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Studies of organic carbon in estuarine and coastal waters involving size fractionation and carbon isotope techniques

by Simeon Ll.D. Jones

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ABSTRACT
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Studies of organic carbon in estuarine and coastal waters involving size fractionation and carbon isotope techniques

by Simeon Llywelyn Degwel **JONES**

The recent controversy over the accuracy of the higher dissolved organic carbon (DOC) concentrations reported by Sugimura and Suzuki (1988; Marine Chemistry 24, 105-131) obtained using high-temperature catalytic oxidation (HTCO) methods has been further critically examined during inter-calibration exercises between HTCO and ultra-violet (UV) persulphate DOC analyzers. The accuracy of these DOC methods is currently still unclear. This study and other work indicates that the HTCO method of DOC analysis may measure 5 to 60 % more DOC than UV persulphate methods. An increase in DOC oxidation efficiency (6 to 8 %) was achieved by adding low concentrations of Hg salts (0.004 M in the actual sample; $Hg(NO_3)_2$ and $HgCl_2$) to the sample stream in a UV persulphate DOC analyzer. This increase in DOC oxidation efficiency implies that there is a fraction of DOC that is not measured by the UV persulphate method of DOC analysis, when used without the addition of Hg salts. The increase of DOC concentrations approaches the higher levels of DOC measured by the HTCO method.

To investigate the size fractionated distributions of organic carbon in the estuarine system a two year field study was undertaken in the Beaulieu estuary during 1990 to 1992; the Beaulieu river system is characterized by an organic rich drainage basin and is situated in the southern part of Britain. Ultrafiltration methods were developed for isolating colloidal organic carbon (COC) fractions from natural waters using the unique properties of Anodisc Anopore filters. Sources of organic carbon were differentiated using $\delta^{13}C$ signatures and background parameters were also measured to aid interpretation.

Colloidal organic carbon represents a large fraction of carbon in the estuarine waters; up to 3.6 x "true" DOC (TDOC; i.e. the DOC concentration after filtration through Anodisc Anopore 0.02 μm filters) and up to 12 x POC concentrations. The mean value of COC as a fraction of the TDOC in the river was 63 % and in seawater the mean value was 59 %. However, the relative standard deviation (RSD) was large for these mean values, being 186 % and 100 % respectively. These high COC concentrations, previously unreported, indicate that during certain times COC is the most important size fraction in the estuary and this may be due to the high content of humic substances found in the estuary.

Dissolved organic carbon was essentially transported conservatively to the coastal zone and was unaffected by high levels of primary productivity in the water column or by high turbidity. The primary source of organic material to the estuary was terrestrial and DOC levels in the river depended on the dynamics of organic matter mobilisation in the catchment area and input into the river. The amount of riverine DOC transported annually into the coastal zone was 54.6 tonnes of carbon for 1991 and 38.4 tonnes of carbon for 1992. The difference is consistent with a higher fresh water discharge for 1991. High phytoplankton activity and high levels of suspended particulate material which introduced particulate organic carbon (POC) into the water column had little affect on the concentrations of COC in the estuary. It was found that degradation events effectively introduced COC into the estuary.

Terrestrial and sedimentary sources were identified for the coarse COC (CCOC; particles in the size range 100 to 700 nm) and fine COC (FCOC; particles in the size range 20 to 100 nm). These were in particular *Spartina* grass growing on the edge of the estuary and *Spartina* debris in the sediments. In contrast to earlier work which showed that *Spartina* did not impart a dominant signal to organic matter in the estuarine environment, the FCOC fraction was found to be most representative of the cellulose material of *Spartina* ($\delta^{13}C$ = -11 to -12 ‰) whilst the CCOC fraction contained material from *Spartina* and POC sources ($\delta^{13}C$ around -13 ‰). Earlier studies may have missed the *Spartina* signal since it exists predominantly in the smaller colloidal fraction and it may be masked by other more dominant signals (such as the phytoplankton signal). Sources of peat ($\delta^{13}C$ = -27 ‰) and soil organic material ($\delta^{13}C$ = -24 ‰) for the POC and CCOC fractions have also been identified in the river whilst other sources for POC include phytoplankton activity ($\delta^{13}C$ signatures around -35 ‰ in freshwater and $\delta^{13}C$ = -20 to -22 ‰ in coastal waters). Terrigenous POC is the most important source in the river whilst *in-situ* phytoplankton POC production is the most important source in the coastal zone.

Sinks for estuarine organic material were identified as bacterial degradation and respiration. Bacterial degradation events in the water column were particularly evident in the area influenced by a small sewage input in mid-estuary, during periods of higher water column temperatures and at the end of phytoplankton bloom events. Possible aggregation of DOC occurred at low salinities (0 to 5). The removed fraction for summer 1991 was estimated to be 17 % of the riverine DOC.

High levels of ^{14}C found in the Beaulieu estuary have not been reported previously and it was supposed that they were due to either anthropogenic contamination or contamination introduced into the samples via handling and preparation.

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List of abbreviations used	
BOD	biological oxygen demand
ca.	circa
CCOC	coarse colloidal organic carbon
COC	colloidal organic carbon
COM	colloidal organic matter
DOC	dissolved organic carbon
DOM	dissolved organic matter
FCOC	fine colloidal organic carbon
HTCO	high-temperature catalytic oxidation
LIN	liquid nitrogen
LOCC	low organic carbon content
%M	percent modern
MRI	meterological research institute
PML	Plymouth Marine Laboratory
POC	particulate organic carbon
POM	particulate organic matter
RO	reverse osmosis
RSD	relative standard deviation
SD	standard deviation
SPM	suspended particulate material
SUDO	Southampton University Department of Oceanography
UCNW	University college of North Wales
UV	ultra-violet
WCO	wet chemical oxidation

Chapter 1:

Introduction: Organic carbon in the coastal and estuarine environment.

1.1. Introduction.

Estuaries and the coastal seas are important when considering organic matter in the natural environment. They provide a key link for the transport of organic matter from terrigenous sources to the oceanic organic matter pool. Quantification of the flux of terrestrial organic matter transported into the open ocean (see for example Mantoura and Woodard, 1983) is important in understanding the global cycling of organic matter (Farrington *et al.*, 1992). Organic matter cycling is intensified in estuaries and the coastal zone due to rapid changes in physical-chemical properties and the high biological productivity found in these areas (Jickells *et al.*, 1991). Therefore, the study of processes affecting organic matter in this area is complex, however, knowledge of these processes is needed to gain a better understanding of organic matter cycling in the ocean. The coast is also heavily utilized by man and this has a large impact, principally through input of pollution into the coastal zone. Therefore, it is of advantage to better understand organic matter cycling in this area in order to improve the quality of the coastal seas.

1.2. Definition of POC, DOC and COC.

Organic matter in natural waters covers a continuous size spectrum ranging from free monomers via macromolecules and colloids to aggregates and large particles (Spitzy and Leenheer, 1991).

For practical reasons, the organic matter reservoir is usually subdivided into two major pools by operational definition. On filtration of a water sample the organic matter that is retained on a Whatman GF/F (mean pore size 0.7 μm) filter is termed particulate organic carbon (POC). The organic matter that passes through the filter into the filtrate is termed dissolved organic carbon (DOC).

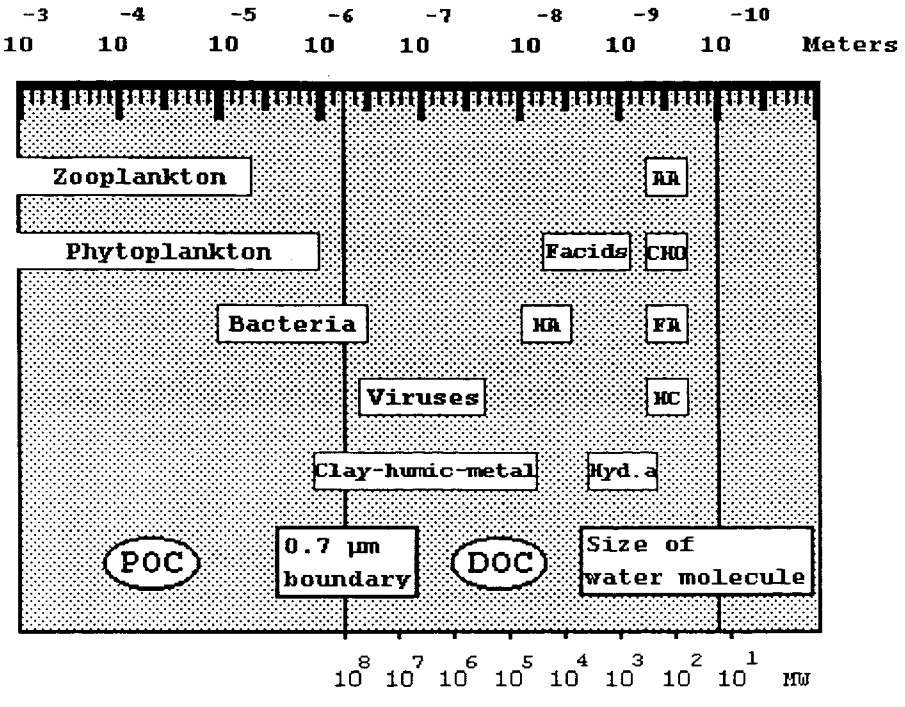
The terms DOC and POC apply only when considering the organic matter's carbon. The terms dissolved organic matter (DOM) and particulate organic matter (POM) also include the organic matter's nitrogen and phosphorus and other elements associated with carbon. Therefore the amount of DOM in organic matter in natural waters is roughly twice the amount of DOC (Hedges *et al.*, 1993).

Organic pollutants found in natural waters (such as poly-chlorinated biphenyls) or phytoplankton exudates (which include proteins and carbohydrates) would best be described using the term DOM. However, when measuring the concentration of DOC in natural waters the term DOC should be used, as this is actually the parameter measured.

Colloids can be defined as a disperse phase comprising of particles or macromolecules which are smaller than 1 μm, but larger than 1 nm in diameter. The classical science of colloids deals with particles that are so small that they behave in some respects like molecules and with molecules so large that they behave in some respects like particles (Yariv and Cross, 1979). When dealing with COC in the ocean the disperse phase is made up of the organic compounds (solid) whilst the dispersing medium is seawater (liquid). However, there is no precise or necessarily useful definition for COC in seawater and it can be operationally defined by the isolation methods employed (Brownawell, 1991).

The term COC applies only when considering the organic matter's carbon and, as for DOC and POC, the term colloidal organic matter (COM) includes the organic matter's nitrogen and phosphorus and other elements associated with carbon.

The size ranges of various organic carbon species in natural waters, including colloids, can be seen in **Figure 1.1.**



Key: AA = amino acids; CHO = carbohydrates; FA = fatty acids;
HC = hydrocarbons;
Facids = fulvic acids; HA = humic acids;
Hyd.a = hydrophylic acids.

(Based on Thurman, 1985)

Figure 1.1. Continuum of particulate and dissolved organic carbon in natural waters.

1.3. The role of marine organic carbon in the global carbon cycle.

1.3.1. Introduction.

The extremely complex interplay of biological, geological and chemical processes by which materials and energy are exchanged and re-used at the Earth's surface are known as biogeochemical cycles. They operate on timescales of microseconds to eons and occur within domains that range in size from a living cell to the entire atmosphere-ocean system. Driven by solar or geothermal energy these cycles are concentrated at interfaces where living organisms catalyze chemical reactions and facilitate material exchange (Hedges, 1992).

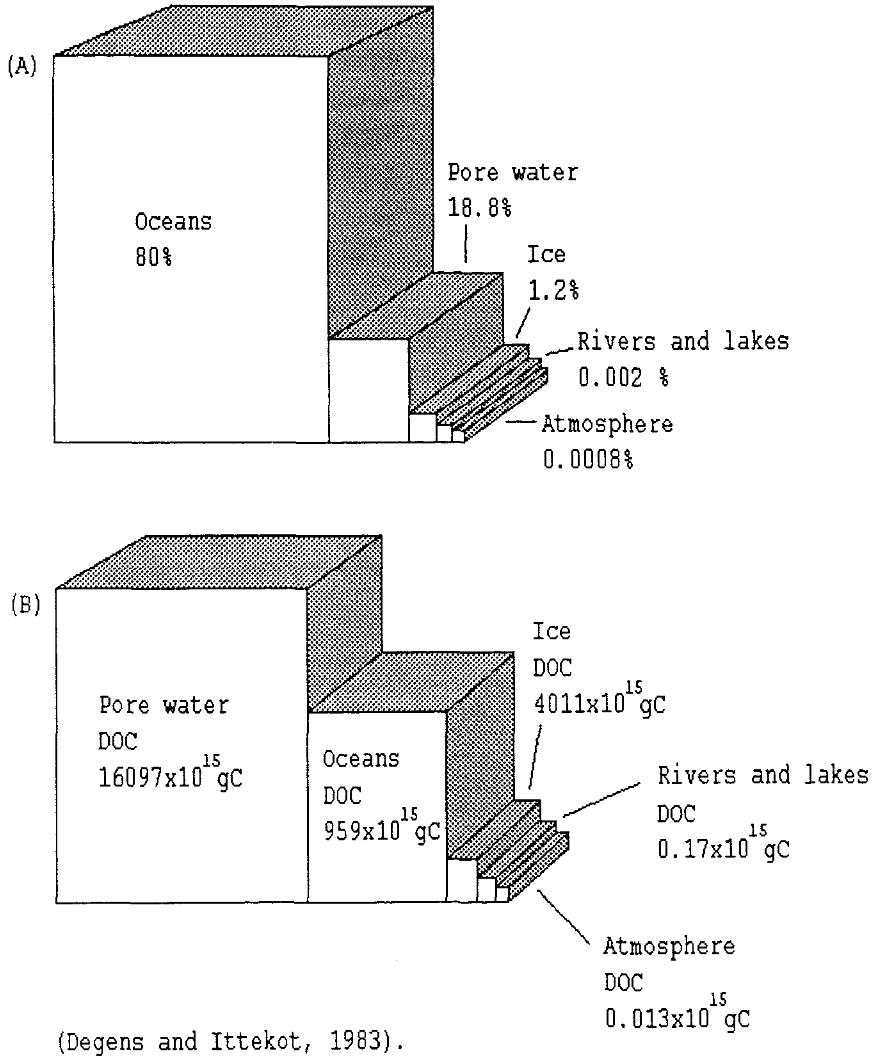
Knowledge of the quantity and variety of oceanic organic components and their interactions with other systems, including food chain dynamics, ocean productivity, paleoceanography and the global carbon budget is important for understanding global cycles of a variety of elements, including carbon itself. Also, detailed information on oceanic organic chemistry is required to determine how and to what extent anthropogenic perturbations may alter oceanic processes (Farrington *et al.*, 1992).

1.3.2. Global carbon reservoirs.

The main reservoirs of carbon in the Earth's crust are sedimentary rocks which hold approximately 60,000 x 10^{18}g of inorganic carbon and 15,000 x 10^{18}g of organic carbon (Olson *et al.*, 1985). However, most carbonaceous materials in these rock reservoirs are isolated by deep burial and only affect biologically mediated cycles on geological time scales.

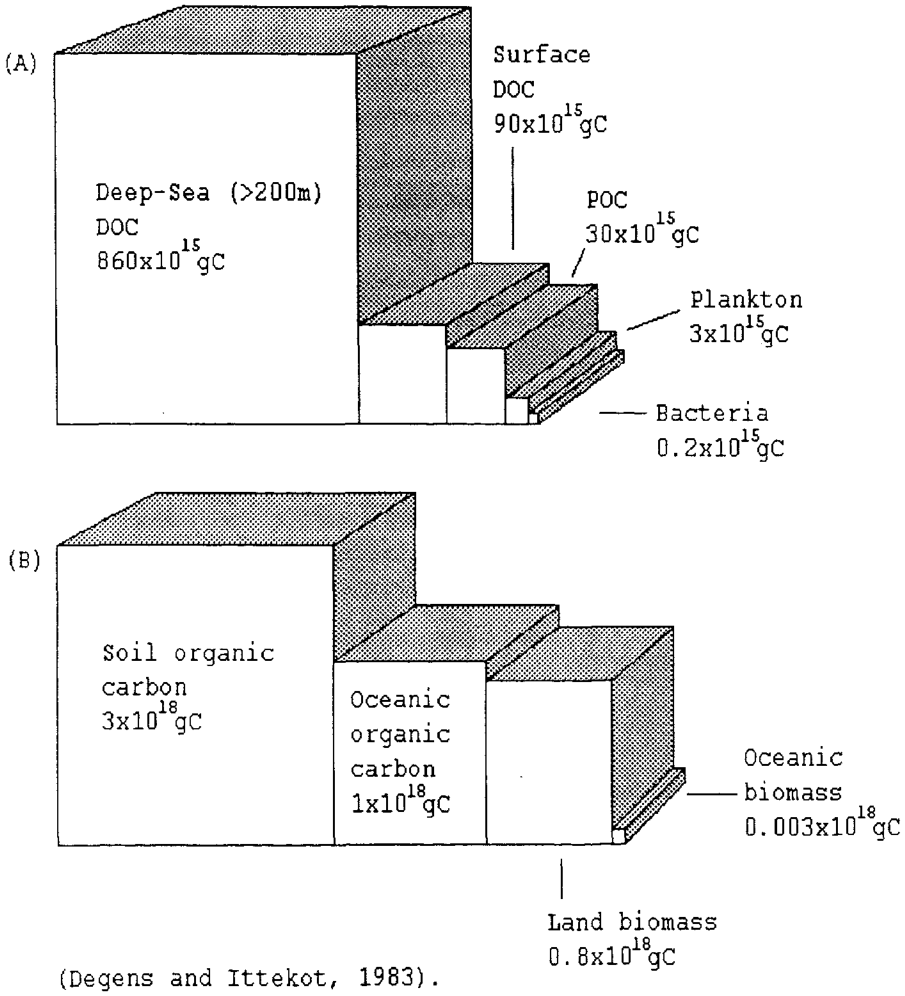
Although smaller, carbon reservoirs at the Earth's surface are numerous and dynamic (see **Figure 1.2 (B)** and **Figure 1.3 (B)**). By far the largest of these exchangeable reservoirs is dissolved inorganic carbon (DIC) in seawater (*ca.* 40 x 10^{18}gC) (Berner, 1989). Seawater DIC corresponds to only about 0.06 % of the carbon in carbonate rocks, but is over an order of magnitude greater than all other "exposed" pools. The mixed layer of the ocean holds about 25 % of all marine DIC and is in active exchange with the other contemporary carbon pools on time-scales of decades and thus may act as a substantial sink for atmospheric carbon dioxide. The deep ocean continually exchanges carbon with the atmosphere via upwelling and this process takes around 1000 years for all the carbon in the deep ocean to be exchanged (Broecker and Peng, 1982).

Non-living DOC in seawater is the biggest reservoir of organic carbon in the ocean (see **Figure 1.3 (A)**). This DOC may have been underestimated by roughly 50 % if the higher concentrations obtained by the high-temperature catalytic oxidation (HTCO) method (Sugimura and Suzuki, 1988) of DOC analysis proves accurate (see discussion in **Chapter 2**). The organic carbon content of living marine organisms (*ca.* 0.002 x 10^{18}gC) is trivial compared both with seawater DOC and non-living POC (Olsen *et al.*, 1985). The DOC in all the remaining reservoirs i.e. ice, rivers and lakes (see **Figure 1.2 (A)**) are all small compared to the global oceanic DOC reservoir and the global pore water DOC reservoir, however, they are vital in maintaining the global water cycle and in transferring carbon to the major DOC reservoirs (Degens and Ittekkot, 1983).



(Degens and Ittekot, 1983).

Figure 1.2. (A) Different compartments of the present hydrosphere (%). (B) Distribution of DOC in various hydrospheric compartments.



(Degens and Ittekot, 1983).

Figure 1.3. (A) Distribution of organic pools in the ocean. (B) The major pools of organic carbon of land and in the sea.

1.3.3. Importance of the coastal zone.

The coastal zone directly receives the flux of dissolved and particulate material carried by rivers and although the surface area of the coastal region represents only 8 % of the global ocean, its contribution to the global production of the ocean is between 18 % and 33 % respectively if the highest estimate (Martin *et al.*, 1987) or the lowest estimate (Berger *et al.*, 1987) is considered.

Some of the more important features in relation to carbon in the coastal zone are the relatively high biological productivity due to the river input of nutrients, upwelling of fertile deep waters, the absence of losses of nutrients below a compensation depth and the close coupling of the benthic and pelagic systems. These features therefore mean that detailed attention needs to be paid to the carbon cycle in the coastal area when global cycles are considered (Wollast, 1991).

The importance of continental margins in the marine biogeochemical cycling of carbon has been stressed (Jahnke *et al.*, 1990; Walsh, 1991). Walsh (1991) has suggested that the continental margins and deep-sea are equally important in the carbon biogeochemical cycle, whilst Jahnke *et al.* (1991) report higher benthic derived carbon fluxes at the sea-floor near ocean margins where half of the input of organic carbon to the sediments occurs within 500 km of the continental slope.

1.3.4. Definition of an outer and inner margin.

Jickells *et al.* (1991) report that physical forcing by rivers, the atmosphere and oceans is a major determinant of all processes on continental margins and that biogeochemical cycles in marine systems are controlled mainly by interactions between dissolved and particulate phases.

Therefore they distinguish between two general areas of margins on the basis of the dominance of certain physical forcing. At the outer oceanic boundary of the margin, exchange with the open ocean is the dominant process. At the inshore boundary, the interaction with fluvial systems dominates, producing another unique biogeochemical system.

1.3.5. Biogeochemical processes occurring in the coastal zone.

In order to construct suitable models of carbon fluxes the quantification of the major pathways in biogeochemical cycles need to be determined, as well as identification of the many chemical substances involved and the variable rates and stoichiometries that are characteristic of environmental reactions (Hedges, 1992). The biogeochemical processes that affect dissolved organic compounds in water have been reviewed by Thurman (1986).

Autochthonous inputs of organic carbon into the water column occur via primary production by algae and from autotrophic production by photosynthetic and chemosynthetic bacteria. Terrigenous input of organic matter via rivers is also an important allochthonous source. The decomposition of carbon begins with the release of POC and DOC from plant matter. The second event is the selective colonization of coarse particulate matter by micro-organisms, such as bacteria, fungi and protozoa. Hence coarse POC is converted to fine POC. The DOC may be quite labile and is susceptible to rapid decomposition of simple organic compounds, such as amino acids, sugars and fatty acids. The higher molecular weight compounds, such as humic substances, take a much longer period of time to degrade, from weeks to months, compared with hours for the labile simple organic compunds. Sedimentation of algae and detritus also occurs and as oxygen is depleted in the interstitial water the microbial community changes to an anaerobic one. The microbial transformations of carbon include inorganic carbon assimilation from photosynthesis and chemosynthesis and aerobic and anaerobic decompositon. The carbon dioxide from decomposition and from diffusion from the atmosphere is a source of carbon for the autotrophs, mainly algae, but also autotrophic bacteria. These organisms convert inorganic carbon to various forms of organic carbon and their cellular debris is often carried to the sediment. Hence, carbon is cycled actively through the water column.

Figure 1.4 summarises the processes affecting the distribution of organic carbon in the ocean. Organic compounds can be transported from terrestrial sources to the ocean adsorbed onto particles and carried by the wind or via land drainage and rivers. Once in the ocean a number of marine inputs are important with DOC and POC occupying a central position. Some of the more important sources controlling the distribution and flux of organic molecules are phytoplankton, bacteria and microbiological processes. The sediments also play an important role where sedimentation and diagenesis of organic matter provide a sink for these compounds.

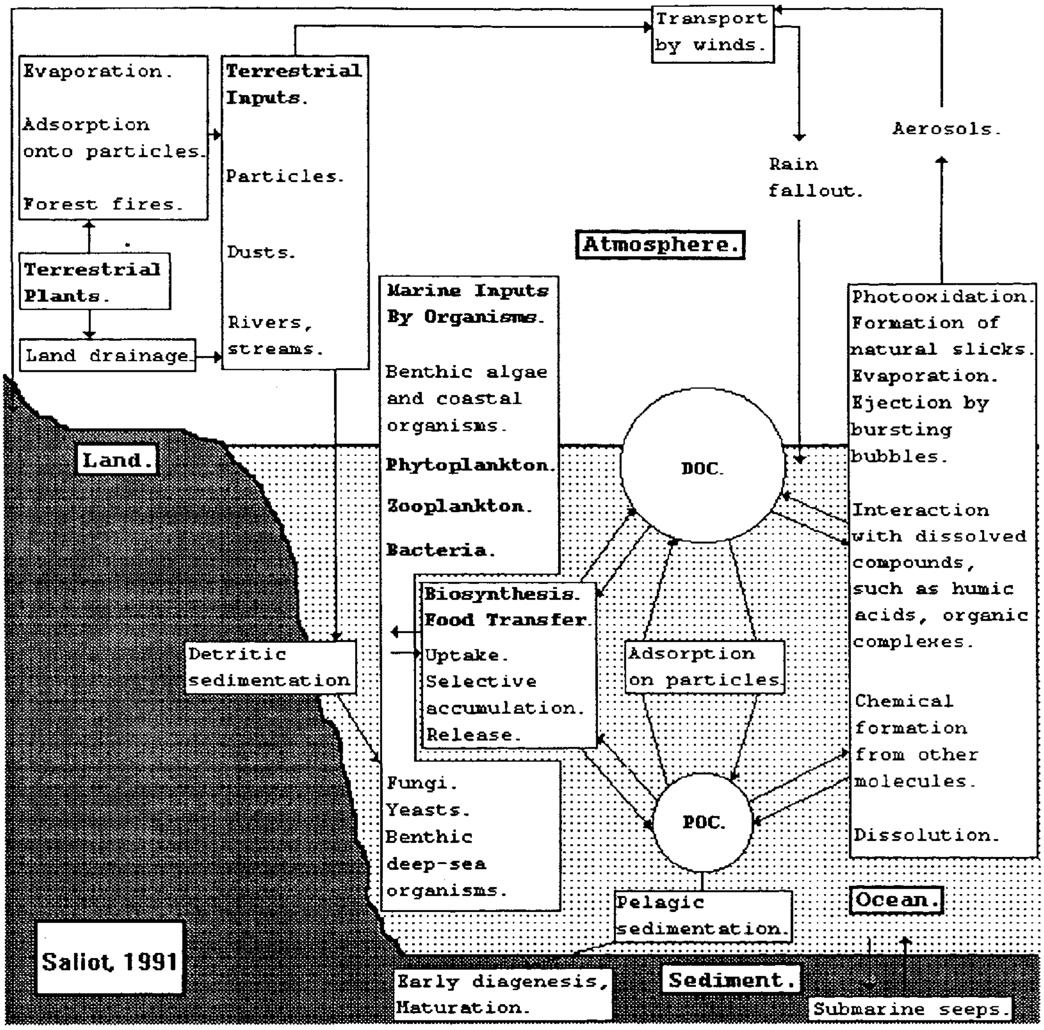


Figure 1.4. Processes affecting the distribution of organic carbon in the ocean.

1.3.6. Sources of organic matter in the coastal and estuarine environment.

Transfer of organic matter from the land to the sea is a key link in the global carbon cycle because it provides a link between the large reactive pools of organic carbon on land and in the sea. It is also the most important pathway for ultimate preservation of terrigenous production in different environments (Hedges, 1992) (See **Table 1.1**).

Type of organic material and process	**Flux**	**Reference**
Kerogen Weathering *	0.1	Berner (1989)
Terrestrial primary production	60	Olson *et al.* (1985)
Riverine DOC discharge	0.2	Meybeck (1982)
Riverine POC discharge	0.2	Ittekkot (1988)
Eolian input to the ocean	0.1	Romankevich (1984)
Marine primary production	50	Martin *et al.* (1987)
Marine POC rain at 100m	7	Martin *et al.* (1987)
Seawater DOC turnover	0.1	Williams and Druffel (1987)
Burial in marine sediments	0.1	Berner (1989)

Table 1.1. Organic carbon fluxes in global biogeochemical cycles (10^{15}gC year^{-1}) (Hedges, 1992). (* Estimated to balance marine sediment burial).

The amount of terrestrial organic matter in the ocean is one of the most poorly known factors in the global carbon cycle (Likens *et al.*, 1981). However, estimates suggest it represents a significant input to coastal areas and hence to the biogeochemical processes occurring at ocean margins. Although present evidence suggests that terrestrial material is only a small fraction of the total organic matter in the ocean, its signature is detectable in sediments and in the DOM pool in pelagic regions (Farrington, 1992). Degens and Ittekkot (1983) have taken an average DOC concentration for river water transported annually by rivers into the world ocean and found that it would take 3300 years to produce an amount of DOC equivalent to that present in the sea today. This value is the same as the average age of deep water DOC (Williams, 1969) and an interpretation of this data is that the observed DOC pattern in the sea could represent a steady state between input of DOC via the rivers and the oxidation of deep sea DOC. However, the homogenous distribution of DOC throughout the deep ocean could also be a consequence of physical mixing processes. The average replacement time of abyssal waters is 500 years (Stuiver *et al.*, 1983) and therefore this suggests that the core of marine DOC has been recycled several times. Therefore either riverine DOC discharge alone, or a few tenths of a per cent of marine primary production are quantitatively sufficient to support the average turnover rate of the marine DOC pool (Hedges, 1992).

The bulk of organic matter in seawater is produced *in situ* by marine organisms. Most of the material biosynthesized in surface waters is degraded in the upper several hundred meters of the water column where biotic processes dominate the fate of organic materials, however, abiotic processes also play a role.

Sediment pore-waters and marine sediments are essentially the only source from which "old" authigenic DOC may be directly introduced into seawater. Williams and Druffel (1987) have suggested input from sedimentary pore-water as a possible explanation for unusually negative $\Delta^{14}C$ in DOC from near-bottom Pacific Ocean water samples. Another possible means of introducing pore-water DOM into seawater is by thermally driven convection of water through sediments along the flanks of ocean spreading centres.

1.3.7. Sinks for organic matter in the coastal and estuarine environment.

Effective sinks for DOC are as obscure as the sources (Hedges, 1992). High rates of heterotrophic bacterial production throughout the surface ocean mean that a substantial portion (20-40 %) of photosynthetic production passes through a "microbial loop" (Azam and Fuhrman, 1984). However, this large DOC flux leads rapidly and almost quantitatively to remineralization and as a result the steady-state concentrations of reactive substrates are extremely small (Lee and Wakeham, 1988). The removal of the "old", well-mixed, structurally uncharacterized components of seawater DOM is less well understood (Hedges, 1992).

Mass balances alone indicate that DOC is destroyed somewhere in the ocean (see **Table 1.1**). The flux of DOC out of the ocean is comparable with the total burial rate of organic carbon in marine sediments, however there is little compositional evidence to suggest that most organic matter preserved in sediments came from seawater DOM (Emerson and Hedges, 1988). One process that is receiving growing support as a potential removal mechanism for seawater DOM is photochemical degradation to carbon dioxide (Mopper *et al.*, 1991).

In general, however, remineralization (oxidation of organic matter to carbon dioxide) is the most common fate of recently biosynthesized carbon and thus is the dominant sink term in the global organic carbon balance (Hedges, 1992).

The amount of organic matter that is produced in the ocean and transported landward may also be important, for example to be sequestered in coastal wetlands (Farrington *et al.*, 1992).

At ocean margins there is a steep gradient in salinity, light, ventilation and bacterial activity. These areas are therefore expected to be areas of degradation of DOM from terrestrial sources (Jickells *et al.*, 1991). An important feature of coastal carbon cycling in the coastal zone, in the context of the global carbon budget, is the large fraction of organic carbon exported from the water column either to the sediment or to the slope and open ocean (Wollast, 1991). The exact processes occurring are not well understood, however it is true that most organic matter (more than 80 %) is at present preserved in deltaic and coastal marine deposits and not in ocean upwelling regions or euxinic basins (Berner, 1982). The detrital organic carbon reaching the bottom of shallow areas is not necessarily buried and preserved in the coastal sediments. The shelf is a zone of high turbulence under the influence of vigorous currents where fine material is unable to accumulate. Storms can efficiently resuspend the sediments and on narrow shelves, dominated by coarse sand deposits, there is a strong tendency for export of the detrital organic carbon off the shelf and thus only a small fraction of this organic carbon is accumulated in the coastal sediments (Wollast, 1991). There is also reason to expect that a significant fraction (about 20 %) of the total organic matter in pelagic marine sediments may also be of terrestrial origin (Prahl and Muehlhausen, 1989), however, Gough *et al.* (1993) report that the near shore continental shelf acts as a major sink for terrestrial carbon introduced by fluvial discharge, with more than 90 % of the total depositing lignin occurring on the shelf.

Hedges (1992) has stated that the fraction of preserved terrigenous organic matter has been greatly underestimated. If preservation of terrigenous organic matter in marine sediments is larger than expected then estimates of the fraction of local primary production that is preserved in sedimentary organic matter would have to be corrected. Also the presence of land-derived organic components would have a strong influence on the types and amounts of organic substances that might be present (Hedges, 1992).

1.3.8. Summary.

The present perception of the global carbon cycle (see **Table 1.1**) presents a perplexing contrast. The great mass of recalcitrant organic materials exposed in weathering shales, exported from soils by rivers and cycled as dissolved macromolecules in seawater are all apparently remineralized efficiently somewhere in the ocean. In contrast, seemingly labile, recently formed plankton remains comprise the bulk of organic matter preserved in pelagic marine deposits, where preservation should be least favoured (Emerson and Hedges, 1988). In many cases, the physical transport terms (e.g. continental denudation rates, river discharge and sediment accumulation rates) are reasonably well known.

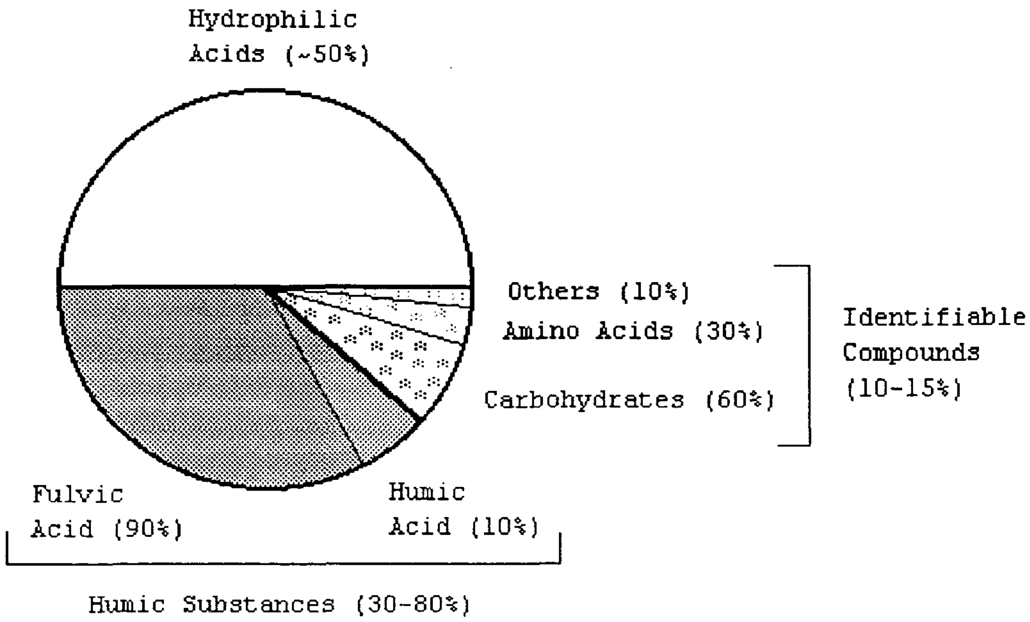
The major unknowns needed for flux and residence time estimations are the concentrations of organic species (ideally for each individual compound) in the different reservoirs and the first-order rate constants for their degradation within the different environments. This is difficult due to the many chemical substances involved and the variable rates and stoichiometries that are charateristic of environmental reactions (Hedges, 1992).

This point has also been made by Jickells *et al.* (1991) where the problem of distinguishing the fluxes depends on the boundaries considered. If there is a clear physical boundary, such as the sediment-water boundary, the determinations are relatively straightforward. Whilst the physical forcing on some margins are quantifiable it is not yet possible to quantitatively predict how they drive the biogeochemical cycle on the margins. In particular, it is not known how the rates of primary productivity are controlled in many shelf areas and these cannot be predicted from physical variables.

Understanding of the cycle of organic carbon in margin systems is very limited and whilst it is clear that organic carbon is being deposited in large amounts in this environment it is not understood what ultimately controls the amount finally buried.

1.4. Composition of DOC in natural waters.

The composition of DOC can be divided into three distinct groups. These are humic substances, identifiable compounds and hydrophilic acids (see **Figure 1.5**). The proportions of the various groups can vary greatly depending on the water type, nevertheless the diagram does help to indicate the relative proportion and importance of the different groups.



(Thurman, 1986; Degens and Ittekkot, 1983; Malcolm, 1985)

Figure 1.5. Composition of DOC in natural waters.

1.4.1. Humic substances.

Humic substances make up between 30 and 80 % of DOC in natural waters depending on the type of water (Malcolm, 1985) and are composed of three major fractions which when isolated are operationally defined in terms of their solubilities (Aiken *et al.*, 1985). Humin is that fraction of humic substances which is not soluble in water at any pH value. Humic acid is not soluble in water under acid conditions (below pH 2), but becomes soluble at greater pH and fulvic acid is that fraction that is soluble under all pH conditions. Fulvic acids have a lower molecular weight (1000 to 10,000 Daltons) than humic acids (18,000 to 100,000 Daltons) and are essentially aliphatic in nature (Degens and Ittekkot, 1983). The proportion of humic acid to fulvic acid varies. Fulvic acids may account for between 80 and 90 % of humic substances (Malcolm, 1983).

Polyunsaturated triglycerides present in seawater have been suggested as precursors in the formation of humic substances (Harvey *et al.*, 1983) where various cross-linking reactions by functional groups in the aliphatic chains produce a compound of higher molecular weight. Initially fulvic acids are produced then further cross-linking produces humic acid type compounds of higher molecular weight. Degens and Ittekkot (1983) have proposed a structure for the bulk of DOC extracted from natural waters. The structure consists of an aliphatic core with a molecular weight of around 350 Daltons. Surrounding this are various compounds such as fatty acids, phospholipids and carbohydrates which include various functional groups, such as amino acids and urea.

Humic acids, especially the ones of higher moleculare weight, are most likely to be colloidal in nature. It has been shown that the primary organic components of estuarine COC are carbohydrates and peptides (Sigleo *et al.*, 1982). The results were consistent with the composition of phytoplankton (approximately 50 % protein, 30 % carbohydrate, 10 % lipid and 10 % nucleotides by dry weight) and POC although the proportion of some of the components differed.

New light is thrown upon the possible structure of COC by Lee and Henrichs (1993). They suggest that the sources of DOC to the oceans should provide clues as to the nature of this material and identified the major sources as phytoplankton exudation, phytoplankton losses due to cell damage or lysis, the "microbial loop" (including bacteria and microheterotrophs), zooplankton excretion, the land largely via rivers and sediment porewaters (see for example Lee and Wakeham, 1988). By considering the structural and chemical properties that make molecules biologically refractory a structure for COC is proposed that comprises of a multiple layered 3-D structure of proteins and/or carbohydrates from recent biological sources. These structures would be most likely to occur in the colloidal size range. The structure would be biologically labile but would inhibit oxidation and acid hydrolysis and hence may include macromolecular materials with organic subunits, sulfur or other constituents that inhibit oxidation.

1.4.2. Identifiable compounds.

The amount of identifiable compounds present in natural waters vary between 10 and 15 % (Thurman, 1986). The main components generally remain the same, however, the trace components may vary. Carbohydrates are the main group present accounting for approximately 60 % whilst amino acids account for around 30 %. Other compounds present include hydrocarbons and fatty acids. Trace compounds found include chlorophyll and other pigments, urea, sterols, phenolic compounds, alcohols, aldehydes, ketones, organic bases, phosphorus compounds, organic sulphur compounds and pollutants (e.g. plasticisers and polychlorinated biphenyls).

1.4.3. Hydrophilic acids.

One of the largest pools of organic compounds present in most natural waters are the hydrophilic acids accounting for about 50 % of DOC. This percentage, however, can vary greatly (Thurman, 1986).

Little is known of the structure of these materials although they are thought to contain both simple organic acids, such as volatile fatty acids and complex polyelectrolytic acids that probably contain many hydroxyl and carboxyl functional groups. Leenheer (1981) reported that the infra-red spectrum of this fraction showed strong hydroxyl and carboxyl character.

1.4.4. Problems associated with identifying DOC.

Little is known about the structure of the bulk of DOC because of the difficulty involved in isolating these compounds. This is especially true of the hydrophilic acids which have only recently been effectively extracted from the water and inorganic salt matrix in which they are dissolved (Leenheer, 1981).

Conventional extraction and isolation techniques for individual classes of organic compounds from natural waters like acid hydrolysis and solvent extraction only release the peripheral compounds from the bulk DOC (Degens and Ittekkot, 1983). This would probably account for the low percentage of DOC which has been characterized by conventional techniques (Williams, 1975).

The approach of investigating the composition of COC should be of help in characterizing part of the DOC since this fraction is relatively easy to isolate (in sufficient quantity for various analytical methods) and makes up a substantial proportion of DOC (10-50 %). The humic acids that have classically been characterized by isolation, e.g. on XAD resin (see for example Malcolm, 1990) make up a proportion of the COC. Fox (1983) has reported that less than half of the ultrafilterable material (100,000 Daltons, 100 nm) was shown to be composed of humic acid. Therefore, it should be possible to characterize many compounds or aggregates in COC, hence providing further information on the composition of DOC (as defined by filtration through GF/F 0.7μm filters).

1.4.5. Molecular weight range of DOM in the ocean.

The molecular weight range of DOM in coastal and north Atlantic waters has been investigated by Carlson *et al.* (1985). They found that most of the DOM was in the less than 1000 Daltons molecular weight range, 34 % of the DOM had molecular weights greater than 1000 Daltons, 6 % had molecular weights greater than 30,000 Daltons and only 1 % had molecular weights greater than 100,000 Daltons. Sugimura and Suzuki (1988) estimated that molecular weights of DOM in the north Pacific ranged between 2000 and 60,000 Daltons, with more material in the higher range. They also found that the concentration of the higher molecular weight material decreased from surface waters to deep waters.

1.5. Origin of DOC.

The hydrology of major world rivers has a significant influence on the origin of DOC. Rivers which drain large lakes, such as the St Lawrence and MacKenzie Rivers, can be expected to have significant phytoplankton inputs to DOC. Rivers such as the Nile, Indus and Missouri have been altered by the construction of reservoirs which decreases sediment concentrations and increases the autochthonous carbon contribution to DOC. Rivers which do not have lakes or reservoirs and have high sediment concentrations which suppress primary carbon production, contain a great fraction of DOC of allochthonous origin. Examples of such rivers are the Amazon and Changjiang Rivers. The hydro-geology of a river can affect the origin of DOC as is the case of the Elbe River which flows across a lignite coal deposit (Spitzy and Leenheer, 1991).

1.5.1. Main sources for DOM in fresh waters.

Dissolved organic matter in natural waters originates from three main sources. These are *in situ* formation (autochthonous prodution), leaching of soil organic matter and anthropogenic inputs (allochthonous production). Other minor sources include atmospheric inputs, groundwater inputs (Leenheer *et al.*, 1974), input from sediments and diffusion of DOM from the river bed during periods of high fluvial discharge (Malcolm, 1985).

1.5.2. *In-situ* formation.

Dissolved organic matter can be released into the aquatic system by *in-situ* formation. This consists of (1) excretion of metabolic products from aquatic organisms such as fish, plankton and bacteria etc. (2) release of cellular components during grazing of phytoplankton by zooplankton (3) death and decomposition of the organisms (Singleton, 1990).

The nature of the material excreted by phytoplankton has been summarised by Lancelot (1984) and includes mono-, oligo- and polysaccharides, amino acids, proteins and polypeptides, fatty acids, glycollic acid, tricarboxylic acids, hydroxamic acids and vitamins. Cell lysis and grazing of phytoplankton by zooplankton also releases DOM into the water system (Hellebust, 1974). In addition DOM is released directly by zooplankton (Johannes and Webb, 1965). Production of polyphenolic compounds by algal communities has also been reported (Sieburth, 1969) together with the excretion of organic substances into water by a variety of marine organisms (see review by Ehrhardt, 1977).

Death and subsequent decomposition of plants and animals also releases DOM. In addition to containing labile compounds such as amino acids, sugars etc., a proportion of this material has been found to be resistant to biological decay (Otsuki and Hanya, 1972).

The formation of organic-rich colloids is associated with the condensation of monomeric or macromolecular material of biogenic origin. This may occur by physical processes such as flocculation (Sholkovitz, 1976) or abiological binding (Carlson et al., 1985), chemical processes such as photochemically initiated cross-linking (Harvey *et al.*, 1983) and biochemical processes such as direct exudation of colloidal material (Sigleo *et al.*, 1982). For example Brophy and Carlson (1989) showed that monomeric amino acids at natural seawater concentrations were transformed biologically into higher molecular weight (700 to 1400 Daltons) dissolved materials through the uptake of monomers by bacteria and excretion of polymeric material.

1.5.3. Terrigenous sources.

Soil organic material is transported into rivers and lakes via direct leaching of soils or slow extraction after percolation through the soil. The DOM which results is a soluble fraction of soil organic matter and should therefore resemble this material.

The nature of soil organic matter has been extensively reviewed (Stevenson, 1982) and similar material has been extracted from river (Gjessing, 1976) and lake water (Christman and Minear, 1971). It results from the degradation of plant material, consisting of both simple compounds, such as sugars, amino acids and fatty acids and compounds which are difficult to degrade such as lignin, cellulose and humic substances.

The study of aquatic organic matter resulting from soil leaching must be performed in areas where there is a large degree of surface run-off and low productivity, so that the contribution from *in situ* formation is low.

1.5.4. Anthropogenic sources.

Organic pollutants can enter natural water systems through a variety of routes including river run-off, discharge of industrial wastes, sewage (piped off-shore from land or direct input from ships), and oil pollution (either from leakage during off-shore mining or during transportation) (Singleton, 1990).

Anthropogenic contributions to the total DOC content are generally small compared to natural production, but some of these materials are resistant to biochemical degradation (e.g. alkylbenzene sulphonates, aromatic hydrocarbons etc.) and thus may persist in the environment for long periods.

Hydrocarbons present in natural waters can be derived from both living organisms and pollution. The occurrence of marine hydrocarbons, both from biogenic sources and as a result of pollution has been briefly reviewed by Parrish (1988). Natural hydrocarbons are often derived from fatty acids and as naturally occurring fatty acids generally have an even number of carbon atoms and are unbranched, a predominance of odd-numbered n-alkanes results. By comparison, there is no preference for odd-numbered carbon atoms in pollutant hydrocarbons. In addition, they tend to have slightly lower molecular weights than biogenic hydrocarbons (Barbier *et al.*, 1973) although overlap does occur. Chlorinated hydrocarbons are also indicators of pollution (Parrish, 1988).

Aromatic hydrocarbons, although present naturally in the environment, can result from oil pollution and, in addition, are products of fossil fuel combustion. Levels of marine alkylbenzenes have been correlated with the seasonal consumption of fossil fuels (Mantoura *et al.*, 1982).

Large concentrations of polynuclear aromatic hydrocarbons are also indicators of pollution as they are synthesised by very few organisms. They have been found in sewage, mussels, tap water (Kveseth *et al.*, 1982), estuarine water (Readman *et al.*, 1982) and in snow samples (Peters, 1992).

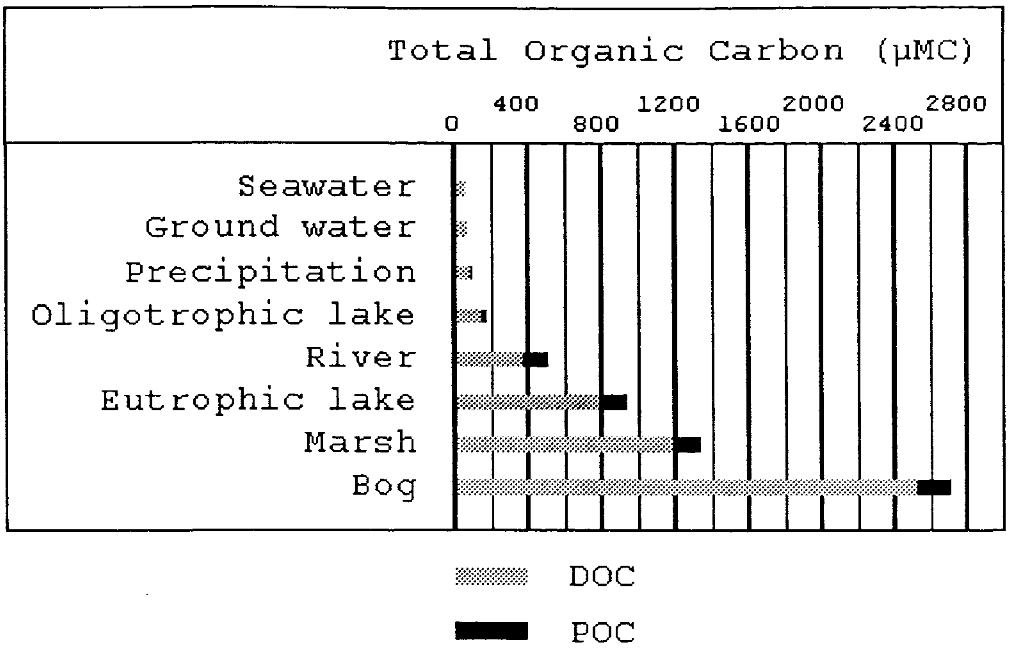
Other hydrophobic pollutants occurring in the aquatic environment include polychlorinated biphenyls, pesticides and phthalic acid esters (Peters, 1992).

1.6. The distribution of DOC and POC in natural waters.

The distribution of DOC and POC in natural waters depends on the type of water. **Figure 1.6** shows the "average" concentrations of DOC and POC in surface and ground waters. It should still be valid to compare the relative magnitudes of the DOC concentrations despite the recent controversy over the measurement of DOC in natural waters (see **Chapter 2** for a discussion of this).

Dissolved organic carbon concentrations vary with the type of water from *ca.* 40 μM C for ground water and seawater to over 2500 μM C for coloured water from organic rich bog and swamp areas. Seawater has the lowest DOC concentration with a median concentration of 40 μM C. Some pristine streams have low concentrations of DOC from 80 to 250 μM C. Rivers and lakes typically contain greater DOC concentrations ranging from 150 to 800 μM C, whilst swamps, marshes and bogs have concentrations of DOC from 800 to 5000 μM C (Thurman, 1986).

Particulate organic carbon also varies with the type of natural water. Ground water and interstitial waters contain very little POC as does precipitation (both as rainfall and snow) where concentrations range from less than 8 to 40 μM C. Seawater contains 0.8 to 8 μM C of POC which consists mostly of algal detritus. Large rivers can also contain large amounts of POC which is usually about a half the concentration of DOC. Particulate organic carbon may also equal DOC in the largest rivers and during times of high discharge (Thurman, 1986).



(Thurman, 1986)

Figure 1.6. Approximate concentrations of DOC and POC in natural waters.

1.6.1. Concentrations of DOC in Rivers.

Concentrations of DOC in rivers range from less than 80 μM C in alpine streams to more than 1600 μM C in some tropical or polluted rivers and rivers draining swamps and wetlands (see for example Naiman and Siebert 1978; Mulholland and Kuenzler, 1979). In terms of concentration, seasonal DOC variations within rivers are usually within an order of magnitude where most rivers show a "flushing effect", i.e. increasing DOC with increasing discharge. The flushing effect has been verified in many temperate zone studies (for example see Moore *et al.*, 1989) as well as in tropical and subtropical rivers (Richey *et al.*, 1991). A dominant soil and plant organic matter source of riverine DOC is therefore indicated. Climate has a pronounced affect on DOC levels in rivers and Thurman (1985) has given estimates of mean DOC concentrations for various climatic zones (**Table 1.2**).

As can be seen in **Table 1.2** rivers containing the lowest DOC concentrations are found in Arctic and alpine regions. Rivers in regions with cool temperate climates and arid climates also have low

DOC concentrations. Rivers containing the highest DOC concentrations are those which drain swamps and wetlands. Wet tropical climate, warm temperate climate and Sub-Arctic rivers have intermediate DOC concentrations. In areas of high pollution the riverine DOC concentration can also be extremely high (Ishiwatari *et al.*, 1980).

Environment	**DOC range (μM C)**	**Mean DOC (μM C)**
Arctic and alpine	80-400	170
Cool temperate climate	160-660	250
Arid climate	160-800	250
Wet tropical climate	160-1250	500
Warm temperate climate	250-1250	580
Sub-Arctic	650-2000	830
Rivers draining swamps and wetlands	400-5000	200
A polluted river (Tokyo, Japan)	2500-27,000	

Table 1.2. Riverine DOC concentrations for various climatic zones (Thurman, 1986).

1.7. Transformation processes affecting organic matter in estuaries.

The processes detailed in this section are those which affect the distribution of organic matter in estuaries and determine whether DOC is conservative or not. Processes affecting DOC include transformations of DOC to POC (flocculation, primary production) and POC to DOC (dis-aggregation of POC, respiration and degradation), desorption from mineral surfaces and adsorption onto mineral surfaces (hydrophobic adsorption and Van der Waals adsorption). Particles aggregate into larger particles via physical transport mechanisms such as Brownian motion, turbulent shear and differential settlement which join smaller particles together. Thurman (1986) lists five biogeochemical processes that are known to transform dissolved organic compounds in water; these are sorption/partitioning, aggregation, volatilization, oxidation/reduction and complexation.

1.7.1. Changes in physical variables (pH, pE and ionic strength).

Changes in the distribution of organic constituents between dissolved and particulate phases during transport through the estuary can be driven by changes in physical-chemical variables such as pH, pE and ionic strength. These variables also affect other processes such as aggregation and adsorption to particulates in the turbidity maximum as well as providing, in some cases, an useful indication of the progress of photosynthesis, respiration and bacterial activity. Variations of pH in estuaries are important in relation to the equilibria between dissolved and particulate species. The pH of oceanic water is around 8.1. Rivers can have highly variable pH values (4 to 9.5) (Baas Becking *et al.*, 1960). For example, rivers draining areas with high organic and low mineral inputs would typically be acidic in nature. Redox conditions in estuarine waters saturated with free oxygen clearly contrast to those found in reducing sediments or anoxic waters. The variations in redox conditions are primarily created by the decomposition of organic material involving different terminal electron acceptors, the most important of these stages being the utilizsation of free oxygen. The settling of organic material to the sediments frequently leads to the depletion of oxygen within the sediments. Geochemical interactions occur when oxic and anoxic waters mix and the resulting change in oxidation states may be associated with transfer from dissolved to particulate forms or vice versa. Coupling of chemical reactions in sediments with processes in overlying waters may also occur in the absence of resuspension, by diffusional fluxes across the benthic interface (Burton, 1988).

1.7.2. End-member composition.

Variation in end-member compositions form one set of factors which influence the extent to which a particular process becomes significant in the estuary. Of major importance is the relationship between the rates of physical-chemical reactions and the residence time of fresh water in the estuary, which reflects river discharge and the hydrographic regime, which in turn is influenced by tidal conditions and topography. Variability in the concentrations of dissolved constituents in river water and in the fluxes of these constituents entering estuaries arises in anthropogenically unperturbed systems through changes in the freshwater discharge. The average concentrations of dissolved constituents tend to decrease with increase in discharge, whereas the actual flux tends to increase (Walling and Webb, 1983). Changes in the master variables such as pH, salinity and both concentration and type of suspended particulate material (SPM) are caused by varying discharge. These would also affect particle/solution interactions and hence the concentration and flux of dissolved constituents in the estuary.

1.7.3. Mixing.

The mixing of river water and seawater gives rise to gradients in ionic strength, composition and pH that are dominantly horizontal, although stratification occurs to a greater or lesser extent, so that pronounced vertical gradients may also be found (Morris, 1985). Changes in concentration of dissolved constituents and particles are brought about by mixing processes and this has been depicted in **Figure 1.7**.

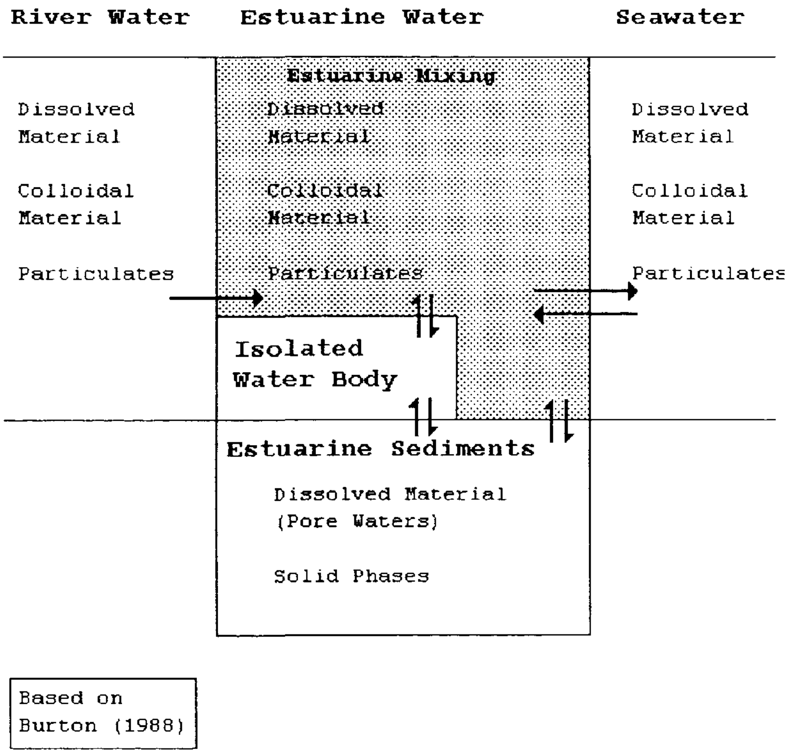


Figure 1.7. Sources and sinks of organic carbon in estuaries caused by mixing processes.

The fluxes of dissolved riverine constituents may be modified by exchanges of material between solution and particles, thus leading to non-conservative behaviour. Due to the greater complexity of particle transport processes and the inhomogeneity of particulate material, the dissolved fraction has generally been used to evaluate fluxes of materials in estuaries (Burton, 1988). However, the simultaneous investigation of both dissolved and particulate fractions and an understanding of the partition of constituents between them via the colloidal phase is increasingly being recognized (Balls, 1989; Turner *et al.*, 1992).

1.7.4. Removal processes for DOC.

A percentage of DOC undergoes aggregation on the mixing of river water with seawater. A substantial part of this is the humic acid fraction of DOC which is affected by flocculation and is likely to precipitate in the estuary (Sholkovitz, 1976). Aggregation of DOC may occur when the pH of water decreases, salinity increases and the polyvalent cation (Fe^{3+}, Al^{3+}, Ca^{2+} and Mg^{2+}) content increases which causes flocculation of bridge-bonded complexes. The subject of organic matter removal in estuaries was studied extensively during the late 1970s and early 1980s and has been covered in reviews by Thurman (1986) and Mayer (1985).

Mantoura and Woodward (1983) suggested that significant removal of DOC by processes such as particulate adsorption or flocculation does not occur in estuaries. Humic acids, which are low in reactive functional groups, invariably flocculate in electrolyte solutions (Theng *et al.*, 1968) and have been shown to flocculate in estuaries (for example see Sholkovitz and Copland, 1981). However, many reports suggest that riverine humic substances are dominated by low molecular weight fulvic acids (molecular weight 1000-10,000 Daltons) (for example see Reuter and Perdue, 1977). Fulvic acids were found to account for 80 % of the DOM in the Severn estuary (Mantoura and Woodward, 1983). Fulvic acids are hydrophilic polyanions, with an abundance of functional groups of high solubility even in seawater. Therefore, aggregation and removal of organics in estuaries is restricted to the high molecular weight, minor constituents of riverine DOC.

Humic compounds extracted from soils exhibit a wide range of molecular weights, with more than 60 % in the range 100,000 to 200,000 (Dell Agnola *et al.*, 1964). These compounds are not widely evident in the riverine environment and this may be due to processes causing molecular weight fractionation at the soil-stream interface. It may be that most of the high molecular weight organic fraction is carried by rivers in particulate form (as POC) or that the high molecular weight fraction is left behind in the soil environment (Degens and Ittekkot, 1983).

A mechanism by which dissolved humics might retain their solubility is seawater has been reported by Mantoura and Woodward (1983). They suggest that the polyanionic sites present in natural water humics are not saturated by the surge in Ca and Mg which occur in estuaries. This net negative charge on the humic molecules not only maintains solvation but also gives rise to electrostatic repulsion between neighbouring molecules and so actually inhibits coagulation. Mantoura and Woodward (1983) also show that particulate adsorption of DOC in the Severn estuary is negligible and contributes less than 1 % of the POC. The order of chemical removal processes of DOC in estuaries is flocculation > van der Waals adsorption > hydrophobic adsorption.

Dissolved organic carbon is well known for its ability to complex trace metals such as copper, cadmium, lead and zinc. If concentrations of these metals in solution are large, such as at mine tailing sites, precipitation of DOC-metal complexes may occur (Spitzy and Leenheer, 1991).

Two additional processes are postulated to cause DOC transformations in surface waters. These are photolysis and abiotic hydrolysis. Reaction rates and specific reactions of photolytic degradation of natural DOC input by rivers into the ocean have been reported (Kieber, 1989) where the alpha-keto acids glyoxylic and pyruvic acid were identified as photolytic breakdown products of terrestrial DOC which are introduced into the ocean. Mopper *et al.* (1991) have considered the photochemical degradation of DOC and its impact on the ocean carbon cycle. They suggest that this degradation pathway is the rate-limiting step for the removal of a large fraction of oceanic DOC.

1.7.5. Processes affecting POC.

Increasing sedimentation of clay and silt particles, which can be coated with organic matter, occurs in the estuarine zone where as much as 75 % of the suspended load may be trapped (Allen *et al.*, 1976). In vertically stratified estuaries such as the Amazon, 95 % of the sediment settles out within the mouth of the river before a salinity of 3 is reached (Milliman *et al.*, 1975). The concentration of POC may change dramatically during a tidal cycle (Laane, 1982) where it may vary by two to four times. The distribution of POC is also affected by increasing riverine discharge which depends on the season (Thurman, 1986).

The conversion of POC to DOC may also be caused by ionic strength increases. This process should occur in the upper estuarine zone and may be partially responsible for loss of POC as reported by Eisma *et al.* (1985). Another source of DOC in the estuary is the biological conversion of POC to DOC. Laane (1982) found that detritus from phytoplankton is rapidly degraded whilst POC from terrestrial sources, which includes soil and plant organic matter, is degraded more slowly. Particulate organic carbon can also increase during times of large algal productivity such as the spring and summer blooms in temperate climates (Laane, 1982).

Microbial decay of organic carbon is an important process (Thurman, 1986). Simple low molecular weight compounds, such as carbohydrates, amino acids and fatty acids are decomposed by heterotrophic bacteria in a matter of hours. High molecular weight dissolved organic substances that are biodegradable, but cannot pass cell membranes until they are broken down by extracellular enzymes or abiotic processes, decompose on a range of timescales from days to months (Saunders, 1976). Straight-chain aliphatic hydrocarbon structures are readily broken down but branched and cyclic aliphatic structures, such as terpenoids and steroids are refractory to aerobic degradation processes. Highly substituted aromatic ring structures, which are found in tannin and lignin residues are also resistant to biological degradation. Eutrophication can increase the concentration of both DOC and POC within the estuary due to the input of nutrient rich waters, where the affects of eutrophication are often greatest at the seaward end of the estuary (Szekielda, 1982).

1.7.6. Sedimentation and the turbidity maximum.

The particulate fractions of constituents in estuarine regimes are affected by sedimentation, often leading to their major retention in estuaries (Dyer, 1986). This is also reflected in modification of the flux of dissolved constituents which may occur through addition (or removal) at the benthic boundary. The concentration of SPM in many estuaries is not so much determined by the concentrations in the riverine and marine end-members as by resuspension of particles from the sediments. In the turbidity maximum the nature of the material is reflected by the effects of erosion, suspension and settling. Turbidity maxima are important regions for the redistiribution of material between particles and solution particularly because of the differences in redox conditions often seen between the bottom sediments and the overlying water column (Burton, 1988). Accumulation and partial transformation of POC in the area of the SPM maximum has been observed by several authors (Cadée, 1982; Cadée and Laane, 1983; Cauwet 1985; El-Sayed, 1988), although the exact mechanism is not well understood. The accumulation of organic-rich particles at the halocline is also favourable to bacteriological activity. According to Azam and Cho (1987), organic detritus is colonized by bacteria attached to the surfaces or remaining in their close vicinity. Bacteria solubilize POM with exoenzymes and then partly utilize the resulting DOM. This results in higher DOC production as a result of autolysis and hydrolysis by the exoenzymes produced by attached bacteria (Cauwet, 1991).

1.7.7. *In-situ* biological processes.

Particles originate in the water column as primary particles or aggregated particles. Primary particles arise directly from either inorganic or dissolved organic sources. In the ocean the majority of primary particles are organic, however, the contribution of inorganic sources to the primary particle pool increases upon proximity to the shore or bottom. Primary particles can be phytoplankton, bacteria or microorganisms, arising from biological action and utilization of DOM as well as inorganic particles from, for example resuspension events. Aggregated particles may be composed of shells, zooplankton, fecal pellets and carcasses arising from animal consumption as well as marine snow and microaggregates arising from coagulation processes (Karl *et al.*, 1991). In estuaries these processes are further affected by the close proximity of the sediments, lateral transport along the estuary, changes in the physical-chemical properties of the water column and the residence time of freshwater in the estuary.

Biological processes can give rise to the production of particles and can affect the conversion of particles to dissolved forms (**Figure 1.8**).

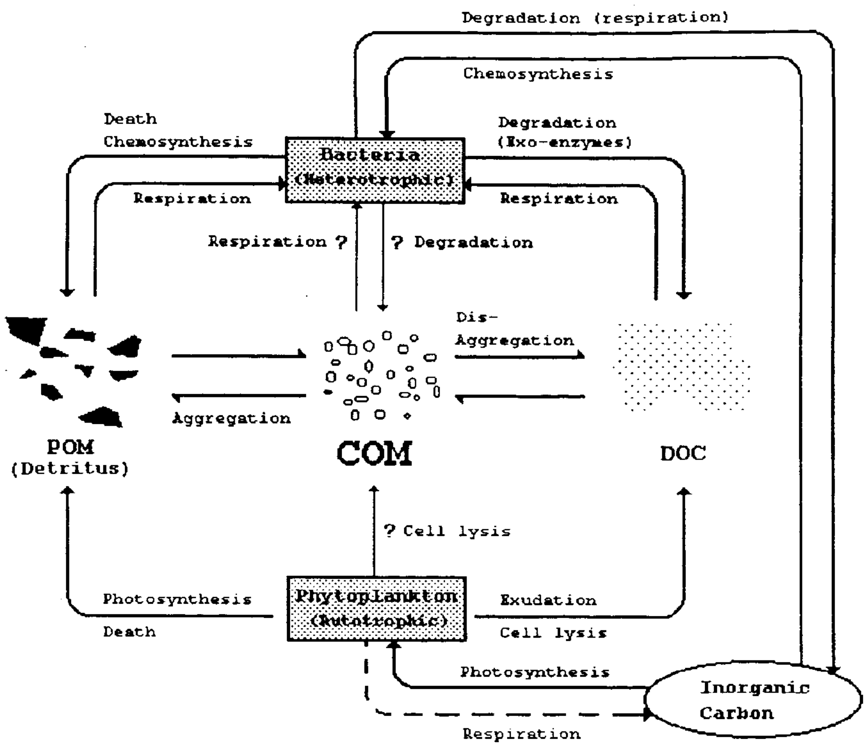


Figure 1.8. Biological processes potentially affecting forms of organic carbon in estuarine waters.

Organic particles arise from a variety of sources but most are produced through photosynthesis by eukaryotic and prokaryotic algae (e.g. phytoplankton and cyanobacteria) where inorganic forms of carbon are transformed into organic particles. Bacterial chemosynthesis may occur, for example, in anoxic waters whilst coagulation processes during phytoplankton blooms can form organic particles (Karl *et al.*, 1991). Death of the various algal forms also produces detritus which is particulate in nature. DOM is produced by bacteria and phytoplankton via exudation of organic compounds (e.g. exoenzymes) and also by bacterial degradation events. Bacteria transform DOM into organic particles (Azam *et al.*, 1983) by respiration whilst POM can also be respired by bacteria into inorganic or dissolved organic forms. Colloidal organic matter (COM) may be involved in these processes and be transferred into particulate or dissolved forms, however, due to the small size of COM and the fact that phytoplankton are particulate in nature the processes affecting COM (e.g. respiration or degradation) are most likely to be influenced by bacterial action.

In general bacteria can be responsible for the production (e.g. degradation or death) and consumption (respiration) of organic matter, whereas phytoplankton are important in producing organic matter (e.g. cell lysis or death). Bacteria are likely to be associated with COM and DOM (and also by attachment to POM) as bacteria are colloidal in nature. Phytoplankton are important to the particulate forms and DOM as phytoplankton are particulate in nature. During periods of elevated phytoplankton activity high levels of POM followed by high levels of DOM are likely to result. With high POM levels in the water column bacterial levels may also be high and this would result in increased DOM and possibly COM production. If DOM concentrations are high this may cause higher COM levels due to bacterial respiration of DOM.

1.8. Objectives of the study.

The oceans are a major global reservoir for organic carbon (see references in this chapter) and knowledge of the cycling and fate of DOC in this environment is important in understanding the oceanic carbon cycle. The overall original goal of the study was to re-appraise existing methods of DOC analysis in seawater in order to improve our understanding of the origin and cycling of organic carbon in the sea. At an early stage in the project major uncertainties became apparent with the DOC analysis method to be used in the study (an ultra-violet (UV) persulphate DOC analysis method) due to the development of a HTCO method of DOC analysis (Sugimura and Suzuki, 1988). Because of the problems associated with demonstrating the accuracy of DOC determinations, greater emphasis was placed on the use of carbon isotopes to study the cycling of organic matter in the marine environment. The work at first centered on the use of ^{14}C as a tracer for studying the sources of organic material in the ocean. This was then extended to the use of $\delta^{13}C$ signatures to investigate the sources and cycling of organic material in the coastal environment. It was intended to use $\Delta^{14}C$ activities to give ages and $\delta^{13}C$ signatures to give sources of organic carbon in marine samples.

The use of XAD resins to separate fractions of DOC was originally investigated. However, due to initial contamination problems with the resins and the emergence of new Anotec Anopore filters which provided a new opportunity to study colloidal carbon phases, XAD work was discontinued.

The fluxes of both particulate, colloidal and dissolved organic substances discharged through rivers are subject to modification in estuaries. Knowledge of such modifications is needed to understand the cycling of these substances in the coastal environment. Therefore, a local estuarine system was chosen (the Beaulieu estuary) which had a high organic matter content so that the transfer of organic matter from land sources into the coastal sea could be studied, and form the basis of an investigation into these processes.

Therefore, the main objectives of this research were:

(1) A continuous investigation of the accuracy of the UV persulphate method of DOC analysis by comparison with the HTCO techniques, including the development of a more efficient method of UV persulphate DOC analysis using mercury salts to aid oxidation of organic material.

(2) The use of $\delta^{13}C$ signatures to follow the transfer of organic material, including colloidal forms of organic matter, from land sources into the coastal sea.

(3) Use of $\Delta^{14}C$ activities to obtain age estimates of colloidal and particulate phases in the estuarine system.

(4) A study of processes influencing the cycling and fate of different size fractions of carbon during estuarine mixing in an organic rich system.

The thesis initially discusses the cycling of organic carbon in the marine system and goes on to consider the current view of recent developments in determining DOC in seawater. The method development involved in DOC analysis is then discussed together with procedures which evolved for the preparation of carbon isotope samples. The main section of the thesis describes the applications of these methods to the Beaulieu estuary and discusses the implications of processes occurring during estuarine mixing on cycling and fluxes of organic carbon to coastal waters.

Chapter 2:

Dissolved organic carbon analysis.

2.1. Introduction

The chapter initially considers DOC analysis and the HTCO method of DOC analysis (Sugimura and Suzuki, 1988). Possible reasons are given for the recent downward re-evaluation of DOC concentrations reported by Sugimura and Suzuki (1988). The current "state of the art" in DOC analysis is reviewed and the accuracy of both the HTCO and wet chemical oxidation (WCO) methods of DOC analysis are questioned.

The general method for analysis of DOC in natural waters is removal of inorganic carbon from the sample by acidification of the sample and sparging with a stream of oxygen gas. The sample then only contains organic forms of carbon which are oxidized to carbon dioxide followed by detection of this carbon dioxide by, for example, an infra-red gas analyzer.

A general review on the analysis of DOC prior to the widespread use of HTCO analyzers has been given by MacKinnon (1981) and on DOC in natural waters by Degens and Ittekkot (1983).

2.2. Literature review of DOC analysis.

Methods of DOC determination can be classified according to the oxidation process. These are wet oxidation methods, dry oxidation methods and direct injection HTCO methods. The wet oxidation method can be further divided into chemical oxidation and UV oxidation or both of these combined.

2.2.1. Wet oxidation methods.

Wet oxidation methods include chemical oxidation by persulphate (Menzel and Vaccaro, 1964: Williams, 1969; Bikbulatov, 1974; Boehm, 1975; Goulden and Anthony, 1978) or photo-oxidation under ultra-violet (UV) irradiation (Beattie *et al.*, 1961: Armstrong *et al.*, 1966; Grasshoff, 1966: Armstrong and Tibbits, 1968; Ehrhardt, 1969; Woelfel and Sontheimer, 1974; Propp and Propp, 1977; Muller and Bandaranayaka, 1983). The use of persulphate and UV oxidation in combination were developed for use in automated systems (Collins and Williams, 1977: Statham and Williams, 1983; Cauwet, 1984).

The use of persulphate oxidation, when used alone requires a high temperature (*ca.* 120^{0}C) and a high reagent concentration, giving high but consistent blank values.

One of the most sensitive analyzers developed so far, using only UV irradiation, was that of Muller and Bandaranayake (1983). This was based on the reactor developed by Graentzel (1980). The efficiency of this sophisticated analyzer relied on the thin sample film developed in the UV reactor. However, using only UV irradiation it showed a lack of oxidation for some nitrogen and sulphur containing compounds.

The combination of the two methods (for example see Statham and Williams, 1983) seems to be able to solve most of the problems and gives higher oxidation efficiencies (Cauwet, 1984).

2.2.2. Dry oxidation methods.

Dry oxidation methods involve dehydration of the sample followed by combustion at high temperature (Skopinstev, 1960: Montgomery and Thom, 1962; Croll, 1972; Gordon and Sutcliffe, 1973; MacKinnon, 1978).

The dry oxidation method is suitable for the analysis of DOC in fresh waters but problems arise for brackish and sea waters. This method also needs a preliminary drying step hence limiting the volume to be analyzed. Therefore a sensitive detection system is required due to the small amounts of CO_2 involved and care must be taken to avoid contamination.

2.2.3. Direct injection methods.

The method of direct injection HTCO was introduced by van Hall *et al.* (1963) for the measurement of DOC in freshwater. The unit assembled by Sharp (1973) was more suitable for the analysis of seawater samples in that the analyzer was not as prone to corrosion and it had a lower detection level. The analyzer of Sugimura and Suzuki (1988) further improved the furnace design and engineering of the whole combustion train coupled with a more sensitive non-dispersive infra-red detector.

2.2.4. The "conventional wisdom" view of the analysis of DOC in seawater prior to 1988.

The differences between wet and dry oxidation methods have been discussed (Sharp *et al.*, 1973: Wangersky, 1975; Skopinstev, 1976; MacKinnon, 1978; Gershey *et al.*, 1979). MacKinnon (1978, 1981) and Gershey *et al.* (1979) confirmed that the persulphate method produces results that are on average 15 % lower than that of the dry combustion method. The difference between dry combustion and photo-oxidation is less than 5 % on average accoring to these authors. The conclusion was that discrepancies between the wet and dry methods are more the result of poor working conditions than differences in the principle (Gershey *et al.*, 1979).

The question of the accuracy of DOC analysis of seawater was reopened by the work of Sugimura and Suzuki (1988), which has demanded a full critical appraisal of the techniques used.

2.3. The HTCO-DOC method of Sugimura and Suzuki (1988).

2.3.1. Introduction.

The direct injection HTCO analyzer (MRI (Meterological Research Institute)-HTCO analyzer) developed by Sugimura and Suzuki (1988) has proved controversial because the DOC concentrations measured by this method are much greater than those obtained by conventional methods and because the inverse AOU-DOC relationship has been interpreted to suggest that DOM, not sinking particulate organic matter, is the main oxygen sink and source of respirative nutrients to the deep western Pacific Ocean (Farrington *et al.*, 1992). Previously the relationship between DOC and AOU was not explained adequately in open ocean water (for example see Williams, 1971).

The overall implication is that DOM may play a much more active role than previously appreciated in the cycling of biogenic material within the ocean (Farrington *et al.*, 1992).

Brewer *et al.* (1986) suggest that the amount of DOC in the ocean may have been underestimated by a factor of two to three. This "new" pool of refractory organic matter which is not oxidized by the UV persulphates oxidation method is concentrated in the surface and deeper layers of the ocean. The closest agreement therefore between the wet chemical results and the HTCO results of Sugimura and Suzuki (1988) are for water from the oxygen minimum zone. The "additional" carbon near the surface and in the mixed layer appears to be actively involved in the biogeochemical cycling of carbon and nitrogen (Druffel *et al.*, 1989).

2.3.2. Implications of higher DOC concentrations.

Whilst higher concentrations of DOC in seawater remains to be fully verified, their presence can have a significant impact on our understanding of carbon cycling in the oceans.

2.3.2.1. Processes on a global scale.

The higher concentration of DOC and the apparent biological lability of the newly revealed fraction have profound implications for not only the size of the oceanic reservoir, but for the degree to which biological processes are involved in the cycling of marine organic matter, the role that the oceans play in terms of modifying the atmospheric concentration of carbon dioxide and the interplay of the oceans and atmosphere in response to the greenhouse effect and global climate modification (Peltzer *et al.*, in press).

2.3.2.2. Evidence that supports higher DOC concentrations in the ocean.

High concentrations of organic matter containing organic acids could explain discrepancies between alkalinities measured in seawater and those calculated from the concentration of the different titrated chemical species in the samples (Bradshaw and Brewer, 1988).

Higher DOC values have been useful in explaining the vertical distributions of nutrients in models where expected particulate fluxes and regeneration have been inadequate (Toggweiler, 1989).

Previous DOC-AOU profiles using UV persulphate did not show a good correlation and there is an imbalance between reduced carbon exported out of the euphotic zone (as measured by sediment traps) and the higher amounts expected from *in situ* production inferred from seasonal oxygen signals (Williams and Druffel, 1988).

An important feature of the higher DOC concentrations measured by HTCO is that in depth profiles in the open ocean AOU and DOC are linearly related to each other (Sugimura and Suzuki, 1988; Peltzer *et al.*, in press). However, Sharp *et al.* (1993) also report that DOC measured by persulphate oxidation showed a correlation with AOU. This implies that the dominant consumption of oxygen after a water parcel leaves the surface is by the oxidation of DOC transported with it (Jackson, 1988). These results suggest that particle sedimentation is less important than previously thought in determining chemical distributions in the ocean (Jackson, 1988). However, recently profiles of DOC distribution, as measured by HTCO, do not show such a clear correlation with those of dissolved oxygen in the western North Pacific and in the southern California Bight (Tanoue, 1992 and Hansell *et al.*, 1993 respectively).

2.3.2.3. Processes in the ocean that do not favour higher DOC concentrations.

Potential significant increases in trace metal complexation could result from elevated DOM concentrations as well as modification of global dissolved oxygen balances (i.e. mineralization of the "new" DOC) in surface waters (Jackson, 1988).

There is no evidence for the requisite increase in dissolved organic phosphorus or dissolved organic nitrogen as would be expected from Redfield C:N:P restraints (Williams and Druffel, 1988; Karl *et al.*, 1993). This discrepancy suggests that either an error has been made in the analysis of one or more of the dissolved element concentrations or that the UV persulphate resistant DOM fraction has a very low phosphorus content compared with average marine plankton (Farrington, 1992). Also, with the higher DOC values much more nitrogen would be potentially available through microbial regeneration than previously thought. If this were the case certain restraints would have to be placed on both the heterotrophic regeneration rate and uptake of nitrogenous substances by phytoplankton to maintain this dissolved organic nitrogen pool.

2.3.3. DOC concentrations reported by Sugimura and Suzuki (1988).

Sugimura and Suzuki (1988) report that for various depth profiles the measured values of the concentrations of DOC in open ocean water have varied from 25 to 250 μM depending on which technique is used to measure the DOC concentration.

The wet oxidation methods give a surface value of between 83 and 167 μM while the dry combustion values range from 125 to 250 μM. The discrepancies between the methods are even more marked in the deep samples, where the persulphate values are close to 40 μM and the dry combustion values range from 83 to 140 μM.

Values for the MRI-HTCO method vary from 186 to 323 μM in surface waters of the western north Pacific Ocean to 100 μM in deeper water, however values as high as 493 μM were reported in the East China Sea (Sugimura and Suzuki, 1988).

2.3.4. Reproduction of the higher DOC values measured by HTCO.

There have been many attempts to reproduce the DOC values measured by Sugimura and Suzuki (1988) using different DOC analyzers and there is a wide cross-section of results for the various analyzers that have been compared. This is evident from the large variability in DOC concentrations (± 40 % of the mean) measured by different instrument types from the Seattle DOC/DON Workshop (Hedges *et al.*, 1993a). Other comparisons have varied from complete disagreement between the HTCO method and the persulphate method as well as disagreement between two types of HTCO analyzer in coastal waters (Sharp *et al.*, 1993) to agreement between two HTCO methods in open ocean water (Bauer *et al.*, 1990). Cauwet *et al.* (1990) found that the HTCO method and the UV persulphate method agreed closely in waters of the Mediterranean whilst De Baar *et al.* (1993) have found no difference between the HTCO method and the persulphate method in waters of the open Atlantic Ocean.

Differences in the DOC concentration measured by HTCO and UV persulphate oxidation methods may not be large in freshwater (Skopinstev *et al.*, 1976; Whitehouse *et al.*, 1989; Suzuki and Tanoue, 1991; Benner and Hedges, 1993; Sharp *et al.*, 1993). Benner and Hedges (1993) reported that a Dohrmann DC-80 UV persulphate analyzer and a Shimadzu HTCO analyzer measured essentially identical concentrations in freshwater samples from the Amazon river, whilst Suzuki and Tanoue (1991) found that the concentration difference between both methods is small in the continental margin (120 %) and quite large in the open sea (320 %). The difference between the two methods became smaller as lower salinities were reached (at around 30).

2.3.4.1. DOC concentrations reported.

Values in the 70-140 μM range found in the open north Atlantic Ocean have been reported (De Baar *et al.*, 1993 and Kepkay and Wells, in press) and for the Sargasso Sea (Bauer *et al.*, 1990). Earlier investigations of the Atlantic Ocean were based on conventional methods of DOC analysis and are either similar to or lower than these results.

Recently reported values for the western North Pacific are also in the same range as these (Ogawa and Ogura, 1992; Tanoue, 1992) except for a distinct surface maximum reaching 170 μM at one station (Tanoue, 1992).

Values quoted for the Mediterranean Sea rarely exceeded 125 µM in surface waters, with deep water values of about 50-60 µM (Cauwet *et al.*, 1990).

However, these values of about 150 µM for surface waters are still below some of the values recently reported by others for the north Atlantic (110-170 µM; Peltzer *et al.* (in press) and 115-250 µM; Suzuki and Tanoue (1991)), for the equatorial Pacific (140-240 µM; Martin and Fitzwater (1992)) and for the Pacific Ocean (180-320 µM; Sugimura and Suzuki (1988)).

Therefore, there seems to be a trend in DOC values for the various comparisons that have been carried out between different DOC analyzers. Hence the MRI-HTCO instrument always shows very high DOC values followed by intermediate values for some HTCO instruments (Sharp *et al.*, 1993; Peltzer *et al.*, in press). Other HTCO instruments and conventional instruments show lower DOC values which are in agreement (Ogawa and Ogura, 1992: Cauwet *et al.*, 1990; De Baar *et al.*, 1993).

2.4. Possible reasons for the elevated concentrations of DOC measured.

A number of reasons for the elevated levels of DOC have been proposed i.e. the so called "new" or "additional" DOC. These include a different colloidal refractory structure for the "new" DOC (Lee and Henrichs, 1993), formation of organic intermediates by chemical oxidation and UV photolysis which are more refractory than the starting materials (Peyton, 1993) and inadequate preservation techniques (Wangersky, 1993).

2.4.1. Colloidal structure for the "new" DOC.

A substantial portion of DOC remains uncharacterized (see **Chapter 1.4**). It has therefore been suggested that the "new" DOC that is not oxidized by the UV persulphate method but is oxidized by the HTCO method has a unique structure and is different in reactivity from the material that is oxidized (Lee and Henrichs, 1993). The "new" material measured by HTCO is biologically labile but chemically resistant (to wet oxidation and UV attack) whereas the UV persulphate oxidizable DOC is more biologically refractory but more chemically reactive (Sugimura and Suzuki, 1988).

By considering the structural and chemical properties that make molecules biologically refractory a structure is proposed that comprises of a multiple layered 3-D structure of proteins and/or carbohydrates from recent biological sources (Lee and Henrichs, 1993). Therefore labile compounds such as proteins and carbohydrates would be present in a chemically resistant matrix. The structure would be biologically labile but would inhibit oxidation and acid hydrolysis and hence may include macromolecular materials with organic subunits, sulphur or other constituents that inhibit oxidation. As the layered polymeric material is subject to chemical attack, the layers may be destroyed one by one with each layer temporarily protecting the one below. These structures would be most likely to occur in the colloidal size range.

2.4.2. Scavenging of sulphate radical anions (SRA) by the chloride ion.

One of the main oxidative species that oxidizes the organic matter in a UV persulphate analyzer is SRA. There is also a competitive reaction for SRA between organic matter and Cl^-. Theoretical calculations indicate that Cl^- would scavenge more than 99 % of SRA, leaving only traces of SRA for radical attack on DOM (Peyton, 1993). However, potassium persulphate oxidizes well defined organic compounds that are added to seawater. These observations indicate that other intermediate free radicals and/or Cl_2 may contribute mechanistically to the UV oxidation of organic compounds in seawater (Williams and Bauer, 1993).

The scavenging of sulphate radicals by chloride has been identified as one of the biggest problems in the oxidation of organic matter by the UV persulphate method (Peyton, 1993). This has been discussed further in **Chapter 3.2.5.**

2.4.3. Formation of refractory intermediates.

The processes of chemical oxidation and UV photolysis are a possible source of refractory organic matter where the oxidized organic intermediates are more refractory than the starting materials. The order of reactivity of organic functionalities with sulphate radicals is aromatic > CH_2 > CO > COOH. Therefore, the oxidation products are more refractory than the parent compounds and this results in a less reactive residue rich in carboxylates. If this occurs with a reduction in the oxidizing capacity of the oxidizing agent, by reaction with the chloride ion, then the oxidized refractory compounds would persist (Peyton, 1993).

Chlorine gas or other reactive species might be produced from the chloride in seawater under the conditions used during UV persulphate oxidation. Both phenolic and non-aromatic constituents of humic substances as well as non-humic substances such as protein react with chlorine (Scully *et al.*, 1988) and may produce refractory compounds. In addition algae and algal exudates have been found to producce organochlorine compounds during the chlorination processes (Karimi and Singer, 1991). A possible mechanism for the formation of a DOC fraction stable to oxidation is the development of a coating of chlorinated material on the surface of organic colloids that protects their interior (Lee and Henrichs, 1993). The formation of refractory chlorinated organics, however, is probably not important due to the low concentration of the chlorine atom in solution (see **Chapter** 3.2.5).

2.4.4. The effect of preservation on the "new" DOC.

It has been suggested that the concentration of DOC can decrease rapidly after sampling, in the order of hours (Sugimura and Suzuki, 1988). The rate of disappearance would be different for samples from different oceanic areas and for different seasons, even for samples that have been preserved by some method (Suzuki *et al.*, 1992). It has therefore been suggested that the "new" DOC would also be found by wet oxidation methods with proper sample preservation (Wangersky, 1993).

2.5. Re-evaluation of the initial high levels of DOC reported by the MRI-HTCO method of Sugimura and Suzuki (1988).

Suzuki (1993) has recently reported that the analytical technique of Sugimura and Suzuki (1988) was inaccurate and on re-evaluating the DOC concentration at two of the stations they found that these earlier values were incorrect. Following the development of a new HTCO analyzer, the "Sumigraph TOC-90" (see for example Suzuki *et al.*, 1992) Suzuki (1993) has made DOC measurements in the equatorial Pacific where the strong relationship between DOC and AOU was not reproduced. Tanoue (1992) concludes that the HTCO method can extract a substantial portion of DOM that was not oxidized by the previously used methods. However, there is no agreement on the magnitude of the difference and it is unclear whether the difference in concentrations of DOC is relatively uniform with depth or is variable and greater at the surface.

2.5.1. Instrumental problems with HTCO analyzers and possible reasons for these high values.

Problems that were encountered with the DOC analyzer of Sugimura and Suzuki (1988) include (1) the sensitivity of the non-dispersive IR detector which was too low (2) insufficient consideration given to the influence of gases other than CO_2 that are generated during the oxidation step (3) blank values that were not subtracted from the DOC data (Suzuki, 1993).

The most important feature appears to be the blank, as blank corrections can be a significant component (50 %) of the total instrument response in waters that contain low concentrations of DOC (Williams and Bauer, 1993). However, evaluating the system blank is not as straightforward (Williams and Bauer, 1993). It is derived from part of the system itself, such as the catalyst, copper oxide, the quartz tube, the connector and carrier gas. Tanoue (1992) suggested that the system blank was not only derived from the contaminating carbon in the internal components of the oxidation column and carrier gas (instrument blank) but also included a "sample memory blank". He suggested that a "running system blank" (instrument plus sample memory blanks) should be quantified during the course of DOC analyses.

A potential source for this blank is the catalyst where a large amount of contaminating carbon is present in a newly prepared column (Tanoue, 1992). This carbon is only gradually oxidized during a series of instrument runs. Benner and Strom (1993) report that a major source of carbon contamination contributing to the blank was derived from the catalyst during the combustion process which decreased to fairly constant levels (10 to 50 μM C) with increasing usage of the catalyst.

2.5.2. The currrent "state of the art" (June, 1993).

Williams and Bauer (1993) have noted that it is not the differences of 5 to 10 % between DOC values determined by HTCO and wet chemical oxidation methods that are of concern. These could be accounted for by correctly applying appropriate blank corrections, subtle differences in signal detection and processing and other operator related factors. It is the discrepancies as high as 100 to 200 % that are important. These differences may be attributable to some procedural inadequacy in previously used methodologies, the inability of previously employed methods to oxidize all forms of DOC or erroneously high results from the HTCO method.

The initial very high levels of DOC measured by Sugimura and Suzuki (1988) may have been due to high blank levels derived from the catalyst (Hedges *et al.*, 1993a; Benner and Strom, 1993; Suzuki, 1993). A "sample memory blank" was also reported (Tanoue, 1992). The magnitude and variability of blanks could easily lead to DOC measurements in ocean water that are 25 to 75 % higher than actual concentrations (Benner and Strom, 1993) and were sufficient to account for much of the variability recently observed among analysts during comparative analyses of DOC in natural waters (Hedges *et al.*, 1993a).

As Hedges *et al.* (1993b) points out, the downward re-evaluation of the data of Sugimura and Suzuki (1988) may not be interpreted to infer that persulphate resistant DOC does not exist. As seen in **Section 2.3.4** a number of researchers have measured elevated concentrations of DOC in seawater using HTCO based analyzers. However, other researchers have measured no difference between wet oxidation methods and the HTCO technique. Hansell *et al.* (1993) report that DOC concentrations measured by the HTCO TOC-90 analyzer (Suzuki *et al.*, 1992) were only 20 to 30 μM C greater than previous estimates using WCO methods, whilst Chen and Wangersky (1993) found that the HTCO method of DOC analysis measured more DOC with most of the results in the range 10 to 40 % greater.

Therefore, the initial very high levels of DOC measured by Sugimura and Suzuki (1988) may have been due to high blank levels derived from the catalyst (for example see Benner and Strom, 1993) and at present the DOC data of Sugimura and Suzuki (1988) is being revised downwards. The extent of this downward re-evaluation is at present not known, however, HTCO methods of DOC analysis may measure 10 to 40 % more DOC (Chen and Wangersky, 1993).

2.6. The accuracy of both the HTCO and UV persulphate method of DOC Analysis.

For DOC analysis, the definition of absolute accuracy is that all of the organic carbon in a sample is oxidized and measured (without the introduction of contamination). The problem with determining accuracy is that there is no comprehensive description of the composition of the DOC pool or which fraction of this pool is difficult to oxidize with the present methods. This point along with the fact that it is difficult to get representative DOC samples has meant that the use of standard reference materials has not been widely employed. Another unknown is the variation of DOC composition in time and space and the variation in its refractory character (Williams *et al.*, 1993). Therefore, there is the possibility that HTCO methods of DOC oxidation do not oxidize all of the organic matter present (Suzuki, 1991).

None of the present methods for DOC analysis (i.e. HTCO or WCO) fulfil the criteria for setting the accuracy, for a method cannot assess itself (Williams and Bauer, 1993). A possible referee method is that of sealed tube combustion or CHN analysis. Sealed tube combustion is known to oxidize very refractory substances such as graphite and diamond and also allows the stable isotope ratio of the combusted material to be determined, which could provide a further check on the completeness of oxidation (Williams *et al.*, 1993). The idea behind this is that any DOC oxidation system that did not oxidize all of the DOC present would introduce fractionation into the sample for ^{13}C analysis and hence give an inaccurate $\delta^{13}C$ signature.

Chapter 3:

Development of methods.

3.1. Introduction.

This chapter considers the accuracy of the UV persulphate DOC analyzer of Statham and Williams (1983), and the use of mercury salts to increase its DOC oxidation efficiency. Further method development included the construction and use of a vacuum line to prepare gaseous CO_2 samples for carbon isotopic analysis. The chapter ends by describing the development of an ultrafiltration method using Anotec Anopore filters to separate COC from natural waters.

3.2. Dissolved organic carbon analysis.

3.2.1. Introduction.

The accuracy of DOC analysis has been open to speculation (**Chapter 2.3.1**) and various DOC concentrations in natural waters have been reported depending on which DOC analysis technique was used (**Chapter 2.3.4.1**). The overall conclusion was that there is no agreement on the magnitude of the difference between DOC concentrations measured by the UV persulphate and the HTCO method of DOC analysis. Some workers have reported that there is essentially no difference between the UV persulphate and HTCO DOC concentrations and this is especially so in freshwater (**Chapter 2.5.2**).

Due to the confusion surrounding the accuracy of DOC analysis a number of experiments have been carried out to determine the relative accuracy of the Southampton University Department of Oceanography (SUDO) UV persulphate DOC analyzer (Statham and Williams, 1983). These include:

(1) Development of clean techniques for the handling of DOC samples.

(2) Participation in the workshop on "The Measurement of Dissolved Organic Carbon and Nitrogen in Natural Waters" (July 14-19, 1991, Seattle, WA., U.S.A.).

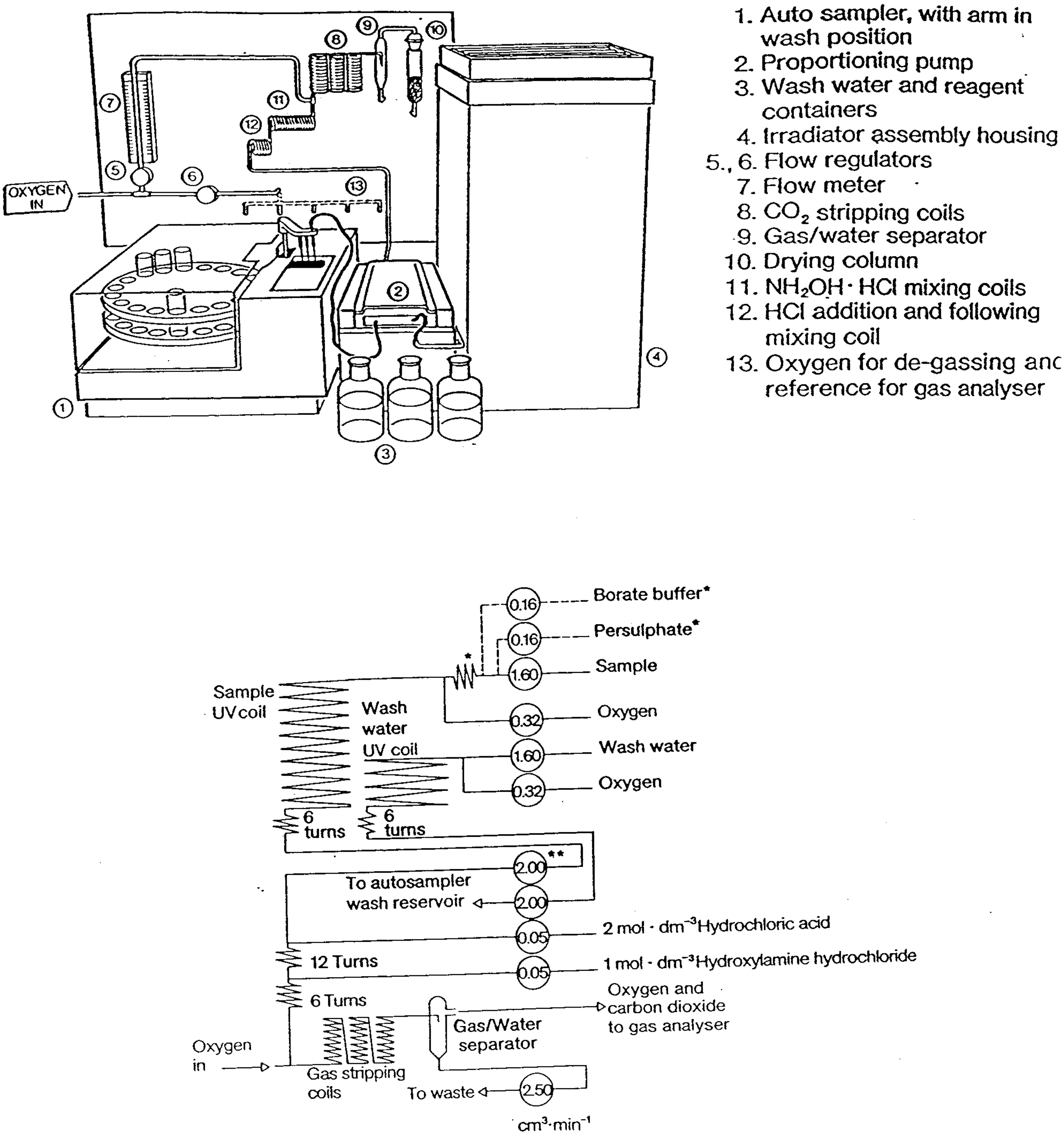
(3) Inter-Comparison between the SUDO DOC analyzer and the HTCO TOC-500 analyzer at PML (Plymouth Marine Laboratory).

(4) The use of mercury salts to increase the oxidation efficiency of the UV persulphate analyzer.

The UV persulphate method of DOC analysis was used in these experiments, based on the analyzer developed by Collins and Williams (1977), and an account of the analyzer and method has been given by Statham and Williams (1983). After acidification (2 M HCl) and removal of inorganic carbon, oxidant (0.09 M potassium persulphate) is added to the sample and the liquid stream passes through a silica coil surrounding a high-intensity UV light source. Organic material is oxidized and the resulting carbon dioxide is subsequently collected in a stream of oxygen (approximately 150 ml min^{-1}) and its concentration measured with an infra-red gas analyzer (Analytical Developments Ltd., Hoddesdon, Hertfordshire, U.K.: type 225). The general lay-out of the DOC analyzer and manifold can be seen in **Figure 3.1.**

The sample irradiation coil is constructed from 30 m of high purity quartz tubing (2 mm internal diameter, 4 mm outside diameter), wound on an octagonal frame (mean diameter 120 mm), whilst the UV lamp (Hanovia 1 kW medium pressure mercury arc, tube type 6751) is axially mounted in the centre of the coil. Low organic carbon content (LOCC) water, which is used for making up reagents and to give an analysis baseline, is prepared by bulk UV photo-oxidation of distilled water using an irradiator similar in design to the one used in the UV persulphate DOC analyzer, but with the coil replaced by 200 ml capacity (35 mm diameter, main body length 255 mm) silica tubes positioned around the lamp.

The analyzer has a sample time of 5.5 minutes and a wash time of 0.5 minutes i.e. 10 samples per hour. There is a 45 minute time lag between sampling and the corresponding gas analyzer recorder trace appearing. Standards are made up from a stock solution of 83 μmC ml^{-1}, prepared by dissolving 0.7669 g potassium oxalate mono-hydrate (analytical grade; BDH Limited, Poole, England) in 100 ml LOCC water. Working standards are prepared immediately prior to use by dilution of the stock in LOCC water. LOCC water blanks are run between sets of standards and samples to give an analysis basline. Standards are usually run after every ten samples. Peak heights, measured from the LOCC water basline, are used for calibration; the best fit line is found by linear regression analysis. The mean overall blank was 6 μM C, with a RSD of 10 % (n = 5), giving a detection limit (taken as 3 SD of the blank) of 2 μM C. Replicate analyses of two stored seawaters gave mean DOC concentrations of 57 μM C (RSD = 6 %, n = 30) and 92 μM C (RSD = 2.4 %, n = 30) (Statham and Williams, 1983).



1. Auto sampler, with arm in wash position
2. Proportioning pump
3. Wash water and reagent containers
4. Irradiator assembly housing
5., 6. Flow regulators
7. Flow meter
8. CO_2 stripping coils
9. Gas/water separator
10. Drying column
11. $NH_2OH \cdot HCl$ mixing coils
12. HCl addition and following mixing coil
13. Oxygen for de-gassing anc reference for gas analyser

* high DOC concentrations only.

** increase to 2.5 $cm^3 \cdot min^{-1}$ if persulphate and borate are added.

Figure 3.1. (a) Lay-out of the DOC analyzer (b) Auto-analyzer pump manifold assembly (from Statham and Williams (1983).

The efficiency of the lamp can be estimated by comparing the standard potassium oxalate with an equivalent standard of thiourea (analytical grade; BDH Limited, Poole, England). Thiourea has been observed to show reduced conversion to CO_2 as the UV lamp ages and the radiation output decreases.

3.2.2. Use of clean procedures in the laboratory for handling and preparation of samples.

The use of clean procedures in the laboratory was essential so that no contamination would be introduced into the samples. Normal procedures were followed for the treatment of glassware, filters and tweezers etc and these have been described earlier in **Chapter 4.2.3.1.**

A further precaution taken was the design and construction of a clean hood in which all handling and manipulation of the samples was undertaken (**Figure 3.2**).

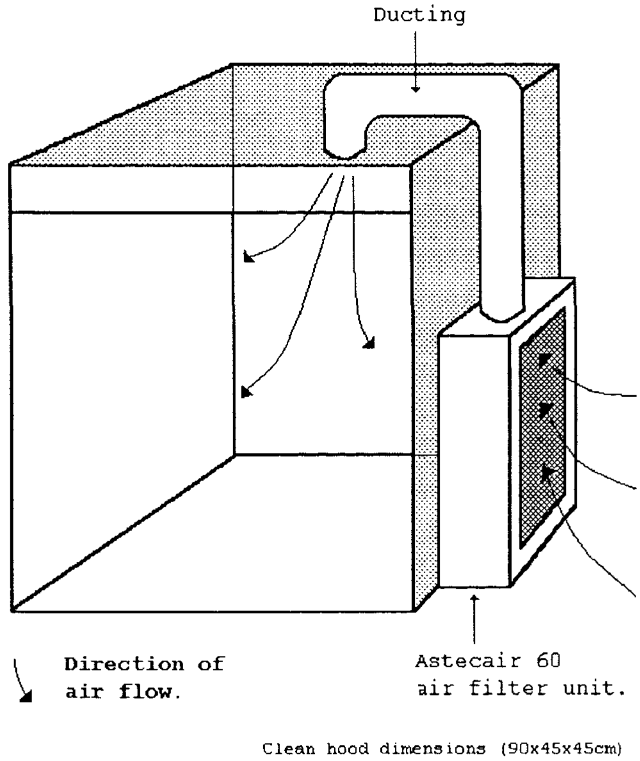


Figure 3.2. Design of the clean hood used for sample handling.

The main aim of the clean hood was to minimize airborne contamination (such as dust particles) of the samples. The clean hood (90 x 45 x 45 cm) was constructed of transparent acrylic sheeting and featured an Astecair air filter unit containing a charcoal filter which directed clean filtered air into the top of the hood and hence out of the hood via the front working space area, so preventing airborne particles coming into contact with the sample. When not in use the front working space area of the clean hood was protected with polythene sheeting to keep the interior of the clean hood dust free.

3.2.3. Results from the workshop on "The Measurement of Dissolved Organic Carbon and Nitrogen in Natural Waters" (July 14-19, 1991, Seattle, WA., U.S.A.).

The workshop included inter-comparisons of the measured DOC contents of four different natural waters carried out prior to the meeting. The study included 34 sets of DOC analyses with over 20 different instruments. The exercise was designed to establish the range of variability in measurement of DOC across the present community of aquatic scientists and to determine if patterns in the measurement values might be related to specific instrument characteristics or data treatments. All data were unidentified as to analyst or specific instrument model.

The seawater samples were collected in the oligotrophic open ocean at Station ALOHA, around 100 km north of Oahu, Hawaii from depths of 4000, 765 and 10 m. The 10 (surface), 765 (mid) and 4000 m (deep) depths corresponded to surface-mixed water, the core of the local oxygen-minimum zone and Pacific Ocean bottom water. The freshwater sample was collected from the lower Waimea River on the north side of Oahu, Hawaii. All samples were filtered through a 0.75 μm pore-size glass fiber filter and acidified to a pH of approximately 2.0 with 11 ml of 85 weight per cent H_3PO_4. The samples were refrigerated and shipped to participating laboratories (Hedges *et al.*, 1993).

The results from the Seattle meeting have been reported by Hedges *et al.* (1993) and a summary of these follows. The precision for individual instruments was good (5.9 % average sample mean deviation), however independently measured DOC concentrations of the same water ranged over an order of magnitude and varied on average approximately ± 40 % of the mean value. Overall mean concentrations and corresponding percent sample mean deviation intervals were 114.2 ± 36.7 % (surface), 73.3 ± 45.9 % (mid), 67.5 ± 45.0 % (deep) and 107.3 ± 29.0 % μM C (river). **Figure 3.3** shows the average DOC concentrations for all test samples. The surface sample had the highest DOC of the three seawaters. The mid and deep seawater values generally plotted close together around 40 to 45 μM C lower. This trend was evident over most of the data range. The river DOC concentrations generally had similar levels to those measured for the surface marine sample. For the surface seawater samples the mean for the HTCO instruments was higher (125 ± 44 μM C) than for the WCO instruments (72 ± 19 μM C), however, there was considerable overlap in the corresponding error bars. The large variation indicates that DOC concentrations cannot be confidently compared among many of the participating laboratories. Due to these conflicting results it was not possible to confidently conclude whether the HTCO and WCO instruments gave consistently different DOC values.

Another explanation for the observed pattern between the mid and deep seawater values was the presence of a blank. It was found that an embedded background signal was present which was constant within each set of four measurements, but which varied among the 34 analyses. This constant background was thought to be most likely due to an instrument blank in the different analyses in the order of ± 25 μM C. Therefore the data suggests that unidentified blanks may be a major cause of variability among individual analyses. However, as mean-subtraction does not perfectly align the data from all analyses, variables in addition to blanks must be involved.

As can be seen in **Figure 3.3** the average DOC concentrations from the 34 sets of DOC analyses were between 24 and 56 % more than the DOC concentrations measured by the SUDO UV persulphate analyzer. The bars show the range of DOC concentrations reported at the Seattle inter-calibration workshop and indicate the wide variation in DOC concentrations measured by the different analyzers. The SUDO DOC concentrations were towards the lower end of the range, and this was also the case for the average DOC concentrations from the 34 sets of DOC analyses. There also appeared to be a constant offset between these data. However, as stated earlier it was not possible to confidently conclude from the exercise whether the HTCO and WCO instruments (includes UV persulphate analyzers) gave consistently different DOC values. The difference between the average DOC concentrations from the 34 sets of DOC analyses and the UV persulphate values measured by the SUDO DOC analyzer are certainly not as large as the two to three times increase in DOC concentrations found by Sugimura and Suzuki (1988).

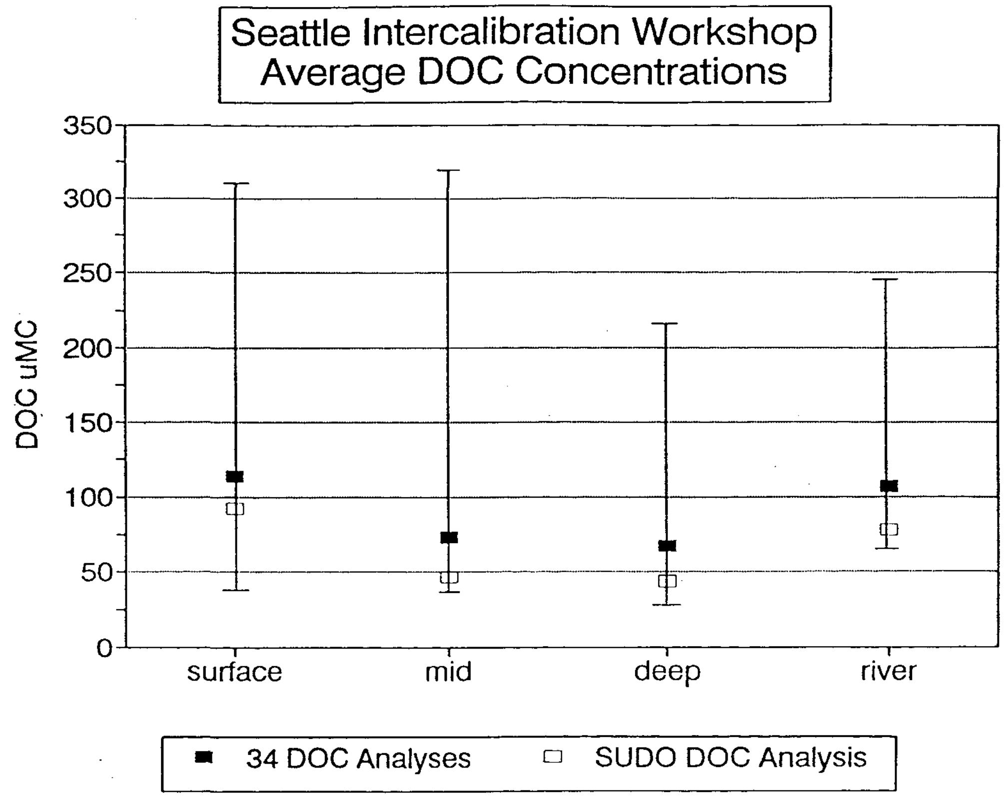


Figure 3.3. SUDO DOC concentrations and average DOC concentrations reported at the Seattle DOC inter-calibration workshop (bars show range of DOC concentrations reported at Seattle).

3.2.4. Inter-comparison between the SUDO UV persulphate DOC analyzer and the TOC-500 HTCO analyzer at PML (Plymouth Marine Laboratory).

In order to gain an idea of whether DOC concentrations measured by the SUDO DOC analyzer were comparable to DOC concentrations measured by a HTCO system, a number of inter-comparisons were made between the SUDO DOC analyzer and the HTCO TOC-500 analyzer at PML (catalyst used was Shimadzu 0.5 % Pt/Al_2O_3). The first such inter-comparison in July, 91 used the data generated for the Seattle workshop. A subsequent inter-comparison was made during autumn 1991 using waters from the Beaulieu estuary.

The DOC concentrations for the same samples for the two analyzers can be seen in **Figure 3.4. Figure 3.5** shows the percentage difference in DOC concentrations between the two analyzers for these samples. There is a large variation (between 15 and 145 %) in the percentage difference, however, this is due to the fact that the DOC concentrations measured for autumn 1991 were higher than those measured for July 91. For July 91 and autumn 1991 the actual difference in DOC concentrations measured by the two analyzers was quite constant (between 40 and 70 μM C). This is close to the blank that was reported to be derived from the catalyst during the combustion process (Benner and Strom, 1993). The blank decreased to fairly constant levels (10 - 50 μM C) with increasing usage of the catalyst.

The catalyst used in the HTCO TOC-500 analyzer for the autumn 1991 inter-comparison was relatively "fresh" i.e. few samples had been run on the analyzer prior to the inter-calibration samples. Therefore, the DOC values analyzed by the HTCO TOC-500 analyzer may have been artificially high due to residual contamination from the catalyst, however, there is no way of telling if this was the case. The value for LOCC water measured by the HTCO TOC-500 analyzer for autumn 1991 was around 100 μM C which suggests that there was a fairly large system blank associated with the analyzer. For the July 91 inter-comparison the catalyst had been used for many other samples prior to the inter-calibration samples and therefore the DOC values reported by the HTCO TOC-500 analyzer should not have been significantly influenced by a catalyst blank. Both inter-comparisons showed a fairly constant offset in the DOC concentrations measured indicating that the possible catalyst blank associated with the HTCO TOC-500 analyzer was more or less constant and, in this case, was not affected by increasing usage of the catalyst.

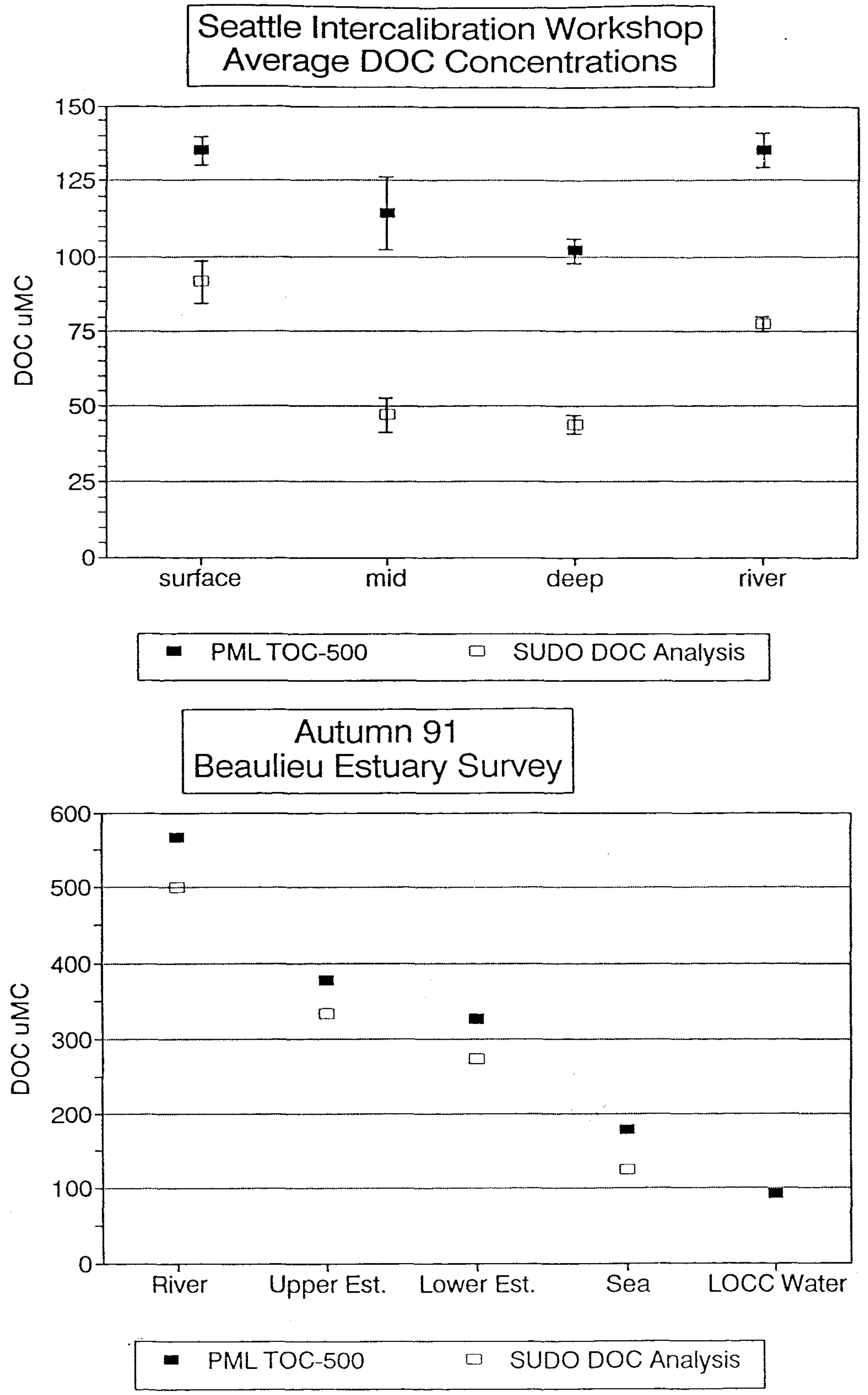


Figure 3.4. DOC concentrations from the PML/SUDO inter-calibration exercise.

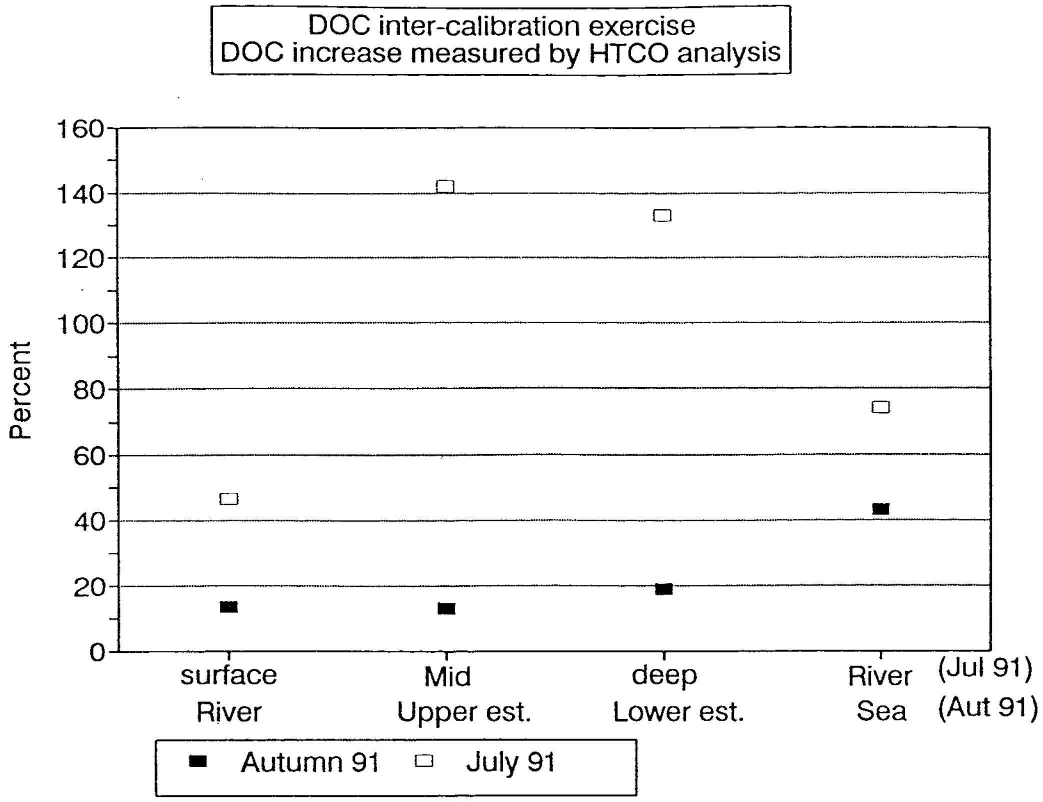


Figure 3.5. Percentage difference in DOC concentrations between the HTCO analyzer and the UV persulphate analyzer in the PML/SUDO inter-calibration exercise.

3.2.5. The use of mercury salts to increase the oxidation efficiency of UV persulphate DOC analysis.

Higher DOC oxidation efficiencies for seawater samples have been found by several workers using certain heavy metal salts (Krogh and Keys, 1934; Plunkett and Rakestraw, 1955; Baldwin and McAtee, 1974; Goulden and Brooksbank, 1975; Skopinstev *et al.*, 1976; Bauer *et al.*, 1991; Benson, 1991; Williams *et al.*, 1993). Bauer *et al.* (1991) used a modified Dohrmann DC-80 photochemical oxidation unit with a 40-W low-pressure UV lamp. The reaction chamber contained a solution of potassium persulphate and mercury salts ($HgCl_2$ and $Hg(NO_3)_2$). They found that by adding Hg^{2+} to the samples, DOC was analyzed "quantitatively" without interference. Williams *et al.* (1993) found that the oxidation of DOC by UV persulphate which included mercury salts (Dohrmann DC-80 analyzer) was increased over UV oxidation alone and approached HTCO levels for open ocean samples.

Silver salts have also been used to increase the oxidation efficiency of UV persulphate DOC analyzers (Skopinstev *et al.*, 1976). It was reported that DOC oxidation by persulphate was inhibited when NaCl was added to natural salt-free waters, but this effect was reversed by the addition of Ag_2SO_4, which removed Cl^- from solution as highly insoluble AgCl. Baldwin and McAtee (1974) and Goulden and Brooksbank (1975) used a silver catalyst for oxidation and determination of DOC in water, where they observed higher oxidation efficiencies for some samples.

Other studies have not shown a significant increase in measured DOC on addition of metal salts. Benson (1991) observed that addition of silver nitrate had no significant affect on the oxidation efficiency for a number of standard solutions. Complexation of chloride with the silver cation (Duursma, 1961) did not result in elevated DOC oxidation using a H_2CrO_4 technique.

The mechanism of the oxidation process has been discussed by Peyton (1993). A brief review of this follows. The production of Sulphate Radical Anions (SRA) is the essential step and the standard textbook approach is to divide free-radical reactions into three stages: initiation, where free radicals are first generated; propagation, where free radicals form other free radicals through chemical reactions; and termination, where free radicals are removed from the system.

Sulphate radical anions are generated by photolysis or thermolysis of the persulphate ion:

$$S_2O_8^{2-} \rightarrow 2\ SO_4^{-\cdot}$$

Sulphate radical anions react with water to yield the hydroxyl radical which can also react with organic matter:

$$SO_4^{-\cdot} + H_2O \rightarrow H^+ + SO_4^{2-} + HO\cdot$$

The reaction of free radicals with organic compounds (propogation) can proceed by a number of mechanisms such as hydrogen atom abstraction and addition to multiple bonds. SRA reacts with certain functionalities such as aromatic rings, amines and carboxylates by electron transfer:

$$SO_4^{-\cdot} + C_6H_6 \rightarrow SO_4^{2-} + C_6H_6^{+\cdot}$$

The reaction with the carboxylate anion is significant because this is probably the sole means by which organic carbon is converted to CO_2:

$$SO_4^{-\cdot} + RCO_2^{-} \rightarrow SO_4^{2-} + RCO_2^{\cdot} \rightarrow R^{\cdot} + CO_2 + SO_4^{2-}$$

The oxidation of macromolecular material has also been investigated where the hydroxyl radical rate constant for the initial reaction was found to be relatively insensitive to molecular type and size and secondary and tertiary structures may not play as great a role in reactivity as might be imagined:

$$OH^{\cdot} + R \rightarrow R^{+\cdot} + OH^{-}$$

(where R = macromolecular material containing alkyl and aromatic structures with various functional groups such as amines and carboxylic acids etc).

It may be that the surface of the macromolecule becomes less reactive but it is difficult to assess the extent of this decrease. In the absence of competition for radicals the reaction will still occur rapidly.

The degradation pathways of organics containing heteroatoms such as sulphur and nitrogen are also not clear, particularly the last steps before mineralization. It can probably be assumed that these compounds are more refractory due to the greater number of available oxidation states.

The order of reactivity of organic functionalities with sulphate and hydroxyl radicals is aromatic > CH_2 > CO > COOH. Therefore, the oxidation products are more refractory then the parent compounds and results in a less reactive residue rich in carboxylates.

Sulphate and hydroxyl radicals react quickly with chloride and bromide to form halogen atoms, hence reducing the concentration of free radicals that can react with organic matter. Due to the high chloride content of seawater, the reaction of the sulphate radical anion (SRA) with chloride is the most important sink of SRA:

$$SO_4^{-\cdot} + Cl^- \rightarrow SO_4^{2-} + Cl\cdot$$

The formation of chlorine by the combination of two chlorine atoms can also occur. The subsequent formation of chlorinated organics could form halogenated organics which are less reactive with free radicals than the corresponding non-halogenated compounds. However atom recombination is probably not important due to the low concentration of the chlorine atom:

$$Cl\cdot + Cl^- \rightarrow Cl_2^{-\cdot}$$

Therefore, the single biggest problem in the UV persulphate oxidation of DOC seems to be the scavenging of sulphate radicals by chloride, hence a means should be found to reduce the amount of chloride (and bromide) by at least two orders of magnitude.

Hydroxyl and sulphate radicals are capable of destroying virtually any organic species of interest and organic material will be mineralized (converted into carbon dioxide) if the competition for radicals is not too great.

The addition of Hg^{2+} to the reacting solution increases oxidation of the organic sample (Bauer *et al.*, 1991). It was also assumed by the amount of Cl^- required to interfere with the oxidation process that mercury complexed chloride with a mean stoichiometry of 1 Hg^{2+}: 2 Cl^-, the two dominant forms of complex being $HgCl^+$ and $HgCl_3^-$ (Turner *et al.*, 1981).

3.2.6. Experimental studies involving the use of Hg(II) salts to increase the oxidation efficiency of the UV persulphate DOC analyzer.

The presence of Hg salts has been demonstrated to increase the oxidation efficiency of UV persulphate DOC analyzers. Therefore, experiments were carried out to improve this technique and apply it to the UV persulphate DOC analyzer of Statham and Williams (1983). Usually high concentrations of Hg salts are added in an attempt to complex the chloride ion and hence increase the oxidation efficiency (see for example Skopinstev, 1976; Bauer *et al.*, 1991; Dohrmann, the manufacturer of the DC-80 analyzer). In the following experiment lower concentrations of Hg salts were added to see if these lower concentrations would also give increased oxidation efficiencies. This direction was chosen because of the uncertainty surrounding the mechanism by which Hg salts increase the oxidation efficiency (House, 1962) and because of the difficulty in introducing high concentrations of Hg salts into the DOC analyzer of Statham and Williams (1983).

A riverine and seawater sample were analyzed (four replicates) from the winter 1992 sampling survey to determine whether salinity had an affect on the oxidation efficiency. The concentrations of $HgCl_2$ and $Hg(NO_3)_2$ varied (see **Table 3.1**) in order to find the optimum Hg salt concentration whilst the concentration of persulphate was kept constant at its usual concentration (0.09 M). Each analysis using the Hg salt solutions included its own set of standards. Blank analyses of the Hg salt solutions showed that no detectable organic carbon contamination was present in the reagents. Blank analysis was carried out by measuring the DOC concentration of the Hg salt/persulphate reagent in the DOC analyzer as for any other sample.

	Analysis 1	**Analysis 2**	**Analysis 3**	**Analysis 4**
$HgCl_2$	0	0.005 M	0.025 M	0.05 M
$Hg(NO_3)_2$	0	0.005 M	0.025 M	0.05 M
Total Hg	0	0.001 M	0.05 M	0.1 M

Table 3.1. Concentrations of $HgCl_2$ and $Hg(NO_3)_2$ used in the experiments.

A problem encountered was that in basic solutions mercury nitrates are reduced to black mercury oxides. To stop this from occurring the solution had to be acidic and therefore hydrochloric acid was also added to the mixture. Therefore, to achieve the required molarities the most concentrated salt solution (analysis 4) contained the following reagents: 3.44 g $HgCl_2$, 4.6 g $Hg(NO_3)_2$ dissolved in 120 ml potassium persulphate solution (0.18 M), 5 ml HCl (2 M) and 125 ml LOCC water (prepared by UV irradiation of MQ water in a bulk irradiator for 6 hours; see Statham and Williams, 1983). The other solutions were then made up by diluting this stock solution by a half and by ten times respectively. This Hg salt/persulphate reagent was added to the persulphate reservoir of the DOC analyzer of Statham and Williams (1983) and was introduced directly into the sample stream.

Figure 3.6 shows the concentrations for the riverine sample and seawater sample for winter 1992 using the different concentrations of Hg salt/persulphate reagent i.e. analysis 1 to 4. The error bars (± 1 SD) are always smaller than the symbol size. Analysis 1 (persulphate only) is the DOC concentration measured in a normal analysis. For the seawater sample the mercuric nitrate concentration of analysis 3 shows the highest DOC concentration measured (79 μM C; RSD = 1.2 %, n = 4) compared to analysis 1 (75 μM C; RSD = 1.5 %, n = 4), however, the most concentrated reagent (analysis 4) shows a drop in DOC concentration (68 μM C; RSD = 1.7 %, n = 4). This indicates that the Hg salt/persulphate reagent of analysis 3 (Hg concentration of 0.05 M) is the most suitable concentration for DOC analysis where a 6 % increase in efficiency was observed. The riverine sample shows the same trend with analysis 3 showing the highest DOC concentration (190 μM C; RSD = 1.6 %, n = 4) compared to analysis 1 (175 μM C; RSD = 1.3 %, n = 4) and a decrease in DOC concentration for analysis 4. The increased DOC concentration reported has a similar magnitude increase (8 %) to that of the seawater sample.

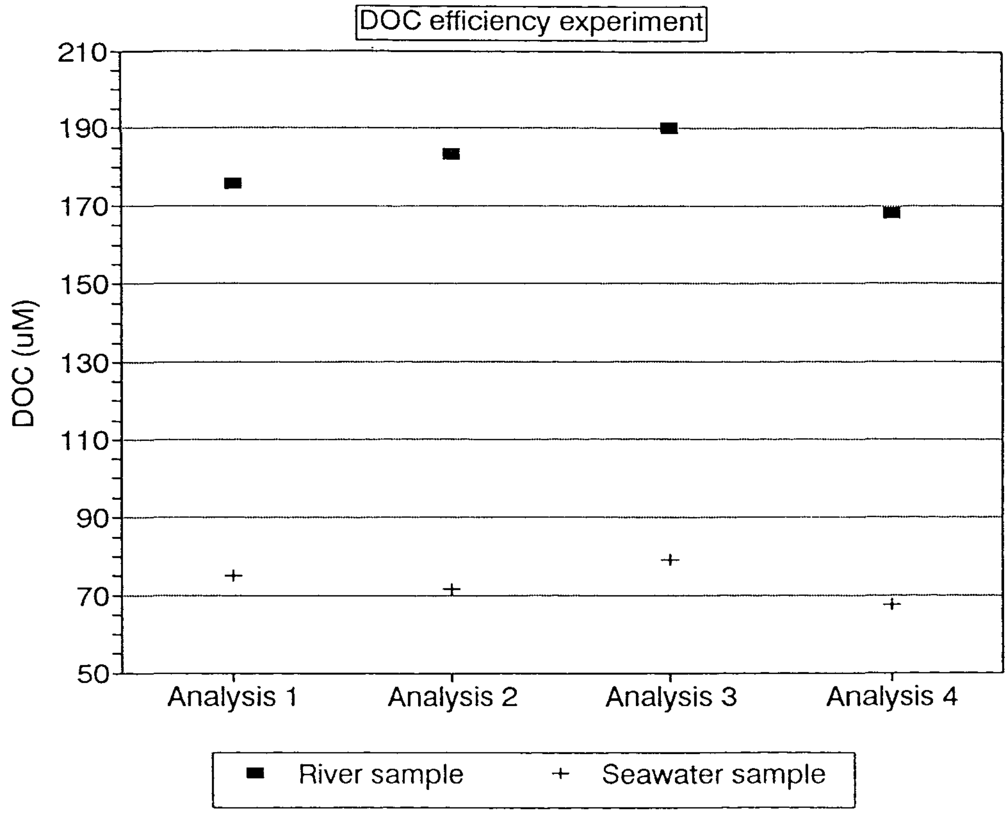


Figure 3.6. DOC concentrations for a riverine sample and seawater sample using different concentrations of Hg salt/persulphate reagent.

These results indicate that there is an increase in oxidation efficiency caused by adding Hg salts (0.05 M) to the DOC sample (between 6 and 8 %).

The concentration of mercury salts added to each individual DOC sample is important when considering the extent to which the chloride ion may be complexed. In the UV persulphate analyzer this can be calculated by considering the flow of the Hg salt/persulphate reagent into the sample stream. The sample stream introduces 8.8 ml of sample into the DOC analyzer whilst the Hg salt/persulphate stream introduces 0.88 ml of oxidising solution into the sample stream i.e. a total of 9.68 ml. Therefore, the concentration of reagents in each sample as it passed through the DOC analyzer was as follows: $K_2S_2O_8$ = 0.008 M, $Hg(NO_3)_2$ = 4 x 10^{-4}, 0.002 and 0.004 M respectively for analyses 2 to 4, $HgCl_2$ = 4 x 10^{-4}, 0.002 and 0.004 M respectively for analyses 2 to 4. The concentration of chloride in seawater is around 0.55 M (8.8 ml sample with a salinity of 34.5), therefore, in the 9.68 ml sample (sample plus Hg salt/persulphate reagent) the concentration of chloride was around 0.5 M.

As the concentration of chloride in the 9.68 ml sample (sample plus Hg salt/persulphate reagent) passing through the analyzer was 0.5 M and the concentration of mercury salts was 0.004 M (analysis 3) the mercury salts could not complex a sufficently large amount of chloride (complexes with a mean stoichiometry of 1 Hg^{2+}: 2 Cl^- (Turner *et al.*, 1981)) to show increased oxidation efficiencies. However, increased oxidation efficiencies were observed up to 8 % using the Hg salt/persulphate reagent of analysis 3 (mercury salt concentration of 0.05 M) and not the most concentrated Hg salt reagent (analysis 4; mercury salt concentration of 0.1 M). The increase in oxidation efficiency was observed for the saline (6 %) and the fresh water (8 %) samples, therefore, it seems unlikely that this increase was due to complexation of the chloride ion by the Hg salts. Another point to note was the response of the calibrating standards, which were made up in UV oxidized Milli-Q water. For analysis 1 (normal persulphate) the standard 250 μM C thiourea peak was 86 % the size of the standard 250 μM C potassium oxalate peak indicating that the UV persulphate analyzer was not performing at full efficiency (this may be taken as an indicator that the efficiency of the UV lamp had decreased). However, on using the Hg salt/persulphate reagent (analysis 2, 3 and 4) the 250 μM C thiourea peak was the same size as the 250 μM C potassium oxalate peak. This suggests that the Hg salts do increase the efficiency of DOC oxidation which again is not dependent on complexation of the chloride ion. This increase in oxidation efficiency may be due to the fact that mercury (II), in the presence of persulphate, undergoes specific reduction/oxidation reactions which enhance the production of sulphate radicals from persulphate, hence increasing the rate of organic carbon oxidation (House, 1962).

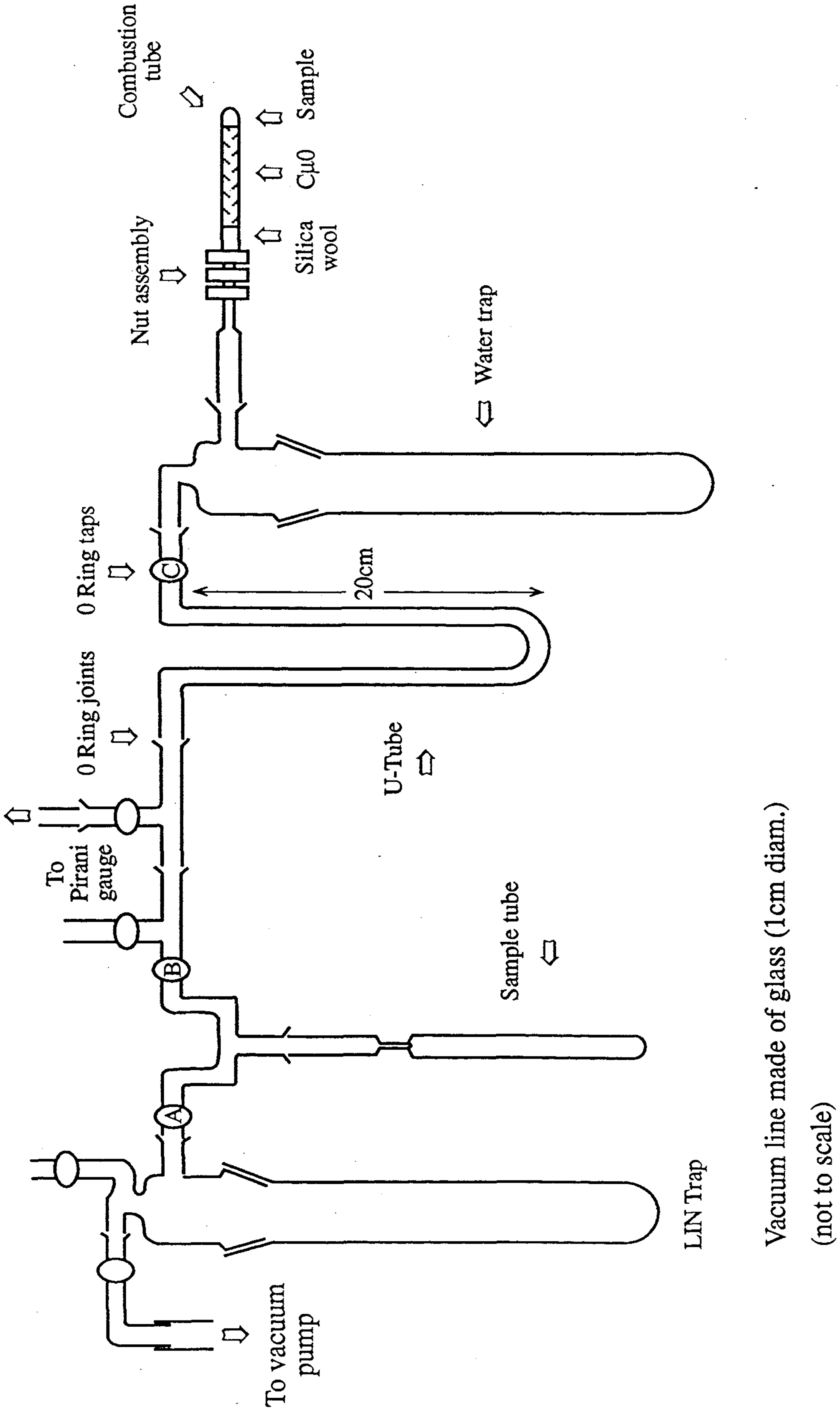
The solubility of $HgCl_2$ is 6.5 g/100 ml (at 20^0C), whilst $Hg(NO_3)_2$ is deliquescent. It should be possible to introduce higher concentrations of Hg salts into the sample stream of the analyzer of Statham and Williams (1983) (due to the high solubility of the reagents and providing there is little interference by other ions e.g. persulphate) to test whether higher Hg salt concentrations would give these increased oxidation efficiencies or whether there would be a decrease in oxidation efficiency when compared to the oxidation efficiency of the optimum Hg salt solution (0.05 M), as suggested from the results of this experiment. The problem of achieving such a high salt concentration in the analyzer of Statham and Williams (1983) is that a small volume of reagent (0.88 ml) is added to the sample (8.8 ml). This is the opposite in the analyzer used by Bauer *et al.* (1991) where a small volume of sample (1 ml) is added to the reagents (88 ml).

3.3. Vacuum line Development for carbon isotope studies.

This section describes the construction and development of a vacuum line for the conversion of organic material (isolated on filters) into carbon dioxide for measurement of the $\delta^{13}C$ and $\Delta^{14}C$ values. The vacuum line should quantitatively convert organic matter samples into carbon dioxide, without introducing contamination into the sample.

3.3.1. Construction of the SUDO vacuum line.

The vacuum line is constructed from borosilicate glass using greaseless "o" ring joints and taps (**Figure 3.7**). The vacuum line consists of a silica combustion tube followed by a large water trap. The U-tube initially traps the carbon dioxide generated from the combusted sample which is then transferred to the sample tube for final collection. The liquid nitrogen trap prevents contamination of the vacuum line with oil from the vacuum pump (or contamination of the pump oil from substances in the vacuum line), hence the vacuum pump was located after the liquid nitrogen (LIN) trap. A Pirani gauge was situated in the middle of the vacuum line to monitor the vacuum.



Vacuum line made of glass (1cm diam.)

(not to scale)

Figure 3.7. Design of the SUDO vacuum line.

3.3.2. Achievement of low blank values in the preparation of samples for isotopic analysis using the SUDO vacuum line.

To ensure that the vacuum line was not introducing carbon contamination into the gas samples, blanks were prepared using the SUDO vacuum line which were then ^{14}C dated at the Radiocarbon Accelerator Unit at Oxford University. The initial test sample was pre-treated (i.e. hydrolysis and acid digestion carried out at Oxford) log cellulose (Chelford peat; > 44,000 years old). The cellulose was derived from peat and hence would contain very low levels of ^{14}C as this would have decayed over time.

The first sample ^{14}C dated had a $\Delta^{14}C$ value of 5.5 %M and this suggested that the sample was contaminated. Subsequently a second sample using a modified combustion method (see below) had a value of 0.5 %M (> 42,380 years B.P.) and this showed that the modified preparation method using the SUDO vacuum line was not introducing organic contamination into the sample.

The preparation of the first sample on the SUDO vacuum line was different to that of the second in that a different combustion tube was used, the two U-tube traps were filled with glass beads and a trap consisting of a LIN/ethanol slurry was used to trap out water instead of a LIN/methanol trap. Also, the removal of contamination before the first sample was prepared was not as thorough and contamination probably originated from the CuO, the combustion tube, the walls of the vacuum line and the silica wool. The preparation of the second sample was modified so as to reduce the possibility of the vacuum line introducing contamination into the sample, principally by more rigorously removing possible contamination on the copper oxide and glass wool (by combustion in a muffle furnace for a longer period) and surfaces of the vacuum line itself (by soaking in "micro" detergent followed by rinsing with water and ethanol). As there was no contamination of the CO_2 sample using the second method, it was felt that this method could be used to prepare samples for ^{14}C analysis at Oxford. This method is described below (**Section 3.3.3**).

A blank sample was also prepared on the SUDO vacuum line for analysis of $\delta^{13}C$ at the University College of North Wales (UCNW). The standard graphite sample (8.14 mg) used at UCNW was combusted in the vacuum line and analyzed for $\delta^{13}C$ at UCNW. The result showed that the combustion process using the SUDO vacuum line did not fractionate or introduce contamination into the sample.

3.3.3. Combustion method using the SUDO vacuum line.

The combustion method using the SUDO vacuum line was as follows; the silica combustion tube (80 x 12 mm for the $\Delta^{14}C$ analysis and 80 x 7 mm for the $\delta^{13}C$ analysis) was attached to the vacuum line, liquid nitrogen was placed in the U-tube trap and a liquid nitrogen/methanol slurry in the water trap. The line was pumped down to a vacuum of *ca*. 0.1 mbar. This took around 10 minutes. Tap C was closed and the combustion of the sample was begun by firstly heating the copper oxide with a Bunsen burner for 10 minutes, then the whole combustion tube was heated for 10 minutes using two Bunsen burners. Tap C was opened for a few seconds periodically throughout the combustion process to bring the vacuum down. Any CO_2 present would be trapped in the liquid nitrogen U-tube trap. Taps A and C were closed and the liquid nitrogen was taken off the U-tube trap and put on the sample tube trap. This was left for 6 minutes (sufficient time for the U-tube trap to warm to room temperature) to allow the CO_2 to diffuse into the sample tube. Tap B was closed and tap A opened and the sample tube was sealed with an oxygen/propane torch. A new combustion tube and sample tube were added and the combustion process repeated. The sample tubes containing the CO_2 were kept at room temperature before isotopic analysis.

3.3.4. Preparation of samples for $\delta^{13}C$ and $\Delta^{14}C$ analysis.

Initially eight samples from the summer 1991 Beaulieu sampling survey were prepared for $\Delta^{14}C$ analysis at Oxford using the SUDO vacuum line. Samples were also prepared for $\delta^{13}C$ analysis at UCNW, using the SUDO vacuum line, from the summer 1991, autumn 1991, winter 1992 and spring 1992 Beaulieu sampling surveys.

After mass spectrometric analysis it became apparent that there were problems with these gaseous samples that made most of them unsuitable for $\delta^{13}C$ and $\Delta^{14}C$ analysis. The problem with the samples for $\Delta^{14}C$ analysis was the quantity of organic carbon present as CO_2 even though the number of filters for combustion had been estimated to give the required amount of carbon. The minimum amount of carbon that can be radiocarbon dated is 1 mgC. All of the samples on preliminary investigation at Oxford had less than 1 mgC in the sample. Two of the samples had 0.7 and 0.9 mgC carbon present. Clearly these could not be radiocarbon dated.

The summer 1991 samples that were submitted to UCNW for $\delta^{13}C$ analysis were suitable for analysis. However, once the analysis of the samples for autumn 1991, winter 1992 and spring 1992 began it became clear that problems existed, with most of the samples showing complex ratio traces indicating that they were contaminated. Therefore, analysis of the remaining samples was discontinued.

Faced with these problems it was decided to re-sample the Beaulieu estuary to ensure that suitable gas samples were obtained for the $\Delta^{14}C$ and $\delta^{13}C$ analyses. Therefore, re-sampling for the $\Delta^{14}C$ data was undertaken during autumn 1991 and it was decided to combust the filters at Oxford using their vacuum line (method the same as that used for combustion of samples on the SUDO vacuum line; **Section 3.3.3**) to make sure that the samples would be suitable for $\Delta^{14}C$ analysis. Re-sampling of the Beaulieu estuary for the $\delta^{13}C$ analysis was undertaken during summer 1992 and again it was decided to combust the samples at UCNW using the vacuum line there (method given below; **Section 3.3.5**), to circumvent problems with the SUDO vacuum line.

The $\Delta^{14}C$ analyses for autumn 1991 were successful and all the samples had sufficient carbon present in the CO_2 samples. The main reason for this was that the volume of aqueous sample processed and hence number of filters used was increased (almost doubled) for each sample, hence ensuring that enough carbon was present for radiocarbon dating of the samples. The amount of carbon present in the CO_2 samples was over 1 mgC. However, the results for the $\Delta^{14}C$ analysis (**Table II.19**) were puzzling due to the enhanced levels of ^{14}C found. This is discussed in **Chapter 5.3** with possible reasons for these enhanced ^{14}C values.

The $\delta^{13}C$ values that were analysed for summer 1992 at UCNW all gave clean ratio traces indicating that the samples were combusted without the introduction of contamination and with no isotopic fractionation. The $\delta^{13}C$ values for the samples from autumn 1991, winter 1992 and spring 1992 that were unsuitable for ^{13}C analysis have been discussed in **Chapter 5.2.2.6**.

3.3.5. Combustion method using the UCNW vacuum line.

The "tube within a tube" method was used for combustion of samples on the UCNW vacuum line. The silica combustion tube was inverted and placed in a larger outer silica tube (160 mm , 14 mm inner diameter), which was attached to the vacuum line and pumped down overnight at 10^{-4} bar. The outer tube was sealed before combustion of the sample in a muffle furnace overnight at 900^{0}C. Following this the outer tube was put into the tube breaker on the vacuum line for isolation and collection of the CO_2 and the vacuum line was pumped down for 20 minutes. The design of the vacuum line at UCNW was essentially the same as the SUDO vacuum line except that in place of the combustion tube on the SUDO vacuum line, the UCNW vacuum line had a tube breaker and the UCNW vacuum line had only one trap that combined as a water trap (liquid nitrogen/methanol slurry) and CO_2 trap (liquid nitrogen). The method of collection and isolation of the CO_2 sample was also similar to the method used for the SUDO vacuum line except in the following ways; the outer tube and combustion tube in the tube breaker were broken and the gases were transferred to the trap which contained liquid nitrogen, hence freezing out all the gases (10 minutes; vacuum of 10^{-4} bar). The trap was isolated, the liquid nitrogen removed and the trap heated with a heat gun to room temperature. A liquid nitrogen/methanol slurry was placed in the trap to freeze out the water (10 minutes). The CO_2 was then transferred to the collection tube (liquid nitrogen trap) and the collection tube was isolated when all the CO_2 had been transferred (as indicated by the Pirani gauge). When the vacuum reached 10^{-4} bar indicating that all the CO_2 had frozen out the collection tube was pumped down (2 minutes) and the collection tube sealed. A new collection tube and combustion tube was placed on the vacuum line and the whole line was opened and pumped down for 20 minutes before the next sample was transferred.

3.3.6. Problems associated with use of the SUDO vacuum line.

Several factors can be highlighted as to why the SUDO vacuum line did not effectively combust the organic carbon isolated on filters into carbon dioxide for isotopic analysis. The process of combustion using the Oxford and UCNW vacuum lines was essentially the same, although there were minor but important differencs.

One of the biggest differences between the SUDO vacuum line and the vacuum lines at Oxford and UCNW was the lower pressures that the latter two vacuum lines achieved. The vacuum generated on the SUDO vacuum line was around 0.1 mbar, whereas the vacuum generated at Oxford was 10^{-3} mbar and at UCNW was 10^{-4} mbar. This was due to more efficient vacuum pumps at Oxford and UCNW and the use of a differential pump in the vacuum line. With a higher vacuum in the vacuum line any non-condensables present would be more efficiently removed. This was especially so at UCNW where adequate time was given for the removal of non-condensables at different stages of the combustion process. These vacuum lines were also left running overnight hence always keeping the line under vacuum and preventing the possible build-up of contamination on the walls of the glass tubing and other components in the line.

The use of accurate Pirani gauges at Oxford and UCNW meant that more accurate measurement of the vacuum could be made and it was possible to tell when the vacuum was at its lowest before combustion began. The use of a pressure transducer at Oxford meant that the amount of CO_2 present could be measured in the vacuum line. The amount of carbon present (in mgC) in the CO_2 sample could then be inferred from this. The advantage of this is that checks could be made on the amount of CO_2 present during combustion and it would be possible to tell if all of the organic sample had been oxidized as no increase in CO_2 pressure with subsequent heating indicates complete combustion.

A more efficent combustion process was used at Oxford where a powerful Bunsen burner was used for the combustion step and for a greater length of time. At UCNW the "tube within a tube" method of combustion was used. This consists of a small silica combustion tube into which the sample and copper oxide are placed, with a strip of tin foil (helps to fully oxidize organic material) and a plug made from a glass-fibre filter. This is then placed (inverted) in a larger glass tube which is evacuated overnight on the vacuum line, sealed with a propane/oxygen torch and combusted overnight at 900^0C. With a more efficient combustion process all of the organic carbon present would be converted into CO_2, preventing fractionation of the sample and the possibility of formation of non-condensables from the organic sample itself would be minimized, hence preventing contamination of the CO_2 sample.

The use of a double trap method at UCNW ensured that all the CO_2 and water were separated. This was done by freezing both the water and CO_2 in the liquid nitrogen trap, allowing both to vapourize and then freezing just the water in a LIN/methanol trap. The CO_2 was then collected in the sample tube in a liquid nitrogen trap. At Oxford the water trap was never in-line with the liquid nitrogen trap for long periods as water may have re-condensed in the liquid nitrogen trap.

It was important to allow sufficient time for the CO_2 to diffuse into the sample tube before sealing the sample tube. If a few % of the $^{12}CO_2$ as the major component was not transferred then it would not have a great influence on the $^{12}C/^{13}C$ ratio, but if a few % of the $^{13}CO_2$ as the minor component was not transferred it would make a large difference to the $^{12}C/^{13}C$ ratio. The end of the transfer process is also the most important as this would be when the heavier $^{13}CO_2$ would be transferred.

3.4. Isolation of colloidal fractions of DOC.

3.4.1. Introduction.

One of the main reasons hampering the analysis and characterization of DOC in seawater is that a fully satisfactory method of separating organic matter from sea water is lacking. Very low concentrations of organic matter in the presence of much higher concentrations of inorganic ions ($>10^4$) make quantitative separation difficult. A pre-concentration step is necessary to obtain sufficient material for physical chemical characterization. Several methods and procedures have been developed over the past decades to pre-concentrate DOM and to extract organic compounds from the marine environment including, for example extraction using XAD resins and ultrafiltration (Pocklington, 1980). Most of these can be used for individual compounds or classes of compounds but none can quantitatively separate all the DOM of sea water. Thus, the information obtained from these methods probably is not representative of the total composition of the DOM.

3.4.2. Development of filtration techniques for the separation of COC from natural waters.

Colloidal organic carbon can be isolated by filtration of water through 0.4 to 1.0 μm pore-sized filters followed by ultrafiltration (Carlson *et al.*, 1985), by gel-permeation chromatography (Sugimura and Suzuki, 1988) or as marine humic substances by solid phase extraction on hydrophobic surfaces (Harvey *et al.*, 1983). Ultrafiltration has been succesfully used to isolate COC (Fuhrman *et al.*, 1988; Barkay *et al.*, 1989; Altabet, 1990; Coffin *et al.*, 1990; Giovannoni *et al.*, 1990; Holigbaugh *et al.*, 1991) and it has been identified as one of the most efficient methods of isolating COC from natural waters (Brownawell, 1991).

Ultrafiltration is more efficient when used with cross-flow filtration and an in-line series of filters. The application of this technique to oceanographic studies has only recently been reported (Whitehouse *et al.*, 1986, 1989; Moran and Moore, 1989; Benner *et al.*, 1992). This system offers a number of advantages such as use of a number of different pore size filters in the same apparatus which increases the total surface area of the filters and means that relatively high flow rates can be achieved. The system is also portable and can therefore be used aboard ship, where sampling and filtration can be carried out *in-situ*.

A recent development has been the use of Anotec Anopore filters for ultrafiltration. For example Altabet (1990) compared the quantity and composition of particles in a depth profile from an oligotrophic open ocean site that were retained on aluminium oxide Anotec Anopore 0.2 μm membrane filters. Although Anotec Anopore filters offer certain advantages over other filter types, they have only recently become available and their use in the literature has not been widely reported. The filter range (0.2, 0.1 and 0.02 μm pore sizes) should be very useful in particulate and colloidal studies because the sieve-like filters have very well defined pore sizes allowing particulate material to be trapped on the surface of the membrane, however due to this small pore size the filtration rate is slow. A further advantage is that the aluminium oxide filters can be heated, up to 900^{0}C if necessary, without a change in pore size, hence allowing thorough cleaning of the filter. A disadvantage is that the filters are very brittle and need to be handled with care.

Ultrafiltration has been succesfully used to isolate COC (see references above) and it has been identified as one of the most efficient methods of isolating COC from natural waters (Brownawell, 1991) where the average fraction of total DOC, recovered as a dry powder by ultrafiltration, was over twice as large as the amount of humic material previously isolated by hydrophobic adsorption onto XAD-8 resin (Ertel *et al.*, 1986). Ultrafiltration isolates the organic fraction on the basis of molecular size rather than hydrophobicity and is likely to recover a more representative fraction of the total DOC (Benner and Hedges, 1993). Solid phase extraction also runs the risk of chemically altering compounds during adsorption and desorption steps and altering the conformation or physical state of organic molecules due to changes in pH. The isolated material would only consist of hydrophobic acids and would not isolate colloids that are too hydrophilic (estimated to account for a significant amount of COC; Sugimura and Suzuki, 1988) to adsorb to the support or colloids which are not reversibly eluted with base or methanol from the support.

Therefore, a method was devised to separate the organic colloidal size fraction of DOC in the Beaulieu estuary. This was achieved using the unique properties of Anotec Anopore aluminium oxide filters. This is illustrated in **Figure 3.8** which shows scanning electron micrographs of the surface of the Anotec Anopore aluminium oxide filter and the Whatman GF/F glass-fibre filter. The Anotec Anopore filters have well defined pore sizes (either 0.1 or 0.02 μm, as used in this study), when compared to GF/F filters and therefore can accurately separate the required size fractions.

The filtration apparatus consisted of a glass Millipore membrane filter holder and manifold (sintered glass frit) connected to a Buchner flask (5 l) with a Quickfit ground glass joint such that the socket was connected to the manifold and the cone was connected to the Buchner flask. This ensured that any spillages from the filter holder would not enter the Buchner flask and contaminate the filtrate.

Three filtration units (consisting of a Millipore membrane filter holder, manifold and Buchner flask as described above) were used; one contained a Whatman GF/F 0.7 μm filter, one contained an Anotec Anopore 0.1 μm filter whilst the third unit contained an Anotec Anopore 0.02 μm filter. The sample was filtered (under a vacuum of 30 cm mercury) using the first filtration unit (Whatman GF/F 0.7 μm filters; a number of filters were used depending on the size of the sample) and collected in the Buchner flask. This filtrate was taken and filtered through the second filtration unit (Anotec Anopore 0.1 μm filters) and again this filtrate was collected and filtered through the third filtration unit (Anotec Anopore 0.02 μm filters). Samples for DOC, POC, SPM and $PO^{13}C$ were taken at various points. The filtration sequence has been depicted in **Figure 3.9**. Therefore, the size fractions isolated were particles > 0.7 μm (mean diameter; isolated on the Whatman GF/F filter), particles in the size range 0.1 to 0.7 μm (isolated on the Anopore Anotec 0.1 μm filter) and particles in the size range 0.02 to 0.1 μm (isolated on the Anotec Anopore 0.02 μm filter). The POC fraction refers to particles > 0.7 μm (mean diameter), the CCOC fraction refers to particles in the size range 0.1 to 0.7 μm and the FCOC fraction refers to particles in the size range 0.02 to 0.1 μm.

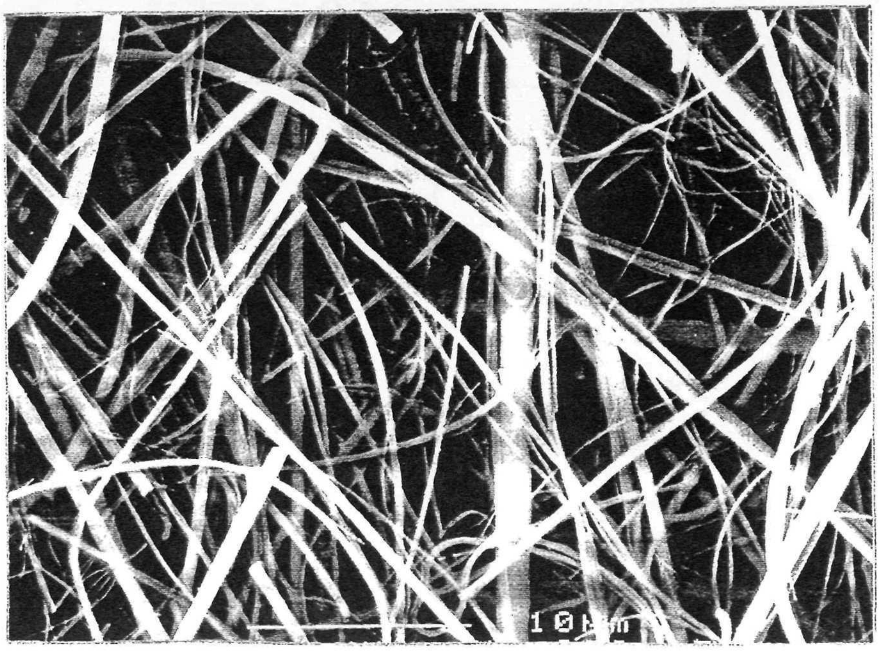


Plate 1

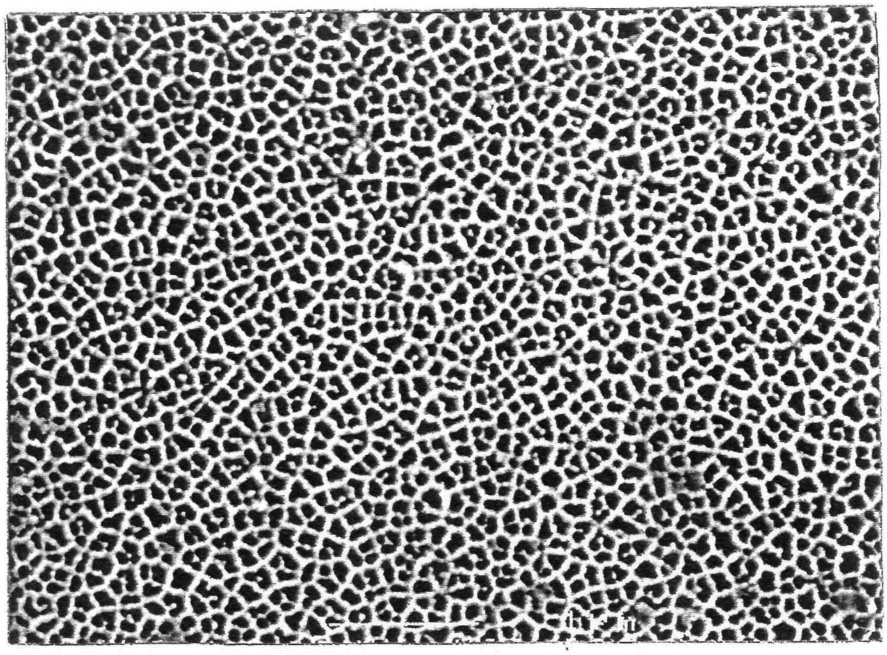
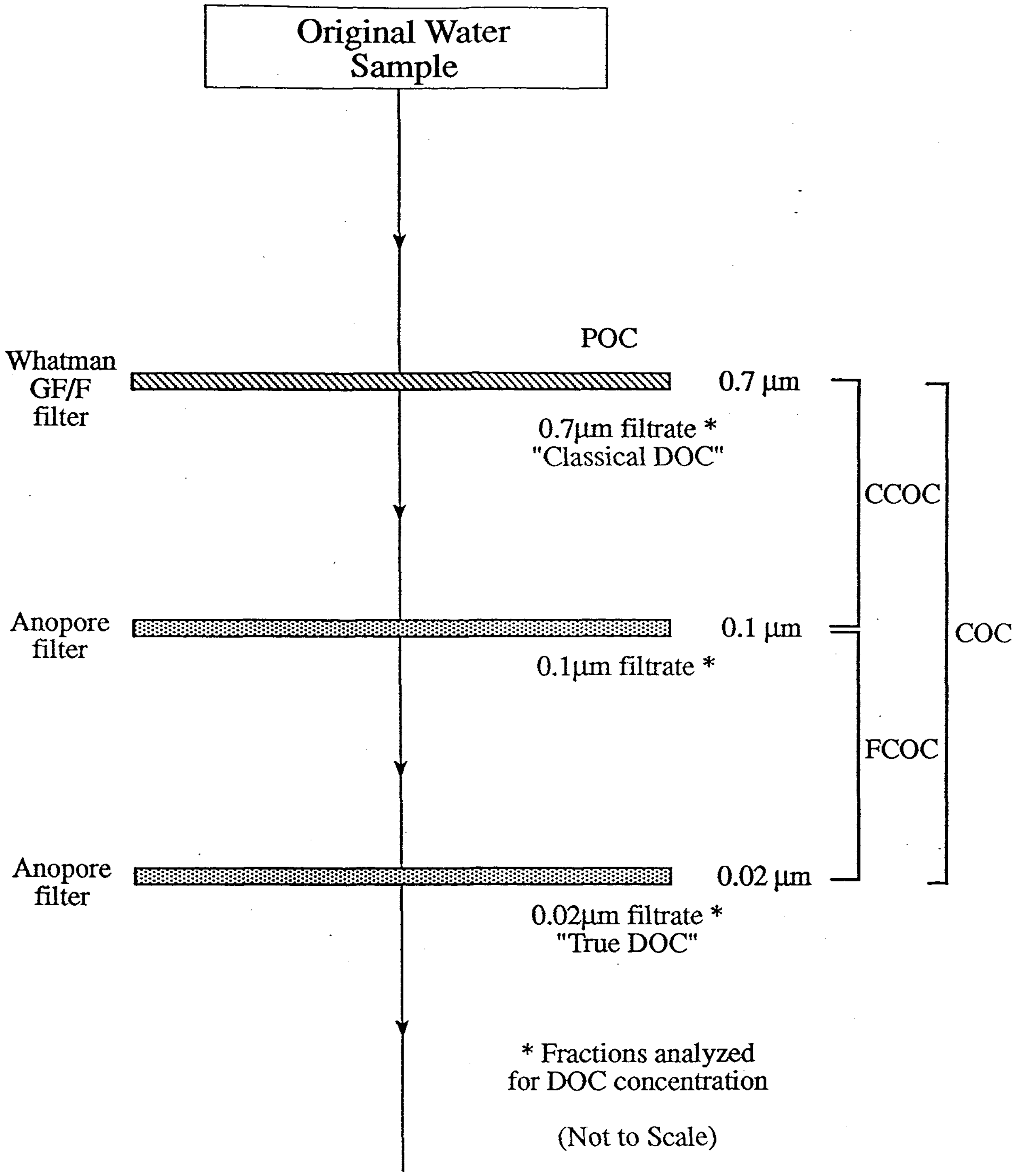


Plate 2

Figure 3.8. Scanning electron micrograph of the surface of the 0.7 µm Whatman GF/F glass-fibre filter (x 400; plate 1) and the 0.1 µm Anotec Anopore aluminium oxide filter (x 20,000; plate 2).



The CCOC fraction was determined as the difference between the DOC and 0.1 μm filtrate concentrations, and the FCOC fraction was determined as the difference between the 0.1 μm filtrate and 0.02 μm filtrate concentrations.

Figure 3.9. Filtration sequence for water samples.

Chapter 4:

An inter-seasonal and inter-annual investigation of organic carbon in the Beaulieu estuary.

4.1. Introduction.

Whilst considerable progress has been made during the past decade towards characterizing the composition of POC and to a lesser extent DOC, there have been few reported studies on the material between the dissolved and particulate fractions i.e. the colloidal material (Farrington *et al.*, 1992). Colloids are an important size fraction, existing between the dissolved and particulate phase, because they have a very high surface to volume ratio, which enables them to interact with dissolved compounds very efficiently. They can be readily transformed into particulate matter by flocculation, which can then actively participate in biological and geological processes. Therefore, they probably act as an intermediary between the more abundant dissolved material and the ecologically more active particulate material (Zsolnay, 1979).

To investigate inter-seasonal and inter-annual variations in the distribution of organic carbon in the Beaulieu estuary, a two year field programme was undertaken. The objectives were to study processes influencing organic carbon in this environment by studying the different forms of carbon, principally colloidal forms, but including DOC and POC, together with carbon isotope studies. Background parameters were also measured to aid process interpretation.

4.2. Study site, sampling and analytical methods.

4.2.1. Study Site.

The Beaulieu estuary is situated in the southeast of the New Forest within limits of 50^0 50′ North to 50^0 46′ North and 1^0 22′ West to 1^0 29′ West (see **Figure 4.1**). The estuary is fed by the Beaulieu river system forming an extensive valley-bog (Tremlett, 1965). The river drains a complex of leached, peaty and fertile alluvial soils including bogs and wet heath in valleys and basins. The most extensive heathland occurs in the southern half of the New Forest, where a gently contoured slope, with a wide and shallow drainage, holds up the percolated water. A rusty red deposition of iron and other minerals can be detected, colouring most of the gravels in the stream (Tubbs, 1968).

The catchment area of the Beaulieu river has a generalized geological structure i.e. the area that is now the centre of the downfold of the chalk known as the Hampshire Basin of the Tertiary Era. The surface rocks of Eocene and Oligocene age are overlain by Pleistocene gravels, sands and clays and are surrounded by low chalk downlands, in which the surface water drainage is held up particularly by the clay beds.

The climate of the Beaulieu river system is generally mild with mean temperatures of 7^0C. The maximum temperature reaches 18^0C and minimum temperatures of 0.8^0C have been recorded. Mean annual precipitation is 951 mm. The mild climate of the heathland area of Hampshire, even during the wet winter months is reflected in the vegetation which has "Mediterranean" and "oceanic" characteristics (Tubbs, 1968).

The Beaulieu estuary is about 8 km long and varies in width from around 30 to 80 m. Its relative depth can be seen in **Figure 4.1.** The estuary is relatively shallow with the depth in the channel ranging from about 1 to 8 m. Much of the estuary is almost vertically homogeneous but at the upstream limits of salt intrusion, which at high tide can reach into the pond at Beaulieu, stratification is observed. In this region a salinity of 1 was observed at the surface whilst a salinity of 19 was found at a depth of 0.5 m (Umnuay, 1981).

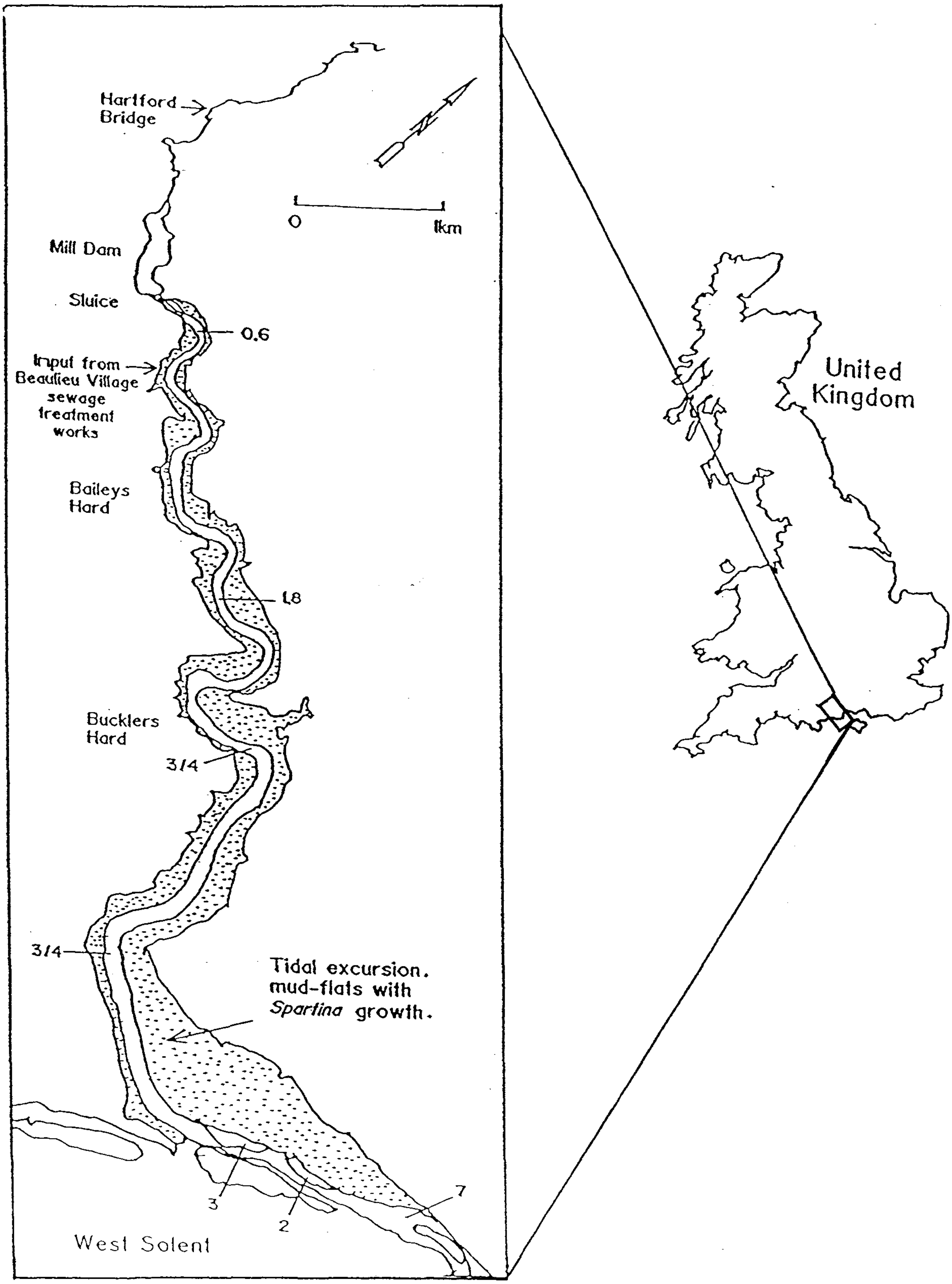


Figure 4.1. Location of the Beaulieu estuary.

The fresh water flow in the Beaulieu river is not extensively surveyed, however, river flow has been measured on discrete occasions. It was found to be higher during the winter and spring months and was typically around 1 m^3sec^{-1}. During the summer and autumn months flows as low as 0.03 m^3sec^{-1} have been recorded. Annual river flow data from the Itchen and Test rivers (both discharge into the Solent) can be used for the period 1989 to 1992 to give an indication of the likely annual variation in river flow in the Beaulieu river (see **Figure 4.2**). Variation in the freshwater discharge for these rivers would generally be the same as the variation for the Beaulieu river. This can be seen by the similarity in freshwater discharge between the Itchen and Test rivers. However, small differences would exist depending on the percolation rate of water through the drainage basin. The peak river discharge for both the Itchen and Test rivers occurred between February and April. High river discharge occurred over the period December to May and the period of lowest discharge was June to November (D. Horne, National Rivers Authority data, personal communication).

The physical-advective diffusive processes in estuaries can be described approximately by the flushing time T (Dyer, 1973), given by:

$$T = \frac{Q}{R}$$

where Q is the volume of freshwater accumulated in the whole or a section of the estuary (m^3) and R is the river discharge (m^3sec^{-1}). Therefore, the flushing time of the freshwater in the estuary can be estimated by dividing the volume of the estuary (6.4×10^5 m^3) by the mean discharge rate (1 m^3sec^{-1}) of the Beaulieu river. This gives a freshwater residence time of around 7 days for the Beaulieu estuary.

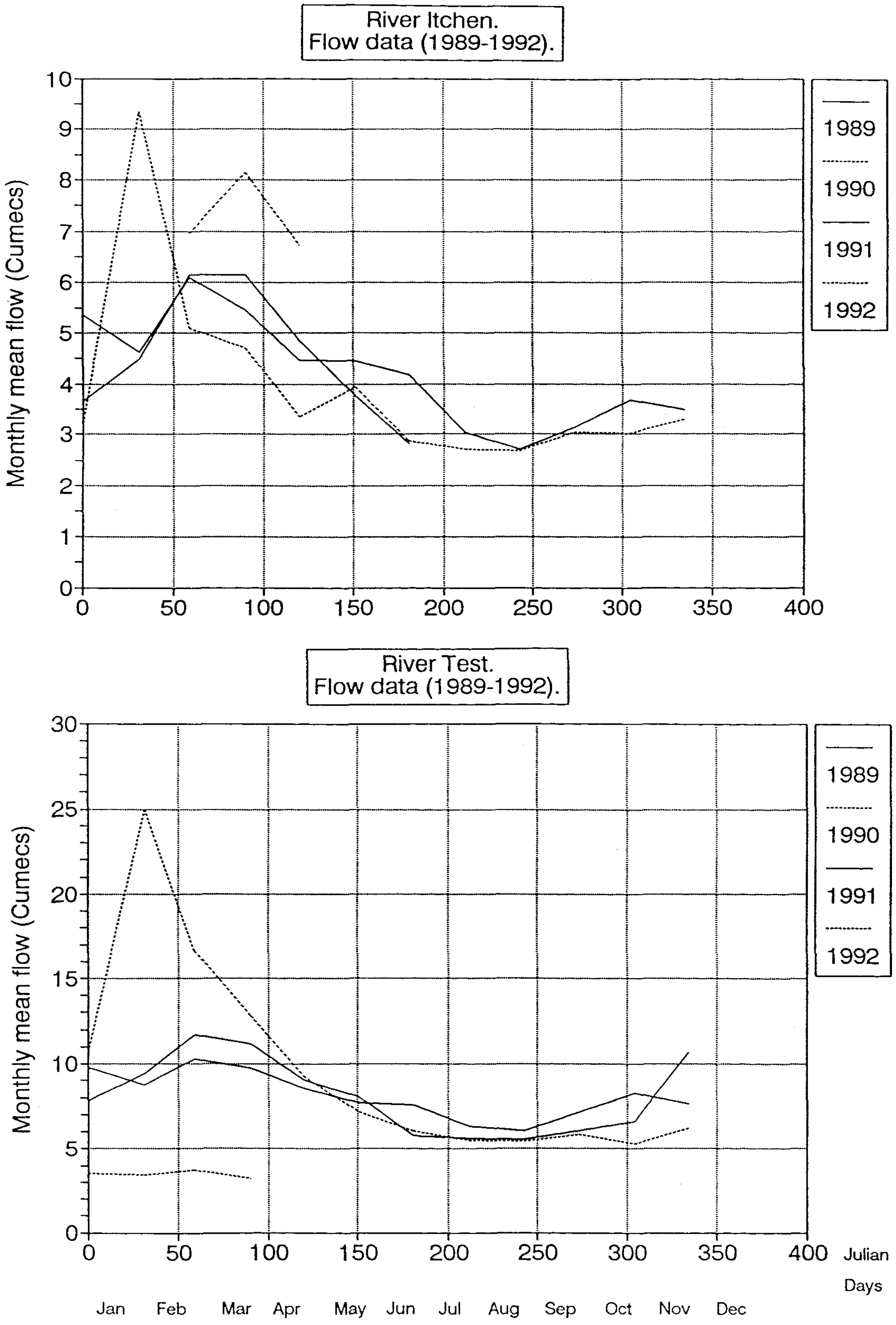


Figure 4.2. Annual river flow data for the Itchen and Test rivers (1989-1992).

4.2.2. Sampling periods and survey methods.

Surveys of the Beaulieu estuary were undertaken on the following dates, November 6th, 1990, February 4th, 1991, May 17th, 1991, July 29th, 1991, November 11th, 1991, February 6th, 1992, March 23rd, 1992 and July 30th, 1992 to coincide with the seasons. Sampling was conducted from *R.V. Bill Conway* on the flood tide by longitudinal sampling transect from the seawater end-member up to Baileys Hard. In order to obtain lower salinity samples a dinghy was used from Baileys Hard to Mill Dam. Sampling positions were chosen to give the largest possible range of salinity over the tidal period. It was decided to sample as far as possible at every 1 to 2 salinity units throughout the estuary to provide good coverage of the estuary, as the behaviour of COC was unknown at this time. This was ensured by use of a portable temperature/salinity probe. Further sampling was undertaken at Mill Dam and at Hartford bridge from the roadside. In these cases every effort was made to sample from the central part of the river in order to obtain samples that would be representative of the main river flow.

4.2.3. Analytical methods.

4.2.3.1. Clean procedures.

A general procedure followed to ensure that contamination was kept to a minimum was to carry out all handling steps in a clean hood. This has been described in **Chapter 3.2.2.** Apparatus used either in the field or in the laboratory was treated to remove possible contamination. All glassware (sampling bottles, filtration apparatus etc) was washed before use in warm water with "Micro" detergent (2 % volume/volume), rinsed with reverse osmosis (RO) water, dried in a heated cabinet (60^0C; to prevent dilution of the chromic acid) and soaked in chromic acid overnight. Following this treatment the glassware was rinsed with RO water, given a final rinse with Milli-Q water and dried in a heated cabinet (60^0C). If the glassware was to be stored it was either wrapped in pre-combusted (4 hours, 450^0C) aluminium foil, placed in sealed polythene bags or if the glassware had a sealable cap, this was tightly sealed. All caps which were used for DOC samples were teflon lined. The teflon was cleaned by soaking in concentrated sulphuric acid overnight, rinsed with RO water, Milli-Q water and dried in a heated cabinet (60^0C). Filters and aluminium foil were heat treated (450^0C for 4 hours) before use and all metal handling implements were cleaned by heating to red heat in a flame.

4.2.3.2. Filtration methods.

The filtration apparatus and method has been described in **Chapter 3.4.2.**

4.2.3.3. Dissolved organic carbon.

Samples were taken just below the surface (0.5 m) by use of a modified lead-weighted Winchester sampling device (Ehrhardt, 1983). A Winchester bottle (2.5 l) was placed in the Winchester sampling device and this was thrown over the side of the boat. On impacting the water the surface was broken hence preventing any surface film from entering the sample bottle and water was drawn in at depth. On retrieving the sample bottle it was tightly sealed.

Filtration through Whatman GF/F filters and Anotec Anopore filters (pre-combusted at 450^{0}C for 4 hours) was performed on the day of sampling on returning to the laboratory. New filters were used for each sample. It has been demonstrated that DOC concentrations decrease in the first few hours after sampling (Sugimura and Suzuki, 1988; Chen and Wangersky, 1993) and after this initial loss the samples are fairly stable. Therefore, instantaneous filtration was identified as desirable and, therefore, filtration was undertaken as soon as practically possible, typically within 5 hours. Filtration was carried out under vacuum (12 bar) in clean conditions using the filtration apparatus described above. DOC samples (300 ml), the 0.1 μm filtrate (150 ml) and the 0.02 μm filtrate (150 ml) were stored at 4^{0}C in the dark.

Analysis of the DOC concentration was carried out as soon as possible after sampling and in most cases this was within a week. It has been shown that this time delay would not result in major changes in DOC concentration (see above). DOC analysis using Hg salts in the UV persulphate DOC analyzer (which has been shown to give increased oxidation efficiencies; this study **Chapter** 3.2.6) was not used for analysis of DOC samples from the Beaulieu estuary surveys because investigation of the technique began half way through the Beaulieu estuary surveys and it was thought best to keep the analytical methods constant throughout the study to prevent any unnecessary disparity in the data.

The UV persulphate method of DOC analysis was used, and an account of this has been given in **Chapter 3.2.1.**

4.2.3.4. Particulate organic carbon.

Water samples were collected in the same way as for DOC analysis (see above) and filtered on the same day as sample collection through Whatman GF/F filters (pre-combusted at 450^{0}C for 4 hours) using the filtration apparatus already described (**Chapter 3.4.2**) until the filter was clogged. The filters were stored frozen in pre-combusted aluminium foil (450^{0}C for 4 hours) placed in individual plastic containers. The filters were handled with metal forceps, cleaned by heating to red heat in a flame.

A carbon analyzer manufactured in the laboratory was utilized for analysis of POC (Williams, 1985). The procedure involves burning the particulate material on the filters in a stream of oxygen (500 ml/min) in a silica combustion tube. The combustion gases are passed over copper oxide (800^{0}C) to ensure complete oxidation and then the oxygen containing the combustion gases is collected in the gas collector unit. The flow rate of the oxygen is arranged such that complete combustion occurs before the gas collector is filled. Once the collector is full, the gas is held for about one minute and then it is forced by the weight of the piston through the infra-red gas analyser (Analytical Developments Ltd., Hoddesdon, Hertfordshire, U.K.: type 225). The gas analyser has cells of three pathlengths enabling a wide range of samples to be analysed. The output of the infra-red analyser is displayed on a chart recorder.

The calibrating standard was a solution of sucrose (Analytical grade; BDH Limited, Poole, England). The stock solution is made up to a concentration of 667 μmC per 100 μl (19.017 g in 100 ml) and dilutions of this are used for calibration ranging from 17 μM C to 83 μM C. The precision of the analysis is high (0.2 μM C, ± 1 SD; Banoub and Williams, 1972).

4.2.3.5. Suspended particulate matter.

The Whatman GF/F filters that were to be used for SPM analysis were treated by rinsing the filter with Milli-Q water (to remove any salts) before heat treatment (450^{0}C for 4 hours) followed by drying thoroughly in a vacuum desiccator over silica gel crystals. The filters were then weighed and stored wrapped in pre-combusted foil (450^{0}C for 4 hours) in plastic containers.

Water samples were collected in the same way as for DOC analysis (see above) and filtered on the same day as sample collection through weighed Whatman GF/F filters (pre-combusted at 450^{0}C for 4 hours) using the filtration apparatus already described (**Chapter 3.4.2**) until the filter was clogged. The filters were rinsed with MQ water (3 ml; to remove any salts) and dried in a vacuum desiccator over silica. The filters were stored frozen in pre-combusted aluminium foil (450^{0}C for 4 hours) placed in individual plastic containers. The samples were then re-weighed, using a Sartorius balance, to a high precision (values are given to within ± 10 μg).

4.2.3.6. Chlorophyll *a*

Whilst in the field a 50 ml water sample was forced through a Whatman GF/F filter (2.5 cm diameter) held in a 2.5 cm diameter in-line filter holder using a 60 ml syringe. These filters were folded, placed in a sealed polythene bag and stored cold in the dark (in a cold box containing ice blocks).

Prior to analysis the filters were soaked overnight in 90 % acetone (5 ml) in the fridge. They were then transferred to centrifuge tubes, manually broken up with a glass rod and centrifuged at 3000 revolutions per minute for 4 minutes. This was repeated and the solution decanted and made up to 10 ml with 90 % acetone in volumetric flasks for fluorescence measurement using an Aminco fluoro-colorimeter. Phaeo-pigment concentrations were determined by acidifying the solution (sulphuric acid; 1 M) and re-measuring the fluorescence. The standard was an Anacystis nidulans algal extract (Sigma Chemical Company). This was dissolved in 90 % acetone solution and the absorbance measured on a spectrometer to calculate the chlorophyll *a* concentration (1.4151 mgChl.*a*/l). Dilutions of this primary solution were made ranging from 1/100 to 1/2000 for measurement on the fluoro-colorimeter. The method has been described by Parsons *et al.* (1984). The precision of the method is 0.05 μg/l chlorophyll *a* at the 0.5 μg/l chlorophyll *a* level.

4.2.3.7. Oxygen

Sampling for oxygen was undertaken in the field in the following way. An oxygen bottle was rinsed twice with water and filled to overflowing with the water sample. This was achieved by submerging the top of the bottle just below the surface of the water and gently allowing the water to fill the bottle, hence preventing any bubbles from entering the sample. The bottle was stoppered to ensure the volume was accurately known and manganous chloride (1 ml, 3 M) was added followed by alkaline iodide (1 ml, sodium hydroxide 8 M/iodide 4 M) from a pipette placed just below the surface of the water sample. The bottle was stoppered, inverted several times to mix the contents and stored in the dark under water.

Oxygen concentrations were determined using the Winkler method with an optical end-point detector and a Dosimat automatic burette. The sodium thiosulphate solution was initially standardized by titration with potassium iodate. Sulphuric acid (1 ml; 10 M) was then added to the sample and the liberated iodine was titrated with the sodium thiosulphate solution. This was based on the method of Williams and Jenkinson (1982). The precision of the method is ± 1 μM oxygen.

4.2.3.8. Salinity.

Samples were collected in the field in soda glass bottles (350 ml) and the caps were tightly sealed. The samples were stored at 4^0C in the dark. Analysis was carried out using a Guildline Autosal analyzer to within ± 0.1 PSU.

4.2.3.9. Isotopic analysis.

Sample collection was similar to that undertaken for DOC analysis (**Section 4.2.3.3**), altough for larger water samples repeated sampling with the Winchester sampling device was necessary to fill the larger sample bottles (4 to 20 l). Filtration through Whatman GF/F filters and Anotec Anopore filters was performed on the day of sampling on returning to the laboratory, apart from the larger samples for isotopic analyses (filtration method described in **Chapter 3.4.2**). In these cases filtration through Anotec Anopore filters continued into the next day and in a few cases into the third day because of the slow filtration rates. It has been shown that this delay would not result in major changes in DOC concentration as the first few hours after sampling, if filtration through Whatman GF/F filters has not been carried out, have been identified as the critical ones (Sugimura and Suzuki, 1988; Chen and Wangersky, 1993). Filters for δ^{13}C and Δ^{14}C analysis i.e. GF/F, Anotec Anopore 0.1 μm and Anotec Anopore 0.02 μm were placed in aluminium foil (pre-combusted at 450^0C for 4 hours) in plastic containers, dried in a vacuum desiccator and stored frozen.

Just before $\delta^{13}C$ or $\Delta^{14}C$ analysis the filters were again dried in a vacuum desiccator and put into silica combustion tubes (heat-treated at 800^{0}C for 3 hours) for combustion on the vacuum line. The size of the silica combustion tubes used were 80 x 12 mm for the $\Delta^{14}C$ analysis and 80 x 7 mm for the $\delta^{13}C$ analysis. Five GF/F filters and between 10 and 14 Anotec Anopore 0.1 μm filters were used for the $\Delta^{14}C$ analysis and 1 GF/F, 2 Anotec Anopore 0.1 μm and 2 Anotec Anopore 0.02 μm filters were used for the $\delta^{13}C$ analysis. The filters were cut into strips (GF/F) or broken into small pieces (Anotec Anopore) and funnelled into the combustion tube. Copper oxide (wire form; BDH Limited, Poole, England; heat-treated at 800^{0}C for 3 hours) was added (around 10 g) and a plug of silica wool (heat-treated at 800^{0}C for 3 hours). The silica tubes were wrapped in heat treated foil for storage before combustion.

Before combustion and isolation of CO_2 the vacuum line was washed in warm water with "micro" detergent, rinsed with RO water and Milli-Q water and dried in a heated cabinet (30^{0}C). The combustion method using the SUDO vacuum line and the UCNW vacuum line has been given in **Chapter 3.3.3** and **Chapter 3.3.5** respectively.

4.3. Processes affecting the distribution of constituents in estuaries.

There are various inputs of organic carbon along the length of an estuary such as input from the riverine and seawater end-member, inputs from anthropogenic sources, pore waters or resuspension events, atmospheric inputs and *in-situ* biological production. Once in the estuarine environment these organic constituents may be affected by a number of processes that alter their distribution. This is dependent on the rates of these processes and on the flushing time of the estuary. Processes which may be regarded include the following: variations in the freshwater discharge, physical-chemical changes in the water column such as salinity, pH or processes occurring on particles such as adsorption, biological production and respiration and processes involving the estuarine sediments such as settlement and resuspension. For a discussion of these processes see **Chapter 1.7.**

4.3.1. Use of mixing diagrams to study the nature of DOC in estuaries.

A basic tool for the study of environmental phase transfer processes has been the estuarine mixing diagram in which the concentration of dissolved constituent is plotted against a conservative indicator of mixing, usually salinity (Burton, 1988). Characterization of estuarine samples with respect to salinity is a standard procedure for chemical investigations in estuaries and is known as the "reactant method" or "survey approach" (Morris, 1985; Burton, 1988).

Recognition of conservative or non-conservative behaviour in a dissolved chemical constituent allows an assessment of gain, loss or conservation of the constituent and an indication of the relative contributions of the species from the separate marine and fresh water sources. The salinity related (and geographical) location of reactivity and the extent of gain or loss of the constituent can also be deduced (Morris, 1985).

The extent of transfer of material between solution and solid phases in estuaries determines whether the flux of a dissolved constituent leaving the estuary differs significantly from that entering. The emphasis on dissolved constituents reflects the difficulties in interpreting data on particulate material because of the complexity of both its composition and transport pathways and also the fact that in some cases mass balance considerations make the dissolved pool a more sensitive indicator of transfer processes (Burton, 1988).

Providing a number of criteria are satisfied, of which steady state is usually the most important (Loder and Reichard, 1981), a linear mixing curve indicates conservative behaviour of the constituent within the estuary. Any other relationship (concave, convex, multiple inflections) indicates addition and/or removal of the constituent.

More quantitative approaches to the assessment of conservative and non-conservative constituents have been developed by several workers. For example Boyle *et al.* (1974) derived a mathematical relationship for the variation of the flux of a given dissolved constituent (Q_c) with salinity. They showed that for conservative behaviour:

$$\frac{dQ_c}{dS} = 0 = \frac{d^2C}{dS^2}$$

where C is the concentration of the dissolved constituent at an iso-haline surface of salinity S. If behaviour is non-conservative:

$$\frac{d^2C}{dS^2} \neq 0$$

and the graph of concentration of the constituent against salinity should be curved.

Marked gradients in constituent concentrations and in ambient physical-chemical conditions (e.g. ionic strength, pH, redox status, suspended particulate material concentration) are characteristic of estuarine mixing zones. Hence, rigorous conservative behaviour of all organic constituents throughout an estuary is highly improbable. For many of the trace constituents of natural waters, it is most likely that linear mixing curves are due to the slow reaction kinetics of these trace constituents and therefore knowledge of the hydrodynamic turnover time of the estuarine water becomes important (Morris, 1990).

4.3.1.1. Limitations of using the conservative and non-conservative mixing approach.

There are limitations to the applicability of this technique. (1) A steady-state condition is assumed in the estuary. However, changes in river water concentrations can give rise to estuarine mixing curves that simulate removal or addition. The precise effects depend upon the magnitude and periodicity of the changes and the integrating time in the estuarine reservoir (Loder and Reichard, 1981). (2) A single fresh water source is implicit although estuaries often have a number of significant water sources, entering at points through the length. (3) Quantitative and qualitative interpretations of mixing curves are severely restricted if the reactive constituent is subject to more than a single unidirectional reaction (Morris, 1985). (4) A constituent which is enriched in the marine end-member may show conservative or non-conservative behaviour in the estuary, but it is difficult to follow processes affecting a low concentration riverine input against a marked gradient of increasing concentration with increase in salinity (Burton, 1988).

These limitations, combined with imprecision arising from variability, make the simple application of the reactant method quite insensitive. Deviations from conservativeness of less than 10 to 20 % will often be unresolved, or poorly characterized, unless they are very localized (Morris, 1985).

Despite these limitations the conservative mixing approach still provides a good basis for the description and discussion of estuarine data sets and has been used in this work with appropriate note being taken of the limitations discussed above.

4.4. Distributions of organic carbon in the Beaulieu river and coastal waters (1990-1992).

4.4.1. Colloidal organic carbon in the Beaulieu estuary (1990-1992).

4.4.1.1. Introduction.

Traditionally studies of organic carbon in natural waters have focused on DOC (i.e. the organic carbon fraction < 0.7 μm in diameter). Few studies have considered the colloidal organic fraction (for example see Whitehouse *et al.*, 1989; Altabet, 1990; Benner *et al.*, 1992). Variations in COC over seasonal cycles in the estuarine environment form a part of the subject and as far as it is known this has not been considered by previous studies.

The fractions of DOC that have been measured in the Beaulieu estuary in this study include the "classical" DOC concentration i.e. filtration through a 0.7 μm GF/F filter, the organic carbon fraction < 0.1 μm and the organic carbon fraction < 0.02 μm.

The mixing curves for these fractions can be seen in **Figures 4.3 and 4.4**. The mixing curves for the organic carbon fraction < 0.1 μm and the organic carbon fraction < 0.02 μm are very similar to the DOC mixing curves suggesting that similar processes are acting on these size fractions.

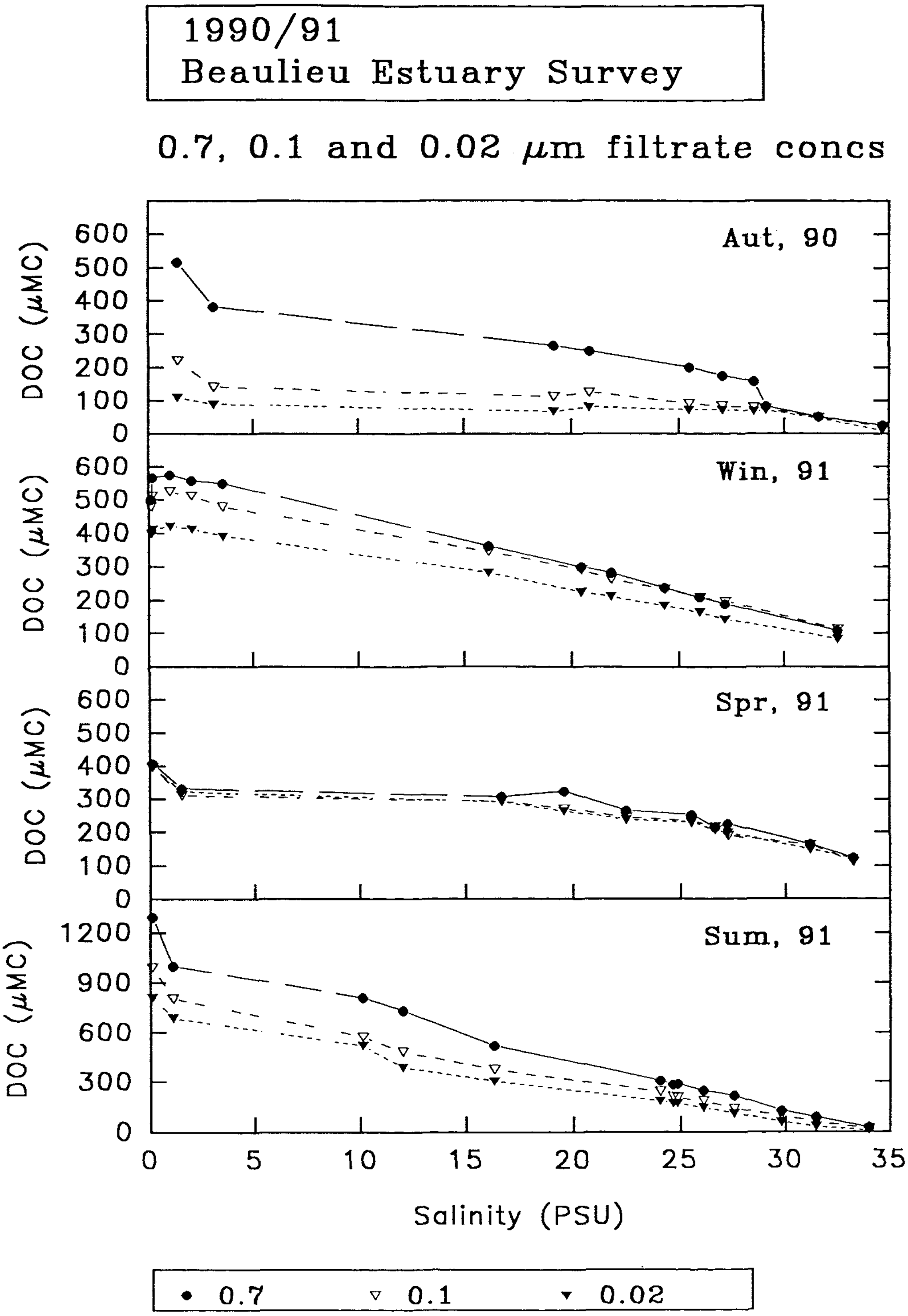


Figure 4.3. Concentrations of the various organic carbon fractions measured in the Beaulieu estuary (1990/1991).

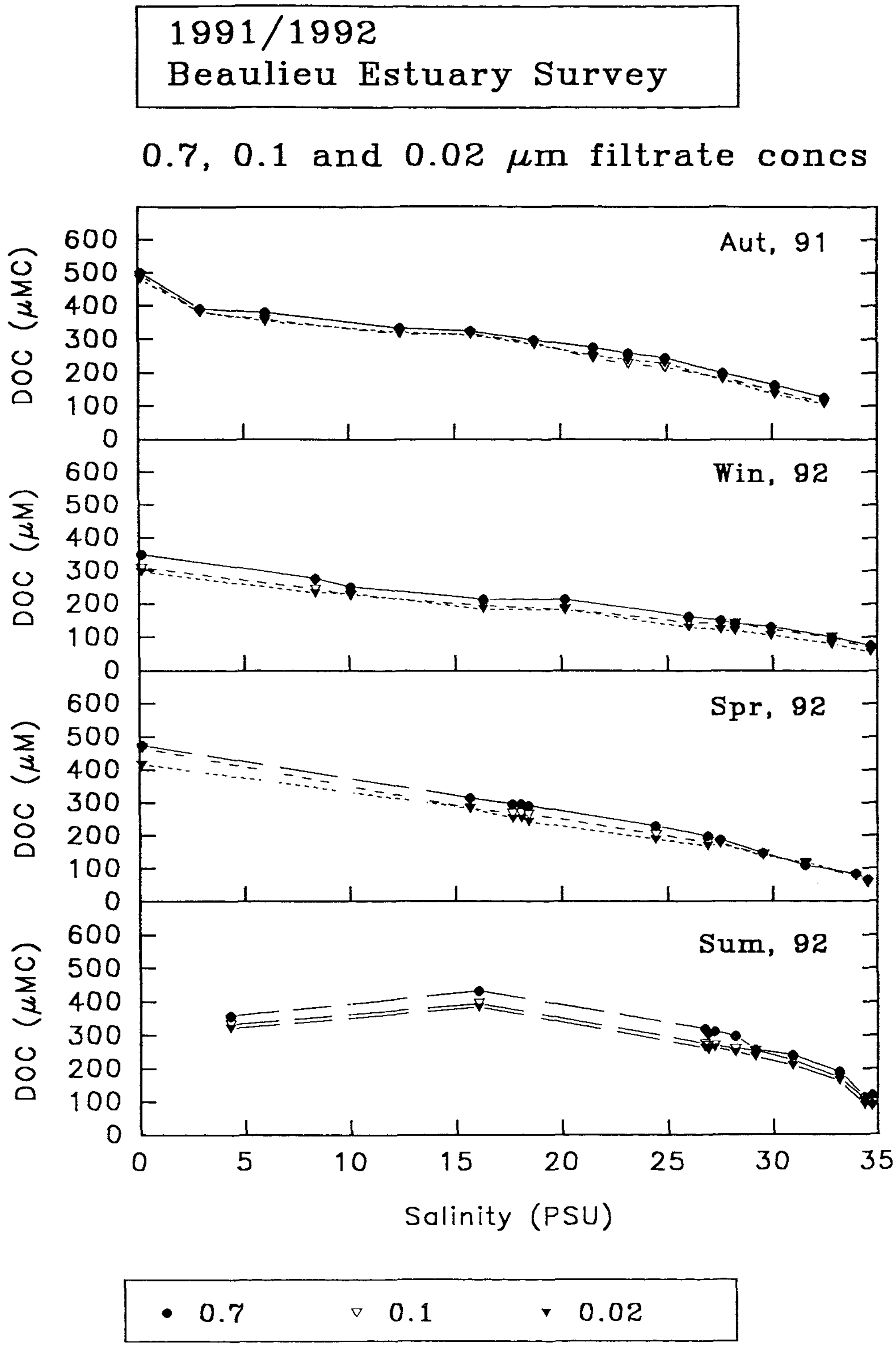


Figure 4.4. Concentrations of the various organic carbon fractions measured in the Beaulieu estuary (1991/1992).

4.4.1.2. COC, Coarse COC (CCOC) and fine COC (FCOC).

The pore sizes of the Anodisc Anopore filters used to isolate the COC fractions were 20 nm and 100 nm. The COC fraction represents particles in the size fraction 0.02 to 0.7 μm, the FCOC fraction represents particles in the size fraction 20 to 100 nm and the CCOC fraction represents particles in the size fraction 100 to 700 nm.

The COC fraction has been derived by subtracting the blank corrected 0.02 μm values from the blank corrected 0.7 μm values. The CCOC fraction has been derived by subtracting the blank corrected 0.1 μm values from the blank corrected 0.7 μm DOC values. Similarly, the FCOC fraction has been derived by subtracting the blank corrected 0.02 μm values from the blank corrected 0.1 μm values. This filtration sequence has been depicted in **Figure 3.9.**

Moore *et al.* (1979) have reported that 18 % of DOC in the Beaulieu river had an apparent molecular weight greater than 10^5 i.e. mainly humic acids. More than 70 % of DOC was in the nominal molecular weight range 10^3 to 10^5. This suggested that a large fraction of the DOM had a molecular weight around 10^4 and was composed principally of fulvic acids.

Fulvic acids (molecular weight 1000-10,000 Daltons, effective diameter 1-5 nm; Thurman, 1986) would pass through the 0.02 μm Anodisc Anopore filter whilst a certain percentage of the humic acids (molecular weight 10,000-100,000 Daltons, diameter 2-50 nm; Thurman, 1986) would be expected to be effectively trapped by the Anodisc Anopore 0.02 μm filters.

Therefore, the FCOC fraction would be expected to contain the highest percentage of the humic acid contents of the Beaulieu river and estuary and would be fairly representative of the humic acid fraction of DOC, especially the humic acids of highest molecular weight around 10^5. Within both the CCOC and FCOC fraction clay-humic-metal complexes and viruses would be found whilst the CCOC fraction would contain some bacteria. The POC fraction would consist of bacteria, phytoplankton, zooplankton and plant debris. The size fraction < 0.02 μm would represent lower molecular weight humic acids, fulvic acids, hydrophilic acids and individual compound classes e.g. carbohydrates and amino acids etc. (see Organic Carbon Continuum, **Figure 1.1**).

4.4.1.3. Concentration of COC compared with the concentration of TDOC and POC.

The COC as a fraction of the "true" DOC concentration (TDOC; see below) can be seen in **Figures 4.5 and 4.6.** The TDOC concentration (i.e. the DOC concentration on filtration through an Anodisc Anopore 0.02 μm filter) contains only particles < 20 nm and would better represent the truly dissolved fraction. **Figures 4.5 and 4.6** also shows the fraction of COC compared to the POC concentration.

The mean value for COC as a fraction of TDOC in the river was 63 % (RSD = 186 %) and in seawater the mean value was 59 % (RSD = 100 %). The mean value for COC as a fraction of POC in the river was 196 % (RSD = 94 %) and in seawater the mean value was 76 % (RSD = 59 %). Values as high as 360 % (summer 1991) were seen for COC as a fraction of the TDOC concentrations and values as high as 1200 % (autumn 1990) were found for COC as a fraction of POC concentrations.

The high COC values compared to both TDOC (up to 3.6 x TDOC concentrations) and POC (up to 12 x POC concentrations) indicate that COC is an important size fraction in the river, estuary and coastal zone and is often the most important. However, low COC compared to TDOC (< 25 %) were also seen showing that values of COC varied within the estuary and that TDOC concentrations in the estuary were also important and in many cases represented the most important organic carbon fraction.

Colloidal organic carbon values reported in the Beaulieu estuary were often a half of the DOC concentration. Similar values have previously been reported in the literature. Carlson *et al.* (1985) and Sigleo *et al.* (1983) have reported that up to half of the DOC in seawater may be colloidal in nature. In interstitial waters of marine sediments, some 50-90 % of the DOC may be colloidal (e.g. Krom and Sholkovitz, 1977; Orem *et al.*, 1986). Other studies indicate that the colloidal fraction represents up to 33 % of the total DOC in coastal and marine waters (Sharp, 1973; Whitehouse *et al.*, 1986; Benner *et al.*, 1992). These studies support the observations in the Beaulieu estuary. However, on occasions, concentrations of COC up to three times the DOC concentration were found in the Beaulieu estuary and COC values as high as this have not been previously reported.

Particulate organic carbon in the estuary is less homogeneous and more non-conservative compared to DOC and therefore COC as a fraction of

POC will be more variable. The COC value as a fraction of POC will therefore probably be most affected by the variability in POC concentrations. This measurement is still useful though in comparing the importance of COC to POC. COC as a fraction of the POC concentration in the Beaulieu estuary stayed below 200 % for most seasons and values rarely fell below 50 %. Previously reported values have shown that the colloidal fraction paralleled the particulate fraction in magnitude and distribution in the MacKenzie river and Beaufort Sea (Whitehouse *et al.*, 1989). The COC levels observed in the Beaulieu estuary were higher and COC concentrations twice as high as POC levels were commonly found whilst the highest COC concentration found was 12 x the POC concentration.

These high COC concentrations, previously unreported, indicate that during certain times COC is the most important size fraction in the estuary. The high COC values found in the Beaulieu estuary may be due to the higher content of humic substances found in the estuary due to a drainage basin rich in humic material.

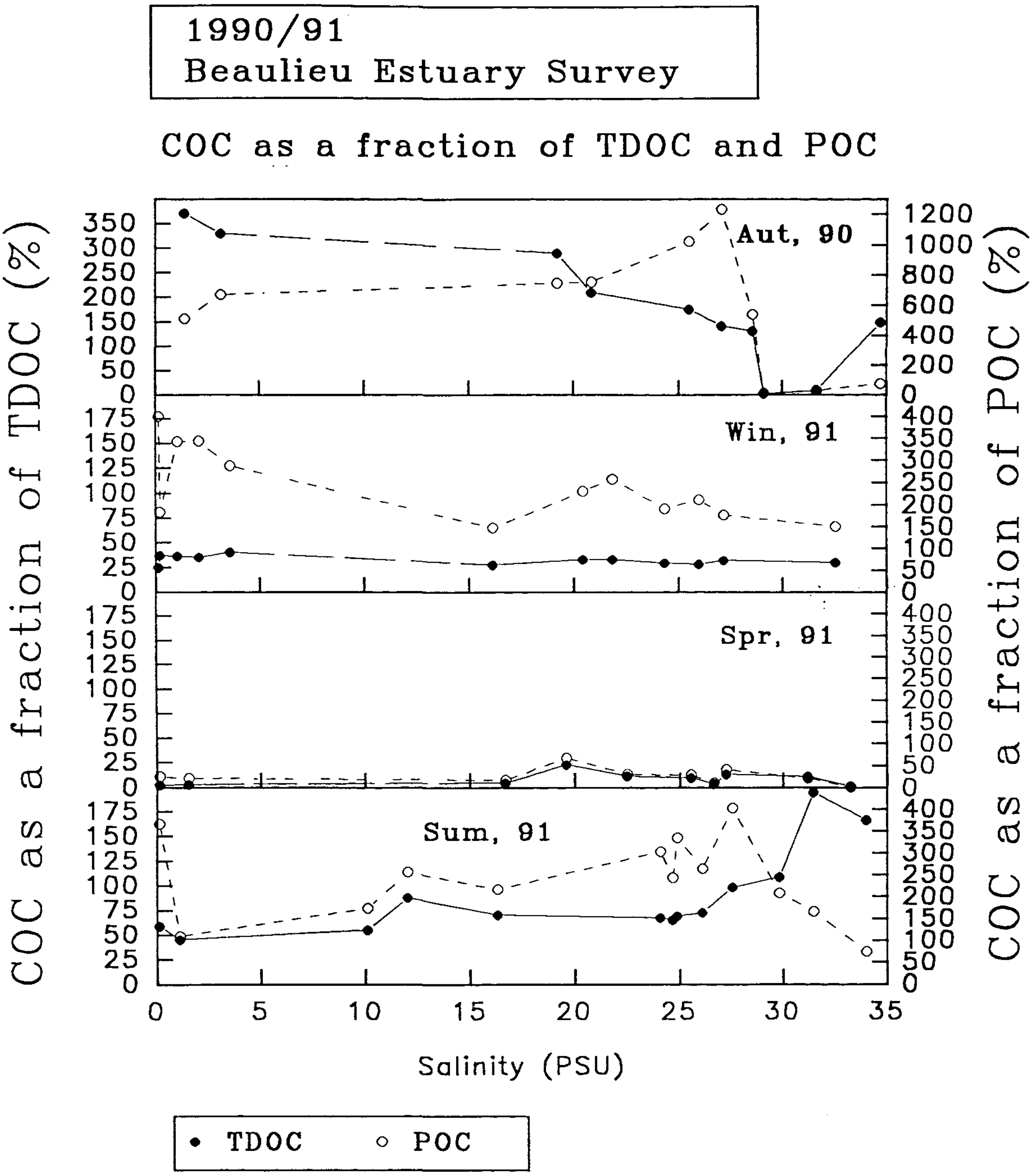


Figure 4.5. COC as a fraction of TDOC (< 0.02 µm) and POC in the Beaulieu estuary (1990/1991).

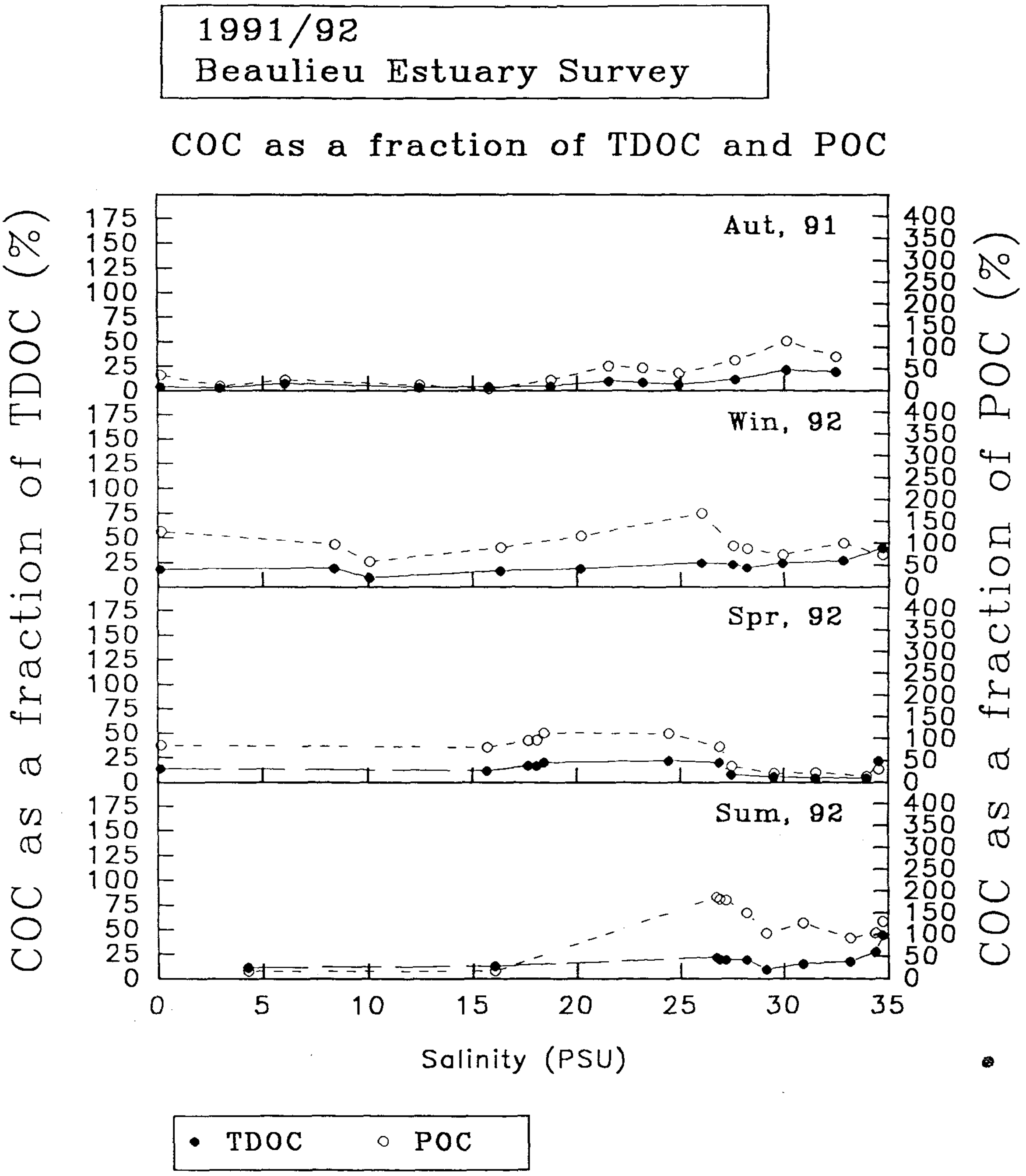


Figure 4.6. COC as a fraction of TDOC (< 0.02 μm) and POC in the Beaulieu estuary (1991/1992).

4.4.1.4. Distribution of COC in the Beaulieu estuary.

The variation of COC compared to salinity can be seen in **Figures 4.7 and 4.8** and for most seasons the COC concentrations varied up to 60 μM C. Higher concentrations were observed during autumn 1990, winter 1991 and summer 1991 where values approached 500 μM C. During these seasons of higher COC concentrations the COC levels were higher in the river water and decreased as the seawater end-member was approached. For the seasons which had lower COC concentrations the mixing curves showed evidence of various input and removal processes. There was no apparent seasonality in the COC concentrations and average end-member concentrations were 18 μM C (RSD = 56 %) in the seawater and 145 μM C (RSD = 120 %) in the river water.

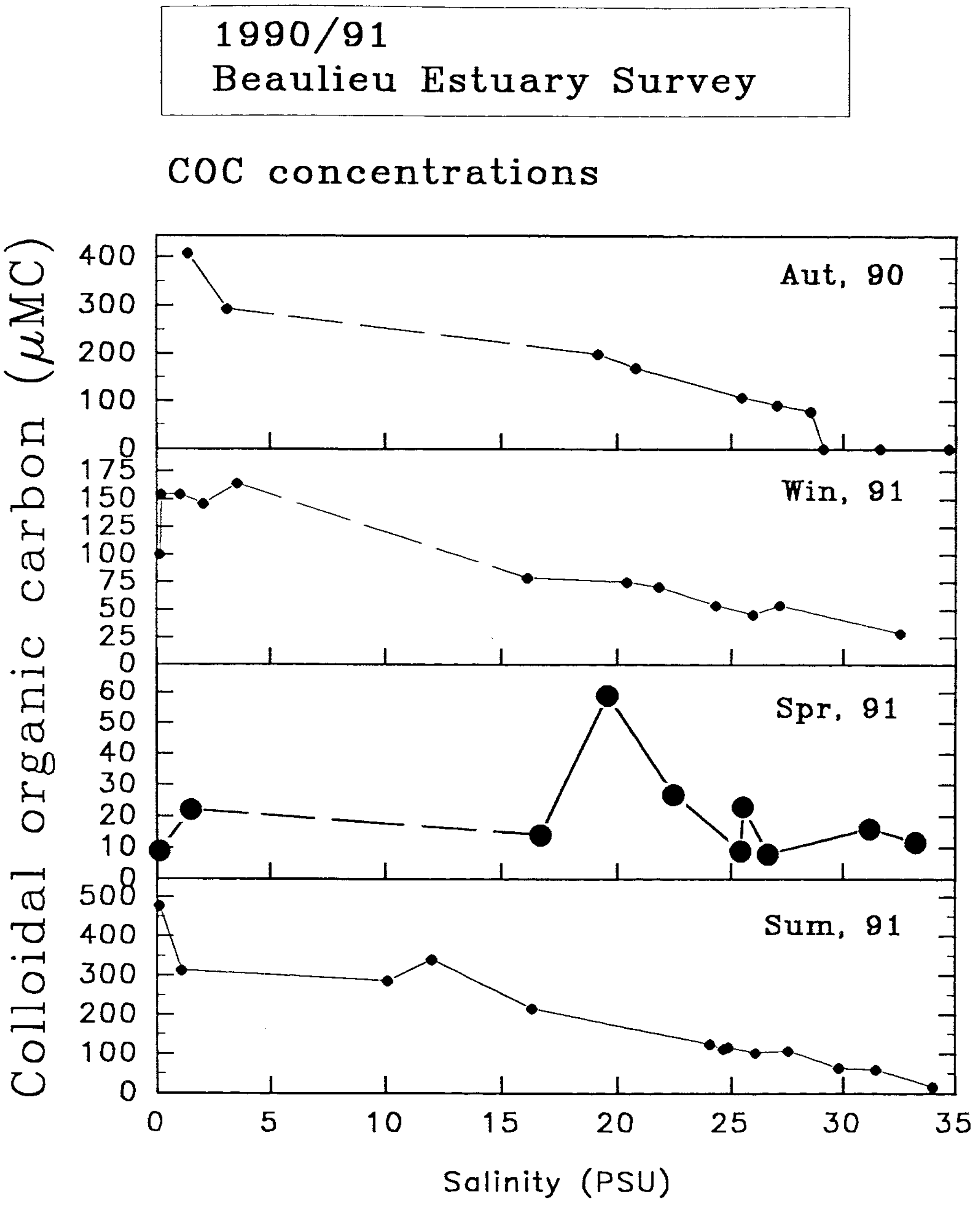


Figure 4.7. COC concentrations in the Beaulieu estuary (1990/1991) (Errors associated with these measurements are within the symbol size).

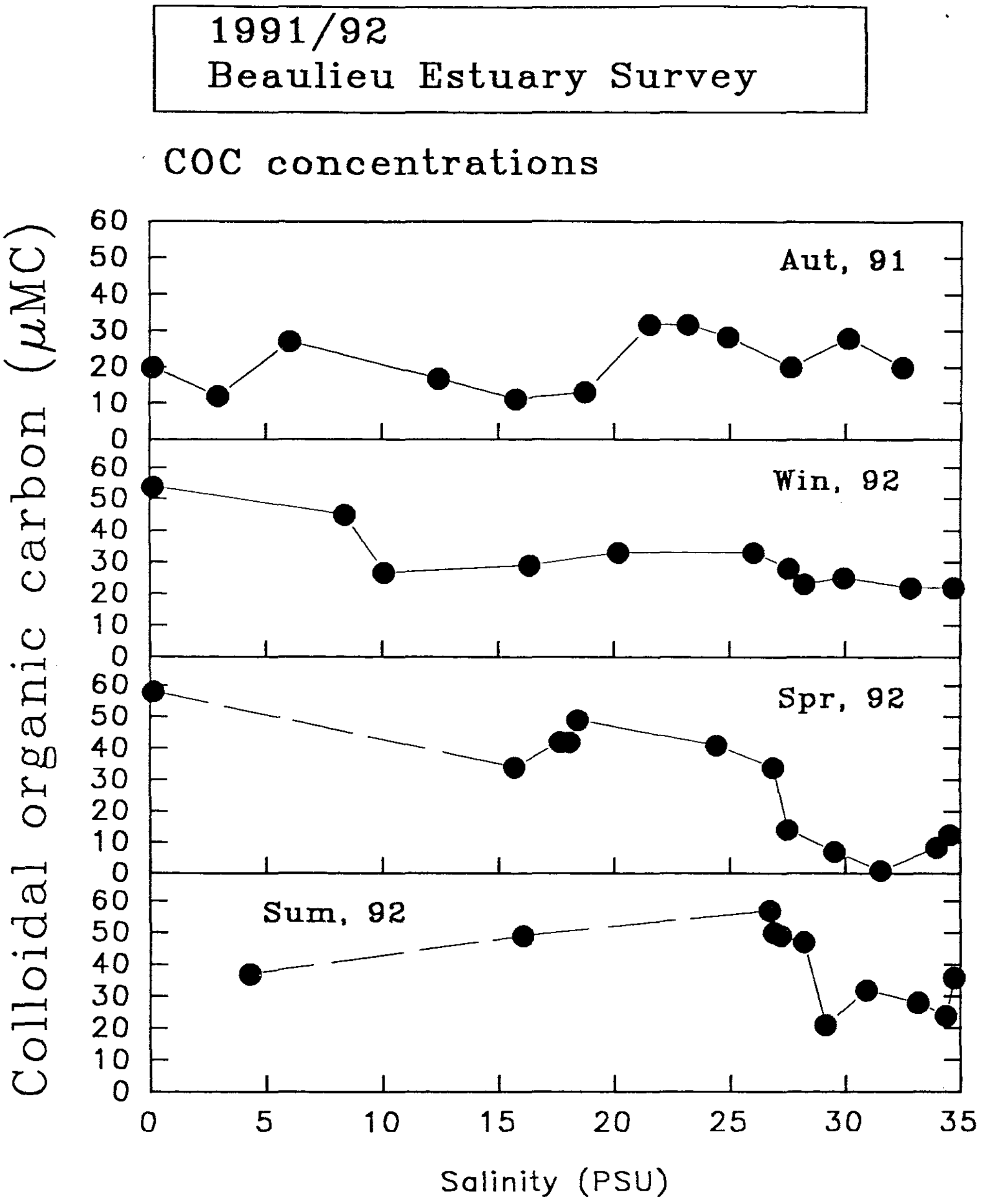


Figure 4.8. COC concentrations in the Beaulieu estuary (1991/1992) (Errors associated with these measurements are within the symbol size).

4.4.1.5. Distribution of CCOC and FCOC in the Beaulieu estuary.

The distribution of the COC fraction shows the behaviour of colloids as a whole (**Figures 4.7 and 4.8**). Further resolution of colloidal distribution is possible by considering the variation of CCOC and FCOC with salinity (**Figures 4.9 and 4.10**). For most seasons the concentrations of CCOC ranged up to 50 μM C (the detection limit is the same as the precision of the DOC measurement i.e. 4 μM C). Values as high as 300 μM C were measured during autumn 1990 and summer 1991, with values just over 75 μM C occurring in winter 1991. The FCOC levels in the estuary approach 100 μM C during autumn 1990, winter 1991 and summer 1991. However, for the other seasons concentrations of FCOC rarely exceeded 30 μM C.

The shape of the CCOC and FCOC salinity profiles showed a somewhat conservative distribution for some seasons although non-conservative distributions were seen for other seasons. Colloids are mid-way between dissolved and particulate fractions in size range and their mixing processes would be expected to be more like the dissolved phase. However, they may be subjected to processes such as flocculation or bacterial degradation and would also show different mixing characteristics. For those seasons of higher concentrations (autumn 1990, winter 1991 and summer 1991) the CCOC and FCOC levels are higher in the river and decrease towards the sea. For the other seasons of average CCOC and FCOC concentrations the levels showed similar values throughout the estuary. CCOC concentrations were higher than FCOC concentrations (apart from a few instances and the whole of winter 1991). The CCOC levels were, on the whole, not systematically higher over the whole period compared to the FCOC levels, indicating the importance of the FCOC fraction i.e. COC particles in the size range 20 to 100 nm.

There was no apparent seasonality in the CCOC and FCOC concentrations. Average end-member concentrations for the CCOC and FCOC fractions were, respectively, 88 and 57 μM C in the river and 10 and 8 μM C in the coastal waters. This also shows the importance of the FCOC fraction which, although consisting of particles in the size range 20 to 100 nm (just over 11 % of the COC size range, using the definition of colloids in this thesis i.e. size range 20 to 700 nm), accounted for 39 % of COC in the river and 45 % of COC in the seawater end-member.

This was the case in the MacKenzie river where it was found that most of the riverine organic colloidal material resided in the low nm to 200 nm size range (Whitehouse *et al.*, 1989). Wells and Goldberg (1991) also found that colloids (composed largely of organic material) less than 120 nm in size were at least three orders of magnitude more abundant than larger submicrometer particles. However, this was not the actual concentration as the colloids were determined by counting the colloid numbers on transmission electron microscope micrographs.

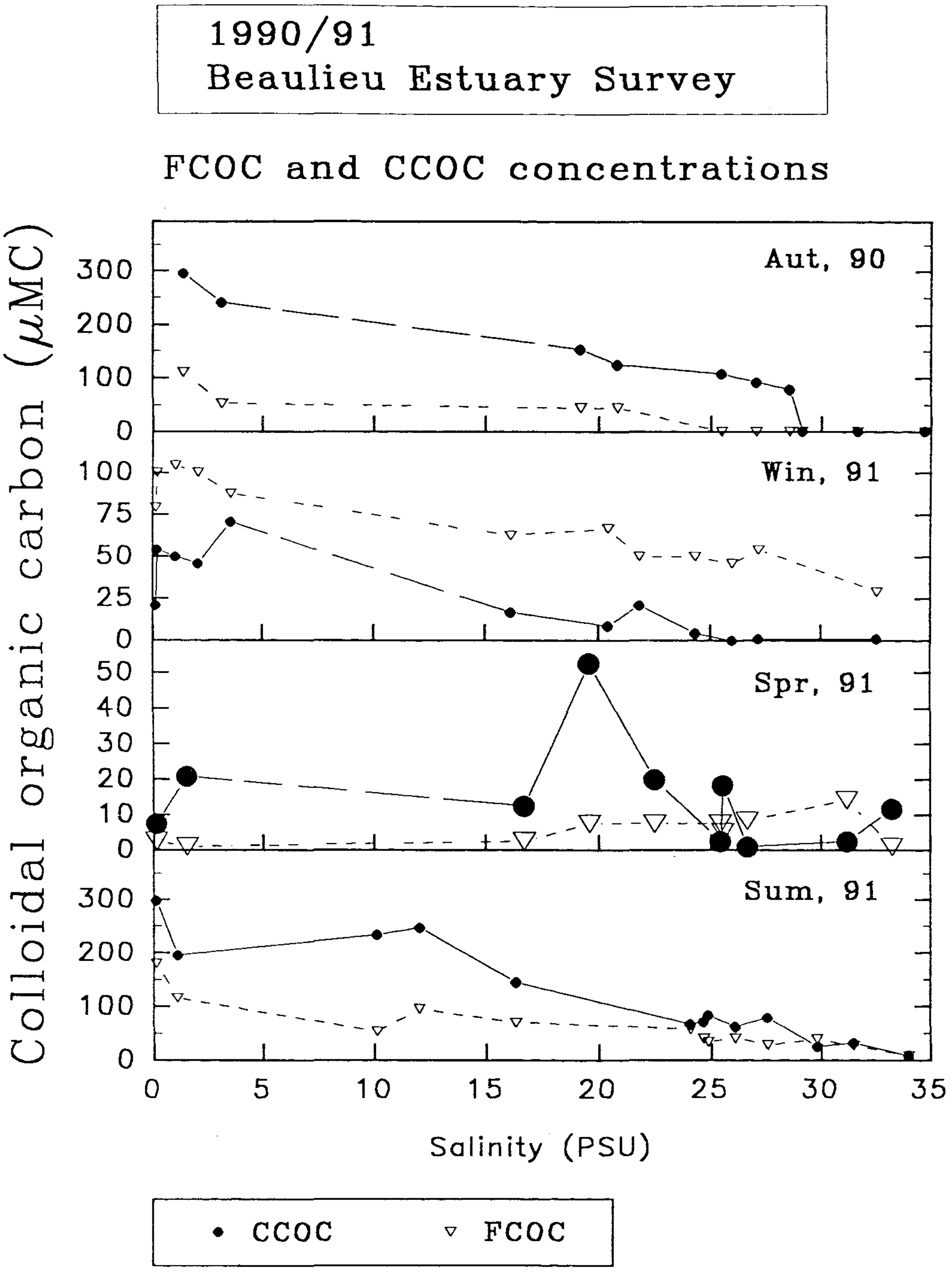


Figure 4.9. FCOC and CCOC concentration in the Beaulieu estuary (1990/1991) (Errors associated with these measurements are within the symbol size).

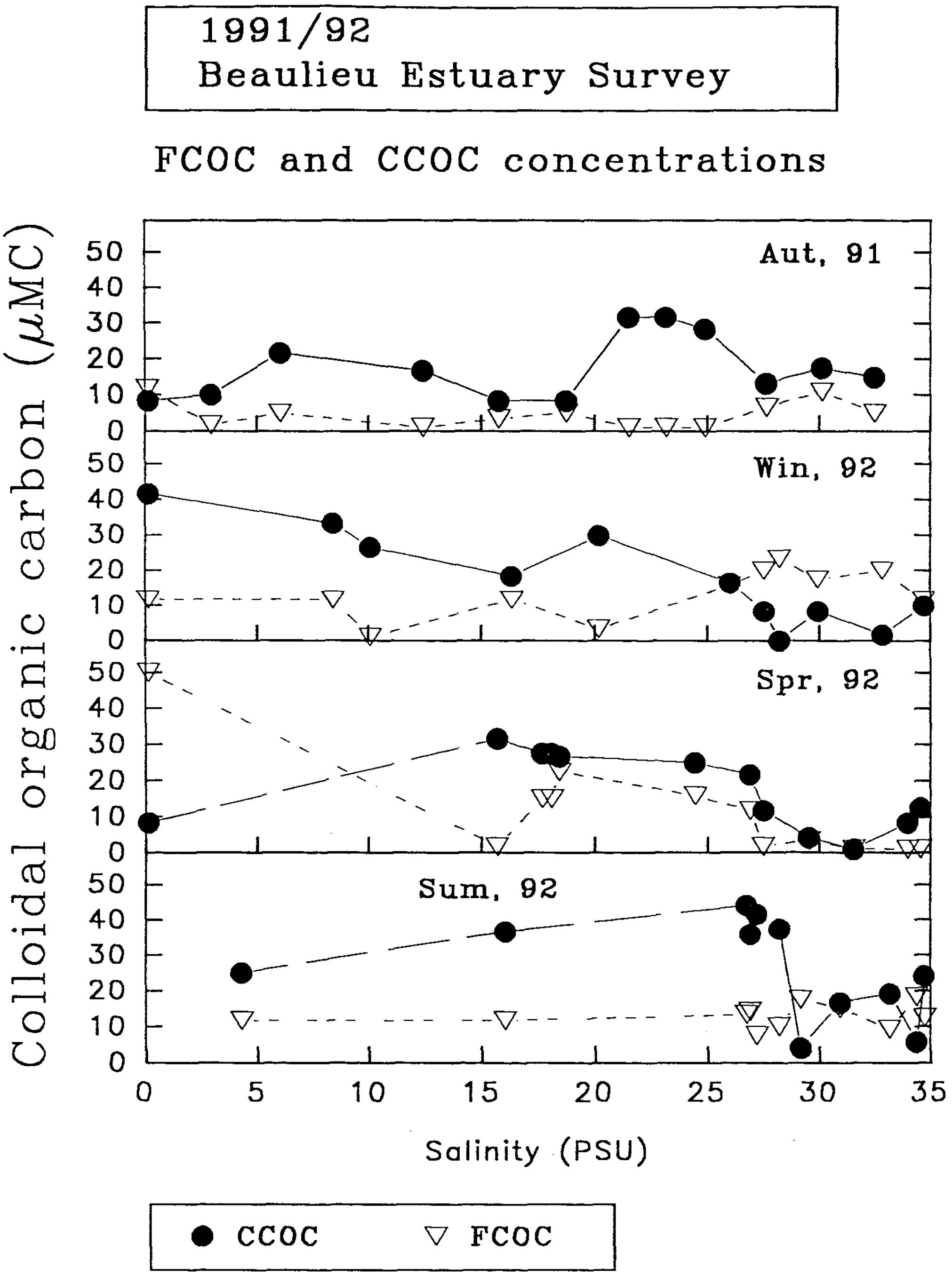


Figure 4.10. FCOC and CCOC concentration in the Beaulieu estuary (1991/1992) (Errors associated with these measurements are within the symbol size).

4.4.2. Dissolved organic carbon in the Beaulieu estuary (1990-1992).

4.4.2.1. General features of the DOC-Salinity profile.

The DOC levels (filtration through GF/F filters) versus salinity found in the Beaulieu estuary during 1991 and 1992 did not deviate markedly from a conservative mixing line, however, minor deviations from linearity can be seen in **Figures 4.11 and 4.12.** The precision of the DOC measurements (**Chapter 3.2.1**) represented in the graphs is always smaller than the symbol size and this is also the case in plots for the other parameters measured (precision given in **Section 4.2.3**).

Comparison of the DOC-salinity profile for each survey show the similarity in the mixing curves (except for summer 1991) with only a small difference in DOC concentrations. This small variation in DOC throughout the year and between years has been observed in the St. Lawrence river, Canada (Pocklington and Tan, 1987), in the Morlaix river (except for peak values in July and December) (Wafar *et al.*, 1989) and for the Elorn river (Aminot *et al.*, 1990) as well as a number of other rivers (for example see Degens *et al.*, 1983 and Degens, 1982).

Conservative mixing of DOC in the Beaulieu estuary has been found previously (Moore *et al.*, 1979). Conservative distributions have also been found in a number of other estuaries (Duursma, 1961; Laane, 1980; Mantoura and Woodward, 1983; Cauwet, 1985; El-Sayed, 1988; Aminot *et al.*, 1990;) but non-conservative distributions have also been found (Sholkovitz, 1976; Hunter, 1980; Ertel *et al.*, 1986).

It is not possible to unambiguously identify areas of non-conservative DOC behaviour either because the data points are too few and far apart (e.g. autumn 1990 and summer 1991 in the salinity range 0 to 5) or because the extent of addition or removal is within the uncertainty associated with the conservative mixing approach (e.g. summer 1992).

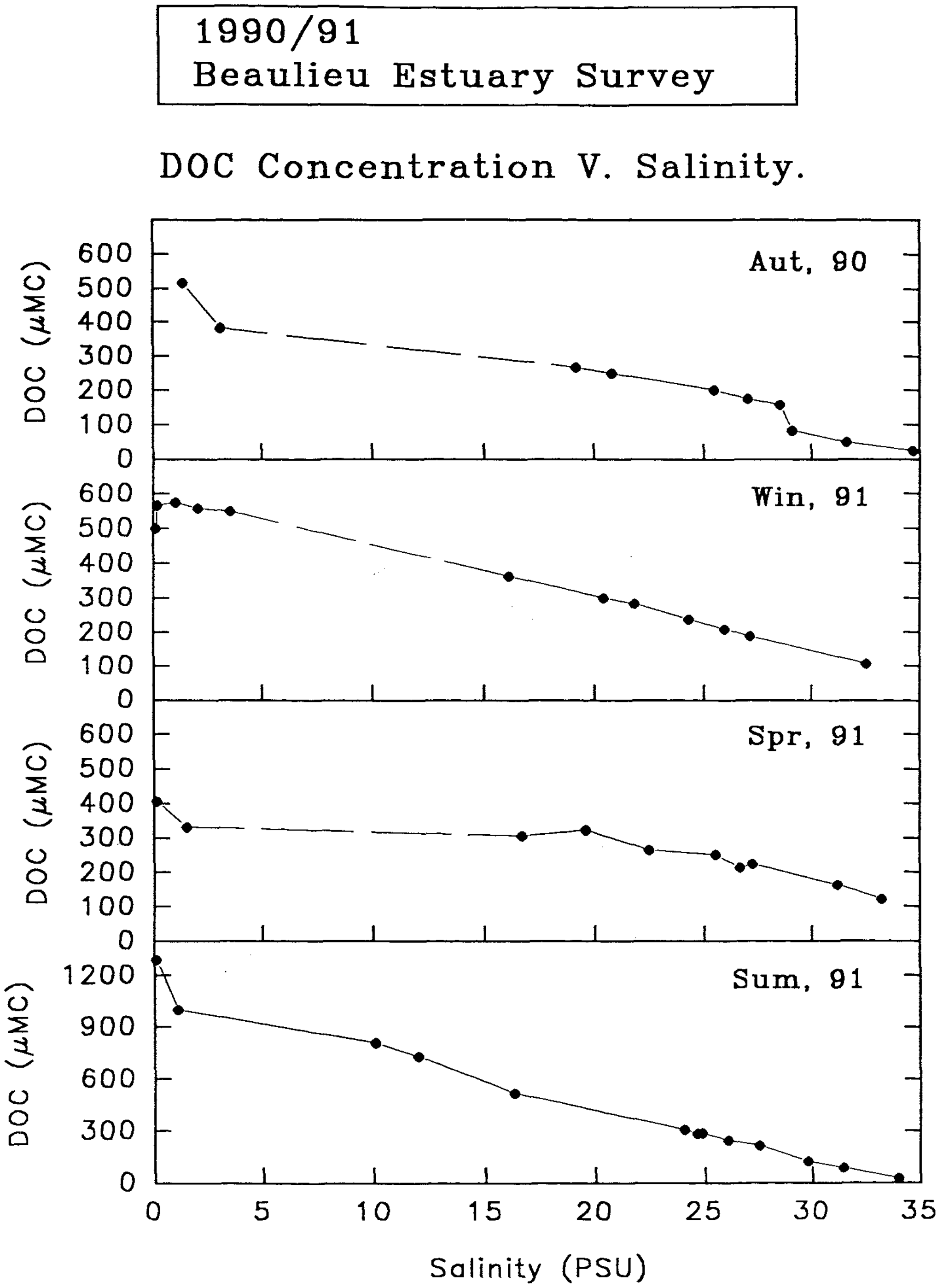


Figure 4.11. DOC concentration versus salinity in the Beaulieu estuary (1990/1991).

1991/1992
Beaulieu Estuary Survey

DOC Concentration V. Salinity.

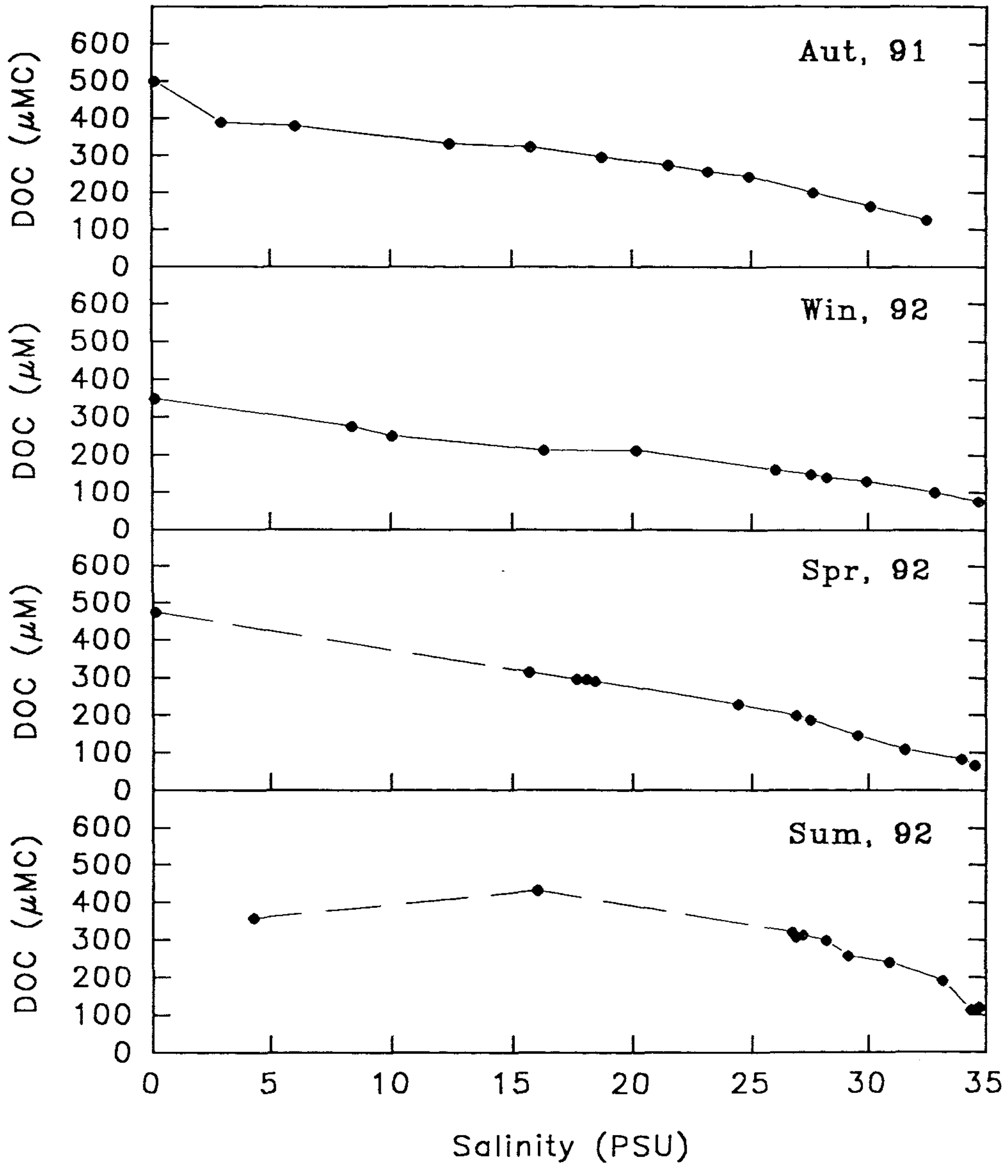


Figure 4.12. DOC concentration versus salinity in the Beaulieu estuary (1991/1992).

4.4.2.2. Variability in the end-member DOC concentrations.

The average DOC concentration found in the Beaulieu river was 576 μM C (excluding summer 1992 where there was no fresh water sample taken). This is well within the range of values of other world rivers where the DOC concentrations can vary between 42 and up to around 4330 μM C (see **Table 4.1** below).

River	DOC (μM C)	Author
North Dawes, Alaska	42	Loder and Hood (1972)
Krka, Yugoslavia	83	Cauwet (1991)
Elorn, France	183	Aminot *et al.* (1990)
St. Lawrence, Canada	313	Pocklington and Tan (1987)
Beaulieu, U.K.	400	Moore *et al.* (1979)
Beaulieu, U.K.	576	This study
Ems-Dollart, Holland	583-1500	Laane (1980)
North, U.S.A.	1250-1670	Zsolnay (1979)
Sopchoppy, U.S.A.	500-4330	Malcolm and Durum (1976)
Williamson, U.S.A.	2500	Gibbs (1979)

Table 4.1. DOC concentrations of selected rivers.

The end-member DOC concentrations in the Beaulieu estuary during 1991 and 1992 were higher in the fresh water than in the seawater for all seasons as would be expected (see **Table 4.2**). The DOC concentrations for the seawater end-member cannot easily be compared due to the varying salinities of the samples. Therefore, a first order regression analysis was performed on the DOC mixing curves and the DOC concentrations were normalized to a salinity of 33. This salinity was chosen to represent the salinity of the coastal water end-member as this was close to the average salinity (33.8) found in the coastal water samples; the precise salinity chosen is not critical as the main objective was to compare relative changes in concentration. This approach assumes conservative behaviour of DOC in the estuary, which appears to be the case.

Season	**River water DOC (μM C)**	**Coastal water DOC (μM C). Salinity in brackets**	**Coastal water DOC (μM C) at S = 33**	**Regression coefficient (%)**	**Slope**
Aut 90	(516)[a]	25 (34.7)	69	96.6	-12.9
Win 91	500	108 (32.5)	118	98.9	-13.7
Spr 91	405	122 (33.2)	176	90.7	-6.7
Sum 91	1290	27 (34.0)	17	98.8	-34.2
Aut 91	499	125 (32.5)	148	97.3	-9.5
Win 92	349	77 (34.7)	103	99.1	-7.2
Spr 92	474	67 (34.5)	107	99.1	-11.9
Sum 92	(358)[b]	120 (34.7)	214	76.6	-8.3

(a) Fresh water end-member had salinity of 1.4 and (b) 4.3.

Table 4.2. DOC concentrations for coastal water (normalized) and Beaulieu river water (1990-1992).

The mean DOC value for the coastal water (normalized to a salinity of 33) was 119 μM C. The normalized DOC concentrations varied by 197 μM C in the coastal water and the DOC concentrations varied by 941 μM C in the river water (167 μM C excluding the high DOC levels of summer 1991). The analytical data for summer 1991, which is very high relative to the other data, was carefully scrutinized and appears asccurate. The relative standard deviation (RSD) for the river water end-member was 52 % (13 % excluding summer 1991) whilst in the seawater end-member the RSD was 51 %.

4.4.2.3. Comparison of river flow with freshwater DOC concentrations in the Beaulieu river.

Comprehensive annual river flow figures for the Beaulieu river are not available for the current study. However, river flow has been measured on discrete occasions (**Table II.22**; National Rivers Authority data, D. Horne, personal communication).

Variations of DOC concentrations in river water are usually related to river flow, where a positive linear relationship characterizes rivers of low or non-existant anthropogenic input where soil leaching is the main source of DOC (Meybeck, 1981). A negative relationship is indicative of significant anthropogenic inputs as increasing river flow produces a dilution effect (Stumm and Morgan, 1981).

The DOC mixing curves for the Beaulieu estuary (**Figures 4.11 and 4.12**) show that simulation of non-conservative behaviour due to increased river discharge over short timescales are not evident in the DOC data. This is due to the fact that there are no unambiguous non-conservative mixing curves for DOC. It would also be unlikely that this process would be seen as the sampling periods were far apart and due to the short residence time of fresh water in the Beaulieu estuary (around 7 days) which would mean that such an event would occur over a short timescale.

In the Beaulieu river there was an increase in DOC concentrtion as the river flow increased (**Figure 4.13**). This relationship between river flow and DOC concentration was only tentative due to the small number of data points and because during summer 1991 and winter 1991 this trend was not followed. Also the DOC measurements and flows were not concurrent. Therefore, a more extensive survey of DOC concentration with river discharge would be needed to fully address this question.

There was no connection between DOC concentration and river discharge for the Elorn river (Aminot *et al.*, 1990) and the Morlaix river (Wafar *et al.*, 1989). Pocklington and Tan (1989) point out that whilst there was no relationship between river discharge and DOC concentration in the St. Lawrence river the mass of DOC transported was correlated with river discharge. In the Severn river, however, Mantoura and Woodward (1983) found that the concentration of DOC did covary with river flow.

Increased river flow has also been cited as the reason for high DOC values in the wet season in many rivers (Wafar *et al.*, 1989 and Martins, 1983) and for high DOC values after storm flood events (Aminot *et al.*, 1990). This is caused by initial leaching of organic matter by the increased river flow. However, the higher concentration is subsequently decreased due to the dilution of DOC with river discharge.

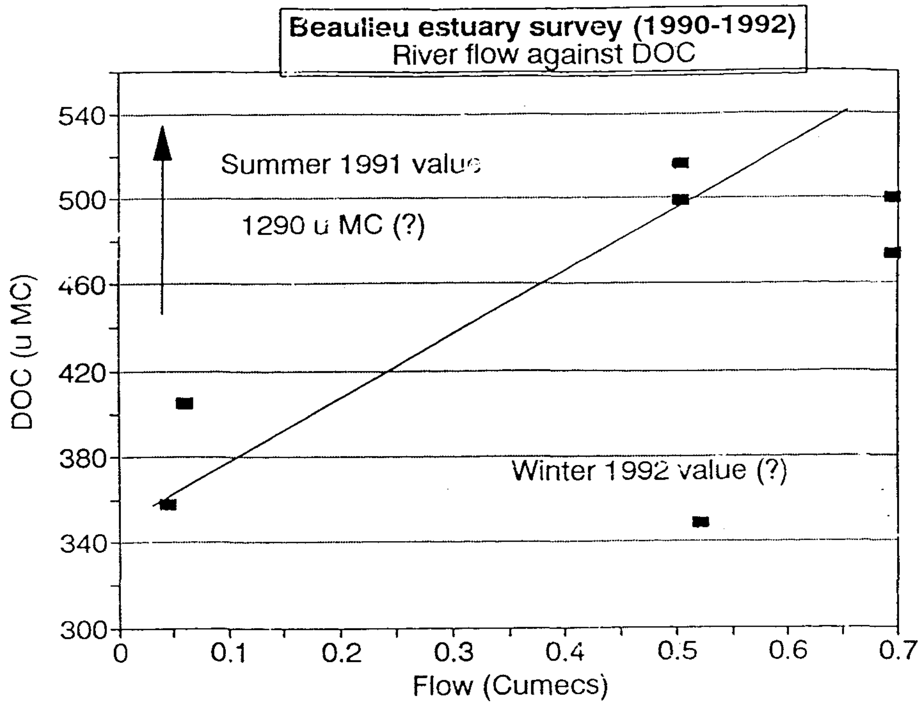


Figure 4.13. Average river flow in the Beaulieu river plotted against DOC concentrations in the river water observed in this study (1990-1992). Line represents a tentative slope of increasing DOC concentration with increasing flow rate.

4.4.2.4. Estimation of the amount of DOC entering the coastal zone from the Beaulieu river (1990-1992).

The mass of a chemical substance transported by a river is defined as the integral of the product of the chemical concentration and the water discharge:

$$M =_0\int^t CQ.dt$$

where M is the transported mass of the substance, C is the concentration, Q is the river discharge and t is the time period. Chemical flux in rivers is commonly estimated by combining flow data with chemical information derived from relatively infrequent sampling periods. This procedure, therefore, introduces uncertainties into the basic calculation, such that resulting estimates may be very unreliable (Walling and Webb, 1985). As DOC is transported conservatively from riverine sources to the coastal zone (Mantoura and Woodward, 1983), the amount of DOC entering the west Solent from the Beaulieu river can be estimated (see **Figure 4.14**).

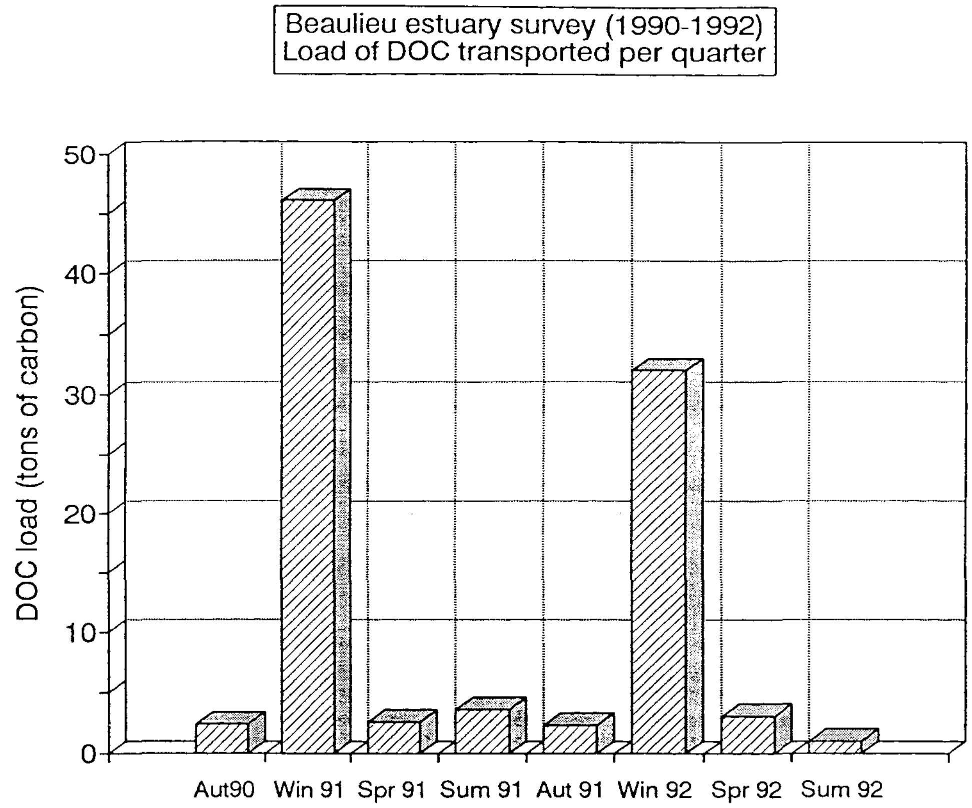


Figure 4.14. DOC load entering the west Solent from the Beaulieu river per quarter (1990-1992).

The flux of DOC in the Beaulieu river has been calculated using the DOC concentration found in the Beaulieu river at that time and an estimation of the river flow using the figures available (D. Horne, National Rivers Authority data, personal communication). Hence, the mass of DOC entering the west Solent has been estimated using the equation above for every quarter and for the whole year. It can be seen that the seasons of highest fresh water discharge (winter and spring) have the largest amount of DOC transported. This is especially true for both winter 1991 and winter 1992 which have a high mass of DOC transported into the coastal zone. For winter 1991 the amount of DOC is over ten times the amount of DOC transported for the other seasons, which suggests that the greatest input of DOC into the west Solent occurs over the winter months, during the period of highest discharge. As there was no apparent connection between river flow and

DOC concentration (this study, **Section 4.4.2.3**) in the Beaulieu river, the increased DOC transported during the winter months is probably due to the increased fresh water discharge. The amount of DOC transported annually was 54.6 tonnes of carbon for 1991 and 38.4 tonnes of carbon for 1992. The difference is consistent with varying fresh water discharge for the two years, where the fresh water discharge for 1991 was probably higher than that for 1992 (an idea of differences in the annual fresh water discharge can be seen in **Figure 4.2** of flow data for the Itchen and Test rivers).

4.4.3. Particulate organic carbon in the Beaulieu estuary (1990-1992).

During all seasons sampled POC values were higher in the freshwater and decreased as more saline waters were reached (**Figures 4.15 and 4.16**). The only exception was spring 1991, where POC values increased with higher salinities.

In the salinity range 25-35, POC levels were fairly constant at 20-50 μM C for all seasons. However, spring 1991 showed POC levels of 60-80 μM C in this salinity range. In the salinity range 0-25 POC levels varied between 20-120 μM C, apart from summer 1991 and summer 1992 where values approached 300 μM C. The average concentration of POC in the river water was 80.7 μM C whilst the seawater had an average value of 35 μM C.

Particulate organic carbon concentrations in the river water were lower during the seasons of high fluvial discharge (winter and spring; average value 43.5 μM C) compared to the seasons of low discharge (summer and autumn; average value 117.8 μM C). This situation is the opposite of that occurring in the St. Lawrence river (Pocklington and Tan, 1987) where POC concentrations were higher in the months of highest discharge and lower in the months of lowest discharge.

The average distribution of carbon between the dissolved and particulate phases was 85 % DOC in freshwater and 70 % DOC in seawater. Meybeck (1982) found that the DOC/TOC ratio for world rivers varied between 0.1 and 0.9 with an average value of 0.6. In the Beaulieu river this ratio was 0.8 (mean) which is slightly higher than the average value for world rivers and indicates low erosion rates in the Beaulieu river and consequent low POC concentrations.

Suspended particulate material concentrations were not higher in freshwater compared to coastal waters as were the DOC concentrations and the majority of POC concentrations (**Figures III.1 to III.8**). The average concentration of SPM was 6.1 mg/l in fresh water and 17.3 mg/l in seawater. Most of the SPM concentrations varied in the range 2-25 mg/l with a substantial number < 15 mg/l. The SPM concentrations for winter 1992 were typical of levels found in the Beaulieu river (around 5 mg/l) and linearly increased to high levels (around 50 mg/l) as more saline water was approached. This affected the POC as a fraction of SPM levels which were low. The high SPM levels may have been due to local sediment resuspension, although, as the levels increased steadily as higher salinities were reached it seems more likely that recent storm activity in the coastal area was responsible. The DOC concentrations were not affected and remained conservative in the estuary. This suggests that even when high levels of SPM are seen in the water column the DOC concentrations remain unaffected and DOC is transferred conservatively to the coastal zone.

The SPM concentrations found in this study are similar to those reported by Moore *et al.* (1979). They found SPM concentrations of 5 mg/l in the river Beaulieu. These rose along the estuary to maximum values of about 15 mg/l in the salinity range 10-28 and decreased at the highest salinities (29-33) to about 7 mg/l. The SPM concentrations in the Beaulieu river are low compared to other world rivers such as the MacKenzie (110.0 mg/l; Telang, 1985), Yukon (88.0 mg/l; Hood, 1983) and Lena (15.4 mg/l; Romankevich, 1984).

Suspended particulate material concentrations were lower during the seasons of high discharge (winter and spring; average value 3.8 mg/l) compared to the seasons of low discharge (summer and autumn; average value 8.5 mg/l). The freshwater had POC as a fraction of SPM values in the range 10-25 %. This is above the average for world rivers (1%; Meybeck, 1982) and is the result of a drainage basin rich in organic matter production and low SPM concentrations in the Beaulieu river.

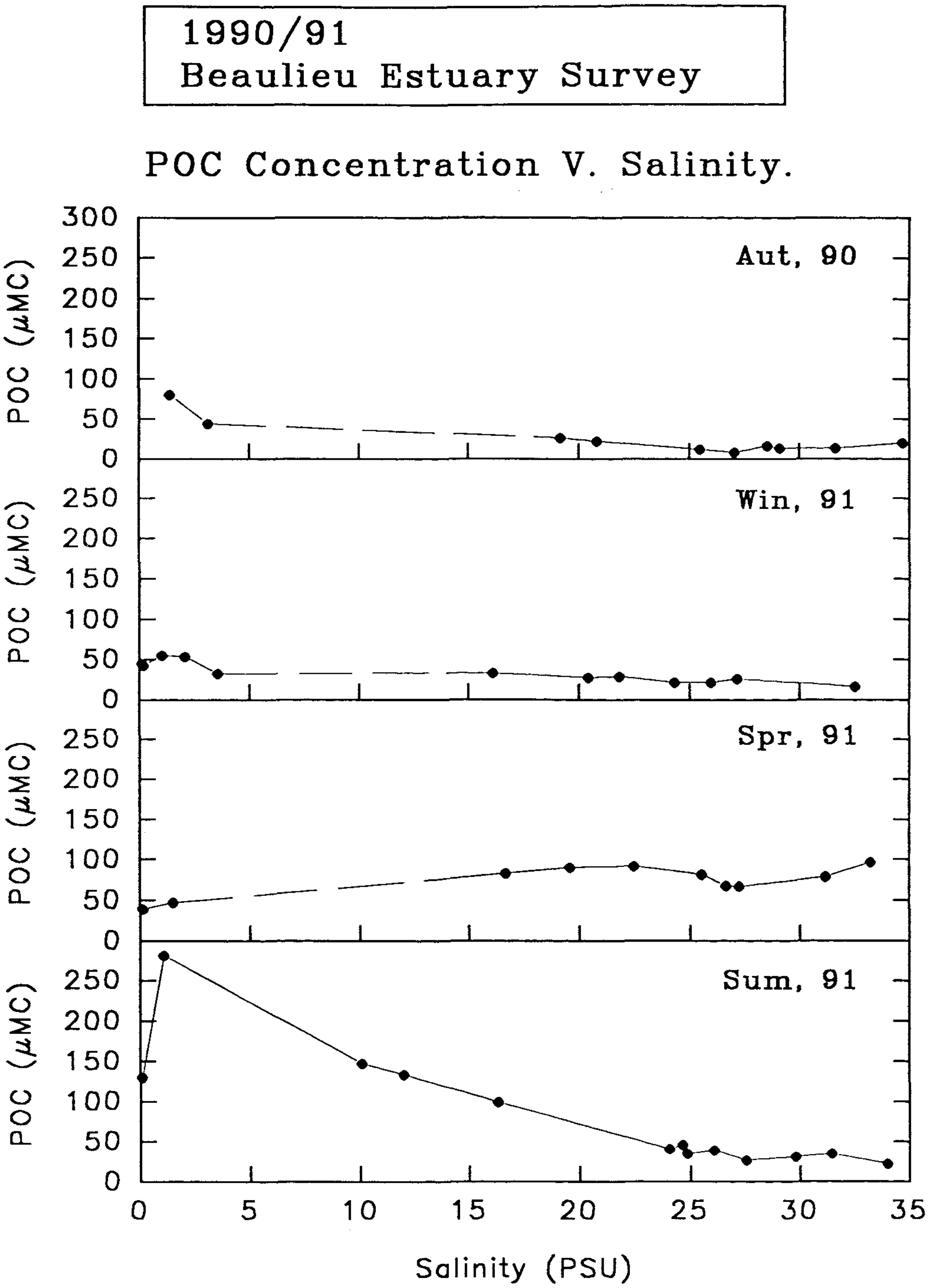


Figure 4.15. POC concentration versus salinity in the Beaulieu estuary (1991/1992).

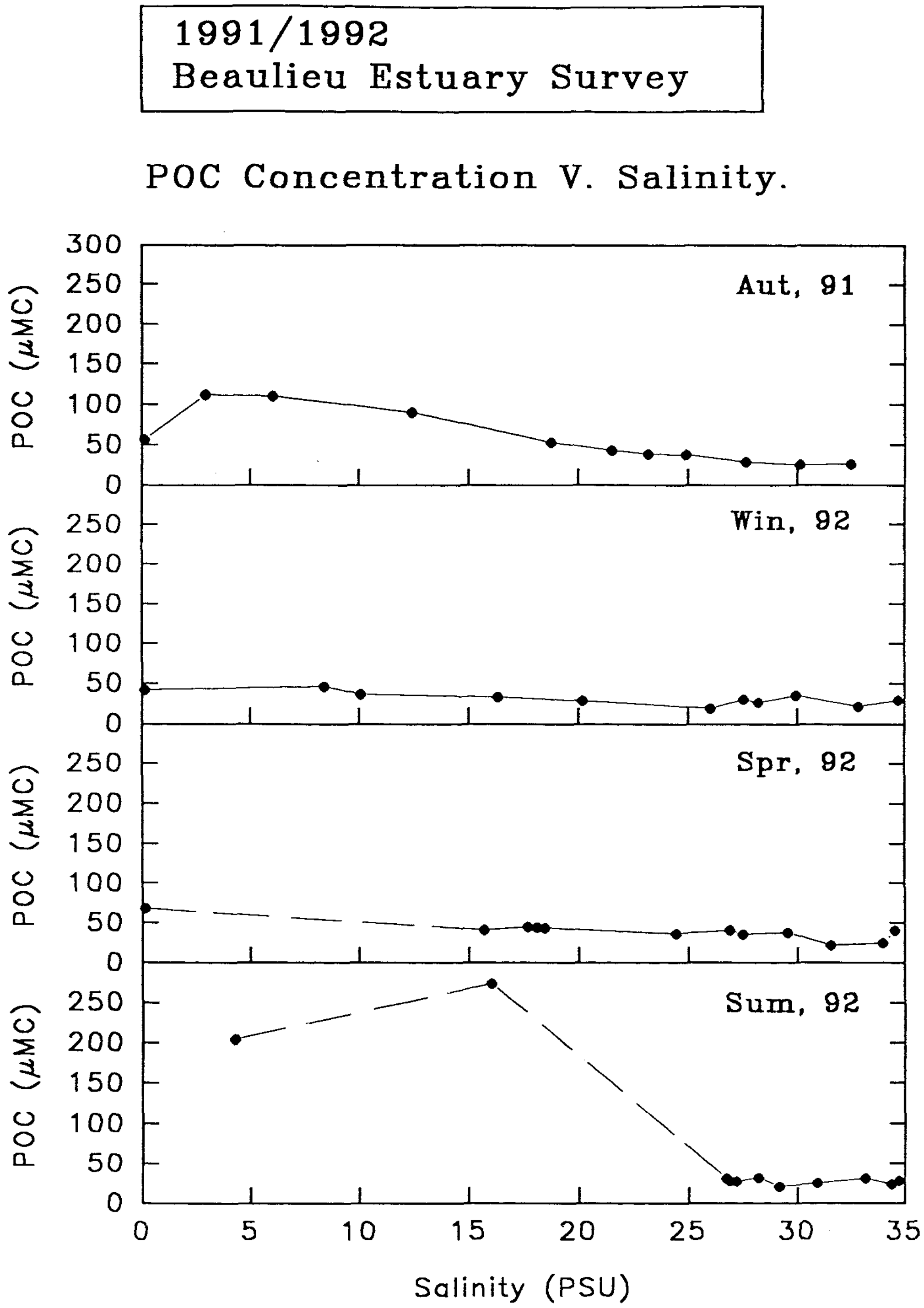


Figure 4.16. POC concentration versus salinity in the Beaulieu estuary (1991/1992).

4.5. Discussion of processes affecting the distribution of organic carbon during mixing between Beaulieu river and coastal waters (1990-1992).

Data tables which include all data generated for the Beaulieu estuary surveys (1990-1992) can be found in **Appendix II.**

The various plots of COC, DOC and POC versus salinity have been included in the text in this chapter. Plots of background parameters measured for the Beaulieu estuary surveys (1990-1992) are given in **Appendix III**; included are plots of SPM levels versus salinity (**Appendix III, Figures III.1 to III.8**) and plots of chlorophyll *a* and oxygen levels versus salinity (**Appendix III, Figures III.9 to III.15**).

4.5.1. Sources of organic carbon in the Beaulieu estuary.

Major sources for organic carbon in estuaries includes terrestrial sources and in-situ sources due to algal activity, either in fresh water or in the coastal zone. Other sources include anthropogenic, pore water and atmospheric sources.

Possible sources of DOC in the Beaulieu estuary have also been identified during bacterial degradation events and this has been discussed below (**Section 4.5.2**). An increase in primary productivity due to anthropogenic inputs of nutrients (eg nitrates and phosphates) is unlikely due to the low levels of nutrients found in the Beaulieu river which were confirmed by the chlorophyll *a* levels found (0.7 μg/l mean). Previously reported chlorophyll *a* values for an unpolluted stream, the Nanaimo river were 0.4 μg/l (Naiman and Sibert, 1978) and for a polluted river, the Morlaix were 4.7 μg/l (Wafar *et al.*, 1989). This was consistent with nutrient pollution in the Morlaix river, where average concentrations of nitrate and phosphate in the Nanaimo river were 1 and 10 % of those in the Morlaix river respectively.

As DOC behaves essentially conservatively in the Beaulieu estuary it may be assumed that the dominant source of DOC in the estuary is from terrigenous sources. Evidence for this is seen in the summer 1991 survey where notably high levels of DOC (up to 1290 μM C) were observed in the salinity range 0 to around 25 and from the summer 1992 survey where pseudo-non-conservative behaviour was potentially identified. During summer 1991 high chlorophyll *a* levels were seen in the upper and mid-estuary (salinity range 0 to around 25) which decreased as higher salinities were reached. These were high at a salinity of 2 in the pond region with high levels in mid-estuary (*ca.* 5 μg/l). High POC levels were again seen in the same salinity range. In the freshwater end-member the chlorophyll *a* level was low (0.34 μg/l) with a lower POC concentration. This suggests that the high DOC levels were caused by high primary production in the estuary, however, this was probably not the principal reason as low chlorophyll *a* levels in the freshwater end-member were recorded which suggests that the high DOC concentrations were source related and dependent on the dynamics of organic matter mobilization in the catchment area and subsequent input into the river. A dominant soil and plant organic matter source of riverine DOC has been verified in many temperate zone studies (for example see Moore *et al.*, 1979) as well as in tropical and subtropical rivers (Richey *et al.*, 1991). River water constituents may also be strongly affected by biotic processes associated with the soil and vegetation cover of a drainage basin ecosystem. Bacterial degradation and a variety of microbial transformations can strongly influence processes of mobilisation and immobilisation of nutrients and other elements in catchment systems (Walling and Webb, 1983).

River flow at this time of year was estimated to be at its lowest (by comparison with flows in the Itchen and Test rivers; **Figure 4.2**) and it has been shown that the average concentrations of dissolved constituents tend to increase with decrease in discharge (Walling and Webb, 1983). A reduced flow in the Beaulieu river may have produced greater concentrations of DOC in the water column via mobilisation of soil organic matter which were further increased by high bacterial degradation in the water column due to higher water temperatures. Therefore, the higher DOC concentrations in the river and estuary during summer 1991 were most likely to be due to higher DOC levels in the riverine source. Possible higher DOC concentrations in the estuary caused by high primary production would be masked by this. A point to note is that even during high biological activity in the water column conservative distributions for DOC were seen in the estuary.

A terrigenous DOC source may also account for the DOC mixing curve during summer 1992 where a possible addition of DOC in the salinity range 4 to 34 occurs, although deviations from conservative behaviour of less than 10 to 20 % across the whole salinity range make it difficult to resolve such behaviour (Morris, 1985). This assumption is critically dependent on one DOC concentration at a salinity of 4.2. Whilst the DOC concentration versus salinity is relatively linear at salinities greater than 15, the salinity value of 4.2 has a lower DOC concentration than anticipated if a linear relation existed for the whole estuary. This may reflect an error in the measurement of the DOC concentration at a salinity of 4.2, as such non-conservative behaviour of DOC was not seen in any other survey. The DOC concentrations above a salinity of 15 were fairly high for the lower estuary compared to other seasons. If the DOC value at S = 4.2 is incorrect (and is not taken into consideration) then the extrapolated fresh water end-member DOC concentration would be around 753 μM C (correlation coefficient = 94.7 %, slope = -17.2). This value approaches the summer 1991 value for the fresh water end-member of 1290 μM C. If the S = 4.2 DOC data point is correct then this would infer a mid-estuary input of DOC.

It may be that there were higher DOC concentrations in the water column for summer 1992 previous to the date on which the survey was carried out, the remnants of which still persisted in the lower estuary. If this were the case then higher DOC levels at the riverine source (as for summer 1991) could account for the higher DOC concentrations. High chlorophyll *a* concentrations were seen in the estuary, which showed a possible input in mid-estuary, although, due to the lack of data to better define the feature this cannot be confirmed. The concentrations of chlorophyll *a* approached 15 μg/l in mid-estuary whilst above a salinity of 25 the concentrations were high for the coastal zone at just below 5 μg/l. The POC levels in mid-estuary during summer 1992 were also very high suggesting phytoplankton activity. However, as seen for summer 1991 the high primary productivity in the estuary was unlikely to be the cause of high DOC concentrations.

Therefore, it is possible that in mid-estuary during summer 1992 pseudo non-conservative behaviour was observed due to the recent cessation of high DOC concentrations in the riverine end-member, possibly caused by an increase in river flow, where the high DOC concentrations were still present in mid-estuary. The reason for these recent high DOC concentrations is likely to be source related in the riverine end-member resulting from lower riverine discharge and possibly higher bacterial degradation rates and not due to phytoplankton activity (similar to summer 1991). Possible higher DOC concentrations caused by high primary productivity would have been masked by this greater DOC signal.

In the coastal zone a small increase in COC as a fraction of TDOC was observed for a number of seasons above a salinity of 30. This increase was primarily due to the CCOC fraction which also showed an increase in concentration in the coastal zone. This indicates that the coastal end-member has a higher ratio of COC to DOC and probably reflects coastal COC including material of *in-situ* marine and other non-estuarine origins, which is different in composition to riverine COC and has not been subjected to removal processes such as aggregation. High phytoplankton activity as seen in the estuary during spring 1991 did not necessarily produce higher concentrations of COC which had average values. This was also the case during a period with high SPM values in the estuary (winter 1992) which had little affect on increasing the COC values.

4.5.2. Degradation of organic carbon due to bacterial activity.

Bacterial action is a key process in the conversion of POC to dissolved organic forms and carbon dioxide. Therefore, when high concentrations of POC exist in the water column, high bacterial activity is also likely. In the Loire estuary, El-Sayed (1988) estimated the maximum production, by various processes, of DOC in the turbid zone would represent 6-12 % of the riverine flux. Microbial decay occurs over varying timescales where simple low molecular weight compounds (such as carbohydrates) are decomposed by heterotrophic bacteria in a matter of hours. High molecular weight dissolved organic substances decompose on a range of timescales from days to months (Saunders, 1976). Laane (1982) found that detritus from phytoplankton is rapidly degraded whilst POC from terrestrial sources, which includes soil and plant organic matter, is degraded more slowly. Highly substituted aromatic ring structures found in tannin and lignin residues are also resistant to biological degradation. However, in the Elorn estuary it was reported that any transformation process in the area of maximum turbidity would be negligible as the residence time of freshwater in the estuary was relatively short (4 days). Therefore, Aminot *et al.* (1990) concluded that bacterial degradation of DOC was of little importance during mixing in the Elorn estuary. This situation would also be expected to occur in the Beaulieu estuary where short fresh water residence times are found (around 7 days) and conservative DOC mixing curves are generally observed.

Degradation due to anthropogenic inputs may occur due to a small anthropogenic input which occurs in the estuary from the Beaulieu village sewage treatment works. This may have contributed to the depletion of oxygen in this region, however, the consented discharge is small (216 $m^3 day^{-1}$; National Rivers Authority data, D. Horne, personal communication). Five day biochemical oxygen demand (BOD) figures are available in the river (Hartford Bridge) and estuary

(Beaulieu village sewage treatment works and Baileys Hard; see map in **Figure 4.1**; National Rivers Authority data, D. Horne, personal communication). During all seasons the BOD in the river was around 30 μM oxygen and at Baileys Hard it was around 60 μM oxygen. The BOD of the sewage input during winter 1991 was around 250 μM oxygen and for summer 1991 it was around 375 μM oxygen. Higher BOD during the summer was probably due to higher temperatures, where a 10^0C rise in temperature roughly doubles the bacterial degradation rate (Lancelot and Billen, 1985).

Seasons which show a drop in oxygen concentration in mid-estuary (where the sewage input occurs) are winter 1991, spring 1991 and summer 1991. During spring 1991 and summer 1991 the chlorophyll *a* values as a fraction of total phaeo-pigments were high indicating that the oxygen drop was not caused by degradation of phytoplankton. The oxygen depletion for summer 1991 was around 60 μM oxygen. The sewage input (BOD 375 μM oxygen) into the estuary is around 8 % of the river flow, therefore, the potential oxygen depletion caused by this input is around 60 μM oxygen (8 % of 375 μM oxygen + 92 % of 30 μM oxygen from the river). However, a number of assumptions have been made in this calculation, including a linear BOD dilution curve, a flushing time of the estuary around the same time as that for the BOD determination, a well mixed estuary, the BOD is attributable only to sewage degradation (this may be assumed because DOC is conservative in the estuary); gas exchange between air and water is assumed to be minimal and any oxygen input from phytoplankton is ignored. Despite these limitations it can be seen that the oxygen depletion occurring during summer 1991 (61 μM oxygen) is consistent with sewage input. This was also the case for spring 1991. Higher temperatures in the water column (21^0C for summer 1991) and a lower river flow (sewage input is 8 % of river flow for summer 1991) would make bacterial degradation more efficient in these seasons. The potential oxygen depletion caused by the sewage input during winter 1991 is around 30 μM oxygen (0.2 % of 250 μM oxygen + 99.8 % of 30 μM oxygen from the river). This suggests that the oxygen depletion caused during winter 1991 (130 μM oxygen) was not caused by the sewage input. Higher flows in the river (sewage input is 0.2 % of river flow) would cause a greater dilution effect and lower temperatures in the water column (3^0C) would not make bacterial degradation as effective. The reason for this oxygen depletion was most likely to be advection of low oxygen content waters, high turbidity and possible sediment resuspension as suggested in **Section 4.5.5**.

Degradation events after a phytoplankton bloom can cause a decrease in oxygen concentrations. This has been observed in the Test estuary, where the collapse of the bloom lowered the oxygen concentration by 10 % saturation (Hugman and Mallet, 1979). Also in Southampton Water a sharp decline in oxygen concentration from 135 to 105 % was associated with the collapse of a bloom event (Kifle, 1989). For winter 1991 the increase in DOC in the salinity range 0 to 3 coincides with a decrease in chlorophyll *a* and chlorophyll *a* as a fraction of total pigments and a small decrease in oxygen concentration. Therefore, it is likely that the increase in DOC concentration is caused by degradation of organic matter, possibly from an old phytoplankton bloom event, as seen by the decrease in chlorophyll *a* as a fraction of total pigments. Chlorophyll degradation products (phaeo-pigments) may form a significant fraction of the total plant pigment in the water column. They result essentially from the digestion process of zooplankton and may be thought of as "dead chlorophyll" (Parsons *et al.*, 1984). Therefore, the presence of phaeo-pigments in the water column indicates old phytoplankton material. In this salinity range there is also a decrease in POC (mass/volume) and SPM, and an increase in POC as a fraction of SPM suggesting that the DOC increase was a POC to DOC conversion process. This supports the concept of the degradation of an old bloom event.

The high COC (both CCOC and FCOC) concentrations seen during summer 1991 coincided with high DOC concentrations in the estuary and also possible degradation events throughout the salinity range. This also occurs during winter 1991 at a salinity of 0 to 3, where high DOC levels were seen possibly associated with a degradation event, producing an input of both CCOC and FCOC. This suggests that degradation events in the estuary give rise to high levels of COC. Although the degradation event during winter 1991 seemed to occur at lower salinities, higher COC concentrations occurred throughout the estuary indicating the likely importance of this event. In this case the degradation event produced greater quantities of FCOC than CCOC (the only time that this occurred). This may have been due to the fact that the degradation event was possibly well in progress.

The apparent non-conservative DOC distribution seen during summer 1992 in the salinity range 4 to 34 was ascribed to the recent cessation of source related high DOC concentrations, the remnants of which still remained in mid-estuary. Possible high degradation rates were also inferred which would produce higher COC levels in mid-estuary. Evidence for this was seen for the CCOC fraction which increased its concentration in mid-estuary (up to a salinity of about 27) to around 50 μM C.

No effective explanation for high COC concentrations during autumn 1990 could be found, however, this phenomenon could have been due to high degradation rates in the water column as observed during summer 1991. These high degradation rates could have been caused, for example, by degradation of a phytoplankton bloom event, or by higher fresh water flows which would release soil organic matter into the water column.

4.5.3. Primary production and organic carbon in the Beaulieu estuary.

Phytoplankton activity can have an important affect on the forms of organic carbon in the water column and high algal activity gives rise to increased POC concentrations where DOC levels increase about 1 to 2 months after the phytoplankton bloom (Wafar *et al.*, 1984). Generally estuaries show reduced levels of primary production with associated *in situ* DOC production situated in the riverine and most saline part of the estuary (Aminot *et al.*, 1990). This is due to the low light penetration caused by elevated concentrations of SPM limiting primary production. High phytoplankton activity has been observed at Calshot, Southampton Water (just outside the mouth of the Beaulieu estuary) where the timing of the seasonal peak of chlorophyll *a* has varied from May, to June to August (Kifle, 1989). Differences in the frequency of sampling or interannual differences in the temperature and solar radiation pattern (which affects chlorophyll *a* concentration) may have been responsible for the observed difference in the timing of the annual chlorophyll *a* maximum (Kifle, 1989). The seasonal cycle of chlorophyll *a* concentration at Calshot showed winter minimum and spring maximum values. A tentative pattern can be seen between autumn/winter and spring/summer where chlorophyll *a* levels in the Beaulieu estuary were higher during spring/summer. A seasonal cycle for the abundance of phytoplankton, with maximum values during spring and summer and low values for the rest of the year has been reported for other north temperate estuaries such as Newport estuary (Thayer, 1971), Lower Chesapeake Bay (Patten, 1963) and Waccassa estuary (Putnam, 1966).

High chlorophyll *a* levels were observed in the coastal zone of the Beaulieu estuary in spring 1991 where chlorophyll *a* levels approached 7 μg/l. High chlorophyll *a* levels were not seen during the other seasons, this being due to the variation in timing of the bloom at Calshot in Southampton Water, which occurred sometime in the period from May to August. Previously reported values of chlorophyll *a* at Calshot, have varied around 3 μg/l with values as low as 1.25 μg/l and as high as 20 μg/l measured during the year (Kifle, 1989).

High chlorophyll *a* (around 5 μg/l) and oxygen levels were also observed throughout the whole estuary during spring 1991. Increasing POC levels as higher salinities were reached supported the view that there was a high algal biomass (this did not happen on any other occasion). An increase in SPM was also observed on increasing salinity which was rather puzzling as this would tend to have an adverse affect on phytoplankton activity. However, as the SPM levels in the Beaulieu estuary were lower than other estuaries (see **Section 4.4.3**) the overall effect of SPM limiting primary productivity in the Beaulieu estuary may not be important.

4.5.4. Physical removal processes occurring in the Beaulieu estuary.

It has been demonstrated that dissolved humic acids are lost from solution during mixing with seawater when using a combination of laboratory mixing experiments and field measurements (Eckert and Sholkovitz, 1976; Sholkovitz, 1976; Sholkovitz *et al.*, 1978). Sholkovitz and Copland (1981) have shown that the extent of humic acid flocculation from river water varies with the salt content and with the pH. The nature of the precipitated material would also vary with changing salt or acidity.

The high molecular weight fraction of the humic substances decreased with increasing salinity (Preston, 1979; Gillam and Riley, 1981) and it was unclear whether this is due to loss of terrigenous material because of aggregation or simply dilution of terrigenous colloidal material by marine organics produced *in-situ*. Fox (1983) and Sharp *et al.* (1982) have shown that riverine humic acids can behave conservatively in mixing experiments combining certain river waters with seawater i.e. there was no evident aggregation. It was reported that the humic acid content of river DOC was 19 % whilst the concentration of COC was 33 %. The amount of organic matter precipitated by seawater was 16 % indicating that only a part of the COC fraction and not even all of the humic substances were precipitated. This result was found for rivers where the field data indicated a loss of dissolved humic acids with increasing salinity. An important implication of this study is that salt induced aggregation may not be the mechanism by which all riverine humic acids are lost from estuarine waters and that other chemical or biological reactions are responsible.

The mechanisms of the aggregation process induced by seawater is not well understood, however the high affinity of humic carboxylate groups for divalent ions (e.g. Mantoura *et al.*, 1978) suggests a strong role of charge neutralization, converting relatively hydrophillic colloids or molecules into relatively hydrophobic ones (Ong and Bisque, 1968; Eckert and Sholkovitz, 1976). Subsequent to this charge neutralization, Van der Waals coagulation may be the driving force responsible for flocculation. Ion-dipole interactions (Theng, 1979) in which Ca or Mg ions bridge and connect functional groups such as carboxylates, may also play a role. Simple flocculation of insoluble Ca and Mg humates (Boyle *et al.*, 1977; Preston 1979) is perhaps an equivalent process. Hydrophobic interactions between the organic materials do not appear to be important (Mayer, 1982; Mantoura and Woodward, 1983). Aggregation of dissolved humic substances can also occur with particulate materials (Preston and Riley, 1982) where adsorption onto kaolinite, montmorillonite and illite increases with salinity and dissolved humic substance concentration. Hydrogen bond formation has been used to explain the adsorption of neutral and other polymers on silica whilst OH^- and COO^- groups are also adsorbed on various minerals.

There seems to be limited aggregation of DOC into particulate forms with increasing salinity in the Beaulieu estuary. On visual inspection of the data a possible localised DOC removal process occurs at low salinity (0 to 5) during the seasons spring 1991, summer 1991 and autumn 1991. The extent of removal can be calculated by performing a first order regression analysis on the DOC versus salinity mixing curves without including the fresh water end-member data point (**Table 4.3**). The Y-intercept would then give the fresh water DOC concentration minus the DOC removed in the low salinity range. The difference between the freshwater DOC concentration and the DOC concentration of the Y-intercept would represent the removed DOC.

Season	River DOC level (A) (μM C)	River DOC level (μM C) with 1st order regression (B)	Error (± 1 SD)	Slope	Regression coefficient (%)	Removed DOC (μM C) (A-B)
Spr 91	405	389	115	-6.4	85.1	16
Sum 91	1290	1070	60	-31.2	99.6	220
Aut 91	499	441	40	-8.7	97.2	58

Table 4.3. Estimation of the DOC removed at low salinity.

Estimated DOC removal at low salinity is between 16 and 220 μM C. The errors associated with the Y-intercept are larger than the removed DOC for spring 1991 and is about the same for autumn 1991, therefore, it is not possible to confirm the potential removal of DOC at low salinities for these seasons. The error associated with the Y-intercept for summer 1991 is smaller than the removed DOC where the removal of DOC is 17 % of the riverine DOC. This, therefore, potentially represents a significant removal of DOC in the estuary. Nevertheless, this conclusion must be treated with caution as only one season shows clear evidence of this DOC removal process at low salinity. The proportion of humic acids to fulvic acids in the Beaulieu river (*ca*. 18 % humic acid; Moore *et al*., 1979) is slightly higher than that found by Mantoura and Woodward in the Severn (*ca*. 10 % humic acid). This indicates that in the Beaulieu estuary a higher proportion of the DOC would flocculate on increasing salinity. Therefore, it would be expected that the removal of a certain fraction of DOC in the Beaulieu estuary would be by aggregation of the humic acid content. It has been shown previously (Holliday and Liss, 1976) that removal of dissolved iron occurs in the Beaulieu estuary in the low salinity range (0 to 15). Proposed mechanisms included precipitation of iron as hydrous oxides onto particles or flocculation of organic material. This would remove the iron as much of the dissolved iron in the Beaulieu river was expected to be in the form of organo-iron complexes. However, Moore *et al*. (1979) found that DOC behaved conservatively in the Beaulieu estuary. They also reported that non-lattice held iron was removed in the same salinity range. In the present study removal of DOC at low salinity, which accounts for around 12 % of the total DOC concentration, cannot be unambiguously assumed; part of the reason for this is due to the small number of data points in this salinity range and the fact that such an extent of removal lies within the uncertainties of the conservative mixing approach (Morris, 1985). The most likely mechanism of DOC removal would be flocculation or adsorption onto particles of the high molecular weight fraction which accounts for 18 % of the DOC in the Beaulieu estuary (Moore *et al*., 1979). This conclusion was also reached by Moore *et al*. (1979) who supposed that iron was removed from the water column along with organic colloids. Changes in physical-chemical variables, for example pH (6.5 in Beaulieu river water and 7.9 at a salintiy of 29; Holliday and Liss, 1976) and ionic strength, would probably effect these changes in the DOC concentration.

The DOC mixing curves for autumn 1990 and summer 1991 that showed evidence of aggregation at low salinities (0 to 5) also showed high colloidal concentrations in the water column. As the colloidal concentrations were high any aggregation of colloidal material would have a greater affect on the DOC concentrations, resulting in the DOC mixing curve showing evidence of aggregation as was the case.

4.5.5. The effect of mixing processes on the distribution of organic carbon in the Beaulieu estuary.

Mixing processes in the estuary can give rise to changes in the concentration of a number of constituents, for example, by the mixing of waters of different origin. During the winter 1991 survey a large fluctuating decrease in the oxygen concentration (around 60 μM) (**Figure III.9**) can be seen in the salinity range 20-25 which is mirrored by the chlorophyll *a* level as a fraction of total pigments (the chlorophyll *a* value as a fraction of total pigments is the amount of chlorophyll *a* compared to the total plant pigments, in this case chlorophyll *a* and phaeo-pigments; see **Figures III.9 to III.15**). This distribution also occurred at a salinity of 22 during spring 1991 and above a salinity of 25 during summer 1992. The decrease in oxygen concentrations is consistent with degradation of POC in the water column. However, there is no similar increase in DOC concentrations as has been observed as a result of bacterial degradation of POM in the turbidity maximum (Cauwet, 1991).

Input of sediment pore waters with a low oxygen content would be unlikely to cause the oxygen depletion as diffusion rates would be small. A possibility is advection of low oxygen content waters. The SPM levels and POC concentrations also show a small fluctuation such that as the SPM level decreases or increases so do the oxygen levels, chlorophyll *a* levels as a fraction of total pigments and to a smaller extent the POC concentrations. Accumulation and partial transformation of POC in the area of the SPM maximum has been observed by several authors (for example see Cauwet 1985). Therefore, it would seem likely that the fluctuation in oxygen concentrations was caused by advection of low oxygen content waters which did not affect the DOC concentrations. High turbidity may have been associated with this event causing sediment resuspension which would cause the fluctuations in SPM and POC. No definite evidence for a turbidity maximum was seen in the SPM levels and for many seasons no variation in oxygen levels occurred suggesting that possible high turbidity in the water column did not occur for every season or it was present during other conditions in the estuary. Aminot *et al.* (1990) found that a turbidity maximum was observed only during low tide in the Elorn and was situated in the upper estuary. However, river flow and tidal amplitude influence both the position of the turbidity maximum and the SPM concentration, therefore, the SPM data for the Beaulieu estuary does not preclude that a turbidity maximum existed.

During autumn 1990 there is evidence for a localized DOC removal process at a salinity of 29 whilst the POC concentration remains constant (mass/volume). There is a fluctuation in the SPM level which shows a sudden increase followed by a decrease at this salinity back to the previous levels. This indicates that there is high turbidity in this region. The mechanism of DOC removal is unlikely to be a DOC to POC conversion process even though there is a small increase in the POC concentrations. However, a DOC to POC conversion process may not be detectable anyway due to the variable nature of POC levels in the estuary. If the process were actually aggregation then during this season there should have been a large amount of colloidal material in the estuary, as flocculation of this material would have produced a decrease in the DOC concentrations. Therefore, it is difficult to determine what affects the change in DOC concentrations with the available data. It may be due to lateral advection of lower DOC concentration waters from the coatal zone. This is supported by the sudden change in SPM levels indicating high turbidity.

An apparent removal process for the COC fraction in the salinity range 20 to 30 is evident for all seasons (except summer 1991). By considering the distributions of the CCOC and FCOC fractions it can be seen that this is primarily due to the CCOC fraction. There was some evidence for a concurrent increase in the POC concentrations (during autumn 1990, winter 1991 and winter 1992) suggesting that aggregation was occurring, however, POC can fluctuate widely in the estuary, for example due to high phytoplankton activity or by sediment resuspension events. This was also seen in the COC as a fraction of POC levels which showed a reduction at this salinity (see for example summer 1992). Whilst the FCOC fraction showed some signs of this removal process (e.g. autumn 1990 and spring 1992) the evidence was not as strong and for a number of seasons (for example summer 1992) the FCOC concentration actually increased as the CCOC concentration decreased. Therefore, if the removal process was aggregation of the CCOC fraction the increase in FCOC concentration could be explained by advection of coastal waters. If this was the case then the decrease in CCOC concentration could also have been caused by advection of coastal waters. Another possible mechanism occurring is a CCOC to FCOC conversion process, however, as these colloids would be hydrophobic in nature dis-aggregation would be unlikely. The reduction in DOC levels at a salinity of 29 is also accompanied by decreases in COC as a fraction of both DOC and POC. This suggests that the process was aggregation of COC, although the reduction seen could also have been caused by advection of low COC content waters from the coastal zone. It may be that the decrease in CCOC seen occurred due to input of low CCOC content waters from the sediments, however, this seems unlikely because the sediments are likely to be rich in these materials. Also in interstitial waters of marine sediments, some 50-90 % of the DOC may be colloidal (e.g. Krom and Sholkovitz, 1977; Orem *et al.*, 1986).

Possible removal of DOC and COC (during autumn 1990 and summer 1991) has been identified at low salinities (in the salinity range 0 to 5) and therefore it would seem unlikely that a further removal process would occur at higher salinities. Hence, it is likely that advection of coastal waters caused these changes in COC concentrations at higher salinities (in the salinity range 20 to 30). This may have been accompanied by high turbidity and sediment resuspension in the water column which has been identified during certain seasons in the same salinity range. Aggregation of the CCOC fraction at these higher salinities, if it did occur, would be an interesting and novel process.

A mid-salinity increase for the CCOC fraction in the salinity range 15-25 occurred during some seasons (for example see autumn 1991 and spring 1992). The FCOC fraction showed some evidence of this (for example during spring 1992). This process did not necessarily coincide with high phytoplankton activity as the chlorophyll *a* levels varied and were not always high indicating that the increase in CCOC concentration were not caused by algal activity. The increase in CCOC levels in the mid-salinity range occurred just before the removal process for the CCOC fraction in the salinity range 25-30 and represented an initial increase in CCOC concentration that was most likely to be due to high turbidity in the water column identified previously. This may have introduced colloidal material into the water column from sedimentary resuspension (see discussion above) or it may simply have represented riverborne re-circulated material.

Chapter 5:

Inter-annual investigation of organic carbon isotopes in the Beaulieu estuary.

5.1. Introduction.

The investigation of organic carbon in the Beaulieu estuary also included carbon isotope studies. The stable carbon isotope (^{13}C) and ^{14}C isotopic compositions have been determined in an effort to further understand the cycling of the various fractions of organic carbon in the estuarine environment, especially the sources and sinks for these fractions and transformation processes between the soluble and particulate phases.

When used in conjunction with each other, $\Delta^{14}C$ and $\delta^{13}C$ can definitively establish the differences among marine, stream and soil organic matter (Malcolm, 1990). Neither $\Delta^{14}C$ nor $\delta^{13}C$ alone can differentiate between organic matter from all three environments. For example, the age of DOC in deep ocean water has been established to be between 3000 to 6000 years before present (47 to 69 % Modern; %M) with $\delta^{13}C$ values of -22 to -23 ‰ (Stuermer and Harvey, 1974; Williams and Druffel, 1987). All stream humic substances and POC analyzed to date have modern $\Delta^{14}C$ values (around 100 %M) with $\delta^{13}C$ values between -25 and -28 ‰ (Hedges *et al.*, 1986). The $\Delta^{14}C$ values for soil humic substances vary almost over the whole range (0 to 100 %M) whilst the $\delta^{13}C$ values range from -25 to -31 ‰ (O'Brien *et al.*, 1981). For example using $\Delta^{14}C$ and $\delta^{13}C$ values in conjunction it would be possible to differentiate between organic matter derived from peat sources, from terrestrial modern plant sources or from marine phytoplankton sources.

The units used for measuring $\delta^{13}C$ signatures and $\Delta^{14}C$ activities are given in **Appendix I.**

5.2. $\delta^{13}C$ signatures as a tracer for organic matter in natural waters.

5.2.1. Introduction.

During photosynthesis, plants preferentially assimilate ^{12}C over ^{13}C (Craig, 1953). A significant difference in the $\delta^{13}C$ value up to around 7 ‰ is also expected between land plants and marine plants, as land plants fix CO_2 (-7 ‰) in the atmosphere, whilst aquatic plants fix CO_2 (-7 to 0 ‰) under an exchange reaction with ambient HCO_3^- (0 ‰). Terrestrial organic matter in temperate estuaries has $\delta^{13}C$ values ranging between -25 to -30 ‰ with a mean value of -26 ‰. In the marine environment, organic carbon $\delta^{13}C$ values vary between -19 and -22 ‰ depending on the hydrologic regime (Sackett *et al.*, 1965; Fontugne and Duplessy, 1981).

The autochthonous stable carbon isotopic compositions of POC, PIC and DIC in natural waters should be closely related. However, carbon in suspended matter and sediment may also originate from other sources and be transported to the sampling location over long distances. In order to be able to help distinguish between the different carbon sources, the $\delta^{13}C$ values of the various contributors needs to be known as well as the isotopic fractionation processes in the water (Mook and Tan, 1991).

Organic carbon in rivers and estuaries is derived from two major sources. These are autochthonous organic matter and allochthonous material. Due to the isotopic difference between plankton and terrestrial organic carbon, it is possible to use stable carbon isotopes to give an estimation of the relative contribution from marine (around -21 ‰) and terrestrial (around -27 ‰) sources of POC to estuarine POC (Fry and Sherr, 1984). For example, the sources, site of deposition and transport of POC in estuarine waters has been studied by testing various classes of autochthonous and allochthonous organic matter (Faganeli *et al.*, 1988; Matson and Brinson, 1990; Rezende *et al.*, 1990; Tan *et al.*, 1991). Fluxes of POC, both to the estuarine floor or exported to the shelf have also been estimated (Fontugne and Jouanneau, 1987; Lucotte *et al.*, 1991). The origin of sediments can be determined in an estuary (Wada *et al.*, 1987; Mulholland and Olsen, 1992) whilst the seasonal nature of POC has been studied using stable carbon isotopes (Pocklington and Tan, 1987; Cifuentes *et al.*, 1988; Lucotte, 1989). A procedure for the analysis of $\delta^{13}C$ in DOC has been devoloped for tracing sources of DOC (Bauer *et al.* (1991); Fry *et al.*, in press).

Few studies have involved particle size fractionation and the determination of $\delta^{13}C$ signatures in organic colloidal material. Rau *et al.* (1990) found differences of up to 5 ‰ in $\delta^{13}C$ size fractions < 3 μm, 3 to 150 μm and > 150 μm. In this case enriched values were found in the larger size fractions and were due to isotopic fractionation associated with plankton cycling. Altabet (1990) has determined $\delta^{13}C$ values in the 0.2 to 0.7 μm size range using Anodisc Anopore filters in the surface layers of the Sargasso Sea. $\delta^{13}C$ values were in the range -26.6 to -21.5 ‰. The $\delta^{13}C$ content of colloidal bacteria in the estuarine environment has also been investigated by Coffin *et al.* (1989).

5.2.2. δ^{13}C signatures of the organic size fractions in the Beaulieu estuary, 1991/92.

δ^{13}C signatures for the organic carbon fractions have been measured for the summer 1991 and summer 1992 seasons. Both summer 1991 and summer 1992 had high algal productivity in the water column which is reflected in high POC concentrations. This does not necessarily impart high COC levels. However, high COC levels were seen during these seasons and these may be due to extensive degradation events seen during summer 1991 and summer 1992 (as inferred from oxygen depletion during summer 1991; **Chapter 4.5.2**). Therefore, these seasons would be likely to have well defined sources for the different organic size fractions. The POC fraction would have a high content of phytoplankton derived material as this is particulate in nature whilst the COC fractions would have a high content of terrigenous material derived from degradation processes as the high organic content in the estuary during summer 1991 was thought to be of terrestrial origin (see **Chapter 4.5.1**).

5.2.2.1. Collection of samples and precision of the δ^{13}C measurements.

The samples for δ^{13}C analysis were collected simultaneously with the samples for DOC analysis and the collection of these samples and subsequent processing are described in the analytical methods section (**Chapter 4.2.3.3**). The combustion method is given in **Chapters 3.3.3 and 3.3.5**. The precision of the δ^{13}C measurement is 0.1 ‰ and all isotope data is quoted to 1 decimal place on the basis of replicate measurements at the UCNW laboratory.

5.2.2.2. PO^{13}C Values.

PO^{13}C values measured in the Beaulieu estuary during summer 1991 were in the range -28.5 to -17.7 ‰, whilst for summer 1992 they varied between -36.3 and -16.0 ‰. Values are plotted in **Figure 5.1.**

The spread of PO^{13}C values for summer 1991 was greater than previously reported ranges for estuarine environments (Fontugne and Jouanneau, 1987; Wada *et al.*, 1987; Salomons and Mook, 1981), whilst for summer 1992 the spread was far greater. For example, Wada *et al.* (1987) gave PO^{13}C values which ranged from -26.5 to -20.7 ‰.

Inter-annually there is very little difference between the mixing curves in the salinity range 26 to 33. In the salinity range 13 to 26 and 33 to 35 the difference approaches 3 ‰. In the salinity range 4 to 13 the difference approaches 7 ‰. The freshwater end-member cannot be compared due to the lack of a freshwater sample for summer 1992, however a difference can be seen in the seawater end-member approaching 3 ‰. The mid-salinity (13 to 26) difference in mixing curves of up to 3 ‰ may not be that significant as this could easily be caused by different mixing regimes in the estuary at the time. Therefore the regions of greatest difference are at high salinity (33 to 35) and at lower salinity (4 to 13) i.e. the end-member values are quite different for the two seasons.

During summer 1992 fluctuations in $PO^{13}C$ in the salinity range 27 to 35 were probably associated with high turbidity identified earlier (**Chapter 4.5.5**). This may also be the case for summer 1991, however, evidence for high turbidity was not as clear. Other processes already identified, such as flocculation of the COC fractions, are not apparent in the $CO^{13}C$ salinity profiles. This may be because the $\delta^{13}C$ value measures ratios and an increase or decrease in amount of material with the same $\delta^{13}C$ signature would not be seen. The $\delta^{13}C$ value would change when material with for example two different $\delta^{13}C$ signatures mix.

Constant values for $\delta^{13}C$ throughout the year for the marine end-member have been reported in the literature. However, seasonal variations in $\delta^{13}C$ values in the sea (by around 1 ‰) can be caused by seasonal variation in sea surface temperature. In the coastal zone and lower estuary the seasonal variation of $\delta^{13}C$ is buffered due to mixing of previously deposited POC and plankton. The freshwater end-member $\delta^{13}C$ values have been reported to show no seasonal variation except during algal blooms when the values become slightly more negative by around 2 ‰ (Fontugne and Jouanneau, 1987; Lucotte, 1989). In mid-stream the $\delta^{13}C$ values are influenced by the importance of the various POC inputs such as terrigenous, planktonic, marine and input from tidal mud-flats and the mixing regime and fresh water residence time in the estuary.

It has been suggested that $PO^{13}C$ values show a progressive change along the estuary (Fontugne and Jouanneau, 1987) and permit an estimate of the proportion of terrestrial carbon in each sample by solving the mixing equation:

$$\delta^{13}C = F_t \, \delta^{13}C_t + F_m \, \delta^{13}C_m$$

$\delta^{13}Ct$ and $\delta^{13}Cm$ refer to the carbon isotopic composition of the terrestrial and marine sources respectively and assumes these end-members are the only significant sources of POC. Ft and Fm are the terrestrial and marine contributions with Ft + Fm = 1 (Fontugne and Jouanneau, 1987). The δ^{13}Ct value is determined from the average δ^{13}C value of POC in the river and the δ^{13}Cm value is determined from the average δ^{13}C value of POC in the seawater end-member. The Ft values for the PO^{13}C values can be seen in **Figure 5.1.** The data suggests that as higher salinities were reached in the Beaulieu estuary very little of the terrigenous POC remained. This indicates that terrigenous POC is lost from the water column, for example by transformation processes of POC to DOC caused by the increase in ionic strength in the estuary (Eisma *et al.*, 1985) or by disaggregation of the POC, bacterial degradation (Laane, 1982), re-mineralization (conversion to inorganic forms) due to respiration or by deposition of the POC to the sediment (adsorbed on mineral surfaces or by direct deposition). The short fresh water residence time for the Beaulieu estuary (around 7 days) would seem to rule out bacterial degradation or transformation processes as a possible sink for POC. Therefore, deposition to the sediments would seem the most likely sink. This process has been observed by Lucotte *et al.* (1991) who reported that about 75 % of the terrigenous POC introduced in the upper estuary of the St. Lawrence river was deposited within the limits of the lower estuary. This figure is comparable with those estimated for the estuaries of the Gironde (80 %; Fontugne and Jouanneau, 1987), the Otsuchi (70-100 %; Wada *et al.*, 1987) and the Amazon (94 %; Showers and Angle, 1986).

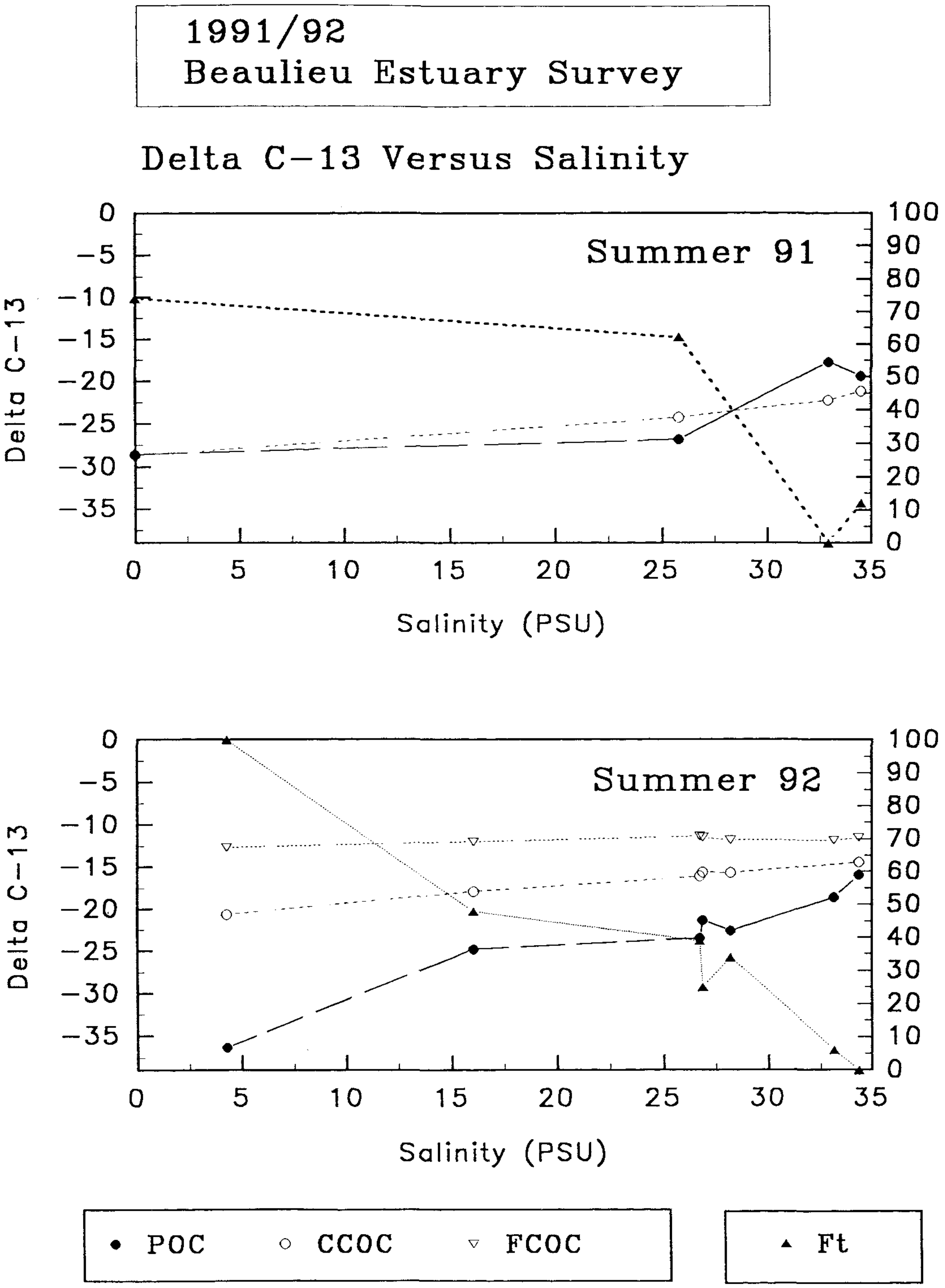


Figure 5.1. $\delta^{13}C$ signatures for the organic carbon fractions and Ft values for the POC fraction in the Beaulieu estuary (1991/1992).

5.2.2.3. CCO^{13}C and FCO^{13}C Values.

The spread of CO^{13}C values lie in the range -28.6 to -21.2 ‰ for summer 1991 and -20.6 to -14.5 ‰ for summer 1992. FCO^{13}C values, which have only been measured for summer 1992, lie in the range -12.7 to -11.3 ‰. Again there is a difference for the CCO^{13}C values for summer 1991 and summer 1992. Both have approximately linear mixing curves; the main difference is that the summer 1992 values are enriched in ^{13}C by around 7 ‰. The FCO^{13}C values for summer 1992 again show a straight mixing curve where the δ^{13}C values are enriched in ^{13}C by around 5 ‰ compared to the CCO^{13}C values for summer 1992. The straight mixing curves for CCO^{13}C and FCO^{13}C points to the lack of major geochemical transformations (production or removal) of these fractions in the estuary i.e. they behave more like dissolved constituents (DOC) in the estuary.

5.2.2.4. Differences between the PO^{13}C and CCO^{13}C mixing curves.

The differences between the mixing curves for the two seasons, as well as the difference in δ^{13}C values compared to other estuarine studies, need to be addressed. Possible reasons for these differences could be in the handling and preparation of the samples or the differences may be real and due to environmental conditions.

Handling of the samples would be unlikely to introduce extraneous contamination as this was undertaken using rigorous clean procedures (see **Chapter 4.2.3.1**). The samples for ^{13}C analysis in this study were not acid treated to remove carbonates as it was considered that these would not be present in high concentrations. Some workers have acid treated filters before measurement of the ^{13}C content (Fontugne and Jouanneau, 1987; Wada *et al.*, 1987; Rau *et al.*, 1990), however a number have not done so (Saliot *et al.*, 1988; Lucotte, 1989; Lucotte *et al.*, 1991). Faganeli *et al.* (1988) acid treated marine sediments but not POC samples for isotopic analysis. Lucotte (1989) reported that carbonates represented less than 0.2 % of the total carbon content, whilst Lucotte *et al.* (1991) reported that carbonates represented less than 0.04 % of the total carbon content in the lower estuarine sediments of the St. Lawrence river.

The preparation of the samples for δ^{13}C determination may have introduced fractionation of the sample by incomplete combustion or inadequate diffusion of the heavier ^{13}C through the vacuum line or the vacuum line may have introduced contamination into the sample. Again this option is unlikely due to the care taken in preparing the samples (making sure blank values were insignificant etc) and by using fully tested procedures (see **Chapter 3.3.5**).

If the $\delta^{13}C$ values are real and due to environmental conditions then mechanisms must exist that produce these extreme values. This is discussed further in the next section.

5.2.2.5. Discussion of processes inferred from the $\delta^{13}C$ signatures reported in the Beaulieu estuary (1991-1992).

$\delta^{13}C$ values for organic matter in rivers and estuaries are subject to local influences such as the nature of the vegetation cover in the drainage basin, the intensity of mineralization and humification processes, the intensity of anthropogenic inputs into the estuary and coastal zone and the effect of temperature (Saliot *et al.*, 1988). The distribution of $\delta^{13}C$ in the Beaulieu estuary may be influenced by a variety of these factors and it is worth noting the $\delta^{13}C$ signatures of possible sources that may contribute to the $\delta^{13}C$ values found.

Sewage effluent has a $\delta^{13}C$ value of around -25 ‰ (Faganeli *et al.*, 1988) and ^{13}C enrichment of freshwater POC has been attributed to increasing concentrations of sewage effluent as the freshwater discharge decreases (Lucotte, 1989). In the Beaulieu estuary the major discharge of sewage effluent occurs downstream of Beaulieu village and is relatively small (216 $M^3 day^{-1}$) (Horne, personal communication) (see map in **Figure 4.1**). This discharge in mid-estuary would help to increase the $PO^{13}C$ values from the low upstream values, however the effect would not be that great due to the low consented discharge.

Vascular plants divide into two groups with respect to their stable carbon isotope ratios. Those with a C_3 pathway of photosynthesis have $\delta^{13}C$ values of -24 to -34 ‰, whilst C_4 plants have less negative values of -6 to -19 ‰. *Spartina* and other grasses growing in salt marshes or on tidal mud-flats have $\delta^{13}C$ of -12.3 to -13.6 ‰, whilst *Spartina* leaf leachates are near -16 ‰ (Coffin et al., 1989, 1990). In seagrass beds eelgrass (*Zostera*) had ratios of -10.6 to -12.2 ‰ (Thayer et al., 1978; Wada et al., 1987). Algae have intermediate values of -12 to -23 ‰ (Smith and Epstein, 1971) whilst benthic diatoms have values of -13.6 to -18.1 ‰ (Haines, 1977; Haines and Montague, 1979; Schwinghamer *et al.*, 1983). $DO^{13}C$ samples taken from a hypersaline microbial mat environment had average $\delta^{13}C$ values of -12.4 ‰ (Bauer *et al.*, 1991). Animals feeding predominantly on one food source have similar $\delta^{13}C$ to that of their food.

Very low DO^{13}C values (-35 to -44 ‰) have been found in the low salinity region (0-2) of a salt marsh estuary (Fry *et al.*, in press). This was due to a phytoplankton bloom where freshwater plankton had δ^{13}C values in the range -30 to -48 ‰ (Rau, 1978; LaZerte, 1983).

During summer 1992 the DOC concentrations were not as high as those for summer 1991 and showed typical values for the Beaulieu river (up to 450 μM C). Chlorophyll *a* levels were also high during summer 1992 in mid-estuary (up to 15 μg/l) and in the lower estuary (up to 4 μg/l); POC levels were elevated in mid-estuary (up to 300 μM C), however, these decreased to normal levels in the lower estuary (around 40 μM C). This suggested high primary productivity. The SPM levels were typical (up to 25 mg/l), although there was a significant increase from around 5 mg/l (27 PSU) to 15 mg/l (35 PSU) in the lower estuary i.e. high turbidity as identified earlier (see **Chapter 4.5.5**). Therefore, the PO^{13}C levels in the estuary should reflect the high levels of primary productivity. This was especially so in the river where the PO^{13}C values were close to freshwater phytoplankton values (δ^{13}C signatures around -35 ‰). The δ^{13}C values at higher salinities were not similar to sea phytoplankton values and were enriched with respect to ^{13}C (up to 15 ‰).

These high values have been previously reported in the literature for estuarine sediments. Along the Dutch North Sea coast, where there is extensive *Spartina* growth, δ^{13}C values of sedimentary organic matter have often been found as high as -12 ‰, especially in the coarse fraction (Laane *et al.*, 1990). However, POC in salt marshes had δ^{13}C values of -19 to -23 ‰ suggesting that the POC was largely derived from phytoplankton (-20 to -22 ‰) and that *Spartina* debris is quickly removed from the water column to the sediments (Haines, 1976; Haines and Montague, 1979; Lucotte, 1989). DO^{13}C values were found to be enriched (-18 to -22 ‰) in a section of salt marsh estuary bordered by *Spartina* (Fry *et al.*, in press); however, these values were not as high as the PO^{13}C found in this study. It is unlikely that any significant transformation of DOC to POC would occur in the Beaulieu estuary, hence enriching the PO^{13}C values, as there are few mechanisms that would produce this effect (see **Figure 1.8**). It has been reported by Marinucci (1982) that benthic diatoms (δ^{13}C value of -13.6 to -18.1 ‰) can significantly contribute to the total net productivity of marshes. However, the Beaulieu estuary does not have extensive marshes and this would be unlikely to cause enrichment of the PO^{13}C fraction.

The enriched $PO^{13}C$ values found in the Beaulieu estuary are therefore probably due to the presence of high turbidity in the salinity range 25 to 35 (as identified earlier in **Chapter 4.5.5**) followed by mobilization of *Spartina* debris from the sediments and mud-flats. There are extensive mud flats where *Spartina* grows at the mouth of the Beaulieu estuary as well as along the edge of the estuary towards Beaulieu village and in the pond region (see map in **Figure 4.1**). Therefore, the region which would be affected most by *Spartina* would be the lower estuary. Seagrass meadows (e.g. *Zostera*) are found at Calshot and further to the west of the Beaulieu estuary and these would not affect processes in the Beaulieu estuary. The influence of *Spartina* plants can persist over long periods due to their very slow biodegradation. These areas of *Spartina* growth, especially in the lower estuary, have been gradually eroding over the last few years and this process would effecively introduce various size fractions of *Spartina* debris into the water column from the mud-flats and sediments especially in the turbidity maximum or following recent storm events.

The $CCO^{13}C$ and $FCO^{13}C$ values for summer 1992 also do not show signatures close to those of phytoplankton. Both CCOC and FCOC are enriched with ^{13}C throughout the salinity range, particularly $FCO^{13}C$ where the $\delta^{13}C$ values are around -11.5 ‰. The $CCO^{13}C$ values were in the range -20.6 to -14.5 ‰. *Spartina* has a $\delta^{13}C$ value of -13 ‰ for bulk tissue. Cellulose and hemicellulose comprise > 70 % of the ash-free dry weight, with $\delta^{13}C$ values of -11 to -12 ‰ (Benner *et al.*, 1987) suggesting that the FCOC fraction is most representative of this cellulose material whilst the CCOC fraction has $\delta^{13}C$ values closer to that of the bulk tissue. The CCOC values which are mid-way between the values for FCOC and POC could reflect material which is derived from both FCOC and POC sources. These values may reflect recently mobilized *Spartina* debris which is colloidal in nature. The $PO^{13}C$ apparently was not influenced by the *Spartina* debris as much as the smaller size fractions. This is probably because the $PO^{13}C$ fraction is more influenced by the signal from phytoplankton (which would mask the *Spartina* signal) and because the *Spartina* debris, having been deposited in the sediments and mud-flats would be most likely to be colloidal in nature (via diagenetic processes such as respiration). Degradation events associated with phytoplankton have also been identified earlier as giving rise to high colloidal concentrations (**Chapter 4.5.2**).

It has been noted that *Spartina* does not impart a dominant signal to DOC in the estuarine environment (Calder and Kearsley, 1977; Fry *et al.*, in press). They supposed that the $\delta^{13}C$ signal is diluted with other algal and riverine carbon or it is metabolized before reaching the estuarine waters. This study suggests that the enriched $\delta^{13}C$ signal from *Spartina* is present in the water column and it exists predominantly in the smaller colloidal fraction (20 to 100 nm). This may be the reason why earlier studies did not observe this signal as it can be masked by other more dominant signals (e.g. from phytoplankton) as this study has also shown in the $\delta^{13}C$ values for summer 1991 when algal productivity was very high in the water column. This study has also shown that the *Spartina* signal was more evident during periods of high turbidity in the water column. Saliot *et al.* (1988) has found higher $\delta^{13}C$ values for smaller particles where particles > 0.7 μm had a value of -22.3 ‰, whilst particles < 0.7 μm had values of -20.7 ‰. Therefore, small particles, such as colloids, were present in the inner estuary with high $\delta^{13}C$ values, however, Saliot *et al.* (1988) suggested that these were closely associated with freshwater algal material.

The DOC concentrations for summer 1991 were very high in the river and estuary (up to 1300 μM C) as were the chlorophyll *a* concentrations in mid-estuary (up to 17 μg/l). However, the chlorophyll *a* values decreased in the lower estuary (around 2 μg/l). POC concentrations were also high (up to 300 μM C in mid-estuary) but these fell to more typical levels (around 40 μM C) in the lower estuary. This therefore pointed to conditions of high phytoplankton productivity in the estuary, being higher than that of summer 1992. The COC concentrations were also high matching the TDOC concentration in the estuary and were around twice the POC concentrations. High COC concentrations were probably caused by degradation processes in the water column (see **Chapter 4.5.2**). Therefore, the $PO^{13}C$ levels in the estuary should reflect this high phytoplankton activity. This was the case, although the $PO^{13}C$ values (-17.74 ‰) at higher salinities were slightly enriched for seawater phytoplankton (-21 ‰). This indicated that the dominant signal for the lower estuary was from phytoplankton, although the *Spartina* signal could also be seen. An increase in colloidal concentration was observed in the coastal zone, suggesting a different source for this colloidal material, possibly from *in-situ* algal activity (see **Chapter 4.5.1**). In the river the phytoplankton activity was low and the $\delta^{13}C$ signatures for POC and CCOC were close to the values for peat (-27 ‰; Mook and Tan, 1991) and soil organic material (-24 ‰; Wada *et al.*, 1987), indicating a terrestrial source for the organic matter as identified earlier (**Chapter 4.5.1**). The $PO^{13}C$ and $CCO^{13}C$ fraction for summer 1991 did not show highly enriched $\delta^{13}C$ values (as for summer 1992) and this may have been due to the higher primary productivity in the water column which partially masked the *Spartina* signal and due to the lower turbidity in the water column (lower SPM values) at higher salinities. During summer 1991 the $\delta^{13}C$ signatures for freshwater POC were higher than for summer 1992 and may have been due to the lower freshwater phytoplankton activity in the water column during summer 1991.

5.2.2.6. $\delta^{13}C$ signatures of the particulate size fractions measured during autumn 1991, winter 1992 and spring 1992.

There is some doubt surrounding the accuracy of most of the $\delta^{13}C$ values measured for autumn 1991, winter 1992 and spring 1992 due to contamination in the samples. Only the samples marked with an asterisk in **Figure 5.2** have ratio traces and mass scans that show clean samples. The samples which are considered to be accurate have $\delta^{13}C$ values in the same range as those reported for summer 1991 and summer 1992 and therefore they increase confidence in the accuracy of the summer 1991 and summer 1992 $\delta^{13}C$ values. However, for autumn 1991, winter 1992 and spring 1992 there are too few of these accurate measurements to make any suppositions on the behaviour of the size fractions during these seasons.

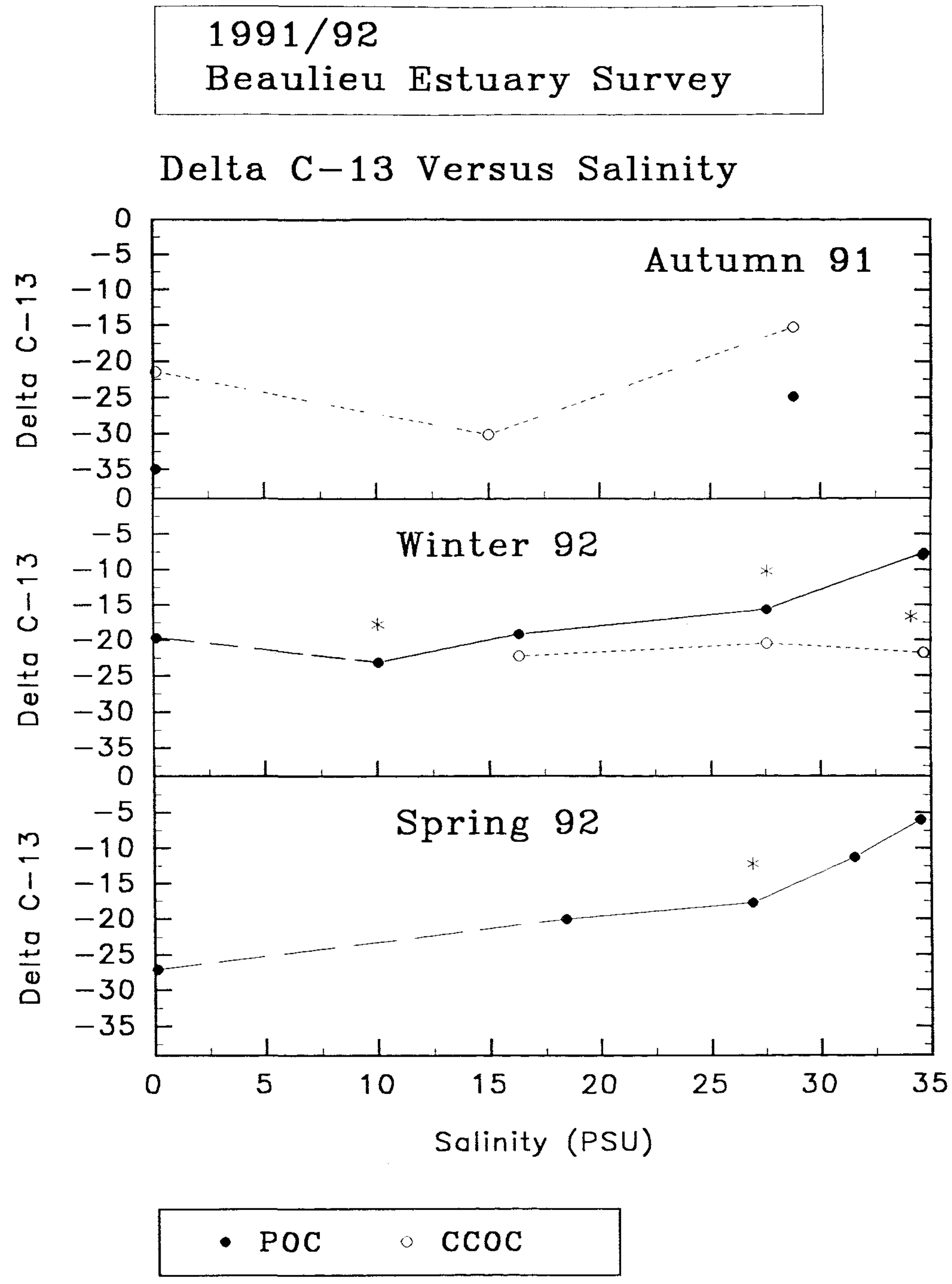


Figure 5.2. $\delta^{13}C$ signatures for the organic carbon fractions in the Beaulieu estuary during autumn 1991, winter 1992 and spring 1992 (samples marked with an asterisk indicate clean samples).

The nature of the contamination in the samples may be identified from the ratio traces and mass scans from the mass spectrometer. For autumn 1991 the CCOC fraction (-15.3 ‰) had a "toothed" ratio trace which is characteristic of the presence of water. This can be seen in **Figure 5.3** which shows an example of a "toothed" ratio trace and a ratio trace that shows a clean sample. The POC fraction (-24.9 ‰) and CCOC fraction (-30.1 ‰) for autumn 1991 were contaminated; most of the gas was not CO_2. The POC fractions during winter 1992 (-7.9 and -7.7 ‰) and spring 1992 (-6.0 ‰) were thought to consist of air samples because the $\delta^{13}C$ values were very close to atmospheric CO_2 and the ratio traces showed showed no contamination. The winter 1992 POC fraction (-19.7 ‰) was grossly contaminated as indicated by the ratio trace. A clean mass scan should show just three peaks at a 44, 45 and 46. The mass scan also showed a number of peaks from 35 to 60 which were probably various organic contaminants either resulting from incomplete combustion of the sample or from non-condensables which were not pumped away. This was also the case for the CCOC fractions (-20.4 and -22.2 ‰) during winter 1992 and for the POC fraction (-27.1 ‰) during spring 1992. Possible reasons for contamination present in these samples have been given in **Chapter 3.3.6**.

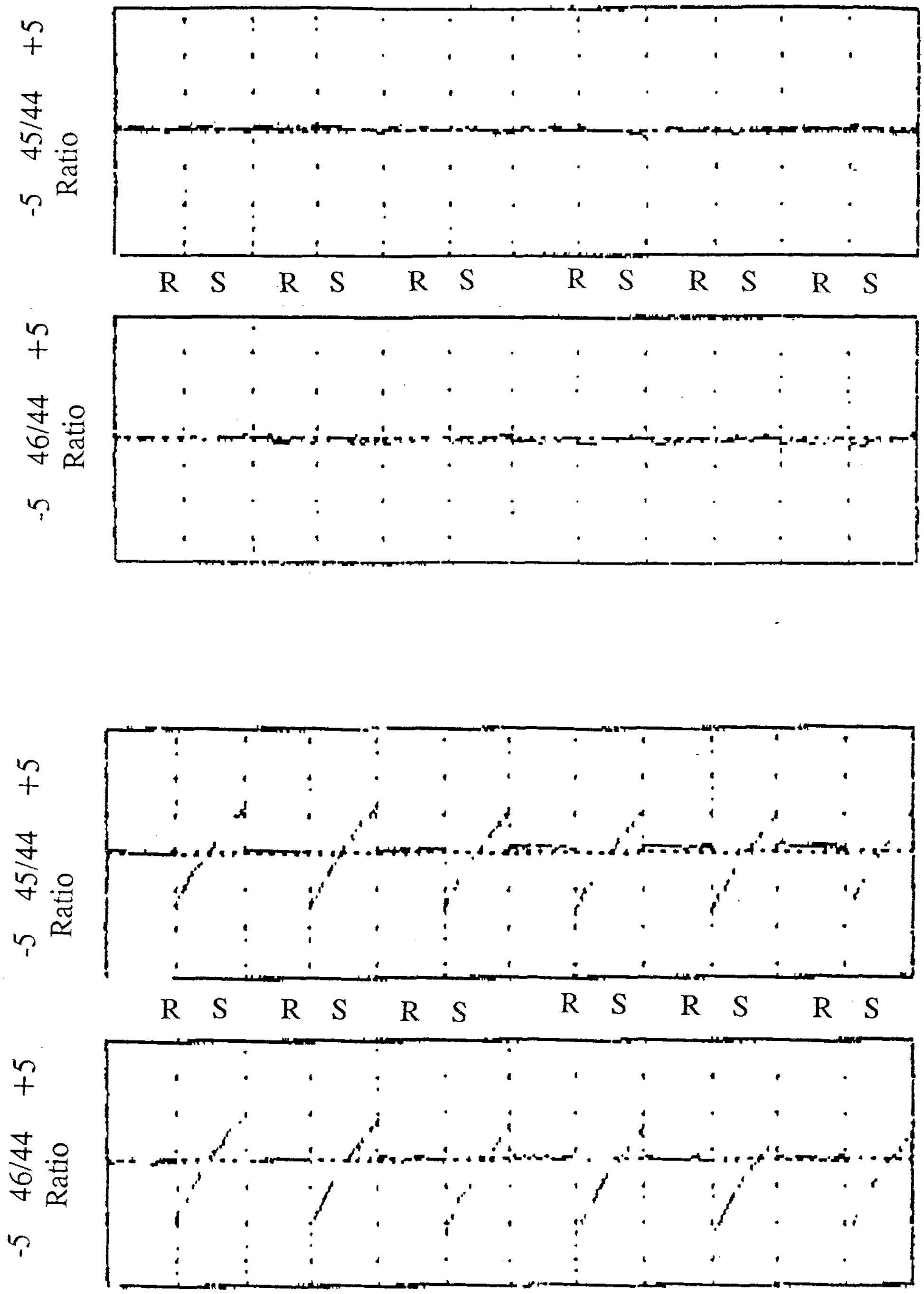


Figure 5.3. Example of a ratio trace showing a clean sample and a ratio trace showing a "toothed" appearance; X-axis = time, Y-axis = ratio, R = reference, S = sample, 45/44 = $^{13}C^{16}O^{16}O/^{12}C^{16}O^{16}O$, *46/44 =* $^{13}C^{17}O^{16}O/^{12}C^{16}O^{16}O$.

5.3. $\Delta^{14}C$ activities as a tracer for organic matter in natural waters.

5.3.1. Introduction.

A recent major development has been the use of accelerator mass spectrometry for the measurement of ^{14}C. This involves separating the ^{12}C and ^{13}C atoms from the ^{14}C atoms in a carbon sample and then counting all the ^{14}C atoms (not just the ones that disintegrate radioactively). Publications first reporting radiocarbon measurements by AMS appeared in 1977 by Nelson *et al.* (1977) at McMaster University and by Bennet *et al.* (1977) at the University of Rochester.

The technique employs a particle accelerator in conjunction with a mass spectrometer which allows the detection of many isotopes down to abundance levels of one part in 1000 trillion (10^{15}) and this apparatus could extend radiocarbon dating to 80,000 years B.P. (before present) (Hedges and Gowlett, 1986). AMS also has the capability for the detection of a number of other important cosmogenic long-lived radionuclides such as ^{10}Be, ^{26}Al, ^{32}Si, ^{36}Cl, ^{41}Ca and ^{129}I (see for example reviews by Elmore and Phillips, 1987; Wolfli, 1987). A general introduction to radiocarbon dating by AMS can be found by Hedges and Gowlett (1986) whilst more detail is given in papers by Hedges (1984) and Litherland (1984). Conference proceedings on AMS and radiocarbon dating have been edited by Henning *et al.* (1982), Stuiver and Kra (1983), Wolfli *et al.* (1984), Stuiver and Kra (1986) and Gove (1987).

5.3.1.2. Radiocarbon dating in oceanography.

The ability to measure the ^{14}C content of microgram samples of carbon is an important breakthrough. Dissolved, colloidal and particulate organic phases in seawater, sediment pore water, river water and precipitation may be dated by AMS as well as bio-carbon derived from mirco and macro-organisms which can be fractionated into individual compound classes and even into individual molecular compounds.

$\Delta^{14}C$ measurements have principally been used to determine the age of oceanic DOC (Williams *et al.*, 1969; Arhelger *et al.*, 1974; Williams *et al.*, 1978; Williams and Druffel, 1987; Bauer *et al.*, 1992). The sources and cycling of POC in the ocean have been studied using $\Delta^{14}C$ measurements (Druffel *et al.*, 1986; Hedges *et al.*, 1986; Druffel and Williams, 1990; Begg *et al.*, 1991). $\Delta^{14}C$ activities of humic substances (Hedges *et al.*, 1986; Druffel *et al.*, 1989; Begg *et al.*, 1991; Bauer *et al.*, 1992) and organisms (Pearcy and Stuiver, 1983; Williams *et al.*, 1987) have also been measured.

5.3.2. Δ^{14C} values of the organic size fractions in the Beaulieu estuary (autumn, 1991).

5.3.2.1. Introduction.

^{14}C activities were determined in the estuary for autumn 1991. Further samples were taken at a sampling site off the Needles and at a sampling site off Lands End later on in the year. The ^{14}C activity of the POC and CCOC samples for autumn 1991 were in the range 147.2 to 431.5 %M and 327.8 to 1226.7 %M respectively. The Lands End sample was taken after the Beaulieu estuary samples in March 1992 and has more oceanic character. The PO^{14}C values were fairly constant throughout the salinity range, with a small increase in ^{14}C activity in the seawater end-member. In contrast the CCO^{14}C values showed a steady increase in ^{14}C activity as higher salinities were reached (**Figure 5.4**). ^{14}C activities reported in the literature are given in **Table 5.1.**

Sample type	Environment	^{14}C activity (%M)	Author
Coarse SPOM (65-3000 μm)	Amazon river	124	Hedges *et al.* (1986)
Fine SPOM (0.5-65 μm)	Amazon river	102	Hedges *et al.* (1986)
POC	Bristol Channel	152	Begg *et al.* (1990)
POC	Sellafield	73	Begg *et al.* (1990)
POC	Beaulieu estuary	147 to 431	This study
Suspended POC	North Pacific, (oligotrophic)	100 to 121	Druffel and Williams (1990)
Sinking POC	North Pacific (oligotrophic)	111 to 118	Druffel and Williams (1990)
CCOC	Beaulieu estuary	328 to 1227	This study
Deep-water DOC	Southern California coast	65	Williams *et al.* (1969)
Deep-water DOC	North Pacific (oligotrophic)	45	Druffel and Williams (1990)

Surface DOC	North Pacific (oligotrophic)	80	Druffel and Williams (1990)
Humic acid	Amazon river	119	Hedges *et al.* (1986)
Fulvic acid	Amazon river	135	Hedges *et al.* (1986)
Total humics	Amazon river	127	Hedges *et al.* (1986)
Humic acid	North Pacific	69	Druffel *et al.* (1989)
Fulvic acid	North Pacific	59	Druffel *et al.* (1989)
Surface animals	North Pacific	120	Williams *et al.* (1987)
Deep-water animals	North Pacific	96	Williams *et al.* (1987)

Table 5.1. Reported ^{14}C activities of organic matter in various marine environments.

As can be seen in **Table 5.1** the levels of ^{14}C activity measured in the Beaulieu estuary are far above the levels measured by other workers. This therefore suggests that the samples were contaminated in some way by a source with highly elevated levels of ^{14}C.

A trend in the $\Delta^{14}C$ values can be seen (**Figure 5.4**) with the CCOC fraction showing consistently higher values than the POC fraction. On increasing salinity there was a large increase in the CCOC $\Delta^{14}C$ values, although the most saline sample (Lands End sample) had the lowest $\Delta^{14}C$ values. Therefore, if the source of ^{14}C were anthropogenic most of it existed in the CCOC fraction (which behaves in a more conservative manner than the POC fraction; see **Chapter 4.4.1**) and increased on going down the estuary into the west Solent.

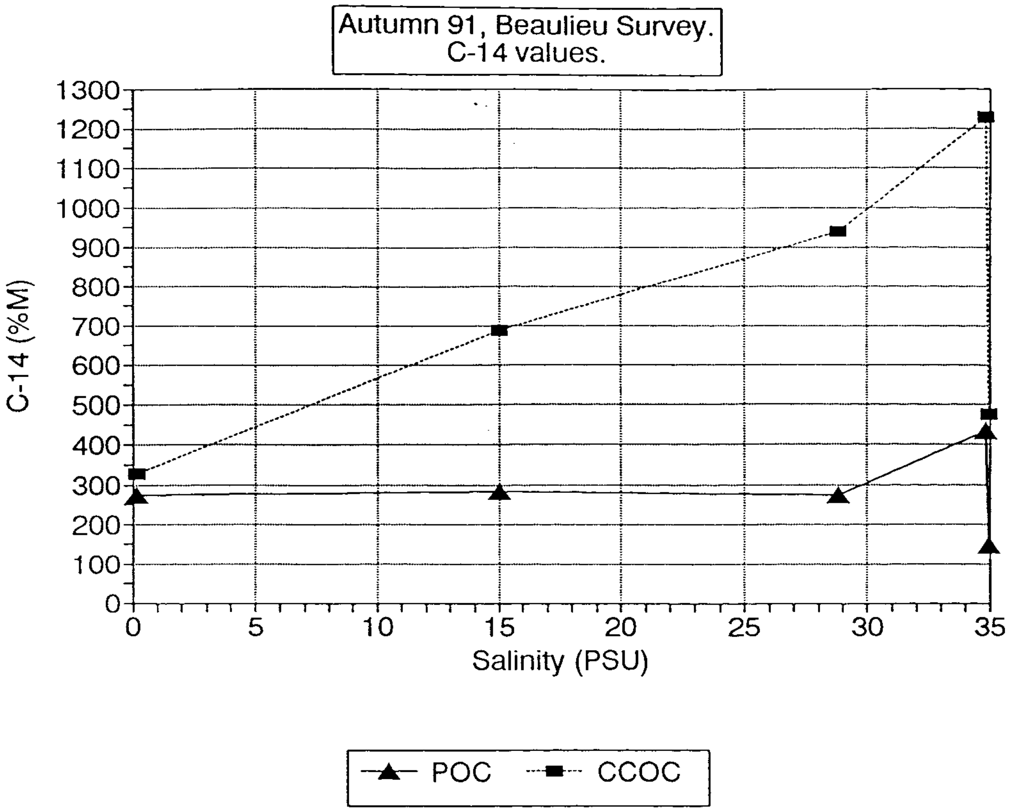


Figure 5.4. $\Delta^{14}C$ activities of the POC and CCOC fractions in the Beaulieu estuary during autumn 1991.

Possible contamination would have to be associated with the material isolated on the filters i.e. particles > 0.7 μm and particles in the size range 0.1 to 0.7 μm. The most obvious suggestion would be that the ^{14}C contamination was part of either the organic fraction or the mineral content retained on the filters, or contamination may have sorbed onto the filtered particles from solution.

The elevated levels of ^{14}C could either be from (1) an anthropogenic source in the environment (2) contamination introduced into the samples via handling of the samples or (3) an analytical artifact. The levels of ^{14}C are too high to be a natural phenomenon because biological fractionation only enriches ^{12}C relative to ^{13}C and ^{14}C by a few percent (Craig, 1953).

5.3.2.2. Possible anthropogenic sources for the highly elevated levels of ^{14}C.

A number of potential anthropogenic sources of ^{14}C in the environment have been considered. The nuclear power plant at Winfrith has a very small discharge of ^{14}C into the atmosphere and the nuclear reprocessing plant at Cap de la Hague has a ^{14}C discharge into the coastal area. Other possible sources are the naval dockyards at Portsmouth and Plymouth. However, it would be very unlikely that these would have any effect due to the distances involved where any discharges would be diluted and dispersed and the fact that the discharges would not be organic in nature. A localised source for the contamination would be far more likely such as discharge from hospitals, industries or Southampton University. Again this would be unlikely to give the highly elevated levels of ^{14}C measured as the discharges are very small and occur in the Solent. Even waters affected directly by ^{14}C contaminated effluent in the Severn estuary did not have POC activities greater than 152 %M (Begg et al., 1990).

5.3.2.3. Introduction of contamination by sample handling.

Carbon-14 contamination introduced during handling stages is another possibility. Possible ^{14}C sources include: the boat used to carry out the work (R.V. Bill Conway), the sampling bottle and containers, glassware in the laboratory used to filter the samples, the filters themselves, dust particles in the laboratory or the combustion tube and copper oxide used to combust the samples. It would be difficult to imagine any of these introducing the elevated levels of ^{14}C seen. The R.V. Bill Conway was newly in service and no ^{14}C tracer studies had been carried out on it prior to this work. All of the glassware was meticulously cleaned with chromic acid and the combustion tube and copper oxide were heated at 800^0C for 4 hours before use to remove any organic carbon contamination. The filters were also heated at 450^0C for 4 hours before use. The laboratory was newly built and again no ^{14}C tracer studies had been carried out in the laboratory.

Accumulated dust in the laboratory and swabs from the glassware have been tested for elevated levels of ^{14}C using a Rackbeta Scintillation Counter (Wallac 1209). No evidence for ^{14}C contamination was found. This was not surprising as the building was new and ^{14}C tracers had never been used in it. It was thought unlikely that the contamination would arise from atmospheric CO_2 as the % of CO_2 gas in the atmosphere is fairly small (0.16 %, *ca.* 320 parts per million) and the CO_2 would also have to diffuse into the water sample and then associate itself with the filter or contents on the filter, which would be unlikely as the sample was only exposed to the atmosphere for a few hours. It has been calculated that for the most contaminated sample (1250 %M) the amount of ^{14}C labelled bicarbonate ($NaH^{14}CO_3$; 2 to 10 mCi/mmol) that would be needed to account for this high level would be around 8 ng. Therefore, it can be seen that a very small amount of substance would be needed to give elevated ^{14}C levels. Whilst the scintillation counter would not be able to detect these small levels (detection limit around 0.2 Bequerels), if contamination was left over from tracer experiments, then it would be expected that higher levels of ^{14}C activity would be present. However, elevated levels were not detected by the scintillation counter.

Five filters were used per sample for the POC fraction whilst between 10 and 14 filters were used per sample for the CCOC fraction. Therefore, there seems to be no correlation between the number of filters combusted and contamination on the filters themselves. If this were the case then the $\Delta^{14}C$ values would be more or less equal for each sample. The volume filtered may be related to the ^{14}C contamination. Samples with a higher salinity had high volumes of filtrate due to lower particulate levels in the water column. The CCOC fraction (**Figure 5.5**) does show an increase in $\Delta^{14}C$ activities on increasing filtrate volume. However, this trend is not repeated for the POC fraction. The order of filtration for the samples showed no relationship with $\Delta^{14}C$ measured and did not decrease on increasing use of the glassware indicating that the ^{14}C contamination was not associated with the glassware.

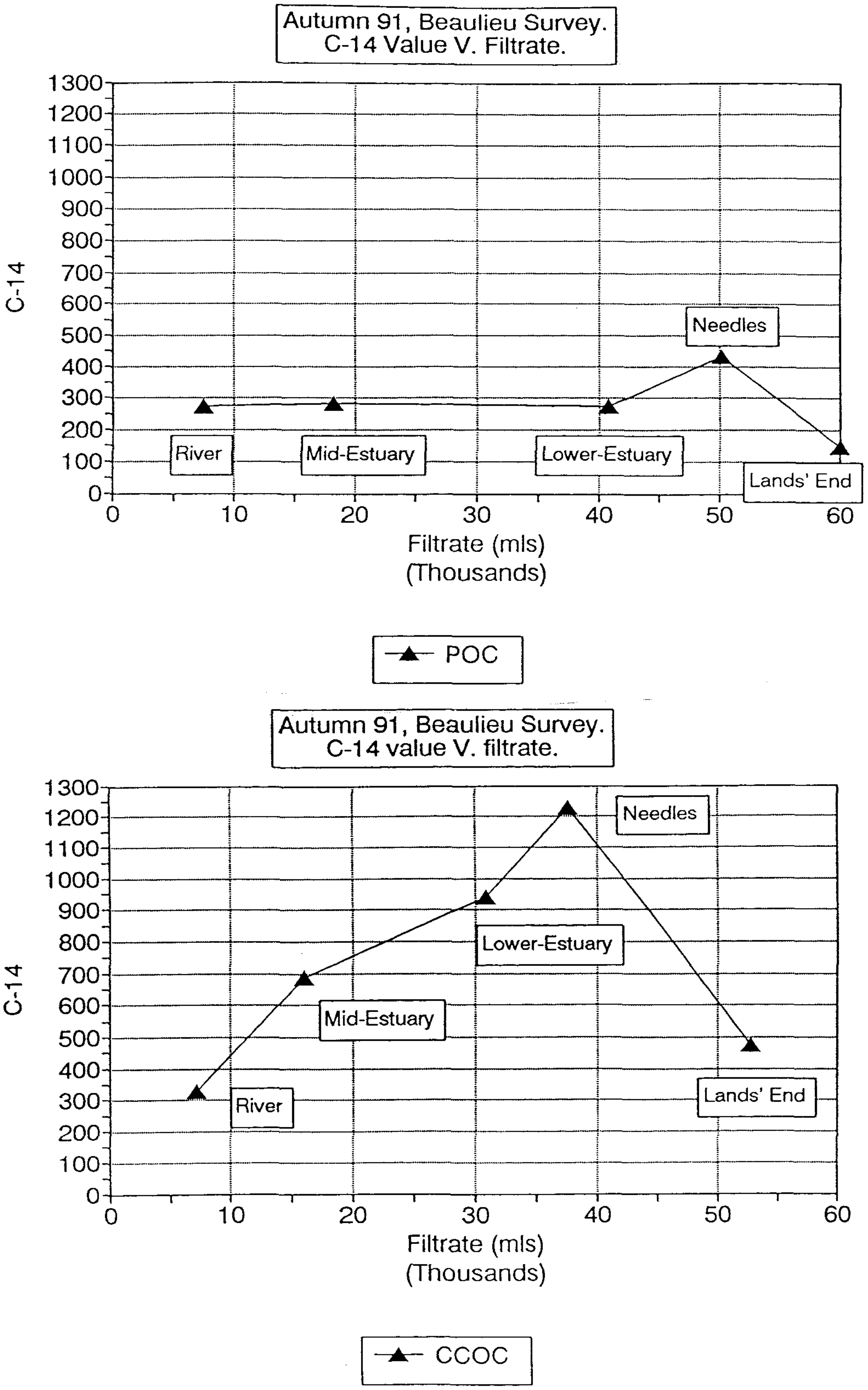


Figure 5.5. $\Delta^{14}C$ activities of the POC and CCOC fractions versus volume filtered in the Beaulieu estuary during autumn 1991.

It may be that contamination was introduced into the samples from the filtration apparatus although with the rigorous cleaning procedures used this seems unlikely. This hypothesis was supported by the $\Delta^{14}C$ values of the last sample taken i.e. the Lands End sample. The POC fraction was filtered on the ship during the cruise (Challenger 90) using different filtration apparatus and had a $\Delta^{14}C$ value of 147 %M i.e. close to the POC values reported in **Table 5.1.** The CCOC fraction was filtered in the laboratory at Southampton using the usual filtration apparatus and had a $\Delta^{14}C$ value of 475 %M. This indicates that the ^{14}C contamination was associated with filtration of the sample using the filtration apparatus in the laboratory at Southampton. However, no obvious source for elevated ^{14}C levels could be discerned.

5.3.2.4. Introduction of contamination due to an analytical artifact.

The high $\Delta^{14}C$ values measured are unlikely to be due to an analytical artifact. The archaeological dating laboratory at Oxford has stringent clean procedures to prevent any adventitious contamination and is world renowned in ^{14}C analytical procedures.

5.3.2.5. Confirmation of elevated levels of ^{14}C in the Beaulieu estuary.

Her Majesty's Inspectorate of Pollution (HMIP) were informed of the abnormally high ^{14}C levels in the Beaulieu estuary and west Solent and could suggest no reasons for the elevated values found as the discharges of ^{14}C into this area were very small or non-existent (G. McLaughlin, personal communication). A further investigation of the area, in an attempt to confirm these elevated ^{14}C levels, would involve measurement of the organic matter in the sediments by scintillation counting.

Chapter 6:

Conclusions and further research.

6.1. Conclusions.

6.1.1. Method developments.

6.1.1.1. Dissolved organic carbon analysis.

The initial very high levels of DOC measured by Sugimura and Suzuki (1988) (200 to 300 % higher than previously measured DOC concentrations) may have been due to high blank levels derived from the catalyst (for example see Benner and Strom, 1993) and at present the DOC data of Sugimura and Suzuki (1988) is being revised downwards (Cauwet *et al.*, 1990; Tanoue, 1992; Ogawa and Ogura, 1992; Chen and Wangersky, 1993; De Baar *et al.*, 1993; Hansell *et al.*, 1993; Suzuki, 1993). The extent of this downward re-evaluation is at present not known. However, HTCO methods of DOC analysis may measure 5 to 60 % more DOC than UV persulphate methods (this study; Chen and Wangersky, 1993).

An increase in DOC oxidation efficiency (6 to 8 %) was achieved by adding low concentrations of Hg salts (0.05 M, $Hg(NO_3)_2$ and $HgCl_2$; 0.004 M in the actual sample) using the UV persulphate analyzer of Statham and Williams (1983). This increased efficiency was noticed in both saline and freshwater samples and did not rely on complexation of the chloride ion.

The 6 to 8 % increase in DOC oxidation efficiency caused by adding low concentrations of Hg salts to the DOC sample (0.004 M concentration in the sample itself) implies that there is a fraction of DOC that is not measured by the UV persulphate method of DOC analysis, when used without the addition of Hg salts. This increase in the DOC concentration approaches the higher levels of DOC measured by the HTCO method of DOC analysis. The magnitude of these higher DOC levels have not yet been adequately quantified (see **Chapter 2.5.2**). Chen and Wangersky (1993) reported that the HTCO technique measured between 5 and 60 % greater DOC concentrations. It was found in this study (**Chapter 3.2.4**) that the HTCO technique measured between 40 and 70 μM C more DOC; most DOC concentrations measured by the HTCO analyzer had values that were between 10 and 60 % greater than the UV persulphate DOC concentrations, although values as high as 140 % were seen. A substantial part of this (10 to 50 μM C; Benner and Strom, 1993) may still be due to a catalytic blank associated with the HTCO analyzer.

6.1.1.2. Vacuum line development.

Blank samples prepared using the SUDO vacuum line showed that the vacuum line did not introduce organic carbon contamination into the samples or fractionate the samples. Therefore, samples were successfully prepared for $\delta^{13}C$ analysis using the SUDO vacuum line, however, problems existed for subsequent ^{13}C samples analyzed, where contamination was found in the samples. The contamination was thought to have been introduced by the combustion process using the SUDO vacuum line, where the main problem seemed to have been lack of a sufficient vacuum. It was not possible on the time scale of this study to upgrade the vacuum line. Due to this further $\delta^{13}C$ and $\Delta^{14}C$ samples were combusted using the vacuum lines at at UCNW and Oxford respectively.

6.1.1.3. Ultrafiltration methods.

An ultrafiltration method was devised to separate the organic colloidal size fraction of DOC (in the size range 20 to 100 nm and 100 to 700 nm). This was achieved using the unique properties of Anotec Anopore aluminium oxide filters which have accurate separation characteristics for various size fractions.

6.1.2. Processes affecting the distribution of organic carbon in the Beaulieu estuary (1990-1992).

Dissolved organic carbon concentrations in the Beaulieu estuary during 1990-1992 were mid-way between the spread of values in world rivers with the RSD for the end-members being around 50 %. The river was also characterized by low SPM values compared to other world rivers reflecting the low errosion rates found in the Beaulieu river and higher than average POC to SPM ratios. This was probably the result of a drainage basin rich in organic matter production and low SPM concentrations in the Beaulieu river. The river and estuary also contained high levels of COC, probably reflecting the high humic acid content found, and at certain times this was the most important size fraction in the estuary.

Dissolved organic carbon was essentially transported conservatively to the coastal zone and was unaffected by high levels of primary productivity in the water column or by high turbidity and high SPM concentrations. The primary source was terrigenous material where DOC levels in the river depended on the dynamics of organic matter mobilisation in the catchment area and input into the river. The riverine DOC input into the coastal zone was highest during the winter months due to increased fresh water discharge. The annual load transported into the coastal zone was 54.6 tonnes of carbon for 1991 and 38.4 tonnes of carbon for 1992.

Terrigenous and sedimentary sources were also identified for the CCOC and FCOC fractions i.e. *Spartina* grasses growing on the edge of the estuary and *Spartina* debris in the sediments, however, other terrigenous material is also likely to contribute to the organic matter content of the water column and these fractions. The FCOC fraction was found to be most representative of the cellulose material of *Spartina* whilst the CCOC fraction was most likely to be comprised of a mixture of material from *Spartina* and POC sources. This probably reflected the fact that *Spartina* debris in the sediments and mud-flats undergoes diagenetic processes hence giving rise to colloidal material. Turbidity in the water column would effectively introduce this material into the water column. An increase in COC levels at higher salinities (above 30) in the coastal zone suggested a source of coastal COC which was more oceanic in nature. This was confirmed by the δ^{13}C values which suggested this material was derived from algal sources. Sources of peat and soil organic material occurring in the POC and CCOC fractions have also been identified in the river whilst other sources for POC include phytoplankton activity and sedimentary resuspension. Terrigenous POC is the most important source in the river whilst *in-situ* phytoplankton POC production is the prominent source in the coastal zone. Periods of high phytoplankton productivity in the estuary would provide important sources of POC within the estuary.

Lateral advection and possible resuspension events occurring in the estuary were associated with high turbidity (high SPM levels) in mid-estuary and in the salinity range 20 to 35. No definite evidence for a turbidity maximum was seen in the SPM levels. The high turbidity caused a decrease in the concentrations of CCOC in the water column (as well as changes in the concentrations of other parameters, for example, oxygen and POC) by introducing material into the estuary from coastal waters (lateral advection) and possibly from the sediments (resuspension). If the decrease in CCOC concentrations at these higher salinities (20 to 30 PSU) was due to aggregation of the CCOC fraction it would be an interesting and novel process.

Sources of organic material (principally POC) were also identified as being due to phytoplankton activity. Primary productivity was low in the river with the estuary showing the highest phytoplankton activity. Only on one occasion was there evidence for the the summer phytoplankton bloom occurring in the coastal zone. Low chlorophyll *a* values reflected the unpolluted nature of the river and estuary. The overall effect of SPM lowering primary productivity in the Beaulieu estuary may not be important and this may be due to the low SPM content of the estuary. Phytoplankton sources of POC have been identified during periods of high productivity in the water column, however, *Spartina* also contributes to POC in the high turbidity regions even during periods of high primary productivity. High phytoplankton activity and high levels of SPM which introduced POC into the water column had little affect on the concentrations of COC in the estuary.

Sinks for estuarine organic material were identified as bacterial degradation and respiration, even though the fresh water residence time was short (around 7 days). This may be explained by the fact that certain materials degrade very quickly such as detritus from phytoplankton. Bacterial degradation events in the water column were due to a small sewage input in mid-estuary, higher water column temperatures and the end of a phytoplankton bloom event. It was found that these degradation events effectively introduced COC into the estuary. Degradation of the phytoplankton bloom introduced FCOC into the water column whilst degradation of terrigenous material introduced CCOC material into the water column. This observation was only tentative and the difference may have been due to other reasons such as the progress of the degradation process. Sinks for POC also included settling to the sediments where most of the POC content of the estuary was deposited within the coastal zone.

A possible aggregation process in the low salinity range (0 to 5 PSU) was most likely to be flocculation due to the high levels of humic acids in the river. Both CCOC and FCOC showed evidence for this removal process as did DOC. Flocculation was most likely to be due to changes in pH, pE and ionic strength.

6.1.3. $\Delta^{14}C$ activities found in the Beaulieu estuary (autumn 1991).

High levels of ^{14}C found in the Beaulieu estuary have not been reported previously and it was supposed that they were due to either anthropogenic contamination or contamination introduced into the samples via handling and preparation. A number of possible anthropogenic sources for the elevated ^{14}C levels in the environment were discounted. It was unlikely that handling of the samples was responsible due to good blank results and clean handling procedures. An analytical artifact was also discounted as a possible reason for the high ^{14}C levels. It is difficult to envisage a source of ^{14}C which would produce the high ^{14}C activities found in the samples. However, it was possible that in some way contamination was introduced via the handling procedures.

6.2. Further research.

A number of areas can be identified for further research work that continues the themes developed in this study. The main thrust would be to use the powerful combination of carbon isotopic analysis with quantification of organic carbon in different size fractions to better understand the sources and cycling of organic carbon in the estuarine environment. Further method development would concentrate on developing the UV persulphate DOC analyzer of Statham and Williams (1983).

6.2.1. Sources and cycling of organic carbon in the estuarine environment.

(1) Testing of the sediments in the Beaulieu estuary and west Solent for elevated levels of ^{14}C using scintillation counting. If high levels are found then this would provide further evidence to support the high $\Delta^{14}C$ values observed for the POC and CCOC fractions in the water column of the Beaulieu estuary (this study, autumn 1991).

(2) Use of the AMS technique to measure $\Delta^{14}C$ values of the POC and CCOC fractions in the Beaulieu river, estuary and coastal zone. This represents a repeat of the work carried out for the autumn 1991 survey. Potentially very useful information could result from such a study where measurement of the $\Delta^{14}C$ and $\delta^{13}C$ values for this material would differentiate between possible sources. For example POC identified as terrigenous from the $\delta^{13}C$ values in the river may be derived from peat sources or from plant material and POC in the coastal zone may consist of organic material derived from the sediments or from phytoplankton. The CCOC and FCOC fraction are thought to be derived from *Spartina* debris (this study), however, whether this material is recent or is older and comes from the sediments is not known. Also it has been suggested that degradation

processes give rise to COC. The material may be derived from peat material, terrestrial plants or from phytoplankton sources. $\Delta^{14}C$ measurements in all these cases would help distinguish between possible sources and cycling processes.

(3) Further sampling of DOC and COC concentrations over the whole salinity would help to confirm the removal processes occurring at low salinity (0 to 5) for DOC and COC (this study; **Chapter 4.5.4**) as well as investigate other regions in the estuary where removal or addition processes may occur. For example, the CCOC fraction showed evidence for a removal process at higher salinities (20 to 30 PSU) in the estuary. It was likely that this was due to mixing of coastal waters in the estuary, however, aggregation of the CCOC fraction at these higher salinites, if it did occur, would be an interesting and novel process. Detailed knowledge of freshwater river flow in the Beaulieu river throughout the year would enable accurate determination of the flux of organic carbon into the west Solent. Processes occur within the turbidity maximum, hence better knowledge of the position of the turbidity maximum (if it existed in the estuary at that time) would be beneficial as a reason for the occurrence of these processes. More intensive sampling of COC over a shorter timescale in the estuary would help to study the production of COC. Most information would be gained if this was undertaken during periods of high degradation rates in the estuary, for example after the occurrence of a phytoplankton bloom or during periods of high organic concentration in the estuary. Measurement of COC concentrations in the coastal zone and out into the open ocean would confirm whether high COC levels persist out into the sea. If $\delta^{13}C$ analysis were included then the extent of the colloidal *Spartina* distribution, and other material derived from terrestrial sources, out into the ocean could be examined. Analysis of the $\delta^{13}C$ signatures of the various size fractions throughout the year would show whether Spartina debris is present in the water column year round and also if other material from other sources is present. Sampling of COC in different estuaries could be undertaken to see if high COC levels are present in other estuaries. Estuaries would be chosen that have differing characteristics such as freshwater discharge, different drainage basin characteristics, higher anthropogenic input, varying freshwater residence times etc.

6.2.2. Method development.

(1) Further development of colloidal separation techniques using Anodisc Anopore filters, perhaps incorporating filters with other pore sizes. A number of improvements to the technique could be made by, for example, increasing the flow rate of the system, having different sized filters in-line (this would reduce sample handling), use of cross-flow filtration methods and making the filtration system portable for use in the field hence reducing the time delay between sampling and filtration which may alter the nature of the organic matter in the sample.

(2) Regular intercalibration exercises between the HTCO DOC technique (which is continuosly being developed) and the SUDO UV persulphate DOC analyzer would provide information on differences between the two techniques.

(3) The use of mercury salts to increase the oxidation efficiency of the SUDO UV persulphate DOC analyzer gave promising results (this study; **Chapter 3.2.6**). Further work should focus on increasing the concentration of mercury salts in the oxidizing solution, the testing of other salts (for example silver salts) to see if they increase DOC oxidation efficiency and studies on the nature of the DOC decomposition process.

Appendix I:

Units used to measure carbon isotopes.

I.1. Units for measuring ^{13}C.

The stable carbon isotope ratio is expressed as the parts per thousand deviation from a standard, according to the equation:

$$\delta^{13}C = \left(\frac{R}{R_0} - 1\right) X\, 1000 \quad ‰$$

where R is the measured ratio of carbon-13 to carbon-12 of the sample and R_0 is the same ratio of the standard; a Cretaceous carbonate, *Belemnita americana* from the Pee Dee formation of South Carolina, known as PDB (Craig, 1953).

I.2. Units for measuring ^{14}C.

Measurement of the carbon-14/carbon-12 ratios (or count rates) for the sample (S; background corrected) and modern standard (M) are needed to calculate the enrichment or depletion of carbon-14 ($d^{14}C$) of the sample with respect to the standard:

$$d^{14}C = \left(\frac{S}{M} - 1\right) X\, 1000 \quad ‰$$

The measured or estimated value for the carbon-13/carbon-12 ratio is then used to normalize ($D^{14}C$) this measured $d^{14}C$ value:

$$D^{14}C = d^{14}C - 2(\delta^{13}C + 25)(1 + 10^{-3}\, d^{14}C) \quad ‰$$

The units for both $d^{14}C$ and $D^{14}C$ are parts per thousand deviation from the modern reference standard. Samples with $\delta^{13}C = -25$ ‰ will have $d^{14}C = D^{14}C$, but all other samples will have $D^{14}C$ different from $d^{14}C$.

The conventional radiocarbon age is then calculated from the equation:

$$AGE = 8033 \ln\left[\frac{1}{1 + 10^{-3}\, D^{14}C}\right] \quad years\ BP.$$

where the constant 8033 is the average lifetime of a carbon-14 atom (derived from the half-life of 5568 years). An age calculated in this way is referred to as a conventional radiocarbon age, which implies that the following assumptions and conventions have been observed:-

(1) 5568 year half-life for carbon-14 (Libby half-life).

(2) 0.95 NBS oxalic acid modern standard.

(3) normalization to $\delta^{13}C = -25$ ‰.

(4) AD 1950 reference year zero.

Conventional radiocarbon ages are expressed as years BP (Before Present) where the present is defined to be AD 1950.

The internationally recognized modern reference standard for radiocarbon age measurements is 95 % of the measured count rate (or $^{14}C/^{12}C$ ratio) of an oxalic acid sample, SRM4990, prepared by the U.S.A. National Bureau of Standards, Known as 0.95 NBS oxalic acid (Gillespie, 1984).

I.2.1. The difference between percent modern (%M) and absolute percent modern (pM).

The quantities associated with %M are $d^{14}C$, $D^{14}C$ and the age BP. The quantities associated with pM are $\delta^{14}C$, $\Delta^{14}C$ and the specific activity (SA) (Stuiver and Pollach, 1977; personal communication, C. Bronk, Oxford University Radiocarbon Accelerator Unit), where:

$$\delta^{14}C = \left[\frac{S}{M_{abs}} - 1\right] 1000 \text{ ‰}$$

(M_{abs} = absolute international standard activity)

$$\Delta^{14}C = \delta^{14}C - 2(\delta^{13}C + 25)(1 + 10^{-3}\ \delta^{14}C) \text{ ‰}$$

When the activity at a particular time (after AD 1950) is needed, for example in 1992, pM (along with $\delta^{14}C$, $\Delta^{14}C$ and SA) are used. %M (along with $d^{14}C$, $D^{14}C$ and the age BP) are normalized to AD 1950 allowing comparisons between ^{14}C measurements. The values $d^{14}C$ and $\delta^{14}C$ are uncorrected for the $\delta^{13}C$ of the sample whereas $D^{14}C$ and $\Delta^{14}C$ are normalized to -25 per mil (in both cases the standard is normalized to -19 per mil). To convert between the two sets it is necessary to know the date of measurement of the calibrating standard:

$$pM = \%M\left(e^{\left(\frac{-(y - 1950)}{8267}\right)}\right)$$

where y is the year of measurement which indicates that if the measurement was made long after 1950, the standard would have decayed a little and so the activity of the sample measured is a little lower in absolute terms.

To convert within individual sets the following conversions are used:

$$D^{14}C = d^{14}C - 2(\delta^{13}C + 25)\left(1 + \frac{d^{14}C}{1000}\right)$$

$$\Delta^{14}C = \delta^{14}C - 2(\delta^{13}C + 25)\left(1 + \frac{\delta^{14}C}{1000}\right)$$

$$pM = 100\left(1 + \frac{\Delta^{14}C}{1000}\right)$$

$$\%M = 100\left(1 + \frac{D^{14}C}{1000}\right)$$

The activity (only relevant to the absolute measurements) for the absolute modern standard is 13.56 dpm/gC which is equal to 226 $\mathrm{BqKg^{-1}C}$. Therefore:

$$pM = 100\left(\frac{SA}{226}\right)$$

To convert between pM and the radiocarbon age the following equation can be used:

$$pM = 100\left(e^{\left(\frac{-t}{8033}\right)}\right)\left(e^{\left(\frac{-(y - 1950)}{8267}\right)}\right)$$

Appendix II:
Data tables.

II.1. Data for the Beaulieu estuary surveys (1990-1992).

(Station numbers correspond to positions in Figures in **Appendix III**).

Autumn 1990					
Station	**DOC (μM C)**	**0.1 μm filtrate (μM C)**	**0.02 μm filtrate (μmC)**	**POC (μM C)**	**SPM (mg/l)**
1	25	21	10	20.4	10.5
2	50	48	46	13.3	5.3
3	83	75	72	13.3	5.4
4	158	80	68	16.7	6.3
5	175	83	72	8.3	14.4
6	200	92	72	12.5	5.2
7	250	125	81	22.5	3.2
8	266	112	68	26.6	5.4
9	383	142	89	44.1	3.1
10	516	221	110	79.9	9.9

Table II.1. Autumn 1990 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Autumn 1990	
Station	**Salinity (PSU)**
1	34.7
2	31.6
3	29.1
4	28.6
5	27.1
6	25.5
7	20.8
8	19.2
9	3.1
10	1.4

Table II.2. Autumn 1990 Beaulieu estuary survey; salinity.

Winter 1991					
Station	**DOC (μM C)**	**0.1 μm filtrate (μM C)**	**0.02 filtrate (μM C)**	**POC (μM C)**	**SPM (mg/l)**
1	108	109	83	16.7	9.4
2	187	189	142	25.8	9.4
3	191			21.6	8.0
4	208	208	162	21.6	8.3
5	237	233	183	28.3	7.8
6	283	262	212	27.5	6
7	296			33.3	8.0
8	300	291	225	32.5	7.1
9	362	346	283	53.3	9.7
10	550	479	391	54.9	6.6
11	558	512	412	42.5	3.0
12	574	525	420	45	3.1
13	566	512	412	84.1	9.1
14	500	479	400	25	1.9

Table II.3. Winter 1991 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Winter 1991					
Station	O_2 (µM)	O_2 (%sat)	Chl.a (µg/l)	Pha. (µg/l)	Salinity (PSU)
1	293	90	0.28	0.22	32.5
2	281	82	0.26	0.29	27.2
3	209	61	0.26	0.24	26.6
4	247	71	0.34	0.33	26
5	302	85	0.43	0.27	24.3
6	234	65	0.34	0.31	21.8
7	306	83	0.52	0.36	21
8	259	71	0.47	0.38	20.4
9	361	95	0.43	0.49	16.4
10	367	89	0.34	0.71	3.2
11	330	79	0.26	0.67	2.1
12	345	82	0.13	0.72	1
13	325	77	2.59	1.04	0.2
14	365	87	0.17	0.2	0.1

Table II.4. Winter 1991 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

Spring 1991					
Station	**DOC (μM C)**	**0.1 μm filtrate (μM C)**	**0.02 μm filtrate (μM C)**	**POC (μM C)**	**SPM (mg/l)**
1	122	111	121	96.6	21.3
2	164	162	147	78.3	24.0
3	225	189	198	66.6	20.6
4	214	215	206	67.4	17.0
5	231	228	221	252	86.7
6	252	234	229	81.6	22.4
7	265	245	237	91.6	26.5
8	322	270	262	90	26.1
9	306	293	291	83.3	20.8
10	331	310	320	47.4	4.7
11	405	398	395	39.1	4.9

Table II.5. Spring 1991 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Spring 1991					
Station	**O_2 (μM)**	**O_2 (%sat)**	**Chl.a (μg/l)**	**Pha. (μg/l)**	**Salinity (PSU)**
1	333	124	7.76	0.06	33.2
2	303	113	7.34	0.07	31.2
3	277	102	5.45	0.31	27.3
4	266	98	4.19	0.64	26.6
5	275	100	1.99	0.63	25.4
6	253	92	4.61	0.94	25.5
7	339	122	4.19	1.16	22.5
8	340	120	6.08	1.43	19.6
9	349	121	6.71	1.11	16.4
10	338	107	5.24	0.32	1.5
11	302	95	1.78	0.53	0.1

Table II.6. Spring 1991 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

Summer 1991					
Station	**DOC (μM C)**	**0.1 μm filtrate (μM C)**	**0.02 μm filtrate (μM C)**	**POC (μM C)**	**SPM (mg/l)**
1	27	18	10	22.5	6.0
2	88	57	30	35	9.7
3	123	98	59	30.8	9.3
4	216	137	109	26.6	6.3
5	246	183	142	39.1	7.3
6	287	204	170	34.9	6.7
7	283	212	171	45.8	8.4
8	308	241	184	40.8	5.1
9	519	375	305	99.1	8.9
10	729	482	388	133	9.4
11	1070	594	488	147	12.4
12	808	574	521	165	13.9
13	999	803	688	281	23.4
14	1290	992	813	130	6.6

Table II.7. Summer 1991 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Summer 1991					
Station	**O_2 (μM)**	**O_2 (%sat)**	**Chl.*a* (μg/l)**	**Pha. (μg/l)**	**Salinity (PSU)**
1	265	115	0.27	0.13	34
2	227	97			31.5
3	209	89	0.96	0.22	29.8
4	204	87	1.05	0.44	27.6
5	208	88	1.36	0.44	26.1
6	204	85	1.47	0.54	24.9
7	204	86			24.7
8	219	92	1.78	0.38	24.1
9					16.3
10	224	97	4.82	0.43	12
11					9.9
12			4.19	0.54	10.1
13	334	129	16.77	1.24	1.1
14			0.34	0.27	0.1

Table II.8. Summer 1991 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

Autumn 1991					
Station	**DOC (μM C)**	**0.1 μm filtrate (μM C)**	**0.02 μm filtrate (μM C)**	**POC (μM C)**	**SPM (mg/l)**
1	125	110	105	25.8	10.1
2	162	145	134	24.9	9.2
3	200	186	180	29.1	9.8
4	245	216	230	37.5	9.2
5	258	226	238	38.3	11.3
6	275	243	250	42.5	9.4
7	296	288	283	53.3	12.0
8	325	316	313	266	35.6
9	333	316	321	89.9	16.8
10	380	358	353	110	15.1
11	391	381	380	112	15.3
12	499	491	479	56.6	5.4

Table II.9. Autumn 1991 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Autumn 1991					
Station	**O_2 (μM)**	**O_2 (%sat)**	**Chl.a (μg/l)**	**Pha. (μg/l)**	**Salinity (PSU)**
1	274	96	0.71	0.26	32.5
2	279	96	0.69	0.2	30.1
3	278	93	0.98	0.25	27.7
4	281	93	1.26	0.34	24.9
5	278	90	0.94	0.44	23.2
6	291	93	1.89	0.58	21.5
7	295	93	2.2	0.89	18.7
8	306	96	2.93	1.18	15.8
9	308	93	4.19	1.57	12.4
10	288	83	3.14	1.49	6
11	289	82	1.47	0.9	2.9
12	274	76	0.4	0.28	0.1

Table II.10. Autumn 1991 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

Winter 1992					
Station	DOC (μM C)	0.1 μm filtrate (μM C)	0.02 μm filtrate (μM C)	POC (μM C)	SPM (mg/l)
1	76	66	54	29.1	48.9
2					
3	101	99	79	21.6	47.8
4	131	123	105	34.9	52.1
5	141	141	118	26.6	40.9
6	149	141	121	29.9	42.4
7	162	145	129	19.1	39.1
8	213	183	179	28.3	31.0
9	213	194	183	33.3	25.5
10	251	224	229	36.6	19.3
11	276	243	231	45.7	20.8
12	349	308	296	41.6	4.9

Table II.11. Winter 1992 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Winter 1992					
Station	O_2 (μM)	O_2 (%sat)	Chl.a (μg/l)	Pha. (μg/l)	Salinity (PSU)
1	306	99	0.36	0.13	34.7
2	310	101	0.33	0.28	34.6
3	314	101	0.42	0.17	32.8
4	327	103			29.9
5	318	99	0.96	0.17	28.2
6	334	104	0.94	0.19	27.5
7	345	106	0.9	0.13	26
8	341	106	0.61	0.28	20.2
9			0.73	0.24	16.3
10	361	100	0.59	0.24	10.1
11					8.4
12	360	95	0.86	0.14	0.1

Table II.12. Winter 1992 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

Spring 1992					
Station	DOC (μM C)	0.1 μm filtrate (μM C)	0.02 μm filtrate (μM C)	POC (μM C)	SPM (mg/l)
1	66	54	54	40.8	25.9
2	83	74	79	24.9	12.9
3	108	112	117	21.6	12.9
4	145	141	138	37.4	16.3
5	186	174	173	35.8	11.1
6	199	178	166	40.8	13.3
7	228	203	188	36.6	12.3
8	291	264	242	43.2	6.6
9	295	268	253	44.1	4.6
10	295	268	253	44.9	5.8
11	314	283	281	41.6	4.6
12	474	466	416	68.2	4.3

Table II.13. Spring 1992 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Spring 1992					
Station	O_2 (μM)	O_2 (%sat)	Chl.*a* (μg/l)	Pha. (μg/l)	Salinity (PSU)
1	290	102	0.67	0.28	34.5
2	289	102	0.93	0.27	33.9
3	282	99	1.07	0.28	31.5
4	282	98	0.96	0.24	29.5
5	284	97			27.5
6	288	98	1.97	0.6	26.9
7	307	101	1.63	0.43	24.4
8	340	109	1.48	0.32	18.4
9	301	98	1.31	0.37	18.1
10	328	107	1.92	0.49	17.7
11	320	103	1.34	0.33	15.7
12	302	88	0.64	0.36	0.1

Table II.14. Spring 1992 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

Summer 1992					
Station	**DOC (μM C)**	**0.1 μm filtrate (μM C)**	**0.02 μm filtrate (μM C)**	**POC (μM C)**	**SPM (mg/l)**
1	119	95	83	28.3	5.8
2	112	106	88	23.3	15.0
3	191	172	163	30.8	14.1
4	241	224	209	24.9	7.3
5	258	253	236	20.8	3.7
6	298	260	250	31.6	4.3
7	312	270	263	27.4	3.7
8	306	270	256	27.4	4.9
9	320	276	263	30.8	3.5
10	432	396	384	273	25.1
11	358	333	321	205	11.8

Table II.15. Summer 1992 Beaulieu estuary survey; organic carbon fractions, POC and SPM levels.

Summer 1992					
Station	**O_2 (μM)**	**O_2 (%sat)**	**Chl.a (μg/l)**	**Pha. (μg/l)**	**Salinity (PSU)**
1	211	90	0.54	0.21	34.7
2			1.89	0.74	34.3
3	226	98	1.78	0.64	33.1
4	259	111	1.26	0.44	30.9
5	257	110	1.05	0.6	27.1
6			2.41	0.52	28.1
7	262	113	2.72	0.46	27.2
8	259	111	1.88	0.38	26.8
9	258	110	0.4	0.18	26.7
10	221	91	15.51	1.47	16
11	236	79	9.01	0.66	4.2

Table II.16. Summer 1992 Beaulieu estuary survey; oxygen levels, chlorophyll a and phaeo-pigment concentrations, salinity.

II.2. Carbon-13 isotope data.

POC (‰)	Precision	CCOC (‰)	Precision	Salinity (PSU)
Summer 1991				
-19.4	0.006	-21.2	0.015	34.5
-17.7	0.009	-22.2	0.004	32.9
-26.8	0.003	-24.3	0.006	25.8
-28.5	0.009	-28.7	0.013	0
Autumn 1991				
				34.8
-24.9	0.03	-15.3	0.07	28.8
		-30.1	0.09	15
-35.0	0.08	-21.5	0.14	0.1
Winter 1992				
-8.0	0.003	-21.7	0.003	34.6
-7.7	0.002	-21.7	0.006	34.7
-15.6	0.005	-20.4	0.298	27.5
-19.1	0.068	-22.2	0.037	16.6
-23.0	0.004			10.1
-19.7	0.062			0.1
Spring 1992				
-6.0	0.087			34.5
-11.3	0.014			31.5
-17.7	0.006			26.9
-19.9	0.212			18.4
-27.1				0.1

Table II.17. $\delta^{13}C$ signatures for the organic carbon fractions for summer 1991, autumn 1991, winter 1992 and spring 1992.

Summer 1992						
POC (‰)	**Precision**	**CCOC (‰)**	**Precision**	**FCOC**	**Precision**	**Salinity (PSU)**
-16.0	0.004	-14.5	0.006	-11.5	0.005	34.4
-18.6	0.006			-11.8	0.009	33.1
-22.6	0.006	-15.7	0.006	-11.8	0.006	28.2
-21.3	0.005	-15.6	0.004	-11.5	0.008	26.8
-23.4	0.007	-16.1	0.005	-11.3	0.007	26.7
-24.8	0.007	-17.9	0.005	-12.0	0.004	16.1
-36.4	0.005	-20.6	0.005	-12.7	0.007	4.3

Table II.18. $\delta^{13}C$ signatures for the organic carbon fractions for summer 1992.

II.3. Carbon-14 isotope data.

Autumn 1991						
Sample site	**Reference no.**	**Filter type**	**Filtrate (l)**	**$\Delta^{14}C$ (%M)**	**Precision**	**Sal. (PSU)**
Lands' End	OXA-3802	GF/F	60	147.2	1	34.9
Needles	OXA-3518	GF/F	50.15	431.5	2.7	34.8
Outer-estuary	OXA-3519	GF/F	40.8	272.4	1.7	28.8
Mid-estuary	OXA-3520	GF/F	18.25	281.6	1.8	15
River	OXA-3521	GF/F	7.45	272.4	1.8	0.1
Lands' End	OXA-3803	Anopore	52.75	475.4	2.8	34.9
Needles	OXA-3522	Anopore	37.65	1226.7	13.9	34.8
Outer-estuary	OXA-3523	Anopore	30.8	939.5	5.9	28.8
Mid-estuary	OXA-3524	Anopore	16.05	687.4	4.3	15
River	OXA-3525	Anopore	7.2	327.8	2.2	0.1

Table II.19. $\Delta^{14}C$ values for the Beaulieu estuary survey, autumn 1991.

II.4. DOC inter-calibration data.

Sample site	SUDO DOC (μM C)	Error bar (μM C)	PML DOC (μM C)	Error bar (μM C)
Seattle inter-calibration workshop, July 1991.				
Surface	92	15	134	8
Mid	47	10	114	23
Deep	43	6	102	8
River	77	5	135	9
Autumn 1991 inter-calibration excercise				
River	500		567	
Upper-estuary	333		377	
Lower-estuary	275		327	
Sea	125		179	
LOCC water			92	
Summer 1992 inter-calibration exercise				
River	358		328	
Upper-estuary	321		300	
Lower-estuary	306		302	
Sea	112		176	
LOCC water			40	

Table II.20. DOC data from the Seattle inter-calibration workshop, autumn 1991 and summer 1992 inter-calibration exercises.

II.5. DOC data generated for DOC analysis using mercury salt solutions.

	River DOC (μM C)	Error bar (μM C)	Sea DOC (μMc)	Error bar (μM C)
Analysis 1	176	2	75	0
Analysis 2	183	7	72	2
Analysis 3	190	8	79	7
Analysis 4	168	0	68	2

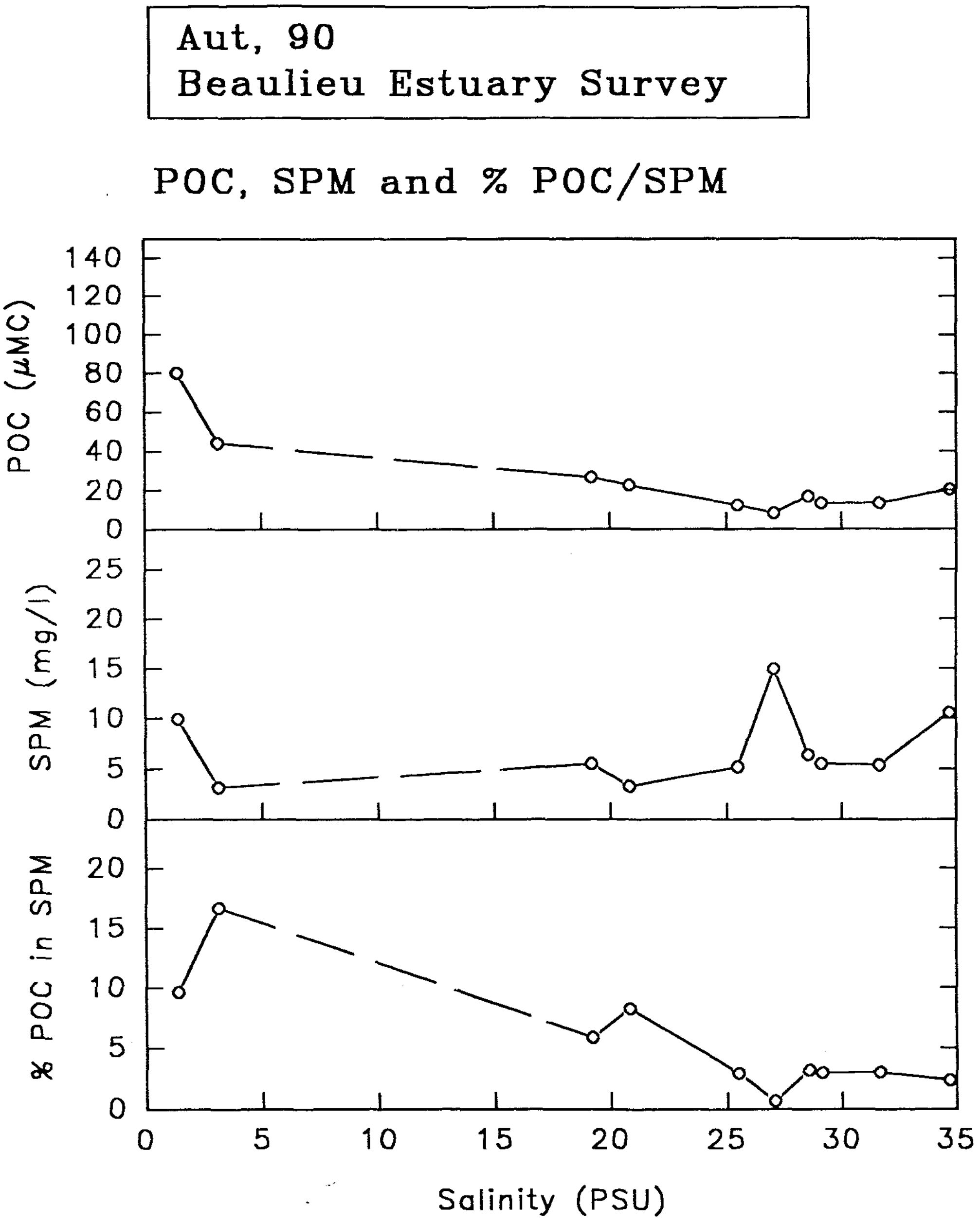
Table II.21. DOC concentrations measured by the UV persulphate DOC analyzer using mercury salt/persulphate oxidizing reagents.

II.6. Flow data for the Beulieu river.

Date	Flow (cumecs)	Date	Flow (cumecs)
9.3.88	0.27	29.9.77	0.06
20.4.76	0.05	16.10.75	0.43
29.9.76	0.99	15.11.74	1.59
27.7.76	0.06	23.10.74	0.31
20.5.76	0.06	29.11.77	0.11
6.10.76	2.4	31.10.78	0.04
11.12.75	0.15	1.10.75	0.06
24.2.76	0.19	18.9.79	0.02
27.1.76	0.13	21.5.80	0.06
23.3.76	0.11	27.7.79	0.02
26.7.77	0.03	19.1.75	0.33
25.11.76	0.17	10.12.74	0.18
28.6.77	0.05	3.9.75	0.02
25.5.77	0.06	30.7.75	0.04
20.4.77	0.13	9.6.75	0.04
13.11.75	0.14	25.1.77	1.1
22.2.77	1.12	14.4.75	0.19
27.10.77	0.11	14.3.75	0.61
16.12.76	0.21	6.2.75	0.27
31.3.77	0.27	12.5.75	0.12

Table II.22. Flow data for the Beaulieu river.

Appendix III.
Beaulieu estuary 1991-1992 plots.



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Beaulieu Estuary Survey

POC, SPM and % POC/SPM

Figure III.1. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, autumn 1990.

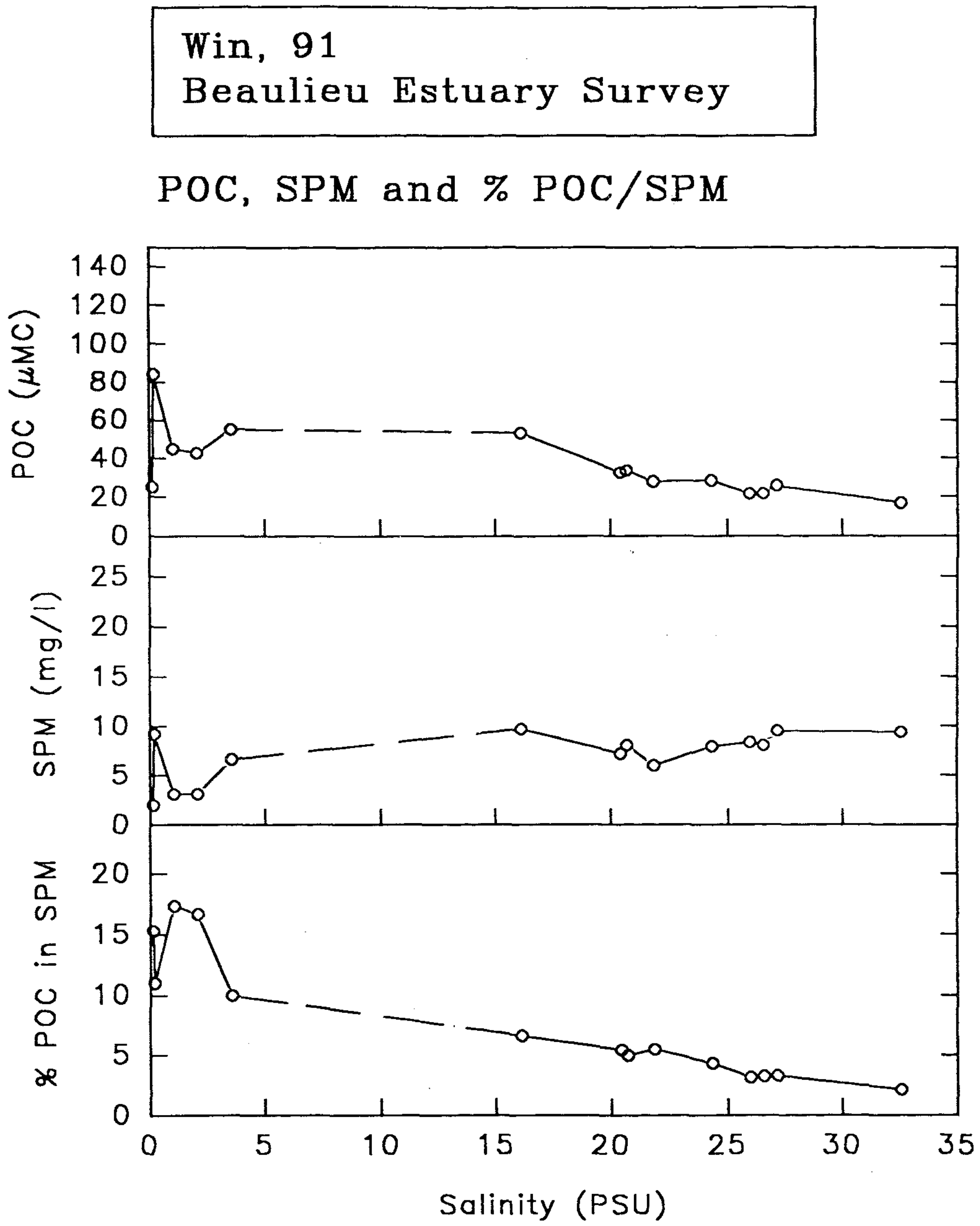


Figure III.2. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, winter 1991.

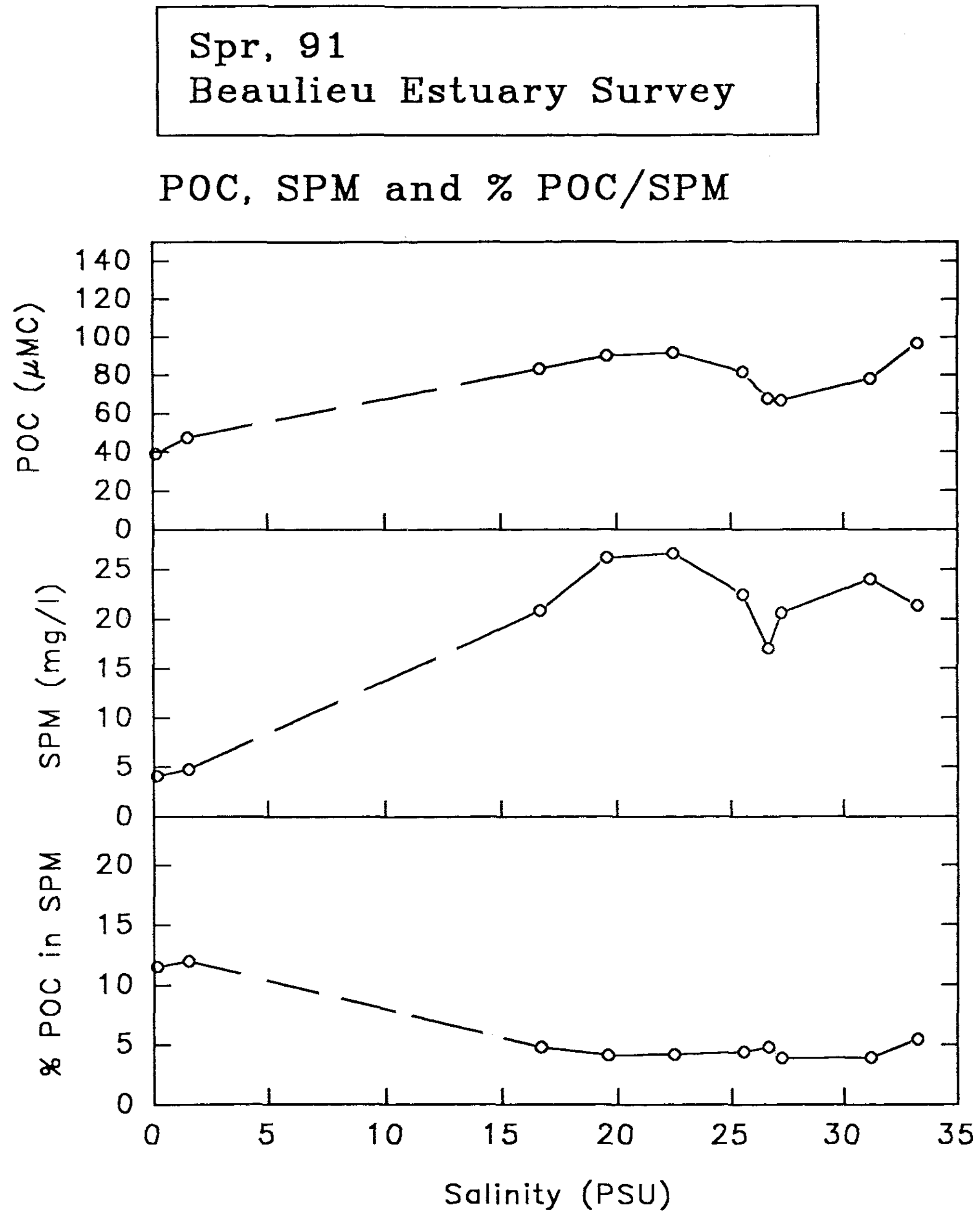


Figure III.3. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, spring 1991.

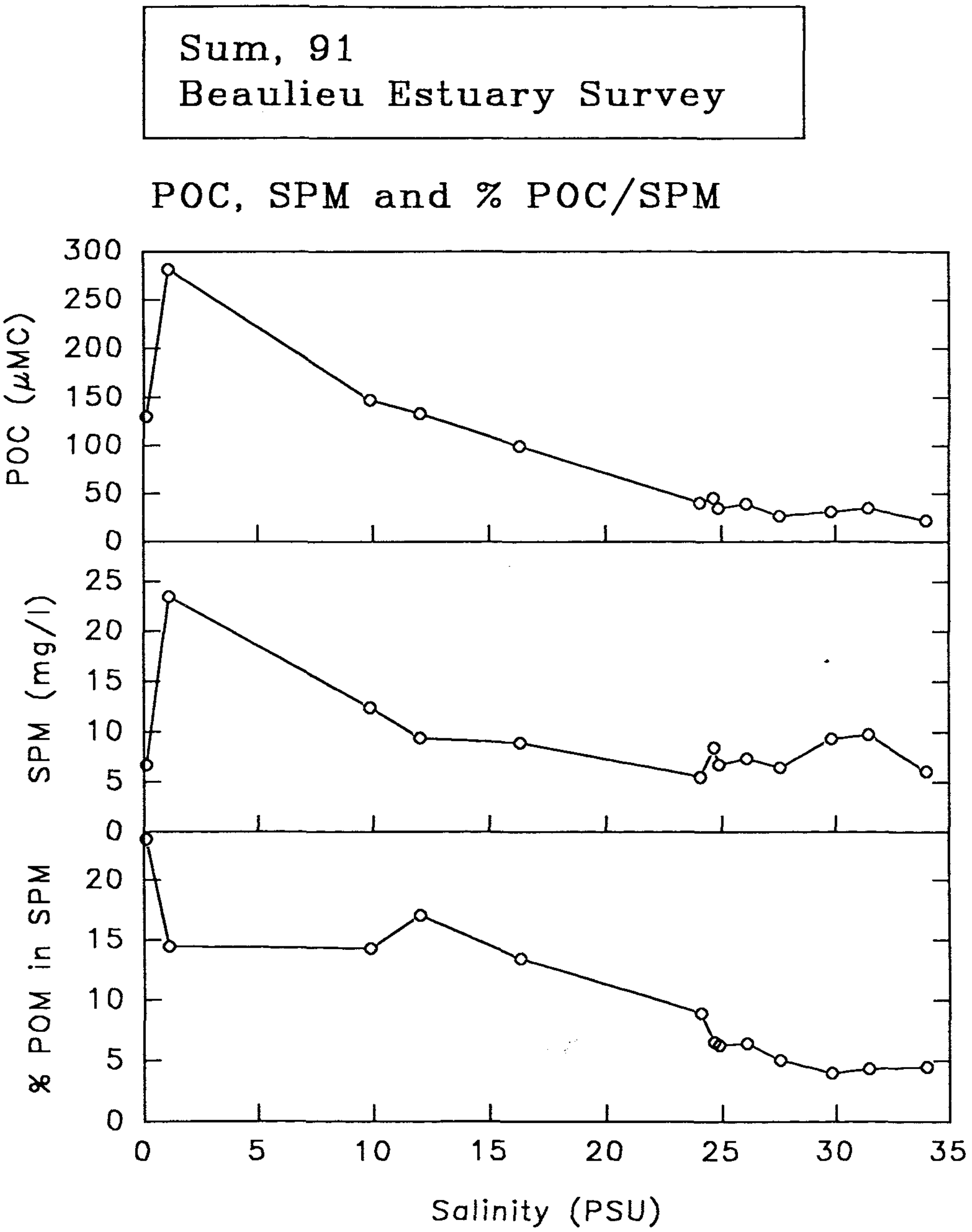
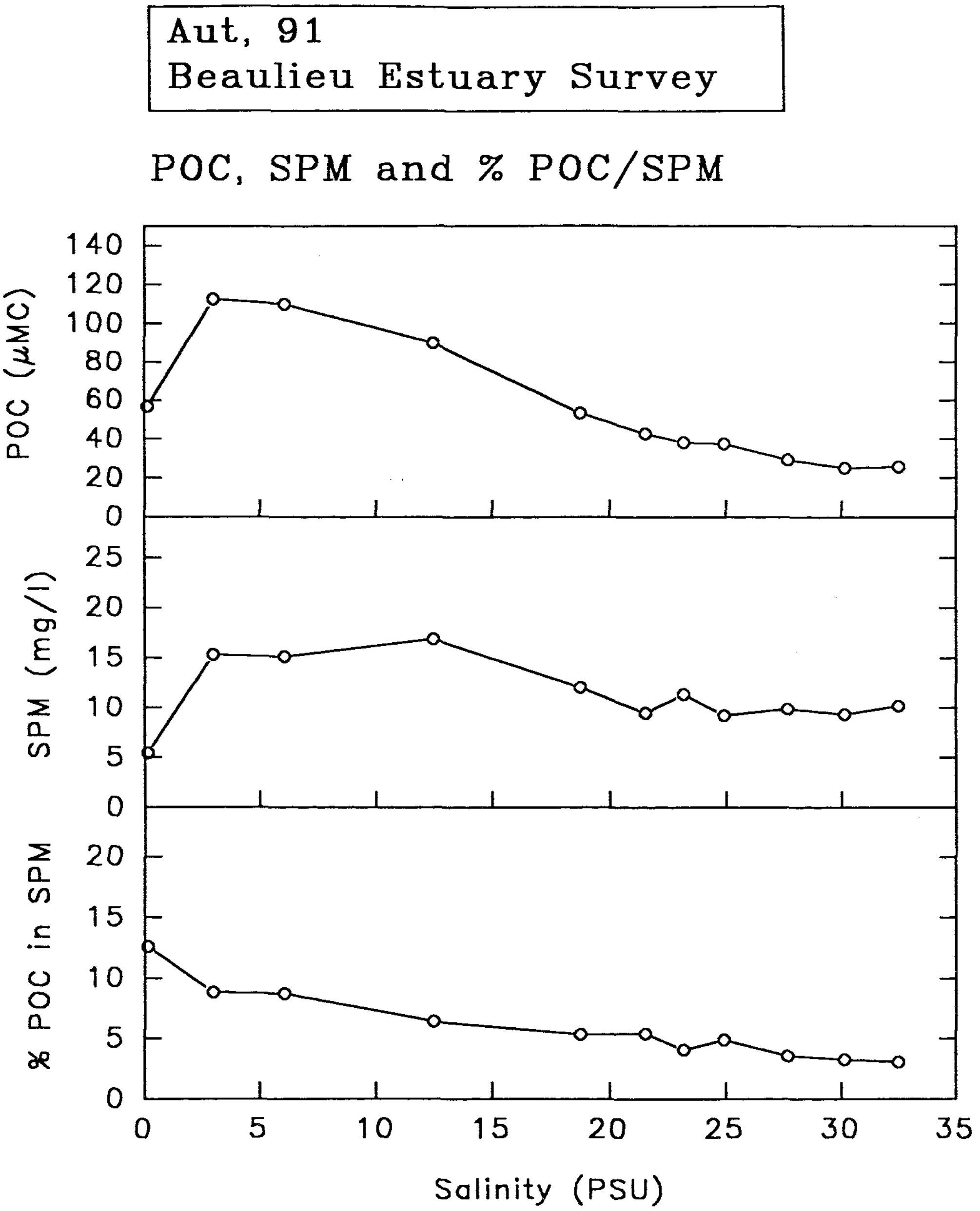


Figure III.4. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, summer 1991.



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Beaulieu Estuary Survey

POC, SPM and % POC/SPM

Figure III.5. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, autumn 1991.

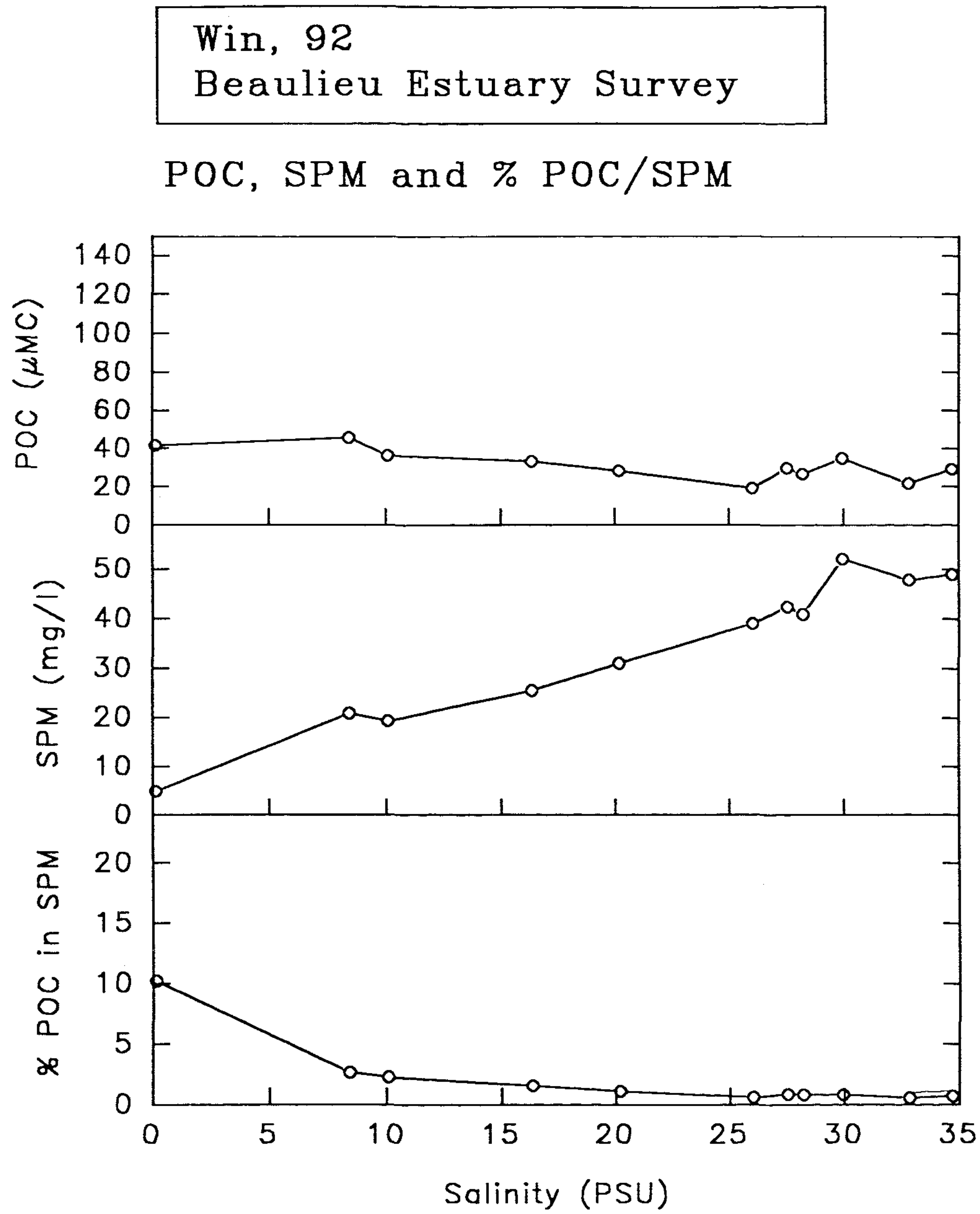

Figure III.6. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, winter 1992.

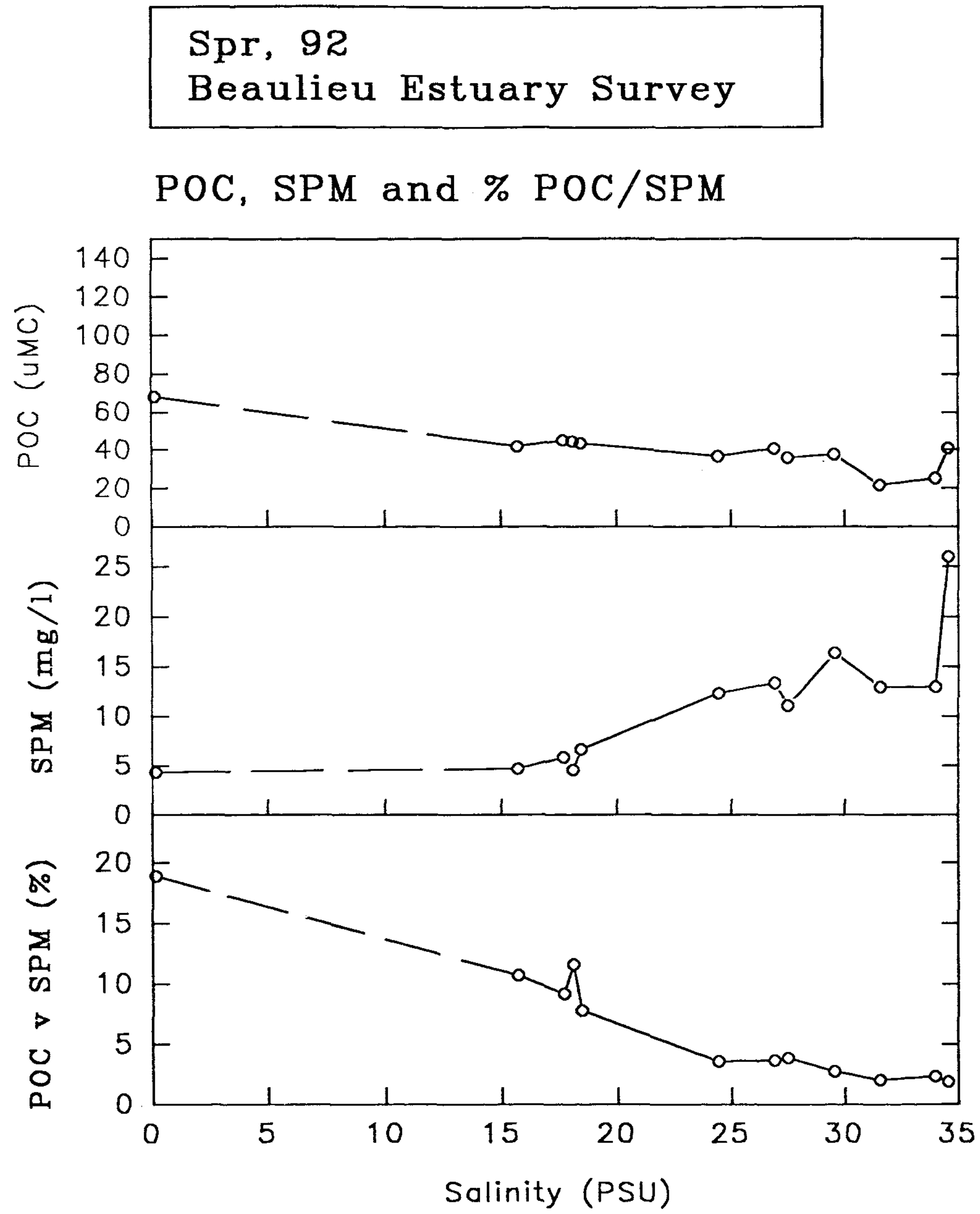

Figure III.7. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, spring 1992.

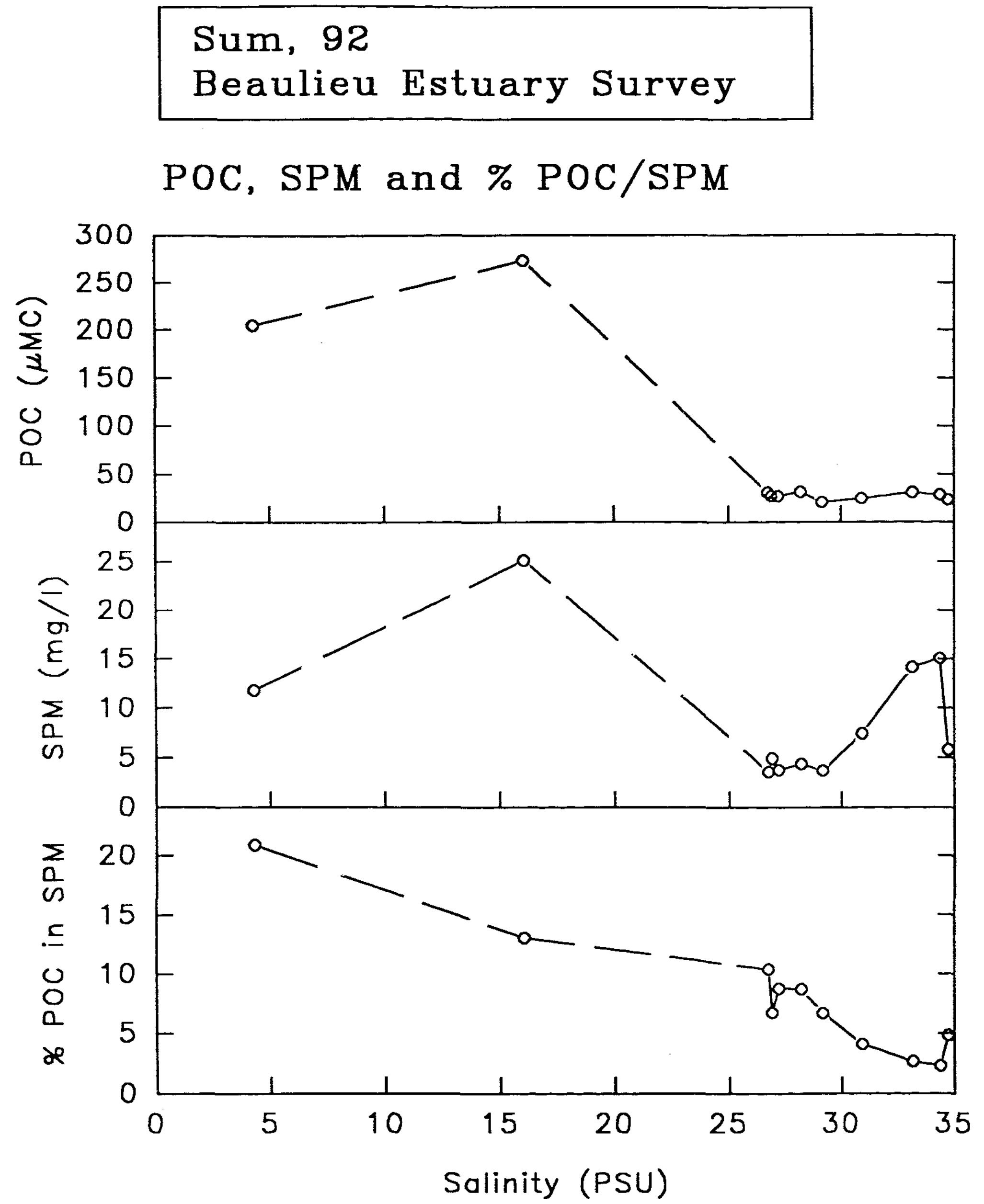

Figure III.8. POC, SPM and POC as a fraction of SPM in the Beaulieu estuary, summer 1992.

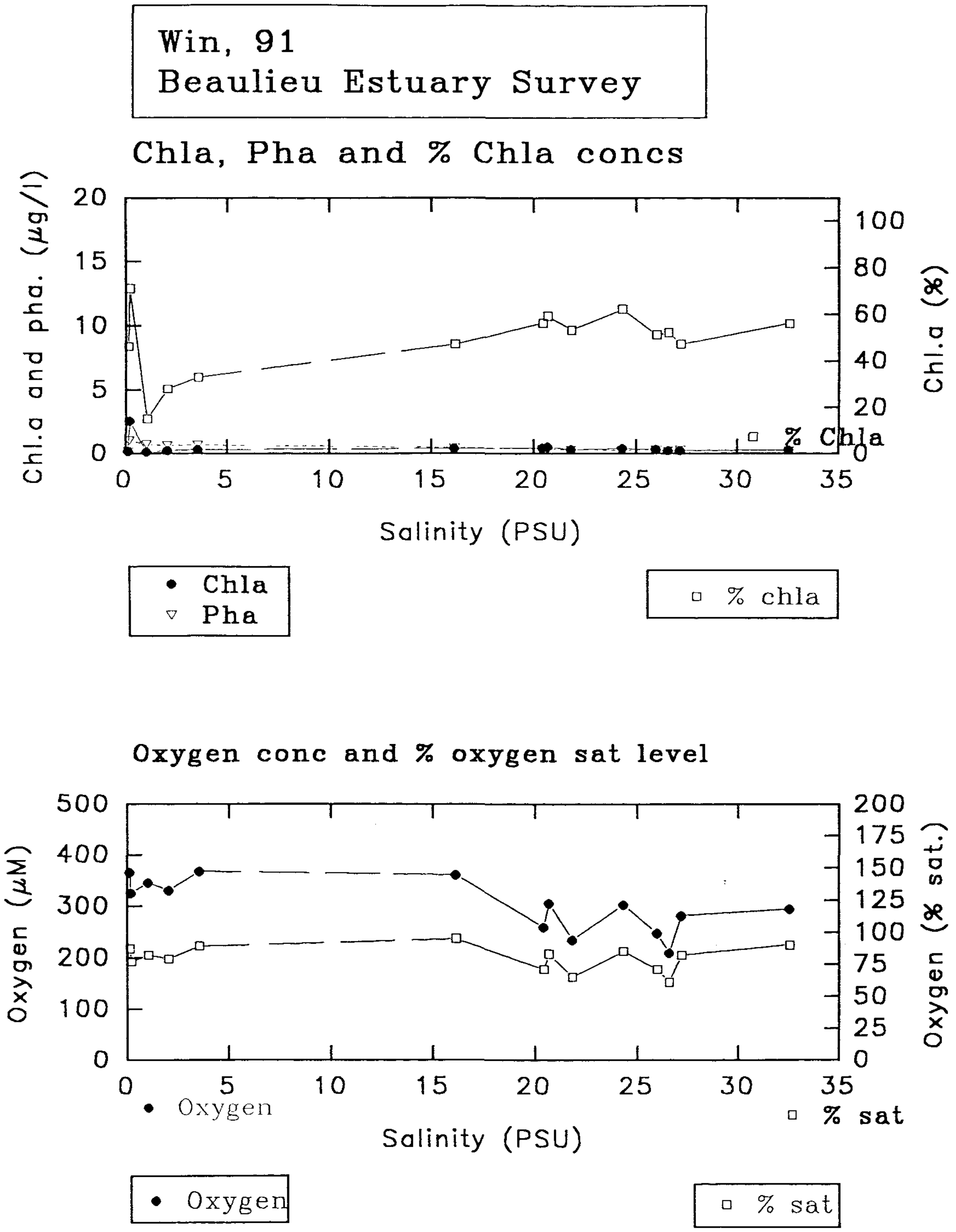

Figure III.9. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, winter 1991.

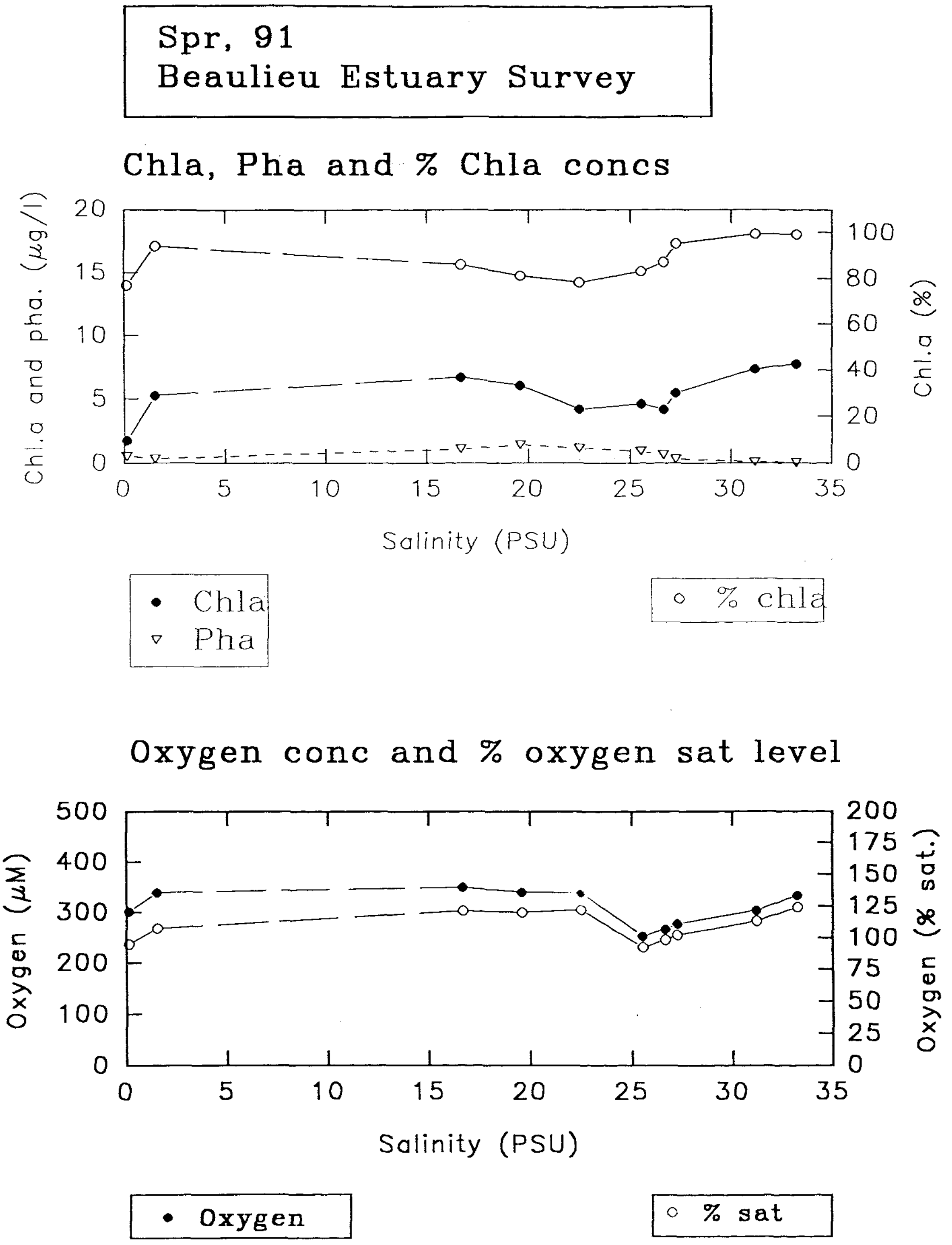

Figure III.10. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, spring 1991.

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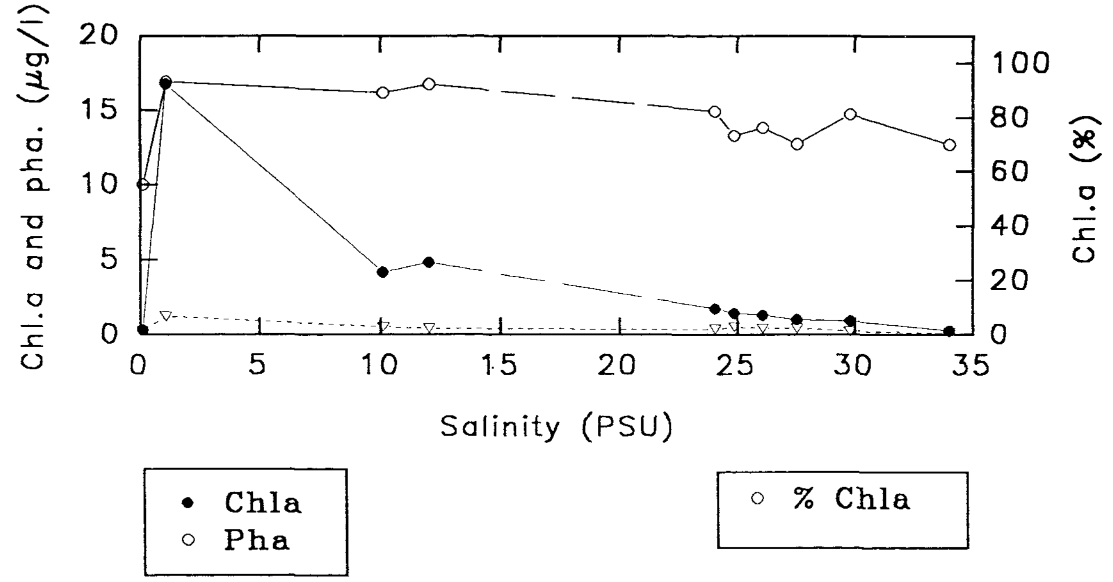
Beaulieu Estuary Survey

Chla, Pha and % Chla concs



Oxygen conc and % oxygen sat level

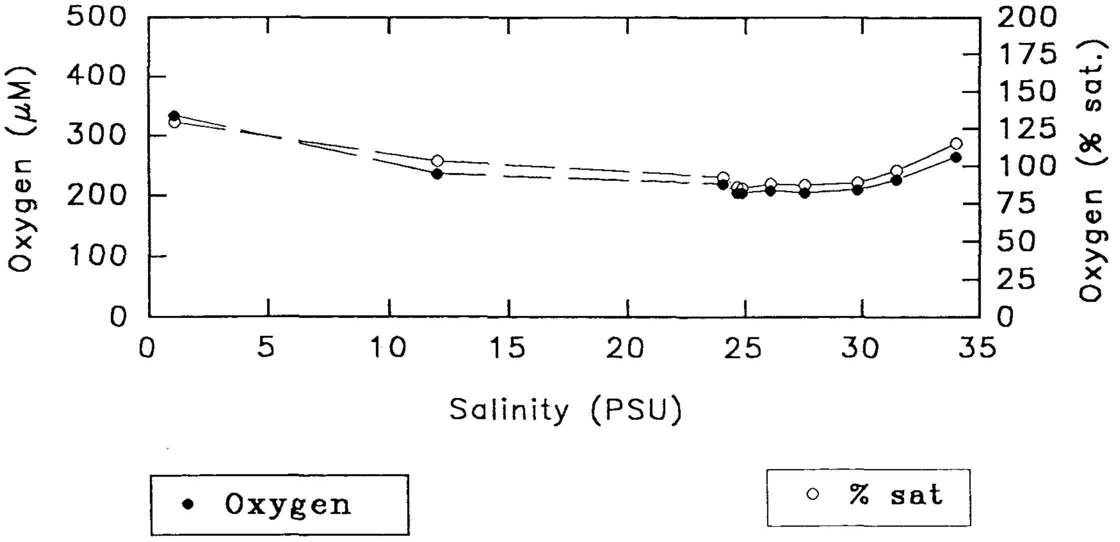


Figure III.11. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, summer 1991.

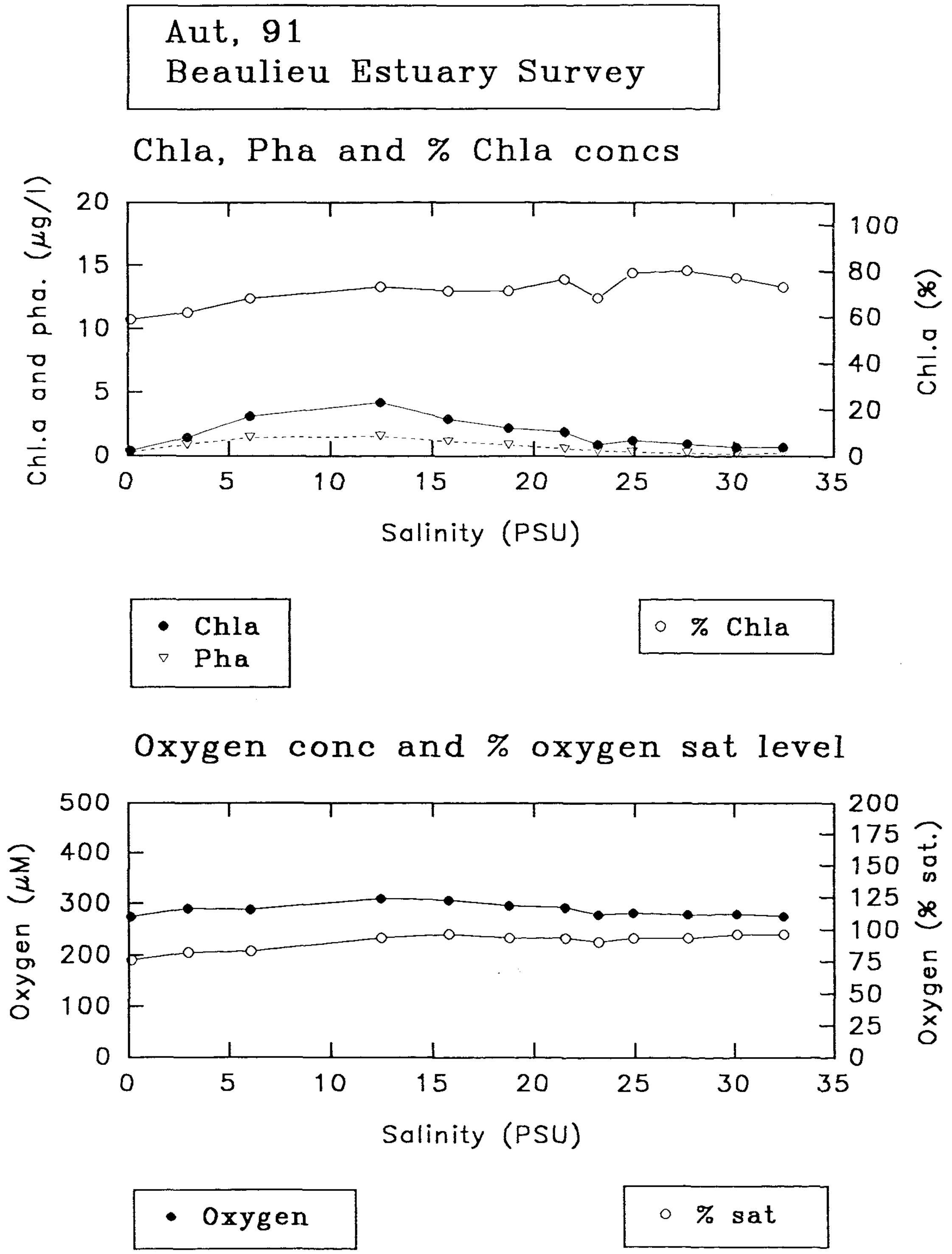


Figure III.12. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, autumn 1991.

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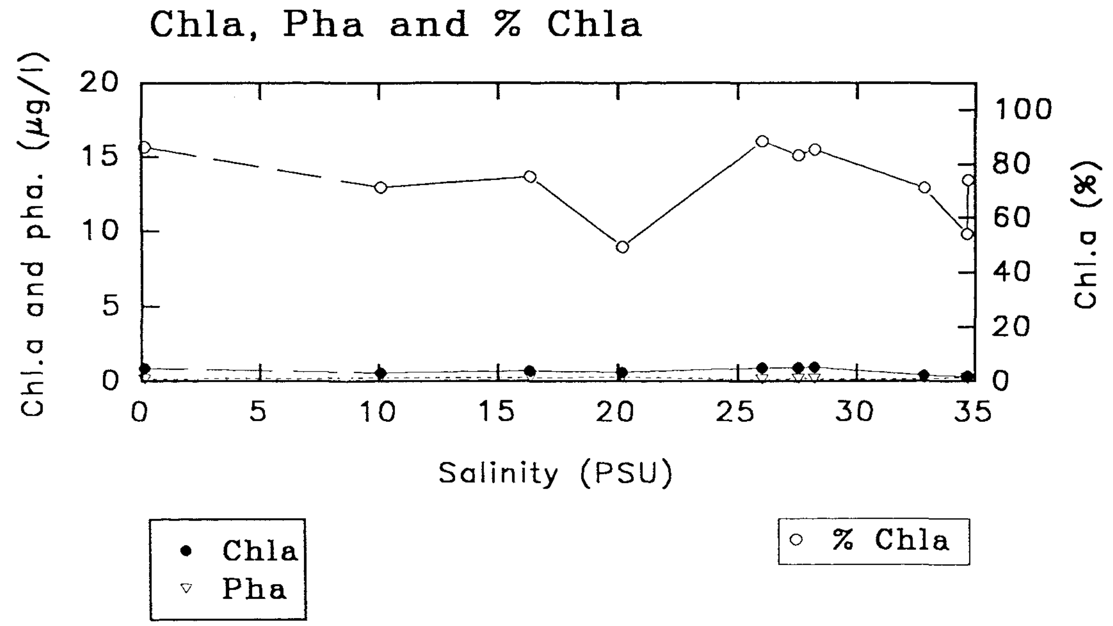


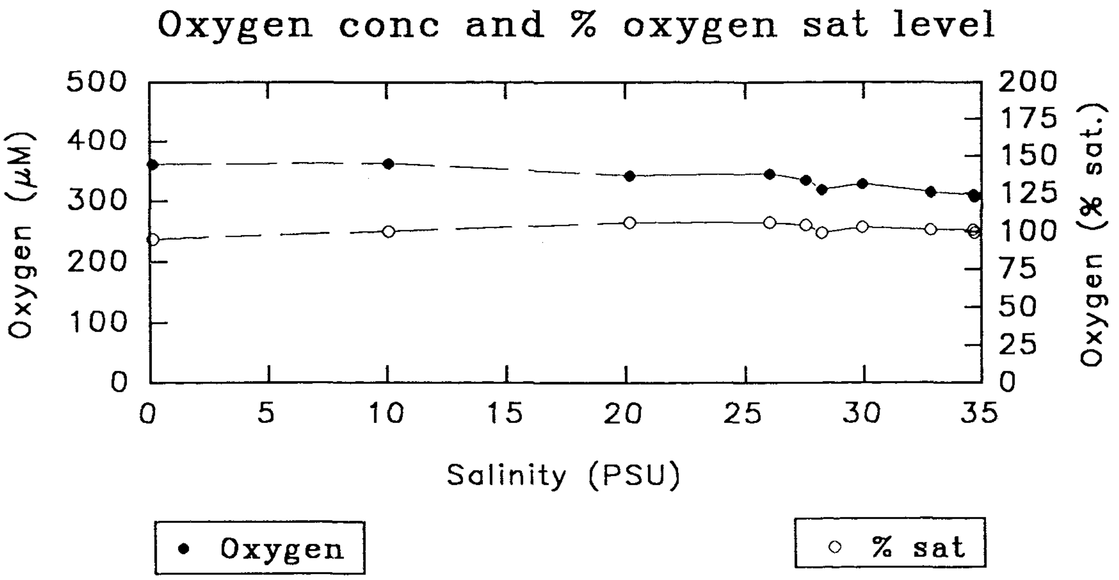


Figure III.13. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, winter 1992.

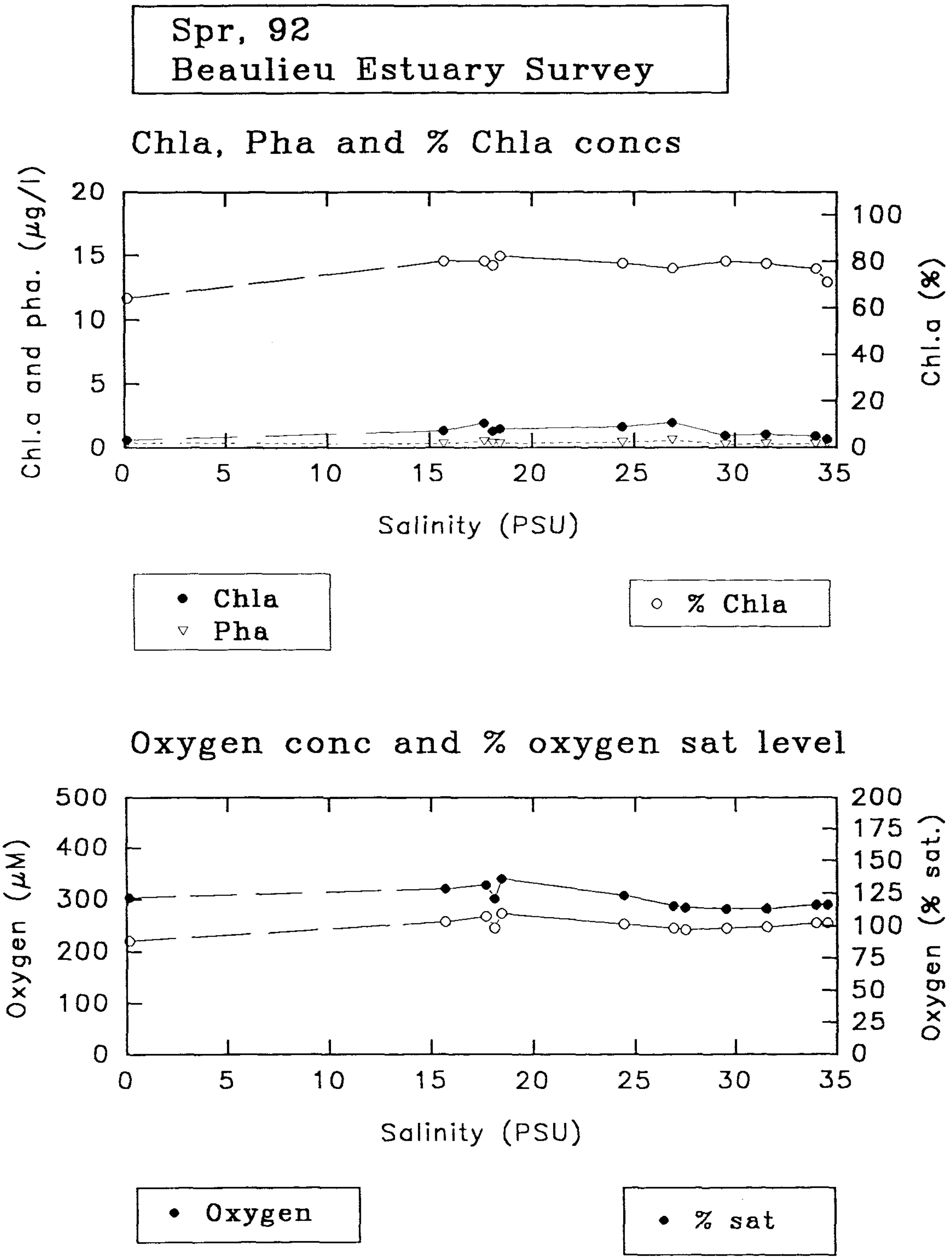
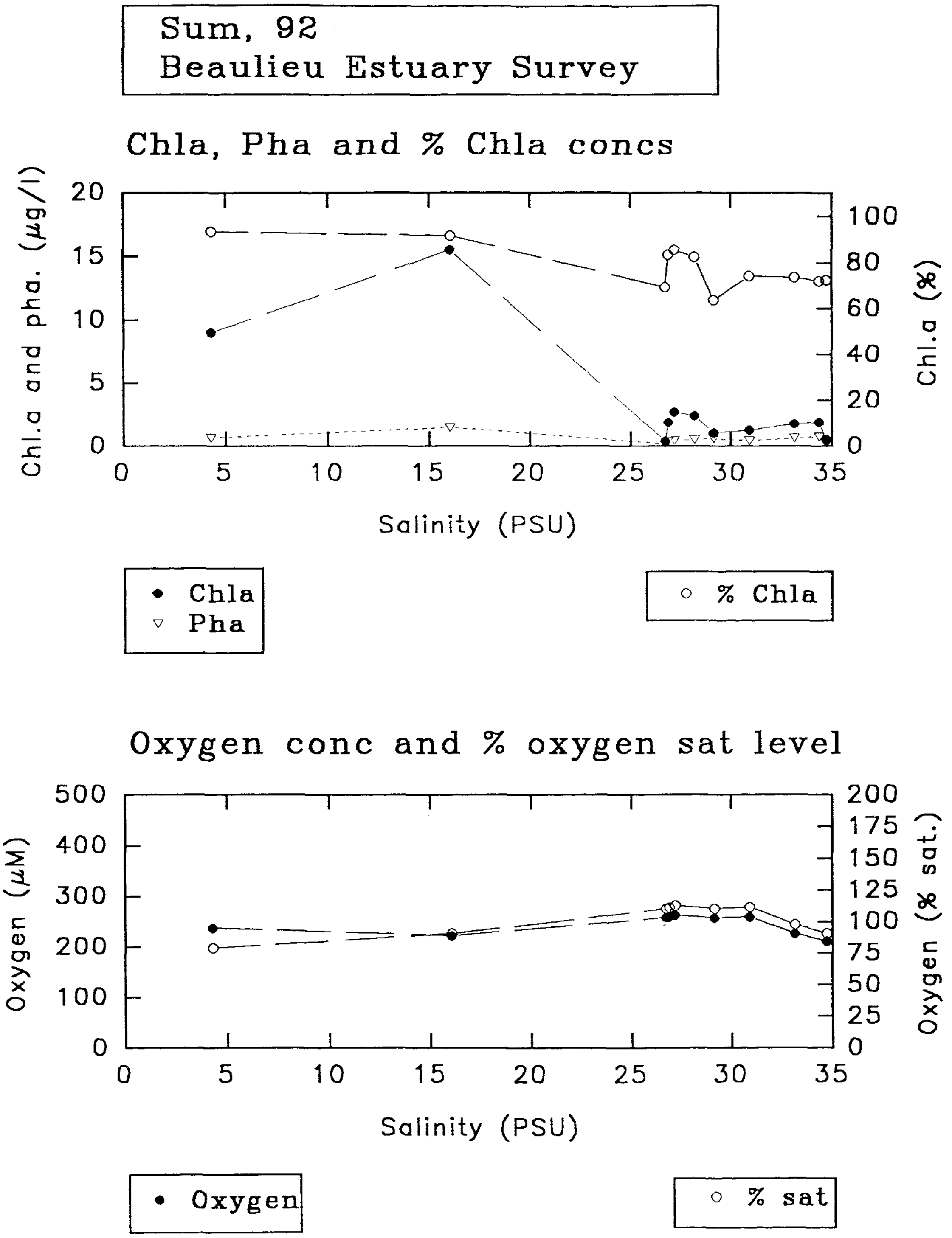


Figure III.14. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, spring 1992.



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Beaulieu Estuary Survey

Chla, Pha and % Chla concs

Oxygen conc and % oxygen sat level

Figure III.15. Chlorophyll a concentrations, phaeo-pigment levels, chlorophyll a as a fraction of total pigments, oxygen concentrations and oxygen saturation levels in the Beaulieu estuary, summer 1992.

References.

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