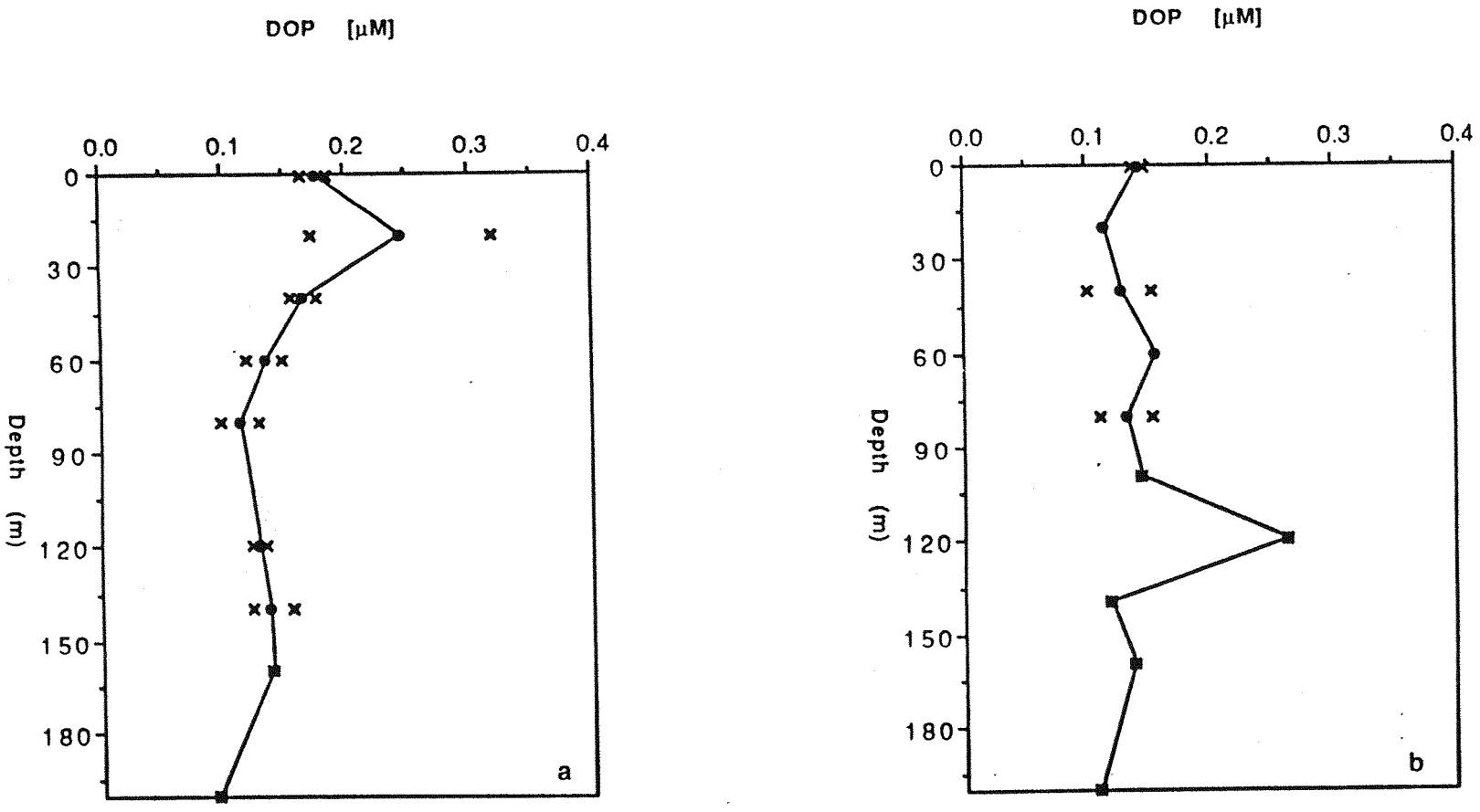


Fig. 4.12 Vertical distribution of DOP in the Sargasso Sea. Station "S". a) 16.Jan. (05:00) and b) 16.Jan. (16:30).

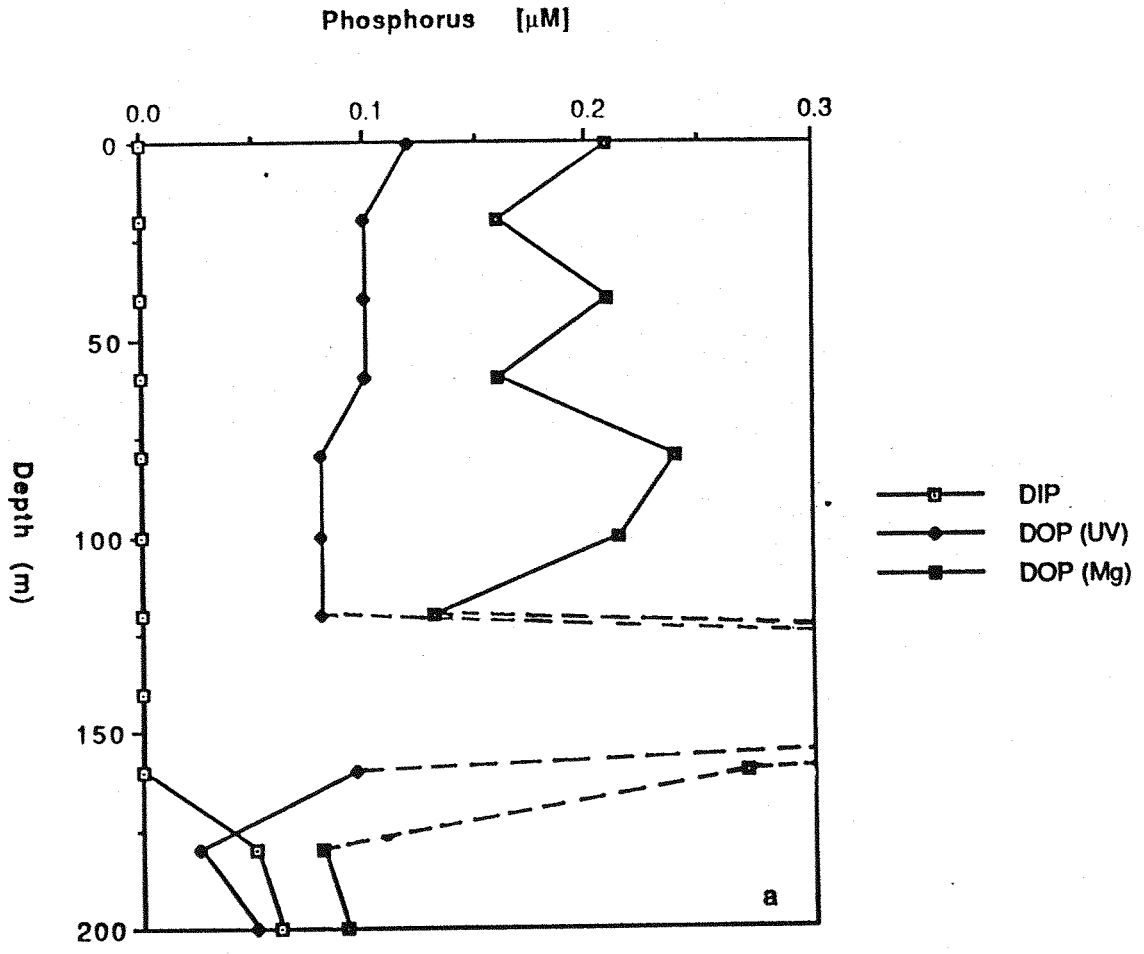


Fig. 4.13 Vertical distribution of DIP, and DOP in the Sargasso Sea. Station "S". a) 16.Feb.
Note that the sample at 140m depth was assumed as contaminated (dashed line). DOP was analyzed by Ultraviolet (UV) and Mg nitrate (Mg) oxidation techniques. Plotted data are average of duplicate analysis.

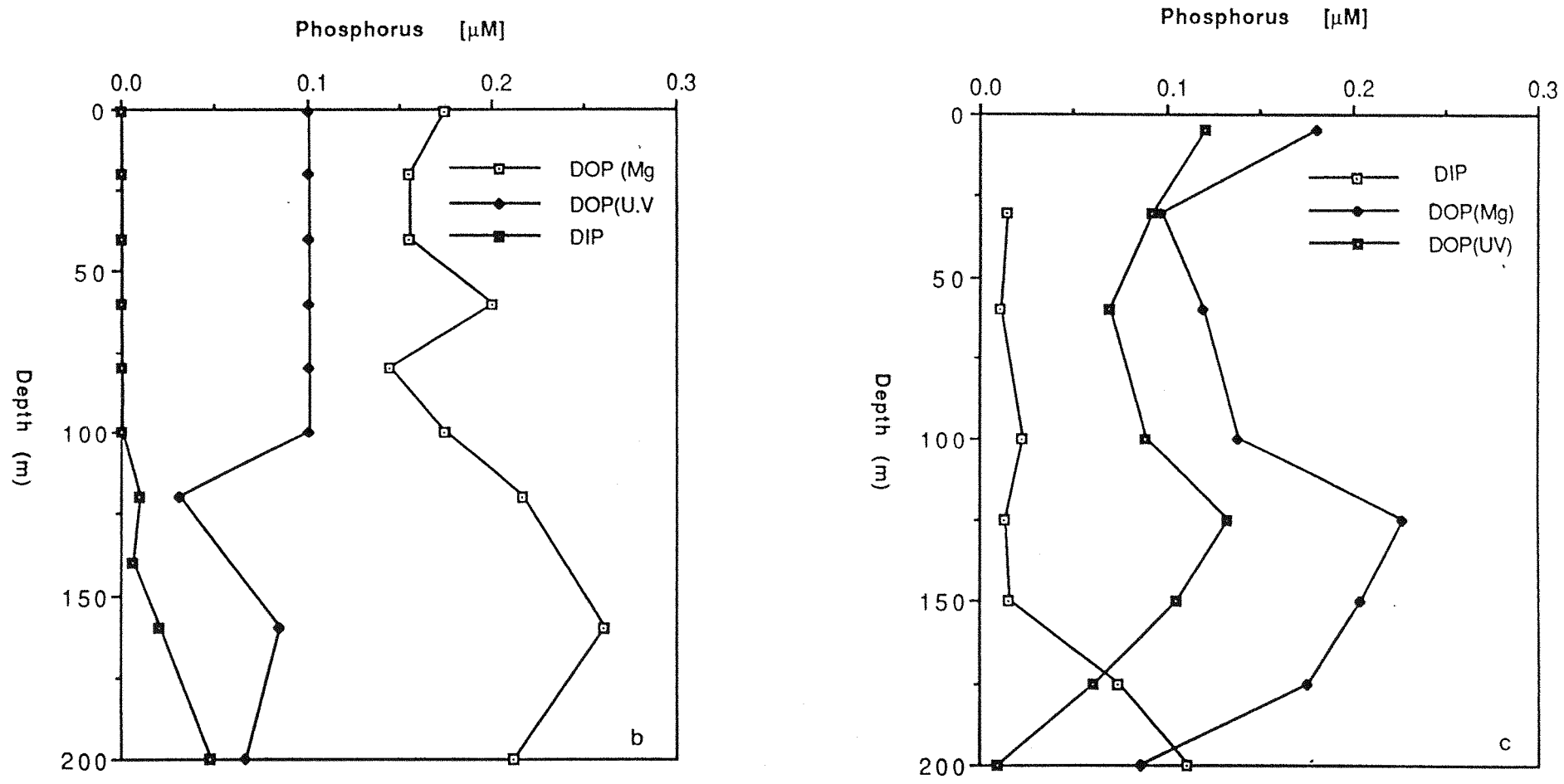


Fig. 4.13 Vertical distribution of DIP, and DOP in the Sargasso Sea. Station "S". b) 21.Feb., and c) 28.Feb. Plotted data are average of duplicate analyses.

4.3.2.3 Particulate total phosphorus

Particulate Total P (PTP) loadings varied markedly over the four cruises (Fig. 4.14 a and b, and 4.15 a, b and c), with the highest concentrations (up to 70 nM) observed in the first cruise, and the lowest ones (3 nM) in the fourth cruise. In all profiles the highest loads were between 20-100m; below this depth there was an abrupt decrease to nanomolar levels. The mass of PTP integrated over the photic layer (0-120m) ranged from 1200 (4th. cruise) to about 6500 $\mu mol.m^{-2}$ (first cruise), thus corresponding about 20 % of the total P budget (Table 4.2)

4.3.2.4 Dissolved poly-phosphates

Dissolved poly-phosphates (PPs) proved to be an important fraction of the total DOP, being almost two times the DOP_{UV}. The vertical distributions of PPs for the last three cruises are shown in Figure 4.16 (a, b and c). Dissolved PPs concentrations are variable through the water column; they do however show a characteristic increase at the bottom of this water layer. The median of PPs concentrations was 120 nM, representing 40-49 % of the DOP concentrations.

Date(time)	DOP	PTP	DIP	Total P
16.Jan.(05:00)	17200	6500	950	24600
16.Jan.(16:30)	16300	7100	460	23900
16.Feb.(05:00)	23200	2800	400	26400
21.Feb.(05:00)	20400	1300	<100	21700
01.Mar.(02:30)	16500	1200	1500	19200

Tab. 4.2 The pools of different forms of P in the 120m upper mixed layer of the Sargasso Sea. Station "S". Mass in $\mu mol.m^{-2}$. Total P is the sum of DIP, PTP and DOP. The average of total P was 23200 $\mu mol.m^{-2}$.

	DIP gradient			mean DIP			
Cruise	$\mu mol.m^{-4}$			$\mu mol.m^{-3}$			
1	1.2	0.7	0.8	1.8	13	26	
2	0.65	2.4		11.	14		
3	0.95			10			
4	2.3	2.3	1.5	45	40	64	31
Average	1.35			27.4			
Range	0.65 - 2.3			10 - 64.2			

Tab. 4.3 DIP gradient and mean concentration in the top 20m of the phosphocline in the Sargasso Sea, Station "S", during Jan.-Mar. 1990. See text for details.

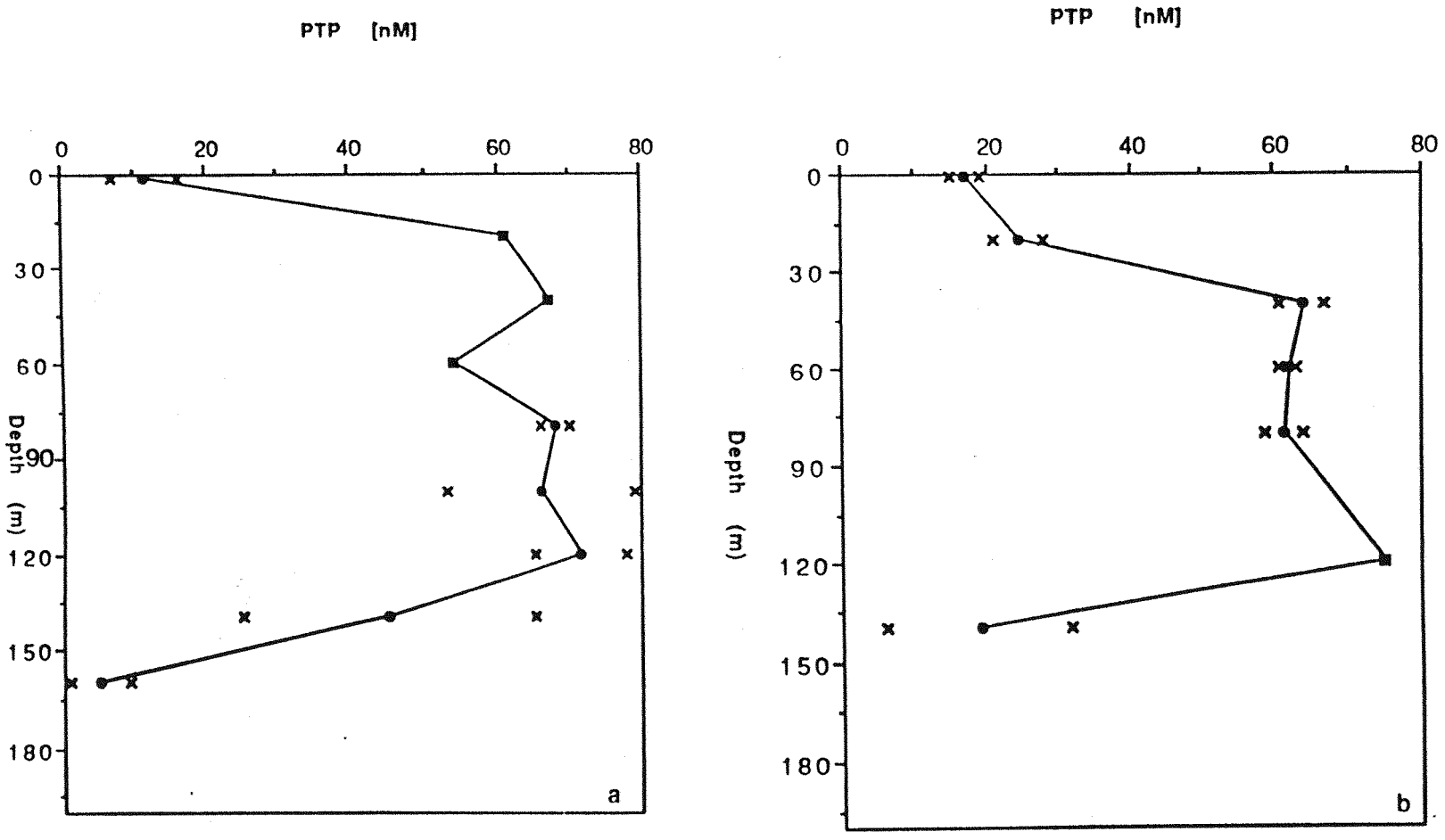


Fig. 4.14 Vertical distribution of PTP in the Sargasso Sea. Station "S". a) 16.Jan. (05:00) and b) 16.Jan. (16:30). Note the slight change in the drawing scale of b.

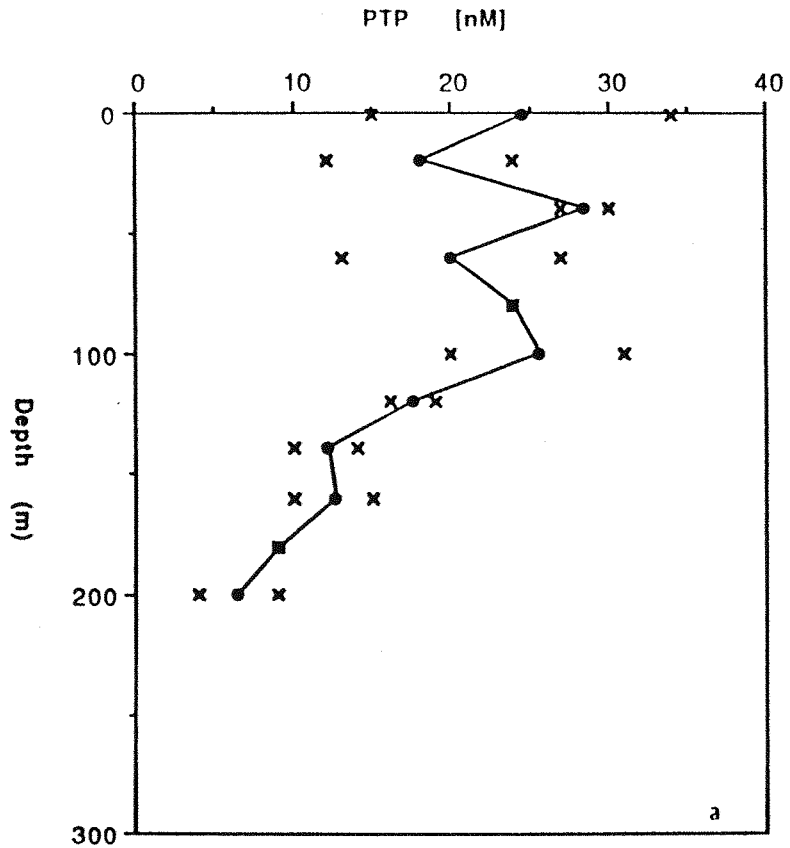


Fig. 4.15 Vertical distribution of PTP in the Sargasso Sea. Station "S". a) 16.Feb.

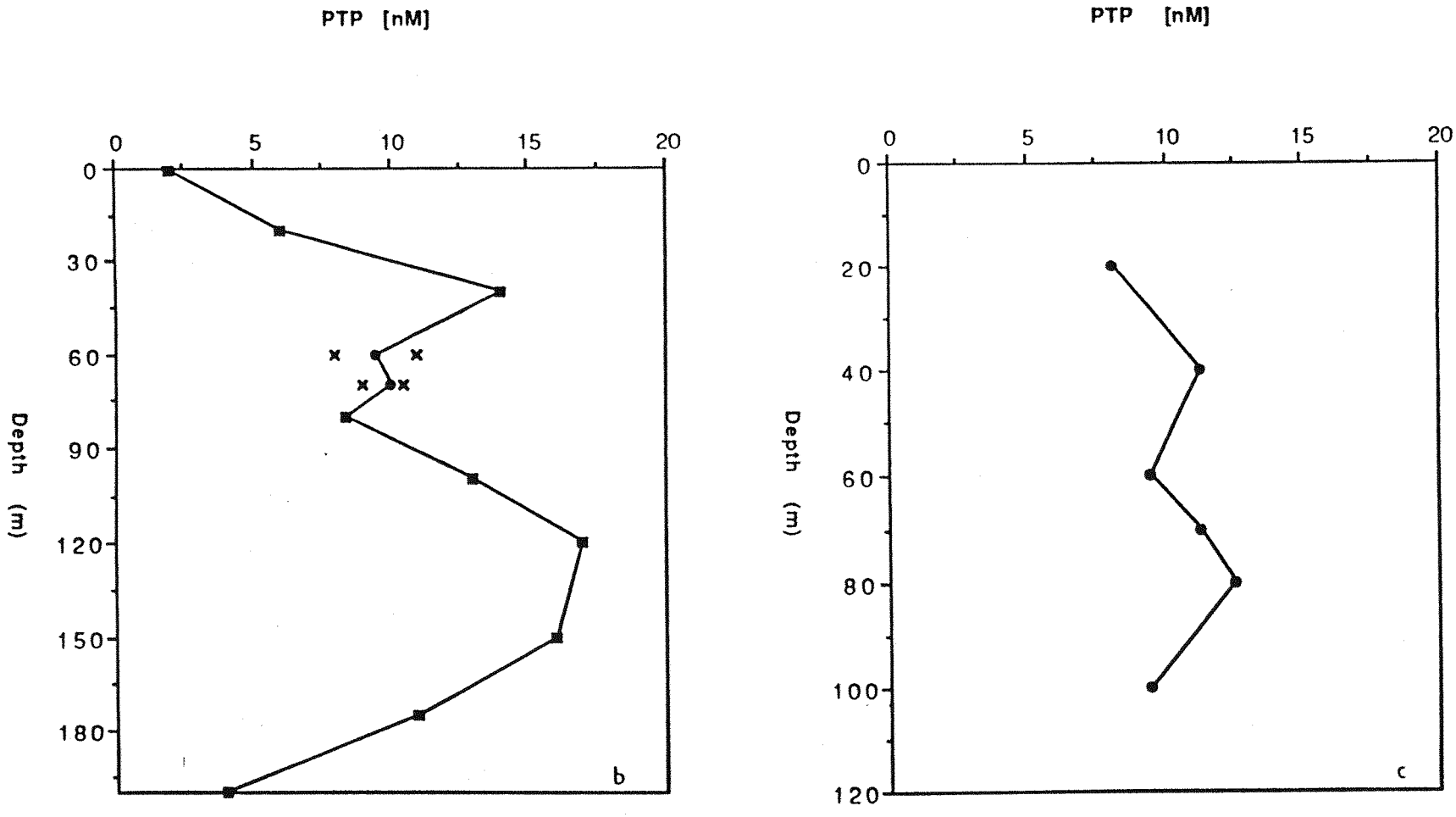


Fig. 4.15 Vertical distribution of PTP in the Sargasso Sea. Station "S". b) 21.Feb. c) 28.Feb.

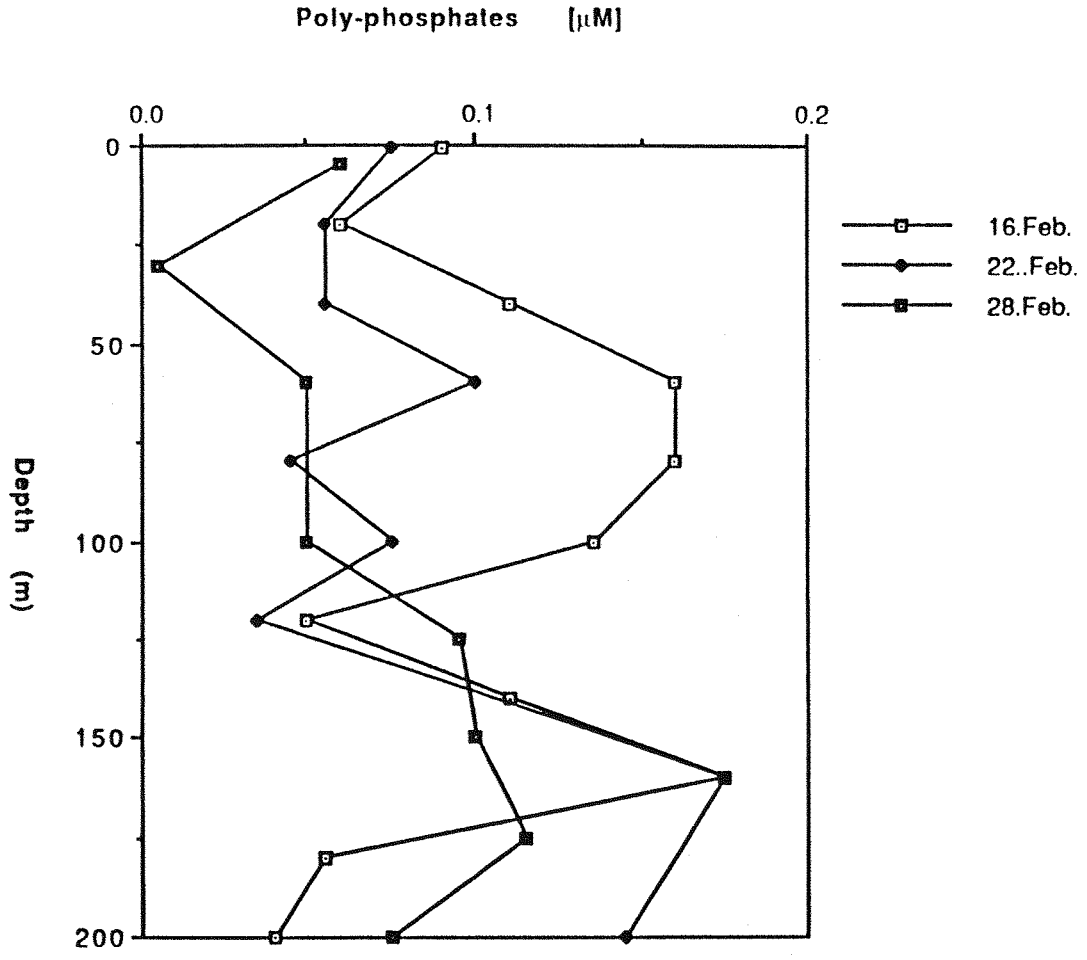


Fig. 4.16 Vertical distributions of PPs in the Sargasso Sea. Station "S" during Feb.90.

4.3.3 Processes affecting vertical P distribution and budgets within the mixed layer

Processes within the mixed layer such as regeneration of DOP by enzymatic reactions, disappearance or net uptake of DIP from solution in a time-series experiments, and removal of P by passively sinking particles and input of P by mainly diffusion from and to the euphotic layer are considered.

Due to the work load on board ship process experiments were only done once, i.e. APA and net uptake of P in the fourth and third cruises respectively, whilst removal fluxes of P in the first and second cruises. Regarding vertical diffusion rates, these were obtained indirectly by measuring the DIP gradient at the bottom of the euphotic layer and then computing them using a Fickian diffusion model, which has been employed by, for example, Garside (1985) and Lewis *et al.* (1987).

Alkaline phosphatase activity was determined three times in 24 hours (Fig 4.17). The three vertical profiles had generally similar structure with activities ranging from 3.8 $nM.h^{-1}$ to the detection limit of the technique (0.06 $nM.h^{-1}$). Vertical profiles show an apparent correlation with phytoplankton distributions (see Chl-*a* data, App. 4.6) and it seemed that 50-70 % of the measure APA was due to autotrophic micro-organisms, because values of APA in the euphotic layer are 50-70 % higher than those at below 120m, where hetero-trophic organisms exist. The remaining APA may have been

bacteria related (see Discussion below). The regeneration capabilities of this non-specific enzyme in the photic layer was in the range 201-387 $\mu mol.m^{-2}.h^{-1}$, thus providing a τ span of 52-99 hours for a typical DOP budget of 20000 $\mu mol.m^{-2}$. Although a decrease of APA from the first profile (Fig. 4.17a) to the second and third (Figs. 4.17b and 4.17c), there was no clear diurnal variation, and thus it appeared the enzyme was acting at similar rates regardless the solar radiation.

The net uptake of DIP in the incubation experiment (Fig. 4.18.a) proved to be patchy in the first 2-3 hours (≈15-<18 h), but after sunset (18-22 h) there was a clear pattern of removal, and the plot of substrate concentration against net uptake rates followed the Michaeles-Menten curve (Fig 4.18b). This curve indicates a maximum measured net uptake (V_{max}) rate of 6 $nM.h^{-1}$ at DIP levels of 50 nM, and V_{max} seems to stabilize just above this DIP concentration. The saturation constant (K_m) was 15 nM under the circumstances described. At environmental DIP concentrations (< 5 nM) the net uptake rates fell quickly to <1.5 $nM.h^{-1}$, which is about half of the APA recycling rate.

The escape of P from the euphotic layer via passively sinking particles was determined to be about 10 $\mu mol.m^{-2}.d^{-1}$ for January and 92 $\mu mol.m^{-2}.d^{-1}$ for February. It should be stressed that they were **single** values with **no replicates** and the sample for January was close to detection limit and therefore will not be taken into consideration for discussion purposes. Problems associated with the efficiency of sediment

traps which are assumed to be collecting falling material at 70-90 % efficiency (Deuser and Ross 1980), have recently received attention because it is believed and in part empirically demonstrated, that cylinder shaped sediment collectors are not sampling at the high efficiency suggested (Drs A. Knap and A. Michael, pers. comm.).

4.3.4 Dissolved organic, inorganic and poly-phosphates in meso- and bathy-pelagic waters

Dissolved inorganic phosphate below the euphotic layer down to 4200m depth (Fig. 4.19 and 4.20) was shown to have the typical characteristic vertical distribution and its concentrations were in the range previously reported (e.g. Jickells 1986), but they were about < 10 % lower than the preliminary profile measured simultaneously by the BBSR scientists. The maximum (1.40 μM) coincided with the oxygen minimum, below which there is a sudden decrease of about 0.20 μM down to 1200m depth, then it is distributed fairly constant down to 4200m depth. The two DTP vertical profiles showed a smooth distribution, but the respective DOP profiles (Fig 4.19 and 4.20) have a quite patchy distribution through the whole water column, ranging from 25 to 400 nM with an average of 202 ± 110 nM. A conspicuous maximum of about 400 nM at 3500-4000 m depth, was observed in both profiles. Profiles showing similar characteristics have been reported by Ljutsarev *et al.* (1977) in the tropical Pacific Ocean. The presence of PPs or/and UV refractory organic-P compounds in the whole water column was found, and they ranged from detection limit (25 nM) to 145 nM and averaging 91 nM, which represents about 7 % of the DIP (Fig. 4.21).

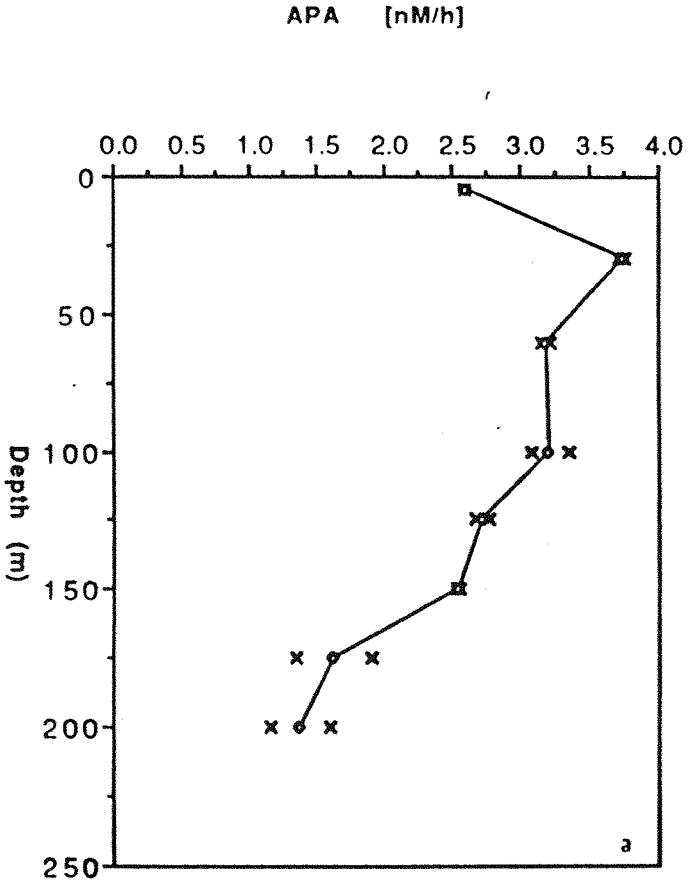


Fig. 4.17 Vertical distribution of APA in the Sargasso Sea. Station "S". a) 28.Feb. (10:45), b) 28.Feb. (16:15), and c) 01.Mar. (02:30). Note the change of scale in the APA axis (a) relative to b and c.

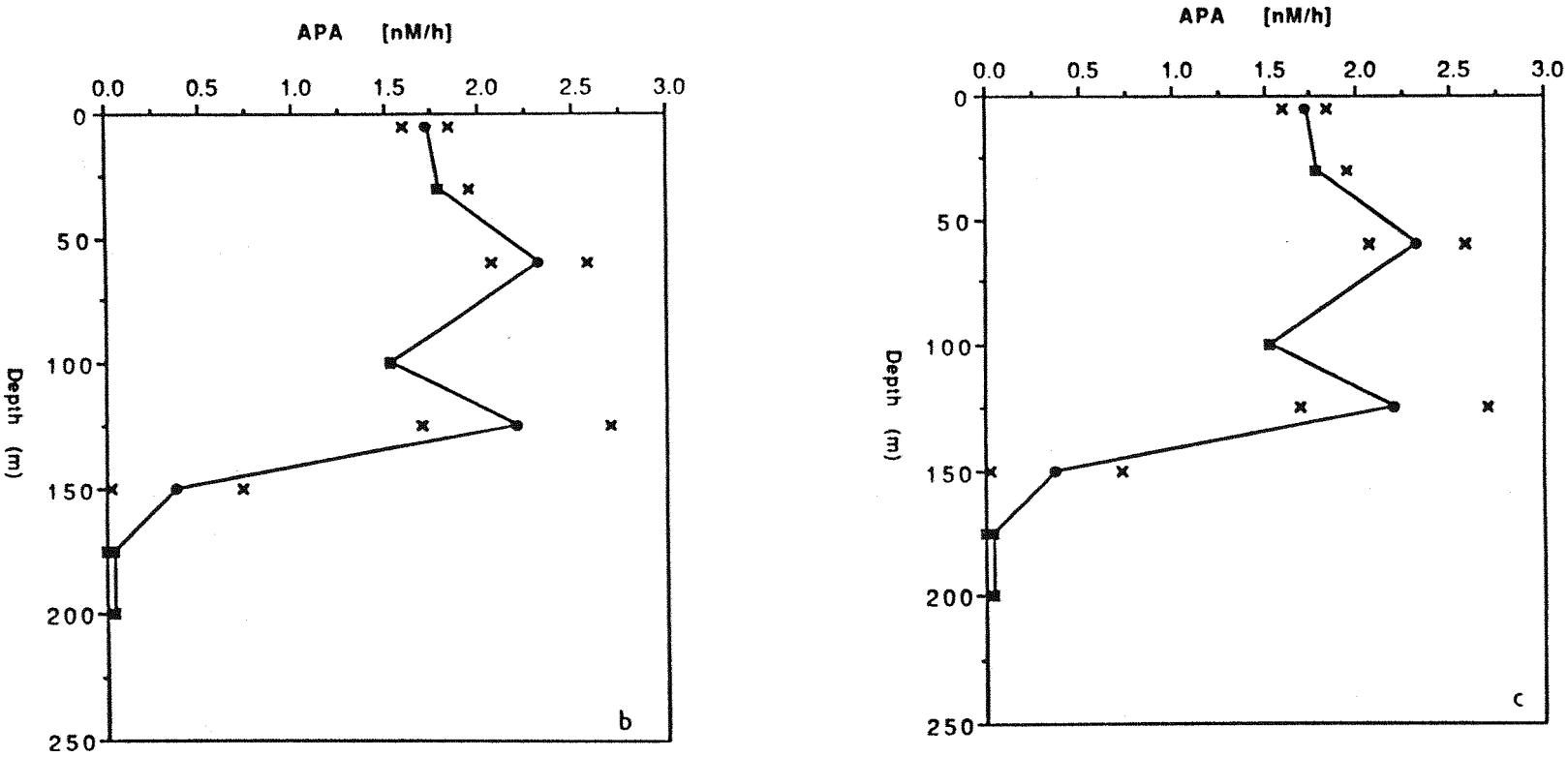


Fig. 4.17 Vertical distribution of APA in the Sargasso Sea. Station "S". a) 28.Feb. (10:45), b) 28.Feb. (16:15), and c) 01.Mar. (02:30). Note the change of scale in the APA axis (a) relative to b and c.

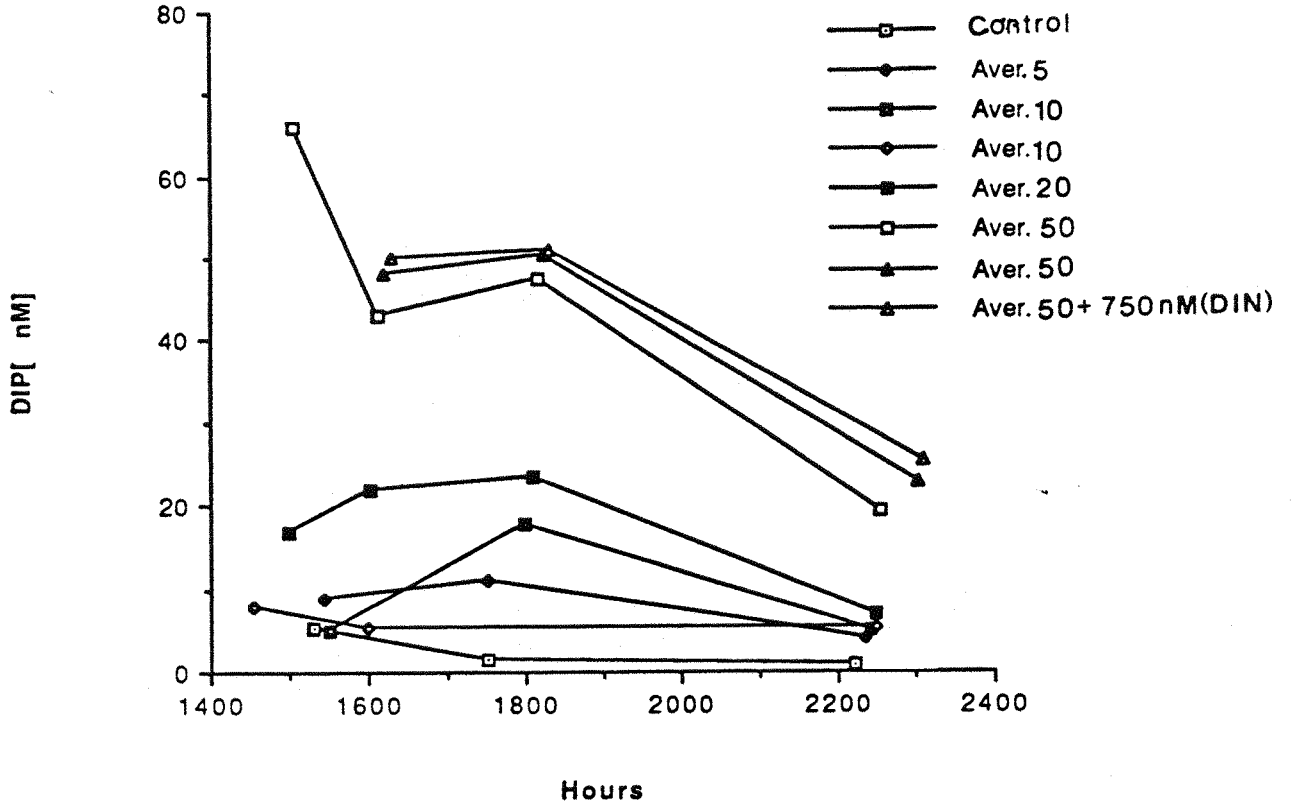


Fig. 4.18 a) Removal or net uptake of DIP in samples incubated "on deck". Sargasso Sea. Station "S". All the analyses were by duplicates (see Appendices 4s). Symbols indicate the amount of the added DIP in nM.

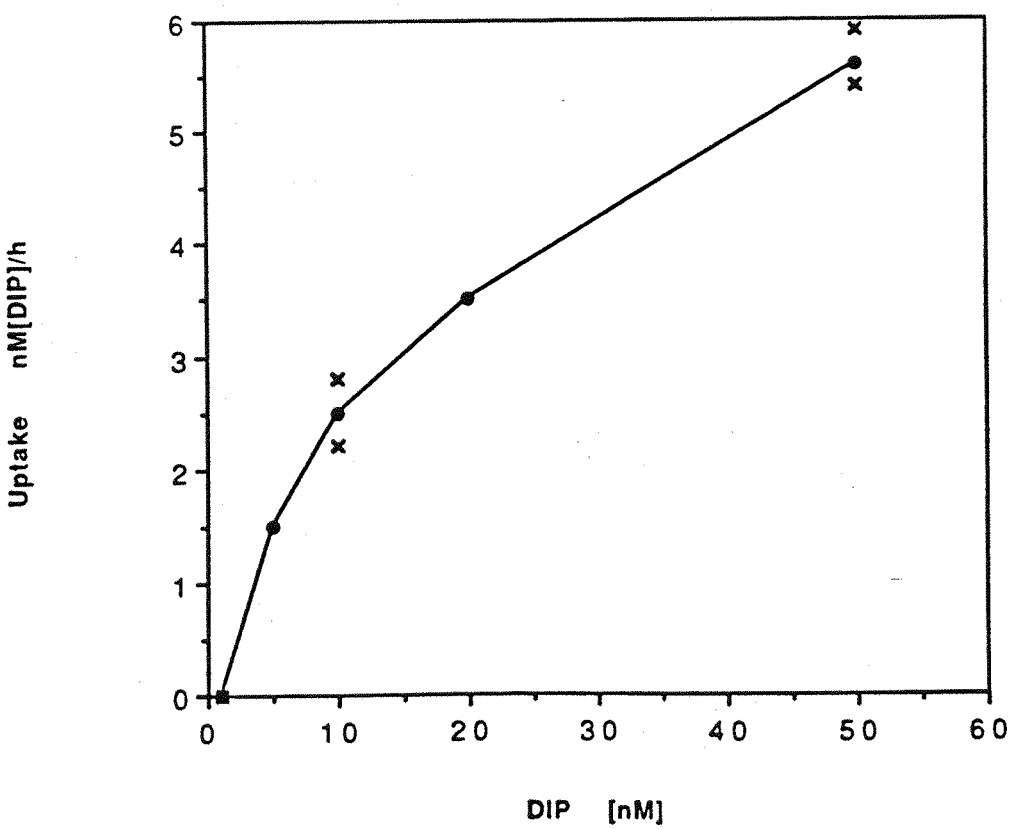


Fig. 4.18 b) Uptake rates *VS* concentrations of DIP for samples incubated "on deck". Sargasso Sea. Station "S". 22.Feb.90.

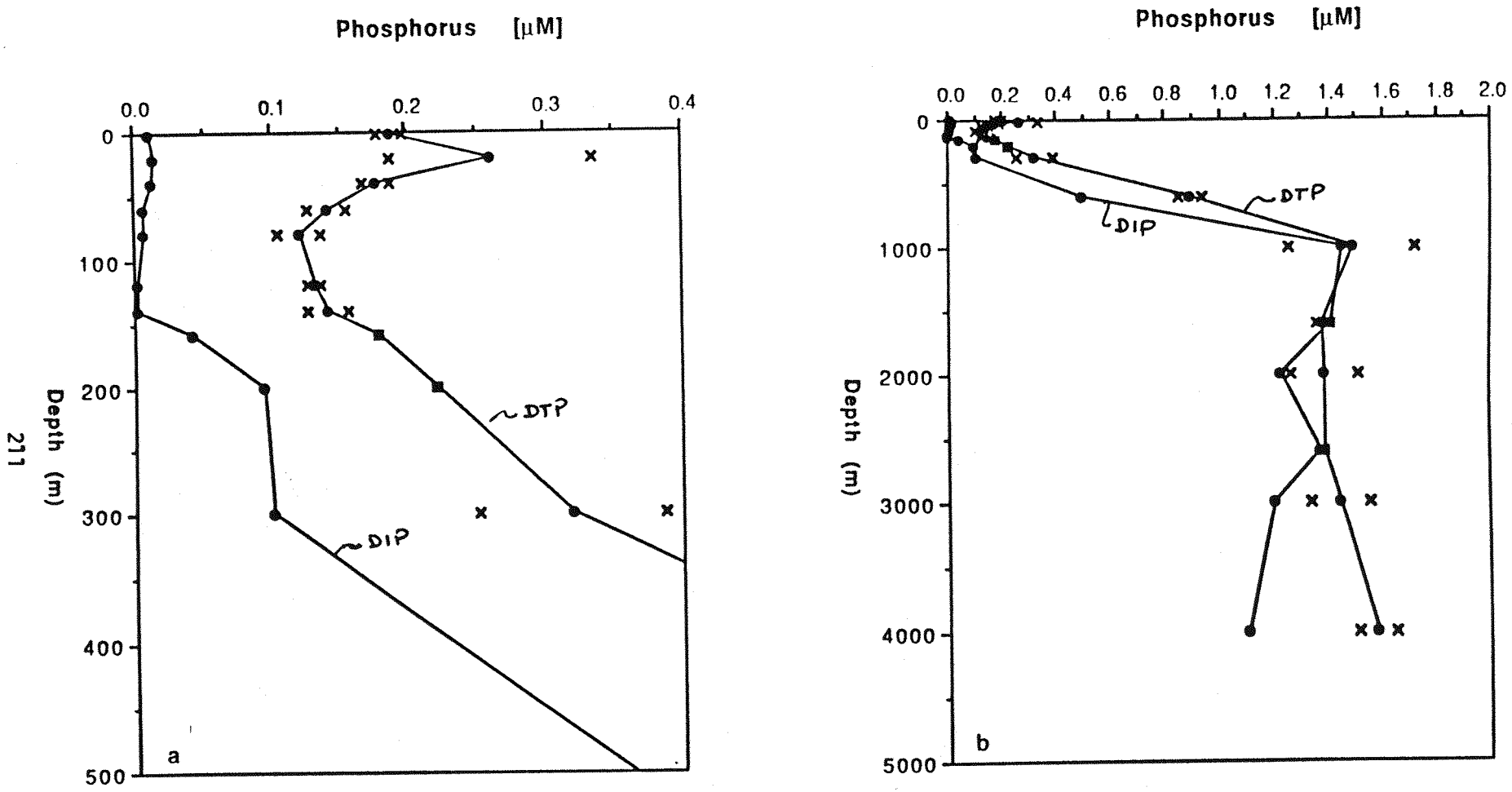


Fig. 4.19 Vertical distribution of DTP (analyzed by Nit-ox technique) and DIP. Sargasso Sea. Station "S". Jan.90. a) 0-500m, and b) 0-4200m.

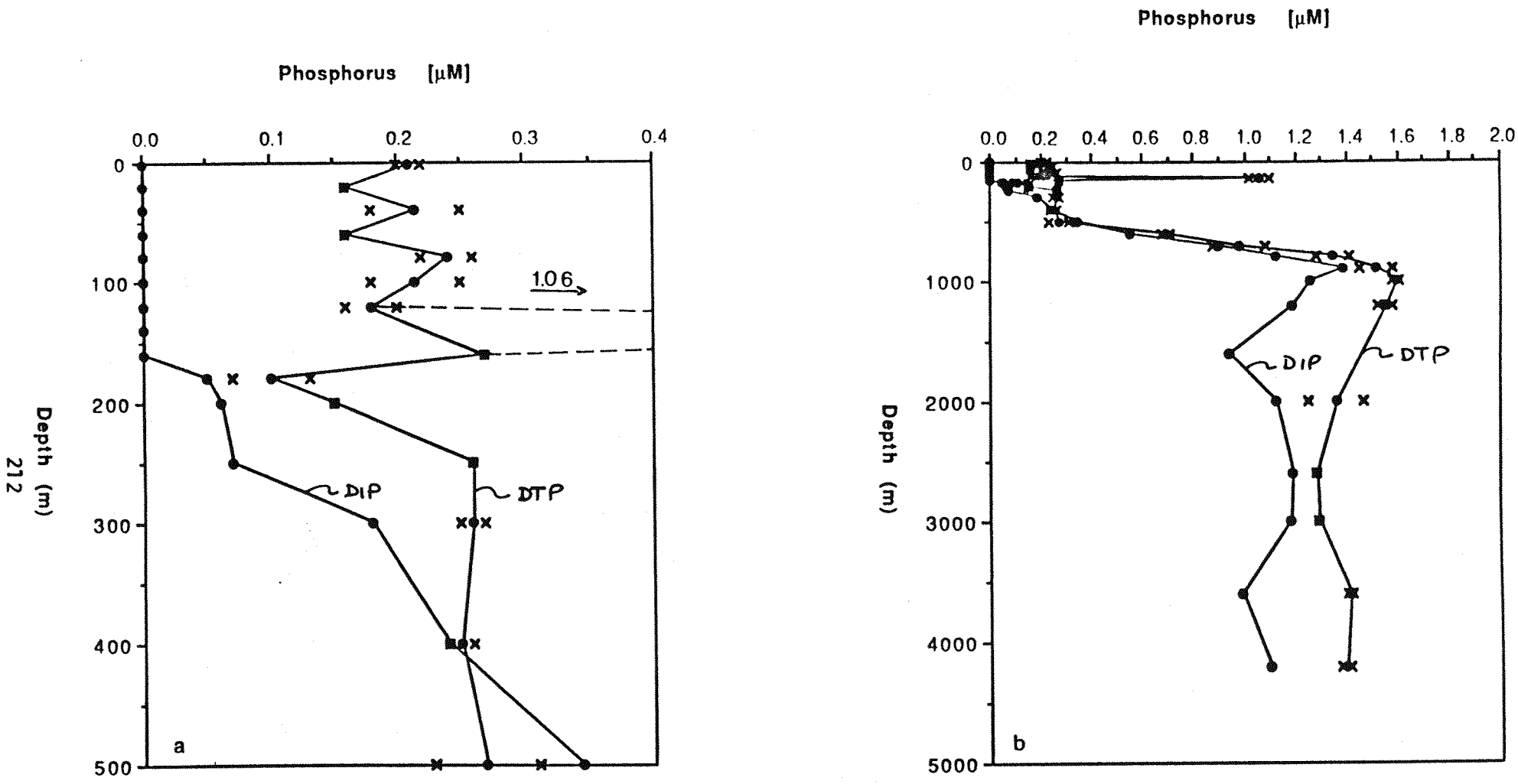


Fig. 4.20 Vertical distribution of DTP (analyzed by Nit-ox technique) and DIP. Sargasso Sea. Station "S". Feb.90. a) 0-500m, and b) 0-4200m. Dashed lines between 120 and 160m indicate that the high value of DOP at 140m was due to comtamination. Unit of the value inserted is in μM.

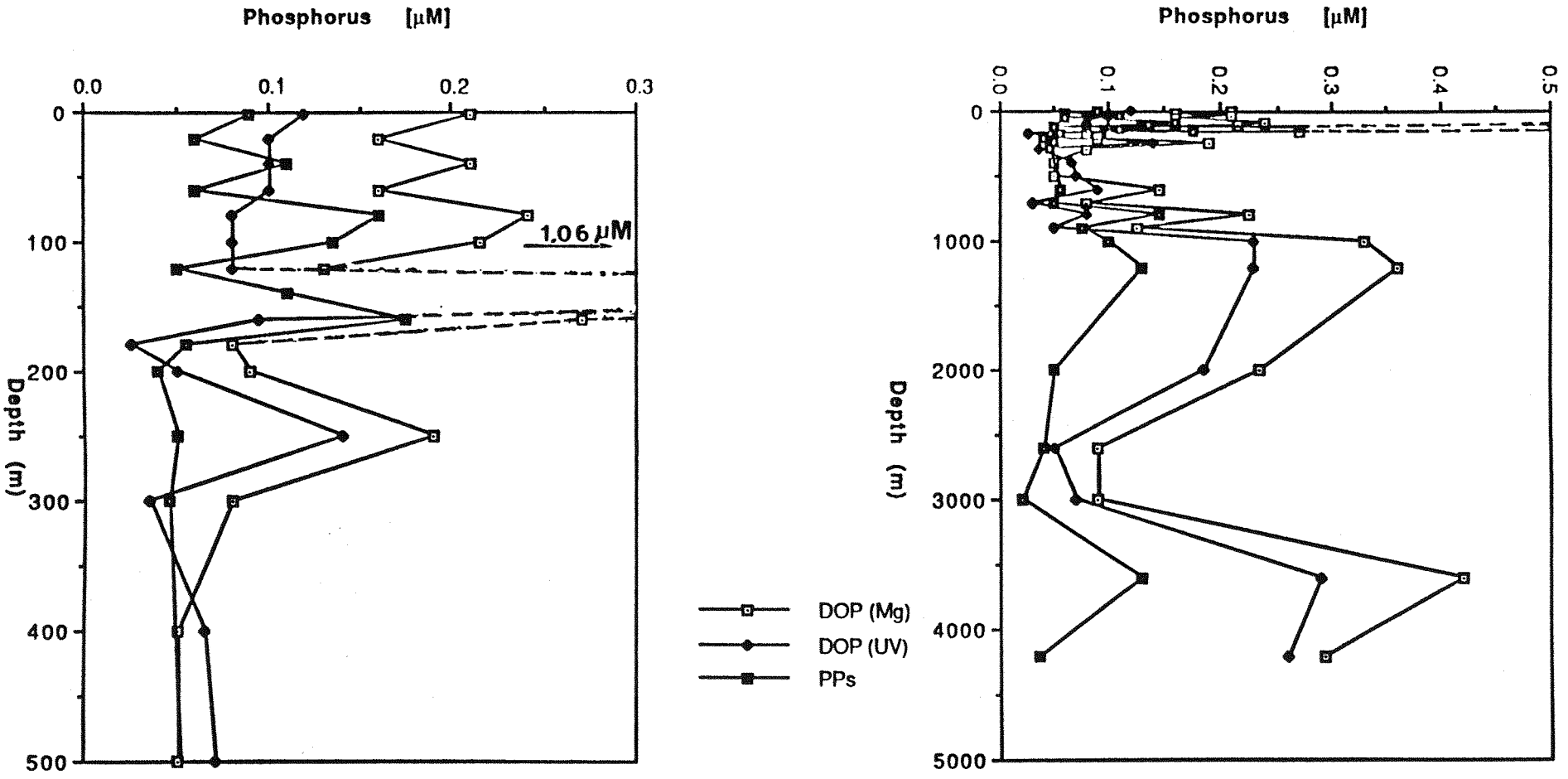


Fig. 4.21 Vertical distribution of DOP determined by UV-ox and Nit-ox techniques, also PPs data is plotted. Sargasso Sea. Station "S". a)0-500m, and b) 0-4200m. Plotted values are averages of duplicate analysis, see Appendices 4s for data.

4.4 Discussion

4.4.1 The phosphorus pools in the euphotic layer

4.4.1.1 Dissolved inorganic phosphate

In this work, the first ever report of nanomolar levels of DIP in oceanic waters is presented. Analytical difficulties have previously preclude scientists from obtaining a clear insight into the behaviour of DIP at these levels. The presented data can be regarded as representative of the DIP concentrations in the water column, because good replicates and an oceanographically consistent vertical pattern were observed. Nonetheless, the question of whether or not DIP (also called soluble reactive phosphate), as measured includes labile short chain poly-phosphates (e.g. Burton 1973, Peters and Bergmann 1982) remains unequivocally unanswered. However, it can be inferred from some points of the presented profiles, in which the DIP was below the detection limit, that the potentially reactive labile poly-phosphates would also have to be below the detection limit of the LCC technique.

Another potential drawback associated with the DIP measurement used here is about the existence of colloidal P, i.e. molecules with a size ranging from about 200 nm to 5 nm (Williams 1986) which could be labile and so reactive to the analytical approach. This colloidal phase is mainly organic and could represent up to 15 % of the actual dissolved organic phosphorus in surface waters (Williams 1986, Moran and Moore 1989). As Nuclepore filters with a 0.45 μm nominal pore size have been used in this work, the possibility of having measured some labile-organic P cannot be categorically discounted, although again this fraction would have had to have been below or at the detection limit of the LCC technique.

A further point to consider in the DIP analysis is the potential positive interference from hydrogen sulphide, silicate and arsenate. The first interferent occurs only in significantly anoxic waters, the other two have a kinetic reaction of formation or reduction or both of molybdo-

arsenic and -silicic acid slower than the molybdo-phosphoric acid counterpart (Chamberlain and Shapiro 1969, Koroleff 1983). The potential inference from arsenate was, in any case, examined during the development of the LCC method and *in situ* (i.e. with North SEA and Sargasso Sea water samples, see Section 2.4). Overall, it can therefore be said with a good margin of confidence that the data presented is accurate and thus representative of naturally occurring levels.

The upper water column vertical DIP profiles at nanomolar concentrations proved to be very dynamic, changing in a time scale of hours to weeks. With some profiles concentrations were below detection limit (1-2 nM), thus giving conclusive evidence that plankton communities can spend energy to actively assimilate these minute amounts of DIP (see Ormaza-González *et al.* 1990e). This point is further corroborated by the DIP uptake experiments.

Overall, the vertical distribution of DIP in the investigated euphotic layer (0-120m) can be divided into three clear sub-layers or zones.

Top sub-layer: Between 0-30m, there were higher levels of DIP than in lower layers (30-120 or <140m). Concentrations ranged from about 10-20 nM at the top (Fig. 4.7), to below detection limit at about 30m. These relatively higher concentration are probably due to the combined action of:

i) phytoplankton activity is partially or completely photo-inhibited by high doses of sun-light close to the surface (e.g. Ryther 1956, Parsons *et al.* 1984), resulting in slow uptake rates. ii) Atmospheric input by dry and/or wet deposition of atmospheric P which could account for fluxes of up to 33-88 $nM.m^{-2}.d^{-1}$ (Duce 1986).

iii) Regeneration of organic P into DIP through enzymatic action of phosphatase (Perry 1972, Siuda 1984), 5'-nucleotidase (Ammerman and Azam 1985), and also possible decomposition of organic P by ultraviolet sun-light (Cullen and MacIntyre 1990).

Intermediate sub-layer: In this sub-layer (30-100m) in which most of the photosynthetic processes take place, DIP levels were generally undetectable (< 1-2 nM). The change of depth of the minimum DIP on a time scale of hours (Fig. 4.7-4.10), reflects the fact that assimilation and regeneration processes were occurring simultaneously. The intensity of both processes has been found to vary with regeneration rates sometimes being higher than those for assimilation, or *vice versa* (Williams *et al.* 1983, Harrison and Harris 1986), but on a large time scale they balance (Harrison and Harris 1986).

During the third cruise the whole euphotic layer was depleted of DIP, i.e. DIP was constantly below detection limit, but in the fourth one (a week later) DIP ranged between 10-20 nM. It is reasonable to suggest that a bloom was occurring in the third cruise, which had crashed a week later. This is supported by data on Chl-*a*, POC and PON, and other nutrients information presented in the appendixes 4.2 - 4.9.

Bottom sub-layer: This sub-layer is the interface between the depleted DIP sub-layer and the top of the phosphocline. It proved to be very mobile and seemed to be affected by both biological (uptake and regeneration) and physical factors. The depth of this sub-layer was controlled by physical mixing processes and it generally was between 100-120m, but 70-90m, and 140-150m depth for the third and fourth cruises respectively. Thus, it was coinciding with the depth of the mixed layer (see Fig. 4.3 and 4.4). A diurnal change was noticed for the first and fourth cruises, which occurred in relatively short time scales. These changes were primarily ascribed to internal waves, that in turn are due to density anomalies which are generally found in the thermocline zone. Internal waves have a wide range of periods, varying from tidal to 20 min or so (Sverdrup *et al.* 1942, Neumann and Pierson 1966). However, turbulent motion and convective circulation could also have been a cause. It is not possible to numerically demonstrate these processes because the lack of detailed information on salinity, temperature and associated parameters such as potential temperature, density etc. Nonetheless small changes of the density surface depths can be observed in the T/S-depth diagrams (Fig 4.5). Changes of DIP

along density surfaces has also been reported by Hulburt (1990). Eppley and Renger (1988) have found that short and moderate meteorological events could enhance the turbulent eddy diffusion, which in turn perturb the top of the nutricline and the phosphate distribution. Vertical upward diffusion also contributes to the observed changes (see below).

Uptake and regeneration processes are certainly occurring at depths in which the sun-light radiation is at or below 1 % of that in the surface. In the Sargasso sea that depth is at or below 120m. Uptake of P and other nutrients could take place in the absence of light (Monheimer 1974, Russel *et al.* 1983), especially at low concentrations of P (Perry and Eppley 1981). In oligotrophic oceans, a second maximum of Chl-*a* has persistently been reported between 90-135 m depth, i.e. just above the nutricline of the Sargasso sea (Menzel and Ryther 1966) and the North Pacific ocean (Venrick 1982, Shulenberg and Reid 1981). Furthermore, Lewis *et al.* (1987) have reported that about 1/3 of the new production, i.e. photosynthetic processes involving nutrients which have been introduced to the euphotic layer rather than recycled within (Dugdale and Goering 1967, and Eppley and Peterson 1979), occurs below the euphotic zone (125 m). This would represent nearly 2-6 % of the total net production, because new production in oligotrophic seas can account for between 6 % (Eppley and Peterson 1979) and 13-25 % (Pace *et al.* 1987, Knauer 1988) of the net production. Net production at station "S" varies from 200 to 840 $mg[C].m^{-2}.d^{-1}$ (Lohrenz *et al.* 1990); taking an average of 400 $mg[C].m^{-2}.d^{-1}$, applying a Redfield ratio C:P of 106:1, and assuming a new production of 3 % of the net carbon production, uptake of P by hetero- and auto-trophic micro-organisms would be about 10 $\mu mol[P].m^{-2}.d^{-1}$. However taking the measured C:P ratio of 35:1 (see ahead) uptake rates in this sub-layer should be above 30 $\mu mol[P].m^{-2}.d^{-1}$. The later uptake rate would be similar to the measured and inferred diffusion rates, thus implying that the potentially new DIP which is not regenerated is trapped in this sub-layer.

Regeneration of DOP into DIP also contributes to the cycling of P in this sub-layer, and it can principally be attributed to heterotrophic communities. Regeneration rates between 125-170m depth varied from 1 to 0.060

nM[P].h^{-1} (Fig. 4.17), which represents the regeneration of DOP into DIP of more than 50 μmol.m^{-2}.h^{-1} in this sub-layer. The APA regeneration would surpass the corresponding uptake rates and in addition other enzymes (e.g. 5'-nucleotidase) are likely to be active in DOP to DIP transformation in this layer. Thus DIP will tend to be accumulated in this sub-layer.

The vertical profiles demonstrate that DIP at nanomolar levels is very dynamic and governed by a combination of physical, chemical and biological processes. The distinct sub-layers described above is a new and important fact, because this gives evidence that 1) the mixed layer is not actually completely mixed in terms of DIP and perhaps other nutrients (see Glover *et al.* 1988) and trace elements (Dr. P. Statham, pers. comm.), 2) different processes in each sub-layer are dominated by one of the driving processes, i.e. physical, physico-chemical, bio-chemical and biological. These facts would suggest new or different approaches to contemplate research in this layer. It should be mentioned that existence of two sub-layers has been already suggested by Jenkins and Goldman 1985.

4.4.1.2 Temporal variability of DIP

The observed diurnal changes in the integrated vertical profiles of DIP allow the estimation of the net removal of DIP on the time scale of hours for the first cruise. Removal or net uptake rates were comparable to those obtained by incubation experiments (see below). Removal rates integrated over the euphotic layer for the first and second cruises ranged up to about 1000 and 450 μmol[P].m^{-2}.d^{-1} respectively. Applying the Redfield ratio (C:P of 106:1) to the P removal rate for the first cruise would imply a carbon fixation 3-6 times higher than that estimated *in situ* during the same cruise. However, when a C:P of 24:1 (see Table 4.5) is used, which is the value actually measured in suspended particles during the cruise, the inferred carbon production is 129-288 mg[C].m^{-2}.d^{-1} which is comparable to that measured by the C-14 technique (see below). It should be noted that the latter carbon production rate deduced from the the P removal, could be under-estimated by up to a factor of 2 because of recycling processes (Harrison and Harris 1986). Thus, if these results

were close to reality, it would indicate that the C-14 technique is underestimating carbon production, thus corroborating what has been suggested by many scientists (e.g. Eppley 1980, Romankevich 1984, etc). In any case this example proves the usefulness of the LCC technique to estimate photosynthetic rates *in situ*, i.e. without using any water sample incubation procedure.

The measured range of DIP levels cannot directly be discussed or compared to other elements (e.g. Cadmium) because of the lack of data taken simultaneously. However, these DIP levels can be compared to previously reported data on nitrate in the Sargasso Sea (Garside 1985 and Glover *et al.* 1988) with appropriate caution as the samples were not collected at the same time. Reported levels of nitrate varied from the detection limit of the technique (2 nM, Garside 1982) to >100 nM[N], with vertical distributions similar to the DIP profiles. Garside (1985) quoted a DIN budget of 1.8-0.2, $mmol.m^{-2}$ from 0 to 30m depth during summer, the corresponding DIP budget ranged from 0.025 to 0.694 $mmol.m^{-2}$ which is a N:P of 8-3:1. With the measured nitrate concentrations during the cruises, the N:P ratios were 3, 35, 96 and 2 for the first, second, third and fourth cruises respectively (Table 4.5).

Crs.	Si	DIN	DIP	PTP	PON	POC	O_2	Chl-a	Phaeo
	mmol.m^{-2}						mol.m^{-2}	mg.m^{-2}	
1	102	2.66	0.87	6.82	21.8	164	56.2	8.95	17.2
2	88.6	16.2	0.46	2.83	21.1	160	55.2	11.5	19.5
3	87.9	9.64	<0.1	1.35	28.2	301	55.6	12.5	16.9
4	109	2.46	1.50	1.16	27.4	225	56.5	12.2	20.9

Tab. 4.4 Pools of nutrients and Chl-a and Phaeopigments (Phaeo) in the 120m upper mixed layer of the Sargasso Sea. Station "S". Crs: Cruises. See text for details.

Cruises	POC:PTP	POC:PON	DIN:DIP	Si:DIP
1	24	3	3	117
2	57	7	35	170
3	232	22	96	869
4	202	24	2	72

Tab. 4.5 The ratios of particulate P, N and C, and dissolved inorganic N, P, and Si in the 120m upper mixed layer of the Sargasso Sea. Station "S".

4.4.1.3 Dissolved organic phosphorus

The concentrations and vertical distribution of DOP (Fig. 4.12 and 4.13) in this part of the Sargasso Sea are reported for the first time here. As with the DIP, they also showed diurnal and monthly variations, although the changes were insignificant relative to the overall DOP budget in the euphotic layer. Concentrations varied from about 100 to > 200 nM. Vertical patterns were patchy at the top 80m, but between 80 and 150-180m depth there was an increase that is explained in terms of the presence of poly-phosphates (PPs) which are exuded by moribund or dead cells.

The use of the term dissolved organic phosphorus (DOP) poses analogous questions as to the precise meaning of the analytically defined DIP. For instance, the presence of colloidal organic compounds could add uncertainty; such materials have been detected in natural waters (e.g. Burnisson 1983, Matsuda *et al.* 1985) and could account for up to 15 % of the true DOP (Williams 1986). Thus the word "dissolved" is probably not the most appropriate term to described the organic-P passing through filters of 0.70-0.45 μm nominal pore diameter. Nevertheless, and given the above caveat, this term will be used hereafter to define organic-P passing 0.45 pore diameter filters used in this study.

Additionally the uncertainties relative to the accuracy of the analytical technique in measuring naturally occurring organic-P compounds have not yet been ascertained unequivocally despite the effort made during the assessment of the various methods (see Section 2.5). This uncertainty on accuracy is not only concerned with DOP, but also with DOC and DON measurements, and poses a question mark amongst marine scientists (see Jackson 1988, Williams and Druffel 1988). With the use of new techniques for DON (Suzuki *et al.* 1985) and DOC (Sugimura and Suzuki 1988, see also Skopintsev *et al.* 1966) and reports of "high" concentrations this question of accuracy

has again been brought into the limelight. Skopintsev and Timoteyeva (1960) and Skopintsev *et al.* (1966) reported the first high values of DOC near the station "S", which ranged from 142 μM in the surface to 116 μM at 1534m. These values were recently largely corroborated by Bauer *et al.* (1990), who reported a range of concentrations of 140 μM in surface and 96 μM in deep waters (4000m). Even higher values are now being reported for other oceanic provinces, for instance 240-280 μM in the North Pacific (Suzuki and Peltzer 1990). If these values are true, and the Redfield ratio (106:1) is consistent in the organic phase (see chapter 1, page 8), then levels of DOP in the Sargasso Sea should range from 1.3 to 0.91 μM. However, the observed range (0.1-0.25 μM) is far too low. The values reported here are double those measured with the UV irradiation technique, which is the technique that has mostly been used to determined DOP in oceanic waters. Nonetheless, the levels of DOP still only represent < 25 % of the expected values based on Redfield ratios. It is difficult to reconcile a difference of 75 % in terms of analytical procedures *per se*, and thus it is demonstrated that the application of the Redfield ratios for dissolved organic phases is not correct.

The range of concentrations of DOP presented here are similar to those reported for the oligotrophic Central North Pacific Ocean (Romankevich 1984, Smith *et al.* 1986, Orret and Karl 1987, Saponizhkov 1981 and 1988, Walsh 1989). The data for the Pacific was obtained using the UV technique that in this work has given nearly half of those obtained by the Mg nitrate procedure; this would imply that naturally occurring DOP values for the Pacific may be higher than reported, because DOC (180-320 μM) and DON (about 40 μM) levels are higher than in the Atlantic (Sugimura and Suzuki 1988, Suzuki and Peltzer 1990; however see Walsh 1989). The higher DOC concentrations in the Pacific relative to the Atlantic may reflect the higher primary production rates in the Pacific (1.8 times greater than the Atlantic, Hinga 1985).

Assuming the DOP analysis is accurate, an explanation of the lower than expected DOP levels could be proposed in terms

of: 1) rigorous application of the Redfield ratio not being appropriate at all in organic phases, and 2) regeneration and production rates for DOP being higher than those for DON and DOC, 3) the water column having a deficit of P in the euphotic layer, and 4) DOP losses occurring during sampling, storage and analysis_procedures.

The first reason is very likely to occur, because the Redfield ratio, which was inferred in order to satisfy the stoichiometric relationship;

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+ \rightleftharpoons C_{102}H_{263}O_{110}N_{16}P + 138O_2$$

It should noticed that the oxidation of organic matter as suggested in this equation may not go to completion in surface waters.

The ratios (C:N:P of 106:16:1) are representative of the average marine biomass as a whole (Redfield *et al.* 1963). However, according to the same authors the average availability of C, N, and P to biota in marine waters are related in an atomic ratio of 1017:15:1 (C:N:P). Interestingly, when the PTP and DOP budgets in the euphotic layer are combined to give 20000 μmol.m^{-2} and the respective DOC (from Bauer *et al.* 1990) and POC (GOFS 1990) data are similarly treated to give 18240 mmol.m^{-2}, the ratio will be 912:1 that is comparable to the above C:P ratio. Lutsarev *et al.* (1977) reported a ratio of 360-547:1 in the North Pacific, but these authors used the UV oxidation technique and the DOC concentrations were 30-50 % more of recently reported levels).

The corresponding budget for nitrogen in this layer, where the combined DON and PON should be at concentrations of *circa* 40 μM in the photic layer (taking data from Sugimura and Suzuki 1988) would be about 4500 mmol[N].m^{-2}, i.e. the ratio C:N would be 240:1. But, if DON concentrations in the oligotrophic North Pacific are 5-6 μM suggested by Walsh (1989), who does not accept Sugimura and Suzuki's data, and

assuming that in the Sargasso Sea DON concentrations are about half that in the Pacific Ocean (PON load are in the order of 0.2-0.3 μM, see Appendices 4.9a and 4.9b) because carbon fixation rates are 1.8 higher in the Pacific (Hinga 1981). Then, the N:C ratio would be 13:1. It should be mentioned that the method employed by Walsh (1989) treated the sample under high temperature (>1000^{0}C) and ultra-pure oxygen conditions. In these conditions probably all organic compounds would be oxidized.

Furthermore, it should be pointed out that Redfield and coworkers did not make clear whether the ratios 1017:15:1 (C:N:P) referred to the total mass of these elements or only the inorganic organic phase. In any case, in here it is assumed that dissolved and particulate organic C, N and P are potentially available to plankton population.

Based on the arguments in the above paragraphs, it appears that the measured DOP values presented here are accurate. However, possible inaccuracies due to the storage procedure have not been assessed in detail because it is difficult to carry out the analysis at sea; problems have been reported for the storage of DOC samples (Sugimura and Suzuki 1988). Problems with the sampling procedures, i.e. contamination or loss of DOP, were not anticipated, but because of logistical constraints it was not possible to examine this factor. A further consideration in looking at the data, is that the Redfield ratio 106:15:1 refers exclusively to the average organic composition of planktonic material only, and thus it cannot be used universally.

All the DOP profiles but one (Fig. 4.12b) showed a slight increase in concentration below 100-180m, followed by a sudden decrease to lower deep water values. These features can be explained in terms of 1) exudation of DOP from the particulate organic matter, and 2) production of PTP and DOP due to nano- and pico-plankton activity, which has been reported in oligotrophic seas (Smith *et al.* 1984, Platt *et al.* 1984). Such organisms are able to produce at least 2 % of the

new production at depths below 125 m (Lewis *et al.* 1987). Oxidation of DOP started to be relevant below 150m depth, thus coinciding with the sharp DIP gradient (See Section 4.8).

4.4.1.4 Dissolved poly-phosphates and particulate total phosphorus

Production of DOP from PTP could be observed in the PTP profiles, especially in the first and second cruises, which showed a sharp decrease of PTP at about 120-150m. POC and PON profiles had similar behaviour (Appendices 4.8-4.9). It has been suggested that moribund or collapsing cell will mainly exude PPs (Solórzano and Strickland 1968), which is part of the measured DOP. This was investigated by determining DOP by UV and Mg nitrate oxidation (DOP_{UV} and DOP respectively) analytical procedures. The difference between the determinations provides an empirical estimation of dissolved poly-, meta-phosphates and also any UV-irradiation refractory organic-P (referred here as PPs only, see Section 2.4). The dissolved PPs (Fig 4.16) proved to be an important fraction of the total DOP, averaging 0.10 μM, i.e. about 50 % of the DOP. Similar fractions have been reported in coastal waters (e.g. Armstrong and Tibbits 1968, Solórzano 1978) where they represented up to 50 % of the total DOP. Solórzano and Strickland (1968) have suggested that PPs are present in the dissolved phase when intense blooms collapse. Polyphosphates are accumulated by cells as a P reserve for when environmental DIP is not available (Watanabe 1988, Watanabe *et al.* 1989) and used by them to, for example, synthesize ATP (Rubtsov and Kulai 1977). The idea of Solórzano and Strickland can be supported by the PPs vertical profiles (Fig. 4.16) pattern, in which an increase of PPs concentration towards the bottom of the euphotic layer can be observed. The increase of PPs coincides with the rapid drop in PTP concentration (from about 70 to 3 nM). Overall, concentrations of PPs did not markedly vary over the three weeks.

It is interesting to note that the reported content of PPs in particulate matter in oligotrophic seas ranges between 20

and 50 % of the PTP load (Perry 1976, see also Section 2.5) and this percentage is very variable in relation to dissolved inorganic P, Mn, Fe levels (Watanabe *et al.* 1989). This would indicate that the difference between DOP_{UV} and DOP is mainly due to PPs. In oligotrophic waters plankton populations are likely to have more efficient adapted mechanisms to assimilate and store very quickly minutes amounts of DIP and trace elements from the water column, than those from upwelled or coastal waters.

The fluctuations found for PTP loads (3-70 nM) are also reflected in the POC concentrations which range from 25 to 3.3 μM (Skopintsev *et al.* 1966, Menzel and Ryther 1966, GOFS 1990). The only PTP previously reported was 87 nM (Harrison and Harris 1986).

4.4.1.5 Phosphorus limiting conditions in the Sargasso Sea

The concentrations of PTP in relation to PON and POC can be considered as an indicator of the physiological state of micro-plankton cells. According to Redfield *et al.* (1963), the average C:P and N:P ratios on plankton cell is about 105:1 and 15:1 respectively (for different ratios see Takahashi *et al.* 1985), but low P:C and N:C ratios (47:1 and 5.6:1 respectively) indicate the presence of healthy or normal cells, whilst high ratios (231:1 and 31:1) implies P-starved cells. This has subsequently been confirmed by, for instance, Rhee (1978) and Vadstein and Olsen (1989). The variable pattern of the P:C and N:C ratios for the measured PTP, POC and PON (Table 4.4 and 4.5), suggests that in the first cruise the plankton community was in a healthy state, (C:P and N:P of 24:1 and 3:1). By the second cruise (a month later) the ratios increased to 57:1 and 7:1 respectively, clearly indicating that micro-plankton (hetero- and auto-trophic) had utilized the intracellular pool of P (presumably present as PPs) to increase fixation of C and N. The carbon fixation in February (0.5 $g[C].m^{-2}.d^{-1}$) was nearly two times higher than in January, and it was also observed that DIP budgets for February were lower than in January with a ratio DIN:DIP of

35:1. By the third cruise (a week later) PTP was even lower, with C:P and N:P ratios of 232:1 and 22:1, i.e. below Redfield ratios, thus indicating cells were P-starved. DIP was undetectable (<1-2 nM) throughout the upper water column with a DIN:DIP ratio of 96:1 and DOP was slightly lower than the previous cruise. For the fourth cruise the PTP budget was similar to the third, but whilst POC fell by 1/3, PON did not. The C:P and N:P ratios were similar to the previous one. On the other hand, DIP increased by a factor of 12-15, and DOP was 20 % less than the preceding budget, making a ratio (DIN:DIP) of 2:1. Taking into consideration Chl-*a* (Appendices 4.6a and 4.6b), this was steadily increasing from the first to third cruise (8.9 to 12.5 $mg.m^{-2}$), then slightly decreasing in the fourth cruise (12.2 $mg.m^{-2}$). Degraded Chl-*a* (phaeopigments) increased by 22 % from the third to the fourth cruise. These facts support information and P data together lead to the hypothesis that a bloom (or simply primary production) started to develop by January in the second week of February it was more intense, reaching a peak a week later. By the end of February either enzymes were activated to begin conversion of DOP to DIP and thus the bloom further develops, or it collapses with subsequent increase in bacterial activity. Tentatively, the first possibility seemed to have occurred, because oxygen content increased from 55.6 in the third to 56.5 $mol.m^{-1}$ in the fourth cruise. However, this increase could have been caused by aeration of the mixed layer due to consistent and high speed winds during the second fortnight of February, that deepened and oxygenated the mixed layer and consequently disguised any loss of oxygen due to bacterial utilization and chemical oxidation. On the other hand, the perceptible decrease of Chl-*a* associated with the increase of silicate (25 %) and phaeopigment (22 %) from the third to the fourth cruise (Appendixes 4.4 and 4.7) would further sustain the second possibility, i.e. the bloom collapsed.

If the bloom collapse idea is true, DIP would be the limiting nutrient and not nitrate as has constantly been suggested in the literature (e.g. Perry and Eppley 1981, Platt

et al. 1989). In the third and fourth cruises nitrate was measurable and even increased during the latter cruise supporting the DIP limitation theory. The ratios of Si:P were 869:1 and 72:1 respectively; a ratio above 80 is regarded as indicative of P limitation (Sommer 1983). Yet other evidence of P limitation is the presence of APA (see below) and the ratios of APA:Chl-*a* (Fig 4.22), which, for oligotrophic and eutrophic waters, have been reported to range between 0.49-1.8 and 0.0002-0.008 $nM.dm^3.(\mu g.h)^{-1}$ (Perry 1972 and Kobori and Taga 1977 respectively). The ratios here ranged from 0.41 to 0.62 $nM.dm^3.(\mu g.h)^{-1}$, with an average about 0.50, thus falling within the previous range reported for an oligotrophic ocean. These ratios also indicated P-limitation.

Although P (as DIP or DOP, see below) was shown to be the limiting nutrient, it should be stressed that the collapse of a bloom cannot solely be ascribed to nutrient limitation but also combined lack of other important elements such as Mn, Fe, Zn (e.g. Martin and Fitzwater 1988, Watanabe *et al.* 1989). Limitation of productivity by P has been suggested for isolated or confined water bodies such as lakes or bays (Smith and Atkinson 1984 see Introduction), and from one point of view oligotrophic seas can be regarded as an immense confined water body.

The limiting characteristic of P has been shown, but the question whether DOP or DIP was the limiting form is open. Speculating, DOP could have been the limiting P fraction rather than nitrate if the potentially available or enzymatically labile fraction of DOP was depleted. This hypothesis would be valid if the rates of converting labile DOP into DIP are higher than those of producing this labile fraction. According to literature information production rates of DOP are similar to those APA regeneration. But there is not information on production rates of labile DOP.

To summarize, the behaviour of phosphorus and others elements and biological pools during the investigated period strongly suggests that 1) nanomolar concentration of DIP are

assimilated by plankton micro-population very quickly, and then stored intracellularly to be utilized in accordance to future needs. 2) Phosphate is the limiting nutrient, at the time and under the circumstances of this study.

4.4.2 Phosphorus cycling processes in the euphotic and mixed layers

4.4.2.1 Regeneration of DIP from DOP by alkaline phosphatase activity

The presence of APA and low (<80 nM) or previously "undetectable" DIP concentrations (< 30 nM) have been suggested as indices of P limitation (Perry 1972, 1976, Siuda 1984, Veldhius and Admiraal 1987, Veldhius *et al.* 1987, Boni *et al.* 1989, etc). When APA was assayed, DIP levels were at 5-20 nM and plankton cells appeared to be P-starved because both particulate C:P and N:P ratios were high (202:1 and 24:1). These conditions are thus strengthening the argument for P limitation.

The APA ranges and vertical pattern (Fig. 4.17 a-c) presented are in accordance with those previously reported in the oligotrophic North Pacific Ocean (Perry 1972). The three APA vertical profiles (Fig 4.17) taken at 8 hourly intervals give strong indication that 1) APA does not significantly change over 24 h, and regeneration of DOP into DIP rates were up to 3.8 $nM.h^{-1}$, 2) its origin was probably shared between small size fraction auto- and hetero-trophic communities, with perhaps some fraction in the dissolved phase, and 3) enzymatic activity on phospho-monoesters, -diesters and short linear chains of PPs contribute the main bulk of the P required for particulate organic matter production.

Due to the work load, it was not possible to measure APA in all the cruises, and thus ascertain if APA increases with concentrations lower than 1-2 nM. However the presented data corroborates that phosphatase enzymes are active when cells

are P-starved, i.e. without PPs reserves, as suggested by Myklestad and Sakshang (1983), and Vargo and Shanley (1985). Applying this idea, it can be inferred that APA was present during the third cruise, with perhaps higher DIP regeneration rates than 3.8 $nM.h^{-1}$, because the particulate C:P and dissolved inorganic N:P ratios were even higher than the fourth cruise, i.e. 232 and 96 respectively (Table 4.4). This is possible because higher rates (7.8 $nM.h^{-1}$) were reported in the oligotrophic North Atlantic by Perry (1972), and even rates up to 21 $nM.h^{-1}$ have been found in the Southern part of the North Sea (Davies and Smith 1988).

The origin of the observed alkaline phosphatase enzyme is considered to be associated with the nano-size and smaller hetero- or/and auto-trophic populations, whose distribution in the water column is greater at the top of the euphotic layer, with decreasing numbers and increasing size with depth (Davies and Smith 1988). At the bottom of the euphotic zone bigger and autotrophic plankton are present as a constantly present peak of Chl-*a* is found (Appendices 4.6a and 4.6b). Thus, by comparison with the APA profiles, there is an indication that the main producers of the enzyme should be between 20-80m depth. This criterion is supported by the magnitude of the ratios APA/Chl-*a* and their relationship (Fig. 4.22). The ratios varied from 0.41 to 0.62 $nM.h^{-1}.\mu g^{-1}.dm^3$ (0-120m), which corresponded to the maximum and minimum respectively of Chl-*a*. The ratio was well correlated to Chl-*a* with a r^2 of 0.740, thus supporting the idea that APA was produce by auto-trophic micro-organisms. Furthermore, the vertical distribution of APA indicates a peak between 20-50m depth, suggesting that the enzyme was related to highest autotrophic activity, because the greatest carbon production was found in this layer (M. Tuel, pers. comm.)

Whether or not the APA was generated by auto- or/and hetero-trophic organisms was investigated by studying the inhibition of the enzyme by DIP. It has widely been found that APA, produced by autotrophic organisms, is immediately suppressed when DIP is added to give equal to or higher

concentrations than naturally occurring DIP levels (Perry 1972, Veldhuis and Admiraal 1987, Davies and Smith 1988). However, APA generated by bacteria and fungi is not regulated by DIP concentration (Forsberg and Chang 1980, Alvarez *et al.* 1982, Halamejko and Chrost 1984), and this appeared to be the case in this study, as the APA in the two sets of samples (Fig. 4.23, only one set shown) from the surface (1-2m) did not show a distinct inhibition with a series of DIP spikes (see Section 4.2), which increased natural concentrations by several times. Therefore, it can be argued that between 30-50 % of the detected APA was produced heterotrophically, because 1) rates at the surface were 30-50 % relative to the maximum APA, and moreover 2) surface rates were the same as those at 130-150m depth, where high bacterial activity is expected and increases in phaeopigments concentrations inferred bacterial activity. This heterotrophically produced APA is another indication that a bloom had partially or mostly crashed by the end of February because bacterial activity is enhanced afterwards a bloom collapses (see Iriarte *et al.* 1990).

4.4.2.2 Removal of DIP from the water column by photosynthetic processes

Uptake rates of P and carbon fixation by populations of microorganisms in natural waters have traditionally been estimated using radio tracers techniques. Although information obtained has been used to interpret the major cycles of these elements in natural waters, the use of these techniques and interpretation of data have resulted in a widely argumentative and profuse literature (Eppley 1980, Grande *et al.* 1989, Platt *et al.* 1989). For instance, operational problems of the techniques were related to e.g. contamination from bottles (especially glass, Fitzwater *et al.* 1982), confinement effect on micro-organisms (Williams *et al.* 1983), and filtration damage to cells (Goldman and Dennet 1982). Additionally, the assumption that the tracer only moves from dissolved inorganic to particulate phases, and that there is negligible recycling during experiments (which generally last 12-24 hours), has been questioned because cycling processes are actively

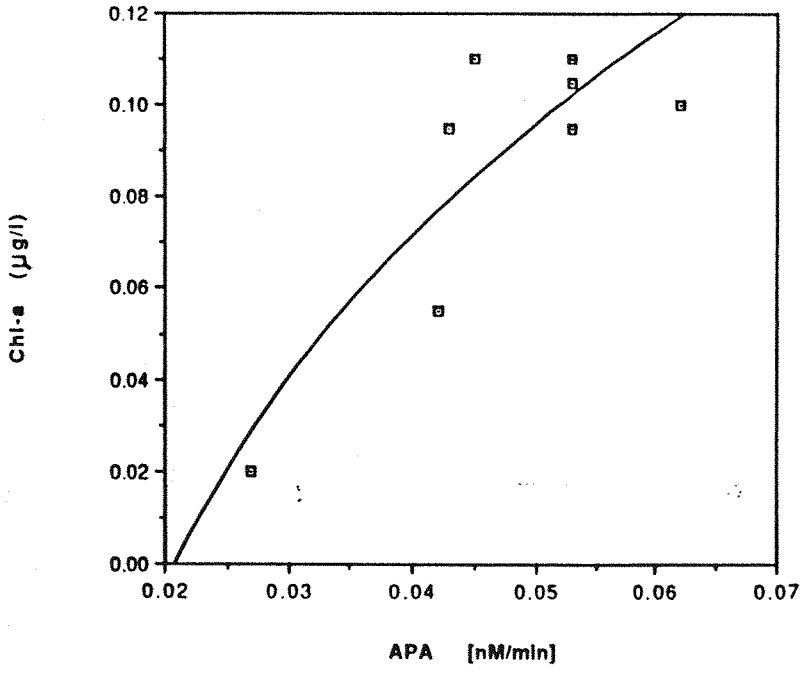


Fig. 4.22 The relationship between APA and Chl-*a*. Sargasso Sea. Station "S". 28.Feb.90. The regression coefficient (r^2) is 0.740.
Data for Chl-a were taken from Appendix 4.6 d.

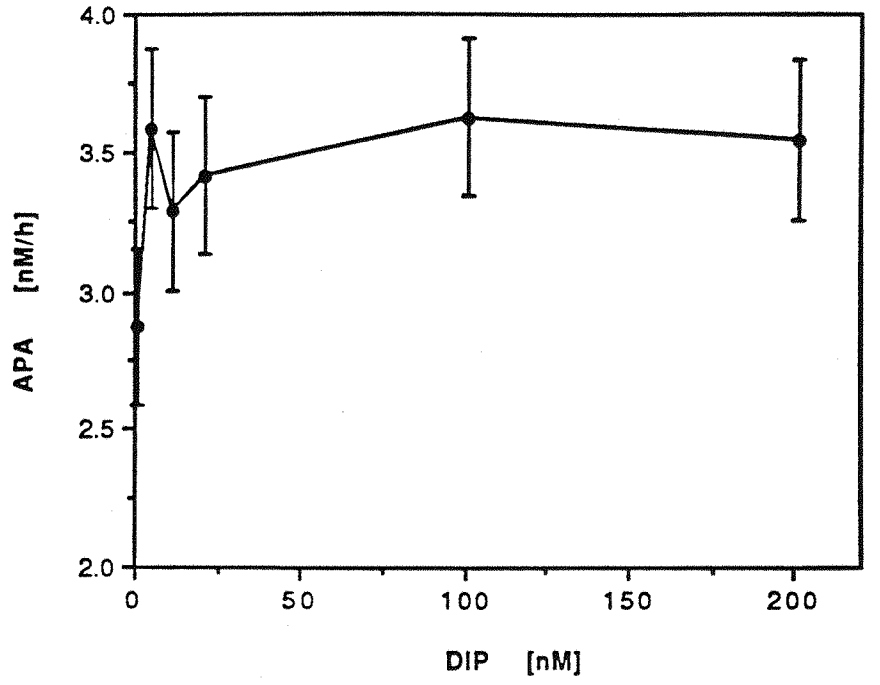


Fig. 4.23 Dissolved inorganic phosphate effect on the APA rates. Analysis by triplicate. Sargasso Sea. Station "S". 28.Feb.90. Note the APA axis offset

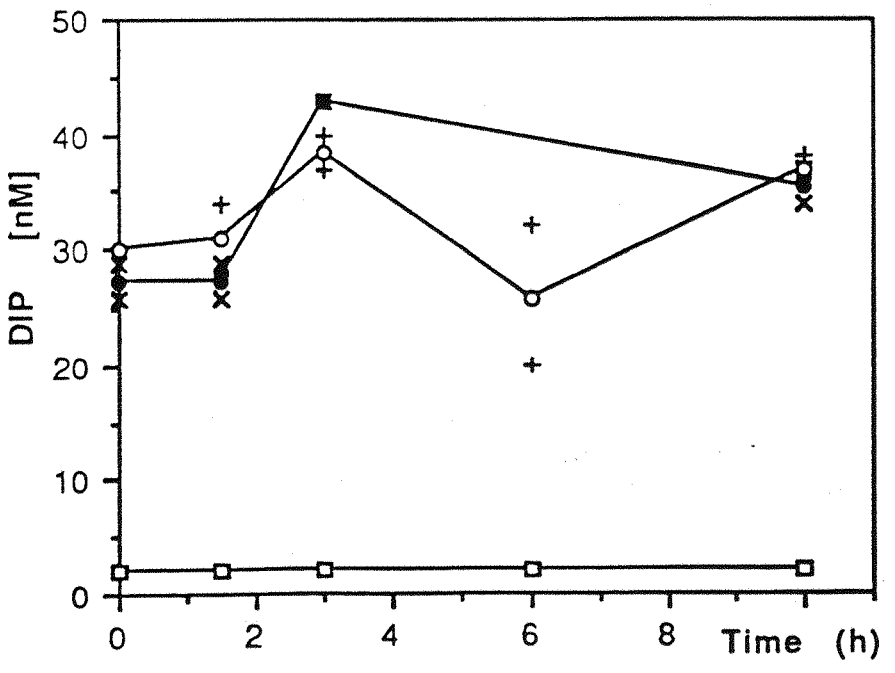


Fig. 4.24 Adsorption of DIP on poly-carbonate bottles. The filtered (Filt.) water sample has a DIP concentration of ≤ 2nM. There were duplicates bottles for samples Filt. and Filt.+30 nM. Symbol + and X indicate duplicate analysis.

occurring on a time scale of hours (e.g. Glibert 1982, Smith 1982, Harrison 1983a and b, Smith and Platt 1984, Smith *et al.* 1984, Harrison and Harris 1986). These rapid internal processes have probably led to an underestimation of carbon fixation and uptake rates of phosphorus and nitrogen. Eppley (1980) and Sapozhnikov (1988) have hinted the underestimation of carbon fixation rates by 2 and 10 times respectively, whilst many others authors have suggested values in between. This underestimation has been indirectly inferred by researchers in order to justify high values for a) pools of DOC (Skonpintsev *et al.* 1966; Sugimura and Suzuki, 1988), and DOP (Sapozhnikov 1988); b) the export flux of particulate organic matter from the euphotic layer (Jackson 1988, Williams and Druffel 1988), and c) atmospheric influx of carbon dioxide. A recent inter-comparison of many popular radio-isotope techniques (Grande *et al.* 1989) indicates operational errors should be small. However in order to provide a resolution of this controversy, an independent way should be resorted. For P, the high sensitivity method for DIP described in here provides such a method.

Radio-isotope techniques were designed and employed, because 1) natural concentrations of C, and especially P which is at low concentrations in most productive waters or below detection limits of analytical techniques, and more importantly 2) uptake and recycling rates occur at nanomolar rates that cannot be determined by current analytical procedures. The LCC technique used in here is able to measure nanomolar levels and thus to determine DIP net uptake directly without the use of radio-isotopes. Under analogous criteria, the use of a very sensitive technique for measuring nitrate (Garside 1982) has been reported as an alternative procedure for estimating N net uptake as well (Eppley and Renger 1986, Ward *et al.* 1989), although assimilation of different forms and more intricate cycling complicate interpretation.

The effect of polycarbonate containers on DIP concentration, i.e. adsorption and/or desorption was investigated. The time series experiment on filtered, and

filtered P-spiked water samples did not show any significant pattern of sorption processes (Fig. 4.24), and it was therefore deemed there were no bottle interferences.

In the time series of incubation experiment to monitor DIP behaviour, at the beginning of it net uptake was not evident and was probably masked by regeneration processes (Fig 4.18a), but after two hours removal of DIP was clear in all the samples, and it seemed that net uptake or disappearance rates increased with time. The most probable cause of this uptake behaviour is that the bulk of the micro-organism population was in the small size (probably in the pico- and femto-plankton range, reported by Platt *et al.* 1984) having a heterotrophic nature as the incubated water samples were from about 10m depth. This type of plankton has been shown to have uptake rates increasing exponentially with time (Cuhell *et al.* 1983). It was indirectly demonstrated from the APA study that surface waters are significantly populated by this type of micro-organism (and accounting for 30-50 % of the APA), which are able to sequestrate DIP more efficiently than autotrophic micro-organisms under low concentrations of DIP (Vadstein and Olsen 1989). However, uptake behaviour described here differs from that reported by Perry (1976) and Harrison and Harris (1986), who reported a linear increase (Central North Pacific Ocean) and exponentially diminishing uptake rates in time (Sargasso Sea) respectively. This difference could be explained in terms of the variety of mixed micro-organisms populating natural waters, where the experiments were carried out. The slow initial DIP disappearance could be due to light intensity, because the incubations were in shade and not screened as done by Harrison and Harris (1986). A more reasonable explanation of the observed behaviour is that under P-limiting conditions non-linear rates of removal take place (Taft *et al.* 1975).

The net uptake of DIP as a function of different concentrations of DIP in the samples ranged from 1-6 $nM.h^{-1}$ and fitted the rectangular hyperbolic model of Michaeles-Menten well (Fig. 4.18b). The slope of the curve was not quite

steep, thus suggesting some species were obtaining P by diffusion through cell walls, whilst others were actively taking up phosphate. Deviations from the Michaeles-Menten model have been reported before (Cembella *et al.* 1983). Previous uptake rates of inorganic P in the surface waters of the Sargasso Sea, as measured by the radio-isotope technique, have been reported to range between 3-12 $nM.h^{-1}$ (Harrison and Harris 1986), with an average of about 3 $nM.h^{-1}$ for most of the time-series experiment, when the environmental DIP was 30-50 nM. Thus the net uptake rates reported here are in line with previous values.

Uptake rates of DIP seem to be independent of the presence of relatively high nitrate concentrations, because the uptake rate from the duplicate sample spiked with 50 nM of DIP and 750 nM of nitrate was almost the same as the other sample which was only spiked with 50 nM of DIP (see Fig. 4.18a). Perry and Eppley (1981) also found that neither ammonium nor nitrate spikes altered uptake rates of P in waters samples from the North Pacific. This fact would suggest that the micro-plankton population is dependent on P (as DIP) rather than N (as ammonium or nitrate), thus P would potentially become a limiting nutrient.

The half saturation constant (K_m in nM) which indicates the substrate concentration (DIP in this case) at which 1/2 of the maximum uptake (V_{max}) occurs, was 15 nM (Fig. 4.18b). This is 5 times lower than the 75 nM found by Perry (1976). The difference can be attributed to: 1) The use of ^{32}P or ^{33}P techniques and interpretation of results may be inaccurate because of the unknown levels of environmental DIP that will affect the shape of the DIP *vs* uptake rates curve and consequently the K_m estimate (Cembella *et al.* 1983).

2) Underestimation of rates due to high internal recycling, e.g. enzymatic and photochemical regeneration of DOP into DIP. In oligotrophic waters uptake rates should be higher than in inshore waters in order to support prolonged growth and metabolism (Cembella *et al.* 1983). Harrison and Harris (1986)

have suggested, that when using radio-isotope techniques, there may be an underestimation of 1.5 to 1.8 times if the regeneration processes are not taken into consideration.
3) the derived K_m here is for the whole natural plankton population, whilst Perry's K_m was for a monoculture with clones isolated from oligotrophic waters.

The lower K_m found in the study reported here would overall indicate that the natural population in the oceanic water parcel investigated had a high affinity at nanomolar concentrations of DIP below 30 nM. A high affinity for the very low substrate concentrations of DIP is typical of bacterial populations (probably includes very small autotrophic plankton), which can compete more efficiently than phytoplankton for DIP (Vadstein and Olsen 1989). The presence of a dominantly bacterial population has already been inferred in this study, from the study of the APA origin.

Turnover rates $(\tau)^{-1}$ of 0.33 h^{-1}, which implies a residence time of 2.9 hours for the DIP pool under the experimental circumstances were observed. These turnover rates are much shorter than those previously reported, thus indicating that in the Sargasso Sea internal processes are much more dynamic than other oligotrophic oceans.

Despite regeneration and net uptake rates not being simultaneously measured, it can generally be observed that at DIP levels of 2-15 nM the net uptake would range from undetectable to about 3 $nM.h^{-1}$, whilst regeneration rates at the surface were 1.5-2.5 $nM.h^{-1}$. Under these circumstances regenerated DIP would provide between 50-100 % of the DIP requirements for the primary production processes. In oligotrophic seas *in situ* regeneration processes have been thought of as being the main source of inorganic nutrients (e.g. Eppley 1980, Jenkins and Goldman 1985). Glibert (1982) found that up to 100 % of the regenerated nitrogen was utilized as a net source of N in the Sargasso Sea, whilst for DIP requirements, 60 - $\geq$ 100 % could be obtained by DOP regeneration (Harrison 1983a, Ammerman and Azam 1985). The DIP

in excess to cellular needs would presumably be released to the water column.

The use of the LCC method to trace net uptake rates of DIP has achieved results that are comparable with those previously reported, has given evidence that on average, natural populations of micro-organisms in the waters of the Sargasso Sea have an extremely low saturation constant, which indicates the capabilities of this population to assimilate nanomolar concentrations of DIP, and has also indirectly corroborated the hypothesis of P limitation.

From the analytical point of view, when measuring DIP uptake, the use of the LCC technique as an alternative to non-radioactive methods has proven to be reliable. Advantages are: 1) exact DIP concentrations are measured provided they are not below 1-2 nM. 2) It uses much safer and "environmental friendly" chemicals. 3) It can be used to measure *in situ*, i.e. without incubations and confined samples, the net uptake of DIP, and 4) if coupled to techniques that measure regeneration rates, the actual rates of DIP and phosphorus cycling can be determined with more confidence. Carbon fixation can confidently be extrapolated by using rates of P and pools of the dissolved and particulate phases of P, N, and C measured simultaneously.

If the technique is applied as described here, further work needs to be carried out as this alternative method potentially has similar problems to the radio-active procedures, i.e. possible consequences of the *in vitro* experimental conditions, such as confinement and container effects.

4.4.3 Vertical fluxes of P into and out of the mixed layer

4.4.3.1 Export fluxes

The assumption that the euphotic or mixed layer has C, P, N, O_2, etc budgets which are in steady state has widely been employed by marine scientists to estimate, for instance, the

cycling of elements and new production rates. This assumption is simply based on the idea that everything that escapes from the mixed layer, has equivalent renovating fluxes, i.e. by advective and convective processes. In oligotrophic waters, the application of this assumption seems to be relatively straightforward because 1) horizontal inputs can largely be disregarded and 2) vertical inputs are dominated by upward vertical diffusion from deeper waters enriched in nutrients, whilst downward atmospheric fluxes could be considered negligible (Knap *et al.* 1986). Therefore, the use of the so-called one box model associated with upward-downward fluxes appears to be adequate, and forms the basis of the treatment here.

In the Sargasso Sea a net flux at 150m depth of 4.76 and 0.73 $mmol.m^{-2}.d^{-1}$ of carbon and nitrogen respectively, and primary production of 67.5 $mmol[C].m^{-2}.d^{-1}$ has been reported for February/1989 (Lohrenz *et al.* 1990) implying an export flux of P of 0.045 $mmol.m^{-2}.d^{-1}$ if a C:P ratio of 106:1 is used. Unpublished data for February/1990 (M. Tuel, pers. comm.) indicates an export flux of 2.87 and 0.36 $mmol.m^{-2}.d^{-1}$ for C and N respectively and a carbon production of 41.5 $mmol.m^{-2}.d^{-1}$, which gives roughly half of the February/1989 P export (0.027 $mmol.m^{-2}.d^{-1}$). The datum obtained from a portion of the sample used by M. Tuel to determine C and N, was 0.092 $mmol[P].m^{-2}.d^{-1}$. This single value appears high relative to the inferred P export flux and that provided by Knauer *et al.* (1979) and Knauer and Martin (1986); 0.014 (at 75m) and 0.035 (at 150m) $mmol.m^{-2}.d^{-1}$ for the North Pacific. This suggests at first sight that P is escaping at two times more quickly from the mixed layer (150m) than C and N. However, examining the vertical distribution of dissolved PPs, it can be observed that a sustained increase of PPs occurs from about 100 to 175m depth (Fig. 4.16). The PPs were presumably derived from sinking dead cells that left the mixed layer. Intracellular PPs can account for up to 60 % of the PTP (Perry 1976, see also Section 2.5). If all or most of PPs are exuded as they sink, a lower flux of PTP could occur below 150 m depth. Therefore, it may be reasonable to assume that the escaping

flux is much lower, thus an *ad hoc* flux of 30 μmol.m^{-2}.d^{-1} will be used for the next discussion. Although, it should be pointed out that this would be purely circumstantial because fluxes out of the mixed layer are dependent in time and space (e.g. Knauer 1988).

4.4.3.2 Vertical diffusive fluxes of phosphorus

Applying the condition of a steady state box model and assuming no horizontal advection, and only vertical fluxes. Thus, the export flux from the mixed layer should be equalled by upward vertical advection and diffusion into it, assuming negligible atmospheric input. Under these circumstances diffusive fluxes of P can be calculated by the Fickian model:

$$F_D = K_z(dC/dZ) \qquad \text{(Eq. 4.1)}$$

where K_z is the diffusion coefficient, which is estimated at 14.7 m^2.d^{-1} (Li *et al.* 1984) and dC/dz is the average gradient in the first 20m of the phosphocline, which was found to average 1.35 μmol.m^{-4} (Tab. 4.3).

The advective flux F_A is simply calculated by;

$$F_A = V_z(C) \qquad \text{(Eq. 4.2)}$$

where V_z is the vertical water speed vector, which is +0.04 m/d for most of the year (Leetma and Bunker 1978), but during winter is -0.08 m/d (Duce 1986); C is the concentration of DIP in the top 20 m of the phosphocline which ranged from 10.5 to 64.2 μmol.m^{-3} with an average of 27.4 μmol.m^{-3}.

The total vertical upward flux F will be,

$$F = F_D + F_A \qquad \text{(Eq. 4.3)}$$

Thus, F_D range between 33.8 and 9.55 with an average of 20. μmol.m^{-2}.d^{-1}, whilst F_A during winter would be -2.2

μmol.m^{-2}.d^{-1} (-0.8 to -5.2), or 1.1 (0.4 to 2.6) μmol.m^{-2}.d^{-1} for most of the year. The latter flux should be qualified as negligible because it represents ± 5 % of the F_D. Thus, it is consequently disregarded because its magnitude is within the analytical error of the vertical flux of P. Thus a typical flux of 20 μmol.m^{-2}.d^{-1} will be taken for discussion.

The main variable in these calculation is K_z, which has been quoted as 1.7 (Rooth and Ostlund 1972), 3.4 (Wunch and Minster 1982), 5.1 (Viecelli *et al.* 1981), and 10 m^2.d^{-1} (Pritchard *et al.* 1971). Here, an even a higher K_z (14.7 m^2.d^{-1}) has been taken for calculations because the determination of this coefficient (Li *et al.* 1985) has involved a more comprehensive study. However and as Broecker *et al.* (1980) cautioned, these coefficients are purely empirical estimates taking into account not only diffusion but also vertical eddy mixing and advection along isopycnal horizons which outcrop at the surface of the ocean. The slope (dC/dz) is calculated from where the phosphocline starts to develop which has been taken as between 120 and 150m depth. The average slope reported here, 1.35 μmol.m^{-4}, is very similar to that reported by Duce (1986); 1 μmol.m^{-4}. This author, took into consideration atmospheric and advective components, and proposed a net flux of P into the Sargasso Sea of 1.7-14 μmol.m^{-2}.d^{-1}, that can be compared to the range given here of 9.55-33.8 which does not consider atmospheric and advective components.

An expected export flux of nitrogen of 320 μmol.m^{-2}.d^{-1} (153-540) should be found if fluxes of P range from 9.55 to 33.8 μmol.m^{-2}.d^{-1} and Redfield ratios N:P of 15:1 are used, but averages of 140 (1-890) and 180 (50-310) μmol.m^{-2}.d^{-1} have been reported by Lewis *et al.* (1987) and Duce (1986) respectively. However, applying a K_z of 14.7 m^2.d^{-1} and a gradient of 45 μmol.m^{-4} (Duce 1986) a flux rate of 661 μmol.m^{-2}.d^{-1} for N is obtained, which implies 1) that N is being supplied at rates 30 times higher than those of P, 2) the different values of fluxes exemplify how variable the magnitudes of vertical diffusive flux estimates are.

For P the vertical diffusive flux (20 μmol.m^{-2}.d^{-1}) is only 30 % lower than the respective literature export flux of 30 μmol.m^{-2}.d^{-1}, and thus there is a reasonably good agreement between the fluxes. However, if the export flux actually measured, i.e. 90 μmol.m^{-2}.d^{-1}, is used then the balance is much poorer, and implies a net loss of P from the mixed layer. If a steady state is to exist horizontal diffusion and advection and/or atmospheric deposition must make up this difference.

An analogous situation exists for nitrogen, whose export rates as measured from sediment traps samples are in the range 180-733 μmol.m^{-2}.d^{-1}, whilst diffusive fluxes are averaged at 190 μmol.m^{-2}.d^{-1}. This does not compare to a diffusive flux of 620 mmol.m^{-2}.d^{-1} using the K_z from Li *et al.* (1984) and slope of 0.045 mmol.m^{-4} (Duce 1986). Moreover, these nitrogen fluxes should be much higher up to 2500 μmol.m^{-2}.d^{-1}, to satisfy new production rates according to Jenkins and Goldman (1985).

As a steady state system should exist on medium and long time scales, the information presented here strongly suggests that the application of the Fickian model is inadequate, and therefore a more dynamic and spatial approach must be employed. Redfield *et al.* (1963) proposed the used a dynamical relationship (see Appendix 4.1c) which is more readily employed if it is expressed in terms of Eulerian equations (see Riley 1956).

The need to use this dynamic type of approach is stressed by reports of ring cores intruding into the vicinity of station "S". These cores have diameters up to 170km (Swallow 1971, Brundage and Dugan 1986) and can entrain nutrients (Spitzer and Jenkins 1990). Also entrainment of nutrients due to irregular and unsteady meteorological events, which can occur on a time scale of hours, have to be considered (Klein and Coste 1984). These entrainment effects can be observed in this set of data as shown by an increase in DIP gradients from 1.5 to 2 μmol.m^{-4} (Table 4.3) in the fourth cruise was found. This cruise was preceded by a period of irregular and strong

winds (10-20 $m.s^{-1}$). The low vertical diffusive fluxes of P found in this work (for analogous case see Garside 1985, Lewis *et al.* 1987) support the idea of rejecting the use of Fickian models as a way of determining net import fluxes into the euphotic layer in a short time scale. Models such as that proposed by Redfield *et al.* (1963), plus considerations of, for instance, short but strong pulsations of nutrients into the mixed layer due to meteorological instabilities (Klein and Coste 1984), have to be applied. The steady state concept seems only to be applicable on long-term time scales.

4.4.4 Phosphorus pools in the meso- and bathy-pelagic waters of the Sargasso Sea

Dissolved inorganic phosphate concentration and distribution were shown to be, in both profiles (Figs. 4.20, 4.21), in good agreement with previously reported data (e.g. Jickells 1986). It is interesting to observe that the slope between 200-900m depth and distribution (down to 4000m depth) of the DIP profiles matched in all sets of data. But there was a discrepancy of about 10 % with the set of data obtained by the BBSR scientists from the same cast (See Appendixes 4.3a and 4.3b). This sort of difference can mainly be ascribed to analytical calibration, because Jickells (1986) reported values varying between 1.45 and 1.72 μM (from 900 to 4200m depth), whilst the BBSR scientists found 1.40 - 1.2 μM, and here a range of 1.45 - 1.10 μM is reported. Nonetheless, others reasons may exist, such as seasonal changes in the export flux of particulate matter and horizontal intrusion of different water bodies.

Utilizing information on oxygen and the relationship given by Redfield *et al.* (1963, see Appendix 4.1b) the so-called pre-formed and oxidative phosphate can be estimated. Thus, between 120-200m, all the DIP in this layer was of oxidative origin, whilst from 400 to 900m depth it only represented about 29 % of the DIP. Redfield *et al.* (1963) have reported that just below the euphotic zone all or most of the DIP is of oxidative origin and Sapozhnikov (1977) has suggested that

in oligotrophic waters DIP of pre-formed origin does not intrude to waters shallower than 75-100m depth. In deeper water the oxidative originated DIP only represents 50-25 % of the present DIP. What is found here corroborates the previous statement that > 99 % of the DIP is recycled in the upper water layer. This also would suggest that most of the advected and/or diffused DIP to the euphotic layer is of oxidative origin, thus implying 1) residence times of P must be much longer, because the diffused and/or advected P is "re-entered" the euphotic zone many times, before effectively escaping this layer, and 2) phosphate is not supporting any "new production", i.e. "new production" in terms of P is zero or approaching it.

Although there is some patchiness in the two DOP deep profiles, the DTP distribution was smooth. For both profiles DOP mirrored DIP levels, this was very conspicuous at some points (Fig 4.19 and 4.20). Both profiles of DOP did not have a defined pattern and had an average of 0.202 ± 0.11 μM and evident increase at 3600-4000 m depth. These features are comparable to a profile from the Tropical Pacific Ocean presented by Ljutsarev *et al.* (1977), which also showed a variable DOP distribution and had a maximum of 0.83 μM at 3000m depth. The author has also found similar concentrations and vertical pattern in the Equatorial Eastern Pacific (unpub. work).

The increase with depth of DOP would match a similar pattern in the DOC concentrations (Baeur *et al.* 1990). However, these DOP profiles differed to that reported by Smith *et al.* (1986) and Orret and Karl (1977), whose DOP values were under 0.1 μM below 150-200m depth. Romankevich (1984) has reported a DOP range in deep water of nanomolar concentration but also as high as 1-2 μM. Much of this disparity in values may be regarded as of analytical origin.

Figure 4.21 shows the profiles of DOP determined by UV irradiation and magnesium nitrate oxidation, and dissolved PPs. It is noteworthy to see that both techniques gave the

same vertical pattern for most of the depths except at 3000m and 400 and 500m depth, where DOP concentrations were close to detection limit (< 50 nM). This comparative pattern provides confidence to data obtained by the chemical oxidation technique. The difference between both techniques gave a striking evidence that PPs or/and some UV refractory organically bound P material exist in the bathypelagic and abyssal waters of the Sargasso Sea. Dissolved PPs average nearly 0.1 μM, and the most likely origin of this fraction would be euphotic layer produced particulate material that had sunk and was slowly dissolved in time. Romankevich (1984) has suggested that organically-refractory phosphate can reach the deeps of the oceans; Deuser and Ross (1980) and Deuser (1986) have found that particles (generated in the euphotic layer) can reach depths of 3200m. The associated P in these particles will eventually be dissolved before being buried into the deep sediments.

4.5 Modelling P cycling in the euphotic and mixed layers of the Sargasso Sea

4.5.1 Dynamics of P estimated by *in situ* measurements

The DIP, PTP and DOP profiles measured *in situ* for the first cruise (Table 4.1 and 4.2) were used to estimate *in situ* rates of DIP removal, DOP removal and regeneration and PTP production.

The net uptake of DIP converted into C production using the C:P ratios found in the suspended particulate matter (24:1) would be 0.14 $g(C).m^{-2}.d^{-1}$, compared to the measured carbon production rate of 0.256 $g.m^{-2}.d^{-1}$. Because there were regeneration processes taking place which provided 50-100 % of P requirements of the micro-organisms, then the carbon production inferred from P uptake would be 0.38-0.51 $g.m^{-2}.d^{-1}$. Production of particulate phosphorus was in good agreement with the net uptake of P, suggesting that it is possible to measure primary production rates *in situ* using P measurements.

The production of DOP can be estimated by knowing the regeneration of DOP and the total DOP change, i.e.

$$DOP_{change} = DOP_{prod} - DOP_{reg} \qquad \text{(Eq. 4.5)}$$

Taking a regeneration rate of 387 μmol[P]. $m^{-2}.h^{-1}$ (APA) of DOP into DIP, and DOP_{change} of 77 (removal) in the 120m upper surface layer, a production of DOP (DOP_{prod}) of 310 μmol.$m^{-2}.h^{-1}$ can be estimated. Assuming it is more or less constant over this layer, then DOP production will be about 3nM.h^{-1}, which is similar to the rates reported by Orret and Karl (1987) for the North Pacific gyre.

Removal of DIP in the rest of the cruises (Tab. 4.1) was not clear; for the second cruise profiles at 5:00, 11:00 and 16:30 h gave DIP budgets over the euphotic layer of 417, 443, 517 μmol.m^{-2}; for the third cruise DIP was constantly below detection limit; and for the fourth cruise, DIP mass increased from 1203 (28/2-10:45), to 1513 (1/3-02:30) and then 2777 μmol.m^{-2} (1/3-05:30) to decrease to 1440 about 5 hours later (Table 4.1). These facts suggest that regeneration processes rates were sometimes higher than uptake rates and *vice versa*, and also horizontal and vertical influx could also have contributed to this pattern. For the second cruise regeneration and uptake of P were matched.

The obtained information indicates that regeneration of DOP into DIP, production of DOP, and removal of DIP are at similar rates; thus the P cycling within the mixed layer of the Sargasso Sea is largely closed.

4.5.2 Residence times of the P pools

An average budget of DOP of 18707 μmol.m^{-2} (over 120m) will be completely converted into DIP in 2.87 days, by the average APA regeneration rate of 271 μmol.$m^{-2}.h^{-1}$. If other enzymes are active as well the residence time will be shorter. On the other hand a DIP mass of 100-1200 μmol.m^{-2} will disappear in 0.10-1.2 days, if the uptake rate is 40 μmol.$m^{-2}.h^{-1}$.

The residence time of all measured forms of P in this layer (23200 μmol.m^{-2}) would be 2.12-0.71 years, if export fluxes of particulate P of 30-90 μmol.m^{-2}.d^{-1} are considered, and if it is the only removal path. Here residence times are lower (about 5 times) than that reported by Knauer and Martin (1981) in the Northeast Pacific who considered the depth interval of 0-150m. They took into account the export of dissolved organic P which was 71 μmol.m^{-2}.d^{-1} measured in a water sample contained in the trap. Recalculating τ from their data and taking only particulate P fluxes, a residence time of 16.4 years is obtained, which is about 8 times higher than the value given here; the difference can be explained in terms of their higher measured P budget (210 mmol.m^{-2}) which is about 9 times higher than the values presented in this work.

As previously suggested (e.g. Eppley 1980, Jenkins and Goldman 1985) internal regeneration in the Sargasso Sea will provide the P requirements of internal biological processes. The "new" production in terms of external P supply (20 μmol.m^{-2} .d^{-1}) will account for 16 % of a net average carbon production in the Sargasso Sea (400 mg[C].m^{-2}.d^{-1}), thus inferring an actual new production of 25 mg[C].m^{-2}.d^{-1}. Because a flux of 20 μmol.m^{-2}.d^{-1} is regarded as lower than the total export flux, the new production in terms of P should be higher than 16 % of the net production (Pace *et al.* 198) and Platt *et al.* 1989). This corroborates the argument that the new production should be 13-25 % of the net production.

However, if the term "new" production is strictly applied, the diffused DIP into the euphotic zone has to be of non-oxidative origin. According to the study on the origin of P in the 200-900m layer, more than 70 % of the measured DIP in this layer is of oxidative origin. It is reasonable to suppose that a higher percentage of this DIP is present above 200 m depth. This fact would oppose the above idea that "new" production in terms of P is > 16 %, it would actually be less than 3 %. From what is found, it is logical to think that "new" production in oligotrophic waters is well below previously suggested values, which were mainly calculated in

terms of the export flux from the euphotic layer, therefore it is proposed to approach the estimation of the true "new" production in terms of diffusive fluxes, but taking into consideration the origin of the diffused nutrient.

4.6 A simplified model of the P pools in the surface waters of the Sargasso Sea

In the Fig. 4.25 a schematic model of cycling between P pools in the euphotic layer of the Sargasso Sea is presented. This model differs from others in which the DIP is the main pool, as here the main pool is DOP followed by PTP, with the smallest one being DIP (see Table 4.4). The diagram shows that more than 99 % of P mass is recycled in the euphotic layer.

4.7 Conclusions

By applying the LCC technique, the existence of dynamic and well structured vertical profiles of DIP in the euphotic layer of the Sargasso Sea has been demonstrated. The profiles were shown to be affected by mainly biological and enzymatic processes. On the other hand, physical factors are principally defining the depth of the phosphocline. Budgets of DIP ranged from < 100 to 1200 $\mu mol.m^{-2}$, and only represented 3-5 % of the total mass of P existing in the water column. The sort of variability proved that auto- and hetero-trophic micro-populations are able to actively consume nanomolar levels of DIP, thus depleting DIP sometimes below detection limit (1-2 nM). Also, it was observed the presence of three sub-layers of DIP within the mixed layer. This facts hints that new approaches have to be taken when studying DIP in the Sargasso Sea. This would apply to DIN as well.

It was possible to calculate the removal or net uptake of DIP that occurred in the water column, which was 50-100 $\mu mol.m^{-2}.h^{-1}$. These rates matched accordingly to those measured from on deck incubation experiments, which also compared well with previous rates measured by radio-active techniques, thus proving the validity of using the LCC technique as an

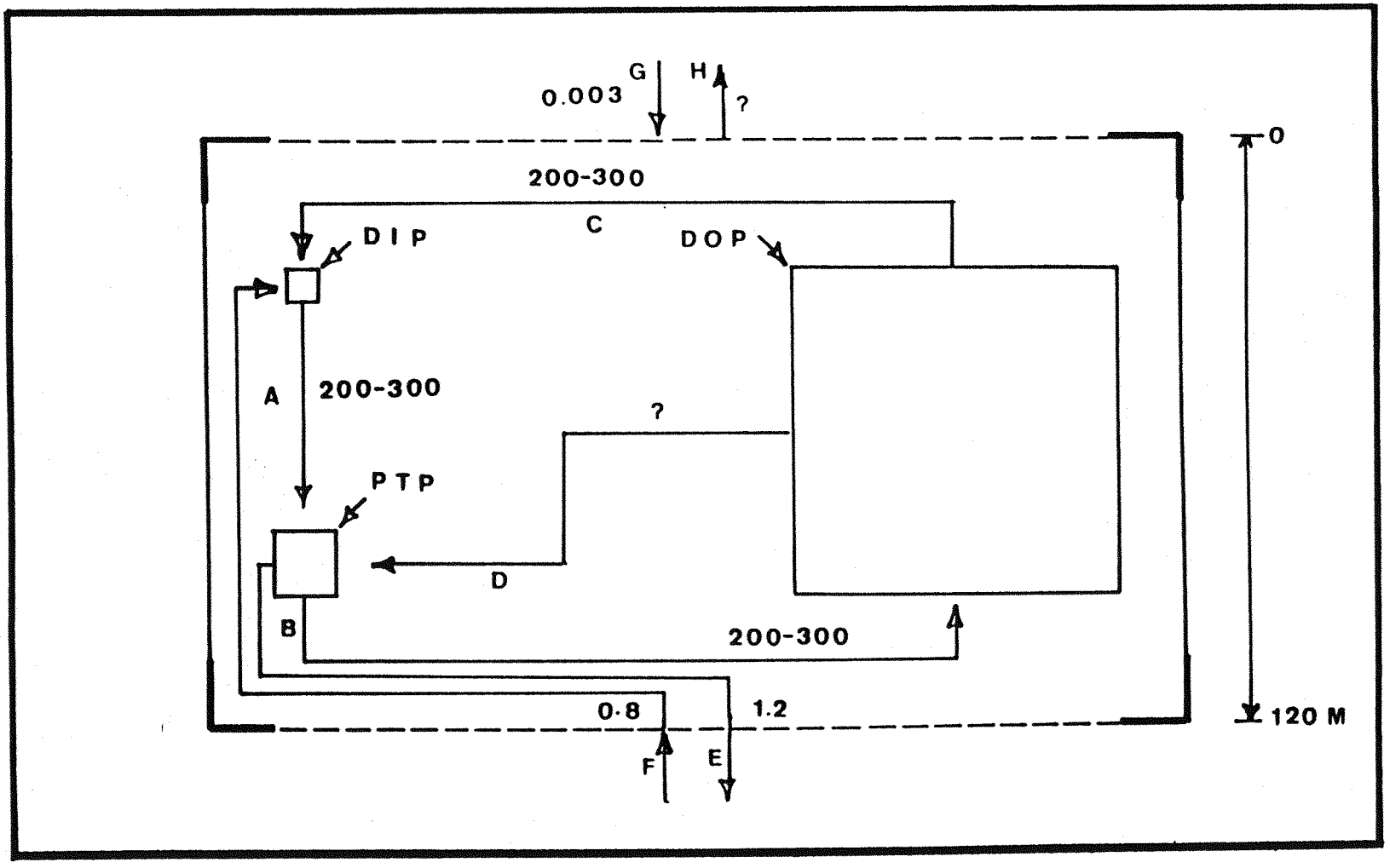


Fig. 4.25 Internal cycle of P pools in the surface waters of the Sargasso Sea (0-120m). Number along arrows are rates in $\mu mol.m^{-2}.h^{-1}$. A: net uptake, B: DOP (also PPs ?) generation, C: regeneration of DOP (PPs?) into DIP, D: uptake of DOP (?), E and F: vertical export and import fluxes, G and H: atmospheric imput and sink. The total budget of all forms of P in this layer averaged: 23200 $\mu mol.m^{-2}$.

alternative procedure to typical methods.

The measurements of DOP, PPs, and PTP proved to be relevant because 1) DOP was the more important pool in the phosphorus cycling, 2) the variability of DOP and PTP were strongly related to biological processes and 3) PPs fraction was a significant and non-variable pool within the DTP mass.

The DOP pool was the main source of P for photosynthetic processes, which together with regenerative ones, regulated the inter-conversion of P forms in the euphotic layer. When the molar ratio of DOP to the new DOC values is calculated there is a large discrepancy with the Redfield ratio. Thus, it is clear that this ratio cannot be applied to dissolved organic phases, mainly because DOP is regenerated much quicker than DON and DOC. However, if average availability ratios of 1017:1 (C:P) are considered, this ratio will be in good agreement. Applying the later ratios for N and P (15:1), the inferred DON pool would be <3 μM, which accordingly matches that reported for the oligotrophic North Pacific (Walsh 1989).

Despite the fact that no clear idea of what role PPs or/and UV refractory organic-P is playing in the euphotic layer processes, the significance of them within the P budget was shown to be important. This important fraction has previously been disregarded, and if PPs are at similar percentages relative to DOP pools in any other natural water body, these pools should be in fact 40-50 % higher than the currently reported DOP levels.

Pools of particulate phosphorus dramatically varied in a time scale of a few weeks; this did not occur with POC and PON (see Appendices 4.8 - 4.9). This fact clearly reflected the P-nutritive state of cells and also demonstrated that DIP is stored intra-cellularly and it is used accordingly to needs.

The comprehensive study of the P pools *in situ* and its processes on mixed naturally occurring populations has given new perspectives or implications, such as, that the plankton

populations are able to actively consume nanomolar concentrations of DIP below 30 nM and their growth is limited by DIP. The possibility that these populations still assimilate DIP even below 1-2 nM of DIP is open. The incubation experiment indicating effective uptake of DIP at 15 nM (K_m), and also the lack of effect on net uptake of DIP when concentrations of nitrate 15 times higher than DIP levels were present, both support the thesis of phosphorus limitation. These are important facts that have not been observed in previous studies due to principally the lack of a sensitive technique for measuring DIP.

The limitation of P was also reflected in the nutricline slope, in which the ratio of DIN:DIP from the euphotic layer bottom to 200 m depth was 22-27. This implies that DIP supply from below the euphotic layer is always in shortage relative to DIN (as nitrate). This also serves as an indication of quicker N regeneration than P in this layer of water. The ratio DIN:DIP at deeper waters approaches to 15.

The detection of APA at rates of up to 3.2 $nM.h^{-1}$ is another indication of the P-limitation conditions during the study. As regards the origin of this enzyme, it was established to be produced by hetero- and auto-trophic micro-organisms, but APA in surface waters seemed to be purely produced by heterotrophic organisms.

The possibility that labile and potentially biologically available DOP rather DIP is the limiting fraction is proposed, thus the term "P limitation" is encouraged to be used instead of "DIP limitation" until this possible dilemma is elucidated.

Although data on the export flux from the euphotic layer was not abundant, reported data suggested that fluxes could be 30-92 $\mu mol.m^{-2}.d^{-1}$, and the "new" production sustained by DIP is about 16 % or more relative to the net production as suggested by some authors. However, because the mass of P imported into the euphotic layer comes from the subjacent layers (120-200m) and it has been demonstrated that this

phosphate has an almost 100 % oxidative origin, phosphate would not support any "new production".

It was found that export flux surpassed the respective import ones, thus suggesting that application of the Fickian model is not the more appropriate when applied under short time scale conditions. This fact leads to the conclusion that the concept of steady state is only applicable at very long time scales.

The presence of measurable DOP in bathypelagic waters is another important finding. The most probable origin of this would be from dissolution of sinking particulate matter produced in the upper surface waters. The two profiles were consistent and similar to previous ones reported for the Pacific Ocean. On the other hand, the presence of PPs is a new fact that needs to be further investigated and confirmed.

C H A P T E R

F I V E

Summary conclusions and future research

5.1 Introduction and background

One of the most salient conclusions to be learnt from the literature review is the lack of a comprehensive knowledge about the magnitude and behaviour of the different pools of P in many bodies of natural waters. Thus, whilst DIP pools have been extensively monitored, the other fractions of P, i.e. DOP and PTP have not been measured as frequently as their carbon and nitrogen counterparts. From the literature it is inferred that DOP has not regularly been determined due to the belief that it is not an important fraction of the dissolved total P, despite many reports having indicated that this fraction could equal or even surpass DIP pools. Under a similar criterion PTP pools have been even more neglected. This is perhaps because when PTP is related to POC and PON using the Redfield ratios, the expected concentrations are very and difficult to measure. As a consequence of this it has been assumed that these fractions are not relevant within the cycling of P in natural waters.

Although measurements of DIP have been regularly made in fresh, estuarine, coastal, and open ocean waters, it is often found that concentrations are below the detection limit (30 nM) of the currently applied techniques. This is particularly true for surface waters of oligotrophic lakes and seas and even sometimes for coastal and enclosed ocean water bodies undergoing extensive and intensive primary production processes. As oligotrophic seas cover about 75 % of the total world ocean area, the absence of DIP information in these regions is indeed significant. Research to solve this problem

is urgently required especially now that scientists are understanding the profound implications and influence that these large and semi-enclosed water masses have in the global cycles of carbon, nitrogen, sulphur, etc.

The literature review also indicated that enzymes such as alkaline phosphatases are associated with low concentrations of DIP, generally higher DOP levels, and intensive photosynthetic processes. The activity of these enzymes has been reported in many types of natural water as both particulate and dissolved phases. Laboratory and field experiments indicate that when low or undetectable DIP concentrations are present and these enzymes are active, hetero- and auto-trophic micro-organisms use the DOP pool to satisfy their P requirements. Furthermore, the recently detected 5'-nucleotidase has been found when concentrations of DIP are above 1 μM, and experiment demonstrated that DOP was regenerated into DIP to satisfy the P supplies for photosynthetic processes.

Regarding the biogeochemistry of P, there is very little information on main sources of P, i.e. riverine and atmospheric inputs, and much of the available information is conflicting in nature. A similar situation exists with the removal processes. The rates are unbalanced and this is ascribed mainly to the absence of data on riverine and estuarine PTP and DOP, although PTP is the most significant fraction of P carried by rivers. Atmospheric inputs although not as important in magnitude as those from rivers, could be the main source of P on a local scale. Atmospheric sinks could also be important in local areas where high primary production processes are reported. This sink is due to the transport of aerosol by wind from surface waters to land or oceanic regions. The content of P in the aerosol increases with high photosynthetic processes.

5.2 Development of techniques for measuring different forms of P in natural waters

The lack of adequate and well established techniques was thought to be another deterrent for scientists addressing the above problems. Thus, problems associated with analytical procedures for low concentrations of DIP and DOP and PTP were investigated. It was found that there were many techniques for measuring DIP at nanomolar levels, but these were generally complicated and involve many handling steps and cumbersome apparatus. Thus, a technique to measure DIP at nanomolar concentrations was developed. The chemistry of the method was the classical phospho-molybdenum blue procedure and the detector was a spectrophotometric system that uses a long capillary cell (LCC). The source of light and detector used were an infra-red light emitting diode and a silicon photo-transistor respectively, which were encased in the ends of the LCC. Under laboratory conditions a detection limit of 1 nM, a range of 1-500 nM and a general relative standard deviation of 6-3 % were achieved. However under ship conditions when variable voltages are likely, the detection limit could be up to 2 nM. No interference from arsenate or labile organic P was found, and the system was applied to fresh and marine waters. The system proved to be easy to construct, cheap, portable, able to work under ship conditions and its components are adaptable to other analytical procedures.

During the optimization of the above method the effect of different surfactants on the chemistry of the phospho-molybdenum blue method was studied. It was found that many reported detergents which are used to improve flushing characteristics in automated systems had a negative interference on the chemical procedure. It was found that one of the surfactants studied (DTMAB) gave adequate flushing in the LCC system without affecting the chemistry of the method.

Five analytical approaches for measuring DTP and consequently DOP in marine waters were inter-compared. Two main sets of experiments were designed to test the decomposition efficiency of DOP by the five methods. 1) Model organic P compounds were added to distilled de-ionized and coastal waters and 2) natural waters samples from the Itchen River and Estuary (England), and North Sea were analyzed. Results obtained indicated that the elegant and popular ultra-violet irradiation oxidation method gave the lowest values of DTP. The most efficient technique was that using magnesium nitrate (Nit-ox) as oxidant. Data from experiments and the literature revealed that dissolved poly-phosphates represent a significant component of the DOP which can account for up to 50 % of it under bloom conditions. The rest of the DOP would mainly be composed by mainly phospho-mono-esters (according to many authors), however part of this material would be un-characterized organic P compounds such as non-refractory geo-polymers. Because the UV technique did not depolymerize PPs into DIP whilst the Nit-ox method did, the difference between the values of DTP measured by each technique can be used to empirically determine the concentrations of dissolved PPs. This fraction would also contain meta-phosphates and perhaps some organic P compounds which are refractory to UV oxidation. The accuracy of the Nit-ox method when applied to decompose natural occurring DOP was studied by inter-comparing this technique with the Inductively Couple Plasma-Mass Spectrometry technique, but no concrete and precise results were achieved although the data suggested a clear trend between both methods; the accuracy problem thus was not unequivocally resolved.

A method to determine PTP in particulate suspended matter in natural waters was developed by modifying the Nit-ox method. When compared with a high temperature oxidation procedure the modified Nit-ox procedure gave a higher content of PTP in natural samples containing biogenic and non-biogenic particles, and also cultured phytoplankton samples. Attempts to determine the accuracy of this modified method were also undertaken without conclusive results.

5.3 The biogeochemistry of phosphorus in four English estuaries

Drawing detailed conclusions about the processes affecting P concentrations and behaviour in estuarine environments from field data only is difficult because of the highly variable and dynamic nature of these systems. However, the following points can be made from the work carried out.

i) The magnitude of DIP concentrations varied greatly from estuary to estuary with a range from 70 to < 1 μM. The higher values reflected the importance of anthropogenic inputs which were ascribed to sewage treatment plants in the Itchen and Thames estuaries. However industrial sources may also be important. Adsorption onto particulate suspended matter through association with flocculated iron, and biological uptake were regarded as the most likely removal processes, although others factors such as transport and cycling in association with calcite may also be important. Non-conservative behaviour due to "buffering" of DIP concentrations was positively identified in the Beaulieu Estuary and can also be suspected to occur in the Humber Estuary.

ii) The concentrations of DOP were similar for the four estuaries ranging from about 1 μM to below detection limit (0.05 μM). However, the relative importance of DOP as a fraction of the DTP fluctuated significantly. In the Beaulieu Estuary it was the major fraction and was also important in the high salinity range studied in the Humber Estuary. In the Itchen and Thames Estuaries the DOP signal was obscured by large inputs of DIP ascribed to sewage treatment plants. Sources for DOP include allochthonous inputs, anthropogenic activities, and *in situ* biological production. Removal processes for the labile and more refractory DOP were not clearly elucidated, but will included biological uptake (for instance bacteria are reported as being able to assimilate phosphonates), and also physico-chemical processes such as

adsorption, and photo-chemical conversion to inorganic forms.

iii) Particulate total phosphorus proved to be an important fraction of the total P particularly in the Beaulieu and Humber estuaries where it was the dominant fraction. For the four estuaries PTP ranged from 6 to 1 μM. The ratio of POC:PTP for the Beaulieu and Itchen estuaries was 3-5 times lower than that previously suggested, based on the averaged POC:PTP ratios for the whole salinity range and over depths. Suspended sediments travelling through the estuaries investigated gradually become enriched with P. These facts challenge accepted concepts of the geo-chemical cycle of this element, and suggest that the estuarine input of P into the oceans is under-estimated.

iv) If major inputs of DIP were not present, as in the Beaulieu Estuary, PTP was the dominant fraction of the total P. Evidently, when adsorption processes are significant in the estuarine environment, the information on PTP is critical in biogeochemical studies of this element.

To summarize, this study demonstrated the potential importance of forms of P other than DIP in estuaries, and the need to consider these other forms if a better understanding of the biogeo-chemistry of P in the estuarine environment is to be attained.

5.4 The cycle of P in the Sargasso Sea

The first profiles of DIP at nanomolar concentrations in the surface waters of the Sargasso Sea are reported here. The vertical structure of the profile was well defined and three sub-layers within the euphotic (generally equivalent to mixed) layer were observed. In each of these sub-layers different processes were governing the vertical pattern. Thus in the top sub-layer, external atmospheric supply of P and enzymatic activity of bacterial origin dominated over photosynthetic processes. In the mid-layer biological uptake processes lowered DIP concentrations to generally below detection limit

although enzymatic activity was detected. The bottom sub-layer structure was basically governed by physical (advection and diffusion) and to some extent enzymatic processes. In this sub-layer the influence of internal waves leading to rapid and dramatic changes in concentrations of DIP was observed. Concentrations of DIP largely varied widely from about 20 to < 1-2 nM. Budgets for DIP in the euphotic layer ranged from < 100 to 1200 $\mu mol.m^{-2}$ and only represented 3-5 % of the total mass of P exiting in the water column. This variability demonstrates that auto- and hetero-trophic micro-organism populations are able to actively consume nanomolar levels of DIP, thus depleting it to sometimes below the detection limit of the LCC technique.

In situ removal or net uptake rates of DIP from the whole euphotic layer were calculated to be 50-100 $\mu mol.m^{-2}.h^{-1}$. These rates matched those from "on deck" experiments, which were executed by using the LCC system to measure DIP net uptake. The rates found compared well with previous values measured by radio-active techniques, thus indicating the validity of using the LCC technique as an alternative procedure to these typical other methods. The "on deck" incubation experiments allowed the determination of the DIP substrate affinity of the natural mixed population in the Sargasso Sea, which was about 15 nM, i.e. about 5 times less than the lowest K_m previously reported.

The simultaneous measurement of DOP, PPs and PTP pools proved to be important because 1) DOP was the largest pool in the phosphorus cycling, 2) the variability of DOP and PTP were tightly related to biological processes and 3) the PPs fraction was a significant and non-variable pool within the DTP.

The magnitude of pools of PTP dramatically varied on a time scale of a few weeks, whilst this did not occur with its counterparts POC and DON, thus the ratios C:P and N:P fluctuated from 24 to 232 and from 3 to 24 respectively. This fact clearly reflected the intra-cellular variability of P

from luxury to starvation, and also demonstrated that DIP is stored intra-cellularly and used according to needs.

Production limitation by P instead of N was positively proved by three facts. 1) DIP was below detection limit whilst DIN was measurable, i.e. the ratio DIN:DIP was well above 15. Information on other water column parameters and different forms of P supported the view that a peak in micro-plankton growth collapsed when DIP was below detection limit, thus indicating that the lack of DIP could have initiated this collapse. After a few days the DIP concentrations dramatically increased from <1-2 nM to above 30 nM and a decrease in POC of nearly 30 % was observed in this time interval. 2) In the "on deck" incubation experiments, net uptake of DIP was unaffected by nitrate which was present at 15 times the concentration of the DIP. 3) The ratio of DIN:DIP at the bottom of the euphotic layer (120-140m) was 22-27, which implies that in this region of diffused/advected supply of nutrients DIP across the nutricline is always in short supply relative to nitrate. 4) A further signal that DIP was the limiting nutrient, was the detection of APA, which was regenerating DOP into DIP at rates similar to that of removal of DIP. This fact also suggests that DOP is ultimately the main source of P for photosynthetic processes, and that DOP could be the limiting fraction of the P present instead of DIP; i.e. when uptakes rates of P are higher than the production of labile DOP and DIP is at levels below 1 nM, DOP will limit the growth. The question about whether labile DOP could be made biologically available by direct uptake or enzymatic action is open.

Data on export fluxes of particulate P out of the euphotic layer were limited, but the information obtained indicated that P is escaping from the euphotic zone to deeper waters at rates of 10-92 $\mu mol.m^{-2}$. However, the import fluxes calculated by a Fickian diffusion model were only up to 20 $\mu mol.m^{-2}$, thus contravening the idea of a steady state of the euphotic layer, if only these sources and sinks were used. The steady state concept is appropriate on a long time scale and thus the

application of the Fickian model appears inadequate on short time scales. Thus in this case, the need for more dynamical model was demonstrated.

The data from the above paragraph would initially corroborate the thesis that rates of "new production" are about 16 % of the total production. However, because the mass of P imported into the euphotic layer originates in the 120-200m layer in which the DIP was found to be almost 100 % of oxidative origin, thus the "new production" supported by DIP is nearly 0 % when the term "new production" is strictly applied (i.e. that production which is dependent on nutrients which are not of oxidative origin).

The first deep profiles of DOP and dissolved PPs demonstrate the presence of measurable amounts of these materials in meso- and bathy-pelagic waters.

5.5 Future work

There is a significant and diverse amount of research to be made in relation to the fate of P in the water bodies studied, however the more urgent research is proposed below.

From the analytical point view of the uncertainties about the accuracy of the DPT and PTP techniques have to be elucidated. Perhaps invoking and applying the principles of the new methods for DOC and DON will help to solve this problem. A detailed study on sampling, handling, and storage of estuarine and marine water samples for DOP and PTP analysis has also to be undertaken.

Determination of DOP and PTP in the estuaries have to be done in a more routine manner. For the case of English estuaries, a trial sampling and analysis of water samples for the most important estuaries could be made in order to find out if results for the estuaries studied in his work are commonplace. The study of the different forms of P has to be continued with a more frequent sampling. The study in the

field of removal of DIP and regeneration of DOP into DIP by biological and chemical processes, and also added to this work, laboratory experiments on physical-chemical removal of DIP would help to understand in a better way the biogeochemistry of P. Inter-disciplinary studies should be encouraged, thus for instance to determine in a detailed manner the relationship between sedimentary, biological and chemical processes.

Regarding the study of P in the surface waters of the Sargasso Sea, the work basically have to be repeated again with more schematic coverage of typical variability in order to answer the following questions:

i) What are the diurnal and seasonal changes in nanomolar concentrations in the Sargasso Sea?

ii) What is the significance of dissolved and particulate (including ATP) poly-phosphates in the regeneration of DIP?

iii) In conjunction with other measurements of chemical and biological parameters, is the Redfield stoichiometry of C:N:P for dissolved inorganic and organic components of the major biologically active elements valid?

iv) What are the dynamics of DIP relative to primary production?

In the latter question, the application of the LCC technique to measuring *in situ et vitro* removal of DIP should be resorted and results compared to those obtained using traditional radio-active techniques. When working on this careful attention must be paid to regeneration processes because it was demonstrated that such processes are acting at very high rates.

The nitrate limitation in oceanic waters concept and "new" production rates previously reported have been challenged in this work. However whilst the set of data presented was

comprehensive it is relatively small. Therefore replication of measurements should be made. It is also suggested to take new directions in studying nutrient limitation and new production. Thus for instance, the origin of the advected/diffused nutrients into the mixed should be elucidated.

The investigation of the chemical structure of the DOP including PPs should be undertaken, especially in this sea, where the DOP is perhaps completely originated *in situ*. Added to this the dynamics of the different fractions of DOP must be studied in relation to biological processes, in order to elucidate the question about DIP *vs* DOP limitation.

Regarding APA its nature and phases (particulate or dissolved) are an important research topics to be undertaken. Spatial and temporal variability of APA have to be elucidated as well as the presence of other enzymes such as the 5'-nucleotidase.

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	X	SD	RSD	DE	n	X	SD	RSD	DE	n
	DDW					NW				
pPn	2.03±.05		2.5	101±0.	6	1.85±.06		3.2	92±3.2	6
G-P	1.75±.41		23	88±2.0	6	1.90±.17		9.2	95±8.7	4
PP	1.97±.04		2.0	99±2.0	6	1.89±.08		4.2	94±4.0	6
MP	1.68±.13		7.5	84±6.4	5	1.75±.29		16	97±3.1	6
G-6-P	1.96±.11		5.7	90±0.0	6	1.96±.14		7.1	89±6.3	6
npP	2.01±.13		6.4	80±5.2	6	1.65±.26		6.4	80±5.2	6
ATP	1.94±.13		6.7	97±6.3	6	1.71±.16		9.3	85±8.0	6
GDP	1.38±.08		5.5	69±4.2	6	2.06±.24		12.	103±12	6

Appendix 2*.a. Performance of method Nit-ox. X Average of the spiked organic-P (in μM) transformed to inorganic P. SD: Standard deviation. RSD relative standard deviation (%). n duplicate number. DE decomposition efficiency (%). DDW and NW are the water background spiked with the organic P, and are distilled de-ionized and natural (from Southampton Water) waters respectively. Symbols for the compounds see section 2.2.

*: Appendix 1 does not exist.

	X	SD	RSD	DE	n	X	SD	RSD	DE	n
Comp.	DDW					NW				
pPn	2.03±.08		4.0	101±0.0	4	1.78±.14		7.8	89±6.9	6
G-P	1.89±.13		7.2	94±6.7	5	2.08±.05		2.8	90±2.7	5
PP	1.80±.08		4.4	89±4.1	5	1.87±.05		2.8	94±2.6	5
MP	1.72±.06		3.7	86±4.2	6	1.94±.06		3.2	97±3.1	6
G-6-P	2.03±.06		3.2	92±2.7	6	2.19±.05		2.4	99±2.5	5
npP	2.23±.06		2.9	89±2.4	6	2.45±.05		2.9	98±2.0	6
ATP	1.87±.04		2.4	94±2.2	6	1.95±.15		7.0	97±5.5	6
GDP	1.48±.17		12	74±8.7	6	1.68±.13		8.0	84±6.7	6

Appendix 1.b Performance for High-t method. Abbreviations similar to 1.a.

	X	SD	RSD	DE	n	X	SD	RSD	DE	n
Comp.	DDW					NW				
pPn	1.83±.16		4.	91±8.1	6	1.38±.11		7.9	89±6.9	6
G-P	1.46±.14		9.5	73± 7.0	6	1.79±.05		2.8	90±2.7	5
PP	1.86±.15		8.0	93±7.0	6	1.16±.09		7.4	58±4.3	6
MP	1.89±.06		4.5	94±8.1	6	1.40±.06		4.5	70±3.2	6
G-6-P	2.05±.09		4.4	93±4.1	5	1.93±.16		8.1	88±7.3	5
npP	2.44±.13		5.4	98±9.6	6	2.12±.06		2.8	85±2.4	6
ATP	2.13±.05		2.4	106±2.6	4	1.81±.11		2.4	91±5.5	4
GDP	1.76±.06		3.4	88±3.0	6	1.48±.16		3.4	74±2.6	6

Appendix 1.c Performance for the method Ac-c. Abbreviations similar to 1.a.

	X	SD	RSD	DE	n	X	SD	RSD	DE	n
Comp.			DDW					NW		
pPn	1.92	±.19	4.9	96±9.0	6	2.00	±.09	4.8	100±4.8	6
G-P	1.66	±.18	11	83±9.1	4	2.03	±.09	4.4	101±4.3	6
PP	1.29	±.14	11	64±7.2	5	1.20	±.07	6.2	60±3.7	5
MP	0.55	±.06	10	27±2.8	4	1.71	±.07	4.1	85±3.5	6
G-6-P	2.06	±.01	0.7	94±0.4	3	2.22	±.07	3.3	101±3.5	6
npP	1.92	±.24	12	77±9.6	6	2.47	±.06	2.6	99±2.4	5
ATP	1.36	±.17	13	68±8.6	5	1.07	±.01	0.8	54±0.4	6
GDP	1.66	±.16	9.8	83±8.6	5	1.24	±.13	10	62±6.3	6

Appendix 1.d Performance of the Al-c method. Abbreviations similar to 1.a.

	X	SD	RSD	RE	n	X	SD	RSD	DE	n
Comp.			DDW					NW		
pPn	2.12	±.06	2.8	106±3.0	6	1.06	±.08	5.1	82±4.2	6
G-P	1.91	±.64	3.8	96±2.0	6	2.08	±.12	5.6	104±5.8	6
PP				18±2.7	6	Not performed.				
MP	0.37	±.09		18±4.6	6	0.06	±.01	16.7	03±0.5	6
G-6-P	2.20	±.05	2.3	100±2.3	6	2.21	±.04	1.9	100±1.8	6
npP	2.27	±.12	5.5	91±4.8	6	Not performed.				
ATP	0.41	±.05	12	20±2.5	6	0.41	±.05	13	21±2.7	6
GDP	0.25	±.11	43	12±6.4	4	0.08	±.02	26	4.0±1.0	6

Appendix 1.e Performance of the UV-ox method. Abbreviations similar to 1.a.

Salinity	DIP [µM]	DTP [µM]	DOP [µM]	PTP [µM]	POC [µM]	POC/PTP	DOC (µM)
0.400	8.070	11.800	3.730	1.060	88.000	83.019	
3.800	19.900	20.400	0.500	1.340	108.000	80.597	325.8
6.700	20.100	21.700	1.600	1.580	107.000	67.722	333.3
8.300	18.700	18.800	0.100	1.810	108.000	59.669	
10.800	12.900	13.000	0.100	1.460	89.000	60.959	
12.300	15.900	16.100	0.200	1.010	73.000	72.277	
15.400	13.600	13.500	0.025	1.020	73.000	71.569	
17.400	12.300	12.000	0.025	1.220	34.000	27.869	431.7 ??
19.600	10.500	10.600	0.100	0.940	40.000	42.553	
20.000	10.400	10.600	0.200	1.360	39.000	28.676	149.2
22.900	9.010	9.010	0.025	1.330	33.000	24.812	
23.900	8.500	8.450	0.025	1.350	41.000	30.370	
24.600	7.410	7.690	0.280	1.340	36.000	26.866	
27.800	5.430	5.760	0.330	1.040	32.000	30.769	130.0
29.000	4.580	4.960	0.380	1.070	27.000	25.234	130.0
30.300	3.020	3.590	0.570	0.930	19.000	20.430	118.3

3.1 a

Append. 3.1 a, b, c, and d. Data for Fig. 3.3, 3.4, 4.5 and 3.6 a and b.

Salinity	DIP [µM]	DTP [µM]	DOP [µM]	DOP/DTP (%)	PTP [µM]	POC [µM]	POC/PTP	Cha (µg/dm^3)	Phae (µg·dm^{-3})	Cha (%)
0.500	26.400	28.300	1.900	6.700	1.090	142.000	130.275	2.540	0.700	78.300
0.930	37.000	41.100	4.100	10.000	1.550	122.000	78.710	2.020	2.320	46.540
2.070	52.600	61.600	9.000	15.000	2.590	154.000	59.459	1.760	2.060	46.000
3.980	50.400	59.900	9.500	16.000	2.320	153.000	65.948	1.180	0.930	22.990
6.000	55.000	64.900	9.900	15.000	2.740	143.000	52.190	3.020	1.650	64.700
9.000	42.800	46.300	3.500	7.600	2.970	135.000	45.455	2.400	2.160	52.630
9.560	32.100	33.500	1.400	4.200	3.120	144.000	46.154	5.280	2.470	68.110
11.940	32.400	32.400	0.000	0.000	3.150	162.000	51.429	5.180	1.660	75.790
16.000	23.800	24.300	0.500	2.000	4.270	162.000	37.939	8.250	2.460	77.040
16.900	22.100	22.300	0.200	1.000	2.260	147.000	65.044	4.030	2.580	60.980
22.560	16.300	17.100	0.800	0.400	2.190	180.000	82.192	13.150	1.440	90.130
22.920	13.700	15.000	1.300	8.700	2.120	207.000	97.642	16.790	2.010	89.310
26.510	9.960	10.500	0.540	5.000	1.390			9.400	1.420	86.870
28.840	7.220	8.170	0.950	1.200	2.440	190.000	77.869	21.110	3.960	84.210
30.180	4.840	5.300	0.460	8.700	2.490	160.000	64.257	17.270	2.100	89.160
31.810	3.370	3.950	0.580	17.000	2.780			14.870	3.360	81.580
32.740	1.350	3.170	1.820	57.000	2.960	151.000	51.014	12.960	2.430	84.210
33.400	0.680	0.880	0.200	23.000	1.450	150.000	103.448	12.480	2.340	84.210

3.1 b

Salinity	Temperature [°C]	DIP [µM]	DIP [µM] error	DTP [µM]	DTP [µM] error*	DOP [µM]
0.02	12.00	19.70	0.01	19.80	0.02	0.10
1.03	13.10	25.50	0.00	26.50	0.10	1.00
4.55	14.10	46.40	0.00	48.20	0.00	1.80
8.07	14.00	36.20	0.02	36.60	0.02	0.10
9.05	14.20	25.60	0.01	26.20	0.05	0.60
13.86	14.60	21.80	0.05	22.60	0.00	0.80
15.72	14.70	18.30	0.00	19.10	0.02	0.80
18.99	15.00	14.70	0.00	15.60	0.04	0.90
22.74	15.20	10.80	0.10	12.41	0.05	1.60
23.99	15.40	12.90	0.00	13.10	0.01	0.20
25.47	15.50	11.60	0.00	12.20	0.00	0.60
28.78	15.90	6.37	0.00	6.09	0.02	< d.l.
30.28	16.10	6.36	0.00	6.93	0.02	0.57
31.93	16.40	5.22	0.00	4.65	0.00	< d.l.
33.91	16.40	3.33	0.00	3.52	0.00	0.19
33.33	16.50	2.75	0.00	2.66	0.02	< d.l.

PTP [µM]	PTP [µM] error	POC [µM]	POC/PTP	Chla [µg/dm^3]	Phae µg·dm^{-3}	Chl-a %
0.24	0.03	68.00	107.46			
0.17	0.00	92.00	73.53			
0.44	0.08		83.87			
0.32	0.00	112.00	31.68	1.44	0.61	70.00
1.45	0.04	97.00	72.03	1.78	1.42	56.00
1.58	0.04		67.69	2.06	1.70	55.00
1.73	0.00	79.00	68.13	2.16	1.72	56.00
1.62	0.40	79.00		2.35	1.35	63.00
		94.00	48.77			
0.91	0.00	62.00	45.66	0.77	1.17	40.00
1.30	0.24	88.00		2.88	0.54	84.00
1.18	0.20	85.00	66.90	1.73	1.12	61.00
1.61	0.02	51.00	350.00	1.66	0.77	68.00
1.02	0.20	75.00		1.73	1.12	61.00
0.62	0.00	52.00	541.18	1.15	0.79	59.00
0.67	0.00	72.00	283.33			

3.1c

* Error is ± ½ of the difference of two duplicates

	Salinity	DIP[µM]	DIP [µM] error	DTP [µM]	DOP [µM]	DOP/DTP	PTP [µM]
1	0.250	7.110	0.000	8.140	1.030	0.127	9.950
2	0.280	7.300	0.030	7.350	0.050	0.007	6.970
3	0.600	10.700	0.030	11.400	0.700	0.061	4.780
4	0.610	10.900	0.100	9.780	0.025	0.003	5.990
5	1.380	13.100	0.000	13.100	0.000	0.000	7.400
6	7.080	11.600	0.110	11.000	0.025	0.002	7.290
7	19.840	9.920	0.070	10.200	0.280	0.027	2.420
8	24.420	8.310	0.050	8.290	0.025	0.003	1.850
9	25.910	5.010	0.010	4.960	0.025	0.005	1.940
10	32.250	3.240	0.000	3.200	0.025	0.008	2.860
11	32.470	2.530	0.030	2.490	0.025	0.010	0.700
12	32.790	2.120	0.000	2.080	0.025	0.012	2.060
13	33.530	1.610	0.010	1.630	0.020	0.012	0.750

	PTP [µM] error	POC [µM]	POC/PTP	Chl-a ($\mu g \cdot dm^{-3}$)	Phae ($\mu g \cdot dm^{-3}$)	Cla-a %
1	0.000	437.000	43.920	12.090	10.130	54.000
2	0.070	412.000	59.110	7.860	5.850	57.000
3	0.000	442.000	92.469	4.460	4.770	48.000
4	2.000	446.000	74.457	5.330	4.250	56.000
5	1.000	425.000	57.432	3.510	4.970	41.000
6	0.500	358.000	49.108	3.170	4.010	44.000
7	0.300	204.000	84.298	1.300	3.150	29.000
8	0.000			0.710	1.170	38.000
9	0.000	122.000	62.887	1.130	0.950	54.000
10	0.000	102.000	35.664	0.540	1.210	30.000
11	0.000	73.000	104.286	0.500	0.630	44.000
12	0.140	77.000	37.379	0.690	0.470	60.000
13	0.000	125.000	166.667	0.860	1.260	40.000

3.1d

Salinity	Jul.89	Salinity	Nov.88	Salinity	Oct.89	Salinity	Dec.89
0.500	6.700	0.400	31.600	0.020		0.250	12.700
0.930	10.000	3.800	2.450	1.030	3.800	0.280	0.700
2.070	15.000	6.700	7.370	4.550	3.700	0.600	6.100
3.980	16.000	8.300	0.530	8.070	0.300	0.610	0.300
6.000	15.000	10.800	0.770	9.050	2.300	1.380	0.000
9.000	7.600	12.300	1.240	13.860	3.500	7.080	0.200
9.580	4.200	15.400	0.000	15.720	4.200	19.840	2.700
11.940	0.000	17.400	0.000	18.990	5.800	24.420	0.300
16.000	2.000	19.600	0.940	22.740	12.900	25.910	0.500
16.900	1.000	20.000	1.890	23.990	1.500	32.250	0.800
22.560	0.400	22.900	0.000	25.470	4.900	32.470	1.000
22.960	8.700	23.900	0.000	28.780	0.400	32.790	1.200
26.510	5.000	24.600	3.640	30.280	8.200	33.330	1.200
28.840	1.200	27.800	5.730	31.930	0.500		
30.180	8.700	29.000	7.660	32.910	5.400		
31.810	17.000	30.300	15.880	33.330	0.900		
32.740	57.000						
33.400	23.000						

Append. 3.2 Data for Fig. 3.4 c.

Depth (m)	DIP [µM]	DTP [µM]	DOP [µM]	PTP [µM]	POC [µM]	POC/PTP
0.000	0.680	0.880	0.200	1.450	150.000	103.448
2.000	0.730	1.950	1.220	4.750	283.000	59.579
4.000	0.930	1.090	0.160	1.840	154.000	83.696
6.000	0.630	0.950	0.320	1.310	139.000	106.107
8.000	0.870	1.050	0.180	2.400	149.000	62.083

	Cha (µg/dm^3)	Phaeo µg·dm^{-3}	Cha (%)
1	12.480	2.340	84.210
2	52.780	9.900	84.210
3	12.570	1.670	88.250
4	9.790	2.520	79.530
5	8.160	2.670	75.350

3.3a

Depth (m)	DIP [µM]	DIP [µM] error	DTP [µM]	DTP [µM]	DOP [µM]	PTP [µM]
0.00	2.75	0.00	2.76	0.02	0.01	0.67
2.00	1.71	0.05	1.81	0.00	0.10	1.37
4.00	1.71	0.05	2.00	0.01	0.29	0.86
6.00	2.47	0.00	2.47	0.02	0.03	1.30
8.00	2.47	0.00	2.75	0.00	0.28	1.02

	PTP [µM] error	POC [µM]	POC/PTP	Chl-a µg·dm^{-3}	Phaeo µg·dm^{-3}	Cha (%)
1	0.00	72.00	107.46			
2	0.08	65.00	47.45	1.06	0.65	61.75
3	0.40	68.00	79.07	0.96	1.15	45.52
4	0.02	71.00	54.62	1.10	1.18	48.42
5	0.08	168.00	164.71			

3.3b

Depth (m)	DIP [µM]	DIP [µM] error	DTP [µM]	DTP [µM] error	DOP [µM]	PTP [µM]
0.00	1.61	0.01	1.63	0.02	0.02	0.75
3.00	1.75	0.00	1.78	0.00	0.03	0.97
6.00	1.77	0.01	1.74	0.01	0.03	1.77
8.00	1.85	0.01	1.85	0.01	0.00	2.15

	PTP [µM] error	POC [µM]	POC/PTP	Chl-a µg·dm^{-3}	Phaeo µg·dm^{-3}	Cha (%)
1	0.00	125.00	166.67	0.86	1.26	40.75
2	0.00	137.00	141.24	0.75	0.96	43.79
3	0.01	142.00	80.23	0.86	1.19	42.11
4	0.00	135.00	62.79	0.81	1.66	32.75

3.3c

Append. 3.3 Data for Fig. 3.7 a, b, and c.

Salinity	DIP [µM]	DTP [µM]	DOP [µM]	PTP [µM]	POC [µM]	POC/PTP	Chl-a(µg/dm^3)	Phae(µg/dm^3)	Cha (%)
16.200	0.060	0.550	0.490	2.580	201.000	77.907	18.230	12.540	59.260
16.310	0.070	0.650	0.580	3.560	256.000	71.910	23.220	20.990	32.990
16.930	0.090	0.560	0.470	3.040	256.000	84.211	31.670	31.670	23.030
17.600	0.060	0.550	0.490	1.710	129.000	75.439	14.870	7.350	66.940
21.500	0.080	0.600	0.520	3.150	180.000	57.143	26.390	6.660	79.850
22.800	0.100	0.730	0.630	2.480	225.000	90.726	16.310	3.060	84.250
23.000	0.180	0.570	0.390	1.700	180.000	105.882	11.230	4.500	71.400
23.930	0.070	0.570	0.500	2.270	194.000	85.463	12.000	1.680	87.720
26.000	0.150	0.670	0.520	2.820	210.000	74.468	21.110	2.820	88.220
30.010	0.100	0.650	0.550	1.940	185.000	95.361	7.560	0.510	93.700
30.370	0.100	0.660	0.560	0.970	87.000	89.691	3.980	1.260	75.970
31.140	0.160	0.790	0.630	0.590	72.000	122.034	2.590	1.570	62.290
31.590	0.170	0.660	0.490	0.980	57.000	58.163	2.780	1.430	66.000
32.230	0.130	0.680	0.550	0.770	69.000	89.610	2.740	1.250	68.570
32.990	0.180	0.670	0.490	1.310	69.000	52.672	2.740	1.940	58.540
34.090	0.260	0.680	0.420	0.480	49.000	102.083	1.200	1.880	38.990
34.570	0.230	0.560	0.330	0.750					

3.4a

Append. 3.4 Data for Fig. 3.8, and 3.9 a, b, and c

Salinity	T (°C)	DIP [µM]	DIP [µM] error	DTP [µM]	DTP [µM]	DOP [µM]
0.280	12.000	0.860	0.000	1.240	0.020	0.380
11.200	12.000	1.800	0.000	2.590	0.000	0.790
18.490	13.000	0.280	0.000	0.620	0.010	0.340
20.270	13.100	0.180	0.000	0.520	0.020	0.340
20.790	13.600	0.210	0.000	0.530	0.120	0.320
23.080	13.700	0.260	0.000	0.670	0.050	0.410
24.790	14.000	0.280	0.000	0.670	0.020	0.390
30.750	14.000	0.690	0.000	1.160	0.050	0.470
31.250	14.600	0.460	0.000	0.920	0.020	0.460
32.180	14.850	0.540	0.000	1.060	0.040	0.520
32.700	14.900	0.530	0.000	0.920	0.010	0.390
33.480	15.100	0.590	0.000	1.230	0.000	0.640
34.910	15.700	0.870	0.000	1.210	0.100	0.340

DOP/DTP (%)	PTP [µM]	PTP [µM]	POC/PTP	Chl-a $\mu g \cdot dm^{-3}$	Phaeo $\mu g \cdot dm^{-3}$
31.000	1.020	0.620	85.980	4.130	1.000
30.000	2.610	0.030	205.390		
55.000	1.990	0.000		8.350	2.820
65.000	1.000	0.030	96.000	5.090	2.320
60.000	2.870	0.000	89.130	6.910	2.550
61.000	2.290	0.000	95.710	6.910	0.840
58.000	1.180	0.060		5.370	2.490
40.000	1.940	0.080	79.060		
50.000	1.900	0.090		1.920	0.700
49.000	1.850	0.020	35.150	1.440	0.550
42.000	1.940	0.060	40.060	1.150	0.560
52.000	0.950	0.000	31.050	1.000	0.480
28.000	1.580	0.000	65.420	1.820	1.250

Cha (%)
80.000
75.000
69.000
73.000
89.000
68.000
73.000
72.000
67.000
67.000
59.000

3.4b

Salinity	T (°C)	DIP [µM]	DIP [µM]$_{error}$	DTP [µM]	DTP [µM]$_{error}$	DOP [µM]
	7.000	0.190	0.000	0.960	0.040	0.770
0.420	7.000	0.300	0.010	1.130	0.110	0.830
0.620	7.500	0.760	0.010	1.030	0.010	0.270
4.390	7.500	0.290	0.050	0.700	0.000	0.410
6.030	7.300	0.560	0.010	1.180	0.070	0.620
8.950	7.600	0.610	0.010	1.040	0.030	0.430
12.310	8.200	0.630	0.030	0.920	0.190	0.290
17.170	8.200	0.870	0.010	0.940	0.010	0.070
21.540	8.300	0.630	0.010	1.060	0.020	0.430
21.970	8.300	0.700	0.010	1.500	0.010	0.800
24.240	8.300	0.560	0.040	1.170	0.030	0.610
26.660	8.300	0.830	0.020	0.970	0.000	0.140
27.860	8.300	0.720	0.020	1.160	0.020	0.440
29.880	8.400	0.700	0.010	1.320	0.010	0.620
33.340	8.600	0.790	0.010	1.170	0.020	0.380
33.800	8.700	0.850	0.010	1.220	0.060	0.370

DOP/DTP	PTP [µM]	PTP [µM]$_{error}$	POC [µM]	POC/PTP	Chl-a (µg·dm^{-3})	Phae (µg·dm^{-3})
0.802	1.470	0.170	148.000	100.680	1.130	2.700
0.735	2.500	0.570	164.000	65.600		
0.262			147.000		7.050	5.770
0.586			292.000		1.470	4.170
0.525			168.000		1.840	3.800
0.413	2.280	0.000	154.000	67.544	2.020	3.910
0.315	1.370	0.000	133.000	97.080	1.940	4.120
0.074					1.500	2.880
0.406						
0.533	1.000	0.000	100.000	100.000	1.380	2.450
0.521	2.740	0.290				
0.144	1.950	0.040	200.000	102.564	0.920	2.090
0.379	1.860	0.110			0.920	1.700
0.470	1.200	0.100	59.000	49.167	1.150	1.720
0.325	2.490	0.000	120.000	48.193	1.380	1.900
0.303	2.180	0.000				

Cha (%)
29.000
55.000
26.000
33.000
34.000
32.000
34.000
36.000
31.000
35.000
40.000
42.000

3.4c

	Salinity	Jul.89	Salinity	Oct.89	Salinity	Dec.89
1	16.200	54.000	0.280	31.000	0.200	80.200
2	16.310	52.000	11.200	30.000	0.420	73.500
3	16.930	55.000	18.490	55.000	0.620	26.200
4	17.600	35.000	20.270	65.000	4.390	58.600
5	21.500	52.000	20.790	60.000	6.030	52.500
6	22.800	33.000	23.080	61.000	8.950	41.300
7	23.000	26.000	24.790	58.000	12.310	31.500
8	23.930	41.000	30.750	40.000	17.170	7.400
9	26.000	61.000	31.250	50.000	21.540	40.600
10	30.010	7.500	32.180	49.000	21.970	53.300
11	30.370	33.500	32.700	42.000	24.240	52.100
12	31.140	41.000	33.480	52.000	26.660	14.400
13	31.590	54.000	34.910	28.000	27.860	37.900
14	32.230	59.000			29.880	47.000
15	32.990	45.000			33.340	32.500
16	34.090	41.000			33.800	30.300
17	34.570	54.000				

Append. 3.5 Data for Fig. 3.9 c.

	Salinity	DIP [µM]	DTP [µM]	DOP [µM]	PTP [µM]
1	19.280	44.600	44.400	0.025	5.200
2	19.490	42.400	43.000	0.600	4.190
3	19.650	45.600	46.400	0.800	5.550
4	19.710	44.800	45.600	0.800	2.600
5	19.770	44.600	45.200	0.600	2.930
6	20.030	43.100	47.200	4.100	6.110
7	21.030	41.800	42.200	0.400	2.760
8	21.340	43.000	43.000	0.025	5.810
9	21.900	40.400	40.900	0.500	6.480
10	22.380	44.600	44.100	0.025	4.880
11	22.960	38.400	38.600	0.200	6.480
12	23.440	37.400	37.400	0.000	5.920
13	25.000	31.100	31.400	0.300	6.160
14	27.860	20.500	20.200	0.025	2.390
15	33.950	2.720	2.520	0.025	0.770
16	33.960	1.720	1.710	0.025	0.380
17	34.010	2.540	2.800	0.260	1.120
18	34.080	1.860	1.830	0.025	1.270
19	34.110	1.910	19.100	17.190	0.310
20	34.150	1.910	1.900	0.025	0.990

3.6a

Append. 3.6 Data for Fig. 3.10 a, b, and c.

	Salinity	Thames	Salinity	Humber
1	19.280	0.000	28.780	54.000
2	19.490	1.400	26.630	41.000
3	19.650	1.700	29.810	45.000
4	19.710	1.700	30.180	59.000
5	19.770	1.300	30.700	54.000
6	20.030	8.700	30.720	41.000
7	21.030	0.900	31.020	33.000
8	21.340	0.000	31.070	7.500
9	21.900	0.200	31.120	61.000
10	22.380	0.000	31.370	41.000
11	22.960	0.500	31.440	26.000
12	23.440	0.000	31.600	33.000
13	25.000	0.900	31.840	52.000
14	27.860	0.000	32.310	35.000
15	33.950	0.000	32.810	55.000
16	33.960	0.000	32.480	52.000
17	34.010	9.200	33.150	54.000
18	34.080	0.000		

3.6c

	Salinity	DIP [µM]	DTP [µM]	DOP [µM]	PTP [µM]
1	28.780	0.400	0.880	0.480	3.310
2	29.630	0.480	0.820	0.340	3.490
3	29.810	0.460	0.840	0.380	6.030
4	30.180	0.360	0.880	0.520	3.020
5	30.700	0.740	1.600	0.860	4.300
6	30.720	0.620	1.060	0.440	4.590
7	31.020	0.940	1.400	0.460	5.550
8	31.070	1.220	0.320	0.025	3.340
9	31.120	0.360	0.920	0.560	3.680
10	31.370	0.900	1.540	0.640	3.840
11	31.440	0.360	0.480	0.120	
12	31.600	0.820	1.220	0.400	1.340
13	31.840	0.680	1.440	0.760	1.390
14	32.310	0.820	1.260	0.440	2.420
15	32.810	0.340	0.760	0.420	1.190
16	32.480	0.860	1.440	0.580	2.240
17	33.150	0.320	0.700	0.380	1.020

3.6 b

Samples	Filters	
	Nuclepore	GF/F
1	5 ± 1	5 ± 1
2	7 ± 2	6 ± 3

Append. 4.1 a) DIP concentration (nM) in water samples filtered through Nuclepore (0.45 μm) and Whatman GF/F (0.70 μm) filters. Samples 1 and 2 were taken at Station "S" (16.Jan.90) and are from 5 and 20m depth. Analysis in triplicate.

Samples	Vacuum pressure (mm-Hg)		
	125	250	375
1	4 ± 2	5 ± 1	6 ± 1
2	6 ± 1	8 ± 3	7 ± 2

Append. 4.1 b) The effect of vacuum pressure filtration on the determination of DIP in the Sargasso Sea. Station "S". Samples as Append. 4.1a. Analysis by triplicate. Concentration in nM.

Appendix 4.1c) Equations given by Redfield *et al.* (1963).

1) Dynamical equation for a non-conservative property.

$$\frac{\partial C}{\partial t} = R + \frac{1}{\rho}\left(K_x \frac{\partial^2 C}{\partial x^2} + K_y \frac{\partial^2 C}{\partial y^2} + K_z \frac{\partial^2 C}{\partial z^2}\right) - \left(V_x \frac{\partial C}{\partial x} + V_y \frac{\partial C}{\partial y} + V_z \frac{\partial C}{\partial z}\right)$$

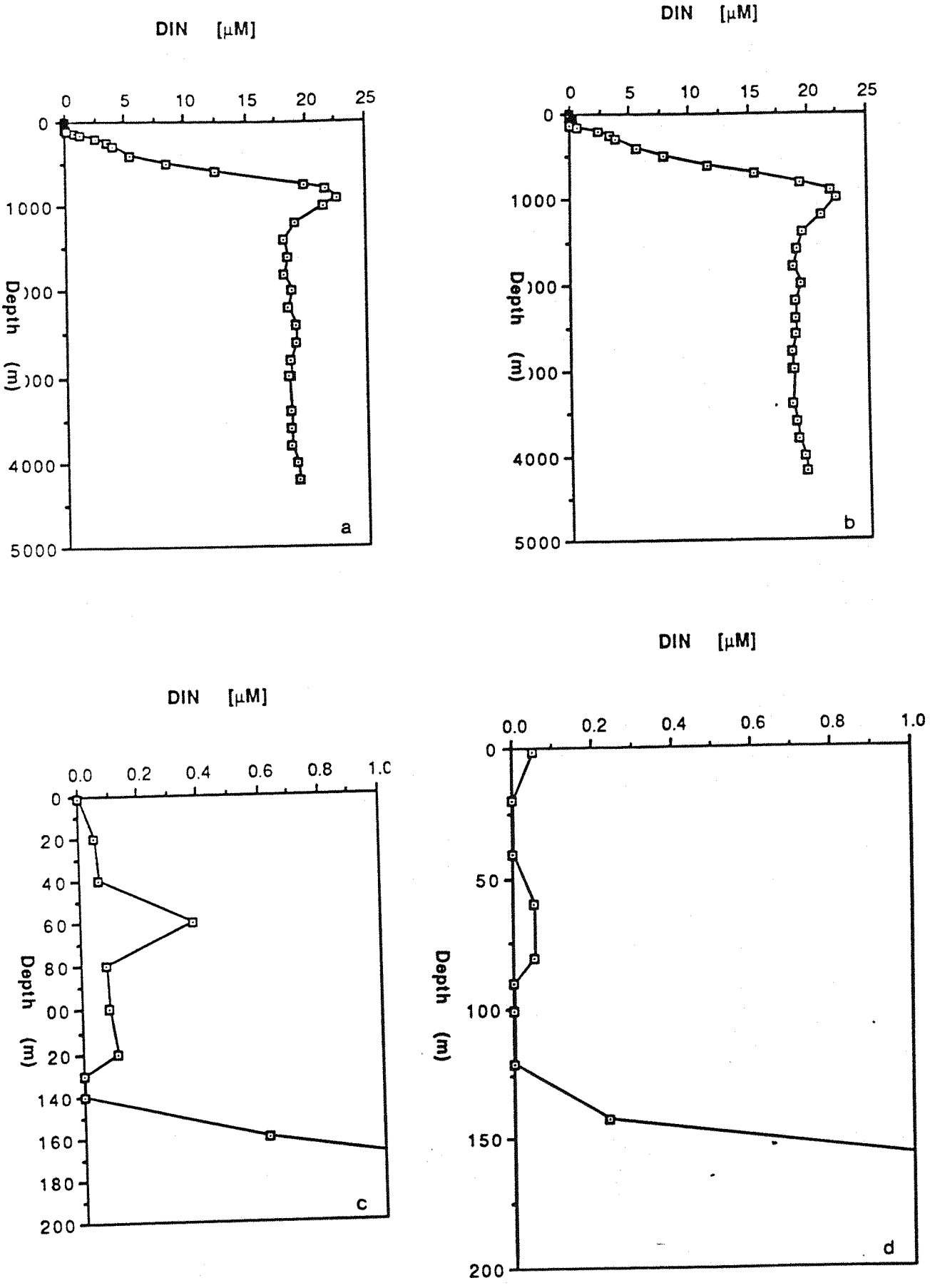
Where:
C: Concentration of a non-conservative property, in this case DIP
t: Time
R:Change of C due to biological processes
ρ: Specific gravity of sea water
$K_{x,y,z}$: Diffusion coefficient in the x-, y-, and z-axis
$V_{x,y,z}$:Component of current velocity in the x-, y-, and z-axis direction

2) Determination of the DIP genetic in sea water

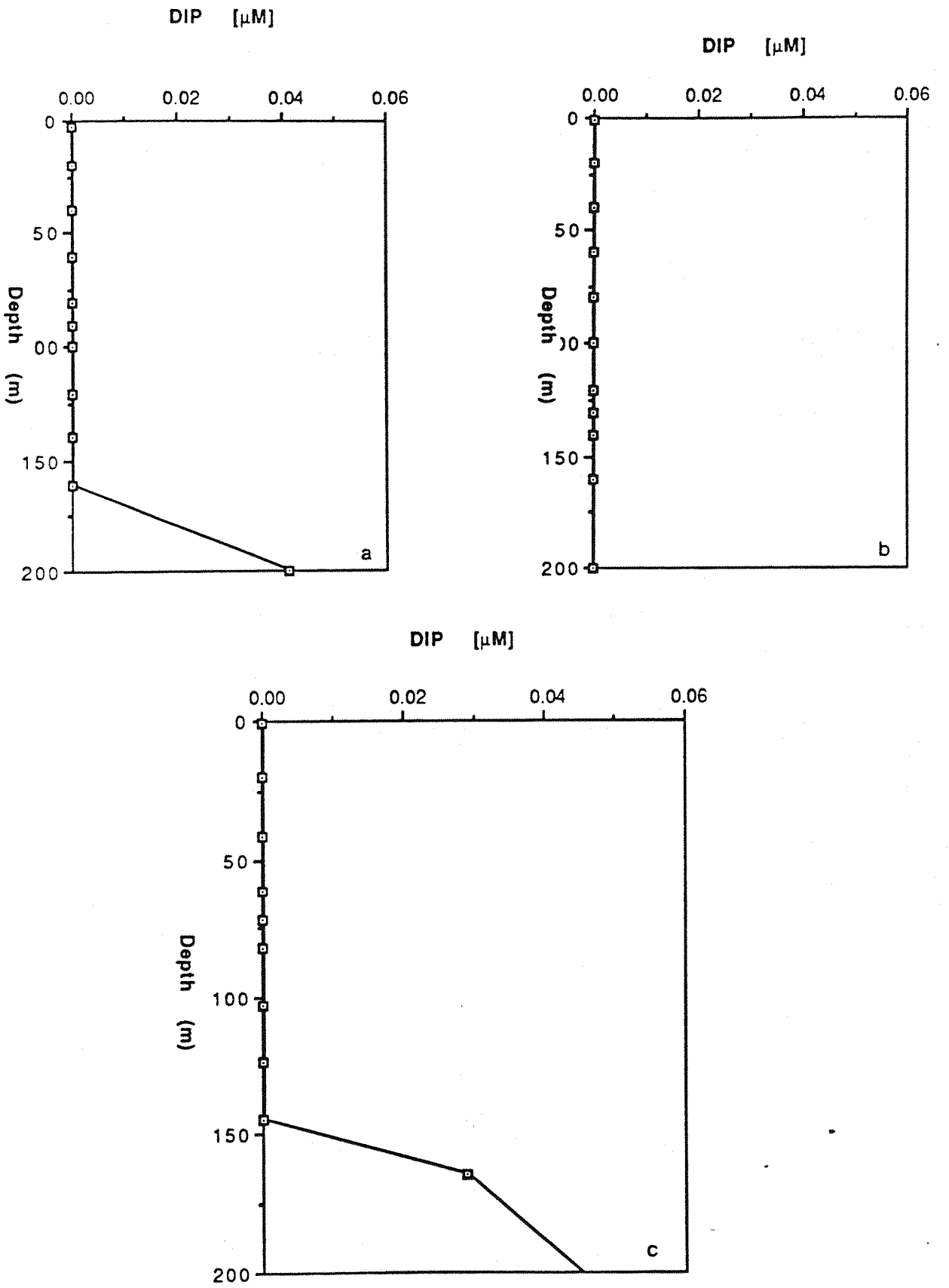
Phosphorus (DIP) of oxidative origin; $P_{ox} = \frac{AOU}{276}$

Preformed phosphorus; $P_p = DIP - P_{ox}$

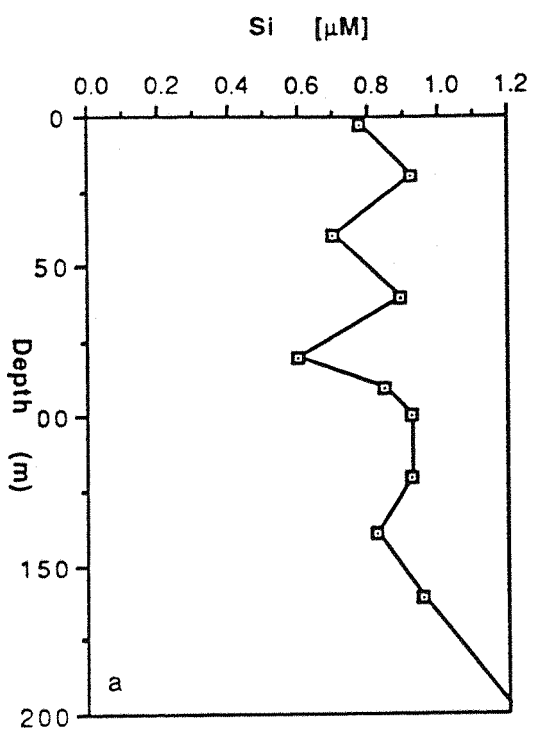
Where:
DIP: is the concentration measured by direct analysis.
AOU: Apparent oxygen utilization.

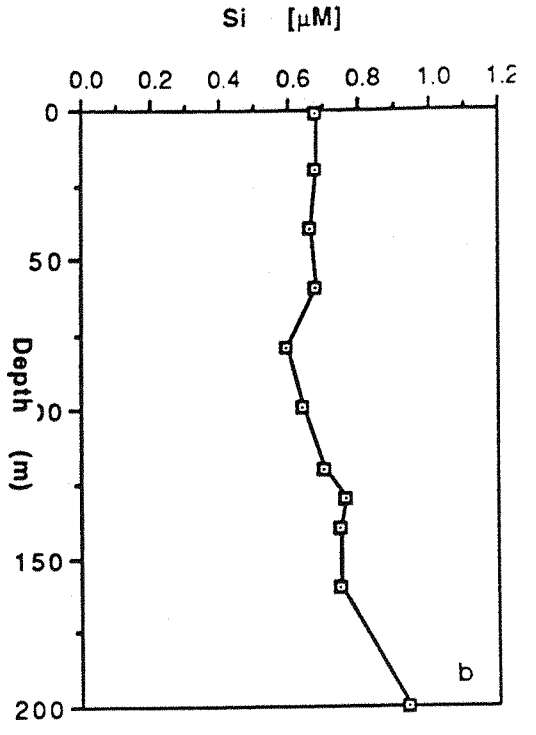


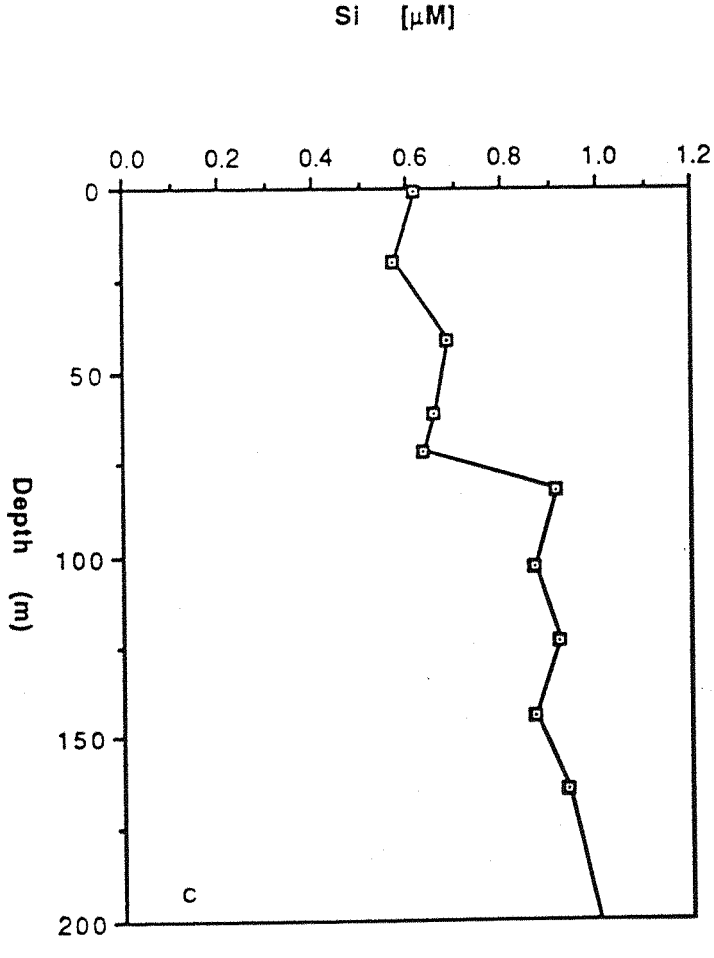
Append. 4.2 Nitrate data. Hereafter a, b, c, and d refer to cruises 1, 2, 3, and 4 respectively. This data is provided by the BBSR and is of preliminary nature.

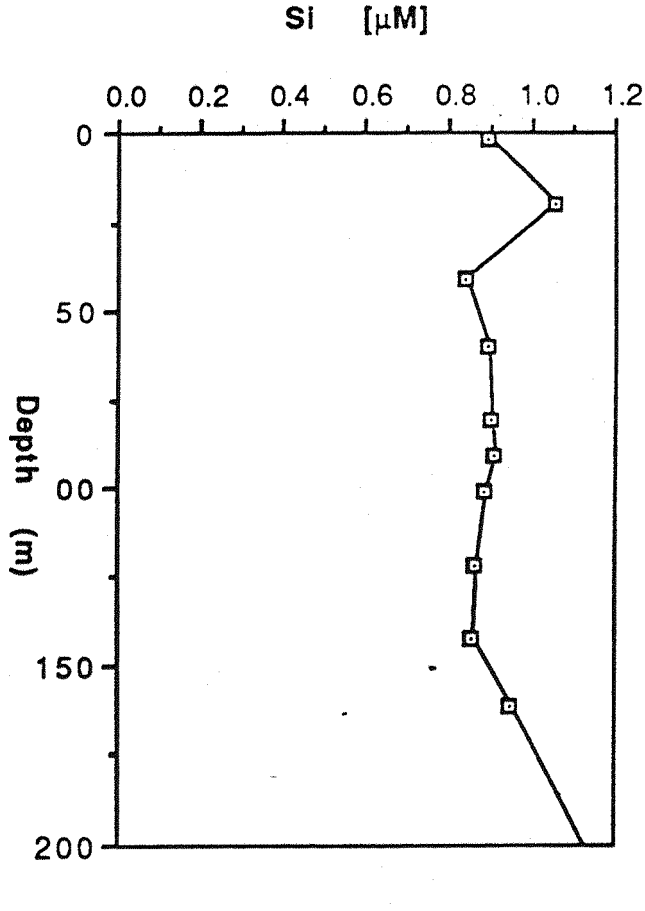


Append. 4.3 Phosphate data.

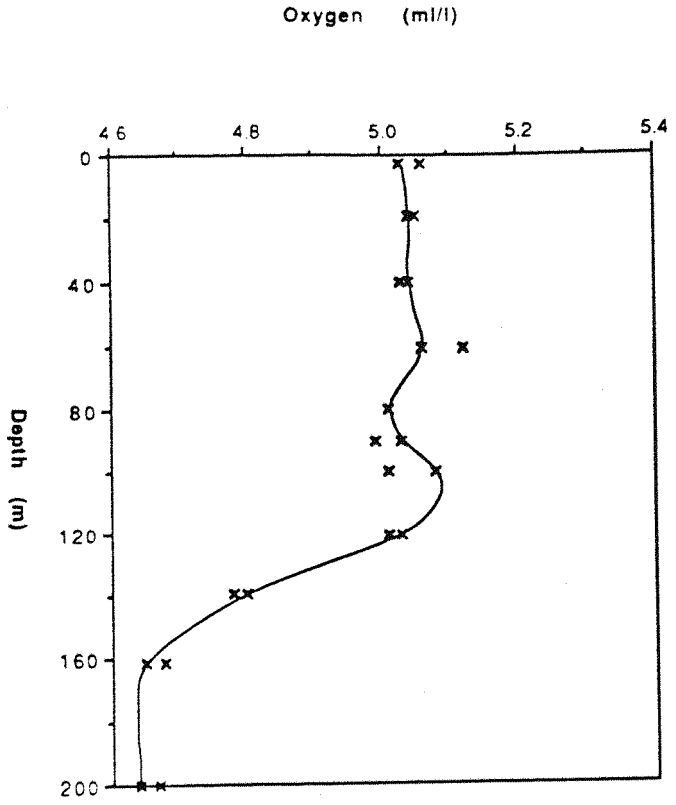


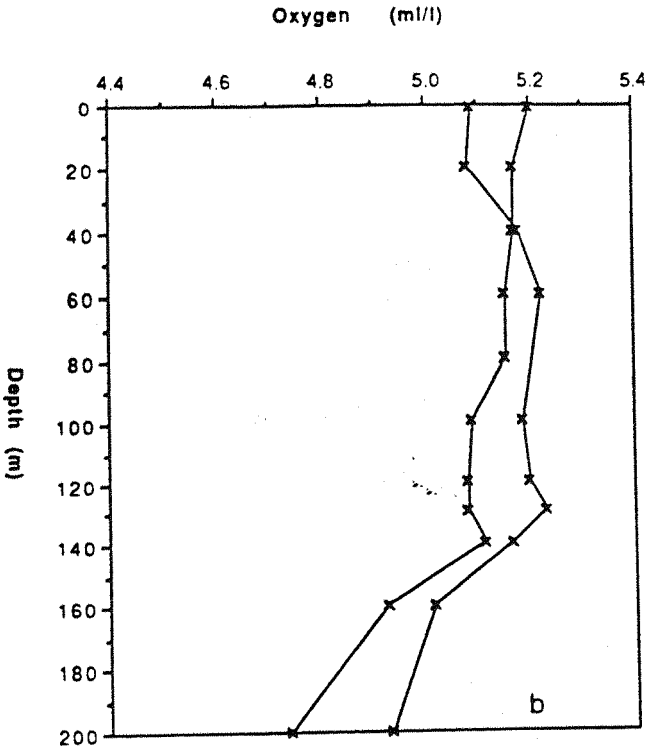


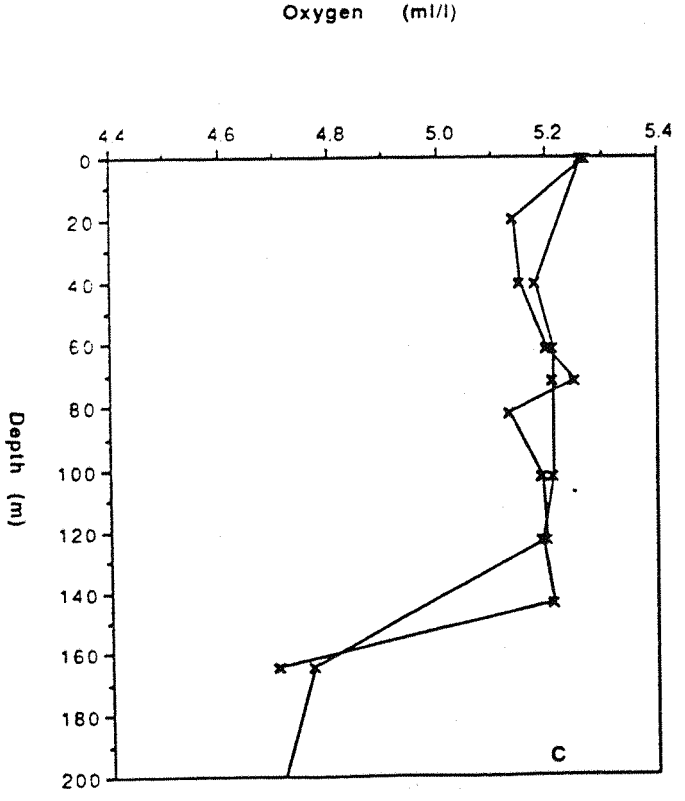


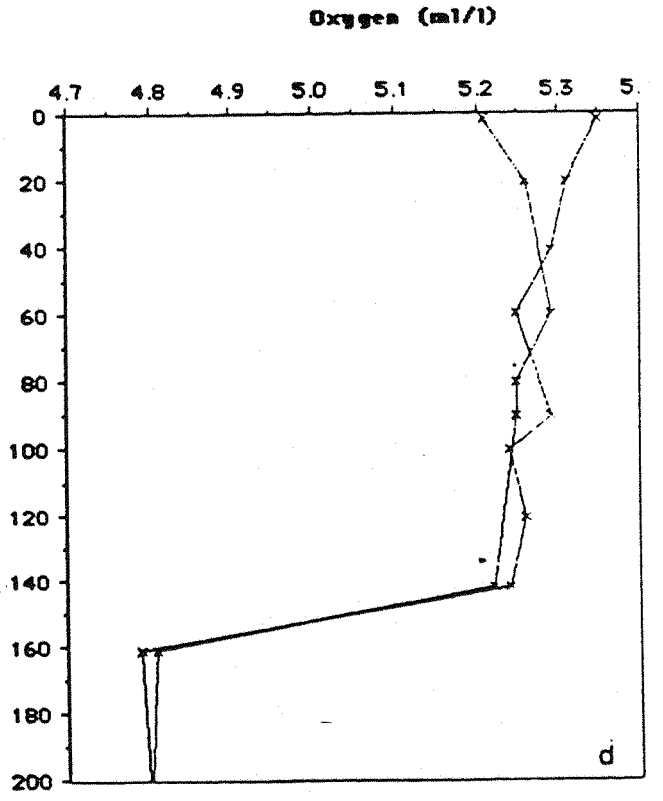


Append. 4.4 Silicate data

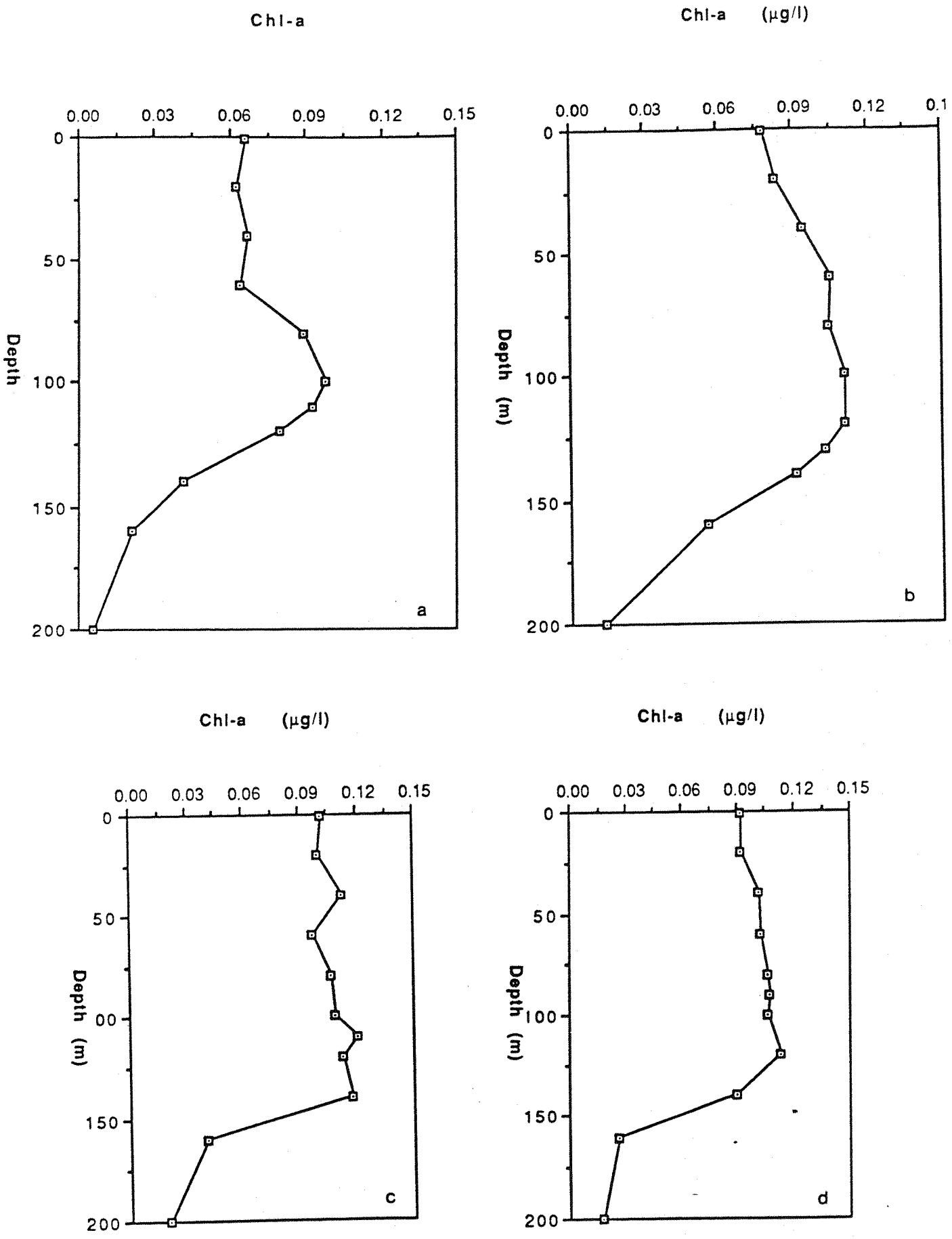




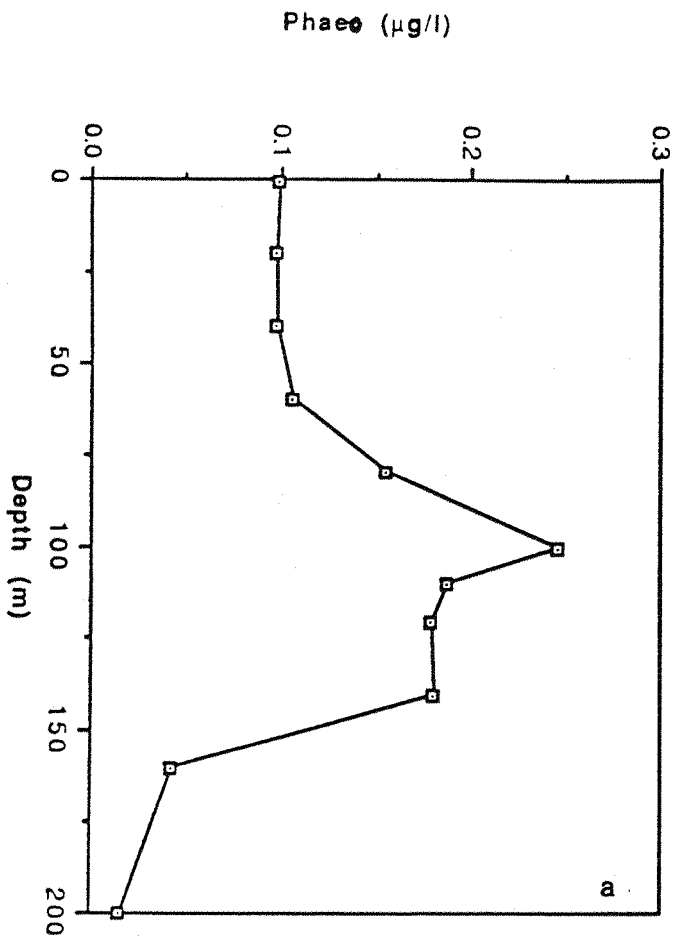


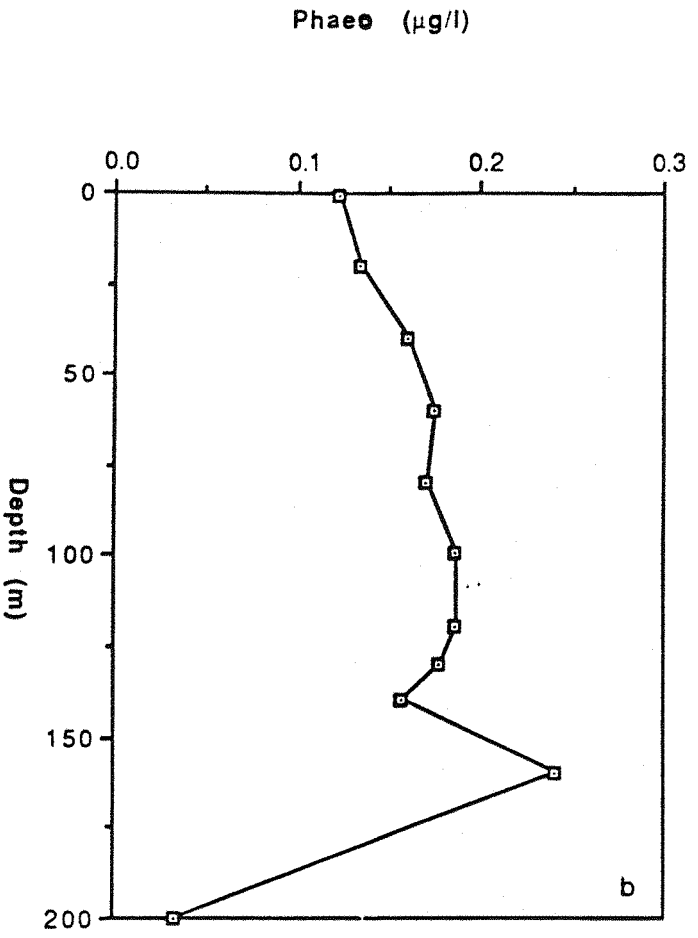


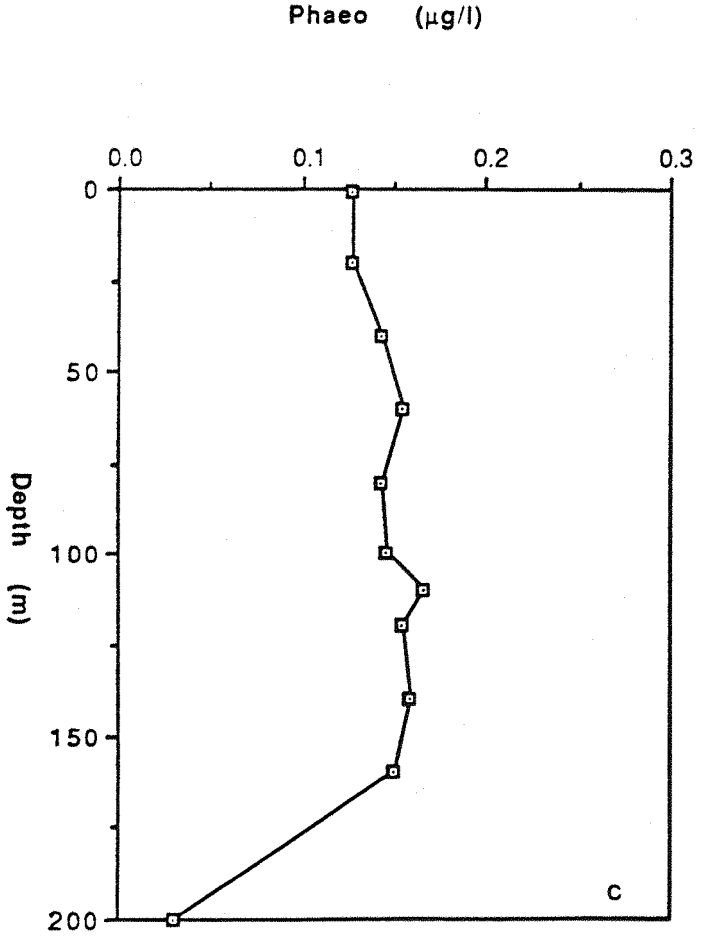
Append. 4.5 Oxygen data.

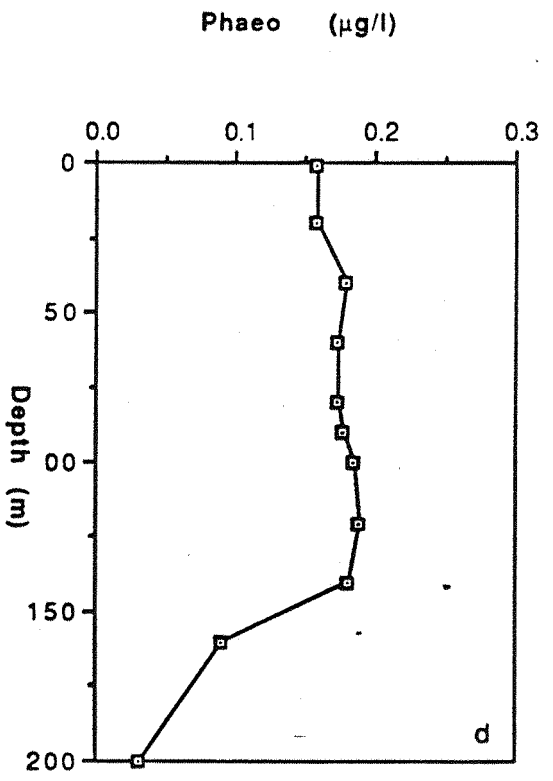


Append. 4.6 Chl-a data.

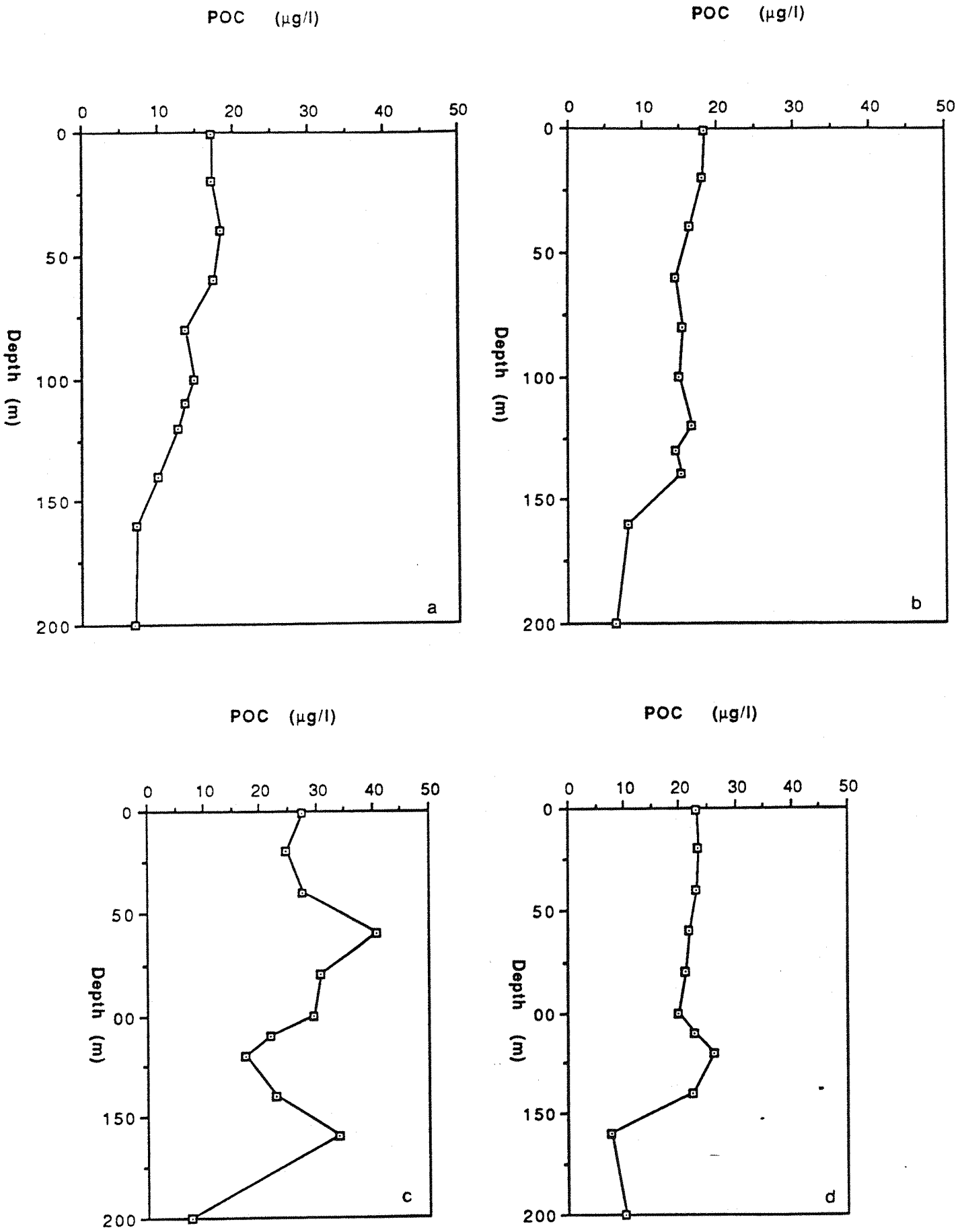




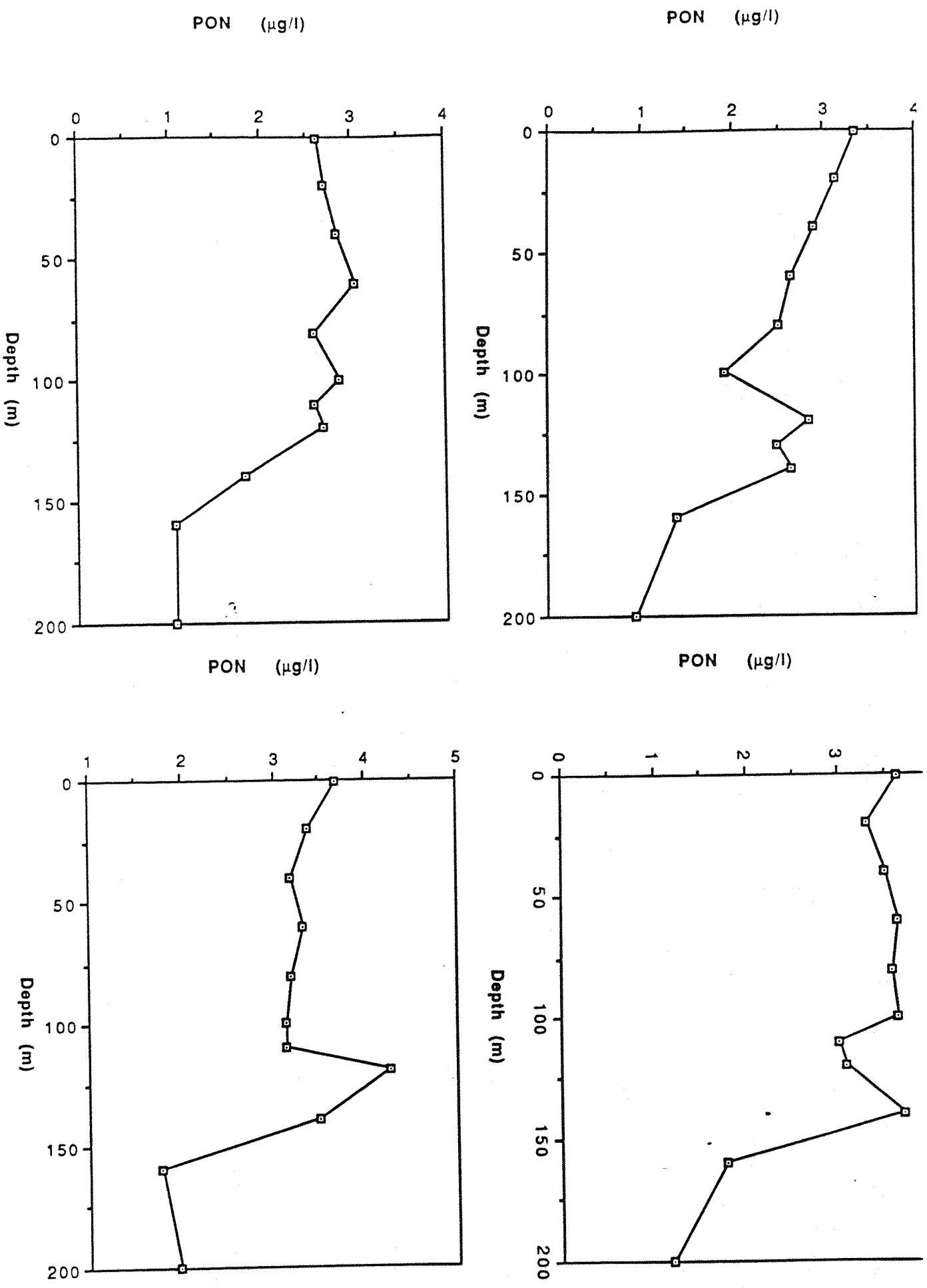




Append. 4.7 Phaeopigments data



Append. 4.8 Particulate organic carbon data.



Append. 4.9 Particulate organic nitrogen data.

	Depth (m)	DIP [μM]	DIP [μM]	DIP [μM] Av.	DTP[μM]	DTP [μM]	DTP [μM] Av.
1	1.0	0.013	0.010	0.011	0.198	0.178	0.188
2	20.0	0.016	0.015	0.015	0.335	0.188	0.261
3	40.0	0.014	0.011	0.012	0.167	0.188	0.177
4	60.0	0.006	0.007	0.006	0.155	0.126	0.140
5	80.0	0.007	0.007	0.007	0.136	0.105	0.120
6	100.0						
7	120.0	0.001	0.001	0.001	0.136	0.126	0.131
8	140.0	0.003	0.002	0.002	0.157	0.126	0.141
9	160.0	0.041	0.041	0.041		0.179	0.179
10	200.0	0.094	0.094	0.094	0.221	0.221	0.221
11	300.0	0.094	0.115	0.100	0.251	0.388	0.320
12	600.0	0.490	0.490	0.494	0.940	0.850	0.890
13	1000.0	1.450	1.450	1.450	1.720	1.260	1.490
14	1600.0	1.420	1.400	1.410		1.410	1.410
15	2000.0	1.240	1.220	1.230	1.510	1.270	1.390
16	2600.0	1.360	1.380	1.370	1.390	1.390	1.390
17	3000.0	1.200	1.210	1.200	1.550	1.340	1.440
18	4000.0	1.100	1.120	1.110	1.650	1.510	1.580

	DOP[μM]	DOP [μM]	DOP [μM] Av.
1	0.187	0.166	0.176
2	0.320	0.173	0.246
3	0.155	0.176	0.165
4	0.149	0.120	0.134
5	0.129	0.098	0.114
6			
7	0.134	0.124	0.129
8	0.155	0.124	0.137
9	0.138	0.138	0.138
10	0.127	0.127	0.127
11	0.158	0.273	0.215
12	0.450	0.360	0.405
13	0.270	0.270	0.050
14		0.000	0.000
15	0.270	0.050	0.160
16	0.031	0.011	0.020
17	0.350	0.130	0.240
18	0.550	0.390	0.470

Append. 4.10 Data for Fig. 4.19.

	Depth(m)	DIP [µM]Av.	DTP (Mg) 1	DTP(Mg) 2	DTP(Mg) Av.	DTP (UV) 1	DTP (UV) 2
1	1.0	0.000	0.220	0.200	0.210	0.120	0.120
2	20.0	0.000	0.160	0.160	0.160	0.100	0.100
3	40.0	0.000	0.250	0.180	0.215	0.100	0.100
4	60.0	0.000	0.160	0.160	0.160	0.100	0.100
5	80.0	0.000	0.220	0.260	0.240	0.080	0.080
6	100.0	0.000	0.180	0.250	0.215	0.080	0.080
7	120.0	0.000	0.160	0.200	0.180	0.080	0.080
8	140.0	0.000	1.100	1.020	1.060	0.090	0.100
9	160.0	0.000	0.270	0.270	0.270	0.090	0.100
10	180.0	0.050	0.130	0.070	0.100	0.080	0.070
11	200.0	0.060	0.150	0.150	0.150	0.130	0.130
12	250.0	0.070	0.260	0.260	0.260	0.210	0.210
13	300.0	0.180	0.270	0.250	0.260	0.220	0.210
14	400.0	0.240	0.240	0.260	0.250	0.250	0.250
15	500.0	0.345	0.310	0.230	0.270	0.400	0.430
16	600.0	0.550	0.710	0.680	0.695	0.640	0.640
17	700.0	0.900	1.080	0.880	0.980	0.930	0.930
18	800.0	1.120	1.280	1.410	1.345	1.200	1.200
19	900.0	1.390	1.580	1.450	1.515	1.440	1.440
20	1000.0	1.260	1.600	1.580	1.590	1.490	1.490
21	1200.0	1.190	1.580	1.520	1.550	1.420	1.420
22	1600.0	0.940					
23	2000.0	1.125	1.470	1.250	1.360	1.300	1.320
24	2600.0	1.190	1.280	1.280	1.280	1.250	1.270
25	3000.0	1.180	1.290	1.290	1.290	1.250	1.250
26	3600.0	0.990	1.400	1.420	1.410	1.300	1.270
27	4200.0	1.100	1.380	1.410	1.395	1.350	1.370

	DTP(UV) Av.	DOP (Mg)	DOP (UV)	PPs
1	0.120	0.210	0.120	0.090
2	0.100	0.160	0.100	0.060
3	0.100	0.210	0.100	0.110
4	0.100	0.160	0.100	0.060
5	0.080	0.240	0.080	0.160
6	0.080	0.215	0.080	0.135
7	0.080	0.130	0.080	0.050
8	0.915	1.060	0.950	0.110
9	0.095	0.270	0.095	0.175
10	0.075	0.080	0.025	0.055
11	0.130	0.090	0.050	0.040
12	0.210	0.190	0.140	0.050
13	0.215	0.080	0.035	0.045
14	0.250	0.050	0.065	
15	0.415	0.050	0.070	
16	0.640	0.145	0.090	0.055
17	0.930	0.080	0.030	0.050
18	1.200	0.225	0.080	0.145
19	1.440	0.125	0.050	0.075
20	1.490	0.330	0.230	0.100
21	1.420	0.360	0.230	0.130
22				
23	1.310	0.235	0.185	0.050
24	1.260	0.090	0.050	0.040
25	1.250	0.090	0.070	0.020
26	1.280	0.420	0.290	0.130
27	1.360	0.295	0.260	0.035

*: This appendix also includes data for Fig. 13.a

Append. 4.11* Data for Figure 4.20, and 4.21. Mg and UV indicate samples were analyzed by using Nit-ox and UV-ox techniques. Values of DTP and DOP are in µM.

Depth(m)	DIP [nM]1	DIP[nM]2	DIP [nM] Av.
1.0	13.0	10.0	11.5
20.0	16.0	15.0	15.5
40.0	14.0	11.0	12.5
60.0	6.0	7.0	6.5
80.0	7.0	7.0	7.0
100.0	1.0	1.0	1.0
120.0	1.0	1.0	1.0
140.0	3.0	2.0	2.5

a

Depth (m)	DIP [nM] 1	DIP[nM]2	DIP [nM] Av.
1.0	4.0	4.0	4.0
20.0	1.0	0.0	0.5
40.0	0.0	0.0	0.0
60.0	6.0	5.0	5.5
80.0	5.0	4.0	4.5
100.0	9.0	4.0	6.5
110.0	8.0	8.0	8.0
120.0	6.0	5.0	5.5
140.0	44.0	39.0	41.5
160.0	75.0	79.0	77.0
200.0	114.0	114.0	114.0
250.0	140.0	147.0	143.5

b

Depth	DIP[nM]1	DIP[nM]2	DIP[nM]Av.
1.0	9.0	10.0	9.5
20.0	12.0	11.0	11.5
40.0	17.0	11.0	14.0
60.0	1.0	1.0	1.0
80.0	1.0		1.0
100.0	1.0		1.0
120.0	4.0	8.0	6.0
140.0	18.0	19.0	18.5
160.0	42.0	41.0	41.5
200.0	104.0	103.0	103.0
250.0	142.0	145.0	143.0

c

Depth	DIP[nM]1	DIP[nM]2	DIP[nM]Av.
1.0	9.0	10.0	9.5
20.0	12.0	11.0	11.5
40.0	17.0	11.0	14.0
60.0	1.0	1.0	1.0
80.0	1.0		1.0
100.0	1.0		1.0
120.0	4.0	8.0	6.0
140.0	18.0	19.0	18.5
160.0	42.0	41.0	41.5
200.0	104.0	103.0	103.0
250.0	142.0	145.0	143.0

d

Append. 4.12 Data for Fig. 4.7

	Depth (m)	DOP (Mg)1	DOP (Mg)2	DOP (Mg)Av.	DOP (UV)1	DOP (UV) 2	DOP (UV)Av.
1	1.0	0.180	0.170	0.175	0.100	0.100	0.100
2	20.0	0.150	0.160	0.155	0.100	0.100	0.100
3	40.0	0.170	0.140	0.155	0.100	0.100	0.100
4	60.0	0.210	0.190	0.200	0.100	0.100	0.100
5	80.0	0.160	0.130	0.145	0.100	0.100	0.100
6	100.0	0.200	0.150	0.175	0.100	0.100	0.100
7	120.0	0.230	0.220	0.225	0.040	0.040	0.040
8	140.0						
9	160.0	0.270	0.290	0.280	0.110	0.100	0.105
10	200.0	0.260	0.260	0.260	0.120	0.120	0.115
11	250.0	0.300	0.300	0.300	0.160	0.180	0.170

	PPs	DOP (Mg)	DOP(UV)	DIP [µM]
1	0.075	0.175	0.100	0.000
2	0.055	0.155	0.100	0.000
3	0.055	0.155	0.100	0.000
4	0.100	0.200	0.100	0.000
5	0.045	0.145	0.100	0.000
6	0.075	0.175	0.100	0.000
7	0.185	0.216	0.031	0.009
8				0.006
9	0.175	0.260	0.085	0.020
10	0.145	0.212	0.067	0.048
11	0.134	0.198	0.064	0.106

4.13 b

	Depth (m)	DIP [µM]	DTP (UV)1	DTP (UV)2	DTP (UV)Av.	DTP(Mg)1	DTP (Mg)2
1	5.0	0.014	0.100	0.110	0.105	0.100	0.120
2	30.0	0.011	0.080	0.008	0.008	0.110	0.150
3	60.0	0.022	0.110	0.110	0.110	0.160	
4	100.0	0.013	0.140	0.150	0.145	0.240	
5	125.0	0.016	0.100	0.140	0.120	0.220	0.220
6	150.0	0.074	0.130	0.140	0.135	0.280	0.220
7	175.0	0.110	0.100	0.140	0.120	0.200	0.190
8	200.0						

DTP (Mg)Av.
0.110
0.130
0.160
0.240
0.220
0.250
0.195

Append. 4.13 Data for Fig 4.13 and 4.16 (b and c)

4.13c

	Depth (m)	PTP [nM]1	PTP [nM]2	PTP [nM]Av.
1	1.0	7.0	16.0	11.5
2	20.0	61.0	61.0	61.0
3	40.0	67.0	67.0	67.0
4	60.0	54.0	54.0	54.0
5	80.0	70.0	66.0	68.0
6	100.0	53.0	79.0	66.0
7	120.0	78.0	65.0	71.5
8	140.0	25.0	65.0	45.0
9	160.0	9.0	1.0	5.0
10	200.0			

Append. 4.14 Data for Fig. 4.14 a.

	Depth (m)	PTP [nM]	Depth (m) 1	PTP [nM] 1	PTP [nM] 2	PTP [nM] Av.
1	20.0	8.1	1.0	2.0	2.0	2.0
2	40.0	11.3	20.0	6.0		6.0
3	60.0	9.5	40.0	14.0		14.0
4	70.0	11.3	60.0	11.0	8.0	9.5
5	80.0	12.6	70.0	9.0	10.5	10.0
6	100.0	9.5	80.0		8.4	8.4
7			100.0	13.0		13.0
8			120.0	17.0		17.0
9			150.0	16.0		16.0
10			175.0	11.0		11.0
11			200.0	4.0	4.0	4.0

4.15b 4.15c

	Depth(m)	PTP[nM] 1	PTP[nM] 2	PTP[nM]Av.
1	1.0	14.9	34.0	24.5
2	20.0	12.0	24.0	18.0
3	40.0	27.0	30.0	28.5
4	60.0	27.0	13.0	20.0
5	80.0		24.0	24.0
6	100.0	31.0	20.0	25.5
7	120.0	19.0	16.0	17.5
8	140.0	14.0	10.0	12.0
9	160.0	15.0	10.0	12.5
10	180.0	9.0	9.0	9.0
11	200.0	4.0	9.0	6.5

4.15a

Append. 4.15 Data for Fig. 4.15 a, b and c.

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	Depth (m)	APA 1	APA2	APA Av. a)
1	5.0	2.60	2.60	2.60
2	30.0	3.72	3.75	3.73
3	60.0	3.22	3.15	3.18
4	100.0	3.34	3.07	3.20
5	125.0	2.67	2.76	2.71
6	150.0	2.53	2.55	2.54
7	175.0	1.35	1.90	1.62
8	200.0	1.59	1.16	1.37

4.17a

Depth (m)	APA 1	APA 2	APA Av. b)
5.0	1.26	1.12	1.19
30.0	2.16	1.59	1.87
60.0	1.90	2.46	2.18
100.0	1.82	2.00	1.91
125.0	1.50	1.62	1.56
150.0	1.43	1.33	1.38
175.0	0.36	0.62	0.49
200.0	0.81	0.61	0.71

4-17 b

Depth (m)	APA 1	APA 2	APA Av. c)
5.0	1.84	1.60	1.72
30.0	1.95	1.78	1.78
60.0	2.07	2.58	2.32
100.0	1.53	1.53	1.53
125.0	2.70	1.70	2.20
150.0	0.73	0.02	0.37
175.0	0.00	0.03	0.03
200.0	0.04	0.04	0.04

4.17c

Append. 4.17 Data for Fig. 4.17. a, b, and c refer to same as Fig. 4.17.

	Depth (m)	DIP [nM] 1	DIP [nM]2	DIP[nM] Av.
1	5.0			
2	30.0	14.0	14.0	14.0
3	60.0	17.0	5.0	11.0
4	100.0	17.0	28.0	22.5
5	125.0	13.0	14.0	13.5
6	150.0	23.0	19.0	21.0
7	175.0	76.0	73.0	74.5
8	200.0	107.0	114.0	110.5

4,18a

	Depth (m)	DIP[nM] 1	DIP [nM]2	DIP [nM] Av.
1	5.0	13.0	2.0	7.5
2	30.0	4.0	2.0	3.0
3	60.0	11.0	7.0	9.0
4	100.0	10.0	3.0	6.5
5	125.0	6.0	3.0	4.5
6	150.0	13.0	9.0	11.0
7	175.0	70.0	69.0	69.0

4,18b

	Depth (m)	DIP [nM]1	DIP [nM]2	DIP[nM]Av.
1	5.0	25.0	27.0	26.0
2	30.0	6.0	6.0	6.0
3	60.0	14.0		14.0
4	100.0	10.0	9.0	9.5
5	125.0		12.0	12.0
6	150.0	50.0		50.0
7	175.0	88.0	89.0	88.5
8	200.0	103.0	103.0	103.0

4,18c

Append. 4.18 Data for Fig. 4.10 a, b, c, d, and e respectively.

	Depth (m)	DIP [nM] 1	DIP [nM] 2	DIP [nM] Av.
1	1.0	30.0	22.0	26.0
2	5.0	26.0	20.0	23.0
3	15.0	21.0	21.0	21.0
4	55.0	23.0	23.0	23.0
5	75.0	32.0		32.0
6	95.0		20.0	20.0
7	115.0	24.0	22.0	23.0
8	135.0	31.0	21.0	26.0

4.18d

	Depth (m)	DIP [nM] 1	DIP [nM] 2	DIP [nM] Av.
1	1.0	14.0	10.0	12.0
2	20.0	4.0	2.0	3.0
3	40.0	14.0	10.0	12.0
4	60.0	29.0	27.0	28.0
5	80.0	33.0	24.0	31.0
6	90.0	13.0	11.0	12.0
7	100.0	19.0	26.0	22.5
8	120.0	7.0	8.0	7.5
9	140.0	11.0	11.0	11.0

4.18 d

Append. 4.18 Data for Fig. 4.10 a, b, c, d, and e respectively.

Time (h)	Filt.1	Filt.2	Filt. (Av.) a)	Filt.1	Filt.2	Filt. (Av.) b)
0.0	2.0	2.0	2.0	2.0	2.0	2.0
1.5	2.0	2.0	2.0	2.0	2.0	2.0
3.0	2.0	2.0	2.0	2.0	2.0	2.0
6.0	2.0	2.0	2.0	2.0	2.0	2.0
10.0	2.0	2.0	2.0	2.0	2.0	2.0

	Filt.1 +30 1)	Filt 2 +30 2)	Filt.2 +30	FilT.+30 1)	Filt.+30 2)	Filt.+30 (Av)
1	30.0	30.0	30.0	26.0	29.0	27.5
2	28.0	34.0	31.0	29.0	26.0	27.5
3	40.0	37.0	38.5	43.0		43.0
4	32.0	20.0	26.0			
5	36.0	38.0	37.0	34.0	37.0	35.5

Append. 4.19 Data for Fig. 4.24. Filt.: filtered samples respectively. a and b, and 1 and 2 mean duplicate bottles and analysis respectively. Filt. + 30: Filtered samples plus 30 nM (DIP) added.

Salinity 1	Depth1	Temp. 1	Depth 2	Salinity 2	Temp 2	Depth 3	Salinity 3	Temp 3
36.750	2.810	21.887	2.630	36.746	21.751	1.600	36.736	21.632
36.748	20.810	21.871	19.750	36.745	21.746	30.340	36.736	21.636
36.747	40.940	21.857	40.420	36.747	21.750	61.510	36.701	21.423
36.743	61.090	21.813	61.100	36.740	21.725	101.420	36.660	21.102
36.657	80.940	21.201	80.720	36.716	21.571	161.810	36.576	19.394
36.672	100.490	21.100	91.030	36.679	21.368	251.670	36.508	18.413
36.655	112.310	21.001	100.570	36.652	21.157			
36.630	121.810	20.812	121.220	36.633	20.855			
36.597	142.090	20.081	140.560	36.582	19.575			
36.570	161.360	19.328	162.650	36.552	19.035			
36.539	200.880	18.811	201.450	36.535	18.721			
36.508	251.530	18.398	251.960	36.500	18.363			

Append. 4.20 Data for Fig. 4.11. Numbers 1, 2, and 3 refer to c, b, and d (in the Figure) respectively.

	Depth (m)	DOP Av. a)	DOP 1	DOP 2	Depth 1	DOP Av. b)	DOP 1	DOP 2
1	1.0	0.176	0.187	0.166	1.0	0.143	0.149	0.138
2	20.0	0.246	0.320	0.173	20.0	0.115		
3	40.0	0.165	0.155	0.176	40.0	0.128	0.154	0.102
4	60.0	0.134	0.149	0.120	60.0	0.156		
5	80.0	0.114	0.129	0.098	80.0	0.132	0.153	0.111
6	120.0	0.129	0.134	0.124	100.0	0.145	0.145	0.145
7	140.0	0.137	0.155	0.124	120.0	0.263	0.263	
8	160.0	0.138	0.138	0.138	140.0	0.119	0.119	
9	200.0	0.094	0.094	0.094	160.0	0.138	0.138	
10					200.0	0.109	0.109	

Append. 4.21 Data for Fig. 4.12. a and b in the Figure as in this append.

	depth[m]	DIP[nM]1	DIP[nM]2	DIP[nM] Av.
1	1.0	9.0	5.0	7.0
2	20.0	5.0	6.0	5.5
3	40.0	4.0	3.0	3.5
4	60.0	2.0	2.0	2.0
5	80.0	2.0	2.0	2.0
6	100.0	5.0	2.0	3.5
7	120.0	4.0	5.0	4.5
8	140.0	7.0	7.0	7.0

a

	Depth (m)	DIP[nM]1	DIP[nM]2	DIP Av.
1	1.0	5.0	5.0	5.0
2	20.0	3.0	2.0	2.5
3	40.0	5.0	5.0	5.0
4	60.0	2.0	2.0	2.0
5	80.0	3.0	2.0	2.5
6	100.0	4.0	8.0	6.0
7	120.0	5.0	5.0	5.0
8	140.0	5.0	6.0	5.5
9	160.0	4.0	6.0	5.0
10	200.0	16.0	16.0	18.0

b

	Depth[m]	DIP[nM]1	DIP[nM] 2	DIP (Av.)
1	1.0	4.0	3.0	3.5
2	20.0	11.0	6.0	8.5
3	40.0	7.0	5.0	6.0
4	60.0	2.0	2.0	2.0
5	80.0	2.0	2.0	2.0
6	100.0	4.0	4.0	4.0
7	120.0	9.0	8.0	8.5
8	130.0	9.0	6.0	7.5
9	140.0	13.0	15.0	14.0
10	160.0	24.0	19.0	21.5
11	180.0	69.0	69.0	69.0

c

Append. 4.22 Data for Figure 4.8 a,b & c

	Salinity 1	Depth1	Temp. 1	Depth 2	Salinity 2	Temp 2	Depth 3	Salinity 3	Temp 3
1	36.750	2.810	21.887	2.630	36.746	21.751	1.600	36.736	21.632
2	36.748	20.810	21.871	19.750	36.745	21.746	30.340	36.736	21.636
3	36.747	40.940	21.857	40.420	36.747	21.750	61.510	36.701	21.423
4	36.743	61.090	21.813	61.100	36.740	21.725	101.420	36.660	21.102
5	36.657	80.940	21.201	80.720	36.716	21.571	161.810	36.576	19.394
6	36.672	100.490	21.100	91.030	36.679	21.368	251.670	36.508	18.413
7	36.655	112.310	21.001	100.570	36.652	21.157			
8	36.630	121.810	20.812	121.220	36.633	20.855			
9	36.597	142.090	20.081	140.560	36.582	19.575			
10	36.570	161.360	19.328	162.650	36.552	19.035			
11	36.539	200.880	18.811	201.450	36.535	18.721			
12	36.508	251.530	18.398	251.960	36.500	18.363			

Append. 4.23 Data for Fig. 4.6

time[h]	DIP[nM]	DIP[nM]	DIP (Av.)+0 nM Time'		DIP[nM]	DIP[nM]
1430.0						
1530.0	4.0	7.0	5.5	1545.0	10.0	8.0
1750.0	1.0	2.0	1.5	1750.0	12.0	10.0
2222.0	1.0	1.0	1.0	2235.0	4.0	4.0

DIP (Av.)+5 nM Time'		DIP[nM]	DIP[nM]	DIP (Av.)+10 nM Time		DIP[nM]
9.0					1455.0	8.0
11.0	1550.0	5.0	5.0	5.0	1600.0	5.0
4.0	1800.0	18.0	18.0	18.0	1805.0	
	2240.0	6.0	4.0	5.0	2248.0	5.0

DIP[nM]	DIP (Av.)+10 nM Time		DIP[nM]	DIP[nM]	DIP (Av.)+20 nM Time	
8.0	8.0	1500.0	18.0	16.0	17.0	1506.0
6.0	5.5	1605.0	21.0	23.0	22.0	1615.0
		1810.0	22.0	25.0	23.5	1818.0
6.0	5.5	2250.0	7.0	7.0	7.0	2256.0

DIP[nM]	DIP[nM]	DIP (Av.)+50 nM Time		DIP[nM]	DIP[nM]	DIP (Av.) +50 nM
71.0	61.0	66.0	1510.0			
40.0	46.0	43.0	1620.0	48.0	48.0	48.0
47.0	48.0	47.5	1825.0	50.0	51.0	50.5
17.0	22.0	19.5	2305.0	22.0	24.0	23.0

Time	DIP[nM]	DIP[nM]	DIP (Av.) +50 nM (DIP) +750 nM (DIN)
1515.0			
1630.0	51.0	49.0	50.0
1830.0	51.0	51.0	51.0
2310.0	24.0	27.0	25.5

Append. 4.24 Data for Figure 4.18 a and b.