ENVIRONMENTAL ASSESSMENT OF BIOGEOCHEMICAL CYCLING OF DISSOLVED ORGANIC CARBON (DOC) AND NITROGEN (DON) IN NATURAL WATERS

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ENVIRONMENTAL ASSESSMENT OF BIOGEOCHEMICAL CYCLING OF DISSOLVED ORGANIC CARBON (DOC) AND NITROGEN (DON) IN NATURAL WATERS

by

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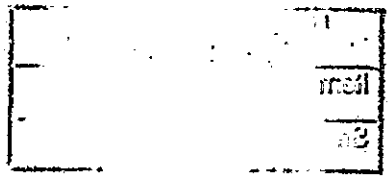

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Environmental assessment of biogeochemical cycling of dissolved organic carbon (DOC) and nitrogen (DON) in natural waters

El-Sayed A. Badr

Abstract

Increases in human population and activities have lead to significantly enhanced inputs of carbon and nitrogen from both point and diffuse sources to rivers, estuaries, and coastal seas, altering the global carbon and nitrogen biogeochemical cycles. This increased load has had a marked ecological impact globally, with detrimental changes to primary production, community structure and water quality. Understanding the nature and cycling of dissolved organic carbon (DOC) and nitrogen (DON), significant components of the global carbon and nitrogen cycles, in estuaries will provide better estimates of C and N transport to coastal and ocean waters. This study involves: (1) optimisation of the high temperature catalytic oxidation (HTCO) analytical method used for reliable analysis of DOC and total dissolved nitrogen (TDN), (2) investigation of spatial and temporal variations of DOC and DON, and other related determinands, in two contrasting estuarine environments, the Yealm and Plym, in south-west England, (3) investigation of DON bioavailability using a bacterial incubation experiment, and (4) preliminary work on the use of stable nitrogen isotope ratios to identify DON sources.

The sampling and analytical protocols required for rapid, precise and reliable determinations of DOC and DON, using the coupled HTCO-chemiluminescence technique, are described in this study. This method gave detection limits of approximately 6.2 μM C and 0.46 μM N, and precisions of < 2-3 % and < 3-5 % (n=3-5) for DOC and TDN, respectively. The mean DOC and TDN of the CRMs analysed, over a period of 2 years, were 48 ± 3.9 μM C and 20 ± 1.5 μM N that were close to the certified values of 44 – 45 μM C and 21 μM N, respectively.

Concentrations of DOC ranged from 61 μM C at the seaward end to 335 μM C at the fresh water end for the Yealm, and 71 – 290 μM C for the Plym. DON concentrations were mainly in the range of 1.8 – 62 μM N for the Yealm, and 4 – 94 μM N for the Plym. The enhanced DON concentrations in the Yealm might be the result of sewage discharges and agricultural run off, while in the Plym they may be due to sewage discharge, run off from the Chelson Meadow landfill and other anthropogenic activities within the urban Plym catchment. Except during a limited numbers of surveys, DOC and DON generally behaved in a non conservative manner in these estuaries. Nitrate and filterable reactive phosphate (FRP) behaved relatively conservatively in the Yealm, but were more non-conservative in the Plym. The spatial distribution of DOC and DON concentrations in the Plym Estuary appeared much more influenced by anthropogenic inputs relative to distributions in the Yealm Estuary. The seasonal variation of DOC and DON was characterised by lower concentrations during winter and a slight increase in spring and summer followed by highest concentrations during late summer and autumn, suggesting a strong link to seasonally variable phytoplankton production.

The contribution of DON to the TDN pool ranged between 4 and 79 % for the Yealm, and 3.5 – 84 % for the Plym. Higher values (53 – 79 %) were observed during late summer, emphasising the important contributions of DON to TDN pool. Incubation experiments using the Plym Estuary water indicated that 30 – 58 % of DON was bioavailable for heterotrophic bacterial utilisation; at the same time nitrate concentrations increased by 9 – 35 %, presumably through mineralization of DON. From the studies undertaken in the present work, it may be concluded that the omission of DON in estuarine and coastal water studies will result in underestimation of the total nitrogen load. As a significant part of the DON appeared to be bioavailable, ignoring this fraction will result in an underestimation of eutrophication pressures on coastal and ocean waters.

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List of abbreviations

Acronym	*Meaning*
AOU	Apparent oxygen utilization
BC	Blank checking
BDOC	Biodegradable dissolved organic carbon
BDON	Biodegradable dissolved organic nitrogen
BGS	The British Geological Survey
BMWP	Biological monitoring working party
BOD	Biochemical oxygen demand
BP	Bacterial production
CBOD	Carbonaceous biochemical oxygen demand
CDOM	Chromophoric dissolved organic matter
CFF	Cross flow filtration
CHL a	Suspended chlorophyll a
COD	Chemical oxygen demand
CRMs	Certified reference materials
CV	Coefficient of variation
CFA	Continuous flow analysis
DA	Devardas alloy
DCAA	Dissolved combined amino acid
DFAA	Dissolved free amino acid
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DPA	Dissolved primary amines
EA	The Environment Agency
EIA	Environmental impact assessment
FDOM	Fluorescent dissolved organic matter
FRP	Filterable reactive phosphate
GFF	Glass fibre filter
GQA	General quality assessment
HDPE	High density poly ethylene
HMW DOM	High molecular weight DOM
HTCO	High temperature catalytic oxidation
HTO	High temperature oxidation

IC	Inorganic carbon
IPB	Indophenol blue
KHP	Potassium hydrogen phthalate
LCW	Low carbon water
LMW DOM	Low molecular weight DOM
LNSW	Low nutrient sea water
LOD	Limit of detection
MTZ	Maximum turbidity zone
NBOD	Nitrogenous biochemical oxygen demand
NCD	Nitrogen chemiluminescence detector
NDIR	Non-dispersive infrared detector
NWC	The National Water Council
ON	Organic nitrogen
PER	Percent extracellular release
PMT	Photomultiplier tube
PO	Persulfate oxidation
POC	Particulate organic carbon
POM	Particulate organic matter
PON	Particulate organic nitrogen
PP	Primary production
SFA	Segmented flow analyzer
SPM	Suspended particulate matter
TC	Total carbon
TDL	Theoretical dilution line
TDN	Total dissolved nitrogen
TDS	Total dissolved solid
TFF	Tangential flow filtration
TOC	Total organic carbon
TSS	Total suspended solid
UF	Ultrafiltration
UV	Ultraviolet
VOC	Volatile organic compounds
WCO	Wet chemical oxidation
WIA	Water impact assessment

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Heartily, I would like to express my cordial thanks and grateful to supervisors Dr Eric Achterberg, Dr Alan Tappin, and Prof Steve Hill whose advice and suggestions have proved both helpful and constructive through out my PhD. I would like to thank the director of my study Dr Eric Achterberg for advising me to work very hard through my PhD so I could finish within the limited time I have! I am practically very grateful to Dr Alan Tappin (second director of my study) for his ongoing guidance and giving me the great opportunity to stay in his office during my very busy months of writing up my thesis. I would like to acknowledge the help and assistance of Dr Charlie Braungardt not only during the first stage of my PhD "with HTCO technique" but also through the whole time of my stay here in Plymouth.

A special thanks to all people who help me with sampling from the Plym and Yealm estuaries including Julian Stapley (the Yealm Harbour Master), Jose Barriada, Sally Madgwick, and Jason Truscott. Thanks Graham Carter for analysis of salinity samples. Big thanks to the Biological Science Technician Team, mainly Peter, who help me to establish my bacterial bioassay experiment in their constant room temperature. Special thanks to Dr David Gaudre who help me with the bacterial count.

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Author's Declaration

At now time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award. The word count of the main text, excluding appendices and references, is approximately 40,000 words. This study was fully financed with the aid of a scholarship from Environmental Sciences Department, Mansoura University and Ministry of Higher Education-Mission Department, Egypt.

A programme of advanced study was undertaken, which included training in the analysis of dissolved organic carbon and nitrogen, nutrients, and chlorophyll a, teaching skills for graduate teaching assistant course, stable nitrogen isotopes analysis training at the British Geology Survey, and European Computer Driving Licence certificate.

Relevant scientific seminars and conferences were regularly attended at which work was often presented, two papers published (one of them from the MSc work) and another paper prepared for publication, with other two papers in preparation.

The stable nitrogen isotopes work was prepared with the collaboration of Dr Tim Heaton (NERC Isotope Geosciences Lab, British Geology Survey). All other data presented in this thesis were completely prepared by the author, whom the ownership and copyright rests with. Before using this data in any presentation or printed publication, a written consent must obtained first from the author.

Signed .....

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Date .01.04.2025.....

Publications

Badr, E.A., Achterberg, E.P., Tappin, A.D, Hill, S.J. and Braungardt, C.B. (2003) Determination of dissolved organic nitrogen in natural waters using high-temperature catalytic oxidation. *Trac-Trends in Analytical Chemistry,* 22 (11), 819-827

Badr, E.A., Cashmore, M. and Cobb, D. (2004) The consideration of impacts upon the aquatic environment in environmental impact statements produced in England and Wales. *Journal of Environmental Assessment Policy and Management,* 6(1), 19-49

Watanabea, K., **Badr, E.A.,** Achterberg, E.P. Conversion efficiency of the high temperature combustion technique for dissolved organic carbon and total dissolved nitrogen analysis. *Marine Chemistry*, in preparation

Badr, E.A., Achterberg, E.P., and Tappin, A.D. Distribution and seasonal variations of dissolved organic nitrogen and dissolved organic carbon in the Yealm Estuary, south-west England, in preparation

Presentations and Conferences attended

03 – 05th September 2001: Progress in Chemical Oceanography (PICO), University of Wales at Bangor, Bangor, U.K. (attended)

09 – 13th September 2002: The Challenger Centenary Conference for Marine Science 2002, University of Plymouth, Plymouth, U.K. (Poster) "Biogeochemical cycling of dissolved organic carbon and dissolved organic nitrogen in a riverine-estuarine system"

22nd January 2003: School of Environmental Sciences Seminars series, University of Plymouth, Plymouth, U.K. (oral) "Determination of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in riverine-estuarine systems, using High Temperature Catalytic Oxidation method (HTCO)"

17 – 18th July 2003: Progress in Chemical Oceanography (PICO V), University of East Anglia, Norwich, U.K. (oral) "Biogeochemical cycling of dissolved organic (DOC) and dissolved organic nitrogen (DON) in the Yealm Estuary, South West England"

06th October 2003: The Challenger Society for Marine Science Centenary Seminar held onboard HQS Wellington, London, U.K. (attend)

22nd October 2003: School of Earth, Ocean, and Environmental Sciences Seminars series, University of Plymouth, Plymouth, U.K. (oral) "Distributions and seasonal variations of dissolved organic (DOC) and dissolved organic nitrogen (DON) in the Yealm Estuary, South West England"

10 – 13th December 2003: 4th European Meeting on Environmental Chemistry, Plymouth, U.K. (Oral) "Biogeochemical cycling of DOC and DON in the Plym Estuary, Southwest England"

13 – 17th September 2004: Challenger Conference for Marine Science 2004, University of Liverpool, Liverpool (Oral) "Water Quality Assessment; Where is DON?"

Associated Activities

The following researchers have been trained in the analysis of dissolved organic carbon and total dissolved nitrogen, using the coupled HTCO-chemiluminescence technique and were given help with running their samples. In addition, DOC/TDN analyses were run for some collaborated projects and scientific institutions.

J. Wang (January – March 2002)	Third year (B.Sc. in Environmental Chemistry) School of Environmental Sciences University of Plymouth
Hossam Nassef Mahamed AbdElazeem (August 2002)	Demonstrators, Chemistry Department Faculty of Sciences at Damietta Al-Mansoura University, Egypt
Rob Spencer Anastasios Anestis (October 2002)	PhD Students, Ocean Research Group School of Marine Science and Technology University of Newcastle upon Tyne
Samantha Pillidge (November 2002)	Third year (B.Sc. in Environmental Chemistry) School of Environmental Sciences University of Plymouth
Kaori Watanabe (January 2003)	MRes Environmental Analytical Chemistry School of Environmental Sciences University of Plymouth
Hadir M. (April 2003)	Kuwait Institute for Scientific Research PO BOX 24885, 13109 Safat, Kuwait
Victoria A. Collins (February 2004)	PhD Student Plymouth Marine Laboratory Plymouth

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Chapter One

Background and literature review

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1.1 Introduction

1.1.1 The global carbon and nitrogen cycles

Since the industrial revolution, human activities have caused significant perturbations to the global cycles of element. Consequently, serious environmental problems such as global warming, acid rain, depletion of the ozone layer, and bioaccumulation of toxic matter have been caused by disruptions of the biogeochemical cycles (Table 1.1). Over the past 200 years, human activities have significantly altered the global carbon and nitrogen cycles. Understanding the consequences of these activities in the coming decades is critical for sustaining our life on the earth (Falkowski *et al.* 2000). Carbon and nitrogen reservoirs and their evolution are considered key parameters in the description of the aquatic environment (Cauwet 1999).

Table 1.1 Examples of human impacts on elemental biogeochemical cycles (Radojevic and Bashkin 1999).

Cycle	Human interference	Environmental consequences
Carbon	Fossil fuel combustion and deforestation	Global warming
Nitrogen	Fertilizers and fossil fuel combustion	Eutrophication and acid rain
Phosphorus	Detergents and fertilizers	Eutrophication
Sulphur	Fossil fuel combustion	Acid rain

Human activities have induced increases of CO_2 (green house gas) in the atmosphere, that is a major cause of global warming (Stumm and Morgan 1996; de Haas *et al.* 2002). CO_2 in the atmosphere traps some of the radiation and cause the Earth's atmosphere to be warmer than it would otherwise be. Global warming and associated potential impacts had lead to focused attention of scientists on the global carbon cycle and quantification of net

fluxes of CO_2 in the atmosphere and ocean (Hedges 1992; Falkowski *et al.* 2000; Druffel 2004).

Carbon is continuously cycled between reservoirs in the ocean, on land and in the atmosphere, where it occurs primarily as CO_2 (Figure 1.1). The pool of carbon on land is primarily in living biota and decaying organic matter, and accounts for approximately 2,000 Gt C (Gt = 10^{15} g). Quantitatively, the largest carbon reservoir is the dissolved inorganic carbon (DIC) pool in the ocean, which holds approximately 38,000 Gt C (intermediate and deep ocean reservoir in Figure 1.1) (Chester 2003). The DOC pool in the ocean is estimated at nearly 700 Gt C. Two main processes are known to play an important role in the global carbon cycle in the ocean: (1) the formation of calcium carbonate skeletons by various types of organisms, and (2) formation of organic matter by phytoplankton during photosynthesis (de Haas *et al.* 2002). The photosynthesis transfers CO_2 from the atmosphere to land plants or oceanic plankton, while respiration releases CO_2 back into the atmosphere. The additional carbon resulting from fossil fuel burning is the main cause of atmospheric CO_2 rises over the last 150 years; and since the beginning of the industrial revolution concentrations of CO_2 in the atmosphere have increased by about 30 %. A recent estimate of the CO_2 reservoir in the atmosphere is 750 Gt C, compared to an estimated 600 Gt C in pre-industrial times (Chester 2003). Therefore, it is of great importance to understand how and why the global carbon cycle has changed over time, and how it may change in the future as human activities continue to add ever-greater quantities of green house gases (CO_2 and CH_4) into the atmosphere (Probst *et al.* 1999). The relative role of the marine environments versus land vegetation and soils as a possible missing sink is still debated (Chester 2003).

A simplified schematic diagram of the nitrogen cycle in the marine environments is presented in Figure 1.3. Nitrogen enters rivers, estuaries and coastal marine ecosystems from natural and, in many cases, anthropogenic sources. As nitrogen is the primary nutrient-limiting element for primary production in estuarine and marine water environments, increases in nitrogen inputs can markedly alter those aquatic ecosystems (Seitzinger *et al.* 2002). Nitrogen has a complex cycle due to the large number of potential oxidation states (-3 to +5) in which nitrogen can exist. Nitrogen in natural waters occurs as nitrogen gas (N_2), nitrate, nitrite, ammonium, dissolved organic nitrogen (DON) and particulate organic nitrogen (PON). In oxidising aquatic environments, *Nitrosomonas* and *Nitrobacter* oxidise ammonium to nitrite and then to nitrate, respectively, through the process of nitrification. Nitrification is initiated by the degradation of organic nitrogen into ammonium by the ammonification process. Nitrification has been reported in the water column, particularly at the maximum turbidity zone (MTZ), and sediments of many estuarine ecosystems (Abril *et al.* 2000). In anoxic aquatic environments with high levels of organic matter such as estuarine and coastal marine sediments, some heterotrophic bacteria can use nitrate as an electron acceptor in order to utilise the organic matter (Seitzinger 1994; Abril *et al.* 2000). Consequently, nitrate is reduced to nitrite and then to nitrogen gas through the denitrification process (Seitzinger 1988; Meybeck 1993; Stumm and Morgan 1996). The major source of nitrate for denitrification in estuarine and coastal marine sediments is the nitrate produced in the sediments by mineralization, and not the diffusing nitrate from the overlying water (Seitzinger 1988). In addition to denitrification in anoxic aquatic environments, anammox bacteria anaerobically oxidise ammonium with nitrite to form nitrogen gas. Recently, it has been reported that the anammox reaction could be a significant, but as yet neglected, sink for marine nitrogen (Dalsgaard *et al.* 2003; Kuypers *et al.* 2003). For instance, the anammox reaction accounts for 19 – 35 % of the total N_2 formation in the water column of Golfo Dulce, Costa Rica (Dalsgaard *et al.* 2003).

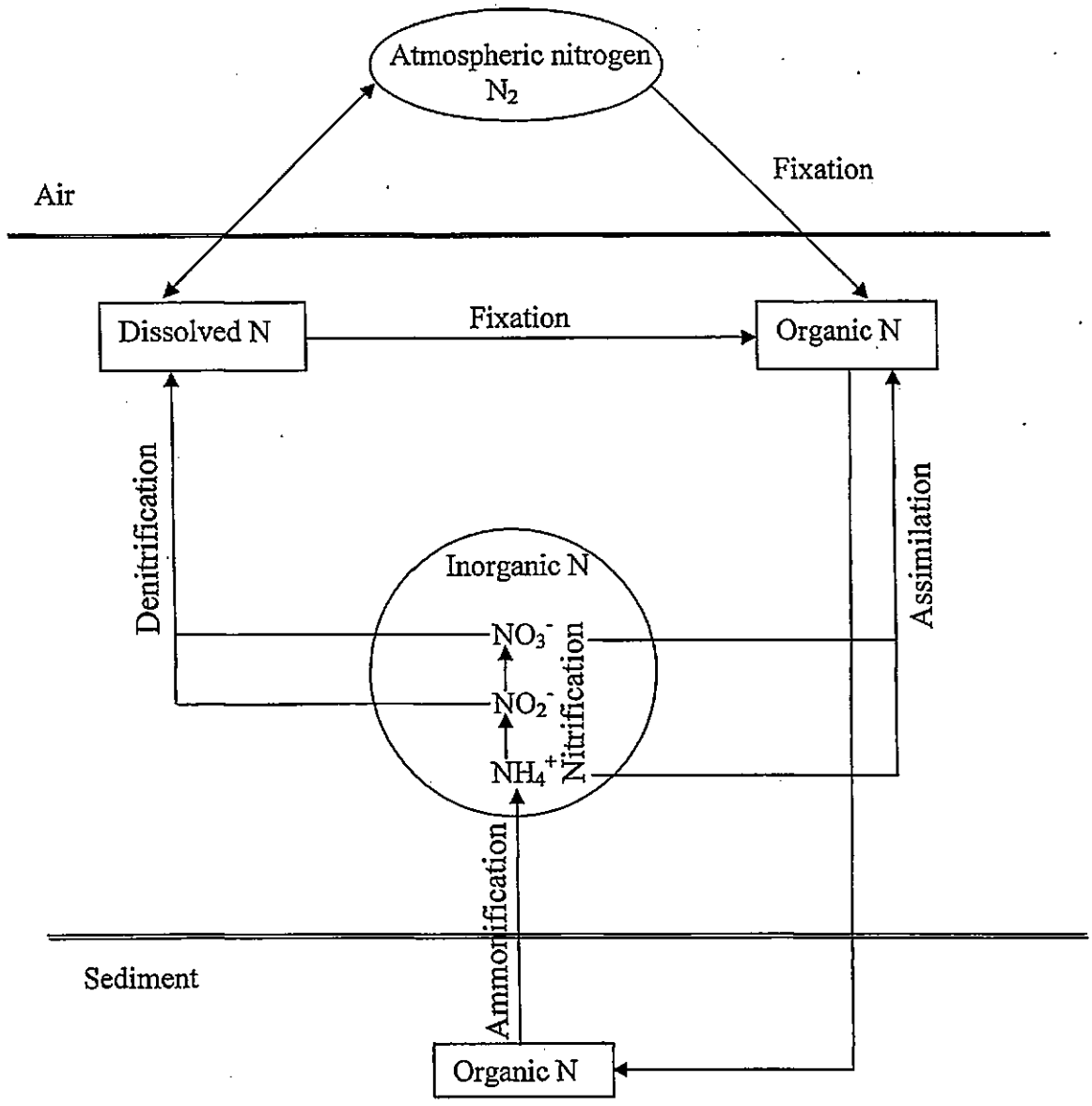


Figure 1.3 A schematic diagram of the nitrogen cycle in the marine environment (adapted from Hanrahan *et al.* 2002).

1.1.2 Environmental impact assessment with respect to water quality

Environmental impact assessment (EIA) is a participatory process employed to identify and evaluate the probable environmental consequences of development proposals in order to facilitate informed decision-making and sustainable development (Glasson *et al.* 1999). The potential benefits of this decision tool have been widely recognised, and the EIA provisions have been implemented in more than 100 countries (Petts 1999). Water impact assessment (WIA) is a component of the EIA process that is concerned with the potential

impacts of development proposals on aquatic environments, including river and marine environments (Brookes 1999). The WIA process follows the same steps as the EIA process, but is solely related to impacts on aquatic systems (Figure 1.4). WIA should be a cyclical process with feedback

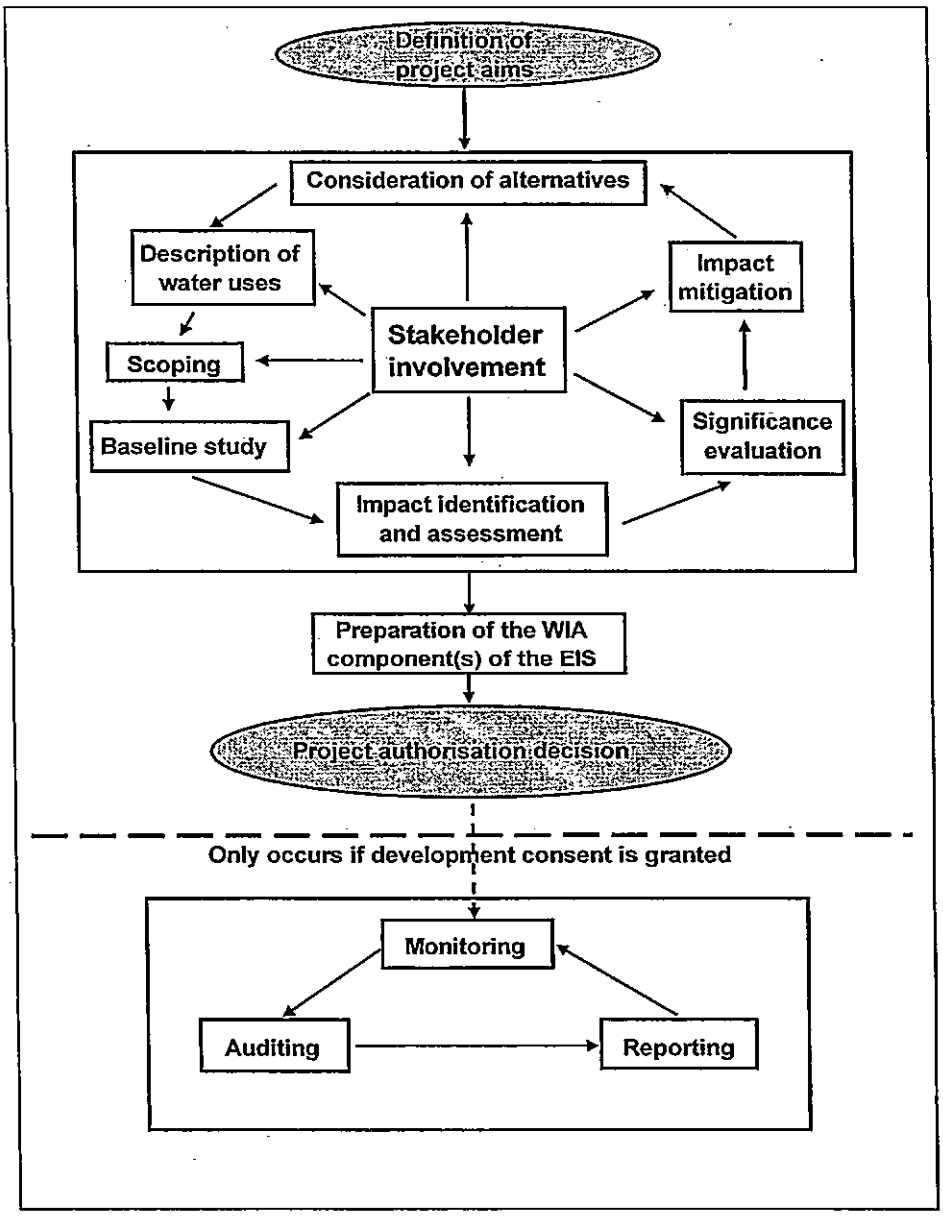


Figure 1.4 Idealised conception of the WIA process (after Badr *et al.*, 2004).

Developments can affect the aquatic environment directly e.g. dams and water abstraction projects or indirectly e.g. industrial, agricultural, and residential activities (Brookes 1999). Both categories of developments can produce a wide range of potential impacts upon the

water environment, from changes in water quantity to changes in water quality including depletion of dissolved oxygen, enhanced concentrations of dissolved organic matter (DOM), eutrophication, and elevated levels of toxic metals and pesticides. Sources of water pollution occur from point and non-point sources. Point sources of pollution, such as sewage outfalls and industrial effluents, are easy to identify and to monitor systematically. Non-point sources, such as agricultural runoff, are diffuse, intermittent and more difficult to control. Consequently, these impacts upon the aquatic environment can potentially affect the biota and cause a cascade of further impacts on socio-economic variables, human health, cultural heritage, landscape, ecology and climate (Morris and Biggs 2001).

A study by Badr *et al.*,(2004) concluded that in England the water quality assessment component is poorer in quality than the overall EIA scheme and indicates the need for further research in improving WIA process. Dissolved oxygen (DO) and biochemical oxygen demand (BOD), and chemical oxygen demand (COD) to some extent, are used in the water quality assessment process to assess the organic load and the extent to which aquatic environments are affected by sewage, industrial effluents and agriculture run-off containing organic matter. Dissolved organic carbon (DOC) is usually ignored. In the same manner, inorganic nitrogen species (nitrate, nitrite and ammonia) are used to assess the nitrogen load in relation to eutrophication, whilst the potential role of DON is not considered. The standard chemical classification used until 1992 was developed by the National Water Council (NWC) which classified waters through England and Wales into classes from 1A, 1B (good quality) to 4 (bad quality) (National River Authority 1994). This chemical classification was based on the concentrations of DO, BOD and ammonia, in addition to other parameters according to the condition of the site under investigation. However, the use of additional assessments of water quality led to some concern over inconsistencies in the way the scheme was operated in different parts of England and

Wales, and from one survey to the next. The Environment Agency (EA) has now replaced the NWC scheme with a more appropriate one, the General Quality Assessment (GQA) scheme. The GQA comprises general chemistry, nutrients, aesthetics and biology. The GQA scheme gives a grade to the water environment under investigation from A (highest quality- very good) through B, C, D, and E to F (lowest quality- bad). The general chemistry is assessed in terms of measuring DO, BOD, and ammonia. The biological component of the GQA is based on the use of the Biological Monitoring Working Party (BMWP) index (Mason 2002). This index uses the diversity of aquatic invertebrate communities as an indicator of water quality. An idealised water quality assessment is based on appropriate monitoring of hydrology, physico-chemistry and biology (Table 1.2). Only organic matter load and nutrients, which are relevant to the current study, will be discussed in the following.

1.1.2.1 Organic matter load

Organic matter load in natural waters can be estimated from measurements of related parameters, as a useful indication of the degree of pollution. These parameters include DO, BOD, COD, DOC and DON (Stumm and Morgan 1996). DO represents the amount of oxygen gas dissolved in natural waters, that is essential for the survival of most aquatic organisms. Sources of the DO in aquatic ecosystems include surface diffusion from the atmosphere and photosynthesis in algae and submerged plants. The amount of oxygen dissolved in natural waters varies with temperature, salinity, turbulence, atmospheric pressure and the photosynthetic activity. The solubility of oxygen decreases as water temperature and salinity increase (Radojevic and Bashkin 1999). A waste discharge that is high in organic matter and nutrients can lead to decreases in DO concentrations as a result of the increased microbial activity occurred during the degradation of organic matter.

Table 1.2 Water quality monitoring parameters, adapted from (Chapman 1996; Morris and Biggs 2001; Hart 2002; Turley *et al.* 2005).

Water quality assessment	Parameters monitored
Physical hydrology	1- Precipitation including rainfall data and related meteorological parameters such as temperature, wind speed and direction, and humidity 2- Water body dimensions and capacity (length, width, depth, water volume) 3- Water body discharge/ recharge rates and flow rate measurements 4- Flushing time and tidal range for estuarine environment 5- Wave, current and tide for oceans and seas
Physiochemistry	1- Temperature, pH, turbidity and suspended particulate matter (SPM), salinity, conductivity and total dissolved solids, alkalinity, hardness, colour, odour, chlorophyll a 2- Nutrients; phosphate, nitrogen, carbon and silica (particulate and dissolved and their ratios) 3- Organic load; DO, BOD, COD, DOC (TOC), DON 4- Major ions; sodium, potassium, calcium, magnesium, chloride, sulphate 5- Trace metals; cadmium, lead, zinc, copper, mercury 6- Oil and hydrocarbon 7- Pesticides, PCBs 8- Surfactants 9- Sediment composition and availability
Biology	1- Phytoplankton (chlorophyll a, productivity, nuisance blooms) 2- bacteriological investigation (number, productivity, indictor species) 3- benthic invertebrates diversity 4- the use of bioindicator species (some algae, fish and aquatic plant species)

BOD is an approximate measure of the amount of biochemically degradable organic matter present in water samples. BOD is defined as the amount of oxygen required for the aerobic oxidation of organic matter by micro-organisms over a specific period. COD is the measure of oxygen equivalent to the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant. The COD is usually higher than BOD, as BOD determines compounds that can be biologically oxidised while COD measures those compounds which can be chemically oxidised (Stumm and Morgan 1996). DOC and DON are the filterable fraction of organic carbon and nitrogen present in natural waters, respectively. Monitoring of DOC and DON in natural and drinking waters has been widely used in the USA to assess organic matter removal, bacterial re-growth / contamination and disinfection by-product, and as a basis for compliance with US EPA regulations for drinking water (Escobar and Randall 2001; Wallace *et al.* 2002; Volk *et al.* 2002; Westerhoff and Mash 2002).

When an aquatic environment is freshly polluted by anthropogenic sources, most of the nitrogen is originally present in the form of organic nitrogen (ON) and ammonia. As the time progresses, the organic nitrogen is gradually converted to ammonia nitrogen which is oxidised later, under aerobic condition, to nitrite and nitrate through the process of nitrification. The progression of these processes found to occur in a manner shown in Figure 1.5 , depending on pollution load and aquatic system conditions (Sawyer *et al.* 1994). So, the discharge of organic and ammonia nitrogen and subsequent degradation can seriously reduce the DO levels in the receiving aquatic environment.

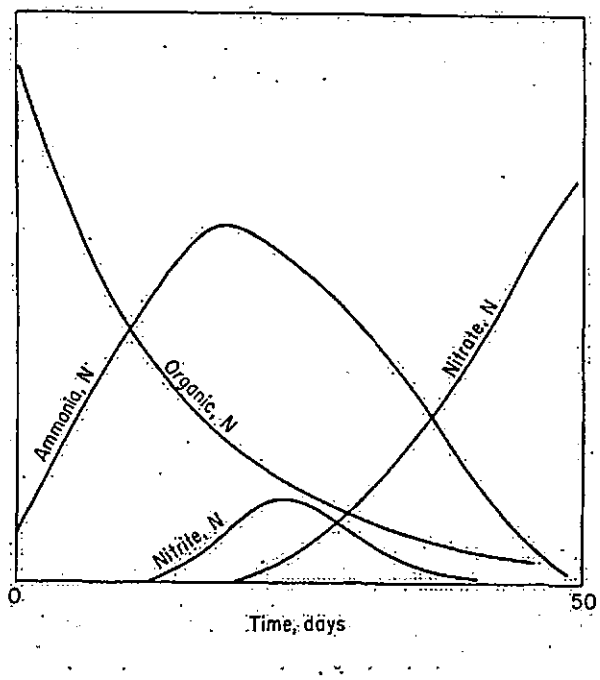


Figure 1.5 Changes occurring in nitrogen species present in recently polluted aquatic environments under aerobic conditions (after Sawyer *et al.* 1994).

1.1.2.2 Nutrients and eutrophication

Nutrients are essential for biochemical reactions and growth and maintenance of aquatic biota, with nitrogen, phosphorus and silicon being the most important and commonly determined macronutrients in aquatic environments (Nedwell *et al.* 1999; Hanrahan *et al.* 2002). Nitrogen is typically the most limiting nutrient to primary production in many estuarine and coastal waters (Seitzinger and Sanders 1997). Anthropogenic sources of nutrients into aquatic environments include stormwater and agricultural runoff and discharges of industrial and sewage effluents (Middelburg and Nieuwenhuize 2000b).

Eutrophication may be defined as the enrichment of the aquatic environment with nutrients, which in turn leads to a marked increase in organic matter production with often detrimental effects. Although eutrophication is a natural process, human activity can greatly increase the load of nutrients, mainly nitrogen and phosphorus, and consequently lead to eutrophication (Johnston and et al. 1998). Eutrophication in estuaries and coastal

waters is one of the best documented consequences of human alterations of the nitrogen cycle (Christian and Thomas 2003). Eutrophication is usually accompanied by an increase in the phytoplankton biomass and a subsequent deterioration of the quality of the water environment. This can cause major ecological changes, including the occurrence of nuisance algae, reduction in species diversity and major changes in community structure (Hanrahan *et al.* 2002). The algae blooms may also reduce light penetration to the extent that benthic communities die. Sharply reduced DO concentrations are frequently associated with this phenomenon, and may contribute to the demise of benthic fauna and flora.

1.2 Environmental significance of DOC and DON research

1.2.1 Why dissolved organic carbon?

DOC is recognised as the largest reservoir of partially reactive carbon that is important in the global carbon biogeochemical cycle (Hopkinson *et al.* 1997b). The global oceanic DOC pool is estimated to be nearly 700 Gt C, a value similar to the inorganic carbon mass in the atmosphere (Carlson 2002). The increasing interest given to DOC distributions in marine environments occurred following the Seattle DOC/DON workshop held in Washington in 1991, and was due to the following reasons (Cauwet 1994; Cauwet *et al.* 1997; Tipping *et al.* 1997). (1) Studying fluxes of organic matter in the marine environment is necessary in order to understand the complex relations between marine production, terrestrial inputs (particularly anthropogenic), and sedimentary accumulation and production of DOM. (2) The enhanced level of CO_2 in the atmosphere and the potential for climate change led to focused attention of scientists on the global carbon cycle. The main question here was whether the equilibrium of the atmosphere was being disturbed due to the increasing emission of CO_2 and other damaging activities (like deforestation and desertification), and to investigate if marine environments could be a

potential sink for the excess CO_2. (3) The debate that developed on the reliability of the DOC analytical methods.

1.2.2 Why dissolved organic nitrogen?

As noted earlier, inorganic nitrogen is often reviewed as the limiting nutrient for primary production in marine waters, whereas excess N inputs may lead to eutrophication. Historically, most studies of N concentrations and cycling in natural waters have generally examined dissolved inorganic nitrogen (DIN) species (nitrate, nitrite and ammonium); DON fraction have largely been ignored (Walsh 1989; Hopkinson *et al.* 1993; Mchale *et al.* 2000; Jones *et al.* 2004). This focus has arisen from the perceived importance of DIN to primary productivity and hence water quality, difficulties in measuring DON and an underlying assumption that DON is biologically inert (Mchale *et al.* 2000). Recent work, using new and improved methodologies, has shown that DON concentrations can be as high as ca. 100 μM N, and that it frequently forms the largest part of total dissolved nitrogen (TDN) in many lake, river, estuarine and surface ocean waters as summarised in Figure 1.6. For instance DON at ALOHA station in the North Pacific represents 95 % of the TDN pool (Church *et al.* 2002). Also, Perakis and Hedin (2002) reported that DON forms 80 % of TDN in South America forest streams. A recent study in the Delaware and Hudson river-estuarine systems showed that inputs of organic nitrogen accounted for 14 to 61 % of the total nitrogen loadings to the systems (Seitzinger and Sanders 1997). Overall, DON averages 60 – 69 % of the TDN pool, excluding deep oceanic waters (Bronk 2002). It has been also demonstrated that large fractions (12 – 72 %) of the DON pool, including urea, and dissolved free and combined amino acids, are bioavailable with turnover times of hours to weeks (Stepanauskas *et al.* 1999; Bronk 2002). Anthropogenic DON sources to industrialised catchments have been reported to be more important than natural sources, by a factor of 2 – 4 for the Thames and Rhine systems (Meybeck 1993).

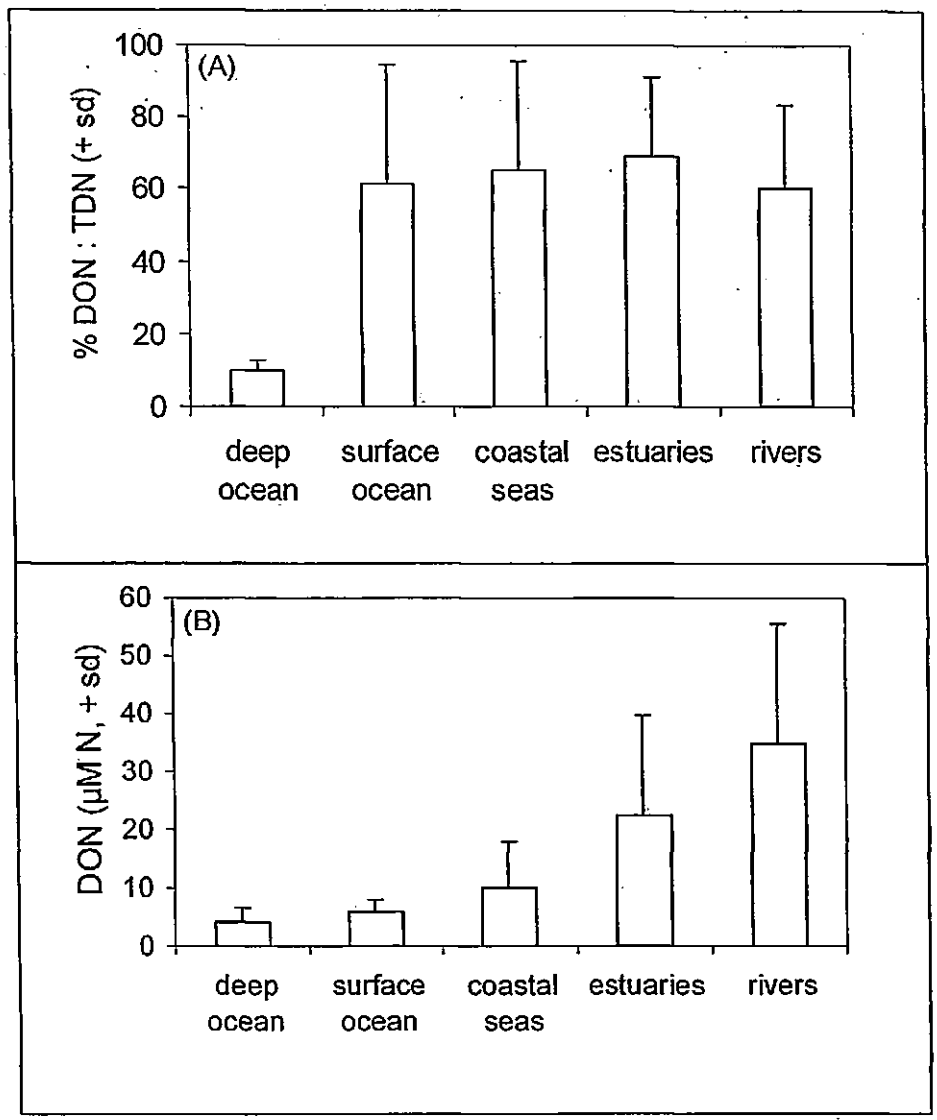


Figure 1.6 (A) percentage of DON to total dissolved nitrogen (DON, nitrate, nitrite, ammonium), (B) mean concentrations (± 1 SD) of DON in natural waters (After Bronk 2002).

1.3 Biogeochemical cycles of dissolved organic carbon and nitrogen

Biogeochemical cycles can be illustrated as a series of compartments (reservoirs) and pathways between them. Each compartment can be viewed in terms of a box model as shown in Figure 1.7 (Radojevic and Bashkin 1999). The flux is the rate of transfer through the reservoir (mass / time). The residence time is the amount of substance in the reservoir divided by the flux. Generally the biogeochemical cycle for C and N includes the uptake and assimilation of nutrients by plankton (primary production), the formation of

particulate organic matter (POM), the sinking of POM out of the water column, the decomposition and release of re-mineralised products from sediment and water column, and desorption of these back to the surface (Kahler and Koeve 2001). Primary production is the ultimate source and bacterial utilization is the principal sink for DOM. This section will introduce the biogeochemical cycles of DOC and DON in the marine environment, focusing on the estuarine system.

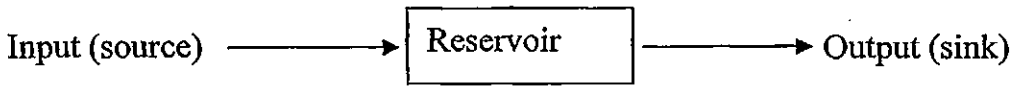


Figure 1.7 The biogeochemical cycle box model.

1.3.1 Sources of DOC and DON

DOM sources in the marine environment are either internal (autochthonous) and mainly comprise primary production or external (allochthonous) comprising anthropogenic inputs.

1.3.1.1 Autochthonous sources

While DOM production is ultimately constrained by the magnitude of primary production, there are several processes responsible for DOM cycling, including extracellular phytoplankton production, grazing induced DOM production, bacterial transformation and release, viral induced cell lysis and solubilization of particles, as illustrated in Figure 1.8.

1.3.1.1.1 Extracellular phytoplankton production

Extracellular phytoplankton release of dissolved organic matter form an important source of DOM (Collos 1992; Myklestad 2000). Extracellular release of DOC and DON has been assessed using ^{14}C and ^{15}N tracer techniques, respectively (Ward and Bronk 2001; Carlson 2002).

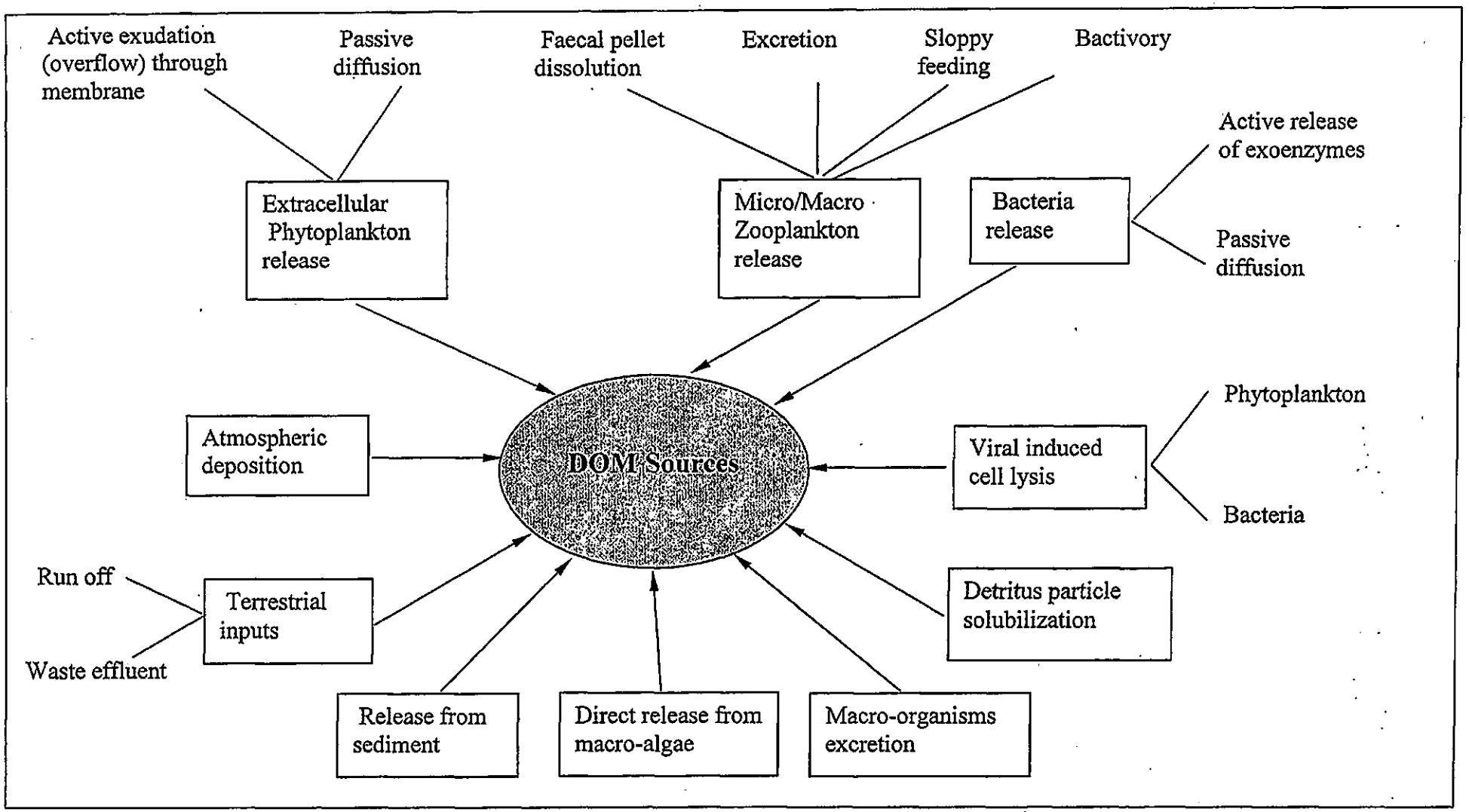


Figure 1.8 Conceptual model of dissolved organic matter sources in natural waters.

The percent extracellular release (PER) of DOM from phytoplankton is likely to be affected by community structure (algal species and physiology), light intensity and estuarine turbidity, nutrient deficiency and temperature (Myklestad 2000).

Smaller plankton release more DOM than larger ones because smaller cells have larger surface to volume ratios (Hasegawa *et al.* 2000). DOM extracellular release rates are low in actively growing cells e.g. at the beginning of spring algal blooms and high when cells are stressed by nutrient limitation e.g. in late summer at the end of the bloom (Bronk 2002). The average percent extracellular release of DOC in marine environments is usually within the range of 10 – 20 % of primary production; for example the PER was estimated to be 13 – 17 % in the Randers Estuary, Denmark (Nagata 2000).

There have been two models proposed to explain extracellular release from phytoplankton, including active release (the overflow model) and passive release (the passive diffusion model) (Lee and Henrichs 1993; Nagata 2000). Active release includes the release of excess photosynthates that accumulate when carbon fixation exceeds incorporation into new cell material due to nutrient deficiency (Bronk 2002). DOM exudation according to the overflow model, should correlate to the photosynthetic rate, the DOM should be composed of both low and high molecular weight (LMW and HMW) DOM and should be absent at night and highly turbid estuaries (Carlson 2002). Examples of DOM exudates compounds are glycollate and polysaccharides (Lee and Henrichs 1993).

In contrast, the passive diffusion model is based on the permeation of LMW DOM compounds through the cell membrane in response to the large concentration gradient that exists between the intracellular and extracellular pools of DOM (Bjornsen 1988). DOM exudation according to diffusion model should reflect the composition of small molecules in the intracellular fluid (Lee and Henrichs 1993). The subsequent uptake of LMW DOM

compounds by aquatic organisms such as bacteria would maintain the diffusion gradient and thus continue leakage from the phytoplankton cell.

1.3.1.1.2 Grazing induced DOM production

Zooplankton grazing on phytoplankton is an established mechanism for inducing the release of DOM to the surrounding water and the bacterial community (Sondergaard *et al.* 2000). Zooplankton grazers (macro-zooplankton and micro-zooplankton) can potentially play an active role in release of DOM, transforming particulate organic matter back into the dissolved phase (Nagata 2000). It has been shown that healthy, exponentially growing phytoplankton are characterised by DOC release rates equivalent to < 10 % of their daily carbon fixation (Dafner and Wangersky 2002b). There are four main processes by which macro-zooplankton may release DOM to the surrounding water: (1) excretory release, DOM compounds are released as waste, (2) egestion, release of unassimilated material, (3) sloppy feeding, large cells are not ingested whole but are broken resulting in release of dissolved intracellular materials and (4) release from faecal pellets via diffusion or dissolution (Lee and Henrichs 1993; Carlson 2002).

In a tracer experiment, it was found that when copepods were fed with diatoms labelled with ^{14}C, 27 % of the radiocarbon ingested appeared as DOC in the external DOC pool (Copping and Lorenzen 1980). It is showed that the major biochemicals (sugars, amino acids, and pigments) made up 57 % of the organic matter in the diatoms (phytoplankton) but only 35 % in fecal pellets produced by *Calanus* (zooplankton) during feeding indicating zooplankton excretion (Lee and Henrichs 1993).

Micro-zooplankton grazing, including herbivory and bacterivory, can release varying percentages of carbon and nitrogen depending on prey type (Nagata 2000). DOM released

by bacterivory recycles DOM while DOM released via herbivory provides new DOM (Nagata and Kirchman 1992). The released DOM consists of high- and low- molecular weight compounds, dissolved free amino acid (DFAA), dissolved combined amino acid (DCAA), as well as dissolved organic phosphorus (Carlson 2002, and references therein). The extent of DOM release is positively correlated with the food concentration and is highest during exponential growth of phytoplankton (Nagata and Kirchman 1992).

1.3.1.1.3 Bacterial transformation and release

Bacteria are generally considered as the main DOM consumers, but they can also be important producers. Heterotrophic bacteria are the dominant consumers of DOM, assimilating it as biomass or converting it to inorganic nutrients whereas photoautotrophic bacteria (cyanobacteria) are net DOM producers (Carlson 2002). Bacterioplankton may directly release DOM in the form of hydrolytic enzymes, which in turn result in solubilization of attached particles and secondary release of DOM (Smith *et al.* 1992; Lee and Henrichs 1993). Bacterioplankton may contribute to the biologically refractory DOM pool by transformation of LMW to HMW dissolved organic matter that are resistant to further bacterial remineralization on the time scale of months in estuaries to thousands of year in open ocean (Ogawa *et al.* 2001).

1.3.1.1.4 Viral induced cell lysis

Viral infection may be a significant cause in the mortality for phytoplankton and bacteria, and thus may be a potentially large contributor to the release of DOM via cell lysis and breakage (Nagata 2000). Viruses may also influence the nature of the released DOM by modifying the metabolic characteristics of the primary and secondary producer communities (Bronk 2002). The majority of the DOM released from bacterial lysis is bioavailable to bacterioplankton over short time scales (Fuhrman 1999). However, viral

lysis of some prokaryotic and eukaryotic organisms may yield DOM compounds that are biologically resistance to degradation (refractory DOM) (Carlson 2002).

1.3.1.1.5 Solubilization of particles

Bacterial ectoenzymes are responsible for the hydrolysis of polymeric DOM and POM and consequently the release of DOM (Carlson 2002). Bacteria attached to aggregates can form a hydrolytic enzymatic reactor which results in the release of DOM (Smith *et al.* 1992).

1.3.1.2 Allochthonous sources

External DOM sources (allochthonous) include terrestrial runoff, leaching from plant detritus and soils into rivers and estuaries, atmospheric deposition and benthic sediment inputs (Cornell *et al.* 1995; Seitzinger and Sanders 1997; Seitzinger and Sanders 1999). Continentally derived materials are transported to the coastal and sea waters by two main routes; via the river-estuaries route and atmospheric deposition, and these transfer mechanisms are important for the fate of organic matter in the global carbon and nitrogen cycles.

1.3.1.2.1 Terrestrial inputs

Terrestrial sources for the estuarine environment include river inputs, sewage and industrial effluents, agricultural run-off and urban run-off. These are the major external sources of DOM to estuarine environment and consequently to coastal waters. It has been estimated that the global annual discharge of terrigenous DOC from rivers to the marine environment is in the range 0.25 – 0.38 Gt C Yr^{-1} (Dafner and Wangersky 2002b). The global discharge of riverine-estuarine DOC is sufficient to sustain turnover of the entire pool of organic carbon dissolved in seawaters.

The quantities and characteristics of DON in estuarine and coastal waters largely depend on riverine inputs, and they may be totally different depending on the nature of the catchment areas being drained. In streams, riverine systems and freshwater reservoirs an important fraction of the DON is often derived from terrestrial leaching and runoff, and consists of an important part of humic substances (Berman and Bronk 2003). Anthropogenic sources are responsible for a significant proportion of the inputs of DON to rivers, estuaries and coastal waters, depending on the level of industrialization and urbanization of the catchment (Seitzinger and Sanders 1997).

1.3.1.2.2 Atmospheric deposition

The importance of atmospheric inputs of DOM has now become evident for both freshwater and the marine environment (Kroeze and Seitzinger 1998; Boyer *et al.* 2002; Berman and Bronk 2003). Recently, DON inputs to estuaries via rainfall have been found to have a biological impact (Seitzinger and Sanders 1999). Net atmospheric deposition was the largest N source (> 50 %) to some USA estuaries such as Barnegat Bay, St. Catherines-Sapelo, Barataria Bay and the forested basins of northern New England (Boyer *et al.* 2002; Castro *et al.* 2003). Rain is a significant source of DOC to surface seawaters and may be equivalent to the magnitude of riverine DOC input to marine environment, where DOC found to be 23 μM C for marine rain and 161 μM C for continental rain (Dafner and Wangersky 2002, and references cited therein). Rainwater provides more bioavailable DOC (13×10^9 g C Yr^{-1}) to the Long Bay than river water (7×10^9 g C Yr^{-1}) (Avery *et al.* 2003). Also, natural fluvial and atmospheric inputs of DON to the marine environments are of similar magnitude (Cornell *et al.* 1995). Therefore, it is important to consider DOM atmospheric deposition when assessing the C and N budgets of aquatic ecosystems (Cornell *et al.* 2003).

1.3.1.2.3 Release from benthic sediment

DOM may be released to the water column from benthic sediments during resuspension and diffusion of pore water DOM (Burdige and Zheng 1998). DOM in sediment pore waters is characterised by heterogeneous compounds ranging in size from relatively large macromolecules such as humic substances and dissolved proteins to smaller molecules such as individual amino acids (Burdige 2002). Much of the total sediment pore water DOC and DON is of relatively low molecular weight and appears to be refractory (Burdige and Zheng 1998). In estuarine and coastal waters sediments can form important sources of DOC and DON to the overlying water column due to the elevated concentration in the pore waters and hence play a vital role in the global carbon and nitrogen cycles (Burdige 2002; Berman and Bronk 2003). For example, in Chesapeake Bay, pore water DOM concentrations were elevated over bottom-water values, up to 160 μM N and 2000 μM C for DON and DOC, respectively (Burdige and Zheng 1998). It has been indicated that sediments play a more important role in the storage of dissolved organic carbon than rain (Dafner and Wangersky 2002b). DON fluxes from the sediments in Chesapeake Bay were reported to be equivalent to river inputs (Burdige and Zheng 1998).

1.3.1.3 Stable nitrogen isotopes as indicator of DON sources

Nitrogen availability in aquatic environments can limit primary production, or in excessive amounts it can stimulate eutrophication and production of harmful algal blooms (Sigleo and Macko 2002). Anthropogenic nitrogen inputs derived from various sources such as fertilizer, livestock waste and sewage have caused eutrophication of many aquatic ecosystems worldwide (Toda *et al.* 2002).

Stable nitrogen isotopes $\delta^{15}N$ have been used to differentiate various sources, namely terrestrial, anthropogenic and *in situ* inputs (Cifuentes *et al.* 1988). Stable nitrogen isotopes

measurements have been used to indicate system level changes at aquatic sites resulting from anthropogenic activities in adjacent watershed (Lake *et al.* 2001). Biogeochemical processes such as nitrification, denitrification, remineralization and algal uptake also affect dissolved nitrogen isotopic composition (Velinsky *et al.* 1990). However, the applications of stable nitrogen isotopes have been used to differentiate sources and *in situ* production of nitrate and ammonium, and the DON fraction ignored due to the analytical difficulty (Feuerstein *et al.* 1997; Bronk 2002). The use of stable DON isotope ratio measurements can (1) reflect long term *in situ* phenomena, (2) provide an independent assessment of dissolved organic nitrogen sources and make the anthropogenic sources identifiable and traceable, (3) reflect the predominate mechanisms controlling DON budget (Feuerstein *et al.* 1997; Kendall 1998). So, the application of stable DON isotope measurements in natural waters provides a new perspective for better understanding of nitrogen biogeochemical cycle (Feuerstein *et al.* 1997).

1.3.2 Sinks for DOC and DON

The four main sinks for DOM are bacterial uptake and mineralization, phytoplankton uptake, photochemical decomposition and adsorption onto suspended particles (Figure 1.9) (Bushaw *et al.* 1996; Sondergaard *et al.* 2000; Middelburg and Nieuwenhuize 2000a).

1.3.2.1 Bacterial mineralization

The biogeochemical role of bacteria within the microbial web is to facilitate the transformation of DOM to POM biomass and/or remineralize DOM back to its inorganic constituents, and hence play an important role in the biogeochemical cycles (Goldman and Dennett 2000). Heterotrophic bacteria are the predominant consumers of DOM and are responsible for the bulk of the DOM decomposition (Sondergaard *et al.* 2000; Becquevort *et al.* 2002). Heterotrophic bacteria are able to directly transport LMW DOM compounds

through their cell membrane via permeases. They can also utilise HMW DOM compounds after hydrolysis to LMW compounds via extracellular hydrolytic enzymes (Sun *et al.* 1997; Carlson 2002). The intermediate compounds that are formed, include amino acids from the hydrolysis of proteins and peptides, and urea from the breakdown of purine. Bacterial production (BP) is a measure represents the net flux of DOM to bacterioplankton that becomes biomass. It has been shown that BP averaged 31 % of local primary production (PP) through a range of geographical locations of marine environment in term of carbon fixation (Carlson 2002).

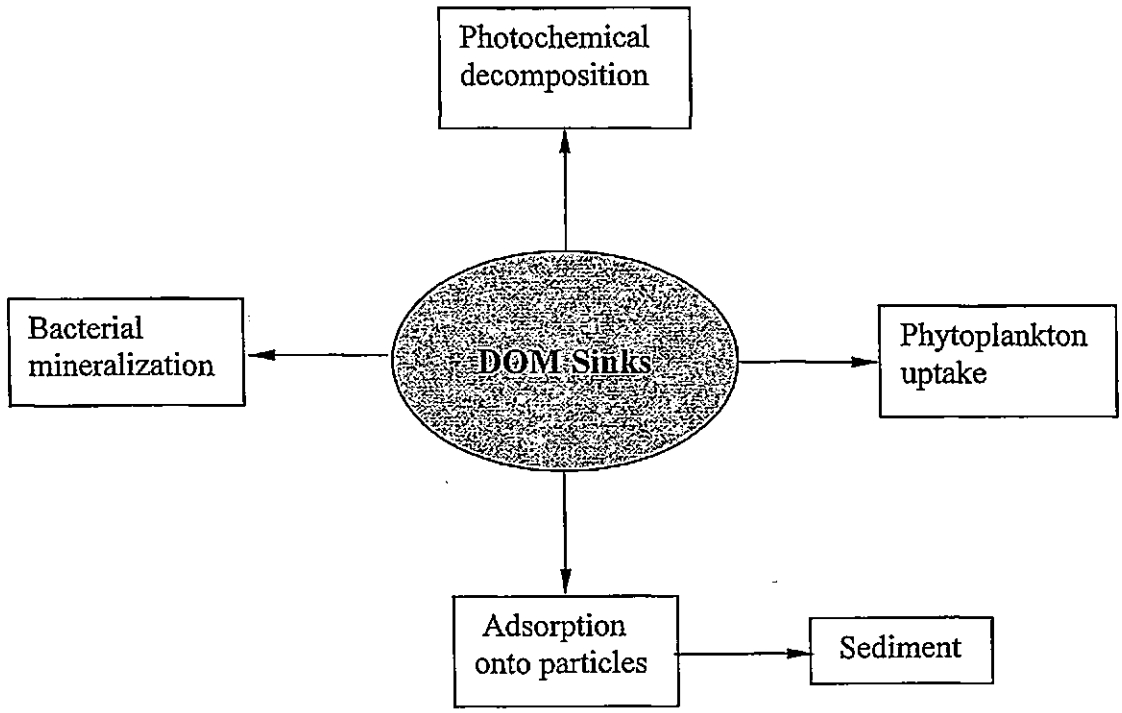


Figure 1.9 Conceptual model of dissolved organic matter sinks in natural waters.

DOC taken up by bacteria is converted to bacteria biomass, and used as an energy source with CO_2 production. So, bacterial mineralization of DOC takes place through the conversion of assimilated DOC to hydrolysed components and ultimately CO_2. The decomposition rate of DOC decreases with time, where the most labile compounds initially

are metabolized first at higher rates, followed by less labile compounds at lower rates (Hopkinson *et al.* 1997b).

Around 70 % of nitrogen transported worldwide by rivers consists of DON but its bioavailability and liability has been poorly investigated (Stepanauskas et al. 1999). These authors showed that marine bacterioplankton assimilated larger fraction of DON than did fresh water bacterioplankton, by a factor of 2.4. This indicates that the susceptibility of DON to bacterial mineralization increases as it is transported from fresh waters into estuarine and coastal waters (Stepanauskas et al. 1999). Bacterial mineralization of DON takes place through the extracellular enzymatic break down of DON and the formation of intermediate compounds such as urea and amino acid due to the break down of the bulk DON. These intermediate compounds are taken up and utilised by bacteria and phytoplankton while further break down will generate NH_4^+ (through the amonification process) (Berman and Bronk 2003). The generated NH_4^+ is then either assimilated directly by micro-biota or oxidised under aerobic conditions to nitrite via *Nitrosomonas* and subsequently to nitrate via *Nitrobacter* (nitrification process). Researchers have demonstrated that heterotrophic bacterioplankton can utilise humic-bound DON and make it available for the growth of phytoplankton (Stepanauskas et al. 1999, and references there in). Bacterial mineralization of DON was observed using incubation experiments in the Delaware Estuary, whereby 40 – 72 % of DON was converted to the biological available DIN within 10 – 15 days (Seitzinger and Sanders 1997). Also, 65 – 85 % of the DON and generated ammonium was rapidly consumed and converted to nitrate in the Elbe Estuary through bacterial mineralization (Kerner and Spitzy 2001).

1.3.2.2 Phytoplankton uptake

There are two possible mechanisms by which phytoplankton take up DOM. First, bacterial decomposition of DOM and subsequent phytoplankton uptake of the released compounds (Bronk 2002 and references therein). The other possible mechanism is the direct incorporation via cell surface enzymes, without bacterial mediation. A number of phytoplankton species have cell surface amine oxidases and amino acid oxidases, which can absorb amino groups from primary amines and amino acids (Palenik and Morel 1990).

Recently, it has been recognised that DON can be a significant source of nitrogen for phytoplankton in the marine environment. It was estimated that, within the equatorial North Pacific, 30 – 50 % of the daily phytoplankton nitrogen demand could be supplied by bacterial remineralization of the DON pool (Benner *et al.* 1997). Some researchers observed that inputs of DON to estuarine and coastal waters stimulate the development of harmful algal blooms, including pelegophyte (brown tide) and dinoflagellates (red tide). Brown tides have been observed along the Northeast Atlantic coast for over 15 years (Bricelj and Lonsdale 1997) and the Long Island's coastal waters (Berman and Bronk 2003), while red tide have been observed along the Egyptian Mediterranean coastal water at Alexandria during the summer of 1998 – 2000 (Mikhail 2001). The nature of organic nitrogen substrates available in estuarine systems could influence the community composition of phytoplankton. Seitzinger and Sanders (1999) found that phytoplankton community structure differed in growth experiments with estuarine water enriched with NH_4 (small monads dominant) or with rainwater DON (dinoflagellates dominant).

1.3.2.3 Photochemical decomposition

Ultraviolet light impacts DOM in the estuarine, coastal and sea waters through photochemical oxidation. This phenomenon plays a vital biogeochemical role in the removal of DOM, as is of increasing interest as incident UV radiation has been increased recently due to stratospheric ozone depletion (Dafner and Wangersky 2002b). Photo-

degradation of DOM is often accompanied by changes in its optical properties (photobleaching) as photo-degradation reduces DOM absorptivity in the UV range (Whitehead *et al.* 2000). There are two photochemical oxidation /degradation pathways of DOM. An abiotic pathway involves the direct photochemical mineralization of DOM into CO_2, NO_2^-, NH_4^+ and to lesser extent CO (Wiegner and Seitzinger 2001). A sequential photochemical / biotic pathway involves the photo-degradation of HMW DOM to LMW DOM compounds, which subsequently utilised by bacteria (Wiegner and Seitzinger 2001; Clark *et al.* 2002). These two pathways together can accelerate both the removal of terrestrially derived DOM in estuarine and coastal waters and the geochemical turnover of old DOM (Mopper and Kieber 2002, and references cited therein). For example, exposure of surface continental shelf of sea water to sunlight for one summer's day can produce DOM photo-degradation products equivalent to > 20 % and 30 % of the bacterial carbon and nitrogen demand, respectively (Moran and Zepp 1997). It is also indicated that 20 – 30 % of terrestrially derived DOC in coastal waters can be rapidly photo-degraded and utilised by heterotrophs (Mopper and Kieber 2002). Photochemical decomposition of dissolved organic nitrogen, in particular humic substances, result in photo production of biologically available nitrogen such as NH_4, DFAA, DCAA, DPA (dissolved primary amines) and NO_2 (Bronk 2002). This photochemical release could contribute to a considerable N flux from DON. Bushaw *et al.* (1996) found that the additional inputs via photochemical release from riverine DON into the SE USA coastal waters increased the terrestrially derived available N by 20 %. In contrast, a mesocosm study in the St. Lawrence Estuary suggested that labile DOM may be transformed into a more refractory DOM by photochemical alterations (Whitehead *et al.* 2000).

1.3.2.4 Sorption onto sinking particles

Sorption of DOM onto suspended particles has been proposed as an important abiotic removal mechanism (Sondergaard *et al.* 2000; Carlson 2002). The consequence of this process may include the following: (1) DOM becomes more refractory because of the close association of the adsorbed DOM with mineral surfaces. More than 90 % of the total sedimentary organic matter, from a wide variety of marine environments, cannot be physically separated from the sediment mineral matrix (Hedges and Keil 1995). Hence, most organic matter preserved in marine sediments is adsorbed to mineral surfaces in protected coatings that are susceptible to degradation only under oxic conditions (Hedges and Keil 1995). However, the close association between DOM and mineral surface may also enhance the degradation of the adsorbed DOM (Keil *et al.* 1994). (2) The DOM may be stored in a slowly degrading sedimentary pool and be available for further modification by geochemical processes on relatively longer time scale. (3) This in turn may de-couple DOM production from bacterial mineralization over the same time scale (Nagata and Kirchman 1996). For example, a significant quantities of organic nitrogen compounds including proteins and amino acids may be removed from solution via sorption onto sinking particles, although they remain susceptible to exploitation by particle–associated bacteria (Berman and Bronk 2003). In summary, it could appear that abiotic complexation of DOM to POM in marine environments is generally reversible as the organic matter is continuously subject to dissolution and bacterial degradation.

1.3.2.5 Accumulation of DOC and DON

DOM is either rapidly consumed through growth and respiration or become resistance to biological utilisation (refractory DOM) (Benner 2002). Understanding how refractory DOM is formed and removed is essential in quantifying the global carbon and nitrogen cycles. Refractory DOM may be produced as a result of biotic (microbial food web) or abiotic (photochemical reaction) transformation (Borsheim *et al.* 1999; Sondergaard *et al.*

2000). Heterotrophic bacteria contribute to the production of refractory DOM (Ogawa *et al.* 2001). HMW DOM compounds are generally more bioreactive than LMW DOM compounds, as enzymatic and photochemical decomposition processes generally decrease the size and molecular weight of DOM (Amon and Benner 1996). Photochemical transformations of DOM occur through free radical reactions which are capable to modify the chemical structure to form photoproducts resistance to biological consumption (Benner 2002). The assimilation of carbon-rich DOM is controlled by the availability of nitrogen; as the consequences of nutrient limitation would be the accumulation of biodegradable DOC (Cauwet 2002). Accumulation has most often been related to algal blooms and their subsequent decay (Sondergaard *et al.* 2000). Physiochemical reactions were proposed as the dominant mechanism for formation of refractory DOM. However Ogawa *et al.* (2001) reported that marine bacteria also rapidly, in < 48 hrs, consume labile DOM (free amino acid and mono-saccharides) and produce DOM that is relatively resistant to decomposition and persist for more than one year.

1.4 Characteristics and composition of DOC and DON

1.4.1 Chemical composition

Data on the concentrations and distributions of individual organic compounds are fundamental for understanding the biogeochemical cycling of C and N. Characterising DOC and DON is complicated because they are composed of numerous compounds that vary widely in space and time within marine environments. The most microbially labile DOC and DON compounds in natural waters are amino acids, simple sugars and urea, followed by polysaccharides, proteins and finally humic substances and fulvic acids (Frankovich and Jones 1998; Murrell and Hollibaugh 2000). Although many DOC and DON compounds have been identified and measured within marine environments, most of the DOC and DON bulk (> 50 %) remains uncharacterised at the molecular level

(Hopkinson *et al.* 1993; Seitzinger and Sanders 1997; Frankovich and Jones 1998; Benner 2002). For example, Hopkinson *et al.* (1993) stated that the DON comprised 25 % amino acids, urea up to 10 % and few percent amino sugars, chlorophyll, amines and vitamins, the reminder was uncharacterized. In estuaries, urea and amino acids comprise 20 % of the DON, with the bulk of DON consisting of uncharacterised complex compounds (Seitzinger and Sanders 1997). Widely identified compounds of the DOC and DON include neutral sugars, amino sugars, amino acids (DFAA and DCAA), urea and humic substances (Bronk 2002; Benner 2002; Berman and Bronk 2003). As listed in Table 1.3, seven neutral sugars and fifteen common amino acids have been measured in marine water. Among those, the most abundant neutral sugars are glucose, galactose, fucose, mannose and xylose (Borch and Kirchmann 1997), while the most abundant amino acids are glycine, alanine, aspartic acid, serine, and glutamic acid (Hubberten *et al.* 1995; Westerhoff and Mash 2002).

Table 1.3 Neutral sugars and amino acids identified in marine waters (Benner 2002).

Neutral sugars	**Amino acids**	
Fucose	Glycine	Tyrosine
Rhamnose	Arginine	Histidine
Arabinose	Alanine	Isoleucine
Galactose	Phenylalanine	Glutamic acid
Glucose	Serine	Lysine
Mannose	Threonine	Methionine
Xylose	Aspartic acid	Leucine
	Valine	

1.4.2 Size classification

Distinctions between major size classes of organic matter in aquatic environments are primarily based on physical separation by passage through filters with varying pore sizes, typically ranged from 0.2 to 0.7 μm. Dissolved organic matter is defined as the fraction of

the organic matter in natural waters which is neither excluded, nor adsorbed by the filter used to remove the particulate organic matter (Wangersky 1993). An 0.7 μM filter does not separate all of the bacteria, viruses and small colloids from the filtrate (Cauwet 1999). Thus, colloids (0.001-1μm size range) fall predominantly in the operationally defined DOM size class using the traditional filtration procedures (Eyrolle *et al.* 1996; Buesseler *et al.* 1996). It has been reported that colloids measurement is important for the characterisation of truly dissolved organic matter (Wells and Goldberg 1991; Benner *et al.* 1997). For example, in the Pacific Ocean, colloidal organic carbon accounts for approximately 25 % of the DOC in surface water (Kepkay 2000). Proteins adsorbed to colloids in natural waters are more slowly degraded than free dissolved protein (Nagata and Kirchman 1996).

Ultrafiltration (UF), also known as tangential- flow filtration (TFF) or cross- flow filtration (CFF), is a valuable tool for investigating the size distribution of DOM, and for separating truly dissolved and colloidal organic matter (Benner 2002). The high flow rates and large membrane surface areas in UF greatly decreases processing times, so sufficient material can be rapidly concentrated and isolated for detailed characterization. DOM can be operationally classified into two classes based on passage through an ultrafiltration membrane with a ~1-nm pore size (ca. 1000 Dalton cut off or 1000 nominal molecular weight) (McCarthy *et al.* 1996). HMW DOM is the fraction retained using a 1000-Dalton cut off membrane, while LMW DOM is the fraction that passes this membrane. This size based distinction between LMW and HMW DOM is quite important for understanding the sources and transformations of DOM. In estuaries, bacteria and some phytoplankton rapidly assimilate very LMW DON compounds such as urea and amino acids (Seitzinger and Sanders 1997). However, recent studies of bacteria in freshwater and marine environments demonstrated that bacteria prefer HMW DOM over those of LMW DOM as

substrates for their heterotrophic processes (Amon and Benner 1996; Sun *et al.* 1997; Seitzinger and Sanders 1997; Kerner and Spitzy 2001). HMW DOM has long term bioavailability and can nearly completely be used as alternative N pool; whilst the LMW DOM includes substances of short term availability, that are preferred over HMW DOM, but at the same time contains significant proportions of refractory DOM (Kerner and Spitzy 2001).

1.4.3 Optical characteristics

Over the past decade, there has been a renewed interest in the optical properties of DOM pool in natural waters (Clark *et al.* 2002; Blough and Del Vecchio 2002). The fraction of the DOM that absorbs light in both the ultra violet and visible ranges is termed chromophoric dissolved organic matter (CDOM) (Rochelle-Newall and Fisher 2002; Stedmon *et al.* 2003) and the fraction of CDOM that re-emits absorbed light at longer wavelengths (fluoresces blue) is termed fluorescent dissolved organic matter (FDOM) (Chen *et al.* 2002a). CDOM has been referred to in the past as yellow substance, gelbstoff, gilvin and humic substances (Nelson and Siegel 2002). CDOM lies at the centre of the photochemical cycles that critically impact the aquatic environment (Clark *et al.* 2002). The recent interest in studying CDOM has a number of reasons.

1- CDOM is of particular interest to remote sensing because it absorbs blue light in the same region of the spectrum as chlorophyll a in coastal and estuarine waters, which can result in overestimation of chlorophyll a concentration by satellite sensors (Rochelle-Newall and Fisher 2002).
2- CDOM in estuarine ecosystems is usually present at much higher concentrations than in other marine systems, originating primarily from terrestrial sources via rivers (Bowers *et al.* 2004).

3- Absorption of sunlight by CDOM also initiates the formation of a variety of photochemical products, which are a sink for DOM; this phenomenon may significantly influence the biogeochemical carbon and nitrogen cycles (section 1.3.2.3).

4- The increased levels of UV radiation due to ozone depletion in polar regions have raised concerns over the possible impacts of UV radiation on both freshwater and marine ecosystems. So the amount of CDOM in surface waters that absorbs light strongly in the UV range controls the penetration depth of radiation that is potentially harmful to aquatic organisms (Blough and Del Vecchio 2002).

5- The remote determination of DOC concentrations in estuaries and coastal waters may be feasible where there are observed correlations between CDOM absorption (or fluorescence) and DOC concentration (Rochelle-Newall and Fisher 2002).

1.5 The estuarine environment

Rivers transport materials from terrestrial sources across the estuarine environment, through the coastal receiving zones and into the open ocean (Mortazavi *et al.* 2001). The sources of the dissolved and particulate materials found in river water, include wet and dry atmospheric deposition, rock weathering, decomposition of organic matter, and anthropogenic inputs (Chester 2003). Water reaches the river environment from rainfall, surface runoff, discharge from aquifers and wastewater.

An estuary is defined as an inlet of the sea reaching into a river valley as far as the upper limit of the tidal rise and within which seawater is measurably diluted with freshwater associated with land drainage (Fairbridge 1980; Elliott and McLusky 2002). Estuaries mediate the transfer of organic matter from drainage basins to coastal and ocean waters (Prahl and Coble 1994; Goni *et al.* 2003). Understanding the nature and cycling of organic

matter in estuarine systems allows better estimates of carbon and nitrogen transport to the coastal waters (Bouloubassi *et al.* 1997; Murrell and Hollibaugh 2000). Estuaries can act as retention pools, where the mixing of riverine and seawater end-members delays the export of non-reactive dissolved continental loads for a period equivalent to the flushing time for freshwaters. In addition, estuaries can function as efficient reactors where physical (adsorption/desorption, flocculation, deflocculation), chemical (oxidation/reduction, complexation) and biological (production/respiration) processes occur. The extent of these processes affects the distribution profile and behaviour of the reactive dissolved organic matter, and consequently their export from the riverine-estuarine system to the coastal waters (Miller 1999). The reactivity of dissolved compounds and flushing rates are dependent on their chemical nature, biogeochemical processes in the riverine estuarine system and its geomorphology (i.e. river flow, tidal range, turbidity, etc.) (Goni *et al.* 2003). Within the estuarine environment, there are usually large spatial and temporal changes in variables such as salinity, pH, DO and SPM, these in turn may affect the chemical reactivity and distribution of other chemical components such as DOM.

The dilution of seawater with river water in the estuarine environment leads to a longitudinal salinity gradient and hence the estuary can be distinguished into three distinct sections; a lower (marine) estuary, a middle estuary and an upper (fluvial) estuary (Elliott and McLusky 2002). The lower estuary is in free connection with the sea. The middle estuary is subject to strong sea water and fresh water mixing. The fluvial estuary is characterised by fresh water inputs but is subject to daily tidal action. Estuaries can be classified, depending on tidal action, into micro-, meso-, macro-, and hyper-tidal with tidal range of < 2 m, $2 - 4$ m, $4 - 6$ m, and > 6 m, respectively (Uncles *et al.* 2002).

Estuaries can be classified, according to water circulation and salinity into the following (Bowden 1980; Chester 2003):

1- Highly stratified estuary or salt wedge estuary; the Mississippi Estuary is an example. The river flow dominates the circulation almost completely. The salt water extends into the river as a wedge under the fresh water flow, the out flowing water remains fresh up to the mouth of the river. In some estuaries the saline layer is not confined to a wedge shape, and in this case the salinity of the upper water layer increase as it moves seaward.

2- Partially mixed estuary such as the Winyah Bay (Goni *et al.* 2003), in which there are still two layer structures, with fresh water at the surface overlying saline water. However, there is a vertical mixing, due to tidal currents, between the mainly inflowing bottom water and the outflowing surface water. This mixing take place via eddy diffusion, with freshwater mixed downward and saltwater mixed upward; and salinity gradually increases from surface to bottom.

3- Vertically well mixed estuary, e.g. the Delaware Estuary. Well mixed system occur when the influence of tidal mixing relative to that of the river flow is very strong; there is little variation in salinity with depth.

Depending on the physical mixing, a dissolved constituent may behave in a conservative and / or non conservative manner (Chester 2003). Physical mixing of fresh and saline waters, of markedly different compositions, is one of the main processes, which modifies riverine transported materials. The physical mixing process results in a linear relationship between the concentration of a component and salinity. If the distribution of dissolved component in an estuary is controlled only by the physical mixing pattern, its concentration along the salinity gradient will yield a straight line (theoretical dilution line, TDL) and the dissolved component behaviour is described as being non-reactive or conservative in

behaviour. In contrast, when the component is reactive and there is addition to or removal from the dissolved state, its concentration will deviate from the TDL and is described as non-conservative behaviour (Figure 1.10).

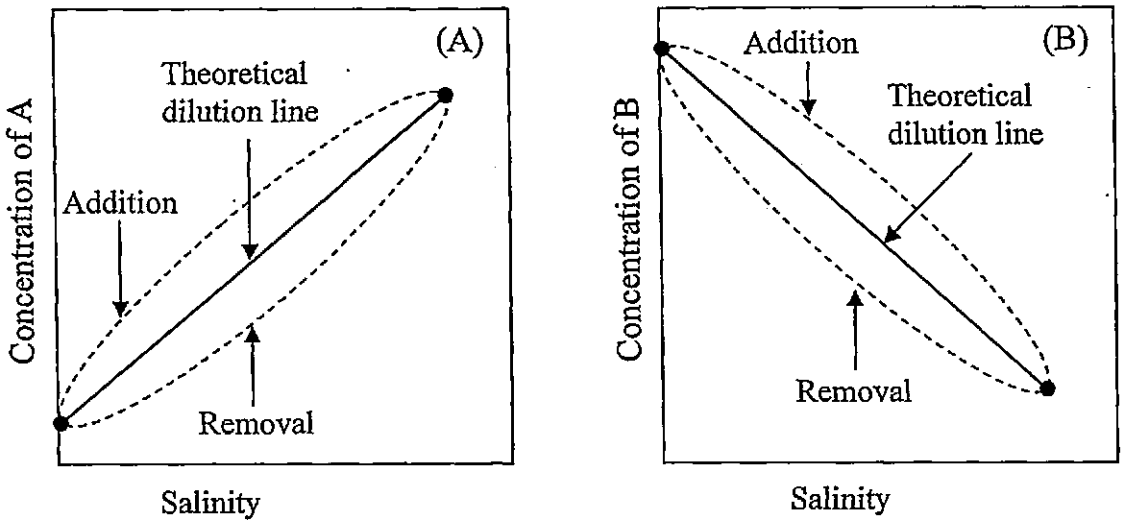


Figure1.10 Estuarine mixing graphs for (A) a component with a higher concentration in the sea water than in river water, (B) a component with a higher concentration in the river water than in sea water.

Processes that are involved in the removal of dissolved components in estuaries include flocculation, adsorption, precipitation and biological uptake; transferring them into particulate phase. The breakdown of organic matter and desorption from particulate surfaces result in the addition of components into the dissolved phase. Anthropogenic inputs through out the estuarine environment also contribute to the addition processes. The flushing or residence time is a measure of the time required to transport a conservative component from a specific location within the estuary to the mouth (Bowden 1980). The flushing time is represented as follows:

$$T\ (\text{day}) = \frac{V\ (\text{volume of fresh water in the estuary, } m^3)}{R\ (\text{river flow rate in to the estuary, } m^3 d^{-1})}$$

1.6 Aims and objectives

The overall aim of this study was to undertake an environmental assessment involving the investigation of the inputs, biogeochemical cycling and export of dissolved organic carbon (DOC) and nitrogen (DON) in contrasting riverine-estuarine systems. To achieve this aim the following objectives were set out:-

1- The optimization of a high temperature catalytic oxidation (HTCO) analytical method for reliable analysis of DOC and TDN in natural waters.

2- The investigation of temporal (seasonal) and spatial changes in DOC and DON concentrations in contrasting riverine-estuarine systems that are influenced to different degrees by anthropogenic carbon and nitrogen inputs, the Yealm and Plym estuaries.

3- Examine the bioavailability of DON within a riverine-estuarine system via laboratory incubations using natural bacteria from the Plym Estuary.

4- 15N / 14N isotope analyses of DIN and DON to identify sources of dissolved N, and *in situ* N transformations.

Chapter Two

Analytical and sampling methods and materials

Analytical and sampling methods and materials

2.1 Introduction

Understanding of the global carbon and nitrogen cycles depends initially on accurate measurements of the concentrations of C and N species in natural waters. Total carbon and nitrogen species include dissolved organic carbon (DOC) and nitrogen (DON), particulate organic carbon (POC) and nitrogen (PON), and dissolved inorganic carbon (DIC) and nitrogen (DIN). DIN comprises ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). Methods for the measurement of DIC and DIN are well developed (Hansell 1993; Cauwet 1994). DOC and DON analyses are a challenge because the samples are composed of complex mixtures of materials with a range of molecular sizes, weights and reactivities. In addition there are challenges related to the methods of analysis, including the possibility of sample contamination, difficulties in estimating blank values, and problems quantifying the efficiency of the oxidation process (Aiken *et al.* 2002). Moreover, there is no feasible method for the direct determination of DON; thus the DON concentration is calculated as the difference between independent measurements of TDN and DIN. The latter is measured using established colorimetric procedures. The high temperature catalytic oxidation (HTCO) method is recognised as the most precise and accurate technique for the determination of DOC and TDN in natural waters (Alvarez-Salgado and Miller 1998b; Spyres *et al.* 2000). However, the difference method (TDN-DIN) requires a good analytical precision and accuracy of both TDN and DIN analyses.

This chapter will outline an approach for the automated, precise and reliable combined determination of DOC and DON in aquatic samples, including sample collection, handling and preservation, decarbonation, analysis and data quality control. The HTCO instrument coupled to a chemiluminescence detector for the simultaneous measurement of DOC and TDN will be discussed, including a historical perspective, the principle of operation,

analytical system description, analytical challenges and quality assurance. Procedures for the measurement of supporting determinands, including master variables (salinity, pH, conductivity, temperature and dissolved oxygen), ammonium, nitrate and nitrite, reactive phosphate and chlorophyll a, are also described. The analytical aspects of bacterial bioassay incubation experiments and stable nitrogen isotope analysis, which were undertaken to improve our understanding of estuarine DOC and DON biogeochemistry will also be discussed.

2.2 Optimisation of HTCO for DOC and DON determination

2.2.1 Sampling protocol

Sample collection protocols for DOC and DON in natural waters should be designed to minimise changes in sample composition resulting from contamination, sorption onto container walls, biological activity and physicochemical flocculation processes (Sharp *et al.* 1993a; Spyres *et al.* 2000). The following sections describe how these issues have been addressed during the course of this work.

2.2.1.1 Pre-sampling preparation

Clean techniques were used throughout all stages of the sample collection and measurement procedures. In this study all glassware and plasticware used for sample collection and filtration for DOC, DON and other determinands were cleaned using the following protocol. A soak in 2% Decon for 24 h, a rinse with ultrapure water (deionised and UV irradiated) (Elga Maxima®, 18.2 MΩ cm^{-1}), a soak in 10 % HCl for 24 h, and finally a rinse with ultrapure water 3-5 times. Glassware and plastic ware were dried in a closed drying oven at 40 °C and subsequently stored in clean sealed plastic bags until use. The glass filtration unit, glass fibre filters, Pyrex glass bottles, HTCO vials, glass ampoules and any other small glass components used for sample collection and manipulation were

wrapped with clean aluminium foil and combusted in a furnace at 450 – 500°C for 4-6 h to remove any remaining organic residues.

2.2.1.2 Sample collection

Niskin, Go-Flo and stainless steel samplers are commonly used for sampling DOC and DON in the water column (Sharp *et al.* 1993a; Spyres *et al.* 2000). Go-Flo bottle produced by General Oceanic (Miami, FL) with an extra-thick Teflon interior coating and with a replacement of rubber and polyvinyl chloride fittings with silicone and Teflon is in common use (Wangersky 1993). Both Niskin and Go-Flo bottles are designed to minimise contamination and can pass the air-sea interface closed (Spyres *et al.* 2000). It is important that the sampler passes through the air-sea interface closed, to prevent sampling of the DOM-rich surface water microlayer. For shallow rivers and estuaries, DOC and DON samples can be collected by hand directly into Pyrex glass bottles. Plastic containers that have been thoroughly cleaned and aged can be used for temporary storage of the sample (Wangersky 1993; Sharp *et al.* 1993a).

Sample collection was undertaken as follows. Polyethylene plastic gloves were worn throughout the sample collection and the following handling procedures. The sample collection bottles for DOC, TDN and DIN were 250 mL Pyrex glass fitted with Teflon lined cap. The samples were collected from the surface water (0.2 m depth) after rinsing the bottles 2-3 times with the sample to reduce sorption to the glass wall, as shown in Figure 2.1 (Sharp et al. 1993a). Filtration and the following procedures were undertaken within 2-3 hours of sampling. Regular sample collection was undertaken in the Yealm and Plym estuaries at high tide and the same geographical locations; in order to understand temporal, spatial and seasonal variations (Sharp et al. 1993a). However, during an early stage of the study (Winter 2002), samples were collected from the Yealm estuary-side by

walking (due to difficulty in arranging a boat) which caused inconsistency in sampling time, where some samples were collected at high tide and others at relatively low tide from shallow water. Hence DOC concentrations for those samples collected from shallow water where the highest among all samples. Therefore, data from the early months (February and March 2003) will not be included in the results and discussion chapter.

The sorption of DOM onto clean and combusted glass surfaces due to surface activation from combustion was investigated. Four 250 mL pre-cleaned Pyrex glass bottles that were used for sampling were filled with Yealm estuarine waters from two locations during the May 2003 sampling campaign. Two of them were rinsed three times with the sample before sampling and the others were filled directly with the sample. Then all bottles were subject to the same sample manipulation including filtration and preservation. Results showed identical DOC and TDN values (within the analytical error of measurement) for each station (Y1 and Y2) (Figure 2.2). These observations indicate that there was no significant sorption of DOM onto the glass surfaces, in agreement with the results from other studies (Tupas *et al.* 1994; Sharp *et al.* 2002a).

2.2.2 Sample filtration and preservation

2.2.2.1 Filtration

Filtration should be undertaken immediately, or as soon as practicable, after collection to minimise changes to DOC and DON from biological activity e.g. microbial consumption, cell exudation/lysis (Spyres *et al.* 2000). Filtration is used to remove living and detrital particulate organic carbon and nitrogen from the sample. DOM is defined as the organic fraction which passes the filter chosen for the separation and is not lost in the removal of the DIC (Dafner and Wangersky 2002a).

A clean, glass filtration unit should be used, with filtration typically undertaken through combusted (450°C, 4 – 6 h) glass fibre filters of nominal pore diameter 0.7 μm (i.e. GF/F), which have been widely used (Walsh 1989; Wangersky 1993; Koike and Tupas 1993; Sharp *et al.* 1993a; Cauwet 1994; Cauwet *et al.* 1997; Ogawa *et al.* 1999; Spyres *et al.* 2000; Murrell and Hollibaugh 2000; Aiken *et al.* 2002). Filtration should be achieved under low pressure to avoid cell lysis (0.1 kg cm^{-1}) (Miller *et al.* 1993a; Dafner and Wangersky 2002a).

The advantages of these filters (GF/F) include low contamination and relatively high flow rates (Sharp *et al.* 2002b). However, the retention characteristics of the filter can be changed at combustion temperatures > 450°C, and significant lyses of the micro-organism cells by the glass fibre can occur, thereby increasing DOC and TDN concentrations in the filtrate. Furthermore, they do not separate all of the bacteria, viruses and small colloids from the filtrate (Wangersky 1993; Norrman 1993; Sharp *et al.* 1993a; Cauwet 1999). With respect to the last point, it has been argued that the fraction of particulate organic matter in the filtrate is negligible compared to the dissolved organic material, and that this is lost in the precision error of the analysis (Wangersky 1993; Cauwet 1999; Spyres *et al.* 2000).

Anopore aluminium oxide filter membranes with pore sizes in the range 0.02 – 0.2 μm can also be used to remove small organic colloids and bacteria. They have a low contamination potential and allow a satisfactory filtration flow rate. The disadvantages in their use include adsorption of humic material, particularly when filtering highly productive or turbid waters, when rapid clogging can occur. Increased back-pressure can also occur with clogging, leading to cell lysis and leaching of additional DOC and DON into the filtrate (Williams *et al.* 1993b). Other filters with small (0.2 μm) pore diameters include polycarbonate and polysulphone filters; however, these are not recommended for

use because the carbon in the membrane matrix is readily leached into solution (Spyres *et al.* 2000).

In the current study, all samples were filtered in the laboratory within 2 – 3 h of collection through precombusted 47 mm diameter GF/F filters (Whatman) of nominal pore diameter 0.7 μm in an all glass filtration unit, (Figure 2.3). Filtration was achieved under low vacuum (≤ 5 psi) to prevent cell lysis. Filtration unit was flushed 3-5 times with ultrapure water and twice with the sample between each sample in order to avoid cross contamination (Cauwet 1999).

An investigation to compare the use of GF/F (0.7 μm pore size, 47 mm Ø) and Anopore aluminium oxide (0.2 μm pore size, 47 mm Ø) filter membranes was carried out on samples collected from the Plym Estuary during the July 2002 sampling campaign. Samples were collected from four stations. One aliquot was filtered through a GF/F filter, and a replicate filtered through a GF/F and an Anopore membrane in series. The results showed that in three out of four samples, the DOC concentrations were similar (within the analytical error of measurements) in both the GF/F and GF/F - Anopore filtered samples, (Figure 2.4). The results indicate there were no significance differences in DOC concentrations between GF/F and Anopore filters for our samples from the Plym Estuary. This indicates that the DOC in the Plym was mainly in the size fraction < 0.2 μm. The results also indicate that the two filter membranes, of significantly different nominal pore sizes (0.7 and 0.2 μm), showed similar filtration efficiencies under the conditions examined here.

2.2.2.2 Acidification and storage

There is no widely accepted opinion on which technique is best for DOC-TDN sample storage. Sharp *et al.* (1993a) and Sharp *et al.* (1995) evaluated and viewed quick freezing as one of the best method for preservation of sample. Tupas *et al.* (1994) found there were insignificant differences between freezing and cold storage when the samples are acidified. Wiebinga and de Baar (1998) observed that acidification and subsequent cold storage up to 15 months did not have any significant effect on the DOC values. Another storage method is the addition of mercuric chloride. However, mercuric chloride is toxic and interferes with the HTCO analysis through the deactivation of the catalyst (Wangersky 1993; Dafner and Wangersky 2002a).

After filtration, of a sample, aliquots were transferred to pre-cleaned High Density Poly Ethylene (HDPE) bottles; one for nitrate plus nitrite and reactive phosphate measurements, and another for ammonium (Figure 2.5). These bottles were preserved via freezing (-20°C) in the dark until analysis. A third aliquot was transferred to a 20 mL clean glass ampoule for DOC-TDN analysis and stabilized by acidification to pH 2-3 using 20 µl of 50 % v/v HCl per 20 mL sample. The acidification process drives off the dissolved inorganic carbon as carbon dioxide, and at the same time arrests biological processes (Cauwet *et al.* 1997; Hopkinson *et al.* 1997; Spyres *et al.* 2000; Murrell and Hollibaugh 2000). Some researchers prefer to use orthophosphoric acid (H_3PO_4) in the same proportion instead of HCl as the chlorine ion, resulted from chloride combustion, may corrode the non dispersive infrared (NDIR) gas detector cell of the TOC analyser. The chlorine should be removed from the carrier gas steam using a halogen scrubber (Lee and Henrichs 1993; Chen and Wangersky 1993; Spyres *et al.* 2000). Our HTCO system includes a halogen scrubber to remove the chlorine ions. The acidification process may cause both the adsorption of atmospheric ammonia and organic amines onto the walls of the ampoules,

and the loss of volatile organic compounds (VOC) by hydrolysis of organic matter. However, the latter phenomenon is expected to be insignificant when ampoules are sealed quickly, as VOC values are usually less than 1 % of the DOC values (Wangersky 1993; Sharp *et al.* 1993a; Spyres *et al.* 2000).

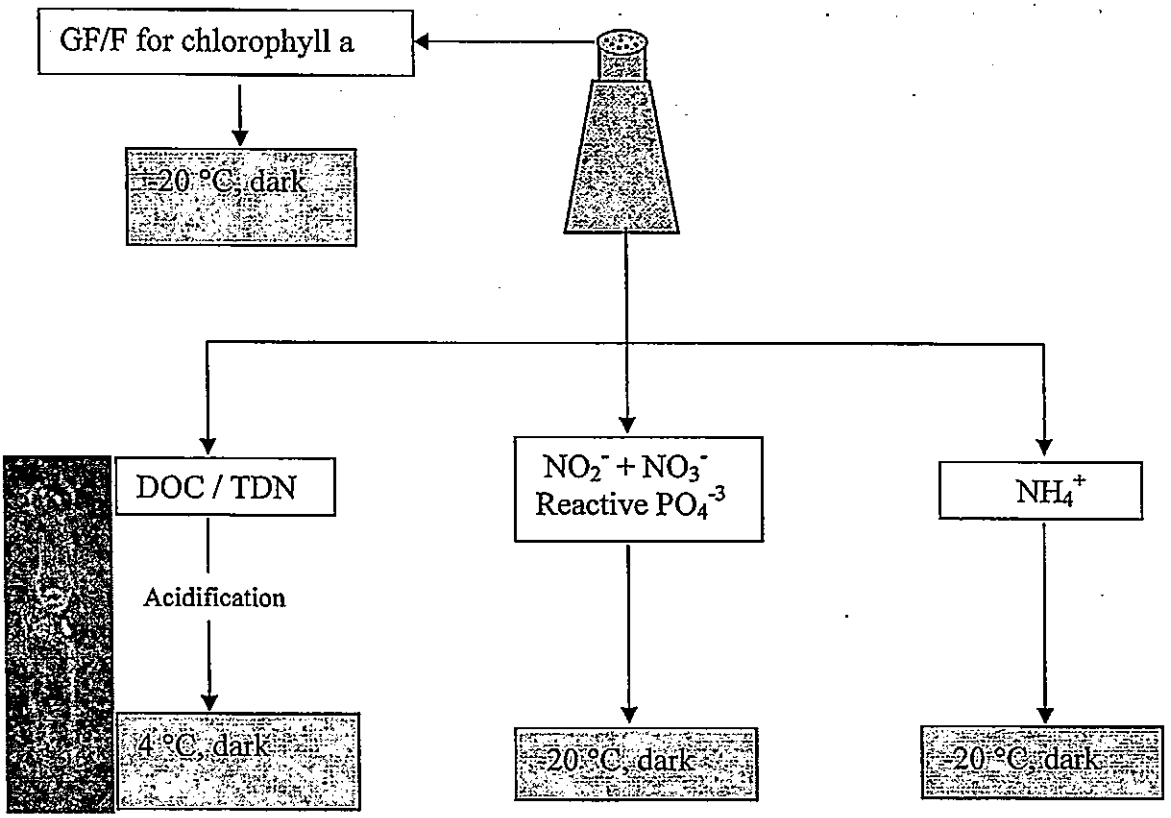


Figure 2.5 Schematic representation of DOC – DON sample preservation.

Following acidification, the glass ampoule is flame-sealed using a butane or propane burner (Miller *et al.* 1993a; Spyres *et al.* 2000). Sealing the ampoules requires care and experience, and contamination of the sample by volatile organics from the flame gas must be avoided. The ampoules are then stored in the fridge (at 4 °C) until analysis. Using this protocol it has been shown that there is neither consistent decrease nor increase in the DOC concentration of the samples due to bacterial break down or sorption onto the wall of the glass ampoule over a four year period (Sharp *et al.* 2002a). The maximum storage time

in the current study was four months for the first sampling exercise in February 2001, until getting reliable and precise data from the coupled HTCO-NCD system in May 2001. Recently, borosilicate glass TOC vials with a Teflon lined cap have become available (I-Chem, Nalgenunc, USA), with a certified TOC blank < 4 μM. These vials also show a negligible TDN blank (1 – 2 μM). These vials were used in some of our experiments.

The potential contamination from the filtration and subsequent steps was assessed by substituting fresh, ultrapure water (i.e. negligible C and N) for the sample. The sample method treatment blank was found to be within the analytical precision and very close to the ultrapure water blank (Figures 2.6 and 2.7). The majority of DOC and TDN values are below 4 μM C and 1 μM N respectively for both sample filtration and ultrapure water blanks. This suggests that there was no significant systematic contamination and supports the measured DOC and DON data quality. Usually, the filtration blanks were subtracted from the measured concentrations. However, high DOC and TDN values (9 – 14 μM DOC and 1.8 – 2.2 μM TDN) were observed for a number of sampling campaigns (27-06-02, 04-07-02, 29-07-02 and 30-07-02) that were possibly due to contamination from the rubber connections of the glass filtration unit used only at that time. As indicated in the Figures 2.6 and 2.7, the higher standard deviations of the measurements were most likely related to the operation of the analytical system near the limit of detection (LOD).

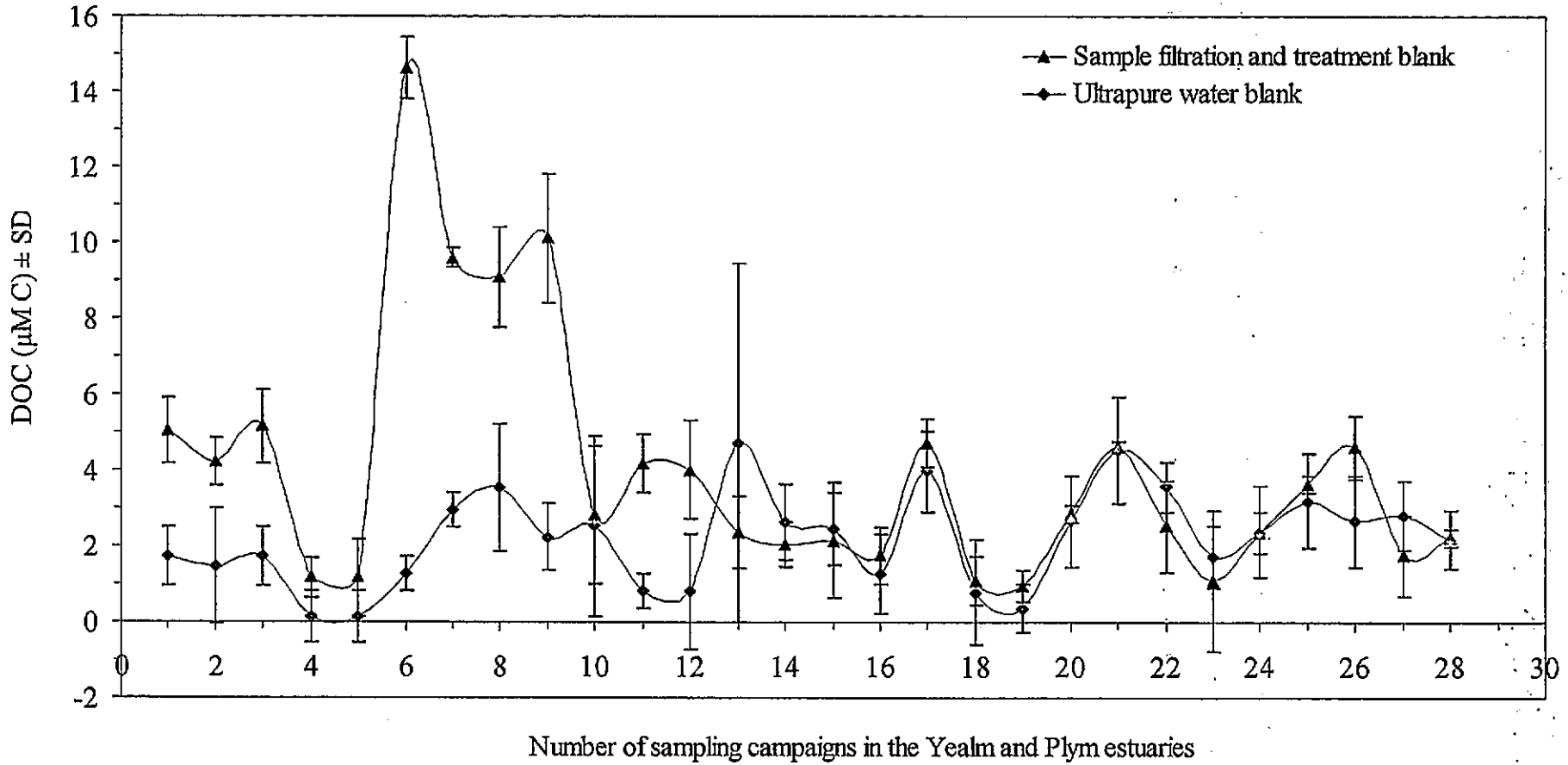


Figure 2.6 DOC sample filtration and treatment blank (µM C) compared with ultrapure water blank for the 28 sampling campaigns undertaken in the Yealm and Plym estuaries (from March 2002 until September 2003).

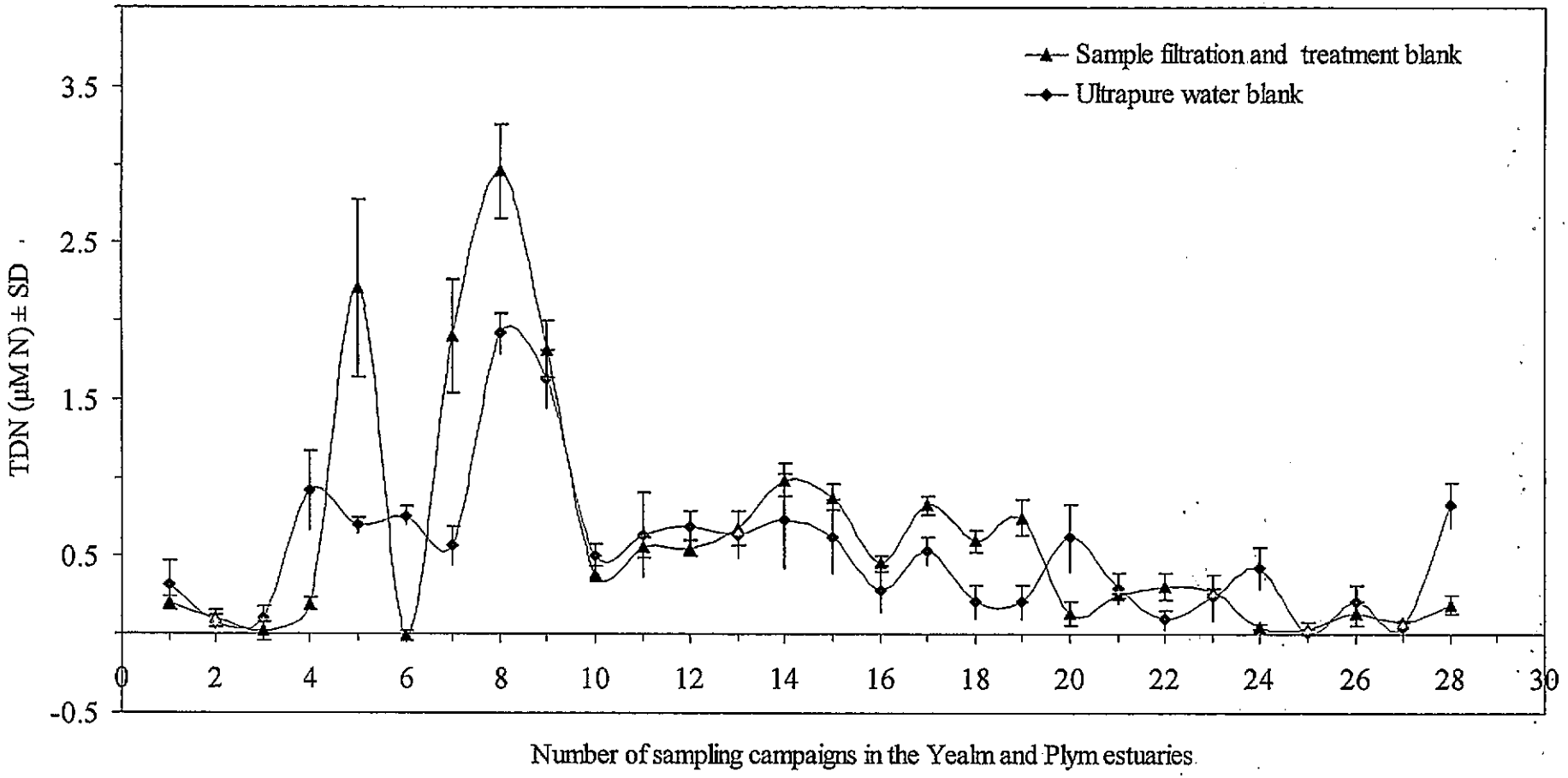


Figure 2.7 TDN sample filtration and treatment blank (µM N) compared with ultrapure water blank for the 28 sampling campaigns undertaken in the Yealm and Plym estuaries (from March 2002 until September 2003).

2.2.3 Analytical method for DOC and TDN determination using HTCO

2.2.3.1 DOC and TDN

For the determination of DOC and DON, one of the following two approaches is commonly used, as illustrated in Figure 2.8. The first relies on wet chemical oxidation (WCO) methods using chemical oxidants (such as persulfate digestion, K peroxide or dichromate in sulfuric acid) and UV photo-oxidation to quantitatively oxidise the DOC and TDN to CO_2 and NO_3^- (Williams *et al.* 1993a; Sharp 1997; Dafner and Wangersky 2002a). The second approach is based on a direct aqueous injection of the sample onto an oxidation column and is in many cases used for simultaneous determination of DOC and TDN. The method relies on the breakdown of all DOC and TDN to CO_2 and NO, which is subsequently measured by infrared and chemiluminescence techniques, respectively (Walsh 1989; Hopkinson *et al.* 1993; Sharp *et al.* 2002b; Peterson *et al.* 2003). The direct aqueous injection approach depends on high temperature oxidation of carbon and nitrogen compounds with (HTCO) or without (HTO) a catalyst (combustion at 680-800°C and 1100°C, respectively). HTO (high temperature oxidation) operates at 1100 °C which causes sublimation of $NaCl$, resulting in damage to the instrument and reduced analytical precision. HTCO, which uses a catalyst (such as Pt on alumina) to oxidise DOC and breakdown TDN, is a key improvement over HTO by obtaining quantitative and reproducible oxidation at lower temperature (680-700 °C).

Through the 1950s and 1960s, WCO methods (mainly persulfate oxidation, PO) and the UV photo-oxidation methods were introduced and became standard approaches for the determination of DOC and TDN in natural waters (see review by Sharp 2002). Each method has advantages and disadvantages as discussed below. Disadvantages of WCO include the following: (i) as a result of the batch nature of the process the oxidant becomes progressively weaker during the procedure. Also the reaction between the oxidant and the

chloride ion in seawater can diminish the oxidising ability of the reagent chosen. (ii) The use of a stronger oxidant can give rise to a higher DOC value (the question of the completeness of chemical oxidation- oxidation efficiency). (iii) The blank determination is considered as the most serious challenge with the PO method. (iv) The methods are time consuming and have sample handling and storage problems (Wangersky 1993; Sharp 1997; Wangersky 2000).

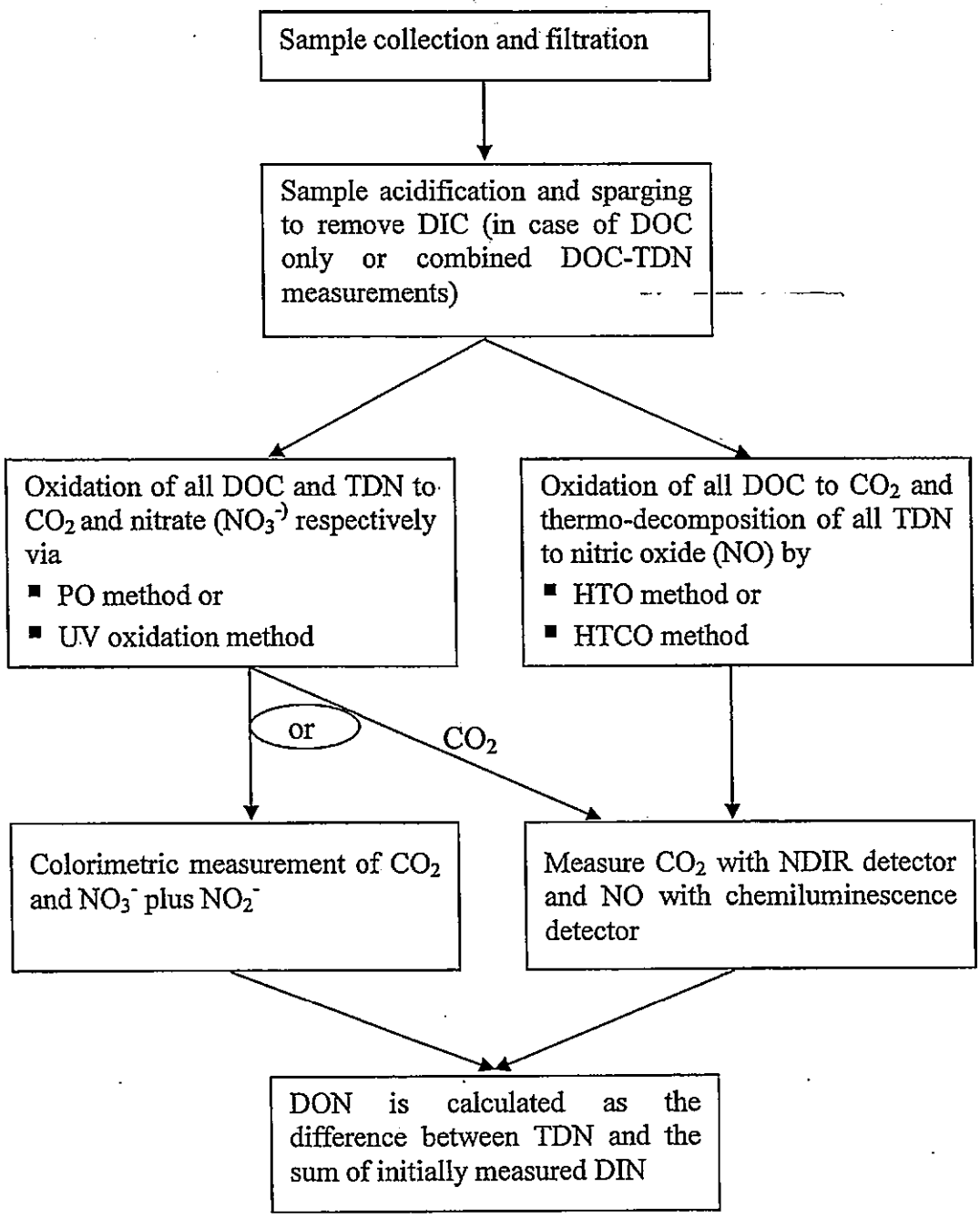


Figure 2.8 Schematic diagram of procedural steps in commonly used DOC and DON analytical methods.

The UV methods have some advantages over WCO. The oxidant is generated continuously, and therefore is not subject to exhaustion. A chemical oxidant can be added to the system to oxidise compounds not readily attacked by oxidants generated photochemically. However, adding chemical oxidant can introduce contamination. Also, the performance of the UV source can degrade with age. The UV oxidation method suffers from the same storage and sample handling problems as WCO (Wangersky 1993)

2.2.3.2 HTCO method

A HTCO method for determination of TDN and DOC in seawater was first reported by Suzuki, *et al.* (1985) and Sugimura and Suzuki (1988), respectively. A number of early studies found that the HTCO method gave higher DOC concentrations than previously observed by PO or UV methods; a controversial finding at that time (Sugimura and Suzuki 1988; Suzuki *et al.* 1992; Peltzer and Brewer 1993; Miller *et al.* 1993a; Williams *et al.* 1993b). These studies indicated that the higher HTCO results were due to a more complete oxidation of all organic matter which meant that HTCO measured additional DOC fractions which are potentially biologically labile but chemically resistant to WCO and UV methods (Miller *et al.* 1993a; Williams *et al.* 1993a; Sharp *et al.* 1993b; Sharp 1997). The controversy over the accuracy of DOM measurements amongst marine scientists lead to the Seattle DOC/DON workshop held in Washington 1991. During this meeting, the HTCO method was recognised as the most precise and accurate technique for the oxidation of DOM in seawater (Hedges *et al.* 1993a; Alvarez-Salgado and Miller 1998b). The workshop brought forward recommendations on the HTCO techniques, including the proper estimation of system blanks, sample collection and storage, and requirements for accurate and precise determinations for both DOC and DON analysis (Wangersky 2000).

Direct comparison between PO and UV methods and HTCO methods showed little or no significant difference between the three methods when freshwater samples were analysed (Benner and Hedges 1993; Sharp *et al.* 1993b; McKenna and Doering 1995). In contrast, in an inter comparison made with seawater samples, higher concentration were measured in samples analysed by the HTCO method (Chen and Wangersky 1993; Tugrul 1993; Miller *et al.* 1993b; Sharp *et al.* 1993b). Later, it was reported that most WCO methods resulted in only few percent lower DOC values compared to HTCO, when the blanks are correctly estimated (Cauwet *et al.* 1997). As analysts, using similar HTCO instrumentation, were unable to reproduce Sugimura and Suzuki's results (Sharp 1997) it was agreed later that the high HTCO values might have been due to poor blank estimation during the first few years of its use (Peltzer *et al.* 1996; Cauwet *et al.* 1997; Cauwet 1999). Eventually, Suzuki requested that the data from his papers be withdrawn and should not used for comparison purposes (Suzuki 1993; Hedges *et al.* 1993b). Sharp *et al.* (2002b) recently reported the first community-wide intercomparison of the three methods for TDN, based on 29 sets of analyses of five water samples. The results suggested that no one approach was grossly inaccurate, although a surprising weakness in the WCO methods was in the relatively poor precision arising from the multiple determinations of nitrate. A couple of broad community inter-calibration exercises were conducted recently for precise and accurate measurement of DOC (Sharp *et al.* 2002a) and TDN (Sharp *et al.* 2004) in marine waters. The DOC inter-calibration showed a composite analytical capability of ± 10 % for a suite of 10 samples distributed to a group of 53 analysts, and using HTCO methods. The use of DOC certified reference materials (CRMs) by 14 participants showed reproducibilities for their laboratories in the 2 – 6 % range. The HTCO instruments also showed the potential to give reliable and accurate TDN values. However the accuracy and precision of the determination of DON is also dependent on the measurement of the DIN fractions of TDN (NO_3^-, NO_2^- and NH_4^+).

Although the HTCO instrumentation needs careful maintenance and a qualified analyst, it potentially provides simultaneous measurements of both DOC and TDN in the same sample. The methods are applicable to all natural waters regardless of salinity, completely oxidises all covalently bonded dissolved organic carbon and dissolved nitrogen, provide high precision and exhibit excellent linearity (Chen and Wangersky 1993; Sharp *et al.* 2002b). The approach is amenable to automated analysis and makes data rapidly available after the completion of the field sampling and analysis, is reliable and relatively easy to operate (Hedges *et al.* 1993a; Alvarez-Salgado and Miller 1998b; Kahler and Koeve 2001; Dafner and Wangersky 2002a; Sharp *et al.* 2002b). Moreover, the availability of reference materials and participation in inter-calibration exercises has resulted in increased accuracy and higher precision (Dafner and Wangersky 2002a). The HTCO methods are currently the preferred analytical technique for the determination of dissolved organic carbon and nitrogen in natural waters and have been well documented and critically assessed by a number of researchers (Cauwet 1999; Spyres *et al.* 2000; Dafner and Wangersky 2002a; Peterson *et al.* 2003; Badr *et al.* 2003).

2.2.3.3 HTCO principle

After removal of DIC by acidification and sparging, as explained in section 2.2.3.4 below, the sample is injected onto the combustion column to oxidise all DOC to CO_2 and thermo-decompose all TDN to nitric oxide NO (probably a combination of NO and NO_2 (Sharp *et al.* 2004)) at 680-800°C in the presence of a catalyst (e.g. platinum on aluminium oxide). $H_2O(g)$ derived from the sample itself is a major source of reactive oxidizing species in HTCO instrument (Chen *et al.* 2002b)

$$\mathrm{DOC\,/\,TDN} \xrightarrow[\text{Catalyst}]{\text{680-800°C}} \mathrm{CO_2 + NO + H_2O}$$

Then, the combusted gases are purified and the CO_2 concentrations are determined using a non-dispersive infrared (NDIR) detector. The signal from the NDIR is recorded (voltage) using a data acquisition/integration system and peak area measurement is used for quantification of DOC concentrations. NO, as the NO_2^* excited state formed following oxidation with O_3, is measured using a chemiluminescence detector (NCD).

$$2\,NO + 2\,O_3 \rightarrow 2\,NO_2^* + 2\,O_2$$
$$NO_2^* \rightarrow NO_2 + h\upsilon$$

The emitted light ($h\upsilon$) is collected by a photomultiplier tube and the resulting signal (voltage) recorded using a data acquisition/integration system. The peak area is used for quantification of the TDN concentration in the injected sample. Figure 2.9 outlines the basic HTCO principle of the DOC-TDN measurements; a more detailed description of the analytical system is presented in section 2.2.4 below.

2.2.3.4 Removal of DIC prior to analysis

Dissolved inorganic carbon removal prior to DOC analysis is critical, as almost all waters with pH > 4 have inorganic carbon from atmospheric and geological sources (Aiken *et al.* 2002). Inorganic carbon (CO_2, CO_3^{-2}, and HCO_3^-) should be completely removed from the sample, by acidification and purging with pure oxygen, before DOC measurement (Wangersky 1993; Peltzer and Brewer 1993; Cauwet 1999). The purging time varies between 3 – 10 min, depending on the gas flow rate and the amount of inorganic carbon in the sample (Dafner and Wangersky 2002a). Sparging conditions should be constant for duplicate or triplicate samples to ensure reproducibility; also the gas flow rate should not be too high in order to avoid sample overflow especially when using vials of small volume. The complete removal of DIC depends on gas flow rate, sparging time, pH of the sample, and sample volume (Spyres *et al.* 2000) and every analyst should establish the optimal

conditions for their system for the complete removal of DIC (Peltzer and Brewer 1993). Purging could remove VOC from the sample, but the VOC fraction lost is usually minor, <1% of total organic carbon (TOC) (Wangersky 1993; Spyres *et al.* 2000).

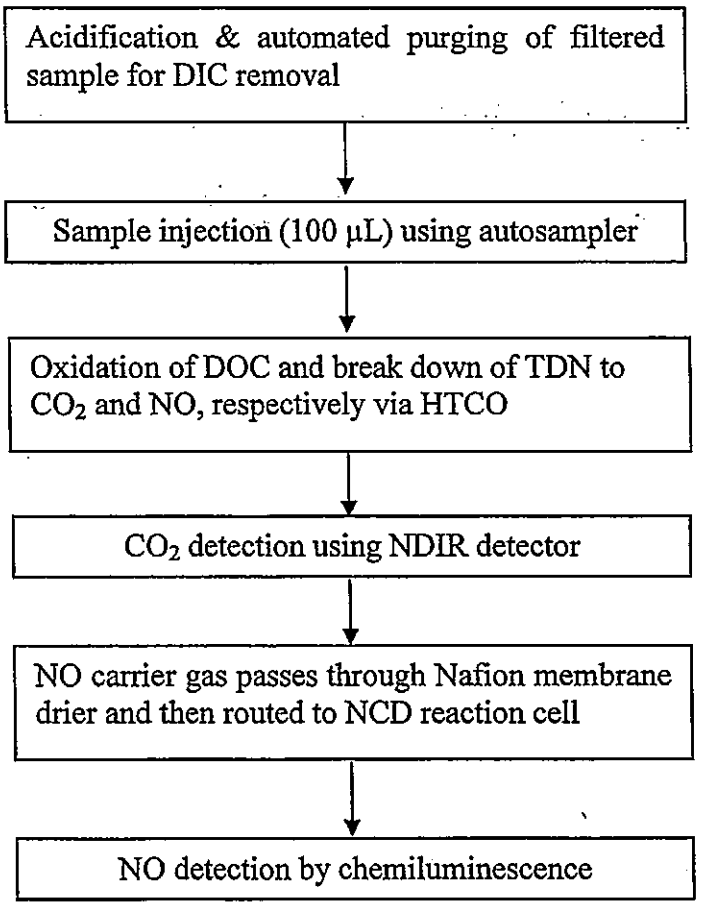


Figure 2.9 Principles of the high temperature catalytic oxidation method for simultaneous determinations of DOC and TDN.

A purging test was performed in the present study using a natural water sample from the Yealm Estuary (21-03-02). The sample was acidified to pH 2-3 using HCl (10 µL of 50 % v/v HCl per 10 mL sample). A constant purging gas flow rate was chosen that is 75 mL min^{-1} ultra high purity oxygen (99.999 %) which is appropriate to the small DOC vials (6 mL) to avoid sample overflow. Then the sample was purged for a series of time intervals (0, 1, 3, 5, 8, 10, 15 and 20 min) and the peak areas for DOC and DIC were recorded. Results for both DOC and DIC showed that under these conditions, at least 8 min of

sparging was necessary for the complete removal of DIC, as illustrated in Figure 2.10. Using this specification, purging for the first minute could remove up to 85% of the DIC in the sample. A constant purging time (8 min), flow rate (75 mL min^{-1}) and volume of acid added for all estuarine samples were followed through the whole time to ensure reproducibility.

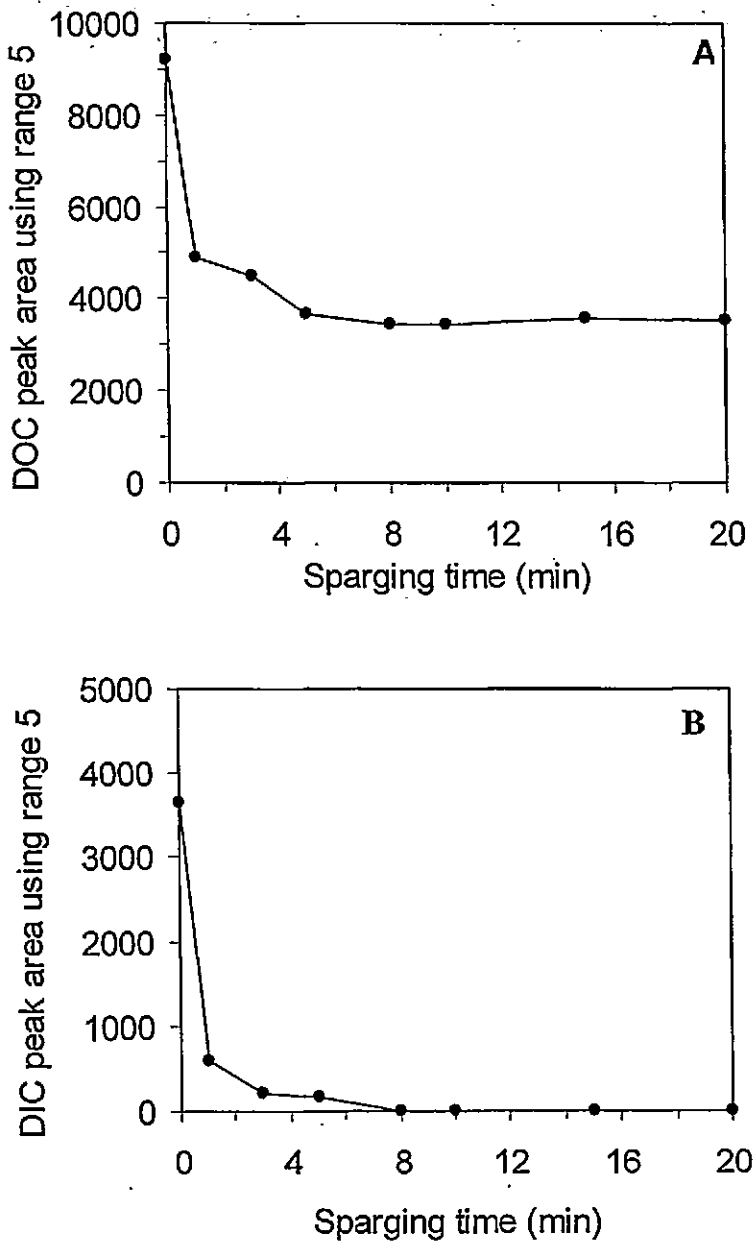


Figure 2.10 Results from sparging trial, representing relationship between sparging time and peak area for DOC (A) and DIC (B).

Table 2.1 Analytical conditions for coupled HTCO TOC-NCD system consisting of TOC-5000A Shimadzu and a NCD-255 (Sievers Instruments).

Instrument conditions	
Carrier gas	Oxygen (ultra pure, 99.999 %)
Carrier gas flow rate through the TOC 5000A	150 ml min^{-1}
Flow rate to the NCD-255	100 ml min^{-1}
Purging gas flow rate	75 ml min^{-1}
Purging time	8 min
Injection volume	100 µL
Number of injections	3 – 5
Catalyst	0.5 % Pt coated aluminium oxide
Furnace temperature	680 °C
Oxidation products	CO_2 and NO
Detection	NDIR (CO_2) and chemiluminescence (NO)
Recording	Peak area
Standards	Mix. of Potassium hydrogen phthalate (KHP) and Glycine (C: N 6:1)

2.2.4.1 Introduction of the sample into the instrument

The sample, after acidification and sparging for DIC removal (section 2.2.3.4), is injected by the motorised syringe of the TOC 5000A into the combustion tube (mounted in a vertical furnace). Three to five replicates of 100 µL sample are withdrawn from the sample vial by an acid-resistant stainless steel needle connected with Teflon tubing to the sample injection valve port. The front of the needle is characterised by ten small pores acting as a filter to prevent needle clogging. Through the use of a double needle arrangement, the next sample to be analyzed is automatically sparged with ultra pure oxygen. The needles are automatically washed with an ultrapure water rinse between sample injections. There is a 4- port valve connected to the motorised syringe. One port is for the sample withdrawal and the second port is for the sample injection onto the

furnace through total carbon (TC) channel in case of DOC analysis. The third port is for the sample injection through inorganic carbon (IC) channel in case of DIC analysis, and the fourth port is for withdrawal of the ultrapure water during the instrument blank check producer (AUX channel). These four channels and other tubes within the instrument are made of Teflon tubing. The syringe and TC injection line are washed four times with ultrapure between samples, and also washed three times with the sample itself before the sample is injected onto the column. Needle blockage due to salt deposit from saline samples was removed by sonication in a low-frequency sonicator, filled with ultrapure water, for 3 – 5 min (Dafner and Wangersky 2002a). Also, the ampoules were shaking well before analysis to make it homogenous.

2.2.4.2 Oxidation

A 100 µL sample is injected into the vertical combustion tube where, by catalytic oxidation at 680 °C, dissolved organic carbon and total dissolved nitrogen compounds are converted into CO_2 and NO respectively. The oxidation products (CO_2, NO, H_2O) are carried by an ultrapure (99.999 %) oxygen gas (BOC Ltd, UK) at a flow rate of 150 mL min^{-1}. Higher gas flow rates decrease the sensitivity, because of the shorter residence time in the combustion tube, and lead to incomplete combustion (Qian and Mopper 1996). Bottled ultrapure oxygen is the preferred sparging and carrier gas because of the guaranteed low contamination (Suzuki *et al.* 1992; Hansell 1993; Alvarez-Salgado and Miller 1998b; Spyres *et al.* 2000). The use of CO_2-free air and low purity bottled oxygen for the TOC analyser, during an initial period of 4 months (September 2000 – December 2000) of this study, resulted in unstable baselines, irregulars peak shapes and low precision and accuracy, as indicated in Figure 2.12.

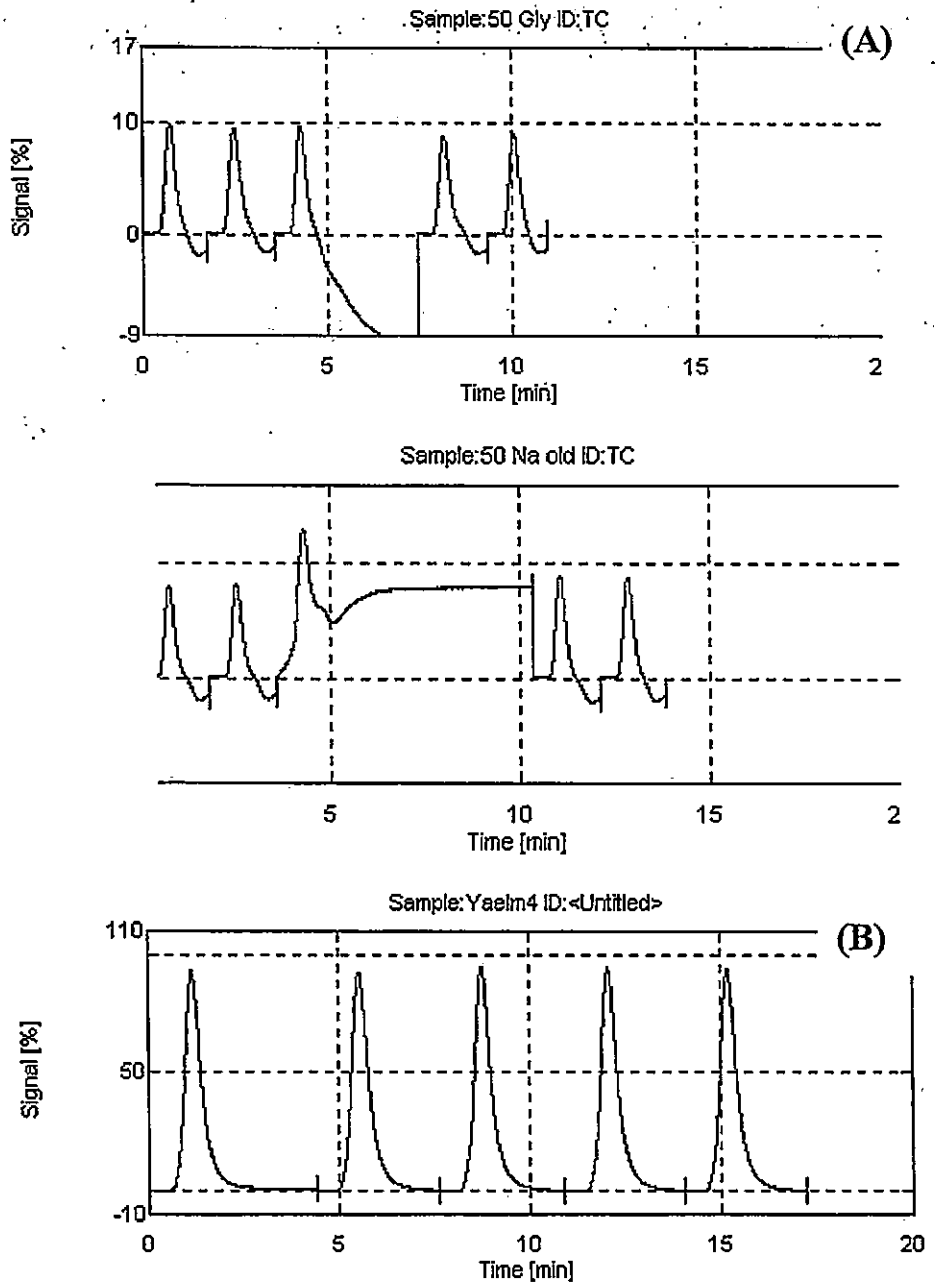


Figure 2.12 DOC peaks shape resulted from the use of CO_2 –free air or low purity oxygen (A) and ultrapure oxygen (99.999 %) (B) as a carrier gas.

The quartz combustion tube is filled with 0.5 % a platinum coated alumina catalyst. The tube is 20 mm in diameter and 230 mm in height, and it is mounted vertically in the furnace. The catalyst particles sit on a platinum net supported by quartz wool (5 mm deep) and the height of the catalyst bed is 130 mm (Figure 2.13). Above the catalyst is a further layer of quartz wool (5 mm deep) which prevents physical disturbance of the catalyst

particles and acts as a barrier to the deposition of salt on the catalyst (Suzuki *et al.* 1992). Injections of ultrapure water between samples was undertaken to eliminate carry over problems due to accumulation of sea salt on the catalyst (Dafner and Wangersky 2002a).

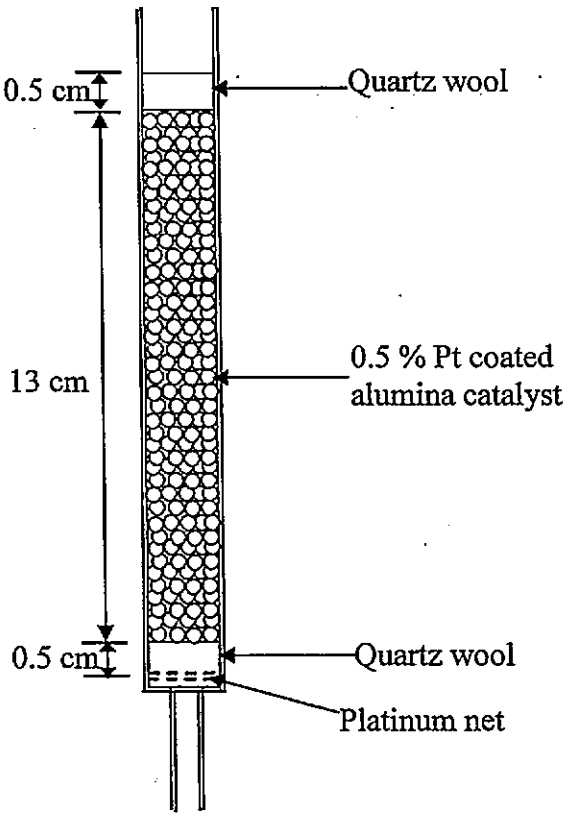


Figure 2.13 The HTCO combustion tube.

Catalyst characteristics

Different types of catalyst, such as Pt on alumina, Pt on quartz, Pt on silica and a mixture of Pt on alumina with CuO and Sulfix, have been used to facilitate DOC and TDN thermo-decomposition. Benner and Strom (1993) found that the alumina support is the primary source of carbon contamination in the platinized-alumina catalyst. The instrument blank associated with the platinized-quartz catalyst is relatively low and would be a better choice for seawater analysis. However, the Pt-quartz catalyst clogs with salt quickly and it cannot be removed from the combustion tube and washed as is the case for Pt-alumina catalyst (Dafner and Wangersky 2002a). Studies indicated that the Pt on silica catalyst has the same

oxidation efficiency as the Pt on alumina, but is usually less contaminated with carbon and gives a lower total blank (Cauwet 1994; Dafner and Wangersky 2002a). A higher blank of the Pt on alumina catalyst is due to the affinity of Al_2O_3, as an amphoteric species, for CO_2, while silica (SiO_2) is an acidic oxide with only a small adsorption capacity for CO_2 (Cauwet 1994; Skoog *et al.* 1997). Also, the Pt on alumina catalyst particles contain carbonaceous impurities that diffuse to the catalyst surface and are oxidized (Skoog *et al.* 1997). However, regardless of the blank signal, all types of catalyst currently in use appear to be equally effective in the oxidation of organic carbon (Dafner and Wangersky 2002a).

In the current study, the Shimadzu 0.5 % platinum coated alumina catalyst was used after conditioning to minimise the blank; see section 2.2.6.1 for details about the system blank. The Pt-alumina catalyst was conditioned as follows: (i) the combustion tube was filled with the new catalyst, (ii) the TOC analyser furnace temperature was heated to 680 °C for 24 h, (iii) ultrapure water was repeatedly injected until the total blank was reduced to an acceptable low value, which was generally a DOC peak area of less than 3000 (Dafner and Wangersky 2002a). The catalyst was changed after the injection of 500 mL of samples plus ultrapure water wash (Qian and Mopper 1996).

2.2.4.3 Purification and DOC detection

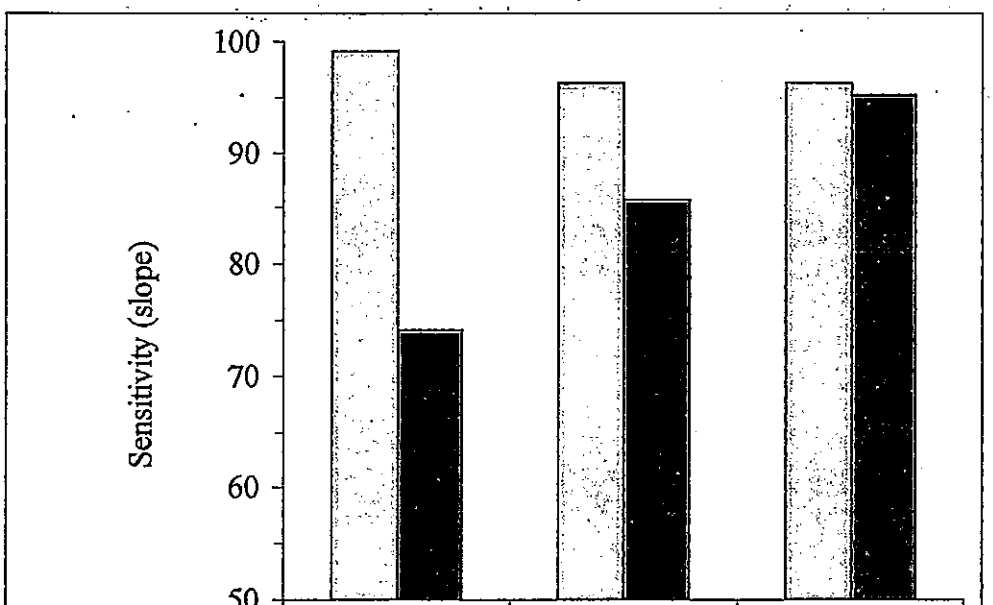
The combustion gases are swept by the carrier gas into a cooling coil and high purity water trap for cooling and H_2O vapour condensation. Then, they are passed through an inorganic carbon (IC) reaction vessel, containing 25% H_3PO_4 (with added $AgNO_3$), which prevents dissolution of CO_2 in water vapour in the stream (Hansell 1993; Alvarez-Salgado and Miller 1998b). The combustion gases are then completely dried in an electronic dehumidifier. Finally, they are further purified by passing them through a halogen scrubber to remove halogens, and a membrane filters to remove particles.

The purified gases are then transferred to the infrared cell for CO_2 detection by a non-dispersive infrared (NDIR) detector. The optical NDIR system consists of a light source, measuring cell, detector and preamplifier. The measuring cell consists of a sample cell and a reference cell. Both have gold plated inner walls. The carrier gas with CO_2 passes through the sample cell while the CO_2-free carrier gas passes through the reference cell. The NDIR principle relies on the fact that CO_2 absorbs infrared radiation in the IR wavelengths, while the monatomic carrier gases (such as O_2 and N_2) do not absorb this radiation. The amount of the infrared radiation absorbed is proportional to the amount of the CO_2 gas, in accordance to the Beer–Lambert Law. The signal (voltage) from NDIR is recorded using a data collection / integration system (TOC 5000A software) and converted to peak area measurements. The peak areas are converted to DOC concentrations using a calibration curve.

2.2.4.4 TDN measurement

TDN was determined by a nitrogen–specific chemiluminescence reaction. Following CO_2 measurements, the combustion gases, including nitric oxide (NO), are pulled into the NCD-255 using a vacuum pump. A gas dehumidifier (Nafion membrane drier) was installed at this stage to remove any remaining water vapour not removed by TOC instrument dehumidifier, because moisture quenches the chemiluminescence reaction and leads to peak tailing (Walsh 1989; Alvarez-Salgado and Miller 1998b). A T-piece has been installed after the Shimadzu NDIR to route ca. 66.6 % of the total carrier gas flow (100 mL min^{-1}) to the NCD, controlled by means of an extra-fine metering valve. The remaining 50 mL min^{-1}, after passing through CO_2 scrubber for removal of CO_2, is passed to the Shimadzu NDIR reference cell and then out to the atmosphere. The gas flow rate to the NCD was optimised at 100 mL min^{-1} in order to maximise the sensitivity of the TDN

measurements whilst not significantly affecting the sensitivity of the DOC measurements, as illustrated in Figure 2.14. This flow rate increased TDN sensitivity by 22 % and decreased DOC sensitivity by only 3 %, compared with the use of only 50 mL min^{-1} for TDN analysis



	50	75	100
DOC slope	99.15	96.26	96.15
TDN slope *(100)	74.11	85.85	95.07

Carrier gas flow rate to the NCD (mL min^{-1})

Figure 2.14 Optimum carrier gas flow rate (mL min^{-1} of the 150 mL min^{-1}) to the NCD detector (tested on 19 and 20th February 2002).

The NO in the combustion gas is reacted with O_3 produced in the NCD reaction cell to give the radical NO_2^* species that chemiluminescence upon decay to its ground state. This reaction take place at low pressure in order to minimise background luminescence and increase sensitivity (Walsh 1989; Alvarez-Salgado and Miller 1998b; Ogawa *et al.* 1999). The emitted light energy is collected by a photo-multiplier tube (PMT) and the resulting signal (voltage) is recorded using data acquisition and integration system (A/D card, Talisman Electronics; slotted into a Pentium PC and controlled using Labview software,

version 6.0). The recorded signal is stoichiometrically proportional to the amount of total dissolved combined N. The TDN peak area is quantified using Labview software. DON concentration is calculated as the difference between independent measurements of TDN and DIN.

2.2.5 Analytical challenges associated with the coupled HTCO TOC-NCD technique

Although HTCO techniques can provide highly precise data, some analytical challenges are encountered.

2.2.5.1 TDN analytical challenges

Potential challenges in the analysis of TDN, using the coupled HTCO TOC-NCD technique, which may lead to poor accuracy and precision, include the following:

1. Residual water vapour; all water vapour must be removed from the combustion gases before entering the NCD because moisture quenches the chemiluminescence reaction, causing peak tailing. The use of chemical driers has been reported, including Drierite (97% $CaSO_4$, 3% $CoCl_3$) (Alvarez-Salgado and Miller 1998b). The use of a Nafion membrane drier (Perma Pure Inc.) in this study resulted in an excellent maintenance-free drying procedure.
2. The peak shape depends on the nature of the nitrogen compound (Alvarez-Salgado and Miller 1998b). The higher oxidation states (e.g. +5 for nitrate) and more refractive compounds show wider peaks, indicating that decomposition to NO may occur through different reaction mechanisms. However, peak area, rather than peak height, was used for quantification purposes as it is relatively unaffected by the nature of the N compounds.
3. In water samples with enhanced TDN concentrations (> 80 μM, e.g. many estuarine and river water samples) a longer peak integration time for the TDN relative to the DOC is required for the coupled TOC-NCD instrument used in this study. The high

TDN concentrations result in increased tailing of the TDN peaks possibly due to slow oxidation of a fraction of the N compounds. Consequently, longer DOC peak integration times and an appropriate dilution with ultrapure water were used for samples with expected enhanced TDN concentrations to allow complete conversion of all N compounds. Recent study indicated that the Shimadzu instruments coupled with the chemiluminescence detectors provide more accurate TDN measurements than the TDN stand alone instruments (Sharp *et al.* 2004) .

4. At a generic level applicable to both the HTO/HTCO and WCO methods, a small relative error in the TDN or DIN measurements can lead to a large error in the estimate of DON particularly when the DON is a small fraction of the TDN (Hopkinson *et al.* 1993; Sharp *et al.* 2002b). Moreover, there is an assumption that the dissolved organic nitrogen is unreactive to standard DIN procedures (Walsh 1989). Therefore quality assurance protocols and an excellent analytical precision are required for the TDN and DIN measurements.

2.2.5.2 DOC analytical challenges

Regarding DOC measurements by the HTCO method, the most significant sources of error are mechanical effects, the estimation of system blank, and the oxidation efficiency (Spyres *et al.* 2000). Mechanical effects include sample injection, salt deposition and memory effects (Qian and Mopper 1996; Alvarez-Salgado and Miller 1998b). Manual injections of samples are intensive labour, time consuming, susceptible to contamination and difficult to reproduce. These challenges are less likely to occur with the use of automated injections (Qian and Mopper 1996). However, the automated injection system may cause problems during analysis of saline samples due to their susceptibility to salt abrasion with time (Spyres *et al.* 2000). The salt abrasion problems were eliminated with regular and careful cleaning and maintenance of the needle, syringe, and injection slide.

Injection of the sample at high temperature results in an expansion introducing volume higher than the void space of the combustion tube. Subsequently a cold zone at the column head may result from higher pressure, causing the deposition of salts (Skoog *et al.* 1997). Salt deposition on the top of the catalyst causes clogging of the column head and then incomplete combustion and memory effects (carry over between samples). Memory effects were evident particularly when injection of sample or standard with a high DOC concentration was followed by a sample or ultrapure water with low concentrations of DOC (Qian and Mopper 1996). The salt residue at the column head can randomly flush through during sample injection, resulting in reduced accuracy and precision. Also, deposition and flaking off of organic carbon in the cold zone results in the appearance of abnormally large peaks at random. A systematic injection of ultrapure water between saline samples was followed in order to prevent salt deposition and eliminate the carry over problem (Dafner and Wangersky 2002a). The top of the catalyst was covered with a layer of quartz wool (5 mm) to support salt deposition instead of catalyst particles (Suzuki *et al.* 1992); as explained in section 2.2.4.2. Also, the following maintenance was frequently undertaken: (i) flushing the system with ultrapure water (up to 50 injections) every day before starting analysis and at the end of the run, (ii) regeneration of the catalyst with 2 M HCl, (iii) removal of the salt deposition on the top of the catalyst, (iv) replacing the catalyst after the injection of 500 mL sample solution. The other two challenges (the instrument blank and oxidation efficiency) are discussed in section 2.2.6 below.

2.2.6 Quality assurance

The reliability of the data from the HTCO method analyses relies upon (i) careful blank determination, (ii) a systematic evaluation of the oxidation efficiency of a variety of organic compounds (including less easily oxidised compounds), (iii) accurate and precise instrumental calibration using easily oxidised standard compounds, and (iv) testing

analytical veracity with certified reference materials (CRMs), in this case deep ocean water with low concentrations of DOC and TDN (Wangersky 1993; Hopkinson *et al.* 1993; Peltzer *et al.* 1996; Sharp 1997; Bouloubassi *et al.* 1997).

2.2.6.1 HTCO method total blank determination

The correct procedure for determination and subtraction of total blank is of key importance for high quality DOC and TDN data. Using the HTCO method, the total blank is derived from (i) the use of ultrapure water, (ii) a reagent blank from the acid added for acidification (iii) the instrument (or system) blank generated from sample line contamination and the catalyst used (Benner and Strom 1993; Williams *et al.* 1993a; Cauwet 1999). The peak area of the injected acidified and purged ultrapure water, obtained during the external calibration, gives the total blank. The acid reagent blank and system blank is common to both standard and samples, while the water blank for standard only (Benner and Strom 1993; Williams *et al.* 1993a).

2.2.6.1.1 DOC total blank

The water and reagent blanks are minor components of the total blank compared to instrument blank (Benner and Strom 1993; Dafner and Wangersky 2002a). The Pt on alumina catalyst is the major source of carbon from the instrument (Sharp *et al.* 1993a; Sharp *et al.* 1995; Cauwet 1999). Inaccurate estimation of the instrument blank was the probable cause of most of the reported high DOC values in 1980s and early 1990s (Hedges *et al.* 1993a; Sharp 1997). High instrument blank may result from (i) the accumulation of carbonaceous residues such as SiC_2 (s) and CaC_2(s) on the surface of the combustion tube between sample injections, (ii) slow diffusive flux of carbonaceous impurities in the catalyst to the surface in the combustion tube during the time interval between sample injections (iii) sorption and desorption of CO_2 by the alumina as it is amphoteric oxide

(Perdue *et al.* 1993; Skoog *et al.* 1997; Alvarez-Salgado and Miller 1998b). However, the instrument blank can be reduced to < 10 μM C after rigorous cleaning and conditioning of the catalyst and systematic injections of ultrapure water during analysis (Wiebinga and de Baar 1998; Alvarez-Salgado and Miller 1998b; Dafner and Wangersky 2002a). The major difficulty with total blank estimation is the lack of a completely carbon-free water to calculate the carbon contamination from the instrument alone (Dafner and Wangersky 2002a).

Although no common protocols for DOC blank have been described, recent literature provides clear guidelines for blank correction (Alvarez-Salgado and Miller 1998b; Cauwet 1999; Spyres *et al.* 2000; Sharp *et al.* 2002a). The first option is to shift the calibration curve to the origin and make a correction to the sample by subtracting the instrument blank (Cauwet 1994) as illustrated in Figure 2.15. Not accounting for the water blank in the calibration curve (using peak area minus total blank then divided by slope without shifting) will result in underestimation of the DOC concentration in the samples by the ultrapure water blank value (Benner and Strom 1993). In contrast not accounting for the instrument blank (using peak area of shifted calibration divided by slope) will result in overestimation of the DOC in the sample (Benner and Strom 1993; Miller *et al.* 1993b). Thus the accurate determination of the instrument blank, particularly for samples of low DOC concentrations, is essential for good blank correction. Some HTCO instruments, including the Shimadzu TOC 5000 A, are provided with a blank checking program. This program involves the injection of ultrapure water onto the catalyst where it is combusted and the collected downstream in a high purity water trap comprising theoretically carbon-free water. Subsequently, this solution is re-injected (five replicates of 100 μL each) and the whole process is repeated 10 times to give 50 injections of theoretically carbon-free water (Alvarez-Salgado and Miller 1998b). The instrument blank is then calculated as the

average peak area of the tenth series of injections (the last five injections in the whole 50 injections); this is converted to a concentration value using a daily calibration curve (Benner and Strom 1993).

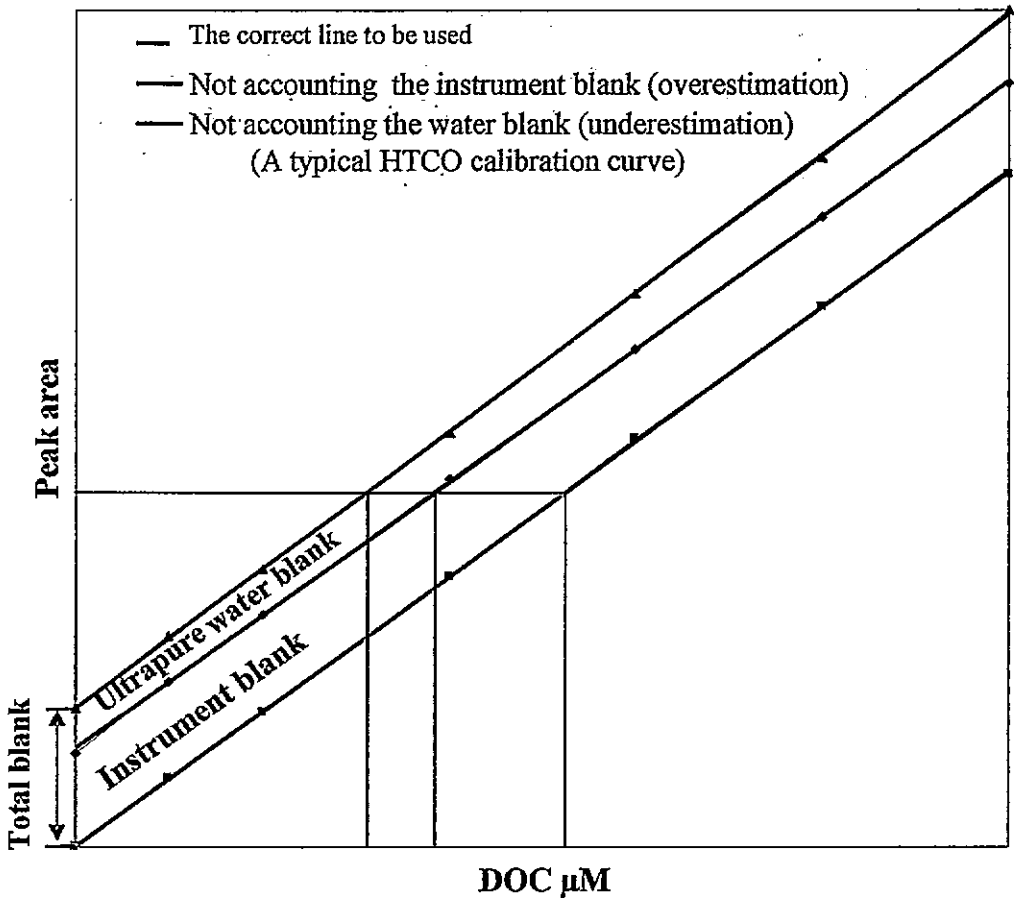


Figure 2.15 DOC calibration curves to illustrate the HTCO method total blank and how to correct for the DOC concentration in the natural water samples.

The automated blank checking (BC) program of the Shimadzu TOC-5000A was employed three times in the first year of this study to determine the instrument blank. Figure 2.16 shows the peak area against the number of injections using the blank check program on 30-01-02 (1st BC), 13-05-02 (2nd BC) and 11-06-02 (3rd BC). The average concentrations of the last four injections, excluding the first, from the tenth series of injections were 3.5, 24.9 and 23.6 µM C for the 1st BC, 2nd BC and 3rd BC, respectively. The first injection of each series of five injections gave abnormal peak areas, very high for the 1st and 2nd BC, and

very low for 3rd BC, and thus they were excluded from the calculation of the instrument blank (Figure 2.17). The 2nd BC value was the highest as a newly conditioned catalyst was used, whereas the catalysts used with the 3rd BC and 1st BC were one month and four months old, respectively. Thus, the instrument blank is relatively high with a new catalyst, and decreases to stable value after washing with aliquots of ultrapure water (Cauwet 1999). However, the above approach was not appropriate for the correction of DOC data in this work because the calculated instrument blank area was higher than the daily total blank using injections of ultra pure water, excluding the 1st BC done before conducting the field study.

The other, common alternative method for blank correction, which was adopted for this study, is the subtraction of a daily average ultrapure water peak area (total blank) from the sample peak area; the resulting peak area is divided by the calibration curve slope (Qian and Mopper 1996; Wiebinga and de Baar 1998; Spyres *et al.* 2000; Sharp *et al.* 2002a; Dafner and Wangersky 2002a).

$$\text{DOC Concentration } (\mu M) = \frac{\text{Peak area of the sample} - \text{Peak area of the blank}}{\text{Slope of the calibration curve}}$$

This correction is appropriate when the blank check program is not available or when the average peak area of the instrument blank using BC program is higher than the daily total blank using an injection of ultrapure water (Spyres *et al.* 2000). However as discussed above, this blank correction results in an underestimation of the DOC concentrations (by the value of the ultrapure water) in samples with low level of DOC, including deep ocean waters. Frequent injections of ultrapure water and certified low carbon water (LCW, 2 μM C) during this study gave approximately identical peak area. This means that the blank of

the ultrapure water used during this study is approximately 2 μM C, and this concentration was added to all measurements of the natural water samples.

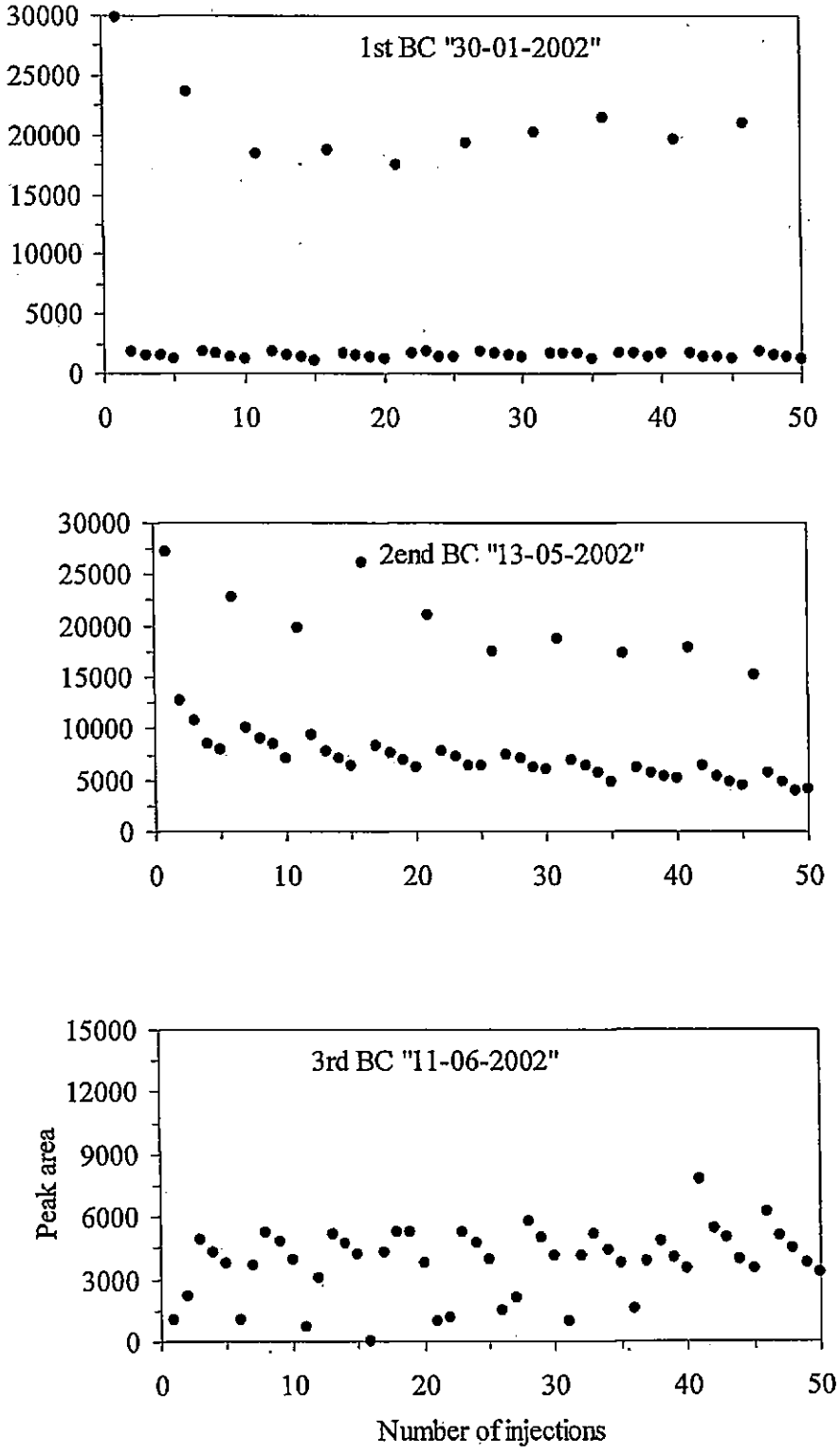


Figure 2.16 TOC 5000A instrument blanks using Shimadzu platinized-alumina catalyst.

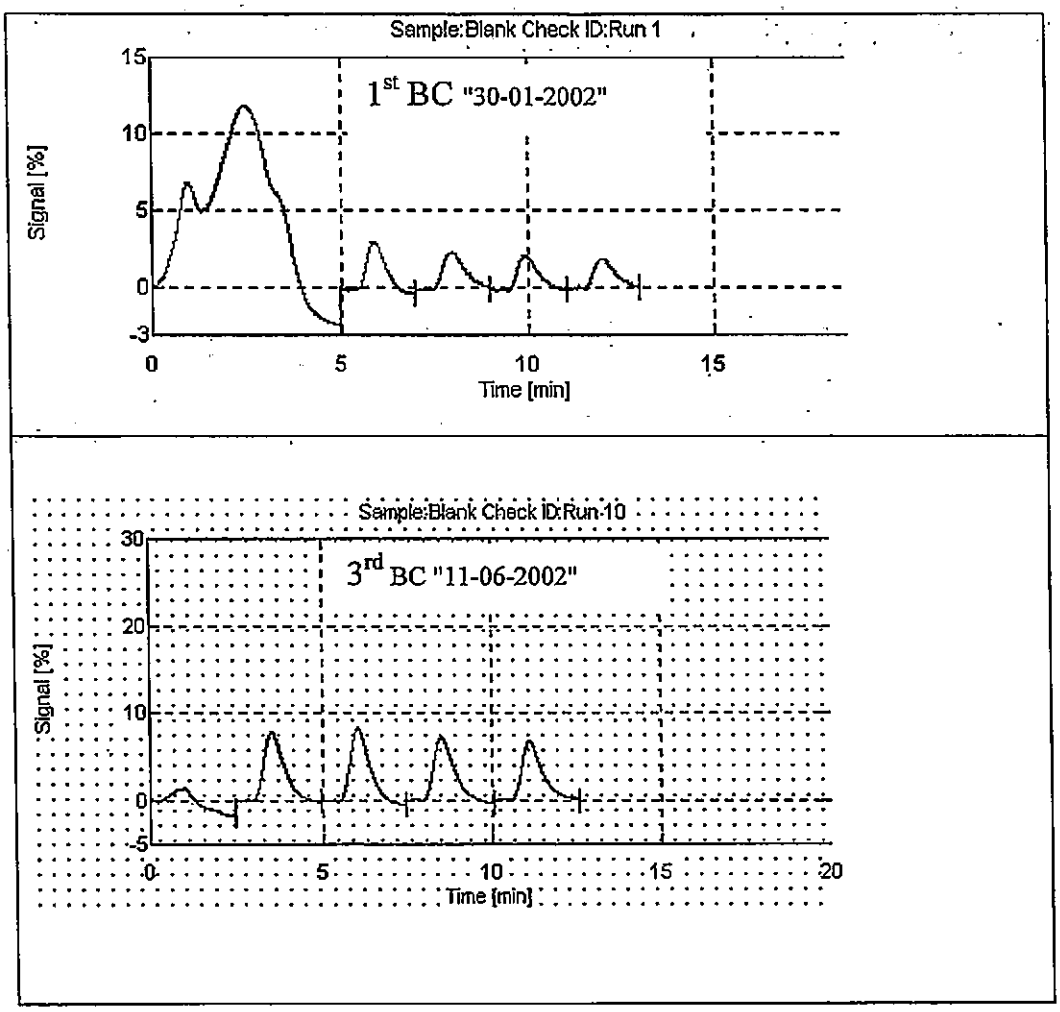


Figure 2.17 TOC 5000A instrument blank peak profiles using Shimadzu platinized-alumina catalyst.

2.2.6.1.2 TDN total blank

In the case of TDN, the combined instrument and ultrapure water blank was typically very low compared with the DOC total blank, at ca. 1 ± 0.1 μM N, and was near or even below the detection limit. This agrees with findings by Hopkinson *et al.* (1993), who estimated that their combined HTCO and HTO total blanks ranged between 1 and 3 μM N, whilst Koike and Tupas (1993) estimated their HTCO combined blank as 2.37 μM N using double distilled water. Walsh (1989) reported that his instrument blank was always below the detection limit, using an HTO method at 1100°C (detection limit not reported). Alvarez-Salgado and Miller (1998) reported a TDN system blank for their coupled HTCO

TOC-NCD system to be in the range < 0.3 – 0.6 μM N. These observations indicate that the combined system and ultrapure water blank should be less troublesome for HTCO TDN analysis compared with DOC, and that special attention should be paid to the quality of the ultrapure water used for TDN standards and blank investigations.

2.2.6.2 Oxidation efficiency

Natural DOM comprises a spectrum of compounds of varying resistance to oxidation. Since the chemical character of DOM remains poorly described, it should not be assumed that any one standard compound is representative of natural DOM. A range of organic carbon and nitrogen compounds of differing refractivities should be investigated to test the ability of the analytical system to oxidise naturally occurring organic matter quantitatively. The degree of oxidation of a range of compounds should initially be determined daily and recoveries established for each method and/or instrument, after which periodic assessment is sufficient (Hopkinson *et al.* 1993; Spyres *et al.* 2000). The HTCO method has been shown to be able to quantitatively recover the carbon and nitrogen from a range of compounds with different degrees of refractivity, including humic materials (Hopkinson *et al.* 1993; Alvarez-Salgado and Miller 1998b; Bronk *et al.* 2000). Some examples are given in Table 2.2.

Table 2.2 Percent recoveries of carbon and nitrogen from standard compounds by HTCO technique.

Analyser / Detector	Catalyst type	Compound	% Recovery	Reference
		(1) Dissolved organic carbon		
Sumigraph N-200 NDIR	Pt on alumina	Thiourea Antipyrine Caffeine Albumin Xylose Glucosamine Cu- chlorophyllin Sulfathiazole	99 98 100 98 99 99 101 63	(Sugimura and Suzuki 1988)
Home made NDIR	5 % Pt on Trion Kawool and Co_2O_3 on alumina beads	Glucose Urea Thiourea Glycine Caffeine Methyl orange Antipyrine Sulfathiazole	98 102 107 106 107 91 111 97	(Chen and Wangersky 1993)
Sumigraph TOC-90 NDIR	3 % Pt on alumina, CuO/ Sulfix	Glucose Potassium biphathalate	95 93	(Tanoue 1993)
Home made NDIR	Quartz beads/CuO/ Sulfix	Antipyrine Caffeine EDTA Glyoxal termeric dihydrate Phthalate Sulfathiazole Thiourea	99.9 102.8 99.7 103.4 99.8 100.7 103	(Qian and Mopper 1996)
		(2) Total dissolved nitrogen		
Antek 703C Chemilumin escence	Quartz wool	Ammonium chloride Potassium nitrate Sodium nitrite Glycine 2,2 Bipyridine Nicotinic acid N-1-Naphthylethylene-diamine EDTA Urea Sulfathiazole Antipyrine Methyl orange	100.2 100.1 100.7 99.6 100.7 100.3 96 101.1 101.2 101.6 97.7 95.5	(Walsh 1989)

Not cited (workshop report)	Not cited	Ammonium Nitrate Nitrite EDTA Urea Glycine Caffeine Thiourea Albumin Nicotinic acid Antipyrine	100 ≥ 90 ≥ 98 ≥ 100 ≥ 90 ≥ 90 ≥ 90 ≥ 90 ≥ 90 ≥ 98 ≥ 90	(Hopkinson *et al.* 1993)
Yanaco TN-7 Chemiluminescence	Manganese dioxide	Urea Thiourea Adenine Caffeine Valine Tryptophan Nicotinic acid Thiamine	100 94 101 102 100 113 98 102	(Koike and Tupas 1993)
Shimadzu TOC-5000 Antek 705D Chemiluminescence	0.5 % Pt on alumina	Ammonium chloride Antipyrine Arginine Caffeine EDTA Histidine Nicotinic acid Sodium nitrite Potassium nitrate Sulfathiazole Thiourea Thymidine Urea	97 85 102 97 102 102 101 101 102 99 96 103 101	(Alvarez-Salgado and Miller 1998b)
Antek 7000N Chemiluminescence	1000 °C	Glycine Urea	99.5 94.3	(Frankovich and Jones 1998)
Antek 7000B-N Chemiluminescence TDN	1000 °C	Urea Ammonium chloride Glycine EDTA Antipyrine Humic acid Potassium nitrate	96.8 95.5 86.2 75.5 79.1 61.0 100.4	(Bronk *et al.* 2000)

Recovery experiments with carbon and nitrogen compounds were performed three times (05-02-02, 28-05-02 and 19-06-02), covering a variety of chemical structures and concentrations. The tested compounds are ammonium chloride, sodium nitrate, glycine, urea, thiourea, EDTA, caffeine, N-1-naphthylethylene-diamine and glucose. Nitrogen and carbon recoveries were in the range 90.5 – 103.6 % (Table 2.3) and 90.3 – 104 % (Table 2.4), respectively, with a low value of 79.5 % (N) for the more recalcitrant compound caffeine. The percent recoveries were calculated relative to a mixture of potassium hydrogen phthalate (KHP) and glycine standard (C: N 6:1 see below). The results are in reasonable agreement with observations reported by other researchers (Table 2.2).

Table 2.3 Percent recoveries of nitrogen from commonly cited nitrogen compounds dissolved in ultrapure water using the coupled HTCO TOC-NCD method (recovery in relation to KHP/glycine standard).

Compound	Concentration (μM)	% Recovery		
		28-05-2002	19-06-02	Average
Ammonium chloride	20	93.4	93.0	94.9
	40	96.9	96.3	
Sodium nitrate	20	99.1	103.6	100.8
	40	99.8	100.7	
Glycine	20	99.6	99.1	99.0
	40	98.1	99.3	
Urea	20	95.2	85.8	92.1
	40	96.2	91.2	
Thiourea	20	97.2	101.8	98.6
	40	96.4	99.1	
EDTA	20	92.2	93.9	92.2
	40	90.5	92.2	
N-1-Naphthylethylene-diamine	20	93.5	95.9	94.3
	40	95.4	92.5	
Caffeine	20	-	81.0	80.3
	40	-	79.5	

Table 2.4 Percent recoveries of carbon from commonly cited carbon compounds dissolved in ultrapure water using the coupled HTCO TOC-NCD method (recovery in relation to KHP standard).

Compound	% Recovery (05-02-2002)						
	50 µM	100 µM	200 µM	300 µM	400 µM	500 µM	Average
Glucose	92.7	93.4	93.9	93.3	93.4	93.6	93.4
Glycine	101.4	99.3	104.0	101.9	100.0	97.0	100.6
Urea	91.9	93.4	98.3	91.7	90.7	91.1	92.9
Thiourea	104.0	92.1	92.6	90.3	92.3	91.9	93.9
EDTA	101.4	100.7	98.2	95.1	94.7	93.2	97.2
N-1-Naphthylethylene-diamine	96.9 (120 µM), 97.5 (240 µM)						97.2
Caffeine	102.4 (40 µM), 98.9 (80 µM)						100.7

2.2.6.3 HTCO method calibration and analytical figures of merit

There are many reference materials that may be used to prepare standards, but the selected material should be widely distributed with high purity, soluble in water, stable to temperature and light, resist microbial degradation and not hygroscopic (Cauwet 1999). A potassium hydrogen phthalate (KHP; $[C_6H_4CO_2(CO_2H)]^-$ K^+) and glycine (NH_2CH_2COOH) mixture is probably the most commonly used standard for combined DOC-TDN calibration (Alvarez-Salgado and Miller 1998b; Cauwet 1999; Spyres *et al.* 2000). Also, a mixed nitrate and KHP standard has been used for calibration by some workers, where DIN is expected to dominate the TDN, as in deep oceanic water (Walsh 1989; Hopkinson *et al.* 1993).

The coupled TOC-NCD system was calibrated daily using a KHP and glycine mixture (C: N atomic ratio 6:1) in ultrapure water. Working standard solutions (30 – 600 µM C and 5 – 100 µM N) were prepared from stock solution (600 mM C and 100 mM N) by the use of a sub-stock solution (60 mM C and 10 mM N) and an appropriate dilution with ultrapure water. The working standard solutions were prepared prior to analysis while the sub-stock solution and the main stock were prepared weekly and monthly, respectively. Linear

regression was used, peak area against concentration, for each daily calibration of DOC and TDN (Figure 2.18). The DOC and TDN concentrations of each sample were defined by the mean peak area of replicate sample injections (n = 3 – 5), subtracted by the average peak area of the total blank (as discussed in section 2.2.6.1) and divided by the slope of the DOC and TDN calibration curves respectively.

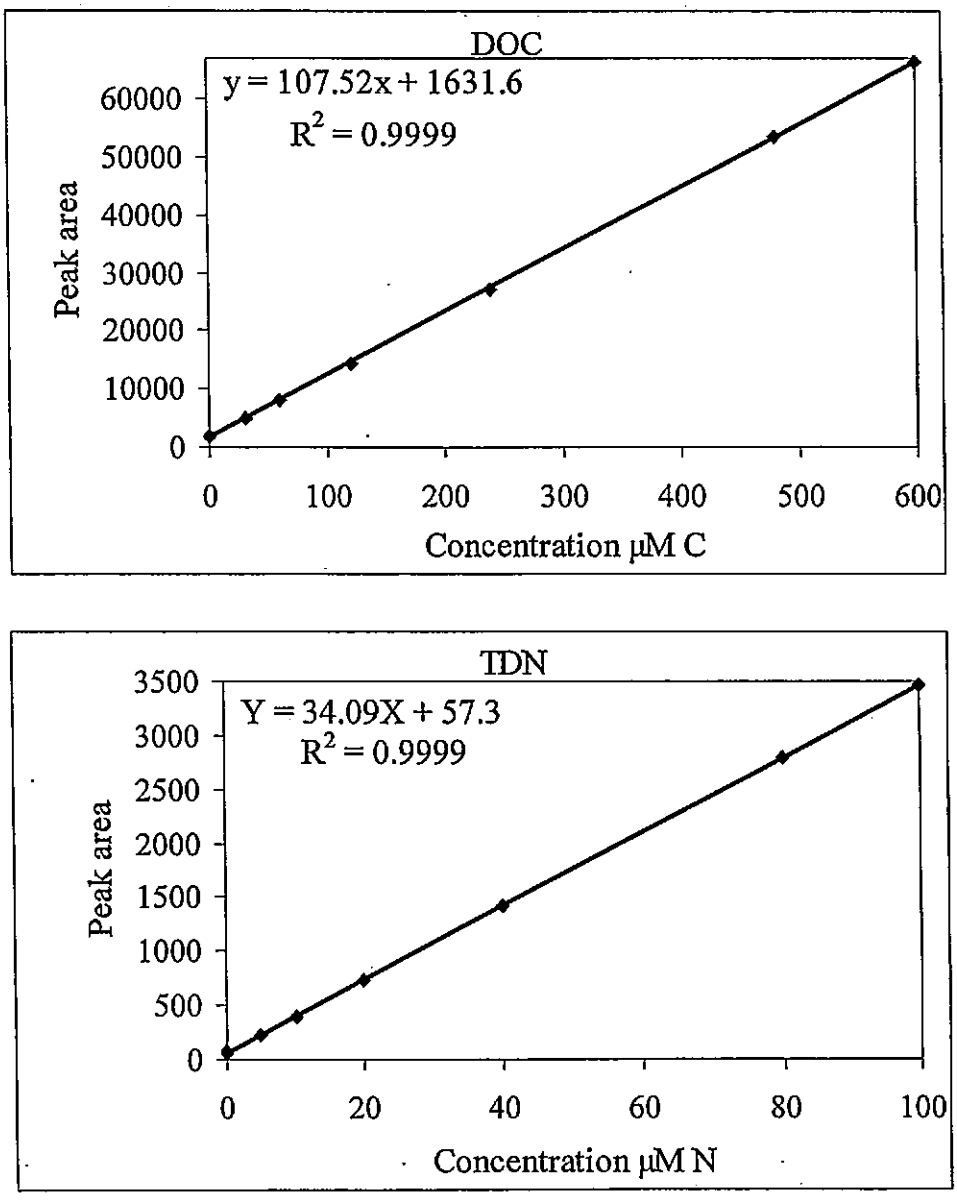


Figure 2.18 DOC and TDN linear regression plot of calibration standards (n = 6, date of analysis 25-10-2002) using HTCO method.

Analytical figures of merit

The linear regression coefficient of the DOC and TDN calibration curves was ≥ 0.999 (n = 6), indicating good linearity between concentration and peak area. Using range 1 of the TOC analyser software, calibration range was linear up to 600 μM and 100 μM of DOC and TDN, respectively. The calibration slopes were used to indicate the stability of the instrument sensitivity. The average slopes of the DOC and TDN calibration curves were 100.3 ± 5.9 and 30.7 ± 3.5 respectively over the period May 2002 until September 2003, indicating good stability for the measurement of DOC and TDN throughout this period. The limit of detection (LOD) was calculated based on average peak area of blank plus 3 standard deviation. The TOC-NCD system used here had a limit of detection of 6.2 μM C and 0.46 μM N, calculated from an analysis done for the North Atlantic samples on 23-03-2003. A standard solution of known concentration (240 μM C and 40 μM N) was systematically re-analysed between each batch of 5 – 10 samples to determine whether on a daily analytical run there was drift of the TOC-NCD system response.

The coefficient of variation (CV) or precision for the combined DOC/TDN analysis was typically < 2 – 3 % and < 3 – 5 % (n = 3 – 5) for DOC and TDN measurements, respectively. The standard deviation (σ) of the DON measurement is estimated from the following equations:

$$\sigma_{DON} = (\sigma^2_{TDN} + \sigma^2_{DIN})^{1/2}$$

$$\text{Where } \sigma_{DIN} = ((\sigma^2_{NO3} + \sigma^2_{NO2}) + \sigma^2_{NH4})^{1/2}$$

The DON concentrations therefore incorporate the combined error of three analyses that are TDN, NH_4^+ and $NO_3^- + NO_2^-$. With a typical CV for DIN of 3 %, it can be seen that the

standard deviation of the DON signal is relatively small when TDN is mainly DON, and larger when DIN dominates the TDN pool.

Thus the analytical figures of merit indicated that the coupled TOC-NCD system used in this study exhibited an excellent linearity for analysis of standard solutions and good precision for standard solutions and natural water samples, allowing the generation of high quality data for natural waters. The accuracy of the TOC-NCD system was regularly tested by the use certified reference materials (section 2.2.6.4).

2.2.6.4 Certified reference materials (CRMs)

CRMs are essential for checking the performance of HTCO techniques, for quantitative validation and accreditation of the measurement, and for comparing the performance of different laboratories (Williams *et al.* 1993a; Sharp 1997; Spyres *et al.* 2000). The CRMs that have been implemented include both low carbon water and deep ocean water (Dafner and Wangersky 2002a). The advantage of using deep ocean water is that it is unlikely to change in DOC, DON, or TDN concentrations on time-scales of decades and can re-sampled as needed. However, a disadvantage is that the concentration of DIN is high and so dominates the TDN measurement; furthermore, it is not expected to contain much labile DOC or DON (Hopkinson *et al.* 1993). The development of a range of CRMs which are more suitable for TDN and DON concentrations encountered over a range of natural waters would be advantageous.

The biogeochemical group at the Division of Marine and Atmospheric Chemistry, Rosenstiel School of Marine and Atmospheric Science, University of Miami, USA, distributes DOC Certified Reference Materials (CRMs); shipping is the only cost to the participating laboratories (www.rsmas.miami.edu/groups/organic-biogeochem/). Both low

carbon water (2 μM C) and deep Sargasso Seawater (44 – 45 μM C and 21 μM N) are available in 10 mL ampoules. They are already acidified and stable for at least one year when stored in the dark at room temperature.

These CRMs were used with every analysis to monitor for bias in the accuracy of the DOC and TDN measurements. The DOC and TDN concentrations of the CRMs analysed between May 2002 and April 2004 (Figure 2.19) showed that the measurements were reliable and accurate. The mean DOC and TDN concentrations of the CRMs analysed (48.1 ± 3.9 μM C and 20.2 ± 1.5 μM N) were not significantly different to the certified values, over a period of two years. Sharp (2002) has demonstrated mean DOC concentrations of 46.1 ± 2.3 μM C for deep Sargasso Seawater over a period of 6 months (Sharp 2002). Preliminary use of DOC reference materials by 14 participants in a broad community intercalibration exercise indicated that most analysts show a mean value that is not significantly different from the expected value (Table 2.5) (Sharp *et al.* 2002a).

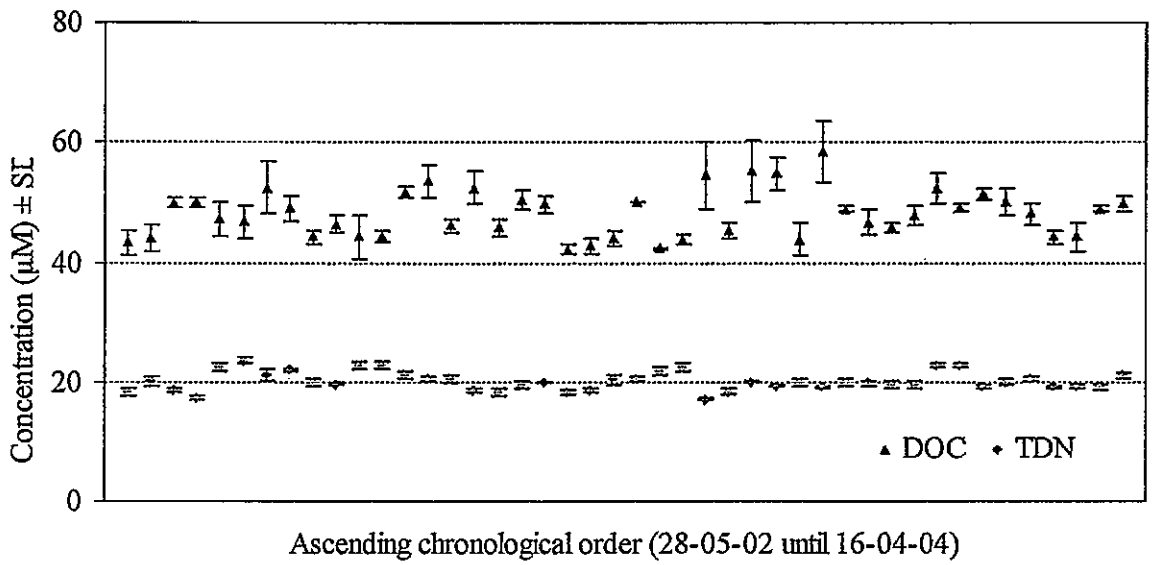


Figure 2.19 Time series plot (n = 44) of the CRMs (deep Sargasso Seawater) measured on daily analytical runs during the period from 28-05-02 until 16-04-04.

Table 2.5 Performance with deep ocean DOC reference materials from 14 analysts plus the current study (data from Sharp *et al.*, 2002a).

Analysts	n*	Range	Mean	SD	CV %
1	31	40.8 to 52.6	46.9	2.8	5.9
2	31	44 to 57	49.1	3.7	7.5
3	11	42 to 53	46.9	3.9	8.3
4	31	40 to 69	45.8	5.9	12.8
5	16	39.7 to 47.1	42.8	2.3	5.3
6	6	46.2 to 48.3	47.2	0.7	1.4
7	27	40 to 60	50.4	4.6	9.1
8	10	34.8 to 50.8	44.0	4.3	9.7
9	55	39.1 to 61.3	49.2	5.2	10.5
10	55	42.9 to 48.2	45.6	0.8	1.7
11	15	38.3 to 75	55.5	11.6	20.9
12	25	39.7 to 53.4	45.6	2.9	6.3
13	9	43.1 to 48.2	45.8	1.5	3.2
14	4	47.4 to 49.1	48.4	0.9	1.8
Current study	44	42.1 to 58.6	48.1	3.9	8.2

Note, Ranges, means, and standard deviations are given as μM C.
*n is the number of times the references were run in the reporting period.

2.3 Measurement of other determinands

2.3.1 Master variables

Master variables measured in this study include pH, temperature, conductivity, total dissolved solids (TDS) and salinity. These variables provide vital information on the state and behaviour of the water environment under investigation. Marked changes in master variables can lead to significant changes in the reactivities, speciation and pathways of other chemical constituents (Howland *et al.* 2000). Measurements of pH and temperature were performed *in situ* using a pH meter (model HI 9025, Hanna Instruments Ltd) that was calibrated for pH before each field campaign using fresh buffer solutions of pH 7.0 and 4.0. Conductivity and TDS were measured *in situ* using a conductivity meter (model HI 9635, Hanna Instruments Ltd). Samples for salinity were collected in clean HDPE plastic bottles and analysed using a salinometer (model 8410, Guildline Portasal) at the Institute of Marine Sciences, University of Plymouth.

The measurement of pH is important in water quality studies as it influences, and can reflect, many chemical and biological processes. pH typically ranges between 6.5 and 7.5 in most natural waters, and extends to 8.3 in marine waters due to the carbonate system (Mckelvie 2002). The pH of freshwater source could affect the pH distribution within the estuarine system (Howland *et al.* 2000). Possible causes of a increase in the pH include algal photosynthesis (autotrophic processes) and bacterial denitrification, whereas possible causes of a decrease include release of strongly acidic waste, influence of acid rain, the release of acid mine drainage water, heterotrophic processes in bacterial nitrification, sulphate reduction and sulphide oxidation (Mckelvie 2002). Low pH can adversely affect aquatic biota directly and can result in the release of heavy metals from the sediments.

Conductivity, expressed in micro Siemens per cm ($\mu S\ cm^{-1}$, $S = \Omega^{-1}$), is a measure of the ability of the water sample to conduct an electric current. Conductivity can be used as an approximate measure of the total concentration of the inorganic solutes (Radojevic and Bashkin 1999). Ions that have a major influence on the water conductivity include Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, H^+, Na^+, K^+, Mg^{2+}, and Ca^{2+}. The term solids refer to the majority of compounds which are present in natural waters and remain in solid form after evaporation. Total dissolved solids (TDS) (mg/l) correspond to the filterable residue of solids while total suspended solid (TSS) to the non-filterable fraction. TDS may be obtained by multiplying the conductivity by a factor, commonly between 0.55 and 0.75, that is specific for each water body (Chapman 1996).

Salinity is important variable in the estuarine environment in order to study the biogeochemistry of various dissolved components. In the past salinity was measured as a function of the total dissolved solids concentration, and so was calculated from chloride concentration, measured by titration, using the following equation; $S\ (‰) = 1.805\ Cl^{-1}(‰)$

+ 0.030 (Chester 2003). Salinity is now measured as function of sample conductivity against standard KCl solution of known concentration, where Salinometers and CTD have replaced the chlorinity titration method for determination of salinity in marine waters (Muller 1999).

2.3.2 Dissolved oxygen and biochemical oxygen demand

Dissolved oxygen (DO; mg L^{-1}, or % of saturation) represents the amount of oxygen dissolved in natural waters and is essential for the survival of most aquatic organisms. DO concentrations vary with temperature, salinity, turbulence and autotrophic / heterotrophic balance activity. DO levels are adversely affected by anthropogenic sources of organic matter pollution and eutrophication. The difference between the theoretical 100 % saturation value and the amount of oxygen actually found is called the apparent oxygen utilization (AOU) (Hansen 1999). DO concentrations and percentage saturations were determined *in situ* using a DO meter (model 55, Yellow Springs Industries).

The biochemical oxygen demand (BOD; mg L^{-1}) is an empirical determination of the amount of oxygen required to oxidise the organic matter in a water sample by micro-organisms. BOD can serve as indicator of the organic pollution load, by measuring the oxygen required for biochemical degradation of organic matter (termed carbonaceous biochemical oxygen demand CBOD). In addition the BOD also measure also the amount of oxygen used to oxidise ammonium (termed the nitrogenous biochemical oxygen demand NBOD) (Radojevic and Bashkin 1999). The NBOD is due to two groups of nitrifying bacteria; one converts ammonium to nitrite, and the second converts nitrite to nitrate (nitrification process). The oxygen uptake is due to nitrification can be eliminated by adding a nitrifying inhibitor prior to the incubation. The BOD is determined by incubating a water sample with naturally present aerobic micro-organisms at 20 °C for 5

days. The dissolved oxygen is measured at the beginning and at the end of the incubation period, and the BOD represents the difference between the initial and final DO concentration.

DO measurements were performed using the Winkler Azide Modification method (Sawyer *et al.* 1994; Rump 1999). The oxygen dissolved in the water sample oxidizes Mn (II) to Mn (IV) which precipitates as a brown hydrated oxide, fixation of oxygen. Then, Mn (IV) oxidizes iodide (I^{-1}) to iodine (I_2). The amount of liberated iodine is theoretically equivalent to the amount of oxygen dissolved in the sample. The iodine is measured by titration with a thiosulphate solution of known concentration.

$$Mn^{2+} + 2\ OH^- + \tfrac{1}{2}\ O_2 \longrightarrow MnO_2\downarrow + H_2O$$
$$MnO_2 + 2I^- + 4H^+ \longrightarrow Mn^{2+} + I_2 + 2H_2O$$

2.3.3 Dissolved inorganic nutrients analysis

Nutrients were measured in GF/F filtered samples using a nutrient auto analyzer (the SKALAR-SAN^{plus} Segmented Flow Analyzer (SFA), Figure 2.20). SFA measurement incorporate widely used colorimetric techniques and measures the resultant coloured solutions spectrophotometrically, (Hansell 1993; Hansen and Koroleff 1999; Gardolinski *et al.* 2001; Hanrahan *et al.* 2002). All chemicals used were Aristar grade and standards were freshly prepared daily. The SKALAR instrument channels were cleaned daily before analysis with ultrapure water, 10 % HCl and then ultrapure water (0.5 h each). All reagents, standards, and samples bottles were cleaned using the cleaning protocol described in section 2.2.1.1. Low nutrient sea water (LNSW, Ocean Scientific International Limited UK) was used for nutrient analysis in estuarine and marine waters in order to minimise errors from the use of artificial seawater (Aminot *et al.* 1997).

waters. Ammonium is generated first from organic matter and is utilized in autotrophic processes in preference to nitrate and nitrite (Ivancic and Degobbis 1984).

The Berthelot's reaction (formation of indophenol blue-IPB, first reported by Berthelot 1859) and its modifications are commonly used for the spectrophotometric determination of ammonium in natural waters (Krom 1980; Searle 1984; Ivancic and Degobbis 1984; Aminot *et al.* 1997; Hansen and Koroleff 1999). The IPB method is appropriate for direct routine analysis of ammonium in both fresh and sea water. Under alkaline conditions (pH 10.5 – 11.5), ammonia and hypochlorite form a monochloramine, which then reacts with phenol in the presence of a sodium nitroprusside catalyst to form an indophenol complex (Figure 2.22). The intensity of the resulting blue indophenol colour is proportional to the ammonium concentration in the sample. The absorption of the complex is measured at 635 nm using a spectrophotometer. Both automated and manual IPB methods were used for the measurement of ammonium during this study.

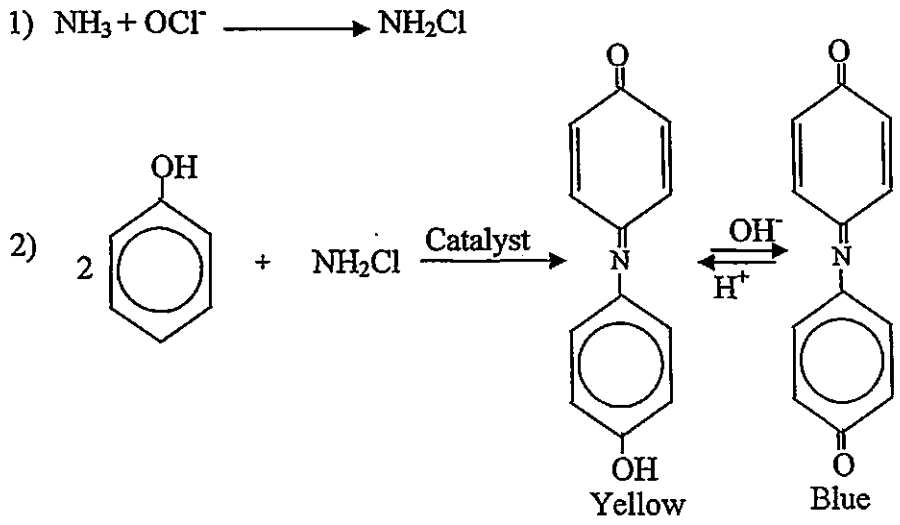


Figure 2.22 The reaction of ammonia with hypochlorite and phenol (IPB method for the measurement of ammonium in natural waters).

Automated determination of ammonium

Automated measurements of ammonium were performed using the SKALAR SFA manifold (Figure 2.23). The linear range was up to 8.57 µM N (120 ppb N).

Reagents

1- Buffer solution: 33 g of potassium sodium tartrate was dissolved in 800 mL ultrapure water. 24 g sodium citrate was added and dissolved. Then, the pH was adjusted to 5.0 using H_2SO_4. The solution volume was made up to 1 L with ultrapure water. 0.5 mL Brij-surfactant solution (30 %) was added and mixed.

2- Phenol solution: 83 g of phenol was dissolved in 50 mL ultrapure water. 40 g sodium hydroxide was added and dissolved. The solution volume was made up to 1 L with ultrapure water. This solution was prepared fresh each time as it stable for one week only at 4 °C.

3- Sodium hypochlorite solution: 200 mL sodium hypochlorite (13 %) was diluted in 700 mL ultrapure water. The solution volume was made up to 1 L with ultrapure water.

4- Sodium nitroprusside solution: 0.5 g of sodium nitroprusside was dissolved in 800 mL ultrapure water. The final volume of the solution was made up to 1 L with ultrapure water and stored in a dark coloured bottle. This solution was prepared fresh each time as it stable for one week only at 4 °C.

5- Standard solution: 0.1909 g ammonium chloride was dissolved in 500 mL of ultrapure water to give 7.139 mM N (100 ppm N). A sub stock solution of 0.7139 mM N (10 ppm N) was prepared from this. Daily working standards of 1.43, 2.86, 4.28, 5.71, 7.14, 8.57 µM N (20, 40, 60, 80, 100, 120 ppb N), were freshly prepared by dilution of the substock solution with a mix of ultrapure water and low nutrient seawater (LNSW) depending on the salinity range of the samples. Main stock and sub stock solutions were prepared weekly and twice-daily, respectively, and kept at 4 °C.

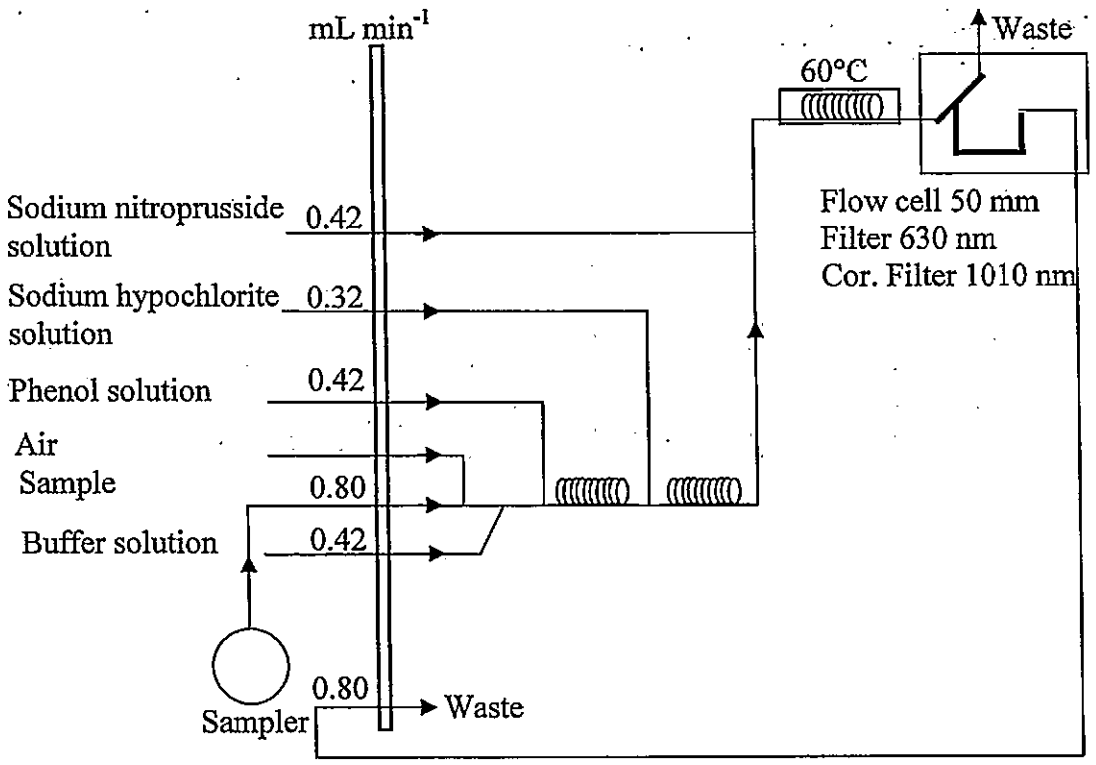


Figure 2.23 CFA manifold for the determination of ammonium in natural water.

Manual determination of ammonium

The manual method was used during the third year of this study, because of instrumental problems with the SKALAR ammonium channel that need to be carefully maintained. The used manual method based on the IPB method published by Ivancic and Degobbis (1984) and recommended in a review by Aminot *et al.* (1997). The concentration range used was 1 – 8 µM N using a 4 cm cell (Figure 2.24). The precision of the manual procedure was quantified by analysis of a 4 µM N standard solution eight times (22/01/2004), and was 3.8 ± 0.069 µM N.

Reagents and procedures

1- Phenol solution: 25 g phenol was dissolved in 250 mL of ethanol (95 % v/v)

2- Catalyst solution: 1.25 g sodium nitroprusside was dissolved in 250 mL ultrapure water.

3- Complexing solution: 100 g trisodium citrate and 8 g sodium hydroxide were dissolved in 250 mL ultrapure water. The citrate solution is to eliminate interferences produced by precipitation of magnesium and calcium ions at high pH (American Public Health Association 1995; Aminot *et al.* 1997).

4- Chlorine source solution: 1 g sodium dichloro-iso-cyanurate was dissolved in 250 mL ultrapure water. Sodium dichloro-iso-cyanurate is a much more stable chlorine donor than hypochlorite solution which is were widely used (Aminot *et al.* 1997).

5- Oxidizing reagent: both complexing solution and chlorine source solution were mixed in equal portions (1:1) immediately before use.

6- Standard solution: 0.1872 g ammonium chloride was dissolved in 500 mL of ultrapure water to give a concentration of 7 mM N. Then a substock solution of 0.8 mM N was prepared by an appropriate dilution factor with ultra pure water. Daily working standards were 1, 2, 4, 6, 8 µM N, prepared by dilution of substock solution with ultrapure water and LNSW depending on the salinity range of the samples. The main stock and sub stock standard solutions were prepared weekly and twice-daily, respectively and kept at 4 °C in the dark.

7- Procedure: 0.6 mL of phenol solution, 1.5 mL oxidizing solution and 0.6 mL of catalyst were added (and mixed) in this order to 15 mL of the sample. The samples were then stored in the dark at 30 °C (using a water bath). Temperatures higher than 40 °C could lead to the decomposition of labile organic nitrogen compounds (Aminot *et al.* 1997). The absorbance was measured within 3 – 5 h at 635 nm using a spectrophotometer (CECIL-1000 Series, School of Biological Sciences-University of Plymouth) with a 4 cm cell.

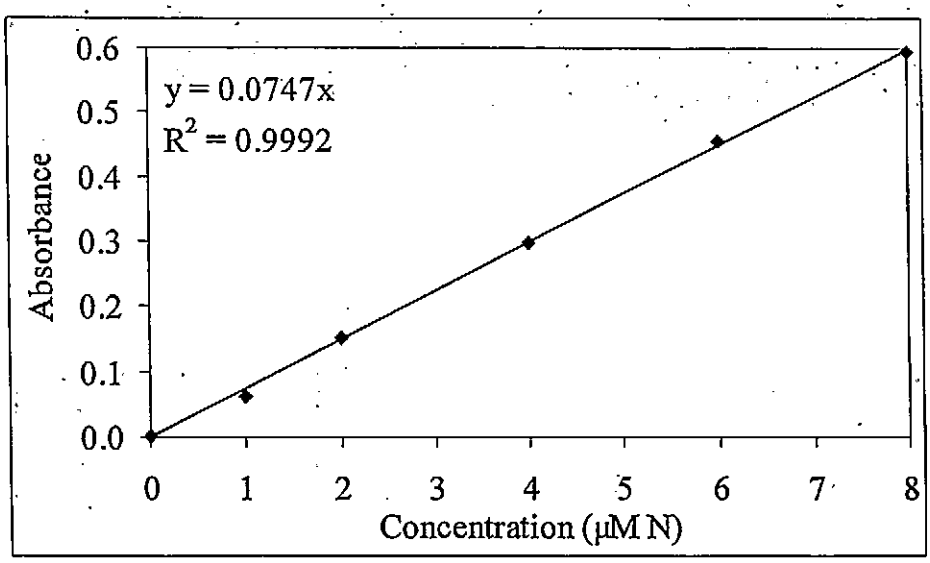


Figure 2.24 Ammonium linear regression plot of calibration standards (n = 6, date of analysis 27-01-2004) using IPB method manual procedure.

2.3.3.2 Nitrate plus nitrite

Nitrate is the final oxidation product and the most thermodynamically stable species of nitrogen compounds in natural waters. Nitrate is derived from rock weathering and anthropogenic sources such as the application of nitrogenous fertilizer (Chester 2003). Nitrification contributes to the natural presence of nitrate, where *Nitrosomonas* bacteria oxidise NH_4^+ to nitrite and *Nitrobacter* bacteria oxidise nitrite to nitrate under aerobic conditions. Nitrite also occurs in natural waters as an intermediate product in the microbial denitrification nitrate which occurs at low oxygen levels. In the current study the term nitrate will be used to refer to both nitrate plus nitrite , where nitrate is the dominant fraction.

The automated nitrate plus nitrite determination is based on the widely used cadmium reduction method (Hanrahan *et al.* 2002). The sample is passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite originally presents plus reduced nitrate is determined by reaction with sulphanilamide and naphthylethylene diamine to form a highly pink-coloured azo dye which is measured at 540 nm (Figure

2.25). There were two concentration ranges used in this study, one for low concentrations 0.14 – 8.57 μM N (2 – 120 ppb N) and the other for high concentrations 7.14 – 142.79 μM N (0.1 – 2 ppm N). The automated analysis of nitrate plus nitrite was performed using the SKALAR SFA manifold (Figure 2.26).

Reagents

1- Buffer solution: 50 g of ammonium chloride was dissolved in 800 mL ultrapure water. The pH was adjusted to 8.2 with 1 mL of ammonia solution (25 %). 5 g sodium hydroxide was then added. The final volume of the solution was made up to 1 L with ultrapure water, then 3 mL Brij-surfactant solution 35 (30 %) was added and mixed. In the case of the higher concentration range (appropriate for river and up estuary samples) sodium hydroxide was not added and only 25 g of ammonium chloride was used.

2- Colour reagent: 150 mL of o-phosphoric acid was diluted in 700 mL ultrapure water. 10 g sulphanilamide and 0.5 g naphthylethylene diamine dihydrochloride were added and dissolved. The final volume of the solution was made up to 1 L with ultrapure water and stored in a dark bottle at 4 °C.

3- Standard solution: 3.0340 g dried sodium nitrate ($NaNO_3$) was dissolved in 500 mL of ultrapure water to give a concentration of 71.39 mM N (1000 ppm N). Then two substock solutions of 7.139 and 3.57 mM N (100 and 50 ppm N) were prepared by appropriate dilution of the standard solution with ultra pure water, according to the expected nitrate and nitrite concentrations in the sample. Daily working standards were 1.43, 2.86, 4.28, 5.71, 7.14, 8.57 μM N (20, 40, 60, 80, 100, 120 ppb N) for samples with relatively low nitrate plus nitrite concentrations, and 7.14, 21.42, 35.70, 71.39, 142.79 μM N (0.1, 0.3, 0.5, 1, 2 ppm N) for samples with relatively high nitrate plus nitrite concentration.

4- The daily working standards were prepared by dilution of substock solution with a mix of ultrapure water and LNSW depending on the salinity range of the samples. Main stock and substock solutions were prepared monthly and weekly, respectively, and kept at 4 °C in the dark.

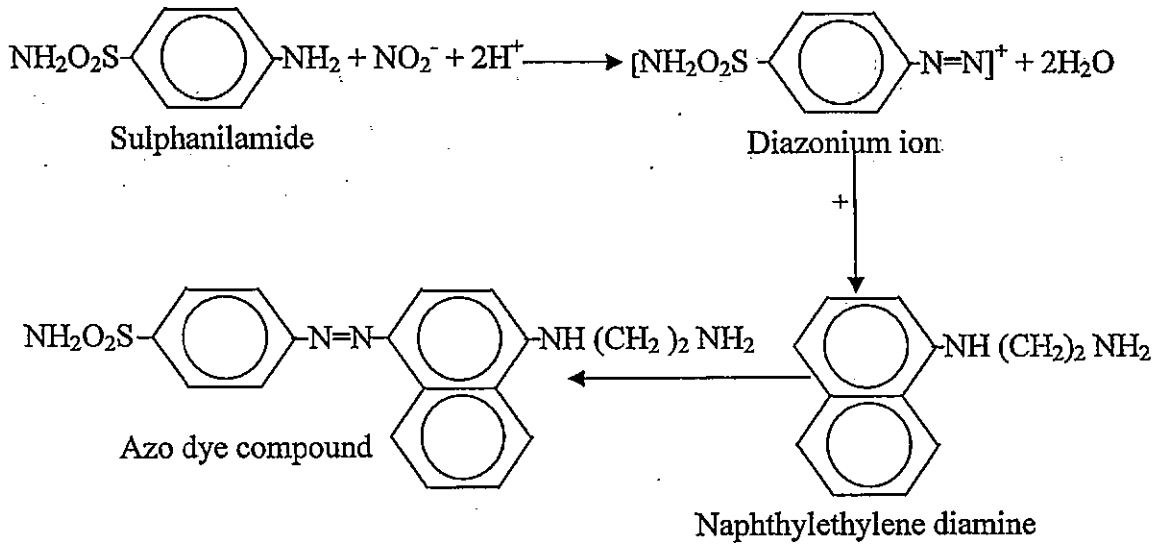


Figure 2.25 The reaction of nitrite with sulphanilamide and naphthylethylene diamine for the determination of nitrate plus nitrite in natural waters.

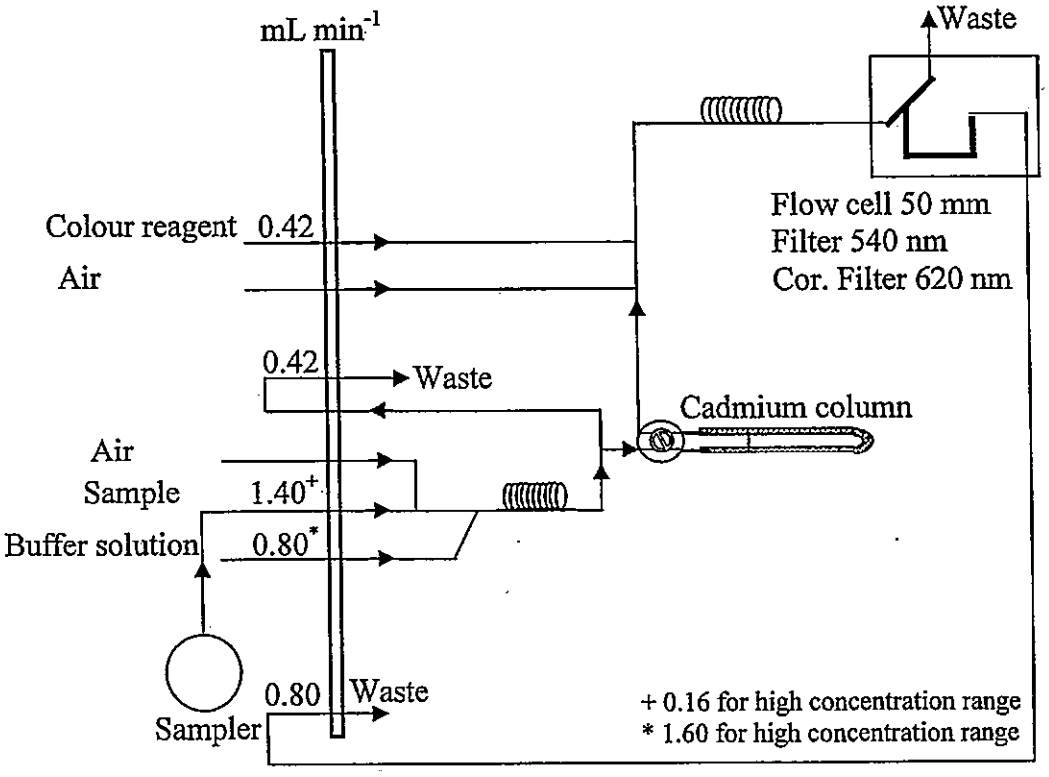


Figure 2.26 CFA manifold for the determination of nitrate plus nitrite in natural water.

2.3.3.3 Filterable reactive phosphate

Natural sources of phosphate are mainly weathering of phosphorus-bearing rocks and decomposition of organic matter. Anthropogenic sources such as domestic wastewater, industrial effluents and fertiliser runoff contribute to the elevated levels of phosphate in the aquatic environment.

The determination filterable reactive phosphate (FRP) is based on the reaction of phosphate with an acidified molybdate reagent to form a phosphomolybdate complex (Figure 2.27) (Hansen and Koroleff 1999; Hanrahan *et al.* 2002). This complex is then reduced by ascorbic acid to form an intensely blue coloured compound which is measured at 880 nm using a spectrophotometer. The linear range is up to 3.87 µM P (120 ppb P). Automated measurements of phosphate were performed using the SKALAR SFA manifold (Figure 2.28).

$$PO_4^{-3} + 12\ MoO_4^{-2} + 27\ H^+ \longrightarrow H_3PO_4\ (MoO_3)_{12} + 12H_2O$$

$$H_3PO_4\ (MoO_3)_{12} \xrightarrow[\text{ascorbic acid}]{\text{Reduction by}} \text{Phosphomolybdenum blue Mo(V)}$$

Figure 2.27 The reaction of phosphate with molybdate and ascorbic acid for the determination of reactive soluble phosphate in natural waters.

Reagents

1- Ammonium molybdate solution: 230 mg of potassium antimony tartrate was dissolved in 800 mL ultrapure water. Then 69.4 mL of H_2SO_4 was added carefully with constant swirling and cooling. 6 g ammonium molybdate was added and dissolved and the solution volume made up to 1 L with ultrapure water. 2 mL FFD6 (wetting reagent) was added and mixed.

2- Ascorbic acid solution: 11 g of the ascorbic acid was dissolved in 800 mL ultrapure water. Then, 60 mL acetone was added and the solution volume made up to 1L with ultrapure water. 2 mL FFD6 was added and mixed. This solution was prepared fresh each time as it was stable for one week only at 4 °C.

3- Standard solution: 0.2197 g potassium dihydrogen orthophosphate was dissolved in 500 mL of ultrapure water to give a concentration of 3.23 mM P (100 ppm P). A substock solution of 0.323 mM P (10 ppm P) was prepared. Daily working standards of 0.65, 1.29, 1.94, 2.58, 3.23, 3.87 µM P (20, 40, 60, 80, 100, 120 ppb P), were freshly prepared by dilution of substock solution with a mix of ultrapure water and LNSW depending on the salinity range of the samples. Main stock and substock solution were prepared monthly and weekly, respectively, and kept at 4 °C in the dark.

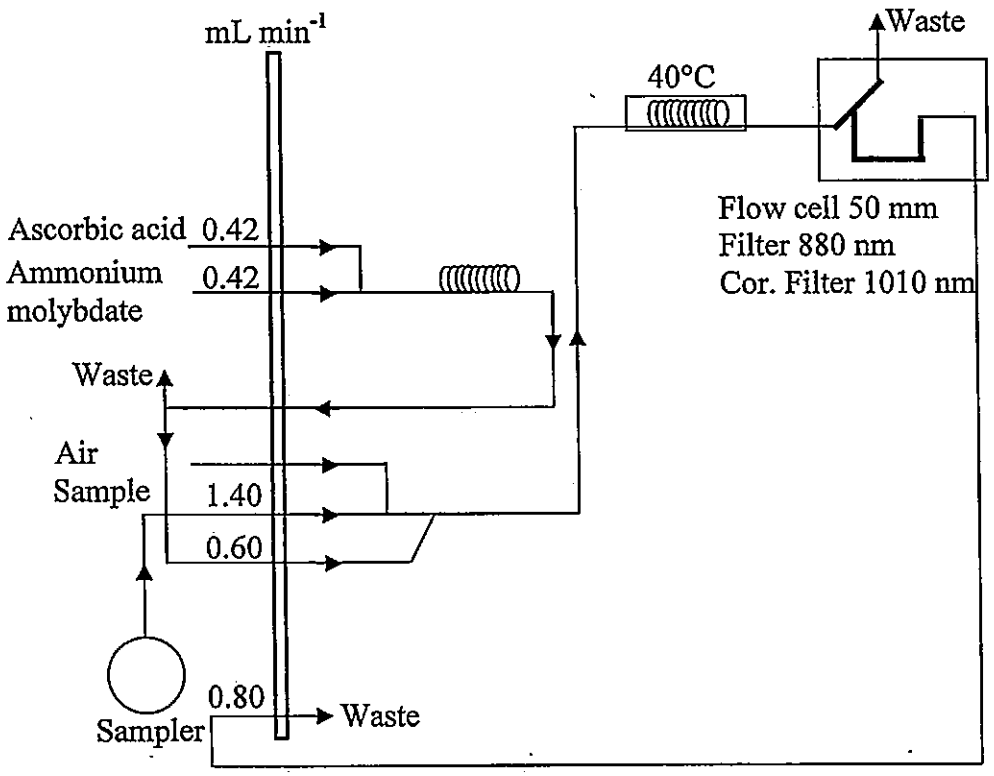


Figure 2.28 CFA manifold for the determination of reactive soluble phosphate in natural water.

2.3.4 Suspended chlorophyll a

Chlorophyll a (CHL a) is present in most photosynthetic organisms and provides an indirect measurement of algal biomass and an indication of the trophic status of the aquatic environment. In Europe the trophic status of waters is classified into oligotrophic, mesotrophic, eutrophic and hyper-eutrophic based on chlorophyll a concentrations of ≤ 2.5, $2.0 - 8.0$, $8 - 25$ and ≥ 25 $\mu g\ L^{-1}$, respectively (Radojevic and Bashkin 1999).

Chlorophyll a concentrations were measured by fluorescence method (Parsons *et al.* 1984; USEPA 1997). All laboratory-ware used for chlorophyll a measurement was cleaned using the protocol described in section 2.2.1.1, with an additional final rinse with 90 % acetone before use. Reagents used were HPLC grade acetone (diluted to 90 %), HCl (0.1 M) and pure chlorophyll a (Sigma Chemical Ltd).

After the water sample was filtered (section 2.2.2), the GFF filter paper was folded once, wrapped in Al foil, and stored in the dark at – 20 °C until analysis. For the analysis, the GFF filter paper was placed in the bottom of a 15 mL centrifuge tube and 10 mL 90 % acetone were added to extract the chlorophyll a pigment. The centrifuge tube was then placed in an ultrasonic bath for 15 – 20 min. The filter paper was allowed to steep in the 90 % acetone for at least 2 h and not to exceed 24 h in the dark at 4 °C to ensure the complete extraction of the chlorophyll a. The tube was shaken at least once during this period. The filter slurry was then centrifuged for 15 min at 675 g (or 5 min at 1000 g). An aliquot of supernatant was transferred to the sample cuvette of the Hitachi F-4500 Fluorescence Spectrometer and the fluorescence was measured before and after acidification with 0.1 N HCl (0.15 mL of 0.1 N HCl was added to the 5 mL sample cuvette and mixed). The acidification step is required in order to account for the combination of phaeophytin (chlorophyll degradation product) to the fluorescence signal. The Hitachi F-4500

Fluorescence Spectrometer was calibrated using chlorophyll a standard solutions (2 – 160 μg L^{-1}, Figure 2.29) that were prepared from stock solution (20 mg L^{-1}). The calibration range was linear up to 250 μg L^{-1}. The chlorophyll a concentration was calculated from the following equation.

$$\text{Chlorophyll a } (\mu g\ L^{-1}) = \frac{(r/r-1) \times (R_b - R_a) \times (V_e)}{(S) \times (V_s)}$$

Where,

r: the before / after acidification ratio of a pure chlorophyll a standard solution.

Rb: fluorescence response of the sample extract before acidification.

Ra: fluorescence response of the sample extract after acidification.

Ve: volume of extraction (L).

Vs: volume of whole water sample (L).

S: calibration curve slope.

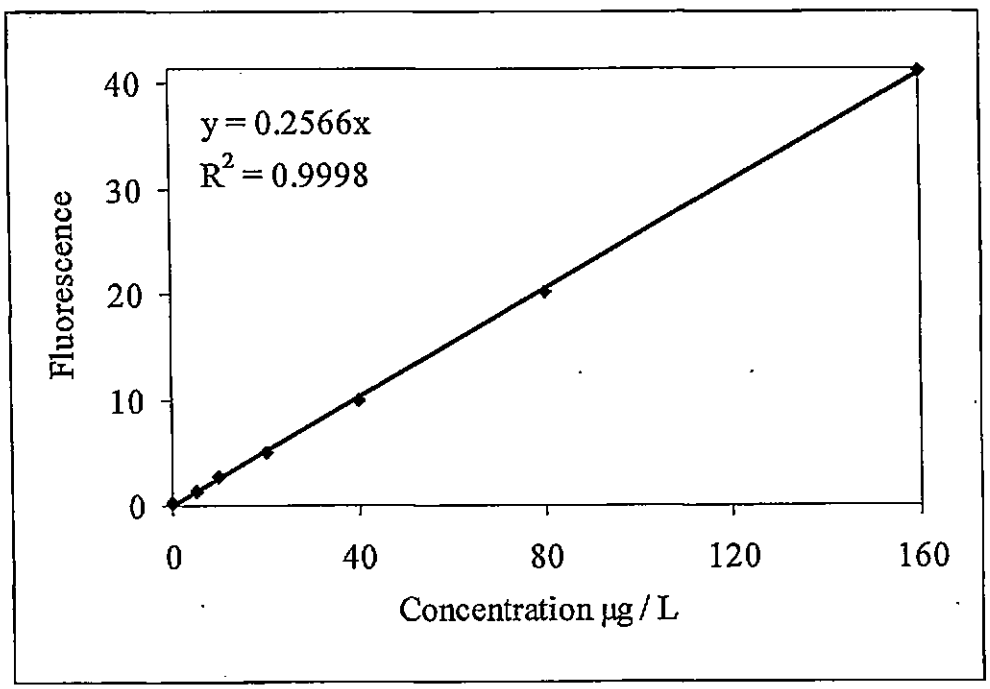


Figure 2.29 Suspended chlorophyll a linear regression plot of calibration standards (n = 6, date of analysis 03-04-2003) using Fluorescence Spectrometer Technique.

2.4 Bioavailability of DON using bacterial bioassay

Identifying the biodegradable fraction of DON and DOC and quantifying their contribution to the heterotrophic bacterial metabolism can improve our understanding of DOM biogeochemistry (Volk et al. 1997). Most of the work done on bulk DON and DOC bioavailability (or uptake rate) has been in fresh water ecosystems using a bacterial bioassay approach (Kaplan and Newbold 1995; Stepanauskas *et al.* 1999; Sondergaard and Worm 2001; Bronk 2002). Concentrations of biodegradable dissolved organic nitrogen (BDON) and carbon (BDOC) over a short timescales can be estimated directly from changes in DON and DOC concentrations following the exposure of natural water samples to micro-organisms, namely heterotrophic bacteria (Stepanauskas *et al.* 1999; Sondergaard and Worm 2001; Seitzinger *et al.* 2002). Also, apparent oxygen utilization, bacterial biomass, CO_2 and NO_3^- production measurements can be used to investigate biodegradable DON and DOC (Hansell *et al.* 1995).

Most BDON and BDOC measurements using bioassay techniques have been carried out in batch cultures of filtered water supplied with indigenous bacteria (Seitzinger and Sanders 1997; Sondergaard and Worm 2001). Some disadvantages of batch cultures include the rather long incubation times and the potential bias from the use of pure strains of bacteria (Sondergaard and Worm 2001). On the other hand, a plug flow biofilm reactor, that is robust and reliable for BDOC measurements (Sondergaard and Worm 2001 and references therein), has been used by a number of workers for studying BDOC in stream water (Kaplan and Newbold 1995; Volk *et al.* 1997), lake (Sondergaard and Worm 2001) and drinking water (Lucena *et al.* 1991; Ribas *et al.* 1991; Frias *et al.* 1992). However, the use of plug flow bioreactors has not been reported in the literature either for estuarine and marine environment or for studying biodegradable DON. So, one objective of this study

was to apply a plug flow bioreactor for bioavailability experiment of DON in an estuarine ecosystem, the Plym estuary.

A plug flow biofilm reactor, Figure 2.30, was colonized by naturally present bacteria to investigate the bioavailability of DON to aquatic biota in the Plym estuarine environment. The bioreactor set-up consisted of three 125 mL glass columns with quartz wool bed supports and filled completely with borosilicate glass beads (Siran, Schott-Germany), Figure 2.31. Siran is an open-pored sintered glass with sphere of 1 – 2 mm diameter and 60 – 300 μm pore diameters that provide a large surface to volume ratio (90,000: 1). The three columns were kept inside a wooden box in the dark and supplied continuously with natural water at a flow rate of 1 mL min^{-1} using a peristaltic pump. All tubing was Teflon. The Plym estuarine water was the source of feed water and bacteria for the biofilm reactor. The water was filtered in-line through a glass fibre filter units (GF2, Fisher Scientific UK Ltd) of 0.65 μm pore size, using a peristaltic pump. The filtered water was stored in two glass reservoirs (up to 200 L capacity), and kept in the dark by wrapping the reservoirs in black plastic film. The water was fed continuously into the three bioreactor columns. All glass reservoirs and bioreactor columns were kept in the dark, to inhibit photosynthetic activity, in a constant temperature room (15 °C) at the School of Biological Science, University of Plymouth.

The three bioreactor columns were colonized with naturally present bacteria beginning in January 2003 until March 2004. Approximately 150 L of natural water was collected on a regular basis (every two months) from the Plym estuary (Plymstock, 5km down estuary of the weir) to feed the bioreactor columns. Water samples from the bioreactor columns inflow and outflow were collected regularly in clean glass ampoules for DOC and TDN analysis and HPDE plastic bottles for DIN and reactive phosphate and were not filtered prior to analysis. The DOC, TDN and DIN were analysed as described previously.

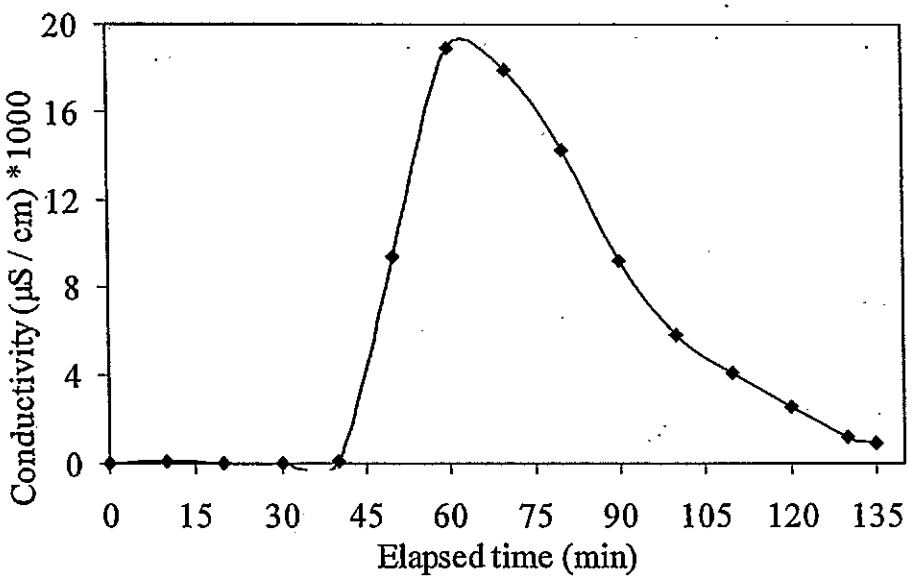


Figure 2.32 Hydraulic residence time for the three bioreactor columns using a pulse of 1 M KCl.

2.5 Stable nitrogen isotope analysis

The use of stable isotopes, such as nitrogen, in marine research is becoming increasingly common to improve our understanding of nitrogen biogeochemical cycling (Holmes *et al.* 1998). The stable isotopic composition of particulate organic nitrogen and aquatic plants is relatively simple and easy to measure (Kendall 1998). However, isotope ratio measurements in dissolved nitrogen pools (NH_4^+, NO_3^- and DON) are more difficult as these dissolved species first must be removed from the solution and concentrated in a form that can be introduced to a mass spectrometer. Methods and procedures for the recovery and isotopes analysis of ammonium nitrate (plus nitrite) and DON are discussed below.

2.5.1 δ^{15} NH_4^+ analysis

In the current study, ^{15}N-NH_4^+ was measured using the ammonia diffusion method described by Holmes *et al.* (1998) and Heaton (2001). The method is applicable to all types of natural waters (marine, estuarine and fresh waters) and allows measurements in samples with low ammonium concentrations. The ammonia diffusion process is based on the

collection of ammonium dissolved in natural water by converting it to ammonia under alkaline conditions and trapping the ammonia gas on an acidified glass fiber filter for ^{15}N isotope analysis. Other methods that have been applied for $^{15}N\text{-}NH_4^+$ analysis include distillation, mercury precipitation, indophenol, ion exchange and microdiffusion / bromite oxidation (Holmes *et al.* 1998). However, most of these methods require relatively high ammonium concentrations and some are specific to fresh water systems only (Holmes *et al.* 1998).

2.5.2 δ^{15} NO_3^- analysis

$^{15}N\text{-}NO_3^-$ was measured using the Devardas alloy / ammonia diffusion method adapted from Sigman *et al.* (1997) and Heaton (2001). The method involves, after removal of ammonium for $^{15}N\text{-}NH_4^+$ analysis, reduction of nitrate to ammonia using Devardas alloy under alkaline conditions, and followed by the gaseous diffusion of ammonia into an acidified glass fibre disk. This procedure was developed by Sigman *et al.* (1997) for low concentrations (≤5 μM N) of oceanic nitrate. The method has also been widely applied to soil extracts (Kelley *et al.* 1991; Sorensen and Jensen 1991; Lory and Russelle 1994), fresh waters (Downs et al. 1999) and estuarine and marine waters (Slawyk and Raimbault 1995; Sigman *et al.* 1997; Holmes *et al.* 1998; Sigman *et al.* 1999; Sigman *et al.* 2000; Middelburg and Nieuwenhuize 2001; Pantoja *et al.* 2002).

2.5.3 δ^{15} DON analysis

For DON isotope measurements, all of the DIN forms first must be removed and the DON pool be isolated with a high efficiency. Currently, there are three basic approaches to isolate the DON, namely dialysis, ion retardation and wet chemical isolation (Bronk 2002).

A technique based on dialysis was developed for isolating DON in freshwater samples for subsequent isotope analysis (Feuerstein *et al.* 1997). The method involved the use of rotary evaporation to preconcentrate the DON which was then followed by dialysis with 100-Dalton cut off to remove the DIN. The sample was then dried on a glass high-vacuum line, and the δ^{15} of the DON measured using a mass spectrometer. Disadvantages of this approach include the excessive amount of time it takes to remove the DIN (calculated 100 h and 216 h for freshwater and marine samples, respectively). Moreover the removal of DIN is never quantitative and the sample is susceptible to bacterial or nitrogen contamination. Dialysis may result in the loss of urea. The most significant problem with dialysis is the adsorption of DOM to the membrane and reduced filtration rates. In that study no data was presented for marine samples. Recently the suitability of this method for marine waters has been questioned (Prof. G. Wolff, University of Liverpool, personnel communication).

The ion retardation method depends on the use of an ion exchange that retards the flow of charged particles. A resin (AG11A8) can quantitatively remove NH_4^+, NO_3^- and NO_2^-, leaving DON to be isolated in the eluant (Bronk 2002). However, the resin may retain variable amounts of DON due to the accumulation of an organic film on the resin beads during manufacturing (Bronk 2002). Even though, this method is not appropriate for nitrate and ammonium isotope analysis in estuarine and marine waters due to the matrix effect of other ions that could be exchanged on the resin (Dr. T. Heaton, NERC Isotope Geosciences Laboratory BGS-Keyworth, personnel communication).

The wet chemical isolation of DON was introduced by Axler and Reuter (1987) to separate DON in oligotrophic lake water for the subsequent isotopic analysis of the $^{15}N/^{14}N$ ratio. With the wet chemical approach, NH_4^+ is changed to the more volatile NH_3 by raising the

pH and the NH_3 is removed by diffusion in a heated oven (Slawyk and Raimbault 1995) or by a vacuum distillation (Bronk and Ward 1999). Nitrate and nitrite are reduced to ammonium by Devardas alloy (DA), and ammonium is subsequently removed in the same way. The DON is then converted to ammonium by wet oxidation using potassium persulphate and subsequent reduction of the resultant nitrate by Devardas alloy (Slawyk and Raimbault 1995). However the removal of NH_4^+ and NO_3^- could potentially lead to a lose of labile DON as a result of base hydrolysis; although this is generally not important (Bronk 2002). Thus, the wet chemical isolation approach was chosen for the current study, as it is appropriate for estuarine and marine waters.

2.5.4 Procedures for nitrogen isotope analysis

The procedures used in the current study for the measurement of nitrogen isotopes in ammonium, nitrate and DON are outlined in Figure 2.33 and explained below. All chemicals used were of reagent grade quality and aqueous solutions were prepared using ultrapure water. Ammonium sulphate ($\delta^{15}N$ +2.6 ‰) and potassium nitrate ($\delta^{15}N$ +5.6 ‰) standards were used and treated in the same manner as samples, except sample preconcentration, to correct for nitrogen fractionation during the DA / ammonia diffusion procedures. The potential contamination was assessed by substituting fresh, ultrapure water for the sample.

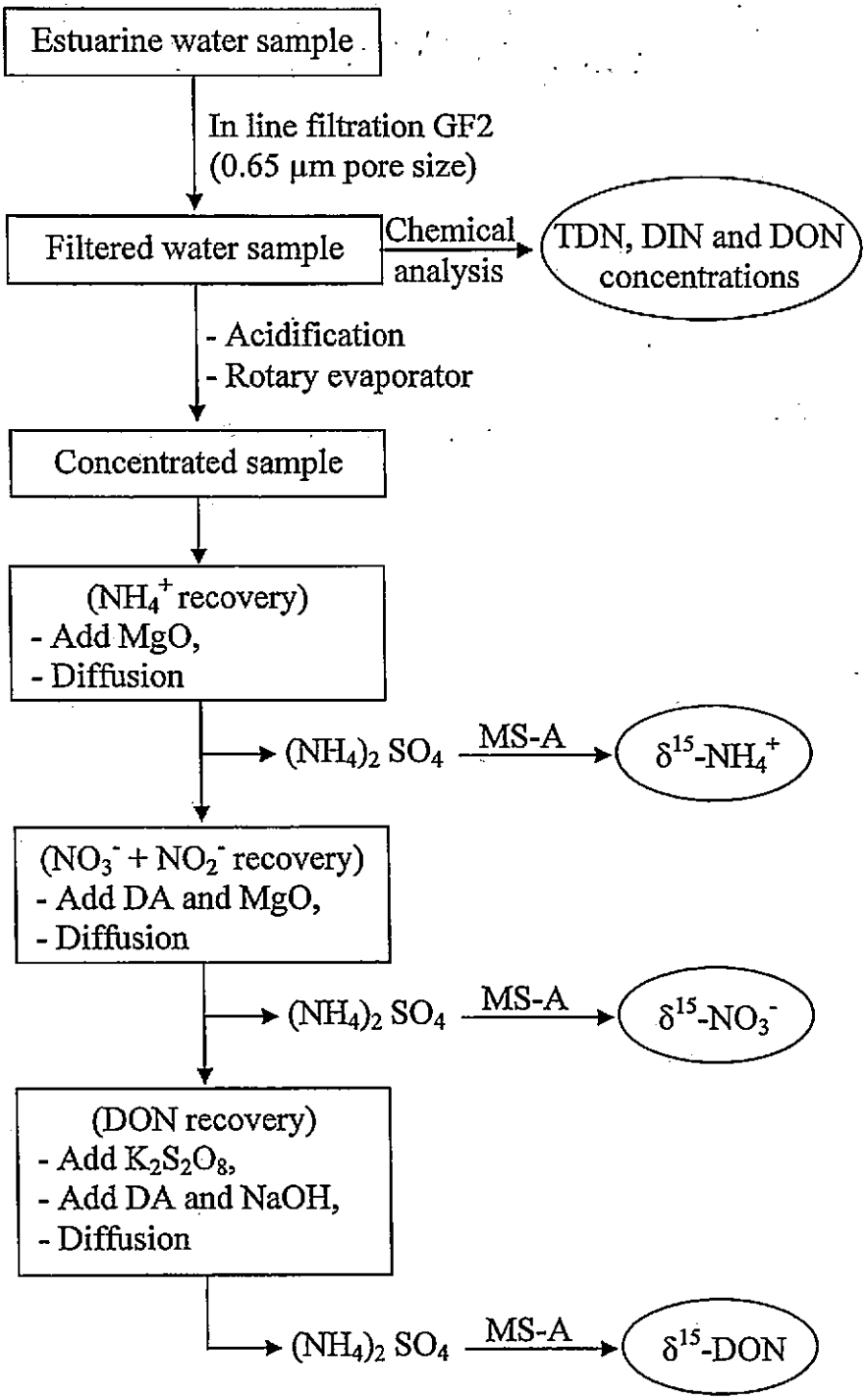


Figure 2.33 Flow diagram of the experimental procedures protocol used to recover ammonium, nitrate and DON from estuarine water for nitrogen isotope ratio analysis. MS-A: mass spectrometer analysis; DA: Devardas alloy.

Sample collection and preconcentration

Water samples were collected from the Plym estuary on 23-03-2004. The samples were collected in 6 L, clean, plastic containers from six stations along a longitudinal transect of

DON isolation take place through (1) removal and isotopic analysis of ammonium and nitrate (plus nitrite) by the above procedure, (2) a wet oxidation digestion of the remaining DON to nitrate using potassium persulphate ($K_2S_2O_8$) and (3) recovery of the resultant nitrate as $(NH_4)_2SO_4$ by adding Devardas alloy and sodium hydroxide. A digestion mixture of 15 g $K_2S_2O_8$ dissolved in 250 mL of 1.5 M NaOH was prepared. Sufficient sample volume to contain 0.2 mg-N from the second diffusion flask was transferred to a third diffusion flask and a stirrer bar was placed in the flask. The diffusion packet was then suspended inside the diffusion flask. 30 mL of the digestion mixture was added and the diffusion flask was autoclaved at 120 °C (at 1 bar) for 2 h. After digestion, 300 mg Devardas alloy and 5 mL of 12.5 M NaOH were added to the sample and immediately the diffusion flask was capped and stirred for 1 h. Then, the procedures described above for ammonium diffusion were repeated.

Chapter Three

Results and discussion

Results and discussion

3.1 Introduction

This chapter will present and discuss the results of an investigation of the seasonal variations in the estuarine distribution of DOC and DON in relation to changes in their sources, sinks and in situ transformations. The studies were undertaken in two contrasting estuarine systems, the Yealm and the Plym, in south-west England. The Yealm catchment is mainly agricultural, while the Plym has a more industrial nature. These estuaries were chosen as they are relatively small-scale and hence relatively easy to survey in a systemically manner using small boats, and are subject to anthropogenic inputs of C and N. Furthermore, there are no published data on the biogeochemical cycling of DOC and DON in these estuaries. The large sediment area to water volume ratio suggests that transformations of nitrogen compounds should be observed, even though the fresh water residence times of these estuaries are relatively short (a few days) (Uncles *et al.* 2002). Results from experiments examining the potential bacterial mineralization of DON will be described in this chapter, as will initial work on the use of stable nitrogen isotopes to identify DON sources. The outcomes of the later studies will be assessed in relation to the observed estuarine distributions of DOC and DON.

3.2 The Yealm Estuary

3.2.1 The study area

3.2.1.1 Site description

The Yealm Estuary is situated on the south-west coast of England (Figure 3.1). Water samples were collected regularly (every six weeks) along an axial transect, as indicated in Figure 3.2, during the period February 2002 – September 2003.

3.2.1.2 Hydrography

The Yealm Estuary is a macrotidal system, with a tidal range of 4.7 m and tidal length of 6.2 km (Uncles *et al.* 2002). The water column is uniformly well mixed with respect to salinity. The estuary has a relatively small input of freshwater, and this allows marine conditions to dominate (English Nature 1996; Hiscock 1998). River flow ranges from a minimum of < 1 m^3s^{-1} to a maximum of 17.1 m^3s^{-1}, with an average daily flow of 1.6 m^3s^{-1}. The lowest flow rates usually occur during June, July and August when there is reduced rainfall. The estimated mean residence (flushing) time for fresh water in the estuary is calculated to be 1.5 days (Uncles *et al.* 2002).

3.2.1.3 Ecology

Plymouth Sound, the Tamar and the Yealm estuaries are designated "Sensitive Marine Areas" (Buck 1997). Hiscock and Moore (1986) stated that the Yealm was a very "natural environment" with "current levels of recreation not appearing to damage its naturalness". The estuary contains areas of sediment (sand with shale gravel and pebbles) which support well established, diverse, rich faunal communities and marine ecosystems of national importance (Hiscock and Moore 1986; Hiscock 1998; Turner and Kendall 1999). Hiscock and Moore (1986) described seven littoral and six sublittoral habitats and communities within the Yealm Estuary illustrating its extensive biodiversity.

3.2.2 Master variables

A complete master variables and chemical measurement data set is given in Appendix 3.1. Correlations (Pearson's product moment) between pairs of chemical determinands were calculated in order to identify any statistically significant correlations (at the 99% confidence level), and thus aid interpretation of the data. The correlations were calculated for the whole data set (Table 3.1) and for different seasons (Appendix 3.2). Because of the

large number of data, only correlation coefficients ≥ 0.7 were considered significant for inclusion in the discussion (Chatfield and Collins 1980). There were no significant differences in the correlation results between the whole data set and different seasons, hence seasonal correlation results are given in Appendix 3.2.

Water temperature exhibited a strong seasonal pattern in the Yealm Estuary. Highest temperatures were recorded in summer 2002/2003, the lowest in winter 2003, with intermediate values during spring 2002/2003 and autumn 2002. During summer 2002/2003 the water temperature was in the range 14.5 – 20.6 °C, while during winter 2003 the range was 5.9 – 8.7 °C. Within the Yealm Estuary, pH ranged from a minimum of 6.1 at the freshwater end to a maximum of 8.5 at the sea water end. Exceptionally high values of 8.5 – 8.7 were observed in fresh water samples collected in April 2004 due to photosynthesic activity of algal blooms (Howland *et al.* 2000; Mckelvie 2002). Salinity ranged between 0.03 for river water and 35.2 for samples taken at the seaward end of the estuary. A strong positive correlation (r^2 = 0.96, n = 130, 99% confidence) was observed between salinity and conductivity; the latter was measured in the field to indicate the approximate salinity of the collected samples. On return to the laboratory, more accurate determinations were made using a Guildline Salinometer. Dissolved oxygen averaged 8.8 ± 1.1 mg L^{-1}, a range expected in this sort of aquatic environment. A strong negative correlation between dissolved oxygen (DO) and salinity was observed for winter 2002 samples (Appendix 3.2), indicating conservative behaviour of DO when phytoplankton and microbial activity was at a minimum.

Table 3.1 Pearson's product moment correlation matrix for the Yealm Estuary samples. In the table, DONTDN and DOCDON refers to the contribution of DON to the TDN pool and the C:N ratio of DOM, respectively.

		SALINITY	PH	BOD	DO	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
SALINITY	Pearson Correlation	1	.540**	.048	-.402**	-.715**	-.127	-.895**	-.901**	-.492**	.546**	-.621**	.276*	.176*
	Sig. (2-tailed)	.	.000	.723	.000	.000	.150	.000	.000	.000	.000	.000	.015	.045
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
PH	Pearson Correlation	.540**	1	.270*	-.086	-.435**	-.409**	-.305**	-.438**	-.487**	.068	-.473**	.129	.040
	Sig. (2-tailed)	.000	.	.043	.395	.000	.000	.000	.000	.000	.443	.000	.259	.652
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
BOD	Pearson Correlation	.048	.270*	1	.312*	.086	.116	-.026	.012	.071	.149	.016	.423*	-.199
	Sig. (2-tailed)	.723	.043	.	.018	.525	.391	.847	.926	.601	.270	.908	.010	.139
	N	57	57	57	57	57	57	57	57	57	57	57	36	57
DO	Pearson Correlation	-.402**	-.086	.312*	1	.153	-.267**	.459**	.381**	.039	-.417**	.163	-.117	-.034
	Sig. (2-tailed)	.000	.395	.018	.	.131	.007	.000	.000	.704	.000	.108	.308	.736
	N	99	99	57	99	99	99	99	99	99	99	99	78	99
PO4	Pearson Correlation	-.715**	-.435**	.086	.153	1	.287**	.714**	.764**	.495**	-.394**	.547**	-.274*	-.178*
	Sig. (2-tailed)	.000	.000	.525	.131	.	.001	.000	.000	.000	.000	.000	.015	.043
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
NH4	Pearson Correlation	-.127	-.409**	.116	-.267**	.287**	1	.046	.162	.255**	-.041	.232**	.214	-.173*
	Sig. (2-tailed)	.150	.000	.391	.007	.001	.	.607	.066	.003	.646	.008	.060	.048
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
NO3	Pearson Correlation	-.895**	-.305**	-.026	.459**	.714**	.046	1	.933**	.357**	-.670**	.521**	-.360**	-.166
	Sig. (2-tailed)	.000	.000	.847	.000	.000	.607	.	.000	.000	.000	.000	.001	.059
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
TDN	Pearson Correlation	-.901**	-.438**	.012	.381**	.764**	.162	.933**	1	.668**	-.485**	.577**	-.353**	-.302**
	Sig. (2-tailed)	.000	.000	.926	.000	.000	.066	.000	.	.000	.000	.000	.002	.000
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
DON	Pearson Correlation	-.492**	-.487**	.071	.039	.495**	.255**	.357**	.668**	1	.134	.411**	-.183	-.438**
	Sig. (2-tailed)	.000	.000	.601	.704	.000	.003	.000	.000	.	.128	.000	.109	.000
	N	130	130	57	99	130	130	130	130	130	130	130	78	130

		SALINITY	PH	BOD	DO	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
DON TDN	Pearson Correlation	.546**	.068	.149	-.417**	-.394**	-.041	-.670**	-.485**	.134	1	-.327**	.346**	-.274**
	Sig. (2-tailed)	.000	.443	.270	.000	.000	.646	.000	.000	.128	.	.000	.002	.002
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
DOC	Pearson Correlation	-.621**	-.473**	.016	.163	.547**	.232**	.521**	.577**	.411**	-.327**	1	.020	.087
	Sig. (2-tailed)	.000	.000	.908	.108	.000	.008	.000	.000	.000	.000	.	.860	.327
	N	130	130	57	99	130	130	130	130	130	130	130	78	130
CHL A	Pearson Correlation	.276*	.129	.423*	-.117	-.274*	.214	-.360**	-.353**	-.183	.346**	.020	1	.167
	Sig. (2-tailed)	.015	.259	.010	.308	.015	.060	.001	.002	.109	.002	.860	.	.143
	N	78	78	36	78	78	78	78	78	78	78	78	78	78
DOC DON	Pearson Correlation	.176*	.040	-.199	-.034	-.178*	-.173*	-.166	-.302**	-.438**	-.274**	.087	.167	1
	Sig. (2-tailed)	.045	.652	.139	.736	.043	.048	.059	.000	.000	.002	.327	.143	.
	N	130	130	57	99	130	130	130	130	130	130	130	78	130

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

3.2.3 Dissolved inorganic nitrogen, filteable reactive phosphate and suspended chlorophyll a

Concentrations of nitrate ranged from ~ 1 to 203 μM N, with lower concentrations observed at the seaward end and highest concentrations in the fresh water samples (Figure 3.3). Ammonium concentrations ranged from 0.20 to 10.3 μM N. Filterable reactive phosphate (FRP) concentrations were mainly in the range 0.06 – 2.9 μM P. An exceptionally high value of 5 μM P was measured in a fresh water sample collected in October 2002 (Figure 3.4). Chlorophyll *a* ranged from 0.06 to 5.5 μg L^{-1} (Figure 3.5).

As presented in Figure 3.3, for many field campaigns, nitrate behaved relatively conservatively with high concentrations at the fresh water end being diluted by mixing with sea waters of low nitrate concentration. The decrease in nitrate concentration as salinity increases is a general pattern observed in many tropical and temperate estuaries (Treguer and Queguiner 1989; Sanders *et al.* 1997b; Mortazavi *et al.* 2001). These results suggest that fresh water inputs and mixing with sea water were the dominant factors controlling nitrate cycling within this estuarine system. FRP distributions showed both conservative and non conservative behaviour. The highest FRP concentrations were observed at the freshwater end during the spring and summer period. The conservative mixing behaviour of both nitrate and FRP was supported by the observed strong correlations between salinity, nitrate and FRP (Figure 3.6). These strong correlations indicate, to an extent, the lack of chemical reactivity, with net conservative mixing processes between fresh water and sea water end member concentrations. In contrast, there was no linear inverse relationship between salinity and ammonium concentrations, indicating that ammonium distribution within the estuary was not dominated by physical dilution with sea water and instead indicated extensive in situ reactivity and multiple sources within the estuary.

An examination of seasonal patterns in concentrations revealed that the lowest nitrate concentrations were observed in summer 2002/2003 and early autumn 2002 (mid estuarine concentrations were below 2 μM N), presumably as a result of biological uptake, while the highest concentrations occurred during winter. A similar trend was reported for the Aulne Estuary in France (Treguer and Queguiner 1989). During summer 2002, nitrate concentrations remained low until October 2002 after which primary productivity decreased, as indicated by reduced chlorophyll a concentrations, and nitrate regeneration began to dominate throughout winter 2003. High concentrations of FRP were observed at low salinity in the spring and summer samples, which were probably due to inputs from sewage treatment outfalls in the river and upper estuary. A strong seasonal pattern was observed for chlorophyll a concentrations (Figure 3.5), with highest concentrations during spring 2002/2003 and summer 2002 periods, and lowest concentrations during late autumn 2002 and winter 2003 (< 0.5 μg L^{-1}) periods. In temperate estuaries concentrations of chlorophyll a are high in spring and summer due to enhanced phytoplankton growth (Jarvie *et al.* 2000). High chlorophyll a concentrations during spring and summer were the result of increased summer solar radiation and a consequence increased photosynthesis (Uncles *et al.* 1998a). Lower chlorophyll a concentrations in autumn and winter were attributed to low phytoplankton abundance due to a combination of grazing by zooplankton (Balbi 2000), lower light levels due to lower solar radiation and enhanced run off with consequent flushing of the estuary. Highest chlorophyll a concentrations were observed at station 5 (Steer Point, 2.9 km down estuary of the Weir) during the June - September 2002 sampling campaigns, possibly due to the accumulation of phytoplankton cells within the high turbidity zone by processes similar to those that result in accumulation of particles or due to the rapid increase in salinity which eventually kill most freshwater phytoplankton (Uncles *et al.* 1998a). Ammonium concentrations fluctuated and seasonal patterns were not

clear, indicating its reactivity. Ammonium is subject to generations and rapid microbial uptake processes. Ammonium distributions exhibited spatial pattern, characterised by relatively low concentrations at both fresh and sea water end members and higher concentrations within the estuary (Appendix 3.1, Figure not shown). This spatial pattern of ammonium concentrations most likely resulted from the biological processes occurring at the sediment-water interface and benthic release from sediment (Treguer and Queguiner 1989; Rocha 1998). In the intertidal sediment of the Sado Estuary, ~ 75 % of the dissolved and sorbed ammonium pool was flushed into the above water column by buoyancy-driven pore water exchange after water flooded the sediment (Rocha 1998).

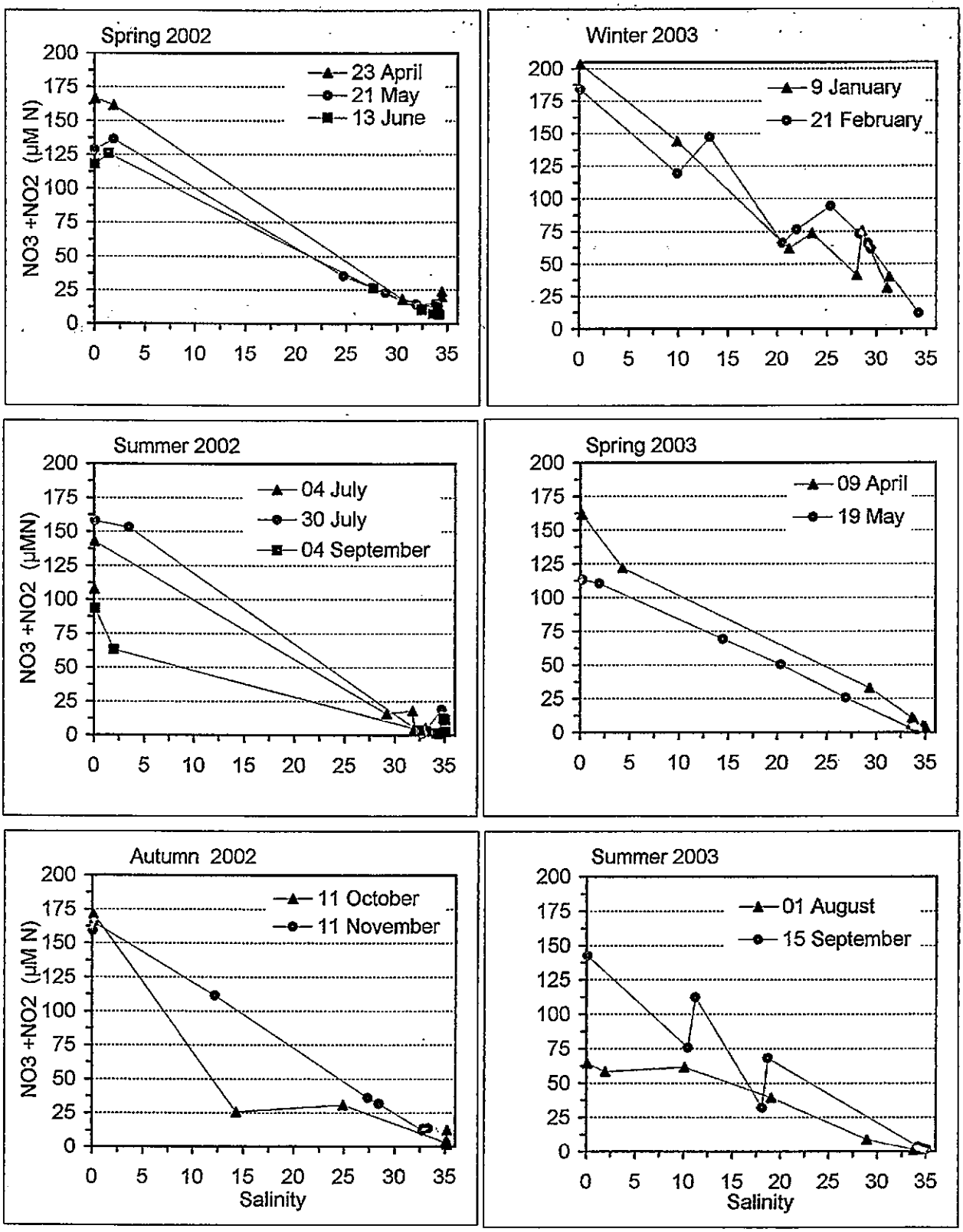


Figure 3.3 Nitrate plus nitrite concentration (µM N) versus salinity in the Yealm Estuary.

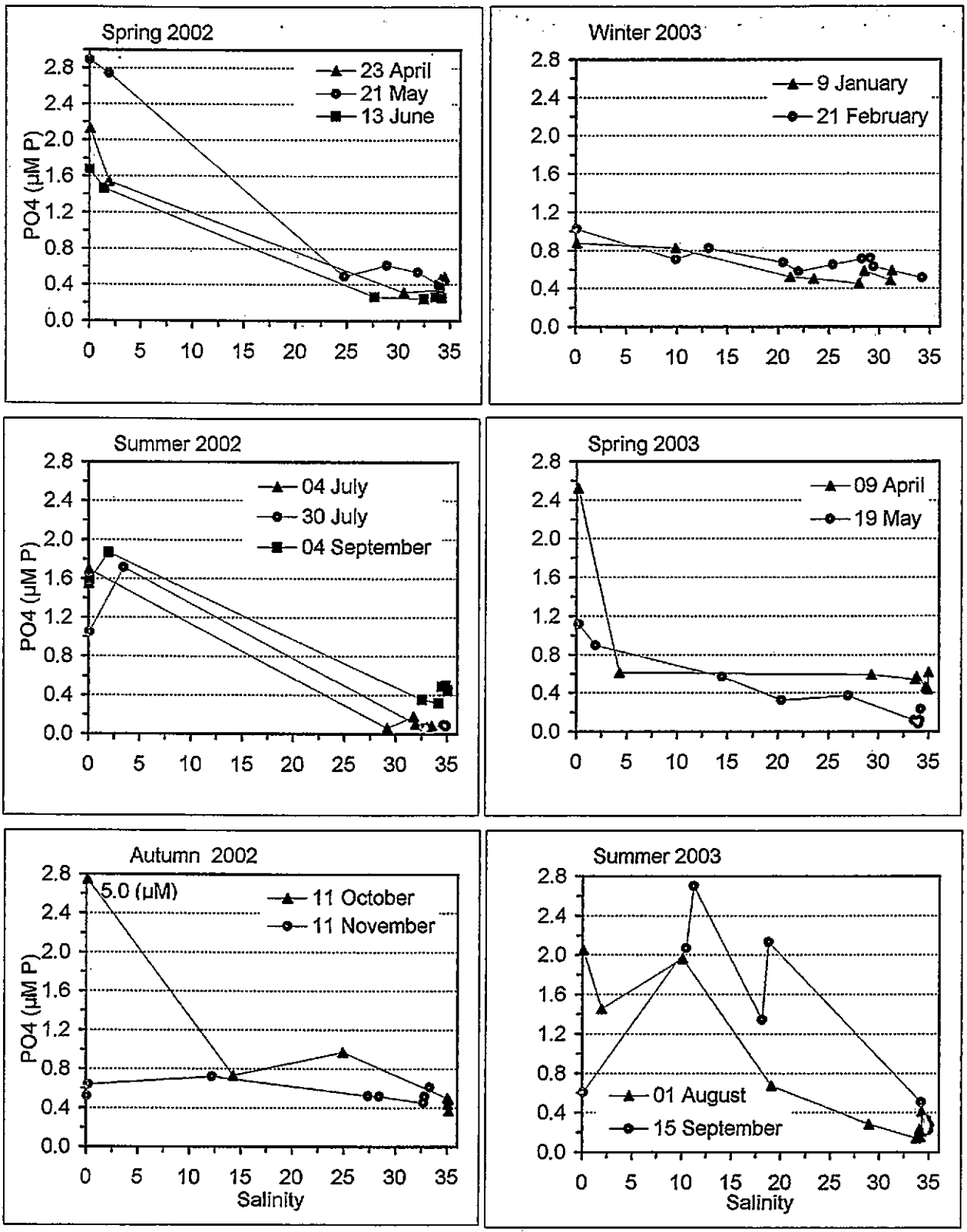
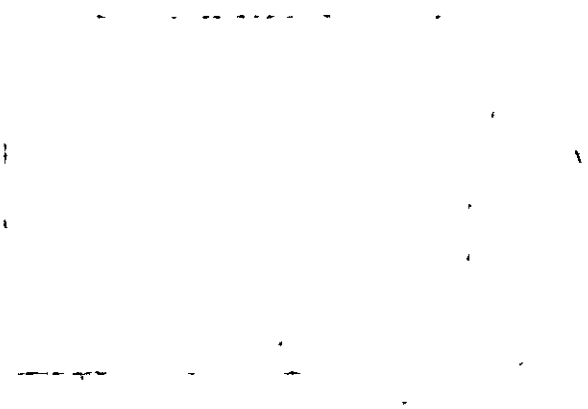


Figure 3.4 Filterable reactive phosphate concentration (µM P) versus salinity in the Yealm Estuary.



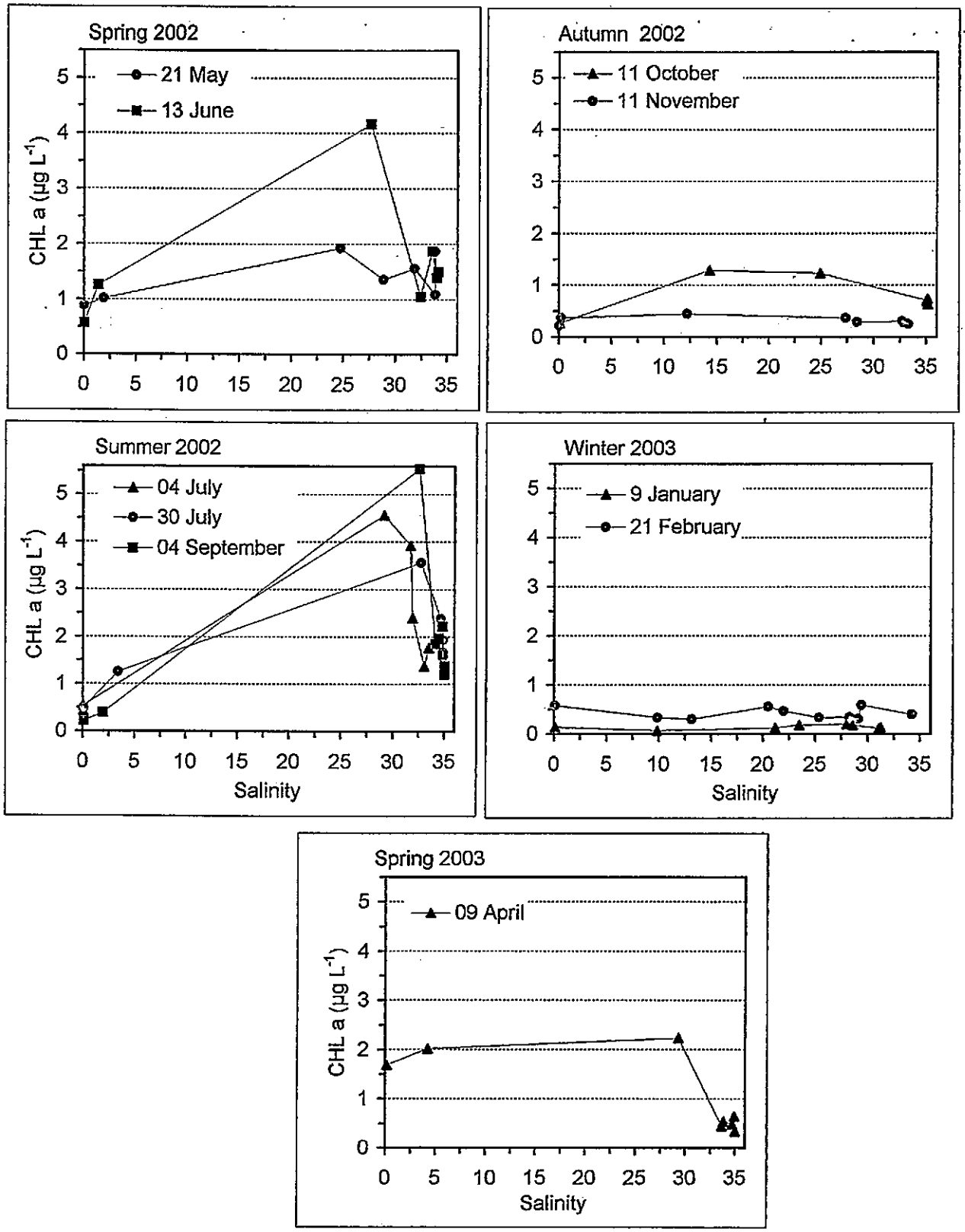


Figure 3.5 Chlorophyll a concentration ($\mu g\ L^{-1}$) versus salinity in the Yealm Estuary.

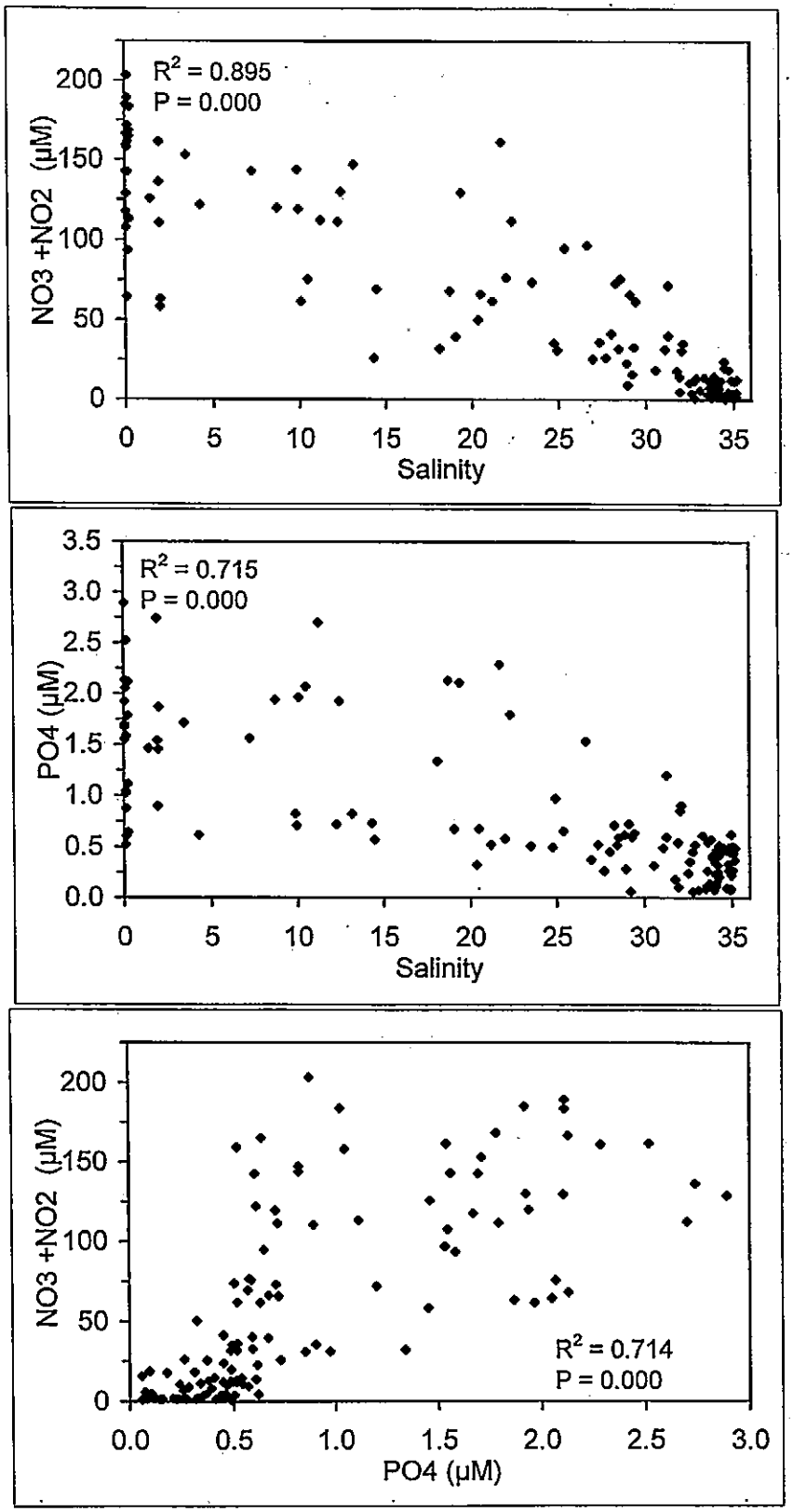


Figure 3.6 Scatter plots showing the correlations between nitrate versus salinity, FRP versus salinity and nitrate versus FRP in the Yealm Estuary for all data, n = 130 (significant correlation at 99 % confidence).

3.2.4 Dissolved organic carbon

Concentrations of DOC ranged from 61 μM C at the seaward end to 335 μM C at the fresh water end (Figure 3.7). This range was relatively lower than observed for other estuarine systems (Table 3.2), and may be due to the relatively small input of fresh water from the River Yealm, the relatively low concentrations of DOC in the river waters and the nature of the catchment area.

Table 3.2 Reported DOC values in estuarine systems

Estuary	DOC range (μM C)	Reference
Amazon River	400	(Benner and Hedges 1993)
Beaulieu Estuary	133 – 1474	(Turner *et al.* 2004)
Chesapeake Bay	153 – 251	(Fisher *et al.* 1998)
Columbia River Estuary	62 – 191	(Prahl and Coble 1994)
Delaware Estuary	90 – 450	(Sharp *et al.* 1993)
Douro Estuary	158 – 208	(Abril *et al.* 2002)
Elbe Estuary	258 – 366	
Ems Estuary	424 – 591	
Gironde Estuary	91 – 208	
Loire Estuary	199 – 291	
Mersey Estuary	149 – 808	(Turner *et al.* 2004)
Rhine Estuary	141 – 258	(Abril *et al.* 2002)
Sado Estuary	300 – 525	
Scheldt Estuary	183 – 516	
Tamar Estuary	130 – 210	(Miller *et al.* 1993)
	110 – 478	(Miller 1999)
York River Estuary	240 – 534	(Raymond and Bauer 2001)

A scatter plot of DOC concentration against salinity, for all data, shows a high degree of scatter ($r^2 = 0.39$, n = 130), suggesting non-conservative behaviour in general (Figure 3.8 A). During April 2003 and to some extent April 2002 and May 2002, DOC concentrations for MTZ (maximum turbidity zone) samples were higher than those for fresh water

samples, implying internal DOC input due to desorption processes (Miller 1999; Abril *et al.* 2002). However, the DOC appeared to be conservative in May 2002, and May and August 2003, period during which biological activity was high. This conservative behaviour implied that the rate of physical flushing of water dominates over biological or physical removal processes of riverine-derived DOC within the estuary (Prahl and Coble 1994). The apparent conservative behaviour of DOC during these months might be also due to the high DOC levels at the fresh water end, implying that the River Yealm was the major source of organic carbon (Abril *et al.* 2002) and / or that DOC become less labile after consumption of the riverine bioavailable fraction. Wiegner and Seitzinger (2001) emphasised the importance of microbial processes in degrading DOC in the riverine environment, affecting the quantity and bioavailability of the DOC exported from rivers to estuaries. They found that riverine microbial degradation removed 6 – 14 % of the DOC from agricultural and forest runoff. A seasonal variation in the apparent reactivity of DOC has been observed in the Humber Estuary (Alvarez-Salgado and Miller 1998). When comparing the DOC distributions in different estuaries, different behaviours can be observed from conservative to an excess or deficit in DOC (Cauwet 2002). Conservative behaviour of DOC has been reported for the Severn Estuary (Mantoura and Woodward 1983), the Humber Estuary (Alvarez-Salgado and Miller 1998), the Columbia River Estuary (Prahl and Coble 1994), the Thames, Elbe, Rhine, Douro and Loire estuaries (Abril *et al.* 2002), the Winyah Bay (Goni *et al.* 2003) and the Long Bay (Avery *et al.* 2003). Non conservative mixing occurs when there are (1) anthropogenic inputs of DOC such as the Scheldt Estuary-polluted by sewage, (2) a large flux of labile DOC due to enhanced aquatic production such as the Gironde and York River estuaries, (3) a supply of DOC from large intertidal areas such as the Sado and Ems estuaries, (4) and/or high rates of DOC mineralization and photochemical break down (Raymond and Bauer 2001; Cauwet 2002; Abril *et al.* 2002). Furthermore, the non-conservative behaviour of DOC is

generally enhanced in stratified estuaries with longer mixing time, vertical gradient in DOC concentrations, and the possibility of photochemical break down of surface DOC (Cauwet 2002).

An examination of the seasonal variations in DOC concentrations in the Yealm Estuary revealed that DOC concentrations were elevated during the summer and autumn relative to winter, as shown in Figure 3.7. Seasonal variations of DOC depend on abiotic and biotic mechanisms such as biological production, inputs of organic matter from anthropogenic sources and also the biodegradability of the newly produced DOC (Sondergaard *et al.* 2000). During spring and early summer the elevated DOC concentrations were partially driven by high river concentrations, presumably due to increased primary production in the catchment area and subsequent biological processing of the fixed carbon, perhaps, coupled to the relatively longer residence time. Also, during the dry seasons, river flow rates decreased and consequently the residence time of fresh water in the estuary increased, delaying the physical dilution of DOC with sea water. In addition, DOC produced during the spring and early summer by phytoplankton blooms and autotrophic bacteria may accumulate in the estuary, to give higher DOC concentrations during the summer and early autumn (Volk *et al.* 1997).

In the Yealm high concentrations of chlorophyll a were observed in both spring 2002/2003 and summer 2003, indicating the presence of phytoplankton blooms (Figure 3.5). Mantoura and Woodward (1983) reported that estuarine accumulation of DOC was apparently related to phytoplankton production in the Severn Estuary. Low concentrations of inorganic nutrients in late spring and summer at high salinity (Figure 3.3) may result in accumulation of organic carbon as nitrogen availability is low (Kahler and Koeve 2001; Al-Azri 2003). Some photoautotrophic organisms can store organic carbon as lipid or carbohydrate

resulting in POC accumulation and higher C:N ratios in the cell, and a subsequent high C:N ratio of DOM in the surrounding water environment (Karl and Bjorkman 2002).

Strong positive correlations were observed between DOC, BOD and chlorophyll a for spring 2002 and 2003 samples, respectively, indicating the relationship between phytoplankton (primary producers) and organic matter (Figure 3.8 B and Appendix 3.1). During productive seasons with high concentration of organic matter, the amount of dissolved oxygen needed for biological oxidation of organic matter by heterotrophic bacteria increased.

At the river mouth, concentrations of DOC in the November 2002 samples were higher than for those in the October 2002 samples. This may have been due to the extensive rainfall that occurred in the week prior to the sampling campaign in November, with consequent enhanced DOC in the run off following a dry period from mid July – October. Concentrations of DOC in early July 2002 samples were higher than those in late July 2002 samples, which may be due to the extensive rain fall first week of July 2002, before sampling. Marked increases in DOC concentrations were frequently observed (April 2002 and April and May 2003) at the mouth of the estuary. These samples were of relatively low salinity, and presumably reflected fresh water inflows from Newton Creek. High levels of DOC, DON and nutrients were observed in the samples collected from the mouth of the estuary in February 2003. These enhanced levels were probably due to surface run off from Noss Mayo (as was observed during sampling campaign). Inflows of fresh water from run off were consistent with fluctuations in salinity along the axial transect at this time (Figure 3.9).

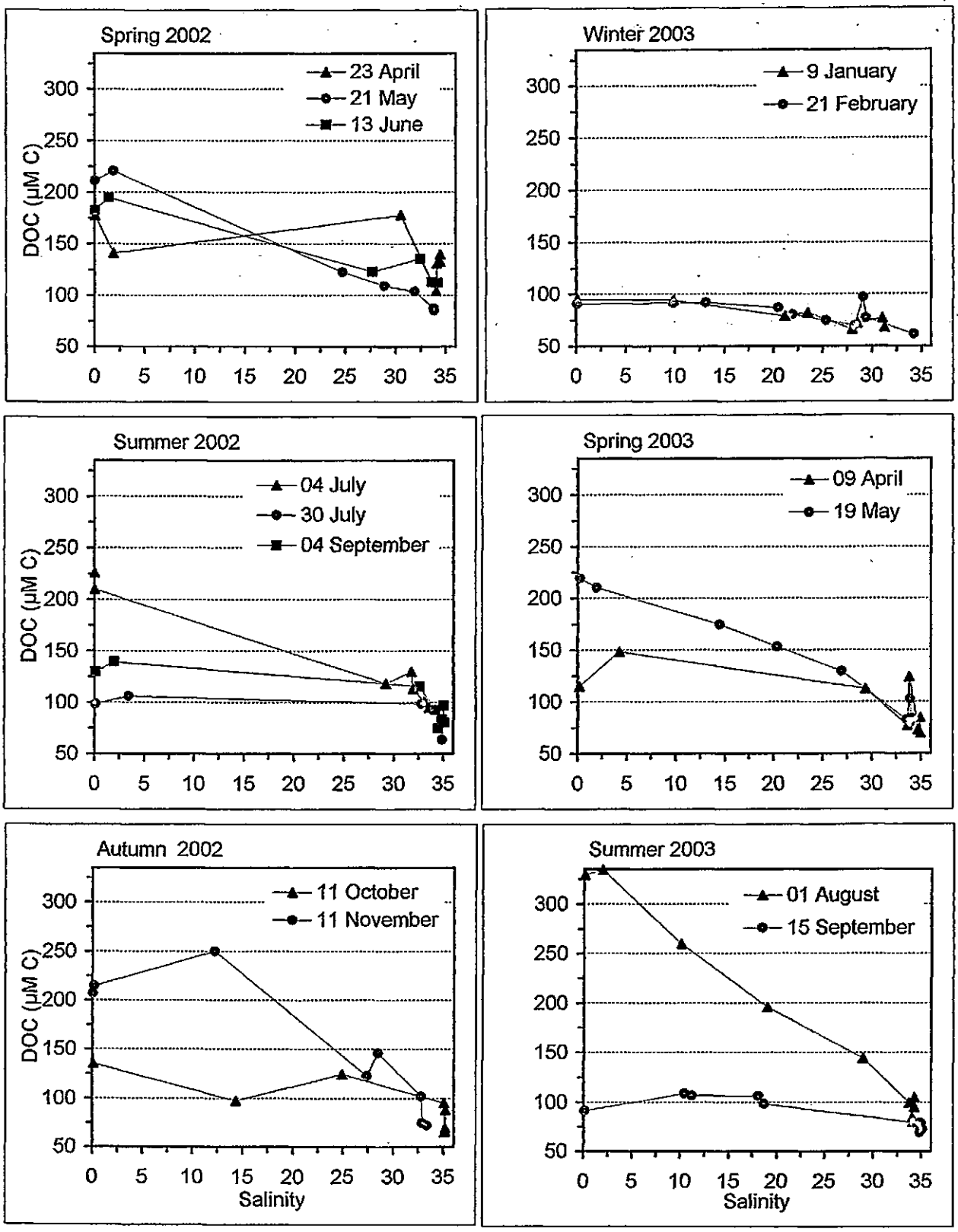
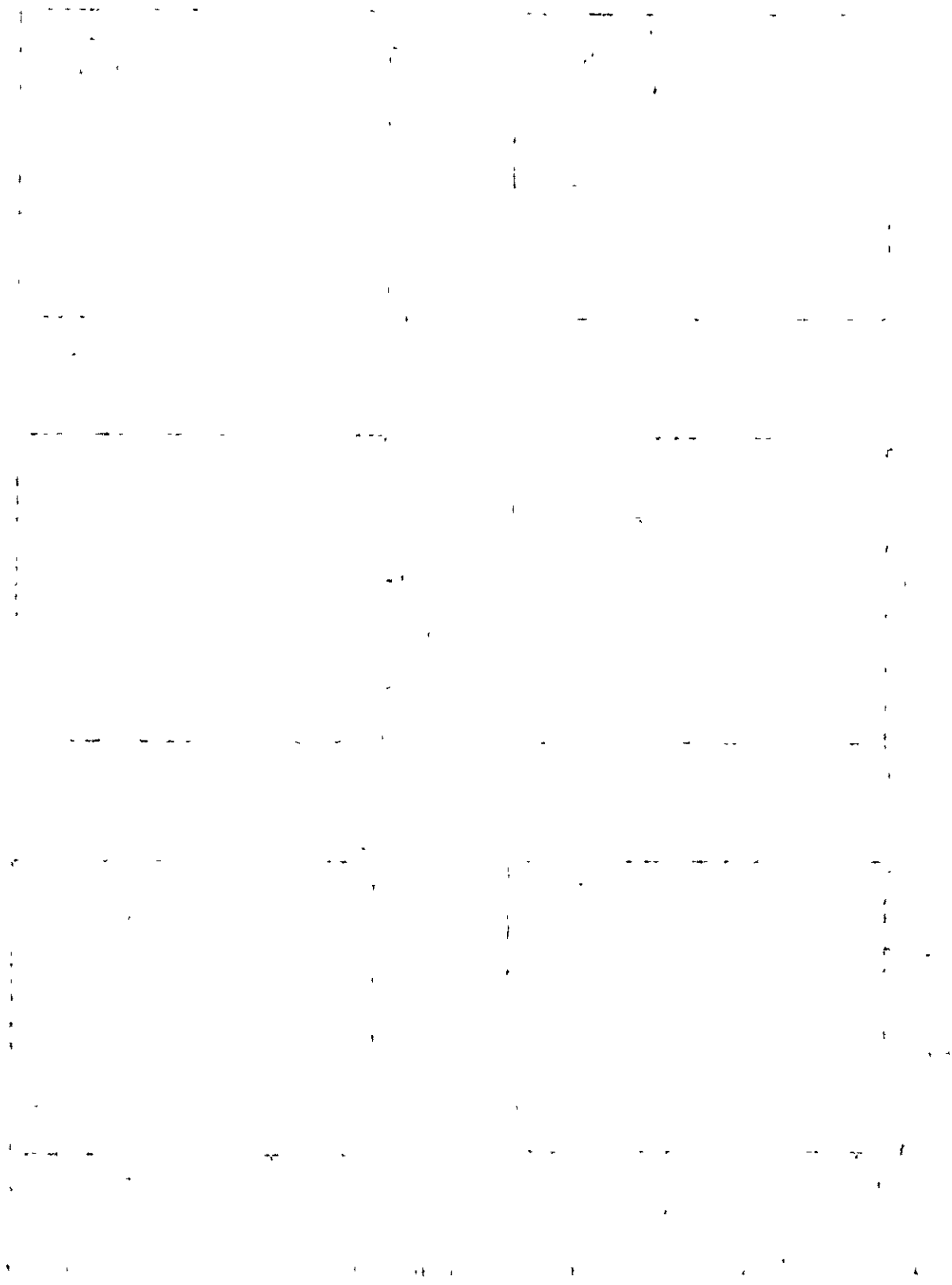


Figure 3.7 Dissolved organic carbon concentration (µM C) versus salinity in the Yealm Estuary.



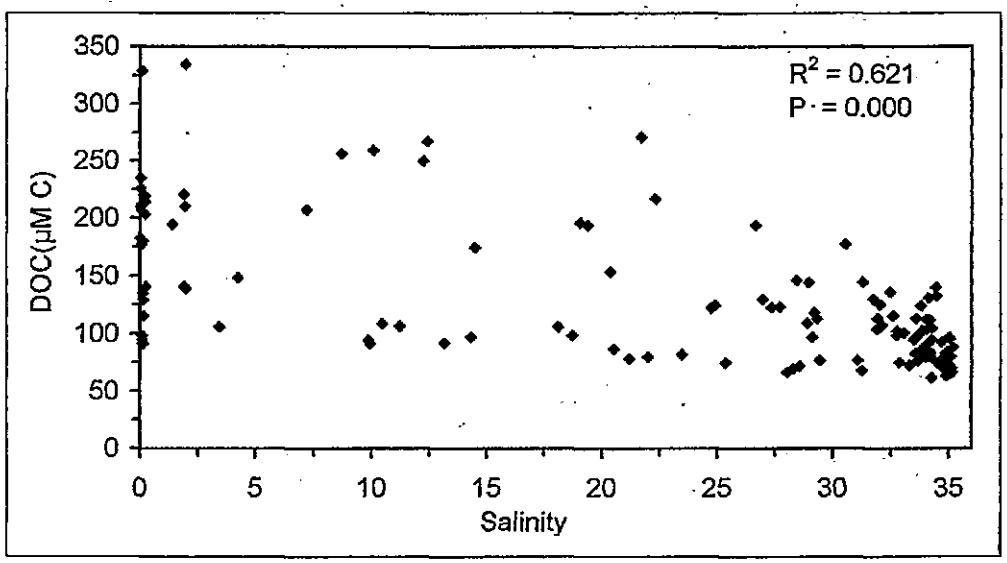


Figure 3.8 (A) Scatter plot of dissolved organic carbon concentration (µM C) versus salinity in the Yealm Estuary for all data.

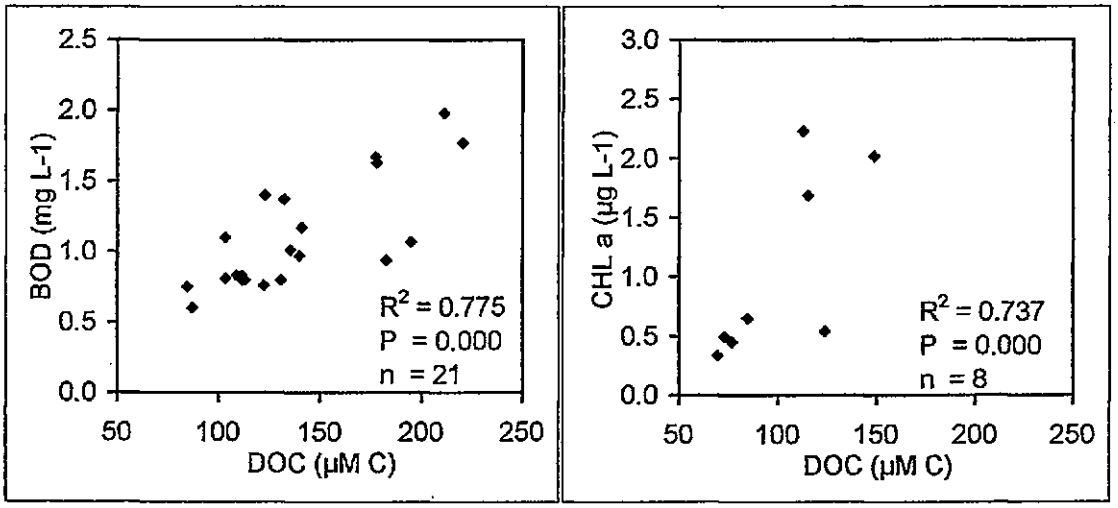


Figure 3.8 (B) Scatter plots showing the correlations between DOC, BOD and chlorophyll a in the Yealm Estuary for spring 2002 and 2003, respectively.

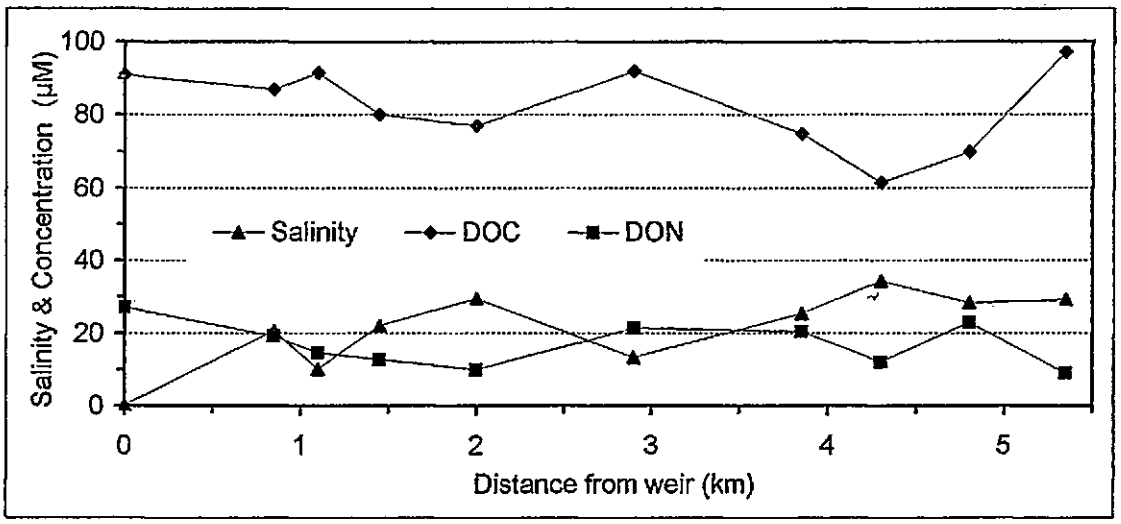
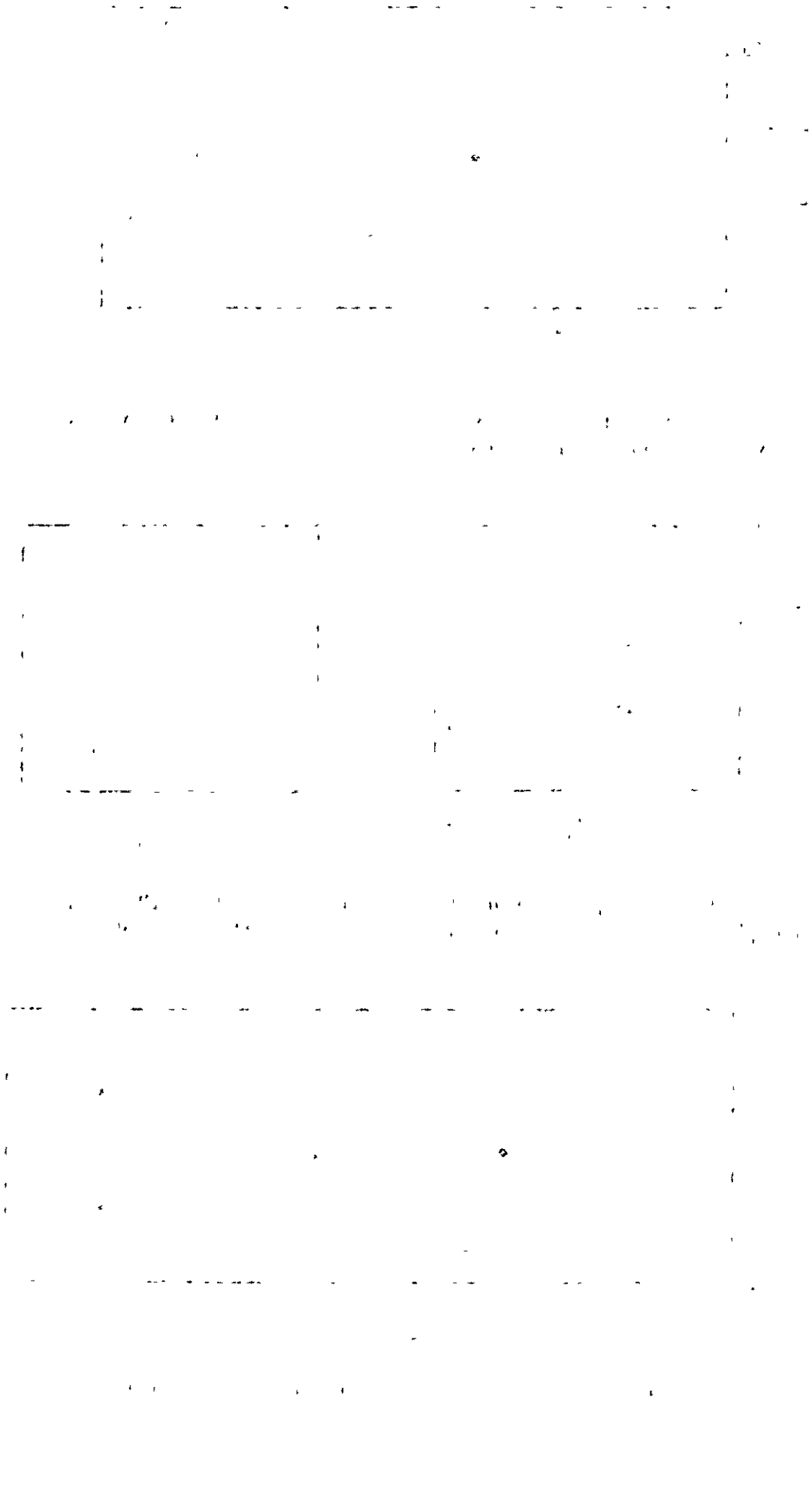


Figure 3.9 Salinity, DOC and DON (µM) in the Yealm Estuary during February 2003.



3.2.5 Dissolved organic nitrogen

DON concentrations were mainly in the range 1.8 – 62 μM N, with highest values in the fresh waters; exceptionally high concentrations of 109 and 185 μM N were measured in non-saline waters in August 2003 and September 2002, respectively. The maximum values for DON reside at the upper end of the range reported globally for estuaries (22.5 ± 17.3 μM N) and rivers (34.7 ± 20.7 μM N) (Bronk 2002). The enhanced DON values might be the result of sewage discharges and run-off of organic agricultural manure into this estuarine system. A plot of DON against salinity for all data revealed a high degree of scatter around the theoretical dilution line (Correlation coefficient = 0.49, n = 130; Table 3.1 and Figure 3.10), indicating that DON behaviour was usually non-conservative in this estuary, and that external sources and / or in situ biological uptake/transformation and benthic inputs have been influential.

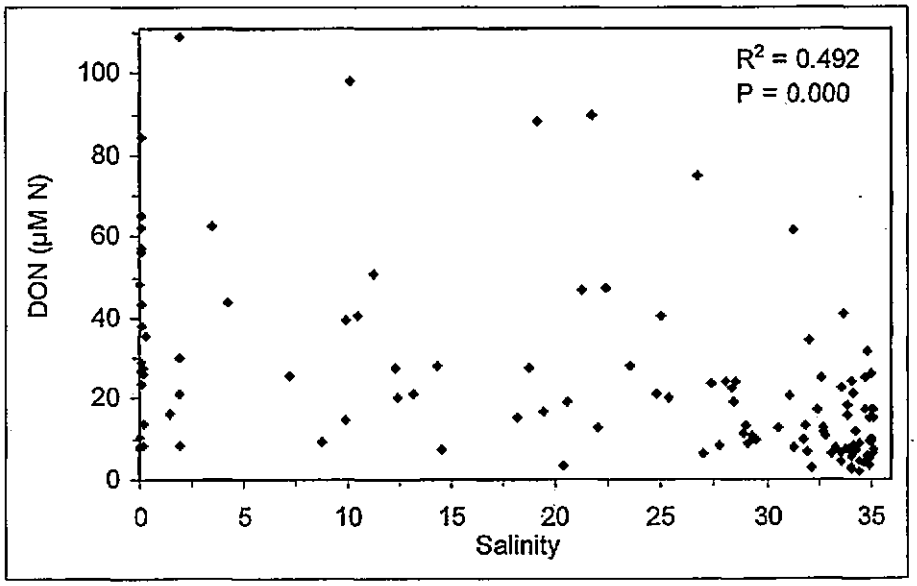


Figure 3.10 Scatter plot of dissolved organic nitrogen concentration (μM N) versus salinity in the Yealm Estuary for all data.

An examination of the seasonal variations in DON concentrations revealed that DON concentrations were lowest during spring and early summer (April and July 2002, April and May 2003) and highest during the late summer (September 2002 and August 2003)

(Figure 3.11). The enhanced DON levels in late summer may have been due to active release of DON from phytoplankton; as nitrate was utilised by phytoplankton growth there was an increase in the concentration of DON (Butler *et al.* 1979; Bronk 2002). So, on seasonal time scale, DON can serve as intermediate nitrogen pool during the transition from autotrophy to heterotrophy in estuarine environments (Mortazavi *et al.* 2001). The high DON values observed in the Yealm Estuary during September 2002 and August 2003 were consistent with the trends observed in the English Channel, i.e. maximum DON in September and minimum nitrate in June (Butler *et al.* 1979). Similar trends, with high DON values in late spring and summer, have been reported in the Gulf of Mexico and Chesapeake Bay (Bronk *et al.* 1998; Bronk 2002). In contrast, no seasonal patterns were indicated in the Santa Monica Basin and the Bermuda Atlantic Time Series site in the Sargasso Sea (Bronk 2002).

During the surveys of April, May and November 2002, and May 2003, there appeared to be removal of DON in the low salinity region of the Yealm, which was sometimes accompanied by increased concentrations of ammonium and/or nitrate (Figure 3.3, 3.5 and 3.11). This behaviour is consistent with bacterial ammonification of DON and nitrification of the released ammonium. Heterotrophic bacteria are responsible for the transformation of organic matter into inorganic nutrients to be used by primary producers. Kerner and Spitzy (2001) observed that between 75 and 100 % of the low molecular weight DON and ammonium (generated from DON) was taken up and converted to nitrate in the Elbe Estuary through nitrification. A detailed discussion about DON bacterial mineralization is presented in section 3.4.

$$\text{ON (organic nitrogen)} \xrightarrow{\text{Ammonification}} NH_4^+ \xrightarrow{\textit{Nitrosomonas}} NO_2^- \xrightarrow{\textit{Nitrobacter}} NO_3^-$$

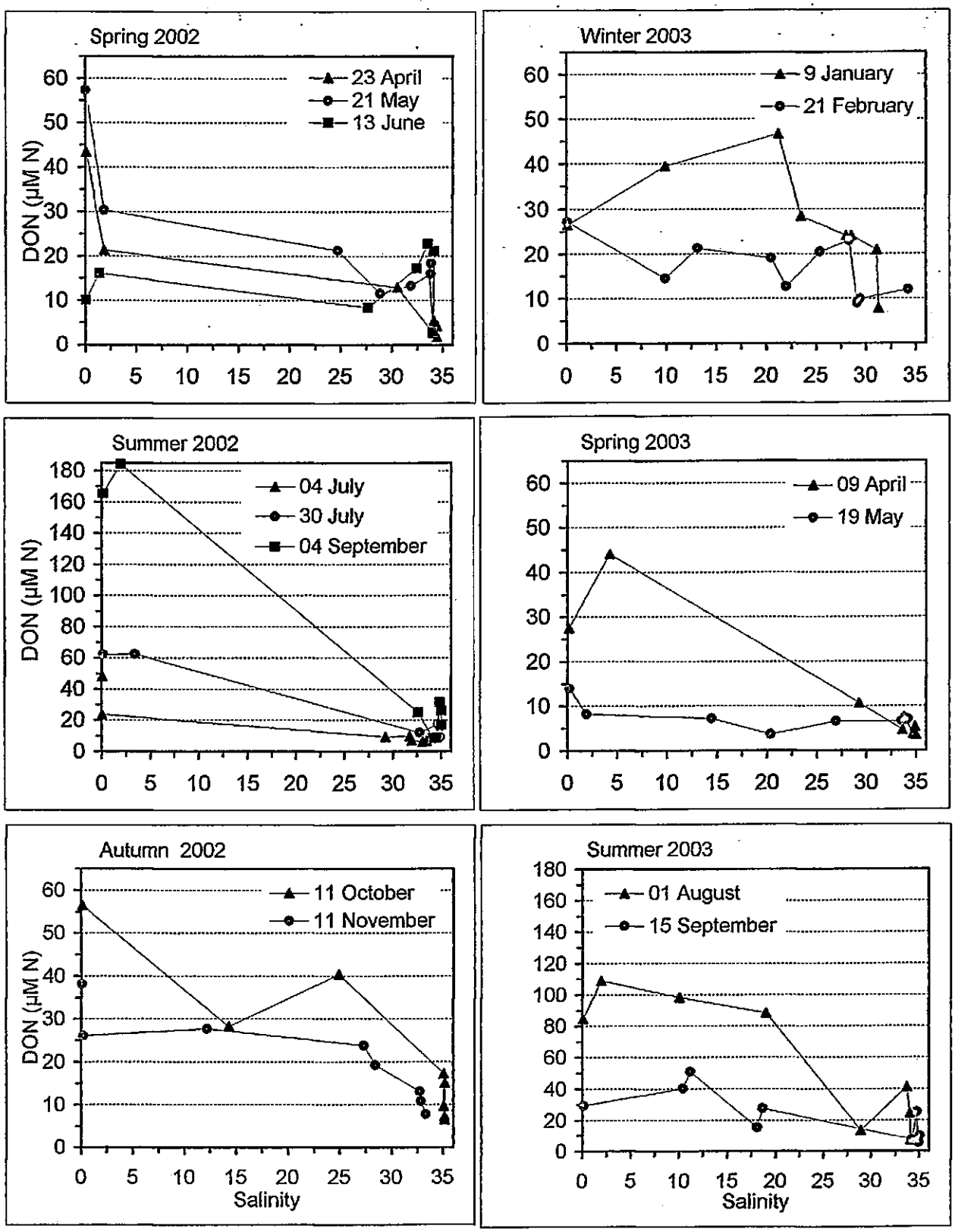


Figure 3.11 Dissolved organic nitrogen concentration (µM N) in the Yealm Estuary (southwest England) versus salinity (different scale used for Summer).

3.2.6 Contribution of dissolved organic nitrogen to total dissolved nitrogen pool

The amount of DON as a percentage of TDN in the Yealm Estuary was between 4 and 79 %, with higher values in the mid-estuarine samples. Generally, high values were observed during the summer and autumn seasons (from ~ 20 % to 79 %), with low values observed in winter and spring seasons (from 10 % to 45 %). As an example, Figure 3.12 shows the important contribution of DON to the TDN pool as the percentage values ranged from 61 % to 74 % and 53 % to 79 % for the September 2002 and August 2003 samples, respectively. The contribution of DON to TDN pool was often lower in fresh water samples than estuarine water samples, indicating that nitrate was the dominant N species in the River Yealm (Treguer and Queguiner 1989).

Phytoplankton uptake of nitrate (to fuel new production, in absence of significant nitrogen fixation) and ammonium (to fuel regenerated production) gives rise to the generation of DON within the biomass. DON may be released from cells either by passive exudation or cell breakage during grazing and may contribute to a higher DON/TDN percentage. Bronk et al. (1994) found that 32 ± 17 % of inorganic nitrogen that was biologically removed was subsequently released as DON in environments ranging from relatively eutrophic estuaries to the oligotrophic ocean. Bronk and Ward (1999) found that the primary fate of nitrogen uptake in Monterey Bay, California was particulate nitrogen (PN) in March and DON in September. The significant contribution of DON to the TDN pool suggests that the omission of DON measurements in water quality studies may result in both an underestimation of the inputs of total nitrogen to natural waters and the extent of eutrophication.

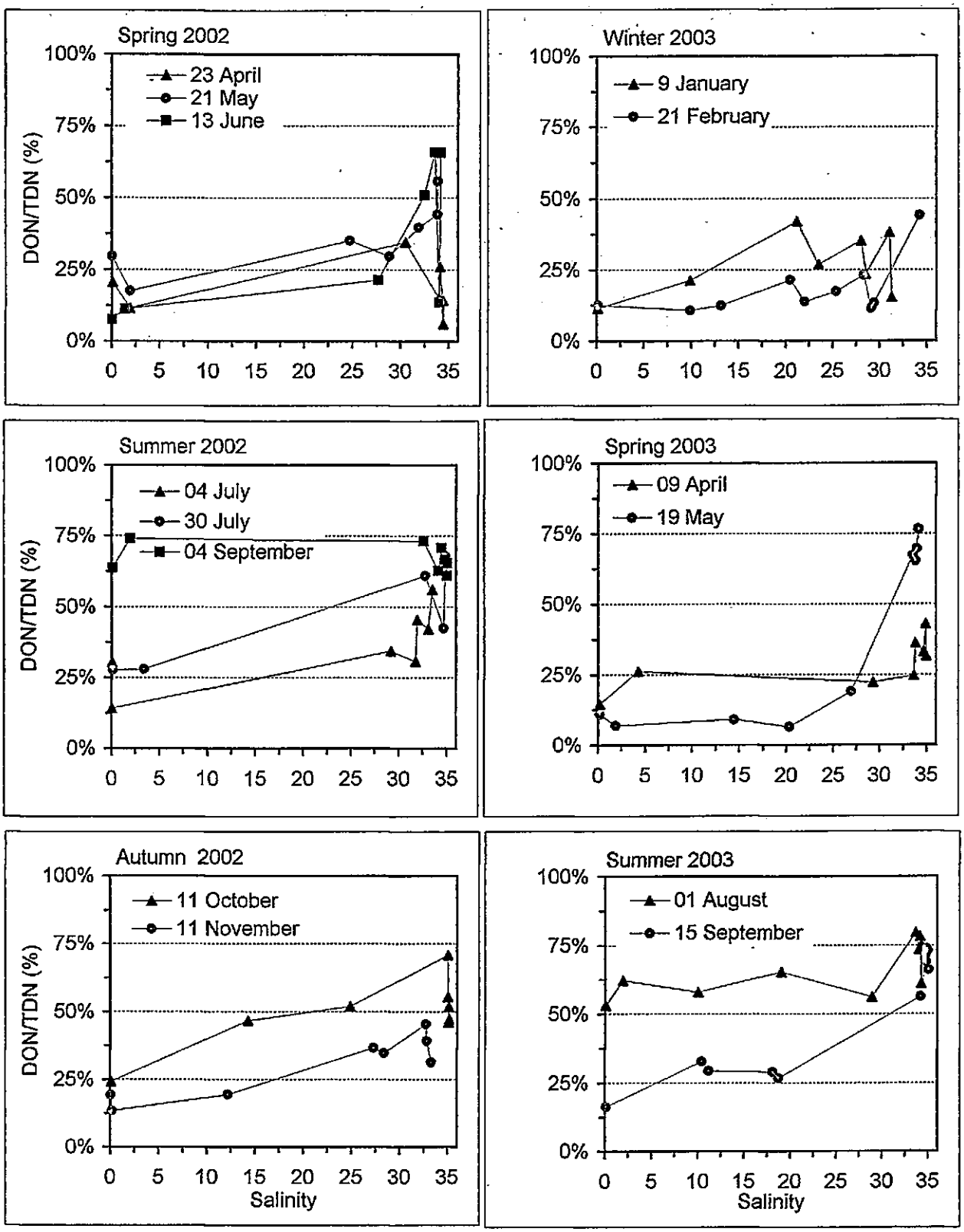


Figure 3.12 The percentage of dissolved organic nitrogen to total dissolved nitrogen versus salinity in the Yealm Estuary.

3.2.7 The C:N ratio of the DOM pool (DOC:DON)

The DOC:DON ratios ranged between 0.75 and 42, with a mean of 9.5 ± 8.3 (± 1SD). This mean is lower than values reported for other aquatic environments. A ratio of ~ 14 in the surface ocean water and a mean ratio of 26 in riverine systems have been reported by Bronk (2002). The low DOC:DON ratio in the Yealm Estuary may be due to the observed high DON values as a consequence of enhanced nitrogen inputs into the system. As shown in Figure 3.13, the DOC:DON ratios were generally higher for spring samples and reside at the lower range (2 – 10) for autumn and winter.

The high DOC:DON ratios observed for spring 2002 / 2003 and early summer 2003 samples, particularly those with high salinity, were due to an enhanced phytoplankton growth as indicated by high chlorophyll a concentration (Figure 3.5 and 3.13). Phytoplankton were the primary source of these C-enriched DOM as observed also in the West Neck Bay, Shelter Island (Gobler and Sanudo-Wilhelmy 2003). They also indicated that, high molecular weight (HMW) organic matter was enriched in phytoplankton-derived carbohydrates and that such fresh DOM frequently has a higher C:N ratios. Phytoplankton growth and the lack of available inorganic nitrogen are the only suggested reasons for accumulation of C-rich DOM (Williams 1995). Therefore, the high ratios of the DOC: DON could be interpreted as the result of the non-proportional increase in DOC relative to DON (Al-Azri 2003). Low DOC: DON ratios during late summer, perhaps, due to the low concentration of inorganic nitrogen and hence the active release of DON from phytoplankton, as discussed earlier.

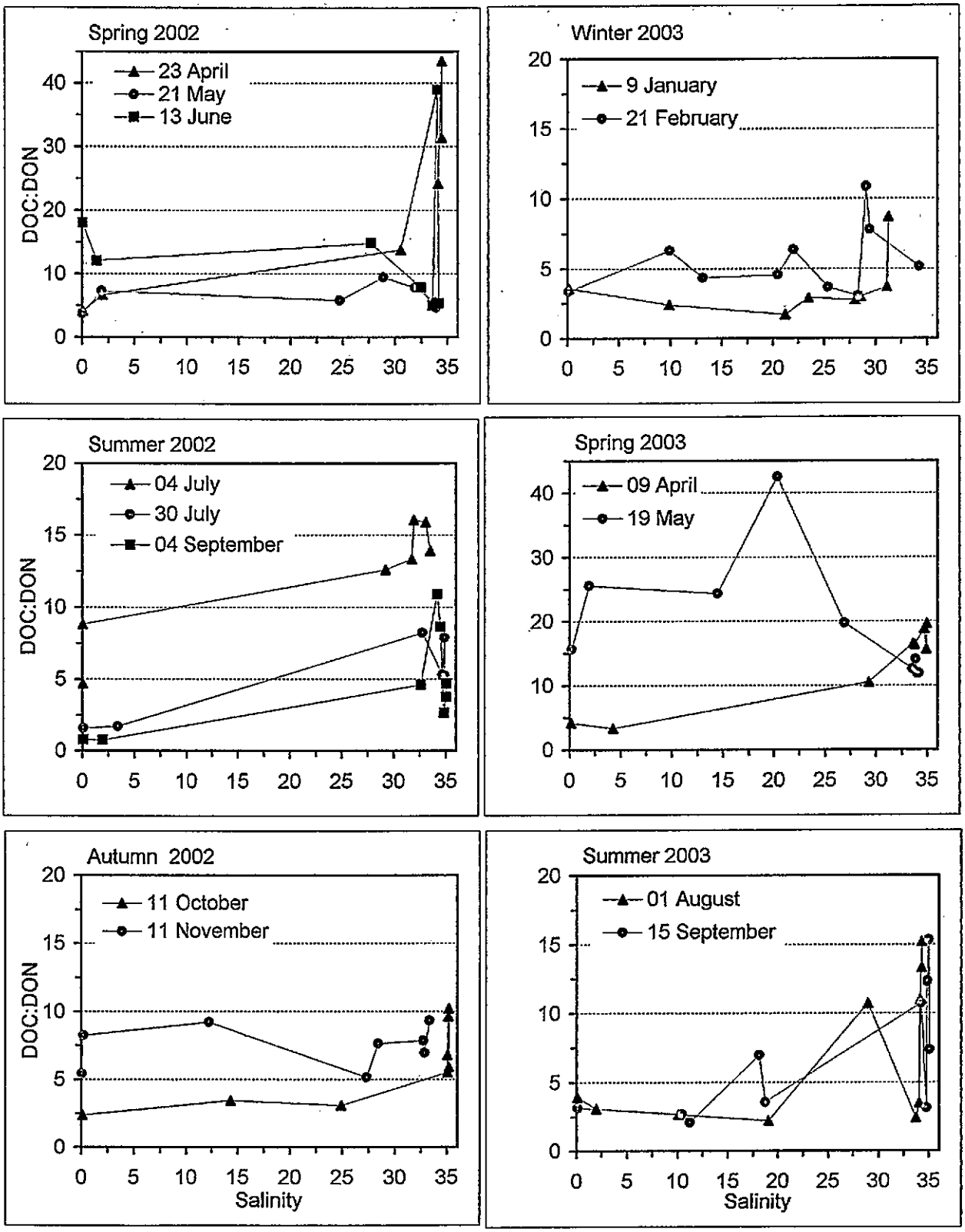


Figure 3.13 Dissolved organic carbon to dissolved organic nitrogen ratio versus salinity in the Yealm Estuary (different scale used for spring).

3.2.8 Relationships between chemical determinands and flow

An examination of the relationships between concentrations of chemical determinands and river flow can provide valuable information on the origin and nature of these determinands. Flow data for the River Yealm, close to the tidal limit, during the study period are provided in Appendix 3.3. Flow data for the period 22-08-2001 to 30-10-2002 (missing from the EA) were calculated from the Plym Estuary flow data using linear regression equation. Fresh water inflow from the River Yealm exhibited a marked seasonal signal, with flows < 0.5 $m^3 s^{-1}$ during July –September 2003 (excluding August 1-7) and > 5 $m^3 s^{-1}$ during the winter. Annual flux of DOC was estimated to be 56 x 10^6 g C Yr^{-1}, compared to an average of 5.6 x 10^9 g C Yr^{-1} reported for 11 rivers in the Humber catchment (Tipping *et al.* 1997), and 1.7 – 2.7 x 10^{10} g C Yr^{-1} for the River Severn (Mantoura and Woodward 1983). Also, the annual flux of DON was 19.4 x 10^6 g N Yr^{-1} compared to 55.4 x 10^6 g C Yr^{-1} of nitrate. World rivers export approximately 0.2 x 10^{15} g C Yr^{-1} of DOC to the Ocean each year (Avery *et al.* 2003)

For DOC and DON concentrations, there were no clear relationships with flow (Figure 3.14), possibly indicating a complex suite of inputs of DOC and DON derived from anthropogenic as well as natural sources. So, a mixture of point and diffuse sources of DOC and DON resulted in the highly scattered relationship with flow. For the River Swales, DOC concentrations were dominated by sewage point sources during base flow and diffuse sources of soil organic carbon during high flow (Eatherall *et al.* 2000). In comparison with the literature, DOC concentrations were diluted under storm flow conditions in the Trent and Aire Rivers, indicating point sources of DOC from sewage effluents (Tipping *et al.* 1997; Jarvie *et al.* 2000). In contrast, the highest concentrations of DOC in the Ouse, Tweed and Derwent occurred under storm flow conditions, indicating diffuse sources of DOC from the catchment surface were dominant (Jarvie *et al.* 2000).

The highest DOC concentration in the Yealm River was observed during the August 2003 sampling campaign when flow rates were high, as shown in Figure 3.15. This might have been due to the flushing of DOC from diffuse sources of organic matter in the agricultural catchment following two months of limited rainfall (river flow ≤ 0.5 m^3 s^{-1}). The highest DON concentrations, coupled with the lowest nitrate concentrations, were observed during the September 2002 and August 2003 sampling campaigns (Figure 3.15). Highest DON concentrations are possibly due to active release of DON by phytoplankton as discussed earlier, and/or due to high river flow rates after a dry period of two months (August 2003 sampling campaign), where DON generated in the catchment was flushed.

FRP and ammonium concentrations decreased with increasing flow in response to dilution of point sources by rain fall (Figure 3.16). As FRP is derived predominantly from sewage effluent inputs, the highest concentrations occurred at base flow and undergo dilution under high flow conditions; this is in consistent agreement with the relationship between FRP concentrations and flow reported for the Mersey and Trent rivers (Head 1985; Jarvie *et al.* 2000). Highest FRP concentrations, coincident with high ammonium concentrations, were observed for the October 2002 sampling campaign reflecting sewage input during base flow (Figure 3.15).

In contrast, nitrate concentrations exhibited positive relationships with flow (Figure 3.17). Increases in nitrate concentrations with flow were possibly related to the diffuse sources of nitrate from the agricultural run off due to fertiliser applications within the Yealm catchment. The trend of increasing nitrate concentrations with flow were in close agreement with the relationship between nitrate and flow reported for the Great Ouse, Mersey and Wear rivers (Head 1985; Neal *et al.* 2000a; Neal *et al.* 2000b). In contrast, for the River Trent, the base flow concentration of nitrate is higher than for storm flow

conditions, indicating that point sources dominate nitrate concentrations in the River Trent (Jarvie *et al.* 2000). As in Figure 3.15 the highest concentrations of nitrate in the River Yealm occurred during the winter period and then decreased during the following spring and summer period; a pattern similar to nitrate behaviour in the Great Ouse (Neal *et al.* 2000b). Minimum nitrate concentration occurred during late summer which also were the result from phytoplankton uptake or biologically mediated denitrification.

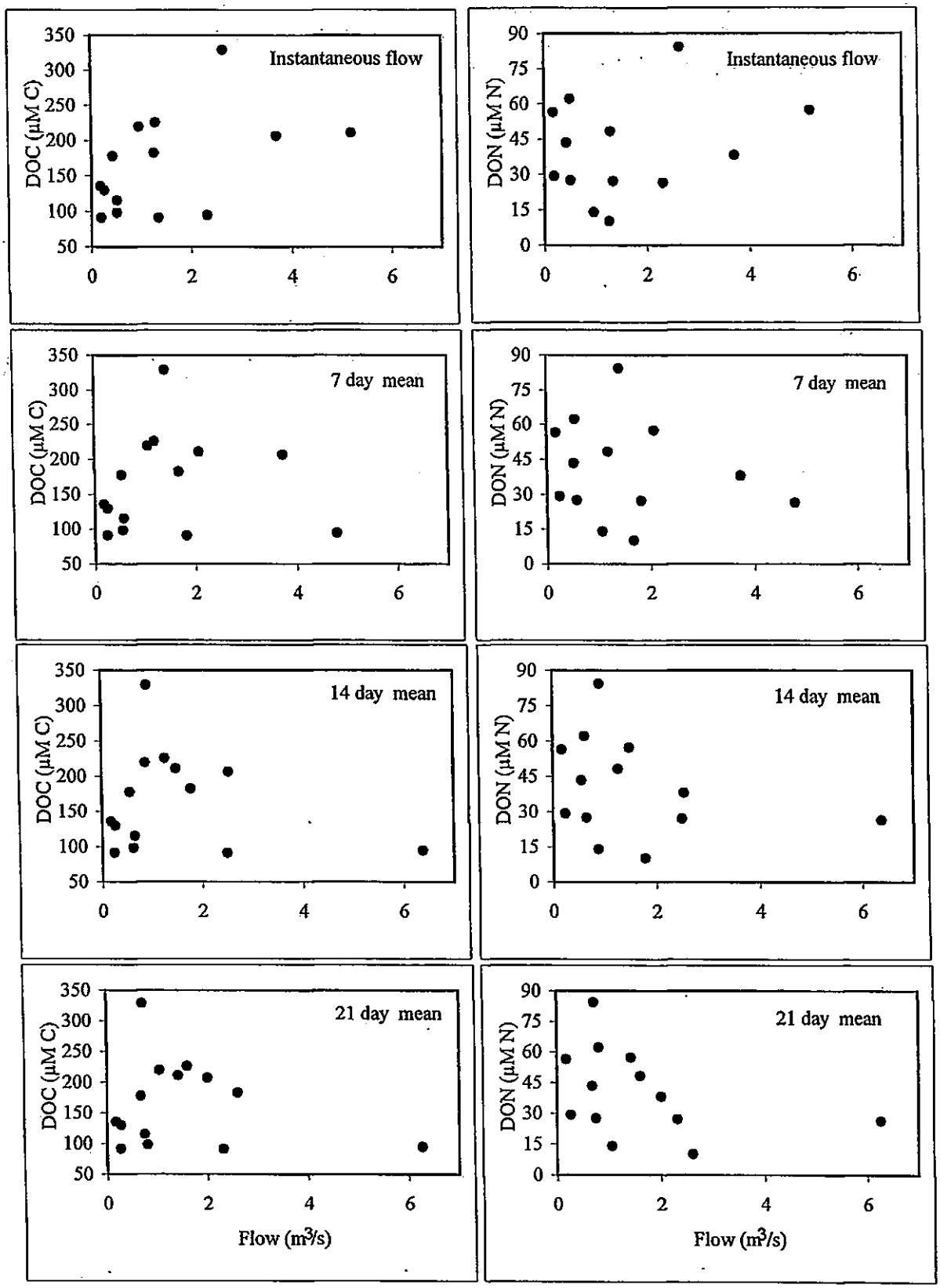


Figure 3.14 DOC and DON concentrations (µM) at the head of the Yealm Estuary (0 km) versus flow (instantaneous and 7, 14 and 21 day mean flow values before the sampling day).

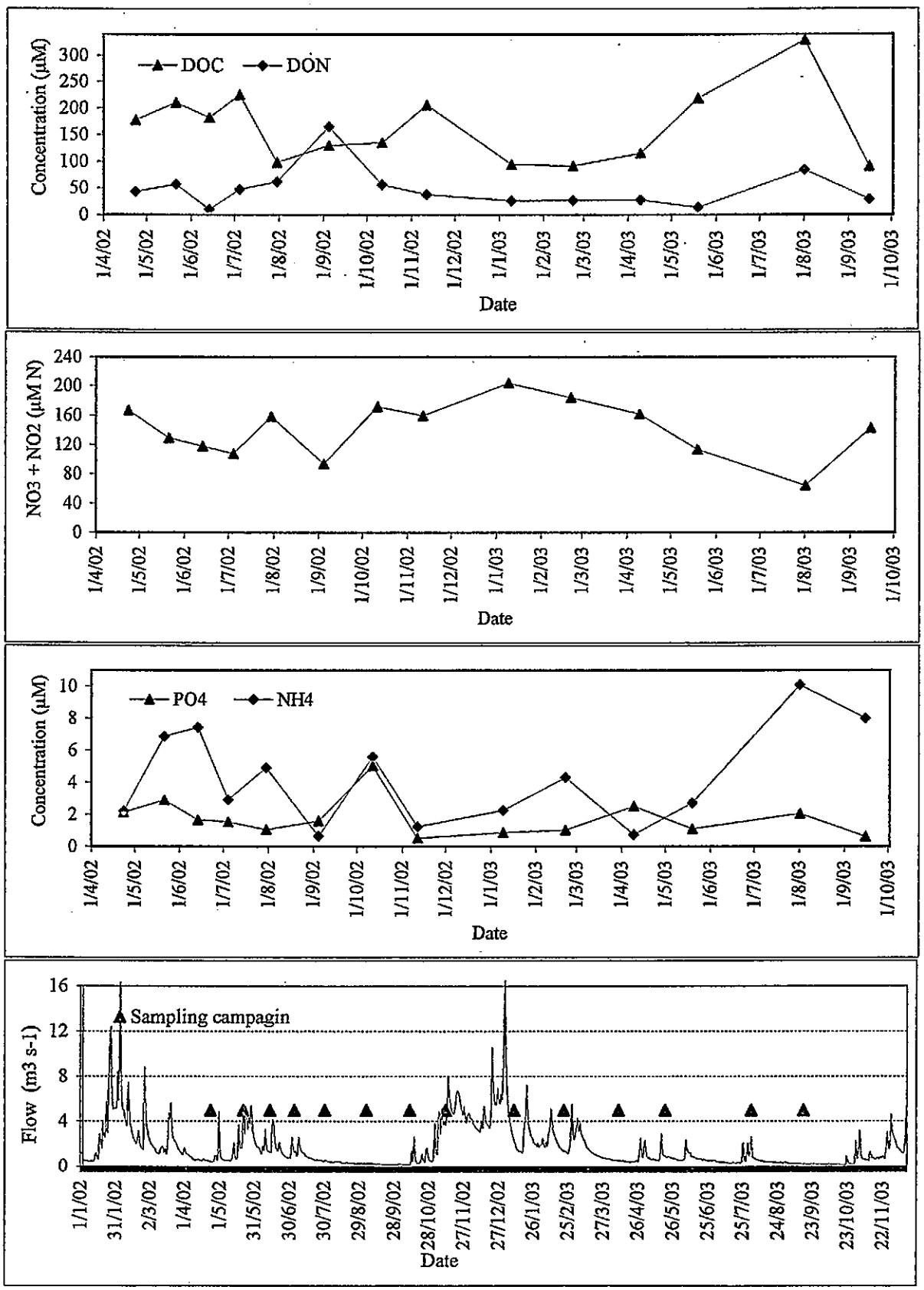


Figure 3.15 Seasonal variations of measured parameters at the head of the Yealm Estuary (0 km) to study the effect of river flow rate.

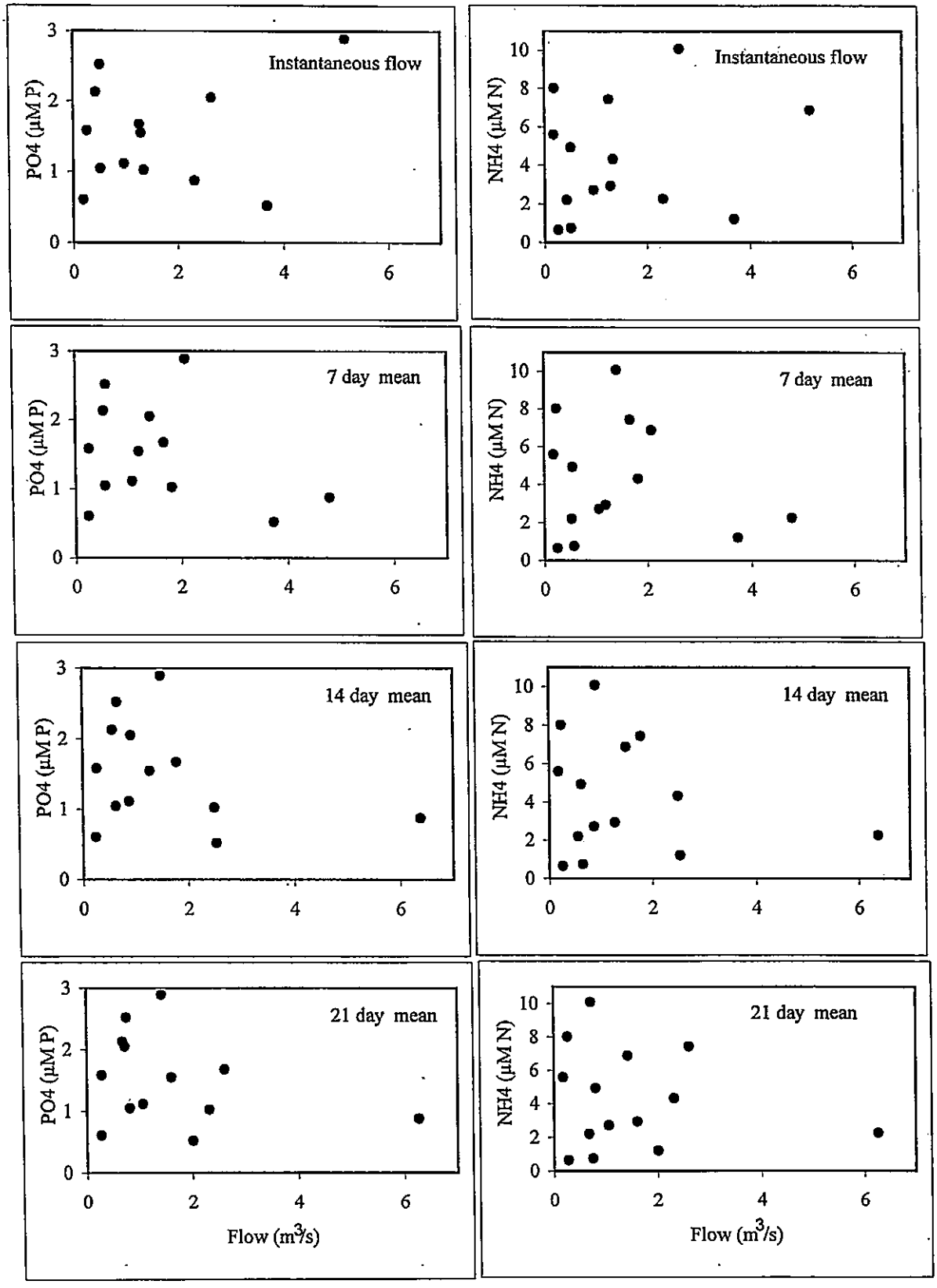


Figure 3.16 FRP and ammonium concentrations (µM) at the head of the Yealm Estuary (0 km) versus flow (instantaneous and 7, 14 and 21 day mean flow values before the sampling day).

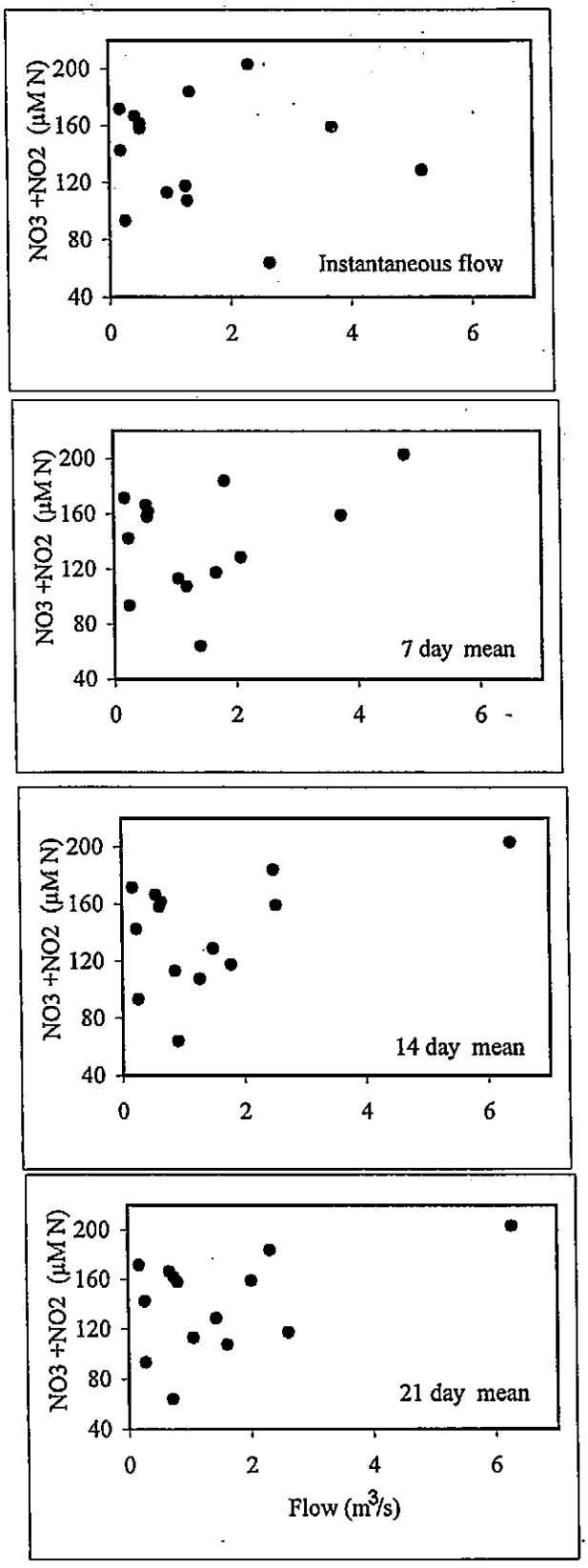


Figure 3.17 Nitrate concentrations (µM) at the head of the Yealm Estuary (0 km) versus flow (instantaneous and 7, 14 and 21 day mean flow values before the sampling day).

3.3 The Plym Estuary

3.3.1 The study area

3.3.1.1 Site description

The Plym Estuary is located on the eastern side of Plymouth Sound, on the south-west coast of England (Figure 3.1). Water samples were collected regularly (ca. every six weeks) at high tide along an axial transect, as indicated in Figure 3.18, during the period June 2002 – September 2003. The catchment area of the Plym is 79.2 km^2. The catchment is urbanised and industrialised by the city of Plymouth, and other land uses include livestock grazing, forestry (Howell 2003). The top of the catchment is characterised by moorland. The River Plym rises on west Dartmoor and flows southward for approximately 21.3 km before draining into Plymouth Sound. Here it joins the Tamar Estuary as it discharges into the English Channel (Environment Agency 1998). As the Plym runs southward from Dartmoor, the river progressively cuts into a landscape of peat and weathered granite. At Plympton Bridge (Marsh Mills), the Plym River is joined by the Tory Brook which carries sediments enriched with china clay waste from Lee Moor (Langston *et al.* 2003).

The Plym Estuary is subject to a number of anthropogenic influences, including (Langston *et al.* 2003): (1) abandoned metalliferous (tin) mining, (2) china clay abstraction, (3) commercial shipping, (4) effluent discharges from the Plympton and Plymstock sewage treatment works, (5) runoff from traffic routes and agricultural activities and (6) leachate discharges from the Chelson Meadow landfill site via two streams known as the North and South Leats. Chelson Meadow is the main landfill site for the city of Plymouth and approximately 765 tonnes of solid waste and 60 m^3 of liquid wastes are deposited daily at the site (Trier 2004). The lower part of the estuary, below the Laira Bridge, is of importance to commercial shipping recreation and fishing industries. Hence the channel is

surveyed and dredged every year if necessary, and rich mudflats are located above Laira Bridge (Hiscock and Moore 1986). The large input of china clay wastes has had a major silting impact in the upper Plym Estuary, where the extensive mudflats have high china clay content and the tidal zone is heavily banked with white clay (Langston *et al.* 2003). Thus the Plym Estuary is very much influenced by urban and industrial developments (Langston *et al.* 2003).

3.3.1.2 Hydrography

The Plym Estuary is a macrotidal system, with a tidal range of 4.6 m and tidal length of 6.9 km (Uncles *et al.* 2002). The water column is uniformly well mixed with respect to salinity. River flow ranges from a minimum of < 1 m^3s^{-1} to a maximum of 27 m^3s^{-1}, with annual mean flow of 2.25 m^3s^{-1}. The lowest flow rates usually occur during June, July and August when there is a reduced rainfall. The estimated mean residence time for fresh water in the estuary is calculated to be 4 days (Uncles *et al.* 2002). The Plym Estuary width above Laira Bridge is 500 m for most of its length (Environment Agency 1998).

3.3.1.3 Ecology

At the head of the estuary, there are dense populations of crustaceans and gastropod molluscs. Further seaward, there is a wide and shallow section, up estuary of the Laira Bridge, which is a haven for wading birds. The Chelson Meadow, the reclaimed land on the eastern side of the Plym Estuary, is drained by a series of ditches which harbour a characteristic brackish water fauna, grading inland into that of fresh water (Langston *et al.* 2003). Fish species found in the Plym include Atlantic salmon, brown trout, bullhead, eel, brook lamprey and three spined sticklebacks (Brooks 2003).

3.3.2 Master variables

A complete master variable and chemical data set is given in Appendix 3.4. Correlations (Pearson's product moment) between pairs of determinands were calculated in order to identify any statistically significant correlations (at the 99% confidence level), and thus aid interpretation of the data. The correlations were calculated for the whole data set (Table 3.3) and for individual seasons (Appendix 3.5). Because of the large number of data, only correlation coefficients (r^2) $\geq$ 0.7 were considered significant for inclusion in the discussion (Chatfield and Collins 1980). There were no significance differences in the overall correlation matrix between the whole data set and the individual seasons, hence the latter are given in Appendix 3.5.

Scatter plots of chemical concentrations versus salinity have been reported in order to be consistent with the treatment of the Yealm Estuary data. However, as the Plym Estuary is heavily subject to additional anthropogenic influences, concentration data have also been plotted against distance along the estuary, in order to aid the interpretation of observed constituent distributions.

Salinity ranged between 0.01 for river water and 34.8 for seaward end water samples. A strong positive correlation ($r^2 = 0.93$, n = 110, 99 % confidence) was observed between salinity and conductivity; the latter was measured in the field to indicate the approximate salinity of the collected samples. Water temperatures exhibited a strong seasonal pattern in the Plym Estuary, with highest temperatures recorded in the summers of 2002/2003, lowest in the winter 2003 and intermediate values during spring 2003 and autumn 2002 seasons. During the summers of 2002/2003 the water temperature was in the range 13.6 – 21.9 °C, while during winter 2003 the range was 4.8 – 9.8 °C. Within the Plym Estuary, pH ranged from a minimum of 6.7 at the freshwater end to a maximum of 8.4 at the sea water end.

Table 3.3 Pearson's product moment correlation matrix for the Plym Estuary samples. In the table, DON/TDN refers to the contribution of DON to the TDN pool.

		SALINITY	PH	PO4	NH4	NO3	TDN	DON	DON/TDN	DOC	CHLA	DOC : DON
SALINITY	Pearson Correlation	1	.492**	-.141	.320**	-.699**	-.674**	-.354**	.291**	-.347**	.078	.181
	Sig. (2-tailed)	.	.000	.142	.001	.000	.000	.000	.002	.000	.503	.058
	N	110	110	110	110	110	110	110	110	110	76	110
PH	Pearson Correlation	.492**	1	-.119	.072	-.375**	-.353**	-.165	.115	.069	-.121	.148
	Sig. (2-tailed)	.000	.	.217	.455	.000	.000	.086	.233	.474	.297	.123
	N	110	110	110	110	110	110	110	110	110	76	110
PO4	Pearson Correlation	-.141	-.119	1	.296**	.592**	.589**	.284**	-.212	.183	-.184	-.039
	Sig. (2-tailed)	.142	.217	.	.002	.000	.000	.003	.026	.056	.112	.685
	N	110	110	110	110	110	110	110	110	110	76	110
NH4	Pearson Correlation	.320**	.072	.296**	1	-.058	.132	.271**	.276**	-.180	-.106	-.318**
	Sig. (2-tailed)	.001	.455	.002	.	.550	.168	.004	.004	.060	.361	.001
	N	110	110	110	110	110	110	110	110	110	76	110
NO3	Pearson Correlation	-.699**	-.375**	.592**	-.058	1	.880**	.295**	-.498**	.310**	-.142	-.164
	Sig. (2-tailed)	.000	.000	.000	.550	.	.000	.002	.000	.001	.222	.086
	N	110	110	110	110	110	110	110	110	110	76	110
TDN	Pearson Correlation	-.674**	-.353**	.589**	.132	.880**	1	.711**	-.090	.405**	-.256	-.414**
	Sig. (2-tailed)	.000	.000	.000	.168	.000	.	.000	.348	.000	.026	.000
	N	110	110	110	110	110	110	110	110	110	76	110
DON	Pearson Correlation	-.354**	-.165	.284**	.271**	.295**	.711**	1	.555**	.381**	-.272	-.580**
	Sig. (2-tailed)	.000	.086	.003	.004	.002	.000	.	.000	.000	.017	.000
	N	110	110	110	110	110	110	110	110	110	76	110
DON/TDN	Pearson Correlation	.291**	.115	-.212	.276**	-.498**	-.090	.555**	1	.011	-.197	-.483**
	Sig. (2-tailed)	.002	.233	.026	.004	.000	.348	.000	.	.908	.088	.000
	N	110	110	110	110	110	110	110	110	110	76	110
DOC	Pearson Correlation	-.347**	.069	.183	-.180	.310**	.405**	.381**	.011	1	.314**	.170
	Sig. (2-tailed)	.000	.474	.056	.060	.001	.000	.000	.908	.	.006	.076
	N	110	110	110	110	110	110	110	110	110	76	110
CHLA	Pearson Correlation	.078	-.121	-.184	-.106	-.142	-.256	-.272	-.197	.314**	1	.655**
	Sig. (2-tailed)	.503	.297	.112	.361	.222	.026	.017	.088	.006	.	.000
	N	76	76	76	76	76	76	76	76	76	76	76
DOC : DON	Pearson Correlation	.181	.148	-.039	-.318**	-.164	-.414**	-.580**	-.483**	.170	.655**	1
	Sig. (2-tailed)	.058	.123	.685	.001	.086	.000	.000	.000	.076	.000	.
	N	110	110	110	110	110	110	110	110	110	76	110

**. Correlation is significant at the 0.01 level (2-tailed).

There was a significant positive weak correlation (correlation coefficient = 0.49, n = 110) between salinity and pH for the whole data set as pH of the saline samples is usually higher than of the freshwater samples (Table 3.3). Dissolved oxygen averaged 8.9 ± 1.4 mg L^{-1}, a range expected in this sort of temperate aquatic environment.

3.3.3 Dissolved inorganic nitrogen, filterable reactive phosphate and suspended chlorophyll a

Nitrate concentrations ranged from 4.4 to 171 μM N, with lower concentrations observed at the seaward end and highest concentrations observed in the upper estuary water samples (Figure 3.19 A). An exceptionally high value of 227 μM N was measured in a water sample collected from station 7 (Saltram House, 1.9 km down estuary of the weir) in September 2003 (Figure 3.19 B). The concentration of ammonium ranged from 0.20 to 9.7 μM N. Filterable reactive phosphate (FRP) concentrations were mainly in the range 0.11 – 8.1 μM P. Exceptionally high values of 10.8, 11.3, 17.7 and 20.2 μM P were measured in water samples collected near sewage treatment outfalls (Figure 3.20 A). Chlorophyll a concentrations ranged from 0.03 to 5.1 μg L^{-1} (Figure 3.21).

In the Plym Estuary, plots of nitrate and FRP concentrations against salinity showed a high degree of scatter, suggesting their behaviour was non-conservative (Figure 3.19 A and 3.20 A). These results suggested that external inputs and in situ biochemical activity were the dominant factors controlling nitrate and FRP distribution within this estuarine system. Nevertheless, a weak but significant negative correlation (correlation coefficient = 0.69, n = 110) was observed between nitrate and salinity for the whole data set, indicating that simple physical mixing accounted for 47.6 % of the nitrate distribution within the Plym Estuary. In June 2002 and August 2003 nitrate was markedly more conservative, although an input of nitrate from sewage effluent was evident at salinities of ca. 6 and 9 in June and

August, respectively (Figure 3.19 A). In contrast, no correlation was observed between salinity and FRP either for the whole data set or the individual seasons (Table 3.3 and Appendix 3.5). Enhanced levels of FRP were measured in water samples collected from stations near sewage treatment outfalls at Plympton and Plymstock, located at the upper and lower ends of the estuary, respectively (Figure 3.20 B). Overall there was a weak correlation between nitrate and FRP (correlation coefficient = 0.58, n = 110), except for summer 2002 and 2003 where the correlation coefficient was 0.73 and 0.90 (n = 22), respectively (Table 3.3 and Appendix 3.5). In summer 2002/2003, the distribution of nitrate and FRP appeared to be dominated by the STW inputs mentioned above. During other seasons, in contrast to FRP, the distribution of nitrate was not significantly influenced by sewage effluent and consequently their distributions were dissimilar. In situ chemical reactivity of FRP may have also contributed to this dissimilarity. Ammonium distributions showed non conservative behaviour. However, ammonium concentrations relatively increased with increasing salinity in autumn 2002 and winter 2003 with higher concentrations for lower estuarine samples, implying a major in situ and/or external sources as English Channel seawater is unlikely source for high ammonium concentrations (Figure 3.22) (Prahl and Coble 1994).

An examination of seasonal patterns in concentration revealed that lower nitrate concentrations were observed in spring 2003 and early autumn 2002, presumably as a result of biological uptake, while higher concentrations were observed during winter 2003. Higher nitrate concentrations were often observed within the mid-estuarine water samples (e.g. June 2002, July 2003, February 2003 and September 2003), presumably as a result of runoff from the Chelson Meadow landfill site and the surrounding agricultural land (Figure 3.19 B). An examination of seasonal patterns in FRP concentrations revealed that relatively high concentrations were observed within the mid-estuarine water samples collected during

June 2002, July 2002, February 2003, June 2003 and September 2003 (Figure 3.20 B). This mid-estuarine FRP peak appears to be a universal feature of estuarine systems (Eyre and Balls 1999) and may have been due to external anthropogenic sewage inputs (Eyre and Balls 1999), regeneration and/or to some extent phosphate release from suspended particles (Uncles *et al.* 1998b). The removal of this FRP peak in the high salinity region may have been due to biological removal of inorganic phosphate (Sanders *et al.* 1997a; Eyre and Balls 1999). A seasonal pattern was observed for chlorophyll a concentrations (Figure 3.21), with higher concentrations during summer 2002, and lower concentrations during the late autumn 2002 and winter 2003 periods. High chlorophyll a concentrations during summer were the result of increased summer solar radiation and increased photosynthesis (Uncles *et al.* 1998a). In temperate estuaries with sufficient light penetration, concentrations of suspended chlorophyll a are high in spring and summer due to high phytoplankton blooms (Jarvie *et al.* 2000). The lower chlorophyll a concentrations in autumn and winter may be attributed to low phytoplankton abundance due to a combination of grazing by zooplankton (Balbi 2000), lower light levels due to lower solar radiation and enhanced run off. Ammonium concentrations fluctuated and the seasonal pattern was not clear, indicating its reactivity as it is subject to generations and rapid uptake processes. During autumn 2002, winter 2003 and summer 2003, the ammonium distributions exhibited a spatial pattern characterised by high concentration within the mid-estuarine samples presumably due to benthic fluxes, biological ammonification and sewage inputs (Rocha 1998; Eyre and Balls 1999).

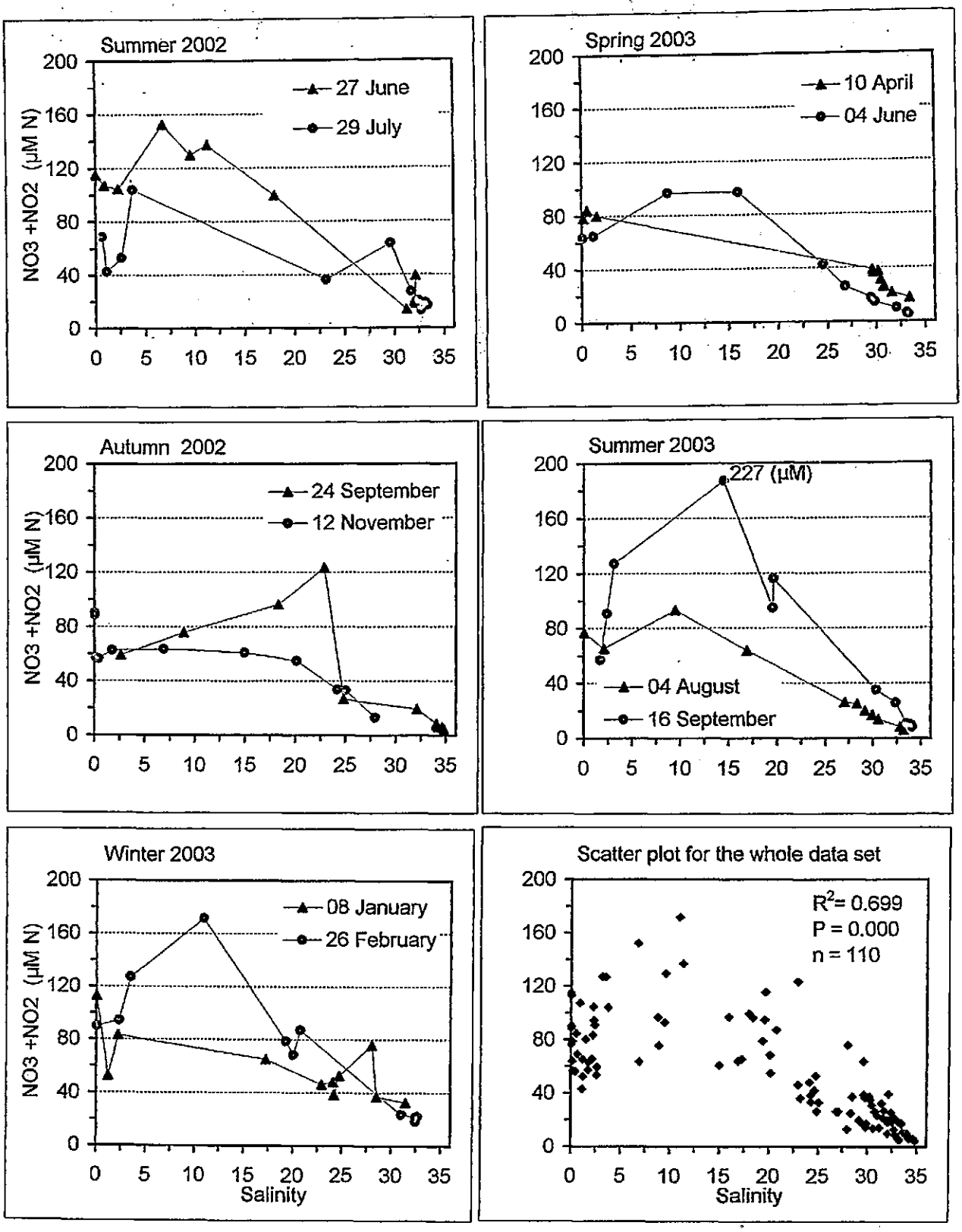


Figure 3.19 (A) Nitrate plus nitrite concentration (µM N) versus salinity in the Plym Estuary.

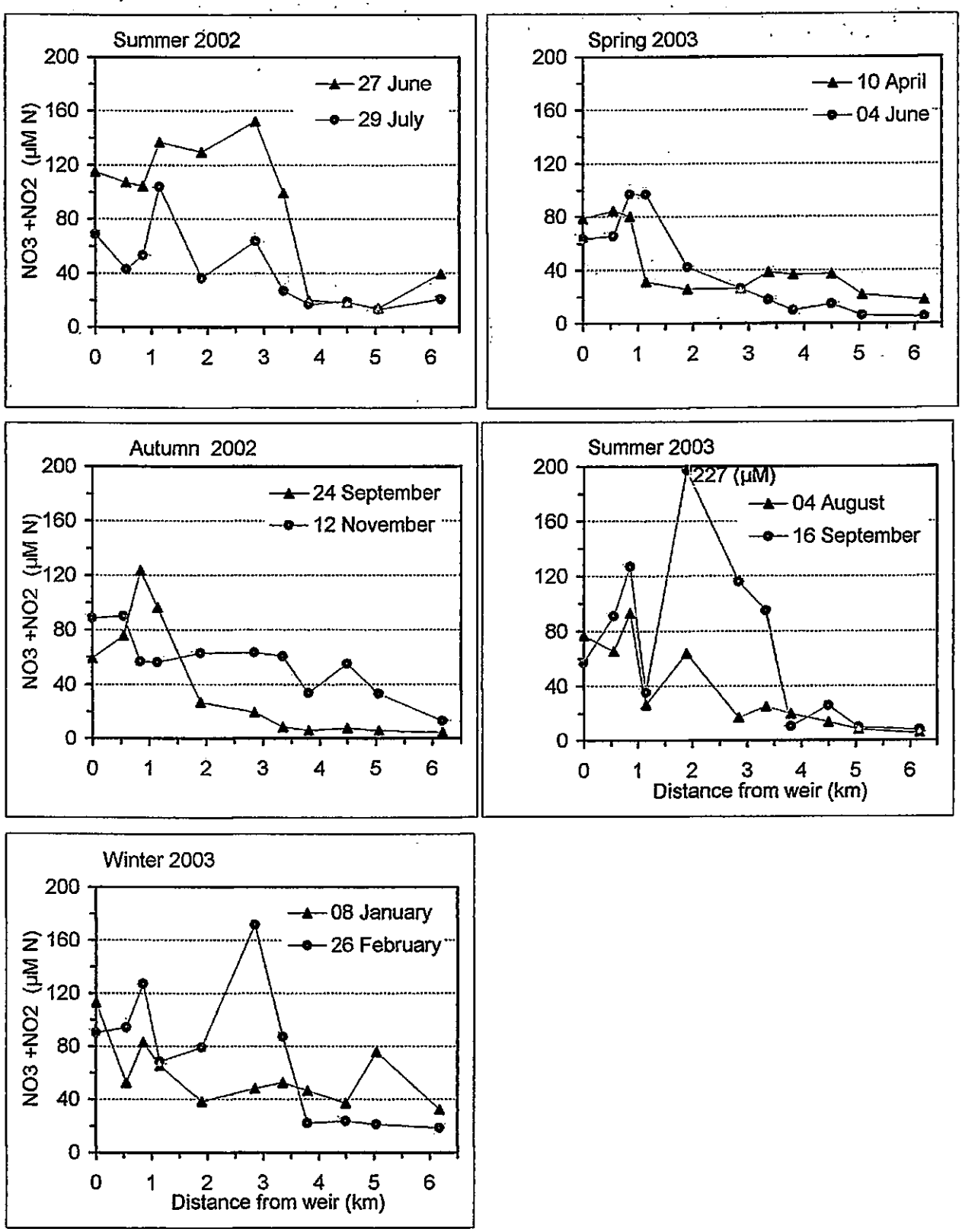


Figure 3.19 (B) Nitrate plus nitrite concentration (µM N) versus distance (km) from the weir in the Plym Estuary.

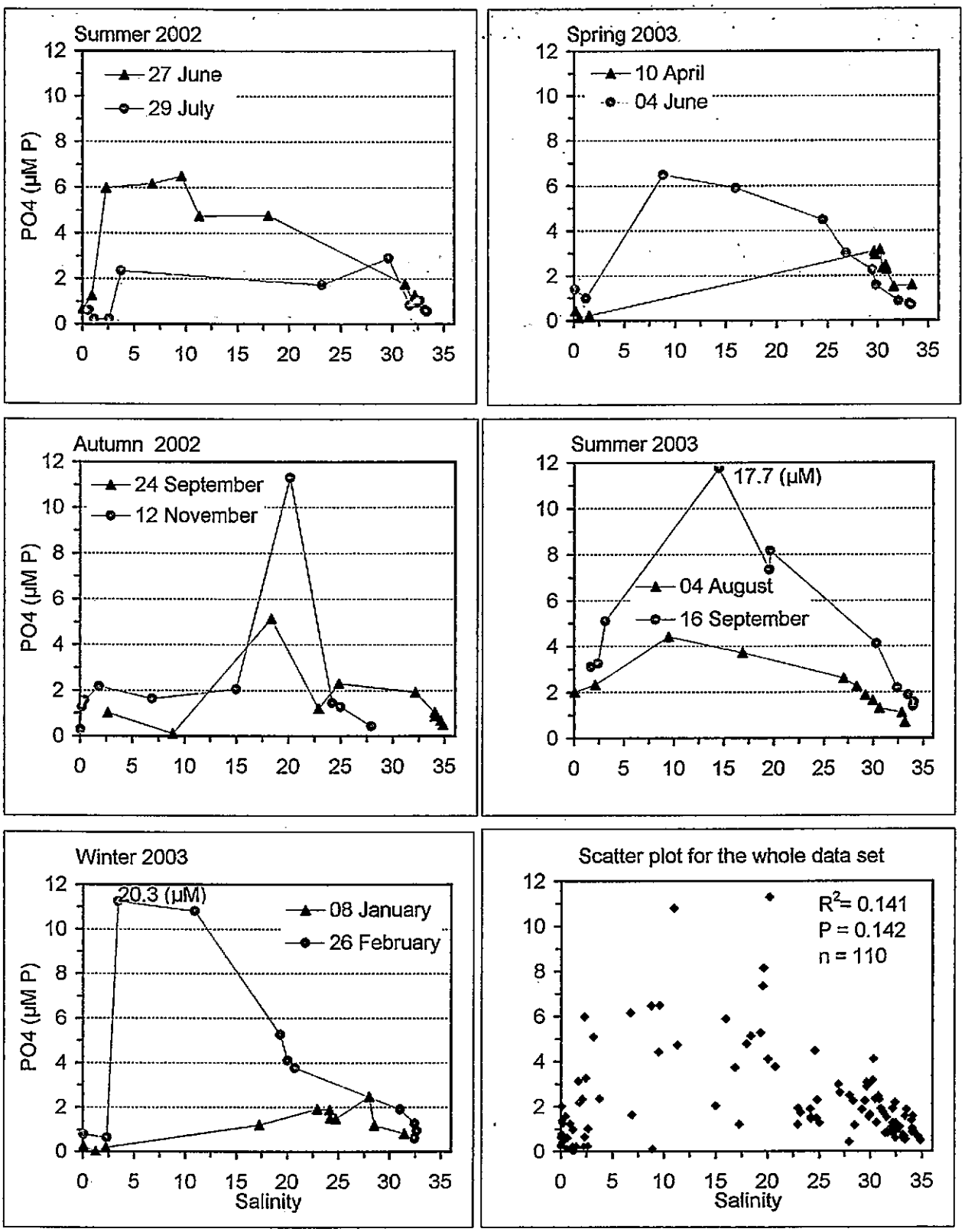


Figure 3.20 (A) Filterable reactive phosphate concentration (µM P) versus salinity in the Plym Estuary.

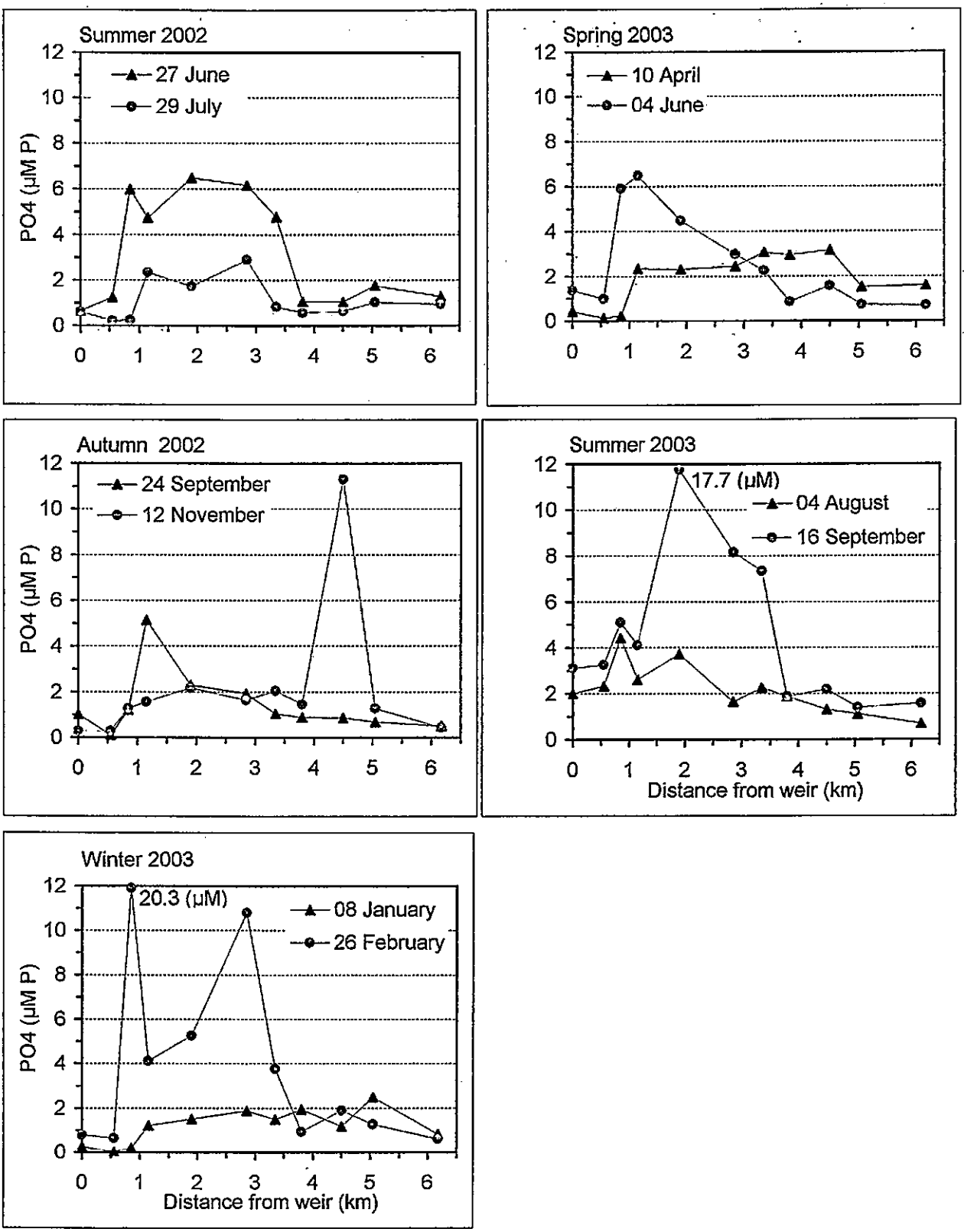
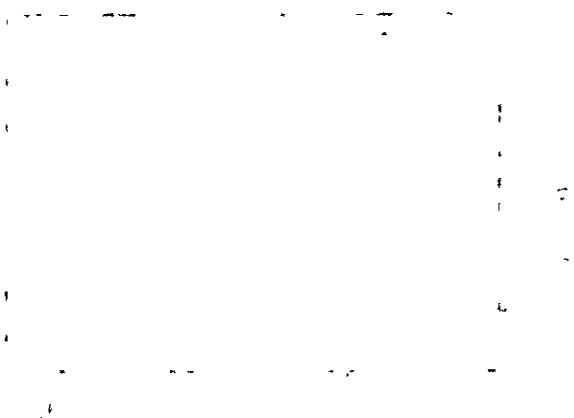
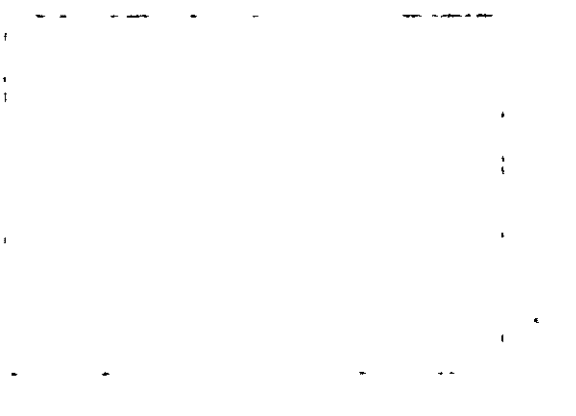


Figure 3.20 (B) Filterable reactive phosphate concentration (µM P) versus distance (km) from the weir in the Plym Estuary.





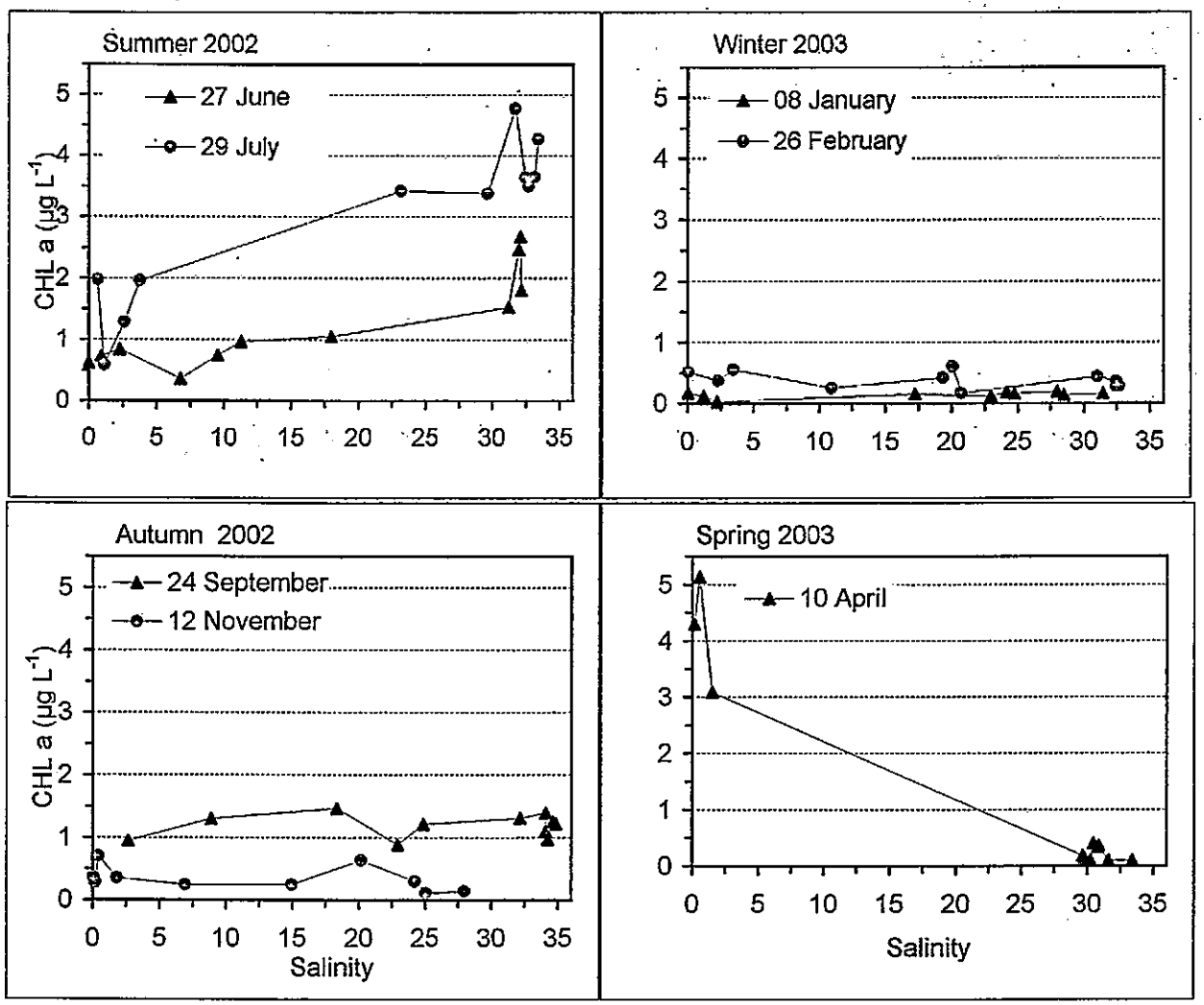


Figure 3.21 Chlorophyll a concentration (µg L^{-1}) versus salinity in the Plym Estuary.

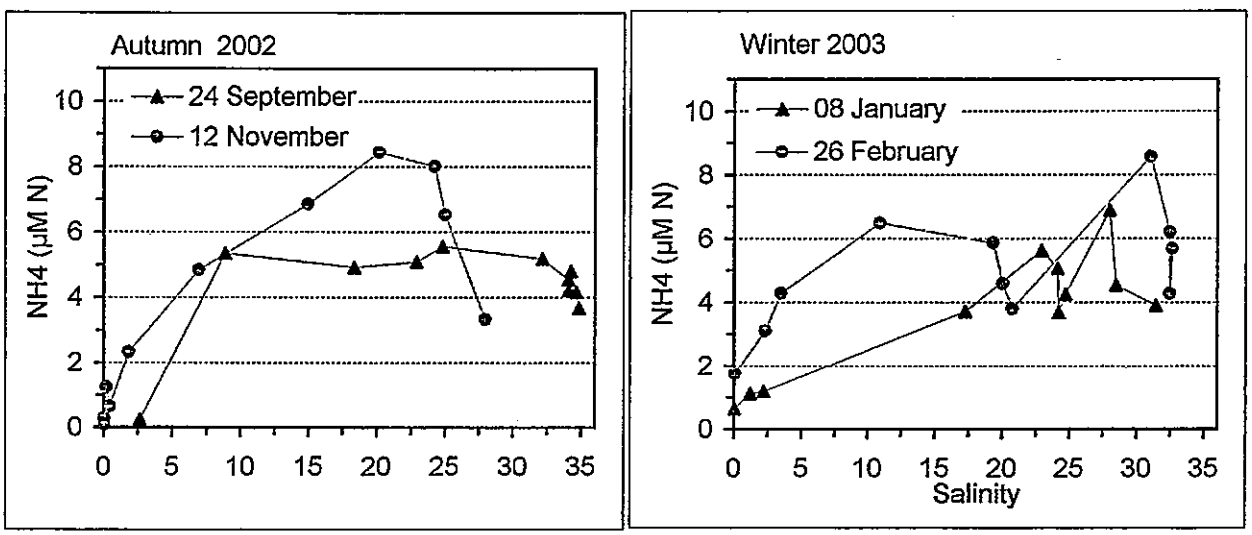


Figure 3.22 Ammonium concentration (µM N) versus salinity in the Plym Estuary for Autumn 2002 and Winter 2003.

3.3.4 Dissolved organic carbon

DOC concentrations in the Plym Estuary were mainly in the range 71 – 290 μM C, with lower concentrations observed at the seawater end and higher concentrations in the upper estuarine water samples (Figure 3.23 A). This range was similar to the range observed for the Yealm Estuary, and relatively lowers than ranges observed for other estuarine systems, as discussed in section 3.2.4.

In the Plym Estuary, DOC behaved relatively in a non conservative manner, as shown in Figure 3.23 A. No correlations were observed between DOC and salinity either for the whole data set or for the individual seasons (Table 3.3 and Appendix 3.5). These results suggest that the distribution of DOC within the Plym Estuary was not dominated by physical dilution with seawater, but instead indicated in situ reactivity and external sources. In contrast, the DOC appeared to be conservative only for the individual months of January 2003 and August 2003. At these surveys, DOC concentrations remained relatively uniform throughout the estuary. However, a linear distribution of DOC versus salinity does not necessarily imply conservative behaviour, since sources and sinks may balance each other simultaneously (Raymond and Bauer 2001; Abril *et al.* 2002). Even, when DOC appears to be conservative, studies on the isotopic composition of the DOC indicated the existence of a dynamic cycle of DOC input and removal (Peterson *et al.* 1994). Apparent non-conservative behaviour of DOC has been observed in other estuaries such as the Scheldt, Gironde, Sado and Ems estuaries, whilst conservative behaviour has been reported for the Severn, Humber, Thames, Elbe, Rhine, Dour and Loire estuaries as discussed in section 3.2.4. Non conservative distributions of DOC have been attributed to: (1) biological processing such as enhanced aquatic primary production e.g. York River Estuary and Chesapeake Bay, and high rates of DOC mineralization, (2) photochemical break down of DOC and (3) anthropogenic inputs of DOC e.g. the Scheldt Estuary (Prahl

and Coble 1994; Fisher *et al.* 1998; Raymond and Bauer 2001; Cauwet 2002; Abril *et al.* 2002). Furthermore, non-conservative behaviour of DOC is generally enhanced in stratified estuaries with long mixing times, vertical gradients of DOC concentrations, and the possibility of photochemical break down of surface DOC (Cauwet 2002).

There was a sharp removal of DOC in the low salinity region during the November 2002 survey campaign which may be related to a high microbial degradability of the catchment-derived organic matter after extensive rainfall (Wiegner and Seitzinger 2001; Abril *et al.* 2002). Elevated DOC concentrations were observed for samples collected near STW effluents outflows, indicating anthropogenic inputs of DOC. For example, relatively high DOC concentrations were observed at station 9 located close to the Plympton STW (0.85 km down-estuary of the weir) during September 2002 and February 2003 (Figure 3.23 B). In addition, high DOC concentrations were observed at station 3 located close to Plymstock STW (4.5 km down-estuary of the weir) during November 2002, February 2003, August 2003 and September 2003 (Figure 3.23 B). At other times (June 2002, July 2003 and September 2003) higher DOC concentrations were observed in mid-estuarine water samples, perhaps reflecting external inputs from the Chelson Meadow landfill site and agricultural runoff. These observations strongly suggested that DOC distributions in the Plym Estuary were markedly influenced by external anthropogenic inputs.

An examination of the seasonal variations in DOC concentrations in the Plym Estuary showed that DOC concentrations were generally higher during summer 2002 and autumn 2002, and lower during winter 2003 and spring 2003 (Figure 3.23 A). During November 2002, concentrations of DOC were higher than those during September 2002, which were probably due to the extensive rainfall that occurred in the week prior to sampling in November 2002, with consequent enhanced DOC in the surface run off, as discussed in

section 3.3.8. DOC produced during the spring and early summer by phytoplankton blooms may accumulate in the estuary, to give higher DOC concentrations during the summer and early autumn (Volk *et al.* 1997; Myklestad 2000). Seasonal accumulation of phytoplankton, that result in DOC accumulation, have been reported in other estuaries (Mantoura and Woodward 1983; Fisher *et al.* 1998). The occurrences of phytoplankton blooms within the Plym Estuary was indicated by the high concentrations of chlorophyll a observed in summer 2002 (Figure 3.21); indeed, the Plym water was observed to be clearly green during this time. A weak but significant positive correlation (correlation coefficient = 0.314, n = 76) was observed between DOC and chlorophyll a for the whole data set, indicating that phytoplankton exudates contribute to the DOC pool as discussed in section 1.3.1.1.1 (Table 3.3).

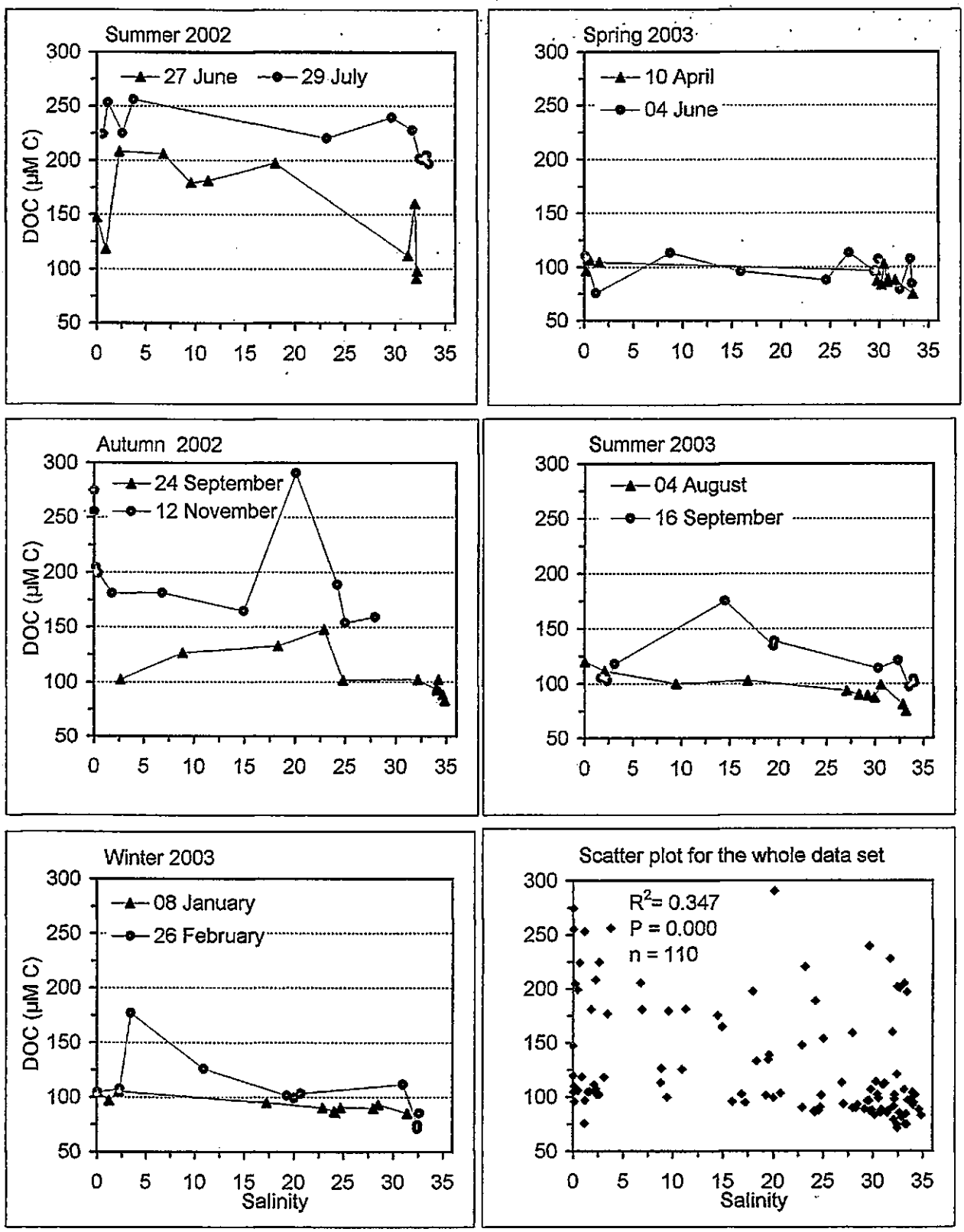


Figure 3.23 (A) Dissolved organic carbon concentration (µM C) versus salinity in the Plym Estuary.

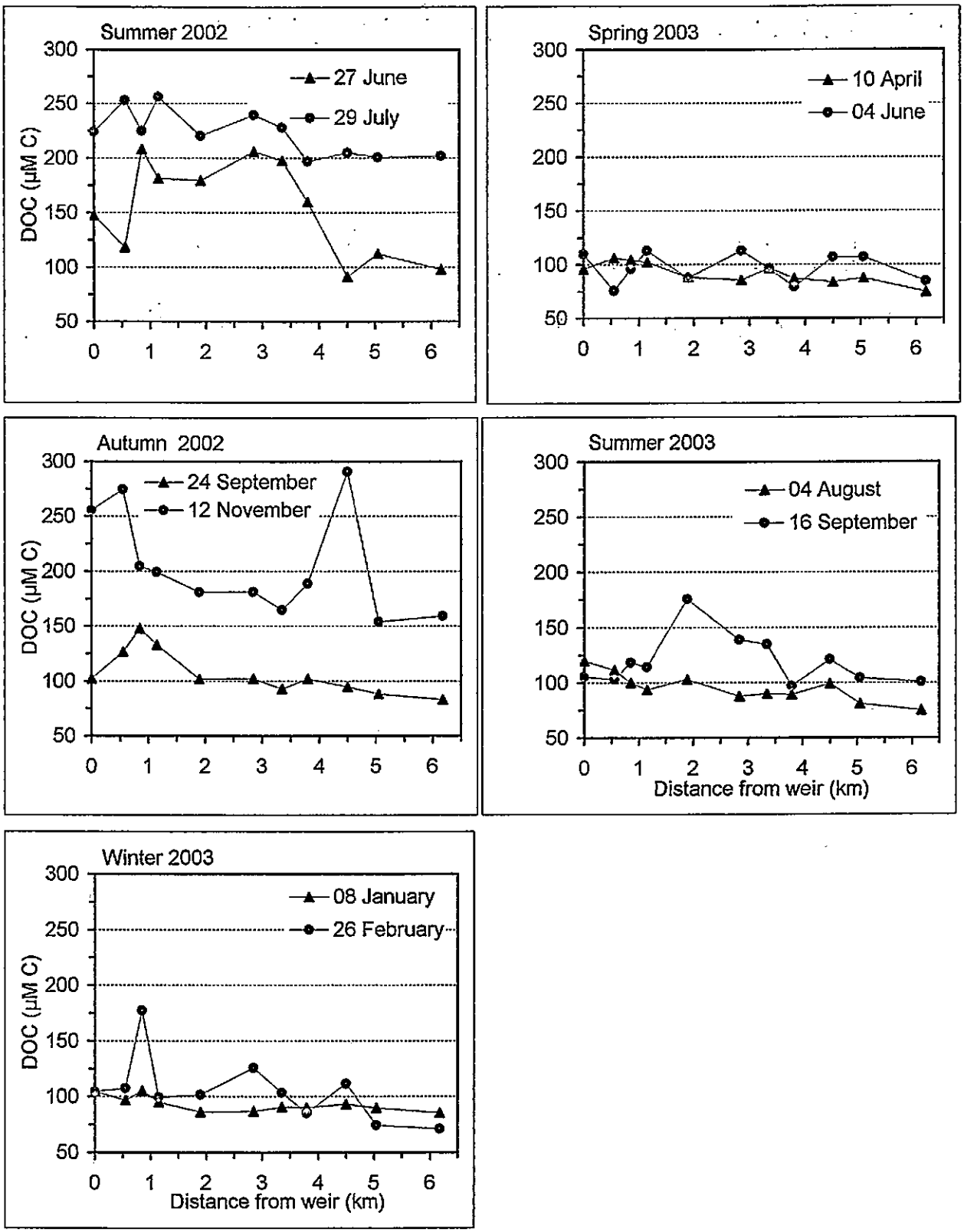
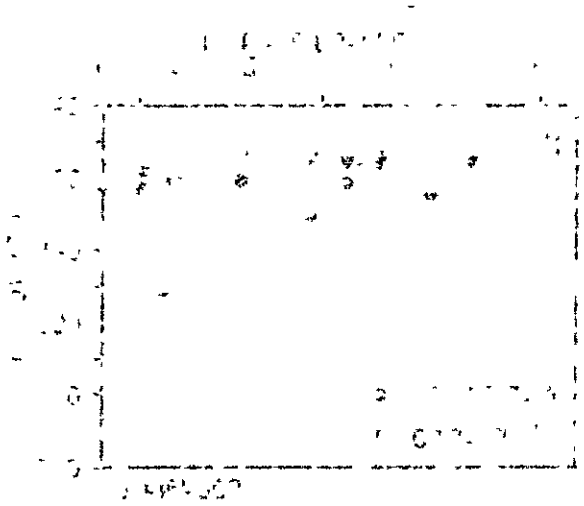
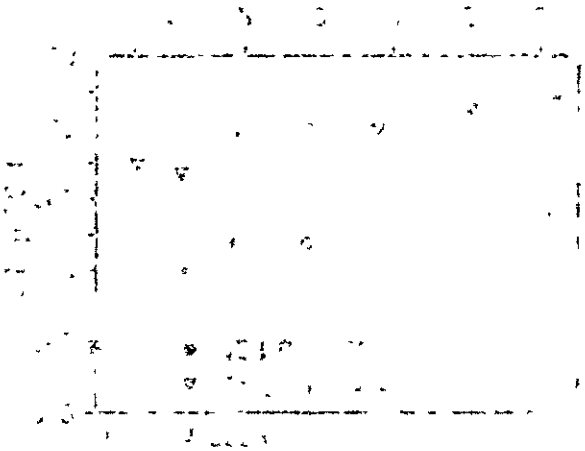
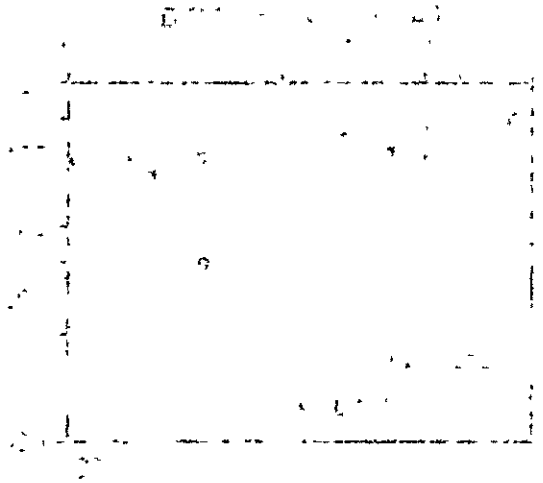
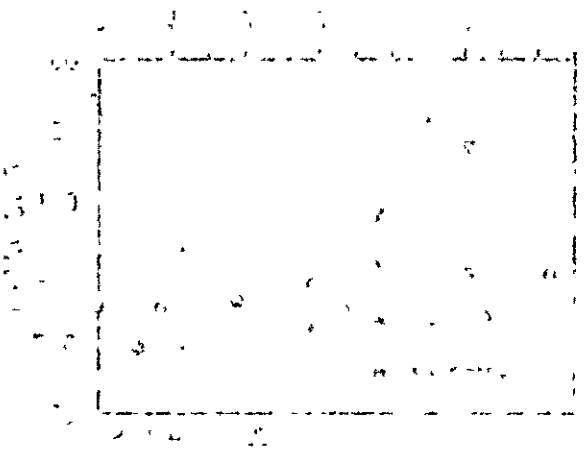


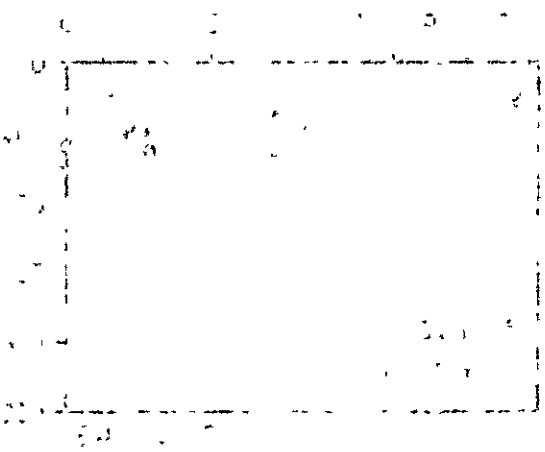
Figure 3.23 (B) Dissolved organic carbon concentration (µM C) versus distance (km) from the weir in the Plym Estuary.











3.3.5 Dissolved organic nitrogen

Concentrations of DON in the Plym Estuary ranged from 4 μM N at the seawater end to 94 μM N at the freshwater end. Exceptionally high concentrations of 110 and 177 μM N were measured in water samples collected at stations close to STW, in September 2003 and November 2002, respectively. The maximum values for DON reside at the upper end of the range reported globally for estuaries (22.5 ± 17.3 μM N) and rivers (34.7 ± 20.7 μM N) (Bronk 2002). Within the Plym Estuary, the enhanced DON values may be the result of sewage discharges, run off from the Chelson Meadow landfill site and other anthropogenic activities within the Plym catchment. A scatter plot of DON concentrations against salinity revealed a high degree of scatter around the theoretical dilution line (correlation coefficient = 0.35, n = 110; Table 3.3 and Figure 3.24 A), indicating that DON was generally non-conservative in this estuary, and that external anthropogenic sources, in situ biological activity and benthic inputs markedly influenced the distributions of DON.

Related high concentrations of DON and ammonium coincident with low chlorophyll a concentrations were observed at station 10 (0.55 km down-estuary of the weir) during July 2002 (Figure 3.24 B). This station was close to the discharge of the Plympton STW, which may be the source of the DON and ammonium. This may also have been due to the release of DON and ammonium from re-suspended sediments, the water column was exceptionally turbid at this time, as observed visually. Where DON and ammonium may be diagenetically released to the water from benthic sediments during re-suspension (Rocha 1998; Burdige 2002; Berman and Bronk 2003). High DON was also observed at station 3 (4.5 km down estuary of the weir) during February 2003, perhaps due to inputs from sewage effluent or from resuspension of bed sediments, as the sample was also high in ammonium. Elevated DON concentrations were observed during November 2002 in the upper and mid-estuarine water samples, perhaps implying anthropogenic inputs of DON

from either the Chelson Meadow landfill leachate, sewage discharges or both (Figure 3.24 B). Anthropogenic nitrogen sources to industrialised catchments have been reported to be more important than natural sources by a factor of 2 – 4 for the Thames and Rhine estuaries (Meybeck 1993). A high DON concentration that was coupled with a low nitrate concentration was observed at station 10 (0.55 km down estuary of the weir) during July 2002 and January 2003 sampling campaigns (Figure 3.24 B).

In the high salinity region of the Plym Estuary during the surveys of June 2002 and September 2002, there appeared to be removal of DON, which was sometimes coupled with increased concentrations of nitrate (Figure 3.19 A and 3.24 A). This pattern is consistent with bacterial mineralization of DON to ammonium and then nitrate. Heterotrophic bacteria are responsible for the transformation of organic matter into inorganic nutrients (Stepanauskas *et al.* 1999). A more detailed discussion of the bacterial mineralization of DON in the Plym Estuary is provided in section 3.4.

An examination of the seasonal variations in DON concentrations revealed that lower DON concentrations were observed in spring 2003 and higher DON concentrations were observed in summer 2003 and autumn 2002, presumably due to in situ recycling of DOM (Treguer and Queguiner 1989) (Figure 3.24 A). Lower DON concentrations observed in spring 2003 may have been due to phytoplankton uptake during early spring bloom as discussed in section 1.3.2.2 (Bronk 2002). Chlorophyll a showed a weak significant negative correlation with DON, but not with nitrate or ammonium, implying, to some extent that DON was relatively the major source of nitrogen for phytoplankton growth (Table 3.3). On the other hand, the high DON concentrations observed in summer 2003 may have been due to release of DON from phytoplankton (Butler *et al.* 1979; Chapman *et al.* 2001; Bronk 2002). Similar trends have been reported in the English Channel, the

Chesapeake Bay and the Yealm Estuary, as discussed in section 3.2.5. A significant fraction (up to 75 %) of DIN consumed by phytoplankton is released as DON into the surrounding water and then recycled by the heterotrophic and autotrophic microbial communities (Collos 1992; Mortazavi *et al.* 2001). Thus, food-web processes, particularly related to phytoplankton growth were important factors contributing to the increased DON concentrations (Mortazavi *et al.* 2001). The inverse relation between DON and nitrate concentrations reported for the English Channel (Butler *et al.* 1979) and Chesapeake Bay (Bronk 2002) implied that phytoplankton DIN uptake was followed by DON release. DON originated from phytoplankton can be released to the surrounding water by excretion, cell death and virus-induced lysis, or sloppy feeding, as discussed in section 1.3.1.1.

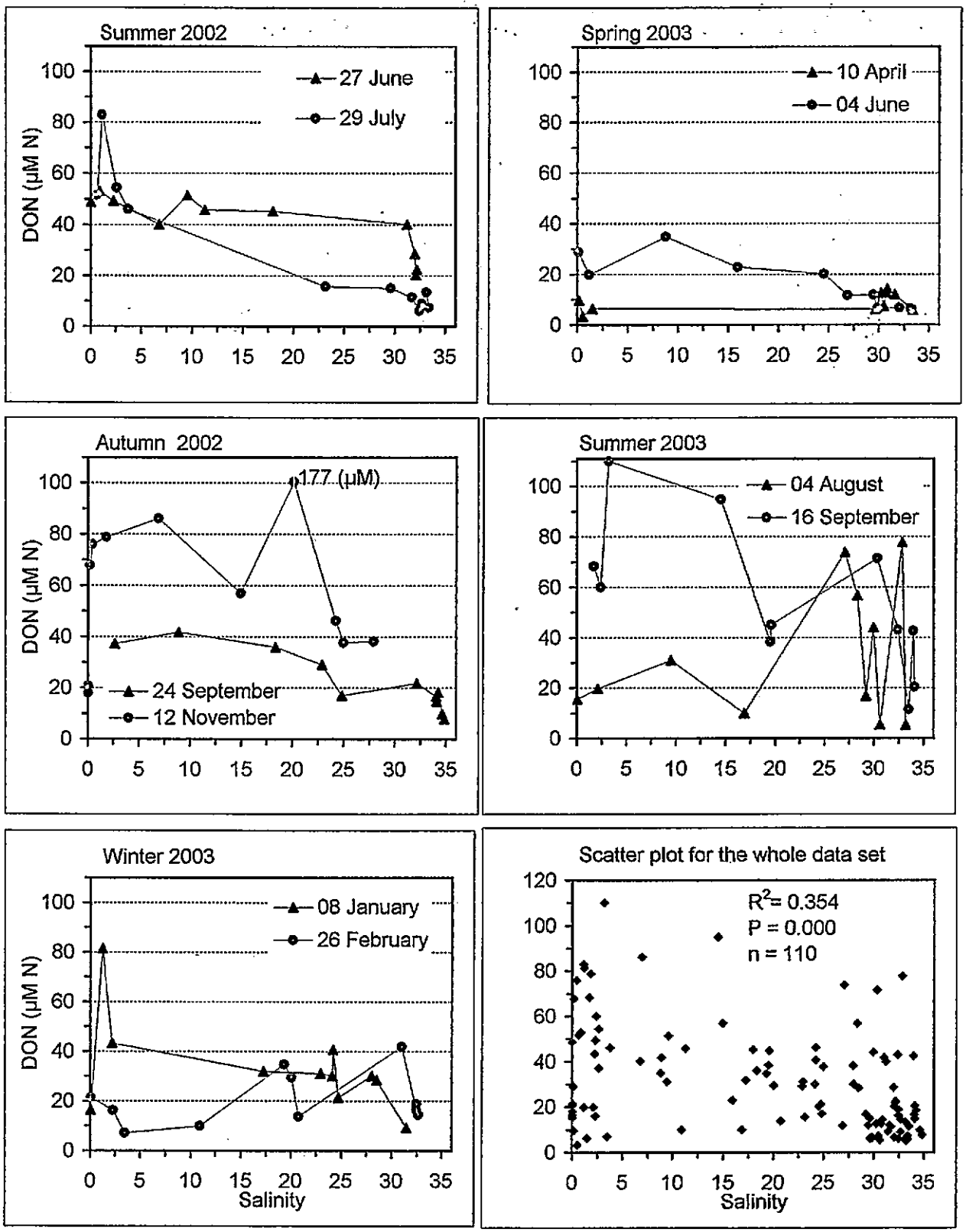


Figure 3.24 (A) Dissolved organic nitrogen concentration (µM N) versus salinity in the Plym Estuary.

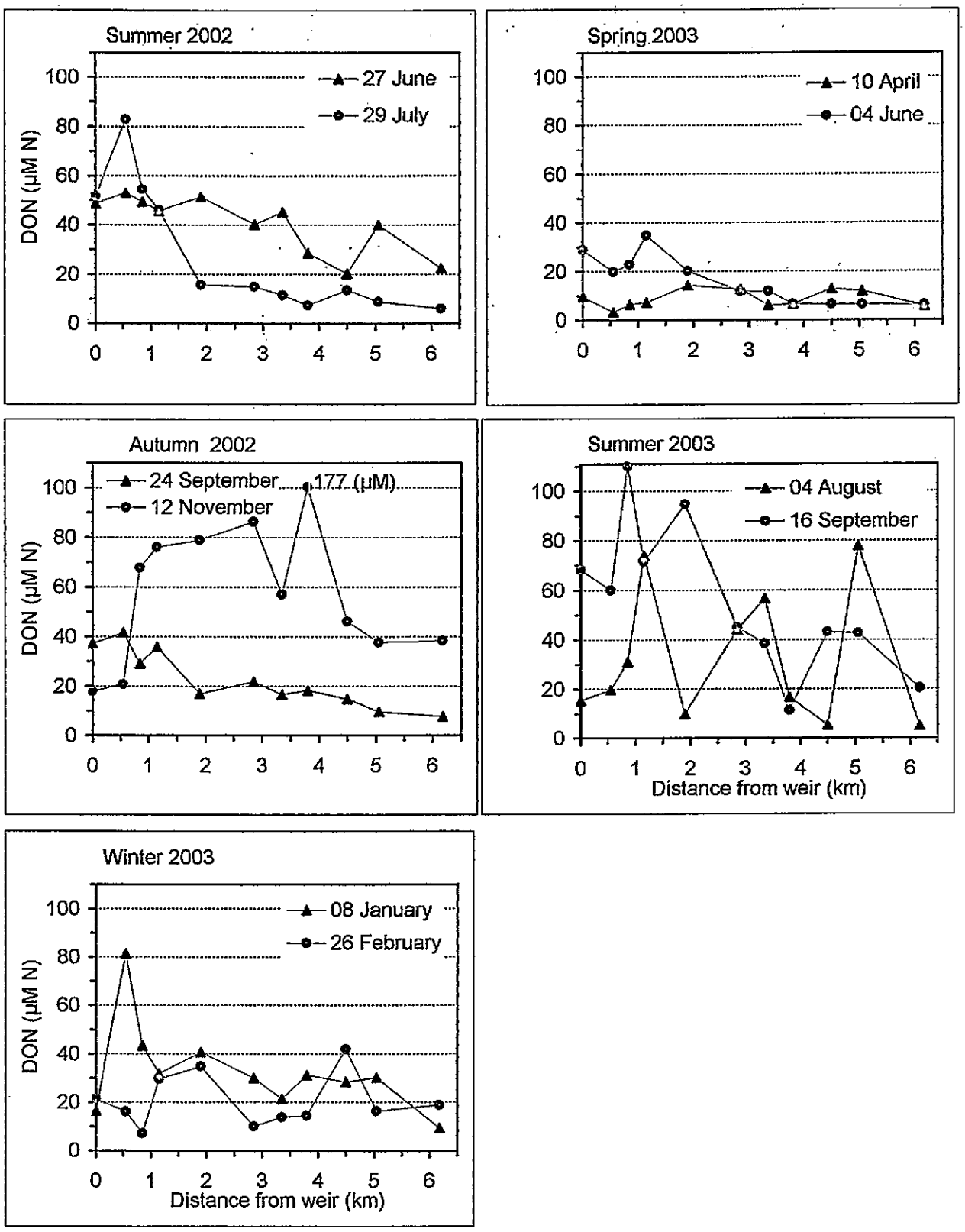
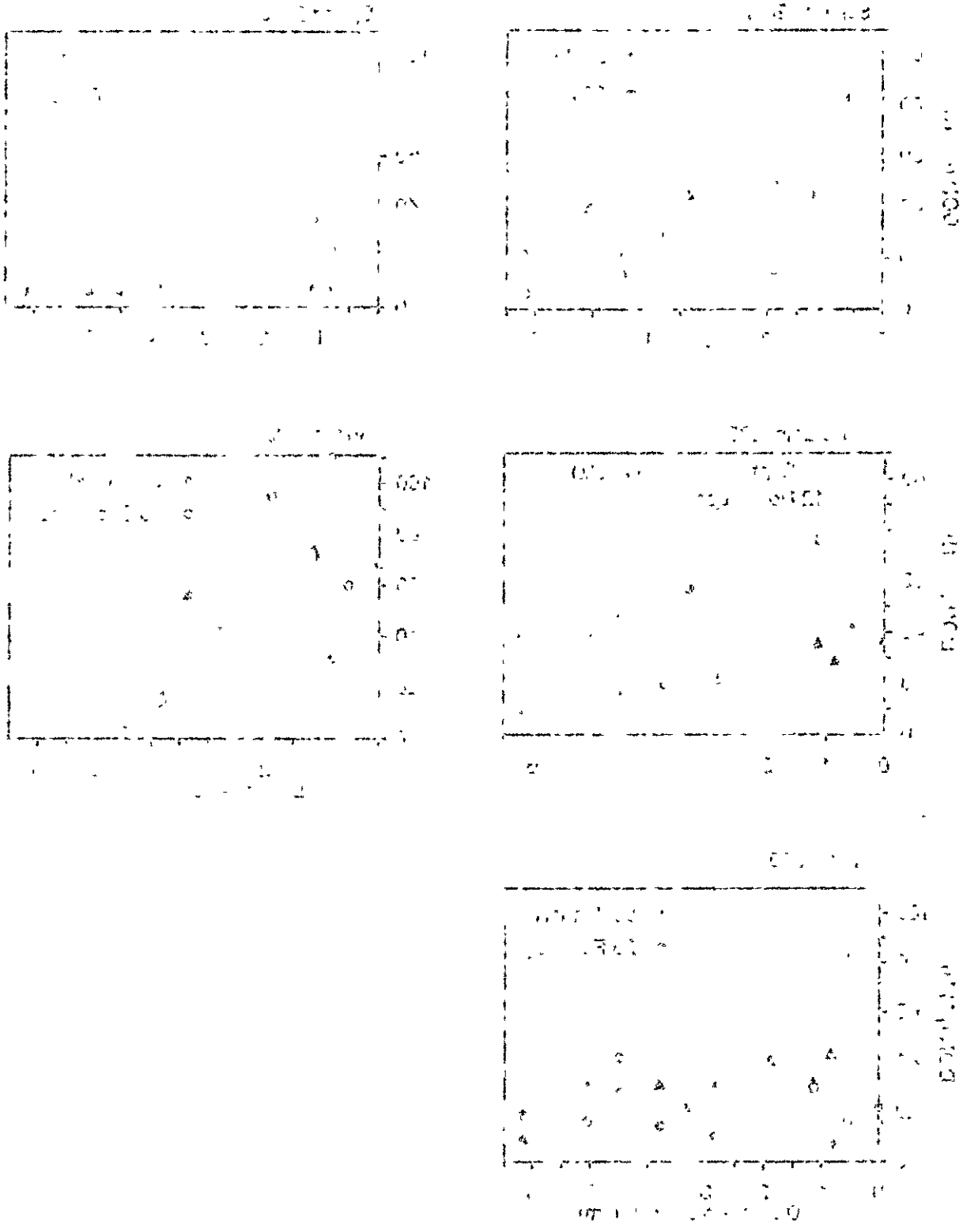


Figure 3.24 (B) Dissolved organic nitrogen concentration (µM N) versus distance (km) from the weir in the Plym Estuary.



3.3.6 Contribution of dissolved organic nitrogen to total dissolved nitrogen pool

The amount of DON as a percentage of TDN in the Plym Estuary varied between 3.5 and 84 %. Generally, higher values were observed during summer 2002/2003 and autumn 2002, and low values in winter 2003 and spring 2003 seasons (Figure 3.25). During the high flow period of November 2002, the contribution of DON to the TDN pool was < 20% in the river water dominated samples, whereas it was in the range of 45 – 74 % in the estuarine water samples. Hence, nitrate was the dominant species of TDN in the river whereas DON was the dominant species in the estuarine samples. Further discussion of the influences of river flows on DON and DIN concentrations is to be found in section 3.3.8.

During summer, the high contribution of DON to the TDN pool was probably due to in situ biological regeneration, as noted earlier (Treguer and Queguiner 1989). Phytoplankton uptake of DIN gives rise to the generation of DON which may be released from cells either by passive exudation or cell breakage during grazing and hence contribute to the higher DON/TDN percentage. It has been reported that, in environments ranging from relatively eutrophic estuaries to the open ocean, 32 ± 17 % of biologically utilised DIN was subsequently released as DON (Bronk 2002). Also, the primary fate of nitrogen uptake in Monterey Bay, California was DON in September (Bronk and Ward 1999). The significant contribution of DON to the TDN pool indicates the importance of including the monitoring of DON concentrations in water quality and eutrophication studies (Chapman *et al.* 2001).

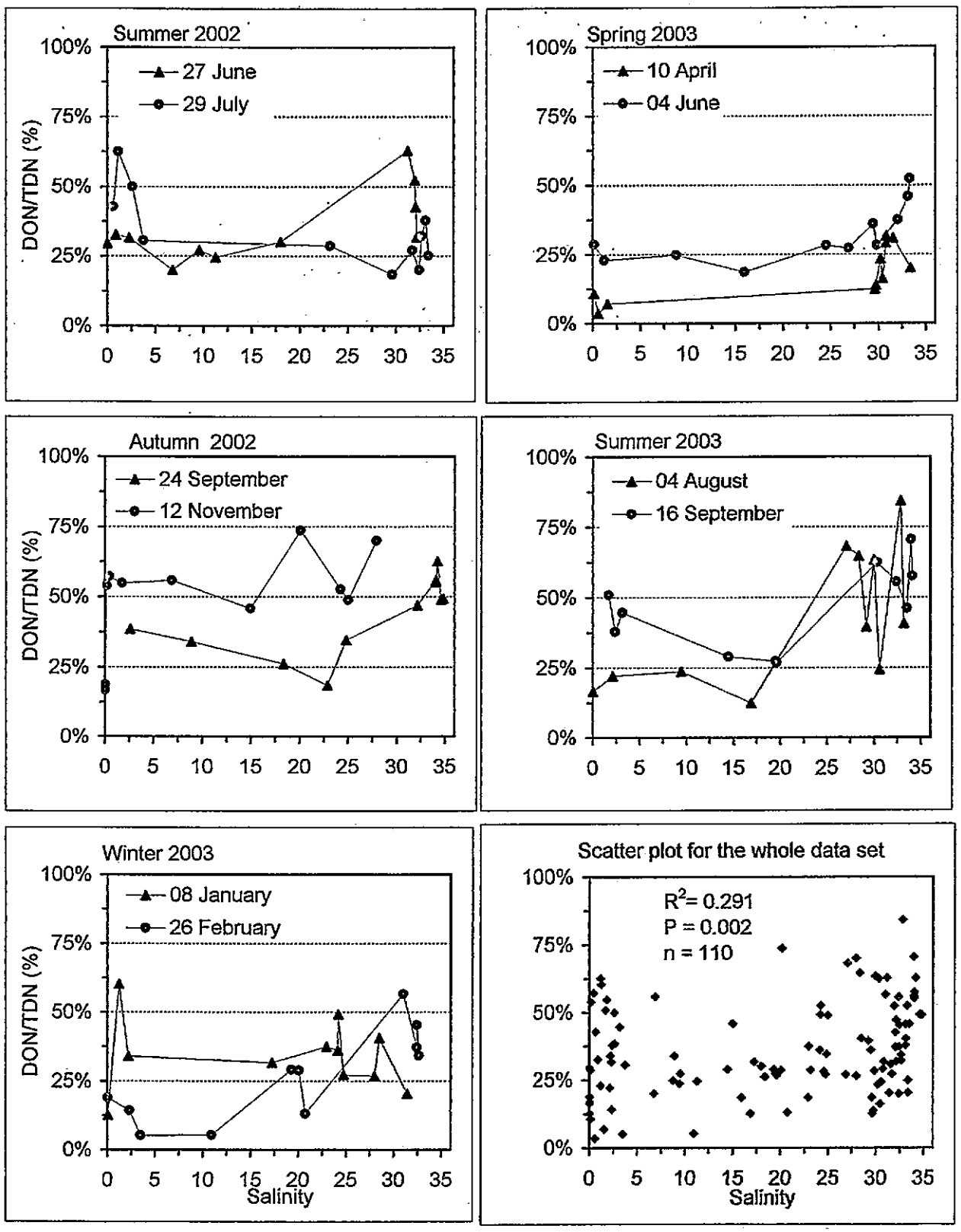
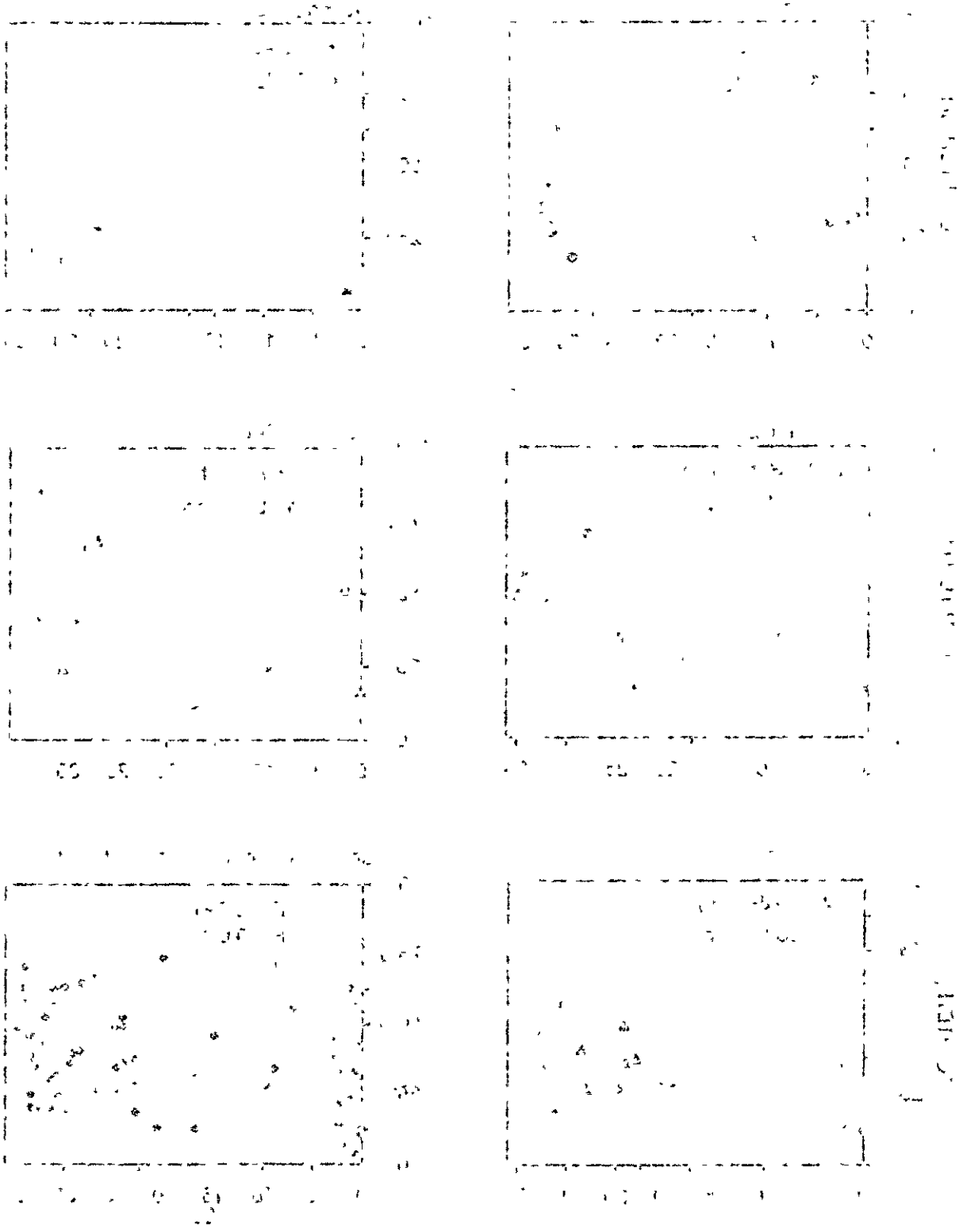


Figure 3.25 The percentage of dissolved organic nitrogen to total dissolved nitrogen versus salinity in the Plym Estuary.



3.3.7 The C:N ratio of the DOM pool (DOC:DON)

The DOC:DON molar ratios ranged between 1 and 33.6, with a mean of 7:2 ± 6.5 (± 1SD). This mean is lower than values reported for other aquatic environments. A ratio of ~ 14 in the surface ocean water to a mean ratio of 26 in riverine systems has been reported by Bronk (2002). The low DOC:DON ratio in the Plym Estuary is most likely the result of the high anthropogenic nitrogen inputs into this system. The generally lower C:N ratios of DOM in the Plym relative to the Yealm reflect the numerous point sources of organic nitrogen. A similar pattern has been reported in the Delaware Estuary relative to the Hudson Estuary (Seitzinger and Sanders 1997).

As shown in Figure 3.26, the DOC:DON ratios were generally higher during July 2002 and spring 2003 and lower during autumn 2002, winter 2003 and September 2003. The low DOC: DON ratios observed during the late summer were, perhaps, due to active release of DON from phytoplankton, as discussed earlier. High ratios of the DOC: DON during July 2002 and spring 2003 may have been due to an enhanced phytoplankton growth as indicated by high chlorophyll a concentrations (Figure 3.21 and 3.26). Phytoplankton growth was, presumably, the main source of C-enriched DOM (carbohydrate), and hence contributing to the accumulation of DOC (Borsheim *et al.* 1999; Myklestad 2000; Cauwet *et al.* 2002; Gobler and Sanudo-Wilhelmy 2003). As shown in Appendix 3.5, a strong negative correlation was observed between DON and DOC: DON for summer 2002/2003 and spring 2003 and strong positive correlation was observed between DOC and DOC: DON only for winter 2003. This indicated that in winter 2003 dissolved organic matter was relatively rich in carbon, while in spring and summer it was relatively rich in nitrogen.

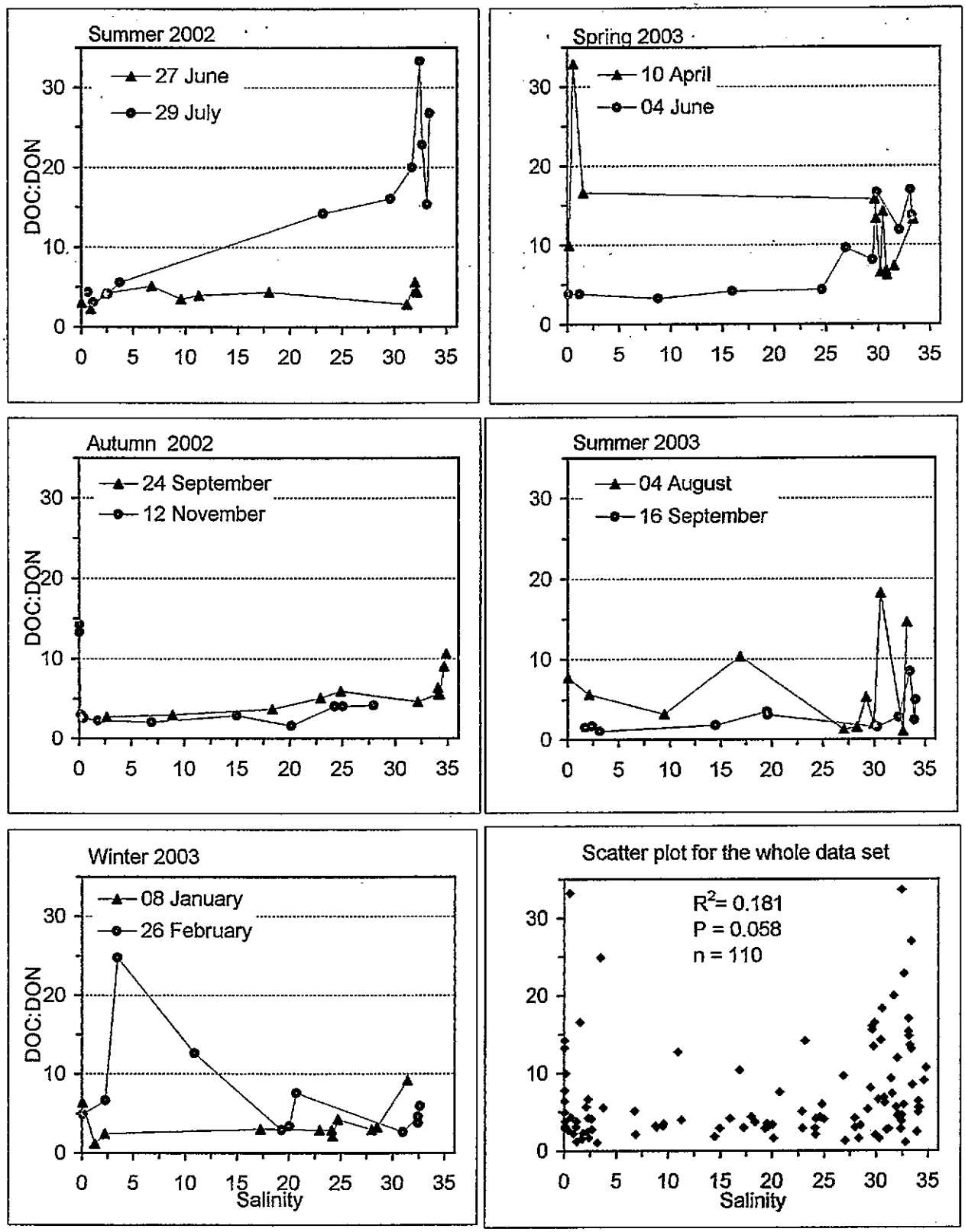


Figure 3.26 Dissolved organic carbon to dissolved organic nitrogen ratio versus salinity in the Plym Estuary.

3.3.8 Relationships between chemical determinands and flow

An examination of the relationships between concentrations of chemical determinands and river flow can provide valuable information on the origin and nature of these determinands. Many dissolved chemical constituents such as DOC, DON, nitrate and FRP vary over time in response to changing flow conditions. Flow data for the River Plym during the study period, close to the tidal limit, are provided in Appendix 3.6. Fresh water inflow from the River Plym exhibited a marked seasonal signal, with flows < 1 $m^3 s^{-1}$ during mid July 2002 – October 2002 and higher flows during winter 2003. Annual flux of DOC was 111 x 10^6 g C Yr^{-1}, compared to an average of 56 x 10^6 g C Yr^{-1} in the River Yealm. Also, the annual flux of DON was 29.8 x 10^6 g N Yr^{-1} compared to 76.6 x 10^6 g C Yr^{-1} of nitrate.

Concentrations of DOC at the head of the Plym Estuary generally increased with increasing flow, indicating diffuse sources of DOC from the catchment surfaces (Figure 3.27). DOC concentrations increased under high flows probably as result of drainage from organic rich soil. Similarly, the highest concentrations of DOC in the rivers Ouse, Tweed, Derwent and Wear occurred under storm flow conditions as discussed in section 3.2.8 (Jarvie *et al.* 2000; Neal *et al.* 2000a). Also, DOC concentrations in the Paine Run Stream, Virginia and the White River inn Muncie, Indian were consistently low during base flow, but during storms mean concentrations were approximately 2 and 3.5 fold over base flow DOC concentrations, respectively (Buffam *et al.* 2001; Volk *et al.* 2002). The highest DOC concentration in the Plym River was observed during the November 2002 sampling campaign when flow rate was highest, as shown in Figure 3.28. This might have been due to the flushing of DOC from diffuse sources of organic matter in the agricultural catchment, supporting the above finding.

In contrast, concentrations of DON and FRP in the River Plym decreased with increasing flows presumably in response to dilution of point sources by rainfall (Figure 3.27 and 3.29). This suggested that in the River Plym, DON and FRP were derived predominantly from anthropogenic point sources including sewage effluents. The trend of diluting FRP concentrations with flow was consistent with the relationship between FRP concentrations and flow reported for the Mersey and Trent rivers (Head 1985; Jarvie *et al.* 2000).

For nitrate, concentrations increased with flow, indicating diffuse sources (run off) from the agriculturally impacted soils of Dartmoor and the lower catchment (Figure 3.29). This behaviour was expected for nitrate due to fertiliser application. As in Figure 3.28, higher concentrations of nitrate in the River Plym occurred during winter 2003 and lower concentrations occurred during spring 2003 and summer 2002/2003. The trend of increasing nitrate concentrations with flow was in close agreement with the relationship between nitrate and flow reported for the Great Ouse, Mersey and Wear rivers and Pain Run Stream (Head 1985; Neal *et al.* 2000a; Neal *et al.* 2000b; Buffam *et al.* 2001). In contrast, for the River Trent, the base flow concentration of nitrate was higher than for storm flow conditions, indicating that point sources dominated nitrate concentrations in this system (Jarvie *et al.* 2000). However, there was no clear relationship between ammonium concentrations and flow for the Plym (Figure 3.30). This mixed response reflects inputs derived both from anthropogenic and natural sources, a mixture of point and diffuse sources.

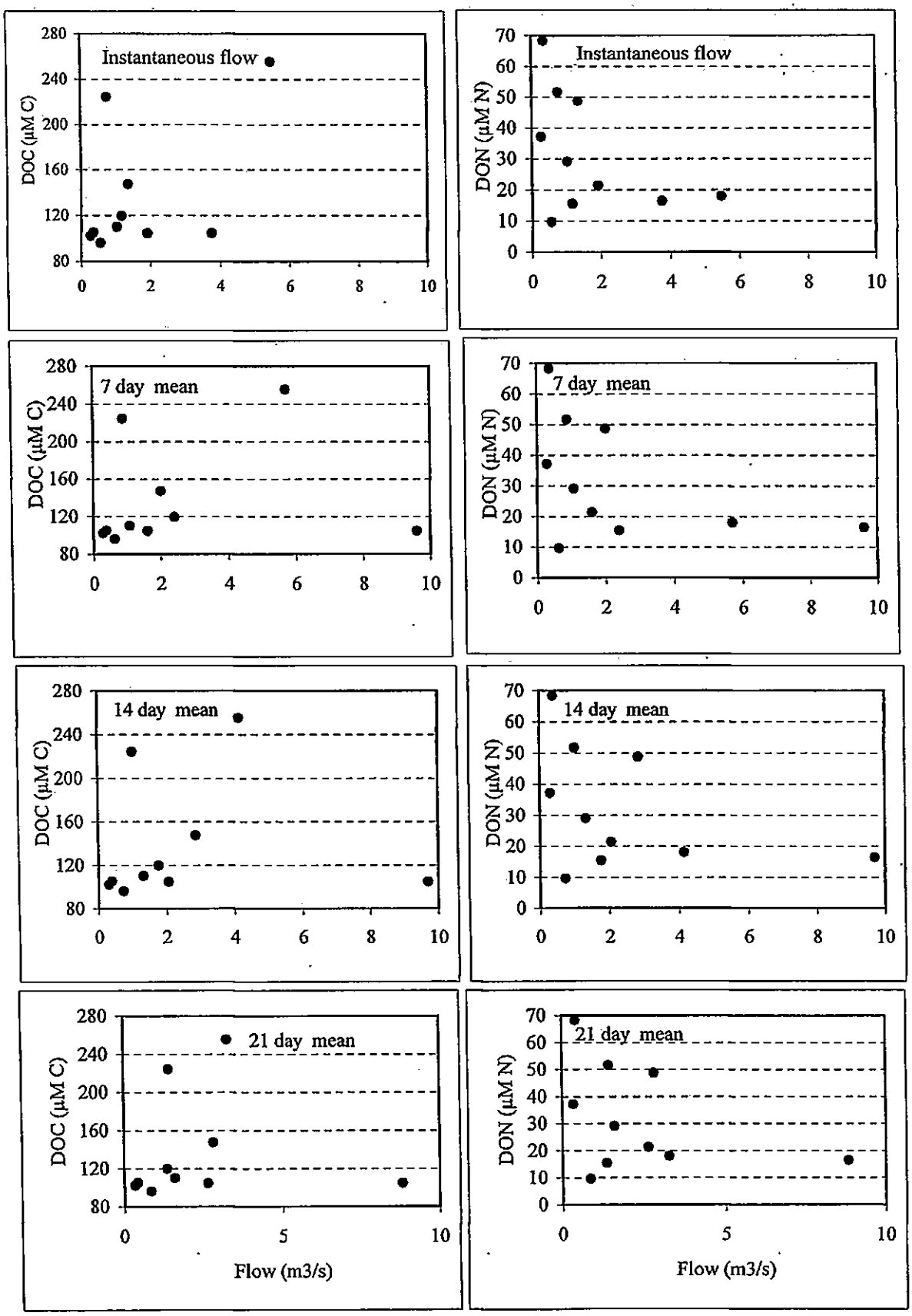


Figure 3.27 DOC and DON concentrations (µM) at the head of the Plym Estuary (0 km) versus flow (instantaneous and 7, 14 and 21 day mean flow values before the sampling day).

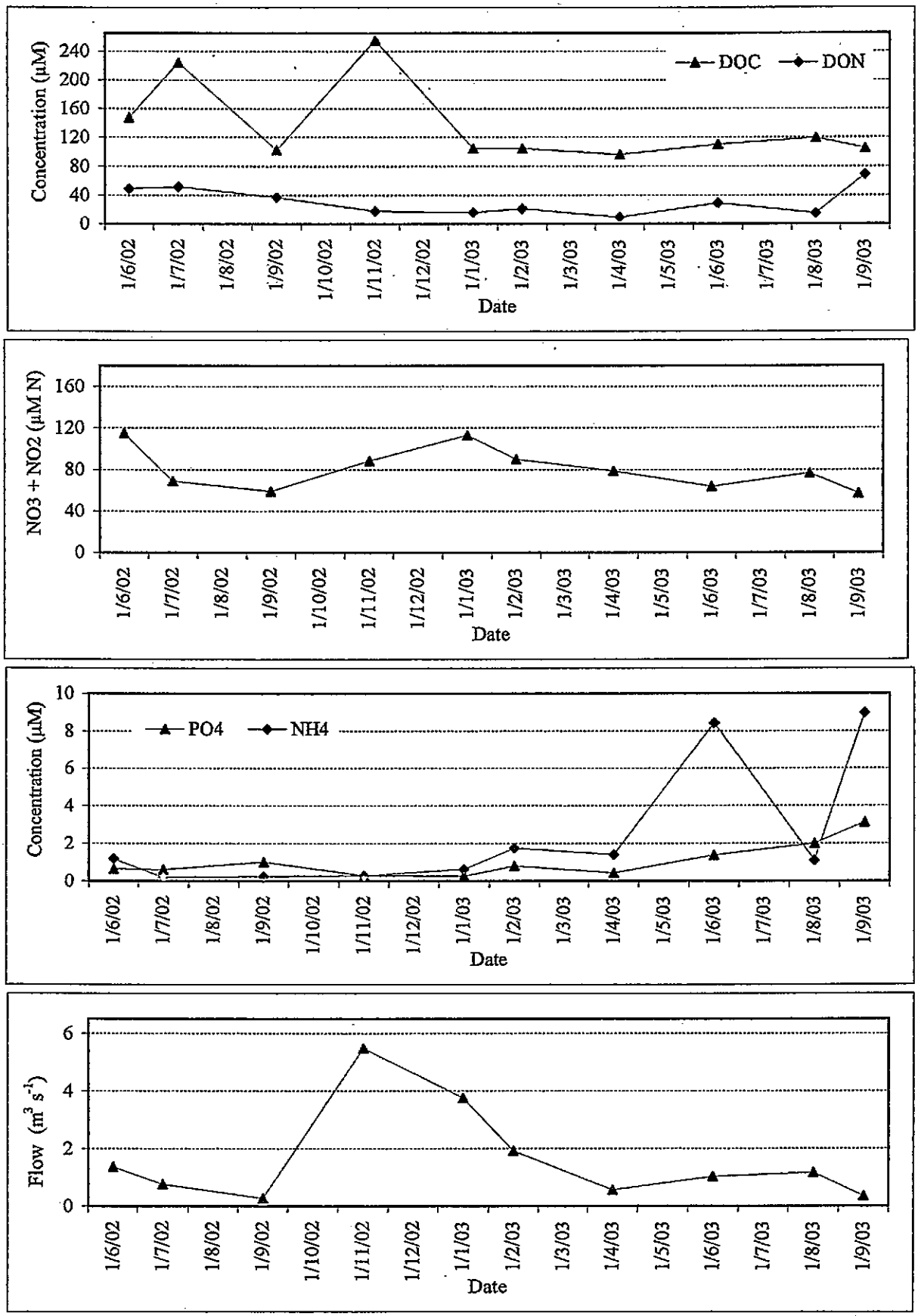
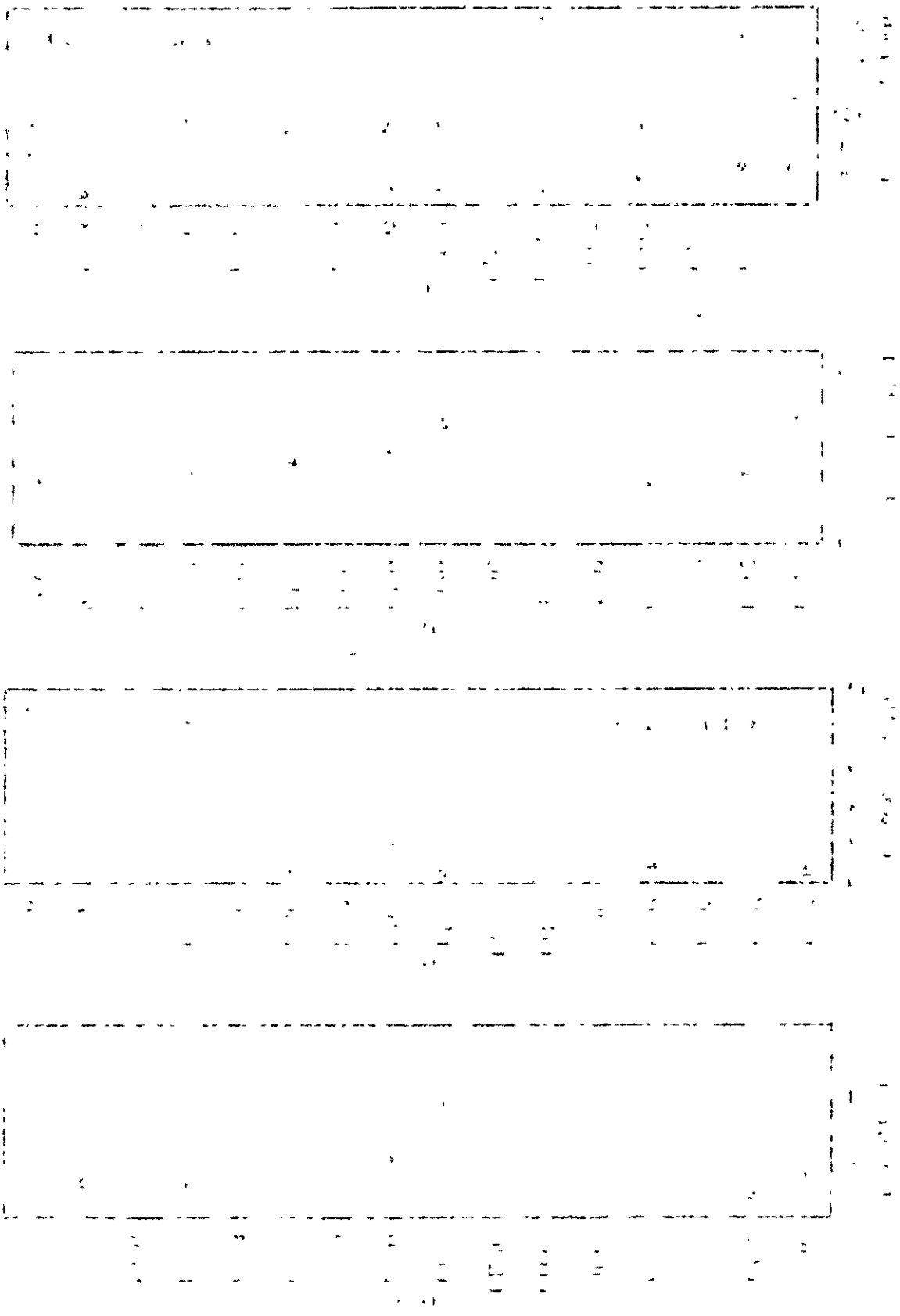


Figure 3.28 Seasonal variations of measured constituents at the head of the Plym Estuary (0 km).



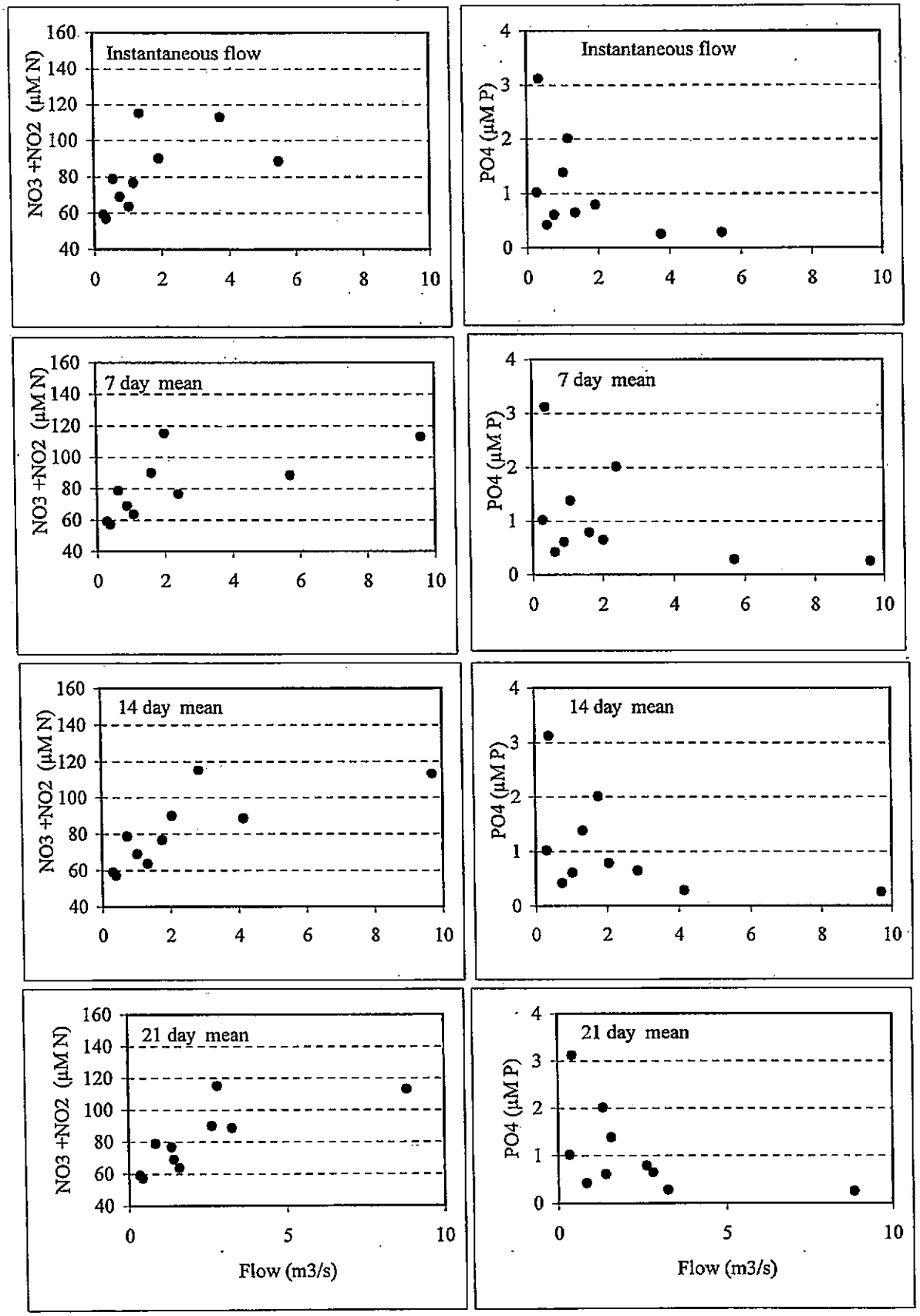


Figure 3.29 Nitrate and FRP concentrations (µM) at the head of the Plym Estuary (0 km) versus flow (instantaneous and 7, 14 and 21 day mean flow values before the sampling day).

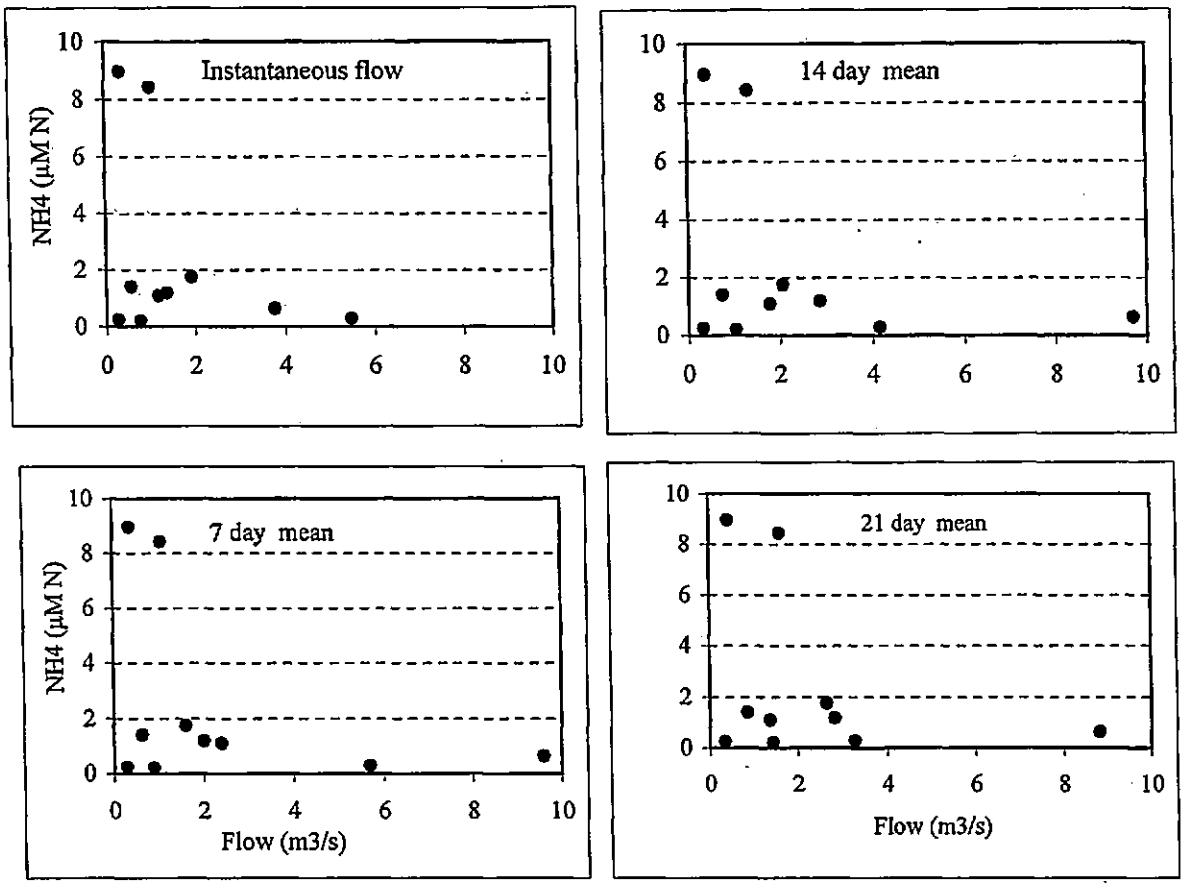


Figure 3.30 Ammonium concentrations (µM) at the head of the Plym Estuary (0 km) versus flow (instantaneous and 7, 14 and 21 day mean flow values before the sampling day).

3.4 Bioavailability of dissolved organic nitrogen in the Plym Estuary

The results from the bacterial incubation experiments are given in Table 3.4. The mean uptake of DON by bacteria was 3.3 ± 2.3 μM N (± 1SD), while the average nitrate regeneration was 4.5 ± 2.3 μM N over the time course of the experiment. A significant fraction of the DON (30 and 58 % average ± 1SD) appeared to be utilised by heterotrophic bacteria that coupled with regeneration of 9 – 35 % of nitrate within an hour, presumably through the processes of ammonification and nitrification of the bioavailable DON (Figure 3.31). Since the hydraulic residence time of the estuarine water in the bioreactors was estimated to be 1 hour (section 2.5), the average bacterial uptake rate in the incubation experiments was 3.3 ± 2.3 μmol N L^{-1} hr^{-1} (Table 3.4). Given that, during the experimental period, the mean River Plym flow and DON concentration were 4.75 x 10^3 m^3 hr^{-1} and 27 mmol N m^{-3} at the head of the estuary, respectively. Then 12.2 % of the river DON flux may be available for bacterial utilisation and ultimate conversion to nitrate within an hour in the Plym Estuary.

During similar incubation experiments in the Elbe Estuary, bacterial consumption of DON was reported to be in the range of 3 – 5.6 μmol N L^{-1} hr^{-1} (Kerner and Spitzy 2001). In addition, these authors showed that between 27 and 64 % of the LMW-DON, which formed 64 – 79 % of the total DON, was consumed by bacterial mineralization. In the Delaware Estuary, an average of 40 – 72 % of DON was available for bacterial utilisation within the 10 – 15 d time course of the batch incubation experiment, with the utilisation range of 11 – 42 % d^{-1} during the first 4 days (Seitzinger and Sanders 1997). Their results suggested that in estuaries with freshwater residence times of weeks to months, such as the Delaware Estuary (80 d), the riverine bioavailable portion of DON would be utilised within the estuary. In contrast, in estuaries with residence times of less than week, such as the

Yealm Estuary (1.5 d), a portion of the bioavailable DON may be consumed with the estuary and a larger fraction exported and presumably utilised in adjacent coastal waters.

The observed variation in the DON uptake rate can be related to the concentrations of DON in the inflowing waters as demonstrated by Kaplan and Newbold (1995) and Kerner and Spitzy (2001). In the present work, a significant positive correlation (correlation coefficient = 0.90, n = 14) was observed between the inflowing DON concentration and the bacterial utilisation rate, showing that bacterial degradation capacity increased with increasing DON concentrations in the substrate (Figure 3.32).

Regarding DOC bioavailability, there was no significant consumption (average 1.8 %) during the incubation experiment, and its concentration varied irregularly, implying that both production and consumption processes were taking place. A similar observation has been reported for the Elbe Estuary incubation experiment, noted earlier. The observed discrepancy between the relatively high DON bacterial uptake and the insignificant DOC consumption indicated that nitrogen rich compounds had been removed from the DOM and utilised by bacteria, whilst the carbon skeleton remained mostly unaffected by the bacterial degradation, or DOC production and consumption were balanced (Sondergaard and Worm 2001; Kerner and Spitzy 2001).

Since phototrophic processes were inhibited in the dark, DON consumed by bacteria was either assimilated into bacterial biomass and/or rapidly mineralised to nitrate through ammonification and nitrification (Seitzinger and Sanders 1997). The rate of nitrification was calculated to be 1.6 – 7.4 μmol N L^{-1} hr^{-1}, the average being similar to that reported for the Elbe Estuary (0.7 – 5.1 μmol N L^{-1} hr^{-1}). The number of bacterial colonies, only in suspension, was 1 – 2 x 10^3 bacterial colonies mL^{-1}.

Table 3.4 Results of the bacterial availability of the DON incubation experiment, in and out refer to the inflow and outflow concentration (μM), respectively.

Estuarine water collection	Sampling date	FRP in	FRP out	NH_4 in	NH_4 out	NO_3 in	NO_3 out	NO_3 regenerated	% NO_3 regenerated	DON in	DON out	DON Utilised	% DON Utilise	DOC in	DOC out
30/01/03	27/03/03	1.83	2.06	7.77	3.15	49.4	61.9	12.42	25%	13.67	5.92	7.75	57%	93.9	83.8
	09/05/03	1.41	1.90	0.56	0.46	52.6	60.3	7.67	15%	16.84	8.60	8.24	49%	91.9	95.9
19/05/03	27/05/03	1.26	1.01	2.39	0.49	21.3	25.5	4.21	20%	3.91	1.37	2.54	65%	105.0	112.9
	17/06/03	0.75	0.77	0.53	1.10	21.4	22.9	1.41	6.6%	2.47	1.42	1.05	43%	129.8	120.9
	09/07/03	0.28	0.35	0.47	1.69	18.2	25.7	7.50	41%	7.75	2.73	5.02	65%	121.2	122.4
10/07/03	15/07/03	0.71	0.77	1.64	0.40	6.1	8.7	2.60	43%	6.83	3.74	3.09	45%	94.3	103.4
	28/07/03	0.83	1.34	1.05	0.48	12.2	17.7	5.50	45%	4.07	2.60	1.47	36%	87.1	90.2
	22/08/03	0.96	1.30	0.39	0.47	13.2	15.9	2.70	20%	3.85	2.61	1.24	32%	95.5	91.2
	08/09/03	0.86	1.07	0.37	1.05	15.7	19.0	3.30	21%	4.18	2.26	1.92	46%	93.6	91.8
31/10/03	02/11/03	0.97	1.57	1.81	0.44	16.5	21.1	4.60	28%	10.55	7.55	3.00	28%	102.8	94.0
	07/11/03	1.32	1.63	0.42	0.33	21.4	24.8	3.40	16%	10.73	6.48	4.25	40%	98.6	88.3
	14/11/03	1.67	1.75	0.36	0.29	27.2	29.9	2.70	10%	7.39	5.64	1.75	24%	100.6	109.1
	26/11/03	1.60	1.76	0.31	0.37	33.8	36.3	2.50	7.4%	5.69	4.02	1.67	29%	89.7	93.8
	23/12/03	1.78	1.78	0.36	0.29	39.8	42.3	2.50	6.3%	5.34	2.02	3.32	62%	114.2	95.1
Average		1.16	1.36	1.32	0.79	24.9	29.4	4.50	22%	7.38	4.07	3.31	44%	101.3	99.5
SD		0.46	0.51	1.97	0.79	14.1	15.8	2.95	13%	4.18	2.36	2.29	14%	12.5	12.2

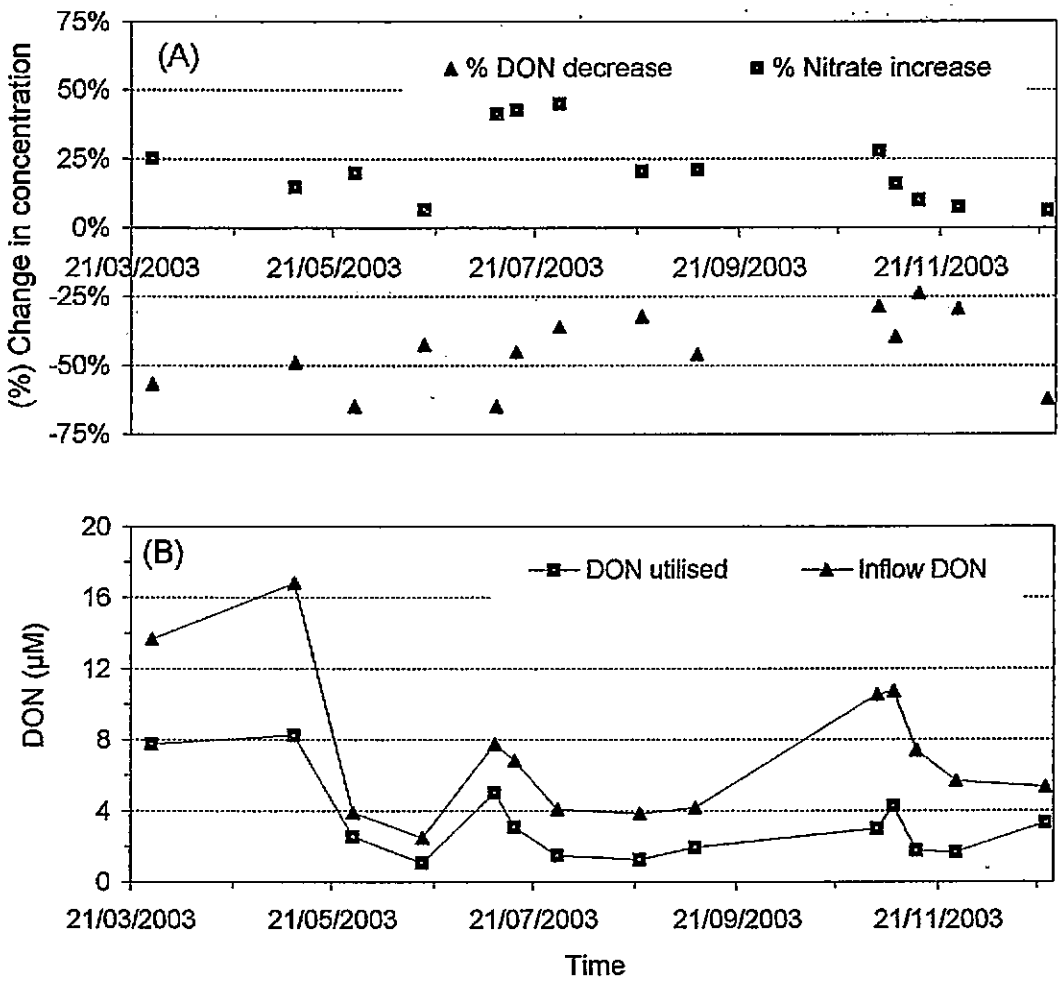


Figure 3.31 (A) percentage decrease in dissolved organic nitrogen and percentage increase in nitrate, and (B) the concentration of the bioavailable dissolved organic nitrogen in the bacterial incubation experiment.

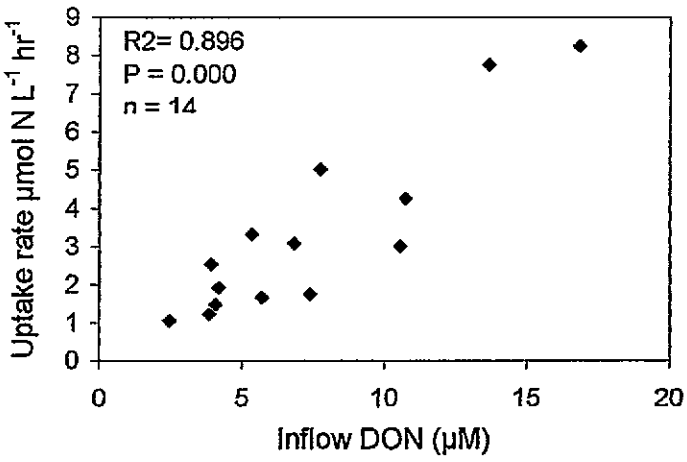


Figure 3.32 Scatter plot showing the correlations between inflow DON concentration (µM) and bacterial uptake rate (µmol N L^{-1} hr^{-1}) in the bacterial incubation experiment.

The conservative mixing behaviour of DOC observed in some estuaries has been used to suggest that riverine inputs of DOC and by implication DON are refractory (Mantoura and Woodward 1983). However, interpretation of the mixing curves should consider all DOM sources and sinks. In addition to the external inputs from rivers and the atmosphere, DON may be produced within estuaries by planktonic organisms and benthic release from sediment (Burdige and Zheng 1998; Bronk 2002; Berman and Bronk 2003) or introduced from external anthropogenic inputs (Seitzinger and Sanders 1997), such as the Plym Estuary. The results from this experiment showed the potential extent of the bioavailability of DON, and is consistent with the distributions of DON observed within the local estuaries of the Yealm and Plym (sections 3.2.5 and 3.3.5).

In summary, the results from the current incubation experiment and other recent studies (Seitzinger and Sanders 1997; Stepanauskas *et al.* 1999; Kerner and Spitzy 2001; Seitzinger *et al.* 2002; Wiegner and Seitzinger 2004) strongly suggest that both internal and external sources of DON are biologically available in estuarine environments. The bioavailability of wetland –derived DON in South Sweden, to marine bacterioplankton varied from 2 % to 16 %, suggesting that DON is an important nitrogen source, mainly in the summer, for the biota of estuarine and coastal waters (Stepanauskas *et al.* 1999). Rapid utilisation of DON released by phytoplankton in estuaries has been demonstrated, where bacteria and some phytoplankton can use LMW DON such as urea and amino acids (Kerner and Spitzy 2001; Bronk 2002; Berman and Bronk 2003). Heterotrophic bacterioplankton may use 30 – 60 % of humic-bound DON and make it available for the growth of phytoplankton (Stepanauskas *et al.* 1999). In addition to bacterial degradation, a portion of DON in estuaries and coastal waters may be photochemically degraded to ammonia, which would then be available for bacteria or phytoplankton utilisation (Wiegner and Seitzinger 2001; Bronk 2002).

3.5 The determination of stable nitrogen isotope ratios

Results from the preliminary work on stable nitrogen isotope measurements are presented in Table 3.5. Examination of the data revealed the following: (1) the measured nitrogen yield and the expected nitrogen yield were very approximate. (2) Ammonium sulphate (SN3) and potassium nitrate (KPN) standards had d15N values lower than the certified values of + 2.6 and + 5.6, respectively. (3) The negative d15N values of the blank might be responsible for the low d15N values of many of the DON samples, and some of the ammonium samples. However, the measured nitrogen yield of the blank was very close to what is usually reported at the NERC Isotope Geosciences Laboratory. (4) In addition, during the isotope measurements, most of the diffusion packets appeared to be wet.

Given these observations, the initial trial of this method was not quantitative in recovery of all dissolved nitrogen species, particularly the standards. When ammonium recovery is less than 100 %, isotopic fractionation occurs and leads to errors in the measured ^{15}N: ^{14}N isotope ratio due to ^{15}N depletion (Holmes *et al.* 1998). Hence, the results can not be used with much confidence for the environmental interpretation of DON cycling in the Plym Estuary. The analytical challenges associated with stable nitrogen isotope measurements of DON are presented and discussed here so that they may be highlighted and addressed during the course of future studies.

During the preconcentration step, the volume of the rotary evaporator flask used was 1 L, and held only 400 – 500 mL of sample. Most of the samples were concentrated from 4 L to 200 mL at an evaporation rate of 1 L per 1.5 – 2 hours. Consequently this lead to preconcentration to 5 % of the original volume, whereas the recommended volume reduction should not be less than 15 – 25 % (Sigman *et al.* 1997). For example, a 16 fold preconcentration lead to poor nitrate reduction efficiency due to the change in the pH of

the natural water sample buffered with MgO as it become more concentrated; pH comes down to 8.4 and hence could keep some ammonium in solution (Sigman *et al.* 1997). Also, the use of relatively small rotary evaporator flask and temperature of 40 – 45 °C might occasionally result in significant loss of nitrogen (DON) due to boiling and transfer of sample to the receiving reservoir. It is recommended that the volume of the rotary evaporator flask should not less than 2 L for preconcentration of estuarine samples with low concentration of ammonium.

The diffusion packets may have been contaminated with ammonia from the laboratory, especially during the H_2SO_4 addition, implying that the diffusion packet might already be saturated with ammonia before the start of the diffusion procedure. It was not possible to control the ammonia concentrations in the local laboratory atmosphere. Furthermore, the diffusion flask used was only 250 mL and the diffusion packet was placed inside the ground glass stopper of the flask (Figure 3.33). Any water vapour that condensed within the ground glass stopper consequently filled the well containing the diffusion packet. This probably reduced surface area available for ammonia sorption, and also the diluted acid might subsequently reduce the efficiency of ammonia recovery. So, an appropriate diffusion bottle should be used.

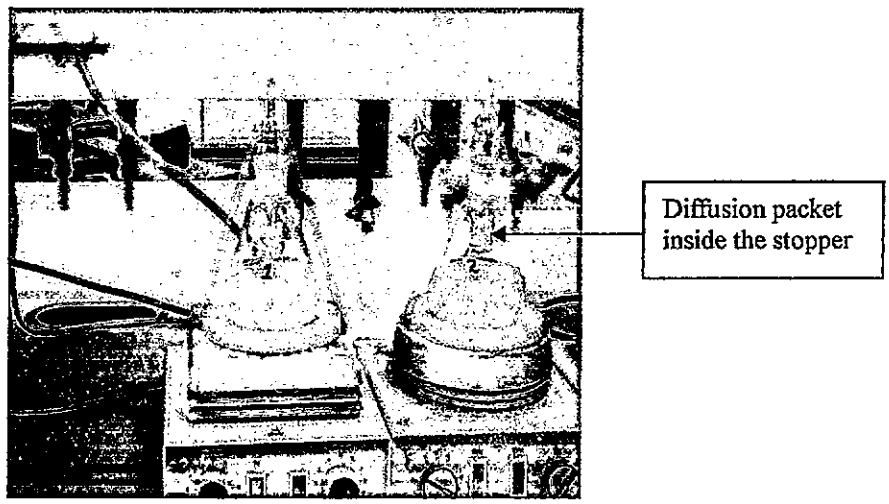


Figure 3.33 Ammonia diffusion process.

Incubating the diffusion flask in the oven at 60 – 65 °C for 4 days might result in the loss of labile DON and hence reduce the recovery efficiency. The loss of labile DON could be reduced by decreasing the incubation period at 65 °C from 4 days to 2 days and consequently increase incubation period with the use of stirrer to 6 days. As the sample must either be heated or shaken continuously during diffusion process (Sigman *et al.* 1997).

Lastly, the sensitivity of the ammonia measurements undertaken by the mass spectrometer method at the NERC Isotope Geosciences Laboratory needs to be reduced from the current 50 μg N to 14 μg N (equivalent to 5 μM concentration in the natural water samples), as is possible elsewhere (Fry *et al.* 1996; Sigman *et al.* 1997; Holmes *et al.* 1998). In this case samples with the lowest ammonium concentrations (1 μM N) will only have to be concentrated from 1 L to 200 mL, i.e. 20 % of the original volume.

In summary, isotopic ratio measurements of stable nitrogen in the DON pool are more difficult than for those in the pools of particulate organic nitrogen (Wu *et al.* 1999; Mahaffey *et al.* 2003), ammonium (Holmes *et al.* 1998; Lehmann *et al.* 2001) and nitrate (Sigman *et al.* 1997; Chang *et al.* 2002). For DON, all of the DIN forms must first be removed and the DON pool subsequently isolated with high efficiency (Bronk 2002).

Table 3.5 Results from the preliminary work on stable nitrogen isotope measurements in 6 water samples collected from the Plym Estuary on 23-03-2004.

Sample	Expected µg N on filter	Fraction of filter used	Therefore expected µg N*	Measured µg N (approx.)	d15N vs. AIR
Ammonium					
Plym Estuary 1	362	1/4	91	50	+0.6
Plym Estuary 2	458	1/5	92	7	+3.5
Plym Estuary 3	105	1/2	53	19	+2.1
Plym Estuary 4	134	1/2	67	86	-0.9
Plym Estuary 5	95	1/2	48	77	+1.3
Plym Estuary 6	103	1/2	52	36	-4.2
MQ blank	600	1		7	-17.2
STD1 (SN3)	600	1/6	100	93	-2.6
STD2 (SN3)	600	1/6	100	123	+0.8
STD3 (SN3)	600	1/6	100	101	+0.9
Nitrate					
Plym Estuary 1	600	1/6	100	18	-4.0
Plym Estuary 2	600	1/6	100	20	+3.8
Plym Estuary 3	600	1/6	100	45	+1.7
Plym Estuary 4	600	1/6	100	90	+3.5
Plym Estuary 5	600	1/6	100	73	+3.4
Plym Estuary 6	600	1/6	100	107	+3.6
MQ blank		1		6	-19.1
STD1 (KPN)	600	1/6	100	18	-10.2
STD2 (KPN)	600	1/6	100	105	+1.6
STD3 (KPN)	600	1/6	100	113	+3.8
Dissolved organic nitrogen					
Plym Estuary 1	116	1/2	58	42	+10.2
Plym Estuary 2	49	1/2	25	14	-5.3
Plym Estuary 3	123	1/2	62	30	-4.4
Plym Estuary 4	54	1/2	27	15	-7.2
Plym Estuary 5	43	1/2	22	23	-8.0
Plym Estuary 6	36	1/2	18	22	-10.9
MQ blank		1		15	-10.5

* The filter was only approximately cut into fractions, and the distribution of ammonium on the filter was not uniform. So expected microgram N was very approximate.

Chapter Four

Conclusion and future work

Conclusion and future work

4.1 HTCO analytical methodology

Understanding of the global carbon and nitrogen cycles depends initially on accurate measurements of the concentrations of C and N species in natural waters. DOC and DON are significant components of the global carbon and nitrogen cycles. Studies on nitrogen in natural waters have generally focussed on DIN, primarily because of the relative ease of DIN analysis, the perceived important influence of DIN on water quality and the analytical difficulty in measuring the DON fraction. However, DON cycling should be investigated, in conjunction with studies of DOC, to obtain a better understanding of the biogeochemical roles of dissolved organic matter in natural waters and their influences on water quality.

In this study, a Shimadzu TOC 5000 A Total Carbon Analyser was coupled to a Sievers NCD 255 nitrogen chemiluminescence detector for simultaneous determination of DOC and TDN. The coupled HTCO-chemiluminescence method forms an excellent analytical technique for the measurement of DOC/DON, as it is simple to perform in the hand of an experienced analyst, is applicable to all natural waters regardless of salinity and exhibits an excellent linearity over a wide concentration range. The approach allows a combined DOC and TDN analysis in the same sample in a single injection, and results in accurate and precise measurements. Detection limits of approximately 0.46 μM N and 6.2 μM C can be achieved and are considered acceptable. However, the reliability of the DOC and TDN data relies upon (1) careful blank determination, (2) a systematic evaluation of the oxidation efficiency of a variety of organic compounds, (3) accurate and precise instrumental calibration, and (4) testing analytical veracity with certified reference materials (CRMs). Any DOC and TDN (DON) analysis should document the use of CRMs for quantitative validation and accreditation of the measurements and to be suitable for peer reviewed publication.

4.2 The biogeochemical cycling of DOC and DON

Knowledge of the DOC and DON reservoirs and their evolution are considered key parameters in the description of the aquatic environment. Riverine-estuarine environments mediate the transfer of dissolved organic carbon and nitrogen compounds from drainage basins to coastal and ocean waters. An investigation of spatial and seasonal variations of DOC and DON concentrations was carried out in two contrasting riverine-estuarine environments. The Yealm and Plym estuaries, in south-west England, were chosen as they are relatively small scale and subject to anthropogenic inputs of carbon and nitrogen. Furthermore, there are no published data on the distributions and cycling of DOC and DON in these estuaries. The Yealm catchment is mainly agricultural, whilst the Plym is urbanised and industrialised by the city of Plymouth. These estuaries are macrotidal systems with freshwater residence times of 1.5 d for the Yealm and 4 d for the Plym. Waters samples were collected regularly (ca. every six weeks) along an axial transect, during the period 02/2002 – 09/2003 for the Yealm, and 06/2002 – 09/2003 for the Plym. DOC concentrations were generally in the range 61 – 335 μM C and 71 – 290 μM C in the Yealm and Plym, respectively. DON concentrations ranged from 1.8 μM N at the seawater end to 62 μM N at the freshwater end in the Yealm. Corresponding DON concentrations in the Plym were 4 and 94 μM N.

Estuarine reactivity

In general, non-conservative behaviour was observed for DOC and DON in these estuaries, except a limited number of occasions. Nitrate and FRP behaved in a relatively conservative manner in the Yealm, whilst they were generally non-conservative in the Plym. Ammonium proved to be a reactive nitrogen component in both estuaries. In the Yealm and Plym estuaries, the longitudinal distribution of DOC and DON reflected a mixture of external inputs and in situ reactivity. Anthropogenic inputs appeared to be particularly

important in the Plym relative to the Yealm. In situ reactivity would have induced physiochemical transformations and biological production and respiration of organic compounds. Whilst, each of these factors may have contributed to the apparent non-conservative behaviours, the specific process of bacterial respiration of DOM may have been identified as a small but significant feature.

Conservative distributions of DOC and DON reflect a physical mixing of river and sea waters containing DOM that is relatively unreactive and biologically unavailable in the time required for transit of dissolved constituents through the estuary. DOC and DON in many estuaries, including the Yealm and the Plym, are behaving in a dynamic reactive way and the linear distribution of the DOM-salinity plot does not necessarily imply a conservative behaviour since sources and sinks might occur simultaneously (Cifuentes and Eldridge 1998; Moran *et al.* 1999; Abril *et al.* 2002). There are several reasons to emphasis the reactivity of DOC and DON in estuarine environments as follows (Peterson *et al.* 1994). (1) Estuarine processes both remove and add DOM. For example, flocculation and precipitation of humic substance by salts remove a portion of riverine derived-DOM while photolysis of complex organic compounds and deflocculation could lead to the production of labile organic matter. (2) There are large and active populations of bacteria assimilating DOM in many estuaries. (3) A variety of processes also produce DOM in estuaries such as phytoplankton growth and related activities. (4) External inputs of DOM from either natural or anthropogenic sources have major influence.

Seasonal variations

An examination of the seasonal variations revealed that DOC concentrations were generally higher in summer and autumn relative to winter and spring in these estuarine environments. On the other hand, DON concentrations were lowest during spring and early

summer and highest during late summer and autumn. Generally, the seasonal variation of DOC and DON was characterised by lower concentrations during winter and a slight increase in spring and summer followed by highest concentrations during late summer and autumn. The seasonality of DOC and DON may be relatively linked to phytoplankton blooms and in situ biological recycling, with an accumulation of DOC and active release of DON during the late summer and early autumn (Williams 1995; Volk *et al.* 1997; Mortazavi *et al.* 2001; Chapman *et al.* 2001; Bronk 2002; Al-Azri 2003; Gobler and Sanudo-Wilhelmy 2003). This was supported by a strong seasonal pattern for chlorophyll a, with highest concentrations observed during spring and summer and lowest concentrations during late autumn and winter. In addition, lowest nitrate concentrations were observed in summer and early autumn, while highest concentrations occurred during winter.

Seasonal variations in the concentrations of DOC and DON in the estuarine environment may also be caused by variations in agricultural drainage, municipal and industrial waste discharge, and river water flow (Alvarez-Salgado and Miller 1998a; Sondergaard *et al.* 2000). Variations in the flow of fresh water to an estuarine environment tend to alter the amounts of dissolved organic materials and nutrients entering the estuary and also alter their distribution by influencing the degree of mixing between the fresh and salt water.

An investigation of the relationships between concentrations of chemical determinands and river flow is an easy and valuable tool for assessing the origin and nature of these determinands at the head of the estuarine environment. These relationships indicated a complex suit of point and diffuse sources of DOC and DON in the Yealm. In the Plym, DOC was affected by diffuse sources form the catchment, while DON was influenced by anthropogenic point sources. In both the Yealm and Plym, nitrate concentrations increased

with flow, indicating diffuse sources from the catchment. FRP concentrations decreased with increasing flow in response to dilution of point sources by rainfall.

Anthropogenic inputs

The Yealm catchment adjacent to the estuary is mainly agricultural, with agricultural run off and sewage discharges influencing the DOC and DON distributions in the estuary. On the other hand, the Plym catchment is urbanised and industrialised by the city of Plymouth. A number of anthropogenic inputs potentially influencing the Plym Estuary include tin mining, china clay abstraction, commercial shipping, sewage effluent discharge, traffic run off and leachate discharge from the Chelson Meadow landfill site. Indeed, the distributions of DOC and DON in the Plym Estuary were influenced by anthropogenic inputs, supported by the following observations: (1) Elevated DOC, DON , NO_3 and FRP concentrations occurred, in several occasions, in samples collected near STW outfalls at Plympton and Plymstock. (2) Higher DOC, DON and NO_3 concentrations were often observed within the mid-estuarine water samples, which might be due to run off from the Chelson Meadow landfill site. (3) The complex pattern of the distributions of the majority of determinands against salinity. Therefore, this study revealed that human activity, through wastewater loads, significantly may contribute to the dissolved organic matter load in the Plym Estuary. This might also be the case in estuaries of other countries, especially in developing countries, where sewage and industrial waste treatment are generally lacking (Abril *et al.* 2002).

4.3 Bioavailability and the important contribution of DON

Nitrogen is a key nutrient in natural waters, where excess inputs lead to eutrophication. DON represents the least understood part of the nitrogen cycle, relative to inorganic nitrogen species (Jones *et al.* 2004). The current study, and recent other works, has shown

that DON is quantitatively significant component (up to 84 %) of the TDN pool and a large fraction (up to 58 %) of the DON is bioavailable for bacterial mineralization within an hour.

The amount of DON as a percentage of TDN varied between 4 and 79 % for the Yealm, and 3.5 – 84 % for the Plym. Higher percentage values were observed in the Yealm Estuary samples for September 2002 (61 – 74 %) and August 2003 (53 – 79 %), and in the Plym Estuary samples for November 2002 (45 – 74 %). These results showed the significant contribution of DON to the TDN pool. During the bacterial incubation experiment, a significant fraction of DON (30 – 58 %) was bioavailable for heterotrophic bacterial utilisation, and appeared to be coupled to the regeneration of nitrate (9 – 35 % of inflow concentrations) within an hour. This finding supports the hypothesised bacterial ammonification of DON and nitrification of the released ammonium to nitrate that appeared to occur during some of the surveys in both the Yealm and Plym estuaries.

The large proportion of the DON that was biologically available in our bacterial incubation experiment, coupled with the significant contribution of DON to the TDN pool in the Yealm and Plym estuaries, suggested that dissolved organic nitrogen inputs may contribute more to estuarine and coastal water eutrophication than was previously suspected. Environmental assessment of DIN inputs underestimate, and total nitrogen inputs likely overestimate, the inputs of biologically available nitrogen to estuarine and coastal waters (Seitzinger and Sanders 1997). Therefore, in order to develop a nitrogen budget and establish a proper environmental monitoring programme for an aquatic environment, DIN inputs plus DON inputs including the portion that is biologically available to aquatic biota must be quantified and equally assessed.

4.4 Future work

The current study provided the bench mark of any future studies on DOC and DON biogeochemical cycling in the estuaries of the Yealm and Plym, and even the DON distributions in the Tamar Estuary. However, more research is needed in order to provide a better understanding of estuarine biogeochemistry of DOC and DON, the most dynamic aquatic environments.

In order to assess DOC and DON sources and sinks in estuaries, many measurements other than the simple distribution of the bulk concentrations are necessary. These include long-term bioassays (Hopkinson *et al.* 1997a; Moran *et al.* 1999), isotopic tracers (Cifuentes and Eldridge 1998) and/or elemental composition of dissolved organic matter (Sun *et al.* 1997; Hopkinson *et al.* 1997a). Bioavailability of riverine DOM can be estimated empirically from the bulk elemental composition of DOM in the water samples (Sun *et al.* 1997).

The use of stable nitrogen isotopes to identify DON sources and in situ production is of significant importance to improve our understanding of DON biogeochemical cycling. A research priority should be given to the quantitative recovery of DON from water samples during analysis and prior to determination by mass spectrometry.

Also, in order to interpret the DOC and DON databases from major world rives, population density, weighted with an index of sewage treatment, should be considered as a master variable in addition to the natural parameters used so far. Such an approach has intensively been used for nutrients but it is much more difficult to assess for organic matter, due to mineralization in river systems (Abril *et al.* 2002).

There are few data on the role of estuarine sediments, particularly in mud-rich systems, with respect to the cycling of DON and its transport through the estuary. Additional studies in this area would provide a better understanding of estuarine biogeochemistry of DON and support the water column studies.

As the case for N, studies on P in natural waters have generally focused on FRP and ignored the dissolved organic phosphate fraction (DOP). It is critical to accurately measure DON and DOP in conjunction with studies of DOC for a better understanding of nutrient limitation and cycling in estuarine and coastal waters (Karl and Bjorkman 2002; Sharp 2002). In particular, marine scientists should also now place more emphasis on the accurate determination of DOP concentrations for better understanding of phosphorus dynamic. Improving DOP methodology will also allow the calculation of C, N, P stoichiometry in DOM, DOC:DON:DOP.

Appendices

Appendix 3.1

Raw data of the chemical determinands measured in water samples collected from the Yealm Estuary during the period February 2002 – September 2003

Results of the Yealm Estuary samples collected on 24 - 02 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	Y3	8.01	#	#	#	32.11	7.20	1.17	0.90	3.48	35.30	38.78	41.60	2.81	67.50%	107.30	lost	38.19
2	4.80	Y5	8.01	#	#	#	32.04	7.31	1.16	0.85	4.00	30.84	34.84	69.55	34.71	49.91%	125.00	in PC	3.60
3	4.30	Y4	8.11	#	#	#	31.28	8.02	1.53	1.20	5.75	71.75	77.50	146.3	61.82	42.25%	144.50	#	2.34
5	2.90	Y6	7.98	#	#	#	26.67	7.34	0.91	1.53	4.70	96.79	101.49	176.59	75.10	42.53%	193.80	#	2.58
6	0.85	Y2	7.54	#	#	#	0.26	8.93	1.56	2.11	2.57	183.45	186.0	221.77	35.75	16.12%	140.70	#	3.94
7	0.00	Y1	7.28	#	#	#	0.14	8.62	1.59	2.11	8.46	189.04	197.50	262.8	65.28	24.84%	180.20	#	2.76

Results of the Yealm Estuary samples collected on 21 - 03 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
ayo (elder	5.50	10:00	8.10	11.70	29.80	19.90	21.72	9.22	1.45	2.29	3.98	161.07	165.05	254.9	89.80	35.24%	271.10	lost	3.02
1	5.35	10:15	8.26	11.70	16.60	10.50	22.33	8.95	1.43	1.79	2.98	111.70	114.68	162.06	47.38	29.24%	217.20	in PC	4.58
2	4.80	10:35	8.40	11.50	25.80	18.40	19.40	8.85	1.19	2.11	2.80	129.55	132.35	149.09	16.74	11.23%	193.70	#	11.57
3	4.30	11:15	8.39	11.90	16.30	11.26	12.42	9.32	1.03	1.93	5.50	129.97	135.47	155.62	20.15	12.95%	267.20	#	13.26
4	3.85	11:30	7.81	12.00	11.60	8.15	8.71	9.27	1.42	1.94	3.28	119.94	123.2	132.49	9.27	7.00%	256.50	#	27.67
5	2.90	14:00	8.03	13.00	9.80	6.80	7.21	10.73	0.90	1.56	3.27	142.93	146.2	171.74	25.54	14.87%	207.00	#	8.10
6	0.85	13:05	7.52	11.90	0.20	0.12	0.23	9.93	0.60	1.78	1.52	168.25	169.77	178.20	8.43	4.73%	203.70	#	24.16
7	0.00	12:35	7.35	11.60	0.05	0.04	0.05	9.79	0.50	1.92	4.03	184.91	188.9	196.64	7.70	3.92%	235.00	#	30.52
8MQ										0.06	0.34	0.34	0.68	ND			5.06		

Results of the Yealm Estuary samples collected on 23 - 04 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	15:15	7.44	13.90	55.00	38.50	34.12	8.82	0.80	0.47	5.80	9.69	15.49	20.90	5.41	25.89%	130.70	#	24.16
2	4.80	16:45	7.55	13.50	55.30	38.80	34.46	8.47	0.97	0.45	4.56	23.82	28.38	30.14	1.76	5.84%	139.80	lost	79.43
3	4.30	16:25	7.46	13.90	55.20	38.60	34.47	9.72	1.37	0.49	5.98	19.82	25.80	30.03	4.23	14.09%	132.40	in PC	31.30
4	3.85	16:03	7.75	14.20	54.60	38.30	34.05	8.87	0.81	0.34	4.16	11.23	15.39	18.05	2.66	14.74%	103.70	#	38.98
5	2.90	18:30	7.44	15.70	49.00	34.40	30.54	9.79	1.63	0.31	6.12	18.35	24.47	37.41	12.94	34.59%	178.00	#	13.76
6	0.85	17:45	8.74	14.40	0.17	0.12	1.92	12.17	1.17	1.54	4.13	161.33	165.46	186.83	21.37	11.44%	141.10	#	6.60
7	0.00	18:00	8.48	14.20	0.10	0.07	0.07	10.53	1.67	2.13	2.20	166.42	168.6	212.01	43.39	20.47%	177.40	#	4.09
8MQ										0.02	0.16	ND		ND			4.23		

Results of the Yealm Estuary samples collected on 21 - 05 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	13:20	8.07	13.70	40.00	30.50	31.90	7.82	1.10	0.54	5.89	14.35	20.24	33.57	13.33	39.71%	103.50	1.56	7.76
2	4.80	13:45	7.88	13.50	38.60	29.60	28.90	7.80	0.83	0.62	4.79	22.65	27.44	39.01	11.57	29.66%	109.12	1.36	9.43
3	4.30	14:30	7.80	13.40	48.00	33.60	33.90	7.58	0.75	0.39	6.53	8.05	14.58	32.90	18.32	55.68%	84.83	1.87	4.63
4	3.85	14:10	7.80	13.40	47.00	33.00	33.87	7.62	0.60	0.41	5.59	14.47	20.06	35.99	15.93	44.27%	87.16	1.09	5.46
5	2.90	15:50	7.51	14.20	6.50	4.49	24.75	7.48	0.76	0.49	4.13	35.22	39.35	60.65	21.30	35.12%	122.47	1.93	5.75
6	0.85	15:10	7.75	13.70	2.68	1.88	1.90	8.62	1.77	2.74	7.36	136.22	143.58	173.89	30.31	17.43%	220.57	1.01	7.28
7	0.00	15:30	7.14	12.40	0.08	0.05	0.05	8.69	1.98	2.89	6.88	128.72	135.60	192.91	57.31	29.71%	210.97	0.88	3.68
8MQ										0.02	0.18	ND		ND			6.23	ND	

Results of the Yealm Estuary samples collected on 13 - 06 - 2002

Station	Distance km-weir	Time	pH	Tempera ture	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:D ON
1	5.35	07:35	8.11	13.90	43.50	30.50	34.04	7.99	0.80	0.38	5.48	12.83	18.31	21.19	2.88	13.57%	111.78	1.39	38.87
2	4.80	07:55	8.01	13.80	50.00	35.00	34.21	7.97	0.83	0.26	4.38	6.69	11.07	32.15	21.08	65.58%	111.70	1.50	5.30
3	4.30	08:30	8.03	14.00	44.50	31.10	33.60	8.04	0.80	0.26	4.47	7.35	11.82	34.60	22.78	65.84%	113.10	1.88	4.96
4	3.85	08:20	8.00	14.20	46.30	32.40	32.49	8.13	1.01	0.24	6.28	10.46	16.74	34.09	17.35	50.91%	135.60	1.05	7.81
5	2.90	09:55	8.20	15.00	39.30	27.50	27.71	8.57	1.40	0.26	4.41	26.25	30.66	38.98	8.32	21.34%	123.00	4.16	14.79
6	0.85	09:10	7.60	13.20	3.50	2.72	1.42	9.24	1.07	1.46	2.74	125.65	128.4	144.54	16.16	11.18%	194.70	1.26	12.05
7	0.00	09:35	6.92	13.00	0.13	0.09	0.03	9.28	0.94	1.67	7.43	117.64	125.07	135.20	10.13	7.49%	182.65	0.57	18.04
8MQ										0.01	0.23	0.00		ND			0.97	ND	

Results of the Yealm Estuary samples collected on 04 - 07 - 2002

Station	Distance km-weir	Time	pH	Tempera ture	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:D ON
1	5.35	12:55	8.04	15.70	45.00	31.60	33.08	9.58	1.88	0.07	3.11	5.57	8.68	14.99	6.31	42.07%	100.20	1.37	15.89
2	4.80	13:15	7.99	15.70	45.80	32.00	33.50	7.89	0.84	0.09	2.27	3.09	5.36	12.18	6.82	55.99%	94.86	1.76	13.91
3	4.30	13:50	7.98	15.30	43.00	30.10	31.94	8.00	1.05	0.10	3.79	4.64	8.43	15.46	7.03	45.47%	113.00	2.39	16.08
4	3.85	13:35	8.00	15.60	43.80	30.70	31.75	8.22	1.36	0.18	4.23	17.76	21.99	31.73	9.74	30.68%	129.80	3.92	13.33
5	2.90	15:00	8.02	18.00	36.80	25.80	29.21	8.67	1.74	0.06	2.00	15.80	17.80	27.16	9.36	34.47%	118.00	4.56	12.61
6	0.85	14:23	7.70	16.40	0.21	0.14	0.05	9.83	0.88	1.69	3.18	142.72	145.90	169.73	23.83	14.04%	209.80	0.54	8.81
7	0.00	14:41	7.05	14.50	0.16	0.11	0.04	8.96	0.70	1.55	2.94	107.59	110.53	158.74	48.22	30.37%	225.80	0.42	4.68
8 MQ										0.01	0.32	0.00		ND			17.24	ND	

Results of the Yealm Estuary samples collected on 30 - 07 - 2002

Station	Distance km-weir	Time	pH	Tempera ture	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:D ON
1	5.35	09:25	7.99	18.30	39.20	27.60	34.71	8.59	1.53	0.09	5.01	18.75	23.76	41.26	17.50	42.41%	92.40	2.38	5.28
2	4.80	09:45	7.93	18.20	38.00	26.80	34.90	8.14	0.72	0.09	3.28	4.30	7.58	23.08	15.50	67.16%	81.56	1.93	5.26
3	4.30	10:17	7.96	18.50	46.40	33.20	34.91	8.48	1.01	0.08	5.55	1.76	7.31	22.82	15.51	67.97%	81.55	1.57	5.26
4	3.85	10:04	7.92	18.20	46.30	34.40	34.90	8.34	0.79	0.08	2.82	1.81	4.63	13.90	9.27	66.69%	63.51	1.67	7.85
5	2.90	11:30	8.15	20.60	45.80	34.10	32.77	10.01	1.65	0.06	6.64	1.04	7.68	19.65	11.97	60.92%	98.40	3.56	8.22
6	0.85	11:00	7.53	18.20	5.06	3.51	3.44	8.25	0.91	1.71	8.25	152.98	161.23	223.7	62.47	27.94%	106.00	1.25	1.70
7	0.00	11:17	7.20	17.80	0.19	0.13	0.11	8.78	0.98	1.05	4.93	157.98	162.91	225.10	62.19	27.63%	98.20	0.49	1.58
8MQ										ND	0.94	0.04		2.95			15.00	0.01	

Results of the Yealm Estuary samples collected on 04 - 09 - 2002

Station	Distance km-weir	Time	pH	Tempera ture	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	BOD mg L-1	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:D ON
1	5.35	17:50	8.34	18.80	47.60	33.30	34.86	8.60	1.52	0.50	3.73	12.13	15.86	47.50	31.64	66.61%	83.25	2.21	2.63
2	4.80	17:45	8.34	18.80	47.70	33.40	35.04	8.60	1.42	0.45	5.43	11.20	16.63	42.65	26.02	61.01%	96.80	1.20	3.72
3	4.30	17:35	8.32	18.80	47.50	33.20	35.09	8.45	1.56	0.44	7.00	2.02	9.02	26.10	17.08	65.44%	80.06	1.37	4.69
4	3.85	17:25	8.30	19.10	47.70	33.40	34.51	8.96	1.26	0.49	3.17	0.40	3.57	12.22	8.65	70.77%	74.44	1.96	8.61
5	2.90	17:10	8.28	19.40	47.40	33.20	34.18	10.05	2.29	0.32	3.45	1.60	5.05	13.54	8.49	62.70%	92.53	1.85	10.90
6	2.00	17:00	8.25	20.10	45.50	31.90	32.60	10.41	2.06	0.35	5.48	3.74	9.22	34.40	25.18	73.20%	115.40	5.54	4.58
7	0.85	16:45	7.30	19.10	5.30	3.71	1.98	9.32	0.98	1.87	1.48	63.01	64.49	248.8	184.31	74.08%	139.00	0.39	0.75
8	0.00	18:30	7.02	16.40	0.35	0.25	0.13	9.25	1.27	1.58	0.65	93.41	94.06	259.4	165.3	63.74%	129.50	0.22	0.78
9MQ										0.03	0.72	0.27		1.26			19.60	ND	

Results of the Yealm Estuary samples collected on 11 - 10 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:10	8.11	15.80	50.60	35.40	35.16	7.32	90.70	0.49	4.55	1.55	6.10	12.65	6.55	51.81%	66.92	0.64	10.21
2	4.80	10:05	8.10	15.80	50.10	35.10	35.18	7.33	88.00	0.49	4.45	12.46	16.91	31.96	15.05	47.09%	88.21	0.63	5.86
3	4.30	10:00	8.10	15.90	51.00	35.70	35.15	7.36	91.40	0.37	4.12	4.41	8.53	15.81	7.28	46.05%	69.95	0.73	9.61
4	3.85	09:55	8.06	15.70	50.00	35.00	35.05	7.09	88.00	0.50	3.78	3.37	7.15	24.41	17.26	70.79%	95.29	0.70	5.52
5	2.90	09:45	8.04	15.60	50.60	35.40	35.08	7.38	91.00	0.44	3.95	3.82	7.77	17.39	9.62	55.32%	65.24	0.69	6.78
6	2.00	09:40	7.78	14.10	17.60	24.60	24.92	8.50	95.00	0.97	6.35	31.01	37.36	77.68	40.32	51.91%	124.53	1.23	3.09
7	0.85	09:35	7.76	14.20	21.70	15.30	14.32	6.70	92.00	0.73	6.51	25.85	32.36	60.56	28.20	46.57%	97.23	1.29	3.45
8	0.00	10:45	7.20	12.90	0.07	0.05	0.14	8.11	77.00	5.02	5.59	171.67	177.26	233.7	56.48	24.16%	135.33	0.26	2.40
9 MQ										0.00	0.57	0.27	0.84	ND			4.17	0.01	

Results of the Yealm Estuary samples collected on 11 - 11 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:25	8.29	15.30	51.40	36.00	33.32	8.08	96.10	0.61	3.60	13.62	17.22	25.01	7.79	31.15%	72.55	0.24	9.31
2	4.80	10:20	8.29	15.40	50.80	35.60	32.87	8.11	96.60	0.52	3.54	13.35	16.89	27.67	10.78	38.96%	74.76	0.27	6.94
3	4.30	10:15	8.27	15.40	50.40	35.30	32.76	7.92	95.70	0.45	3.63	12.13	15.76	28.80	13.04	45.28%	102.10	0.30	7.83
4	3.85	10:05	8.24	15.00	43.40	30.40	28.45	7.56	87.00	0.52	4.40	31.87	36.27	55.47	19.20	34.61%	146.10	0.29	7.61
5	2.90	10:00	8.23	15.10	43.20	30.30	27.36	7.69	88.00	0.52	5.26	36.04	41.30	65.12	23.82	36.58%	122.70	0.37	5.15
6	2.00	09:45	7.99	13.40	20.60	14.40	12.24	8.86	88.50	0.72	3.69	111.31	115.00	142.71	27.71	19.42%	250.00	0.44	9.20
7	0.85	09:35	7.56	13.30	0.03	0.02	0.22	9.90	91.00	0.64	2.50	164.94	167.44	193.53	26.09	13.48%	214.40	0.37	8.22
8	0.00	10:45	7.60	13.30	0.04	0.03	0.08	10.73	98.10	0.52	1.22	159.20	160.4	198.55	38.13	19.20%	206.60	0.21	5.42
9 MQ										0.02	0.71	ND					4.01	ND	

Results of the Yealm Estuary samples collected on 09 - 01 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:55	8.50	8.50	42.60	21.30	31.27	8.80	94.50	0.59	3.54	40.06	43.60	51.43	7.84	15.23%	68.06	0.13	8.69
2	4.80	10:50	8.50	7.30	37.10	18.50	28.56	9.50	95.00	0.59	3.15	75.62	78.77	102.85	24.08	23.41%	71.90	0.19	2.99
3	4.30	10:45	8.49	7.50	38.50	19.30	31.08	9.00	95.00	0.49	2.34	31.56	33.90	54.80	20.90	38.14%	76.86	0.12	3.68
4	3.85	10:40	8.45	6.90	33.00	16.50	28.03	8.90	92.00	0.45	3.01	41.33	44.34	68.43	24.09	35.20%	66.10	0.20	2.74
5	2.90	10:30	8.40	5.90	28.60	14.30	23.48	10.00	98.00	0.51	3.62	73.65	77.27	105.62	28.35	26.84%	82.31	0.19	2.90
6	2.00	10:15	8.30	6.70	27.80	13.89	21.19	10.00	97.00	0.52	2.44	61.80	64.24	110.87	46.63	42.06%	78.60	0.13	1.69
7	0.85	10:10	8.10	6.30	14.10	7.04	9.87	7.70	69.00	0.82	3.07	143.88	146.95	186.42	39.47	21.17%	94.47	0.06	2.39
8	0.00	11:20	7.90	6.00	0.22	0.10	0.12	11.30	93.00	0.88	2.26	203.30	205.6	231.98	26.42	11.39%	94.54	0.14	3.58
9 MQ										0.02	0.58	0.54		0.88			2.11	ND	

Results of the Yealm Estuary samples collected on 21 - 02 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:15	8.02	7.60	37.20	18.60	29.12	8.80	93.40	0.72	4.26	65.69	69.95	78.89	8.94	11.33%	97.09	0.31	10.86
2	4.80	10:10	7.95	7.70	44.70	22.30	28.29	9.00	95.80	0.71	3.47	73.03	76.50	99.39	22.89	23.03%	69.74	0.34	3.05
3	4.30	10:05	7.94	7.80	44.20	22.10	34.26	9.42	100.0	0.52	3.33	11.92	15.25	27.23	11.98	44.00%	61.42	0.40	5.13
4	3.85	09:55	7.87	7.40	31.50	15.70	25.38	10.50	97.40	0.65	3.15	94.58	97.73	118.17	20.44	17.30%	74.79	0.33	3.66
5	2.90	09:50	7.80	7.40	6.44	3.22	13.17	10.90	95.60	0.82	2.85	147.16	150.01	171.23	21.22	12.39%	91.94	0.30	4.33
6	2.00	09:40	7.90	7.60	26.80	13.40	29.43	9.66	97.60	0.63	3.06	61.52	64.58	74.45	9.87	13.26%	77.01	0.59	7.80
6a	1.45	09:25	7.80	7.50	8.17	4.09	21.99	10.00	94.00	0.58	3.25	76.52	79.77	92.38	12.61	13.65%	80.03	0.47	6.35
6b	1.10	09:30	7.80	7.60	15.10	7.59	9.93	9.30	91.40	0.71	2.45	119.28	121.73	136.30	14.57	10.69%	91.51	0.34	6.28
7	0.85	09:20	7.82	7.40	31.90	15.90	20.51	11.20	97.00	0.67	4.48	66.12	70.60	89.70	19.10	21.29%	86.92	0.56	4.55
8	0.00	10:40	7.30	8.70	0.39	0.19	0.12	12.20	100.50	1.02	4.32	183.90	188.2	215.34	27.12	12.59%	90.92	0.57	3.35
MQ														0.46			1.75		

Results of the Yealm Estuary samples collected on 09 - 04 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:15	8.25	10.90	57.80	40.50	34.97	8.80	99.50	0.62	3.51	4.14	7.65	11.19	3.54	31.64%	69.57	0.34	19.65
2	4.80	10:10	8.26	10.70	58.30	40.80	34.70	8.90	100.10	0.46	3.24	4.58	7.82	11.69	3.87	33.11%	72.98	0.49	18.86
3	4.30	10:05	8.26	10.70	58.20	40.80	34.91	9.20	100.2	0.44	3.32	3.86	7.18	12.59	5.41	42.97%	84.70	0.65	15.66
4	3.85	10:00	8.26	10.90	56.20	39.40	33.64	8.80	97.60	0.54	3.51	10.72	14.23	18.87	4.64	24.59%	76.71	0.45	16.53
5	2.90	09:55	8.24	10.60	56.70	39.70	33.80	9.20	102.0	0.57	3.96	9.42	13.38	20.95	7.57	36.13%	124.01	0.54	16.38
6b	1.10	09:45	8.11	10.60	51.60	36.20	29.32	8.90	82.00	0.59	4.58	32.79	37.37	48.09	10.72	22.29%	112.84	2.23	10.53
7	0.85	11:00	8.33	8.50	12.16	8.59	4.26	6.30	55.00	0.61	3.10	121.86	125.0	169.07	44.11	26.09%	148.59	2.02	3.37
8	0.00	09:05	7.65	9.50	0.05	0.04	0.16	9.30	75.00	2.52	0.75	161.56	162.31	189.79	27.48	14.48%	115.21	1.68	4.19
9 MQ										0.02	0.55	0.15		0.60			1.09	0.05	

Results of the Yealm Estuary samples collected on 19 - 05 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	09:50	8.32	13.50	45.50	31.80	33.88	#	#	0.11	0.49	3.39	3.88	11.16	7.28	65.23%	102.53	#	14.08
2	4.80	09:45	8.32	13.30	45.30	32.90	34.21	#	#	0.23	0.24	1.93	2.17	9.19	7.02	76.39%	83.25	#	11.86
3	4.30	09:40	8.30	13.60	45.00	31.50	34.12	#	#	0.12	0.29	1.86	2.15	9.15	7.00	76.50%	83.40	#	11.91
4	3.85	09:35	8.29	13.60	44.70	31.30	33.56	#	#	0.12	0.34	2.86	3.20	9.75	6.55	67.18%	82.41	#	12.58
5	2.90	09:30	8.26	13.60	44.50	31.00	33.99	#	#	0.08	0.38	2.55	2.93	9.64	6.71	69.61%	79.65	#	11.87
6	2.00	09:22	8.16	14.40	36.00	25.20	26.96	#	#	0.37	2.73	25.50	28.19	34.76	6.57	18.90%	129.63	#	19.73
6A	1.45	09:20	8.10	14.30	28.30	19.80	20.37	#	#	0.32	2.58	50.22	52.80	56.41	3.61	6.40%	153.33	#	42.47
6B	1.10	09:15	8.05	14.20	20.90	14.64	14.48	#	#	0.57	3.10	69.32	72.42	79.60	7.18	9.02%	174.63	#	24.32
7	0.85	09:10	7.81	13.80	4.77	3.34	1.93	#	#	0.90	2.47	110.35	112.82	121.07	8.25	6.81%	210.53	#	25.52
8	0.00	10:10	7.50	13.40	0.68	0.47	0.23	#	#	1.11	2.72	113.11	115.83	129.84	14.01	10.79%	219.53	#	15.67
9 MQ										ND	ND	0.06		ND			2.87		

Results of the Yealm Estuary samples collected on 01 - 08 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:13	7.87	16.30	54.30	38.00	34.27	#	#	0.41	0.82	1.54	2.36	9.48	7.12	75.15%	94.91	#	13.32
2	4.80	10:10	7.88	16.30	54.30	38.00	34.27	#	#	0.21	2.49	1.90	4.39	11.28	6.89	61.06%	104.67	#	15.20
3	4.30	10:05	7.83	16.30	53.90	37.80	34.12	#	#	0.16	0.61	1.42	2.03	9.33	7.30	78.24%	80.19	#	10.98
4	3.85	10:00	7.87	16.40	54.00	37.80	34.04	#	#	0.23	7.21	1.60	8.81	33.10	24.29	73.38%	84.73	#	3.49
5	2.90	09:55	7.92	16.60	53.40	37.40	33.71	#	#	0.14	9.32	1.29	10.61	51.75	41.14	79.50%	99.37	#	2.42
6	2.00	09:47	7.62	17.20	46.70	32.70	28.95	#	#	0.28	1.48	9.04	10.52	23.99	13.47	56.15%	144.17	#	10.70
6A	1.45	09:45	7.23	16.90	31.20	21.90	19.07	#	#	0.67	7.45	39.48	46.93	135.21	88.28	65.29%	195.97	#	2.22
6B	1.10	09:40	6.70	16.50	16.10	11.24	10.08	#	#	1.97	9.79	61.60	71.39	169.54	98.15	57.89%	259.57	#	2.64
7	0.85	09:35	6.20	16.30	0.05	0.03	1.96	#	#	1.45	8.01	58.24	66.25	175.34	109.1	62.22%	334.57	#	3.07
8	0.00	08:45	6.09	16.10	0.02	0.01	0.09	#	#	2.05	10.08	64.15	74.23	158.54	84.31	53.18%	329.17	#	3.90
9 MQ											ND			ND			2.53		

Results of the Yealm Estuary samples collected on 15 - 09 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L-1	Salinity ‰	DO mg L-1	DO %	PO4 µM P	NH4 µM N	NO3 +NO2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L-1	DOC:DON
1	5.35	10:08	7.75	17.40	56.00	39.20	34.96	#	#	0.22	0.45	1.45	1.90	7.05	5.15	73.01%	78.80	#	15.31
2	4.80	10:05	7.75	17.40	55.90	39.10	35.06	#	#	0.27	3.51	1.58	5.09	14.95	9.86	65.95%	72.49	#	7.35
3	4.30	10:00	7.74	17.50	55.80	39.00	34.88	#	#	0.25	0.45	1.65	2.10	7.92	5.82	73.51%	71.77	#	12.33
4	3.85	09:55	7.73	17.40	55.80	39.10	34.81	#	#	0.27	0.69	1.87	2.56	8.21	5.65	68.84%	69.40	#	12.28
5	2.90	09:50	7.71	17.30	55.70	39.00	34.78	#	#	0.33	7.94	2.20	10.14	35.31	25.17	71.28%	78.67	#	3.13
6	2.00	09:45	7.64	17.10	54.80	38.40	34.24	#	#	0.50	2.11	3.65	5.76	13.16	7.40	56.25%	79.35	#	10.72
6A	1.45	09:40	7.42	15.30	20.00	14.01	11.23	#	#	2.70	10.32	112.28	122.6	173.47	50.87	29.32%	106.35	#	2.09
6B	1.10	09:35	7.42	15.10	30.90	21.60	18.74	#	#	2.13	8.51	67.96	76.47	104.05	27.58	26.51%	98.35	#	3.57
6C	1.00	09:33	7.35	14.70	26.80	18.70	10.48	#	#	2.07	7.42	75.47	82.89	123.12	40.23	32.68%	108.65	#	2.70
7	0.85	09:30	7.35	15.20	20.60	14.43	18.12	#	#	1.34	5.64	31.97	37.61	52.88	15.27	28.88%	106.15	#	6.95
8	0.00	08:50	7.15	13.40	0.04	0.03	0.14	#	#	0.61	8.01	142.36	150.37	179.59	29.22	16.27%	91.22	#	3.12
9 MQ												ND		ND			1.75		

Appendix 3.2

Pearson's product moment correlation matrix for the Yealm Estuary samples for different seasons

		SALINITY	PH	BOD	DO	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
SALINITY	Pearson Correlation	1	.046	-.575**	-.559**	-.914**	.064	-.978**	-.972**	-.626**	.450	-.805**	.383	.330
	Sig. (2-tailed)	.	.844	.006	.008	.000	.784	.000	.000	.002	.041	.000	.176	.143
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
PH	Pearson Correlation	.046	1	-.055	.263	-.132	-.527	.086	.076	.063	.158	-.340	.538	-.216
	Sig. (2-tailed)	.844	.	.813	.250	.568	.014	.712	.744	.787	.493	.132	.047	.348
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
BOD	Pearson Correlation	-.575**	-.055	1	.467	.692**	.147	.587**	.633**	.601**	-.291	.775**	.008	-.173
	Sig. (2-tailed)	.006	.813	.	.033	.001	.524	.005	.002	.004	.201	.000	.979	.453
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
DO	Pearson Correlation	-.559**	.263	.467	1	.412	-.256	.660**	.596**	.151	-.524	.455	-.162	.005
	Sig. (2-tailed)	.008	.250	.033	.	.064	.263	.001	.004	.514	.015	.038	.580	.982
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
PO4	Pearson Correlation	-.914**	-.132	.692**	.412	1	.133	.904**	.934**	.737**	-.390	.822**	-.461	-.272
	Sig. (2-tailed)	.000	.568	.001	.064	.	.567	.000	.000	.000	.081	.000	.097	.233
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
NH4	Pearson Correlation	.064	-.527	.147	-.256	.133	1	-.148	-.110	-.012	.094	.142	-.437	-.050
	Sig. (2-tailed)	.784	.014	.524	.263	.567	.	.523	.636	.960	.687	.538	.119	.831
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
NO3	Pearson Correlation	-.978**	.086	.587**	.660**	.904**	-.148	1	.987**	.616**	-.489	.764**	-.399	-.268
	Sig. (2-tailed)	.000	.712	.005	.001	.000	.523	.	.000	.003	.025	.000	.158	.240
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
TDN	Pearson Correlation	-.972**	.076	.633**	.596**	.934**	-.110	.987**	1	.735**	-.375	.770**	-.420	-.354
	Sig. (2-tailed)	.000	.744	.002	.004	.000	.636	.000	.	.000	.094	.000	.135	.116
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
DON	Pearson Correlation	-.626**	.063	.601**	.151	.737**	-.012	.616**	.735**	1	.229	.521	-.293	-.598**
	Sig. (2-tailed)	.002	.787	.004	.514	.000	.960	.003	.000	.	.319	.015	.309	.004
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
DON TDN	Pearson Correlation	.450	.158	-.291	-.524	-.390	.094	-.489	-.375	.229	1	-.446	.086	-.537
	Sig. (2-tailed)	.041	.493	.201	.015	.081	.687	.025	.094	.319	.	.043	.769	.012
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
DOC	Pearson Correlation	-.805**	-.340	.775**	.455	.822**	.142	.764**	.770**	.521	-.446	1	-.372	-.097
	Sig. (2-tailed)	.000	.132	.000	.038	.000	.538	.000	.000	.015	.043	.	.190	.677
	N	21	21	21	21	21	21	21	21	21	21	21	14	21
CHLA	Pearson Correlation	.383	.538	.008	-.162	-.461	-.437	-.399	-.420	-.293	.086	-.372	1	.036
	Sig. (2-tailed)	.176	.047	.979	.580	.097	.119	.158	.135	.309	.769	.190	.	.902
	N	14	14	14	14	14	14	14	14	14	14	14	14	14
DOC DON	Pearson Correlation	.330	-.216	-.173	.005	-.272	-.050	-.268	-.354	-.598**	-.537	-.097	.036	1
	Sig. (2-tailed)	.143	.348	.453	.982	.233	.831	.240	.116	.004	.012	.677	.902	.
	N	21	21	21	21	21	21	21	21	21	21	21	14	21

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations matrix for the Yealm Estuary spring 2002 samples

		SALINITY	PH	BOD	DO	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
SALINITY	Pearson Correlation	1	.899**	.445	-.180	-.933**	.189	-.935**	-.958**	-.712**	.482	-.690**	.573**	.473
	Sig. (2-tailed)	.	.000	.038	.422	.000	.400	.000	.000	.000	.023	.000	.005	.026
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
PH	Pearson Correlation	.899**	1	.561**	.014	-.754**	.328	-.789**	-.869**	-.718**	.383	-.575**	.571**	.411
	Sig. (2-tailed)	.000	.	.007	.951	.000	.137	.000	.000	.000	.078	.005	.005	.057
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
BOD	Pearson Correlation	.445	.561**	1	.575**	-.389	.150	-.447	-.397	-.230	.220	-.259	.540**	.225
	Sig. (2-tailed)	.038	.007	.	.005	.074	.506	.037	.067	.303	.326	.244	.010	.314
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
DO	Pearson Correlation	-.180	.014	.575**	1	.212	-.045	.080	.136	.161	.102	.296	.149	-.083
	Sig. (2-tailed)	.422	.951	.005	.	.344	.842	.723	.547	.474	.651	.181	.507	.713
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
PO4	Pearson Correlation	-.933**	-.754**	-.389	.212	1	-.132	.850**	.932**	.758**	-.298	.643**	-.610**	-.584**
	Sig. (2-tailed)	.000	.000	.074	.344	.	.559	.000	.000	.000	.177	.001	.003	.004
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
NH4	Pearson Correlation	.189	.328	.150	-.045	-.132	1	.039	-.144	-.349	-.089	-.270	.204	-.211
	Sig. (2-tailed)	.400	.137	.506	.842	.559	.	.864	.523	.112	.694	.224	.362	.345
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
NO3	Pearson Correlation	-.935**	-.789**	-.447	.080	.850**	.039	1	.888**	.499	-.642**	.588**	-.537	-.459
	Sig. (2-tailed)	.000	.000	.037	.723	.000	.864	.	.000	.018	.001	.004	.010	.032
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
TDN	Pearson Correlation	-.958**	-.869**	-.397	.136	.932**	-.144	.888**	1	.842**	-.321	.515	-.586**	-.627**
	Sig. (2-tailed)	.000	.000	.067	.547	.000	.523	.000	.	.000	.145	.014	.004	.002
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
DON	Pearson Correlation	-.712**	-.718**	-.230	.161	.758**	-.349	.499	.842**	1	.142	.292	-.480	-.627**
	Sig. (2-tailed)	.000	.000	.303	.474	.000	.112	.018	.000	.	.529	.188	.024	.002
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
DONTDN	Pearson Correlation	.482	.383	.220	.102	-.298	-.089	-.642**	-.321	.142	1	-.579**	.119	-.240
	Sig. (2-tailed)	.023	.078	.326	.651	.177	.694	.001	.145	.529	.	.005	.597	.282
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
DOC	Pearson Correlation	-.690**	-.575**	-.259	.296	.643**	-.270	.588**	.515	.292	-.579**	1	-.231	-.033
	Sig. (2-tailed)	.000	.005	.244	.181	.001	.224	.004	.014	.188	.005	.	.301	.884
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
CHLA	Pearson Correlation	.573**	.571**	.540**	.149	-.610**	.204	-.537	-.586**	-.480	.119	-.231	1	.375
	Sig. (2-tailed)	.005	.005	.010	.507	.003	.362	.010	.004	.024	.597	.301	.	.085
	N	22	22	22	22	22	22	22	22	22	22	22	22	22
DOC DON	Pearson Correlation	.473	.411	.225	-.083	-.584**	-.211	-.459	-.627**	-.627**	-.240	-.033	.375	1
	Sig. (2-tailed)	.026	.057	.314	.713	.004	.345	.032	.002	.002	.282	.884	.085	.
	N	22	22	22	22	22	22	22	22	22	22	22	22	22

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations matrix for the Yealm Estuary summer 2002 samples.

		SALINITY	PH	DO	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
SALINITY	Pearson Correlation	1	.855**	-.698**	-.505	.184	-.954**	-.963**	-.806**	.758**	-.782**	.172	.366
	Sig. (2-tailed)	.	.000	.003	.046	.495	.000	.000	.000	.001	.000	.524	.164
	N	16	16	16	16	16	16	16	16	16	16	16	16
PH	Pearson Correlation	.855**	1	-.451	-.699**	-.064	-.808**	-.851**	-.848**	.415	-.472	-.110	.560
	Sig. (2-tailed)	.000	.	.079	.003	.813	.000	.000	.000	.110	.065	.684	.024
	N	16	16	16	16	16	16	16	16	16	16	16	16
DO	Pearson Correlation	-.698**	-.451	1	.048	-.661**	.777**	.734**	.439	-.746**	.760**	-.442	.028
	Sig. (2-tailed)	.003	.079	.	.859	.005	.000	.001	.089	.001	.001	.087	.917
	N	16	16	16	16	16	16	16	16	16	16	16	16
PO4	Pearson Correlation	-.505	-.699**	.048	1	.331	.542	.606	.722**	-.285	.116	-.159	-.522
	Sig. (2-tailed)	.046	.003	.859	.	.210	.030	.013	.002	.285	.670	.555	.038
	N	16	16	16	16	16	16	16	16	16	16	16	16
NH4	Pearson Correlation	.184	-.064	-.661**	.331	1	-.335	-.224	.232	.401	-.374	.651**	-.497
	Sig. (2-tailed)	.495	.813	.005	.210	.	.204	.405	.388	.124	.154	.006	.050
	N	16	16	16	16	16	16	16	16	16	16	16	16
NO3	Pearson Correlation	-.954**	-.808**	.777**	.542	-.335	1	.990**	.756**	-.816**	.811**	-.385	-.242
	Sig. (2-tailed)	.000	.000	.000	.030	.204	.	.000	.001	.000	.000	.141	.366
	N	16	16	16	16	16	16	16	16	16	16	16	16
TDN	Pearson Correlation	-.963**	-.851**	.734**	.606	-.224	.990**	1	.839**	-.771**	.792**	-.307	-.357
	Sig. (2-tailed)	.000	.000	.001	.013	.405	.000	.	.000	.000	.000	.247	.175
	N	16	16	16	16	16	16	16	16	16	16	16	16
DON	Pearson Correlation	-.806**	-.848**	.439	.722**	.232	.756**	.839**	1	-.434	.566	.047	-.743**
	Sig. (2-tailed)	.000	.000	.089	.002	.388	.001	.000	.	.093	.022	.863	.001
	N	16	16	16	16	16	16	16	16	16	16	16	16
DON TDN	Pearson Correlation	.758**	.415	-.746**	-.285	.401	-.816**	-.771**	-.434	1	-.744**	.581	-.109
	Sig. (2-tailed)	.001	.110	.001	.285	.124	.000	.000	.093	.	.001	.018	.687
	N	16	16	16	16	16	16	16	16	16	16	16	16
DOC	Pearson Correlation	-.782**	-.472	.760**	.116	-.374	.811**	.792**	.566	-.744**	1	-.285	-.032
	Sig. (2-tailed)	.000	.065	.001	.670	.154	.000	.000	.022	.001	.	.285	.906
	N	16	16	16	16	16	16	16	16	16	16	16	16
CHLA	Pearson Correlation	.172	-.110	-.442	-.159	.651**	-.385	-.307	.047	.581	-.285	1	-.352
	Sig. (2-tailed)	.524	.684	.087	.555	.006	.141	.247	.863	.018	.285	.	.181
	N	16	16	16	16	16	16	16	16	16	16	16	16
DOC DON	Pearson Correlation	.366	.560	.028	-.522	-.497	-.242	-.357	-.743**	-.109	-.032	-.352	1
	Sig. (2-tailed)	.164	.024	.917	.038	.050	.366	.175	.001	.687	.906	.181	.
	N	16	16	16	16	16	16	16	16	16	16	16	16

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations matrix for the Yealm Estuary autumn 2002 samples.

		SALINITY	PH	DIN	DO	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
SALINITY	Pearson Correlation	1	.586	-.944**	-.531	-.809**	.109	-.945**	-.948**	-.413	.479	-.744**	-.038	.295
	Sig. (2-tailed)	.	.011	.000	.023	.000	.666	.000	.000	.088	.044	.000	.881	.235
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
PH	Pearson Correlation	.586	1	-.584	-.610**	-.735**	-.311	-.579	-.514	.143	.525	-.465	-.740**	-.113
	Sig. (2-tailed)	.011	.	.011	.007	.001	.209	.012	.029	.571	.025	.052	.000	.654
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
DIN	Pearson Correlation	-.944**	-.584	1	.515	.888**	-.089	1.000**	.985**	.337	-.615**	.737**	.021	-.239
	Sig. (2-tailed)	.000	.011	.	.029	.000	.726	.000	.000	.172	.007	.000	.935	.340
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
DO	Pearson Correlation	-.531	-.610**	.515	1	.462	.200	.512	.490	.076	-.280	.288	.500	-.200
	Sig. (2-tailed)	.023	.007	.029	.	.054	.426	.030	.039	.766	.261	.247	.035	.426
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
PO4	Pearson Correlation	-.809**	-.735**	.888**	.462	1	.215	.885**	.842**	.117	-.676**	.706**	.246	.007
	Sig. (2-tailed)	.000	.001	.000	.054	.	.392	.000	.000	.645	.002	.001	.326	.977
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
NH4	Pearson Correlation	.109	-.311	-.089	.200	.215	1	-.101	-.133	-.283	-.230	.079	.531	.318
	Sig. (2-tailed)	.666	.209	.726	.426	.392	.	.690	.598	.255	.359	.755	.023	.199
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
NO3	Pearson Correlation	-.945**	-.579	1.000**	.512	.885**	-.101	1	.986**	.340	-.612**	.735**	.014	-.242
	Sig. (2-tailed)	.000	.012	.000	.030	.000	.690	.	.000	.168	.007	.001	.956	.333
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
TDN	Pearson Correlation	-.948**	-.514	.985**	.490	.842**	-.133	.986**	1	.492	-.501	.717**	-.065	-.370
	Sig. (2-tailed)	.000	.029	.000	.039	.000	.598	.000	.	.038	.034	.001	.797	.131
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
DON	Pearson Correlation	-.413	.143	.337	.076	.117	-.283	.340	.492	1	.377	.194	-.467	-.824**
	Sig. (2-tailed)	.088	.571	.172	.766	.645	.255	.168	.038	.	.123	.440	.051	.000
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
DON TDN	Pearson Correlation	.479	.525	-.615**	-.280	-.676**	-.230	-.612**	-.501	.377	1	-.603**	-.330	-.483
	Sig. (2-tailed)	.044	.025	.007	.261	.002	.359	.007	.034	.123	.	.008	.181	.042
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
DOC	Pearson Correlation	-.744**	-.465	.737**	.288	.706**	.079	.735**	.717**	.194	-.603**	1	.038	.123
	Sig. (2-tailed)	.000	.052	.000	.247	.001	.755	.001	.001	.440	.008	.	.882	.628
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
CHLA	Pearson Correlation	-.038	-.740**	.021	.500	.246	.531	.014	-.065	-.467	-.330	.038	1	.314
	Sig. (2-tailed)	.881	.000	.935	.035	.326	.023	.956	.797	.051	.181	.882	.	.205
	N	18	18	18	18	18	18	18	18	18	18	18	18	18
DOC DON	Pearson Correlation	.295	-.113	-.239	-.200	.007	.318	-.242	-.370	-.824**	-.483	.123	.314	1
	Sig. (2-tailed)	.235	.654	.340	.426	.977	.199	.333	.131	.000	.042	.628	.205	.
	N	18	18	18	18	18	18	18	18	18	18	18	18	18

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations matrix for the Yealm Estuary winter 2003 samples .

		SALINITY	PH	DIN	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	CHLA	DOC DON
SALINITY	Pearson Correlation	1	.801**	-.980**	-.700**	-.067	-.980**	-.963**	-.647**	.660**	-.824**	-.704	.016
	Sig. (2-tailed)	.	.000	.000	.001	.793	.000	.000	.004	.003	.000	.051	.951
	N	18	18	18	18	18	18	18	18	18	18	8	18
PH	Pearson Correlation	.801**	1	-.777**	-.767**	-.070	-.777**	-.713**	-.215	.628**	-.718**	-.395	-.085
	Sig. (2-tailed)	.000	.	.000	.000	.783	.000	.001	.391	.005	.001	.332	.738
	N	18	18	18	18	18	18	18	18	18	18	8	18
DIN	Pearson Correlation	-.980**	-.777**	1	.798**	.098	1.000**	.993**	.719**	-.668**	.723**	.709	-.111
	Sig. (2-tailed)	.000	.000	.	.000	.699	.000	.000	.001	.002	.001	.049	.661
	N	18	18	18	18	18	18	18	18	18	18	8	18
PO4	Pearson Correlation	-.700**	-.767**	.798**	1	.089	.798**	.779**	.492	-.555	.332	.360	-.247
	Sig. (2-tailed)	.001	.000	.000	.	.726	.000	.000	.038	.017	.178	.382	.323
	N	18	18	18	18	18	18	18	18	18	18	8	18
NH4	Pearson Correlation	-.067	-.070	.098	.089	1	.070	.085	-.004	-.675**	.237	-.143	.259
	Sig. (2-tailed)	.793	.783	.699	.726	.	.783	.739	.989	.002	.345	.736	.300
	N	18	18	18	18	18	18	18	18	18	18	8	18
NO3	Pearson Correlation	-.980**	-.777**	1.000**	.798**	.070	1	.993**	.721**	-.651**	.718**	.702	-.119
	Sig. (2-tailed)	.000	.000	.000	.000	.783	.	.000	.001	.003	.001	.052	.639
	N	18	18	18	18	18	18	18	18	18	18	8	18
TDN	Pearson Correlation	-.963**	-.713**	.993**	.779**	.085	.993**	1	.796**	-.619**	.677**	.727	-.193
	Sig. (2-tailed)	.000	.001	.000	.000	.739	.000	.	.000	.006	.002	.041	.442
	N	18	18	18	18	18	18	18	18	18	18	8	18
DON	Pearson Correlation	-.647**	-.215	.719**	.492	-.004	.721**	.796**	1	-.214	.282	.733	-.571
	Sig. (2-tailed)	.004	.391	.001	.038	.989	.001	.000	.	.394	.257	.039	.013
	N	18	18	18	18	18	18	18	18	18	18	8	18
DON TDN	Pearson Correlation	.660**	.628**	-.668**	-.555	-.675**	-.651**	-.619**	-.214	1	-.671**	-.626	-.420
	Sig. (2-tailed)	.003	.005	.002	.017	.002	.003	.006	.394	.	.002	.097	.083
	N	18	18	18	18	18	18	18	18	18	18	8	18
DOC	Pearson Correlation	-.824**	-.718**	.723**	.332	.237	.718**	.677**	.282	-.671**	1	.737	.326
	Sig. (2-tailed)	.000	.001	.001	.178	.345	.001	.002	.257	.002	.	.037	.187
	N	18	18	18	18	18	18	18	18	18	18	8	18
CHLA	Pearson Correlation	-.704	-.395	.709	.360	-.143	.702	.727	.733	-.626	.737	1	-.873**
	Sig. (2-tailed)	.051	.332	.049	.382	.736	.052	.041	.039	.097	.037	.	.005
	N	8	8	8	8	8	8	8	8	8	8	8	8
DOC DON	Pearson Correlation	.016	-.085	-.111	-.247	.259	-.119	-.193	-.571	-.420	.326	-.873**	1
	Sig. (2-tailed)	.951	.738	.661	.323	.300	.639	.442	.013	.083	.187	.005	.
	N	18	18	18	18	18	18	18	18	18	18	8	18

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations matrix for the Yealm Estuary spring 2003 samples.

		SALINITY	PH	DIN	PO4	NH4	NO3	TDN	DON	DON TDN	DOC	DOC DON
SALINITY	Pearson Correlation	1	.873**	-.897**	-.768**	-.700**	-.889**	-.945**	-.744**	.682**	-.696**	.641**
	Sig. (2-tailed)	.	.000	.000	.000	.000	.000	.000	.000	.001	.000	.002
	N	21	21	21	21	21	21	21	21	21	21	21
PH	Pearson Correlation	.873**	1	-.603**	-.646**	-.577**	-.588**	-.804**	-.843**	.344	-.920**	.503
	Sig. (2-tailed)	.000	.	.004	.002	.006	.005	.000	.000	.126	.000	.020
	N	21	21	21	21	21	21	21	21	21	21	21
DIN	Pearson Correlation	-.897**	-.603**	1	.746**	.707**	.998**	.910**	.535	-.818**	.361	-.659**
	Sig. (2-tailed)	.000	.004	.	.000	.000	.000	.000	.012	.000	.108	.001
	N	21	21	21	21	21	21	21	21	21	21	21
PO4	Pearson Correlation	-.768**	-.646**	.746**	1	.676**	.731**	.771**	.587**	-.672**	.492	-.577**
	Sig. (2-tailed)	.000	.002	.000	.	.001	.000	.000	.005	.001	.023	.006
	N	21	21	21	21	21	21	21	21	21	21	21
NH4	Pearson Correlation	-.700**	-.577**	.707**	.676**	1	.662**	.819**	.735**	-.454	.495	-.935**
	Sig. (2-tailed)	.000	.006	.000	.001	.	.001	.000	.000	.039	.022	.000
	N	21	21	21	21	21	21	21	21	21	21	21
NO3	Pearson Correlation	-.889**	-.588**	.998**	.731**	.662**	1	.893**	.503	-.827**	.339	-.616**
	Sig. (2-tailed)	.000	.005	.000	.000	.001	.	.000	.020	.000	.132	.003
	N	21	21	21	21	21	21	21	21	21	21	21
TDN	Pearson Correlation	-.945**	-.804**	.910**	.771**	.819**	.893**	1	.837**	-.596**	.669**	-.769**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.	.000	.004	.001	.000
	N	21	21	21	21	21	21	21	21	21	21	21
DON	Pearson Correlation	-.744**	-.843**	.535	.587**	.735**	.503	.837**	1	-.134	.888**	-.697**
	Sig. (2-tailed)	.000	.000	.012	.005	.000	.020	.000	.	.564	.000	.000
	N	21	21	21	21	21	21	21	21	21	21	21
DON TDN	Pearson Correlation	.682**	.344	-.818**	-.672**	-.454	-.827**	-.596**	-.134	1	-.050	.407
	Sig. (2-tailed)	.001	.126	.000	.001	.039	.000	.004	.564	.	.828	.067
	N	21	21	21	21	21	21	21	21	21	21	21
DOC	Pearson Correlation	-.696**	-.920**	.361	.492	.495	.339	.669**	.888**	-.050	1	-.406
	Sig. (2-tailed)	.000	.000	.108	.023	.022	.132	.001	.000	.828	.	.067
	N	21	21	21	21	21	21	21	21	21	21	21
DOC DON	Pearson Correlation	.641**	.503	-.659**	-.577**	-.935**	-.616**	-.769**	-.697**	.407	-.406	1
	Sig. (2-tailed)	.002	.020	.001	.006	.000	.003	.000	.000	.067	.067	.
	N	21	21	21	21	21	21	21	21	21	21	21

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations matrix for the Yealm Estuary summer 2003 samples .

Appendix 3.3

The River Yealm flow data close to the tidal limit during January 2002 – October 2003

Date	FQ (m3/s)	Date	FQ (m3/s)	Date	FQ (m3/s)	Date	FQ (m3/s)
01/01/2002	1.171	27/02/2002	3.350	25/04/2002	0.450	21/06/2002	2.130
02/01/2002	1.156	28/02/2002	3.230	26/04/2002	0.620	22/06/2002	1.610
03/01/2002	0.694	01/03/2002	2.560	27/04/2002	0.980	23/06/2002	1.290
04/01/2002	0.505	02/03/2002	2.220	28/04/2002	0.930	24/06/2002	1.100
05/01/2002	0.490	03/03/2002	1.990	29/04/2002	0.610	25/06/2002	1.050
06/01/2002	0.498	04/03/2002	1.850	30/04/2002	4.830	26/06/2002	0.919
07/01/2002	0.475	05/03/2002	1.680	01/05/2002	1.260	27/06/2002	0.860
08/01/2002	0.449	06/03/2002	1.550	02/05/2002	0.720	28/06/2002	0.810
09/01/2002	0.489	07/03/2002	1.360	03/05/2002	0.610	29/06/2002	0.750
10/01/2002	0.481	08/03/2002	1.240	04/05/2002	0.620	30/06/2002	0.840
11/01/2002	0.470	09/03/2002	1.170	05/05/2002	0.600	01/07/2002	0.780
12/01/2002	0.487	10/03/2002	1.180	06/05/2002	0.590	02/07/2002	2.590
13/01/2002	0.978	11/03/2002	1.610	07/05/2002	0.580	03/07/2002	1.640
14/01/2002	1.156	12/03/2002	1.740	08/05/2002	0.550	04/07/2002	1.290
15/01/2002	0.851	13/03/2002	1.760	09/05/2002	0.560	05/07/2002	1.400
16/01/2002	0.683	14/03/2002	1.230	10/05/2002	0.510	06/07/2002	0.919
17/01/2002	2.820	15/03/2002	1.610	11/05/2002	0.600	07/07/2002	1.250
18/01/2002	2.260	16/03/2002	1.090	12/05/2002	0.890	08/07/2002	2.580
19/01/2002	1.660	17/03/2002	1.960	13/05/2002	2.070	09/07/2002	1.740
20/01/2002	4.060	18/03/2002	4.710	14/05/2002	0.910	10/07/2002	1.430
21/01/2002	2.690	19/03/2002	4.140	15/05/2002	0.700	11/07/2002	1.230
22/01/2002	2.630	20/03/2002	5.640	16/05/2002	0.820	12/07/2002	1.210
23/01/2002	5.710	21/03/2002	3.210	17/05/2002	3.680	13/07/2002	1.030
24/01/2002	3.040	22/03/2002	2.550	18/05/2002	2.020	14/07/2002	0.970
25/01/2002	7.720	23/03/2002	2.310	19/05/2002	1.640	15/07/2002	0.880
26/01/2002	11.170	24/03/2002	2.160	20/05/2002	1.700	16/07/2002	0.850
27/01/2002	12.230	25/03/2002	1.860	21/05/2002	5.180	17/07/2002	0.760
28/01/2002	6.170	26/03/2002	1.690	22/05/2002	2.910	18/07/2002	0.760
29/01/2002	5.100	27/03/2002	1.450	23/05/2002	5.290	19/07/2002	0.730
30/01/2002	5.140	28/03/2002	1.330	24/05/2002	5.090	20/07/2002	0.660
31/01/2002	5.180	29/03/2002	1.180	25/05/2002	3.320	21/07/2002	0.640
01/02/2002	5.220	30/03/2002	1.060	26/05/2002	4.380	22/07/2002	0.600
02/02/2002	8.340	31/03/2002	1.620	27/05/2002	3.830	23/07/2002	0.630
03/02/2002	6.070	01/04/2002	1.650	28/05/2002	5.390	24/07/2002	0.570
04/02/2002	16.330	02/04/2002	1.170	29/05/2002	3.350	25/07/2002	0.580
05/02/2002	7.140	03/04/2002	1.140	30/05/2002	2.800	26/07/2002	0.550
06/02/2002	5.080	04/04/2002	1.070	31/05/2002	2.340	27/07/2002	0.560
07/02/2002	5.030	05/04/2002	0.905	01/06/2002	1.970	28/07/2002	0.510
08/02/2002	4.310	06/04/2002	0.878	02/06/2002	1.730	29/07/2002	0.480
09/02/2002	3.920	07/04/2002	0.760	03/06/2002	1.720	30/07/2002	0.519
10/02/2002	3.660	08/04/2002	0.670	04/06/2002	1.520	31/07/2002	0.540
11/02/2002	7.430	09/04/2002	0.620	05/06/2002	1.310	01/08/2002	0.460
12/02/2002	4.480	10/04/2002	0.590	06/06/2002	1.160	02/08/2002	0.430
13/02/2002	3.590	11/04/2002	0.620	07/06/2002	1.840	03/08/2002	0.410
14/02/2002	3.030	12/04/2002	0.630	08/06/2002	1.470	04/08/2002	0.390
15/02/2002	2.760	13/04/2002	0.580	09/06/2002	3.270	05/08/2002	0.390
16/02/2002	2.440	14/04/2002	0.570	10/06/2002	1.610	06/08/2002	0.370
17/02/2002	2.220	15/04/2002	0.520	11/06/2002	1.390	07/08/2002	0.380
18/02/2002	1.990	16/04/2002	0.550	12/06/2002	1.400	08/08/2002	0.450
19/02/2002	2.450	17/04/2002	0.650	13/06/2002	1.260	09/08/2002	0.490
20/02/2002	3.170	18/04/2002	0.590	14/06/2002	1.310	10/08/2002	0.410
21/02/2002	1.860	19/04/2002	0.560	15/06/2002	4.110	11/08/2002	0.450
22/02/2002	1.820	20/04/2002	0.510	16/06/2002	3.890	12/08/2002	0.400
23/02/2002	1.610	21/04/2002	0.500	17/06/2002	2.390	13/08/2002	0.350
24/02/2002	1.540	22/04/2002	0.480	18/06/2002	1.840	14/08/2002	0.340
25/02/2002	8.740	23/04/2002	0.430	19/06/2002	1.560	15/08/2002	0.340
26/02/2002	5.100	24/04/2002	0.420	20/06/2002	1.420	16/08/2002	0.310

Date	FQ (m3/s)	Date	FQ (m3/s)	Date	FQ (m3/s)	Date	FQ (m3/s)
17/08/2002	0.320	13/10/2002	1.391	09/12/2002	3.26	04/02/2003	1.84
18/08/2002	0.370	14/10/2002	0.461	10/12/2002	3.13	05/02/2003	1.72
19/08/2002	0.310	15/10/2002	2.642	11/12/2002	3.1	06/02/2003	2.17
20/08/2002	0.300	16/10/2002	0.588	12/12/2002	4.05	07/02/2003	1.77
21/08/2002	0.290	17/10/2002	0.368	13/12/2002	3.44	08/02/2003	2.93
22/08/2002	0.280	18/10/2002	0.336	14/12/2002	5.33	09/02/2003	3.18
23/08/2002	0.280	19/10/2002	0.282	15/12/2002	4.39	10/02/2003	5.1
24/08/2002	0.270	20/10/2002	0.335	16/12/2002	3.94	11/02/2003	3.83
25/08/2002	0.260	21/10/2002	0.661	17/12/2002	3.75	12/02/2003	3.2
26/08/2002	0.250	22/10/2002	1.092	18/12/2002	3.56	13/02/2003	2.88
27/08/2002	0.250	23/10/2002	0.466	19/12/2002	3.42	14/02/2003	2.52
28/08/2002	0.250	24/10/2002	0.395	20/12/2002	4.17	15/02/2003	2.23
29/08/2002	0.255	25/10/2002	1.047	21/12/2002	10.5	16/02/2003	1.98
30/08/2002	0.270	26/10/2002	1.635	22/12/2002	6.75	17/02/2003	1.8
31/08/2002	0.260	27/10/2002	1.032	23/12/2002	6.4	18/02/2003	1.63
01/09/2002	0.240	28/10/2002	0.575	24/12/2002	5.5	19/02/2003	1.55
02/09/2002	0.240	29/10/2002	0.510	25/12/2002	5.51	20/02/2003	1.51
03/09/2002	0.240	30/10/2002	0.476	26/12/2002	6.92	21/02/2003	1.34
04/09/2002	0.260	31/10/2002	0.539	27/12/2002	5.81	22/02/2003	1.37
05/09/2002	0.240	01/11/2002	0.952	28/12/2002	5.56	23/02/2003	1.33
06/09/2002	0.240	02/11/2002	3.83	29/12/2002	6.46	24/02/2003	1.17
07/09/2002	0.250	03/11/2002	1.21	30/12/2002	5.99	25/02/2003	1.14
08/09/2002	0.240	04/11/2002	1.1	31/12/2002	9.66	26/02/2003	1.58
09/09/2002	0.260	05/11/2002	4.82	01/01/2003	17.1	27/02/2003	2.51
10/09/2002	0.260	06/11/2002	4.52	02/01/2003	9.54	28/02/2003	5.52
11/09/2002	0.230	07/11/2002	2.92	03/01/2003	6.79	01/03/2003	2.86
12/09/2002	0.220	08/11/2002	5.15	04/01/2003	5.38	02/03/2003	2.37
13/09/2002	0.212	09/11/2002	3.7	05/01/2003	4.49	03/03/2003	3.05
14/09/2002	0.204	10/11/2002	3.98	06/01/2003	3.78	04/03/2003	3.22
15/09/2002	0.201	11/11/2002	3.69	07/01/2003	3.24	05/03/2003	4.26
16/09/2002	0.199	12/11/2002	4.52	08/01/2003	2.72	06/03/2003	3.08
17/09/2002	0.193	13/11/2002	7.95	09/01/2003	2.31	07/03/2003	3.91
18/09/2002	0.187	14/11/2002	6.95	10/01/2003	2	08/03/2003	2.97
19/09/2002	0.187	15/11/2002	5.77	11/01/2003	1.7	09/03/2003	2.7
20/09/2002	0.185	16/11/2002	5.16	12/01/2003	1.51	10/03/2003	2.45
21/09/2002	0.182	17/11/2002	4.81	13/01/2003	1.42	11/03/2003	2.29
22/09/2002	0.182	18/11/2002	4.67	14/01/2003	1.32	12/03/2003	1.97
23/09/2002	0.177	19/11/2002	5.16	15/01/2003	1.34	13/03/2003	1.76
24/09/2002	0.173	20/11/2002	5.85	16/01/2003	1.19	14/03/2003	1.59
25/09/2002	0.172	21/11/2002	6.63	17/01/2003	1.38	15/03/2003	1.47
26/09/2002	0.174	22/11/2002	6.65	18/01/2003	3.77	16/03/2003	1.36
27/09/2002	0.175	23/11/2002	6.14	19/01/2003	4.07	17/03/2003	1.28
28/09/2002	0.171	24/11/2002	5.46	20/01/2003	7.21	18/03/2003	1.19
29/09/2002	0.168	25/11/2002	4.94	21/01/2003	4.56	19/03/2003	1.13
30/09/2002	0.168	26/11/2002	4.62	22/01/2003	3.42	20/03/2003	1.07
01/10/2002	0.182	27/11/2002	5.3	23/01/2003	2.99	21/03/2003	1.01
02/10/2002	0.207	28/11/2002	4.36	24/01/2003	2.66	22/03/2003	0.96
03/10/2002	0.212	29/11/2002	3.86	25/01/2003	2.56	23/03/2003	0.944
04/10/2002	0.186	30/11/2002	4.61	26/01/2003	2.2	24/03/2003	0.896
05/10/2002	0.179	01/12/2002	4.7	27/01/2003	2	25/03/2003	0.848
06/10/2002	0.188	02/12/2002	4.38	28/01/2003	1.95	26/03/2003	0.812
07/10/2002	0.199	03/12/2002	4.28	29/01/2003	1.75	27/03/2003	0.767
08/10/2002	0.187	04/12/2002	4.17	30/01/2003	2.07	28/03/2003	0.752
09/10/2002	0.170	05/12/2002	3.79	31/01/2003	1.67	29/03/2003	0.729
10/10/2002	0.166	06/12/2002	3.59	01/02/2003	1.94	30/03/2003	0.691
11/10/2002	0.185	07/12/2002	3.44	02/02/2003	1.92	31/03/2003	0.686
12/10/2002	0.195	08/12/2002	3.31	03/02/2003	2.47	01/04/2003	0.706

Date	FQ (m3/s)	Date	FQ (m3/s)	Date	FQ (m3/s)	Date	FQ (m3/s)
02/04/2003	0.653	29/05/2003	0.624	25/07/2003	2.09	20/09/2003	0.186
03/04/2003	0.619	30/05/2003	0.598	26/07/2003	0.736	21/09/2003	0.189
04/04/2003	0.597	31/05/2003	0.582	27/07/2003	0.482	22/09/2003	0.223
05/04/2003	0.578	01/06/2003	0.598	28/07/2003	1.07	23/09/2003	0.192
06/04/2003	0.563	02/06/2003	0.584	29/07/2003	2.08	24/09/2003	0.203
07/04/2003	0.561	03/06/2003	0.544	30/07/2003	0.958	25/09/2003	0.19
08/04/2003	0.527	04/06/2003	0.585	31/07/2003	1.23	26/09/2003	0.173
09/04/2003	0.511	05/06/2003	0.729	01/08/2003	2.64	27/09/2003	0.179
10/04/2003	0.494	06/06/2003	2.38	02/08/2003	0.925	28/09/2003	0.174
11/04/2003	0.5	07/06/2003	1.38	03/08/2003	0.762	29/09/2003	0.186
12/04/2003	0.484	08/06/2003	1.36	04/08/2003	0.711	30/09/2003	0.185
13/04/2003	0.496	09/06/2003	1.2	05/08/2003	0.637	01/10/2003	0.179
14/04/2003	0.482	10/06/2003	1.2	06/08/2003	0.604	02/10/2003	0.19
15/04/2003	0.453	11/06/2003	0.949	07/08/2003	0.582	03/10/2003	0.18
16/04/2003	0.433	12/06/2003	0.855	08/08/2003	0.555	04/10/2003	0.169
17/04/2003	0.41	13/06/2003	0.811	09/08/2003	0.528	05/10/2003	0.165
18/04/2003	0.402	14/06/2003	0.785	10/08/2003	0.498	06/10/2003	0.176
19/04/2003	0.401	15/06/2003	0.738	11/08/2003	0.476	07/10/2003	0.171
20/04/2003	0.409	16/06/2003	0.704	12/08/2003	0.455	08/10/2003	0.175
21/04/2003	0.407	17/06/2003	0.673	13/08/2003	0.436	09/10/2003	0.16
22/04/2003	0.399	18/06/2003	0.679	14/08/2003	0.397	10/10/2003	0.173
23/04/2003	0.411	19/06/2003	0.651	15/08/2003	0.413	11/10/2003	0.182
24/04/2003	0.455	20/06/2003	0.596	16/08/2003	0.403	12/10/2003	0.166
25/04/2003	0.551	21/06/2003	0.57	17/08/2003	0.387	13/10/2003	0.183
26/04/2003	0.444	22/06/2003	0.65	18/08/2003	0.4	14/10/2003	0.178
27/04/2003	1.16	23/06/2003	0.572	19/08/2003	0.377	15/10/2003	0.176
28/04/2003	2.53	24/06/2003	0.524	20/08/2003	0.368	16/10/2003	0.164
29/04/2003	0.866	25/06/2003	0.504	21/08/2003	0.353	17/10/2003	0.151
30/04/2003	0.852	26/06/2003	0.502	22/08/2003	0.344	18/10/2003	0.151
01/05/2003	1.75	27/06/2003	0.53	23/08/2003	0.335	19/10/2003	0.145
02/05/2003	2.31	28/06/2003	0.465	24/08/2003	0.324	20/10/2003	0.152
03/05/2003	1.04	29/06/2003	0.545	25/08/2003	0.319	21/10/2003	0.156
04/05/2003	0.855	30/06/2003	0.559	26/08/2003	0.374	22/10/2003	0.901
05/05/2003	0.806	01/07/2003	0.556	27/08/2003	0.322	23/10/2003	0.517
06/05/2003	0.705	02/07/2003	0.475	28/08/2003	0.3	24/10/2003	0.277
07/05/2003	0.674	03/07/2003	0.417	29/08/2003	0.331	25/10/2003	0.24
08/05/2003	0.629	04/07/2003	0.39	30/08/2003	0.379	26/10/2003	0.215
09/05/2003	0.602	05/07/2003	0.38	31/08/2003	0.322	27/10/2003	0.2
10/05/2003	0.64	06/07/2003	0.361	01/09/2003	0.306	28/10/2003	0.218
11/05/2003	0.574	07/07/2003	0.366	02/09/2003	0.292	29/10/2003	0.618
12/05/2003	0.578	08/07/2003	0.37	03/09/2003	0.267	30/10/2003	2.3
13/05/2003	0.532	09/07/2003	0.359	04/09/2003	0.247	31/10/2003	0.677
14/05/2003	0.51	10/07/2003	0.339	05/09/2003	0.243	01/11/2003	1.34
15/05/2003	0.865	11/07/2003	0.326	06/09/2003	0.239	02/11/2003	3.18
16/05/2003	2.91	12/07/2003	0.314	07/09/2003	0.229	03/11/2003	1.19
17/05/2003	1.2	13/07/2003	0.288	08/09/2003	0.286	04/11/2003	0.788
18/05/2003	0.962	14/07/2003	0.292	09/09/2003	0.246	05/11/2003	0.677
19/05/2003	0.966	15/07/2003	0.325	10/09/2003	0.274	06/11/2003	0.665
20/05/2003	0.837	16/07/2003	0.403	11/09/2003	0.258	07/11/2003	0.569
21/05/2003	0.794	17/07/2003	0.325	12/09/2003	0.232	08/11/2003	0.529
22/05/2003	0.875	18/07/2003	0.316	13/09/2003	0.22	09/11/2003	0.536
23/05/2003	0.87	19/07/2003	0.279	14/09/2003	0.209	10/11/2003	0.526
24/05/2003	0.79	20/07/2003	0.35	15/09/2003	0.199	11/11/2003	1.24
25/05/2003	0.715	21/07/2003	0.347	16/09/2003	0.206	12/11/2003	1.05
26/05/2003	0.712	22/07/2003	0.289	17/09/2003	0.2	13/11/2003	0.74
27/05/2003	0.72	23/07/2003	0.385	18/09/2003	0.195	14/11/2003	0.757
28/05/2003	0.658	24/07/2003	0.436	19/09/2003	0.193	15/11/2003	0.643

Appendix 3.4

Raw data of the chemical determinands measured in water samples collected from the Plym Estuary during the period June 2002 – September 2003

Results of the Plym Estuary samples collected on 27 - 06 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	07:40	8.22	14.80	41.40	29.09	32.17	#	#	1.28	8.62	39.21	47.83	70.23	22.40	31.90%	97.97	1.81	4.37
2	5.05	07:47	8.32	14.70	40.40	28.30	31.21	#	#	1.76	9.74	13.91	23.65	63.70	40.05	62.87%	112.40	1.53	2.81
3	4.50	07:55	8.37	14.80	41.30	29.10	32.08	#	#	1.05	9.13	18.20	27.33	47.60	20.27	42.58%	91.26	2.68	4.50
4	3.80	08:05	8.41	14.60	41.20	28.90	31.97	#.	#	1.04	6.55	19.49	26.04	54.60	28.56	52.31%	159.80	2.47	5.59
5	3.35	08:12	8.15	14.20	18.70	13.08	17.99	#	#	4.78	5.48	99.50	104.98	150.20	45.22	30.11%	197.80	1.05	4.37
6	2.85	08:18	8.30	14.50	9.53	6.61	6.77	#	#	6.17	7.58	152.26	159.84	200	40.17	20.08%	205.80	0.36	5.12
7	1.90	08:24	8.02	15.10	21.20	14.85	9.56	#	#	6.49	7.53	129.58	137.11	188.50	51.39	27.26%	179.60	0.75	3.49
8	1.15	08:30	8.29	15.10	20.80	14.55	11.29	#	#	4.73	3.31	136.9	140.24	186.00	45.77	24.60%	181.60	0.97	3.97
9	0.85	08:45	7.57	14.30	4.32	3.07	2.30	#	#	5.99	2.30	104.45	106.75	156.10	49.35	31.61%	208.40	0.84	4.22
10	0.55	08:55	7.75	14.50	1.21	0.85	0.90	#	#	1.25	2.15	107.31	109.46	162.50	53.04	32.64%	118.40	0.73	2.23
11	0.00	09:20	7.15	14.20	0.07	0.05	0.00	#	#	0.65	1.19	115.09	116.28	165.00	48.73	29.53%	147.40	0.62	3.03
12MQ										0.01	0.34	0.00		2.21			1.18	ND	

Results of the Plym Estuary samples collected on 29 - 07 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	09:35	8.15	20.20	45.00	31.70	32.46	#	#	0.94	3.47	20.59	24.06	30.12	6.06	20.12%	201.90	3.64	33.32
2	5.05	09:45	8.29	21.60	44.10	31.00	32.69	#	#	1.02	5.59	12.89	18.48	27.28	8.80	32.26%	200.80	3.50	22.82
3	4.50	09:50	8.32	20.60	45.10	31.50	33.14	#	#	0.63	3.37	18.64	22.01	35.41	13.40	37.84%	205.00	3.65	15.30
4	3.80	09:55	8.37	20.80	45.60	32.60	33.40	#	#	0.55	5.06	16.90	21.96	29.33	7.37	25.13%	197.00	4.27	26.73
5	3.35	10:05	8.29	21.30	19.10	13.40	31.71	#	#	0.84	3.76	26.96	30.72	42.12	11.40	27.07%	227.70	4.78	19.97
6	2.85	10:10	8.27	21.90	24.70	17.40	29.63	#	#	2.90	2.04	63.68	65.72	80.64	14.92	18.50%	239.50	3.38	16.05
7	1.90	10:20	8.25	21.00	38.20	26.80	23.18	#	#	1.73	2.41	36.31	38.72	54.27	15.55	28.65%	220.50	3.42	14.18
8	1.15	10:30	8.05	21.60	6.32	4.42	3.76	#	#	2.35	0.41	104.1	104.47	150.51	46.04	30.59%	256.10	1.96	5.56
9	0.85	10:35	7.90	21.00	3.55	2.51	2.62	#	#	0.24	1.27	53.21	54.48	108.93	54.46	49.99%	224.70	1.29	4.13
10	0.55	10:45	8.20	20.80	3.90	2.81	1.13	#	#	0.22	6.85	42.86	49.71	132.67	82.96	62.53%	253.00	0.59	3.05
11	0.00	11:35	7.43	21.50	1.81	1.19	0.64	#	#	0.61	0.20	68.94	69.14	120.69	51.55	42.71%	224.20	1.98	4.35
12MQ										0.03	1.50	0.03		1.90			9.61	0.03	

Results of the Plym Estuary samples collected on 24 - 09 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	07:55	7.70	15.70	47.00	32.90	34.84	6.71	86.00	0.51	3.68	4.39	8.07	15.91	7.84	49.28%	83.02	1.22	10.59
2	5.05	08:10	7.86	15.90	46.90	32.80	34.63	6.55	84.80	0.67	4.17	6.01	10.18	19.96	9.78	49.00%	88.29	1.25	9.03
3	4.50	08:20	7.89	15.90	46.40	32.50	34.08	6.40	82.50	0.87	4.23	7.76	11.99	26.81	14.82	55.29%	94.57	1.10	6.38
4	3.80	08:25	7.90	15.00	45.70	31.90	34.23	6.41	82.50	0.89	4.80	6.14	10.94	29.29	18.35	62.65%	102.08	0.98	5.56
5	3.35	08:35	7.90	16.20	46.60	32.60	34.06	6.19	78.40	1.04	4.54	8.60	13.14	29.79	16.65	55.89%	92.59	1.39	5.56
6	2.85	08:40	7.88	14.90	42.80	30.00	32.15	6.30	79.00	1.92	5.18	19.52	24.70	46.58	21.88	46.97%	101.58	1.31	4.66
7	1.90	08:50	7.86	15.00	42.60	29.80	24.83	6.68	82.20	2.30	5.56	26.62	32.18	49.20	17.02	34.59%	101.68	1.21	5.97
8	1.15	09:00	7.83	14.80	29.10	20.40	18.36	6.91	84.80	5.13	4.92	96.42	101.34	137.24	35.90	26.16%	132.88	1.47	3.70
9	0.85	09:05	7.73	14.40	20.90	14.60	22.91	6.85	75.00	1.21	5.08	123.45	128.53	157.57	29.04	18.43%	147.88	0.88	5.09
10	0.55	09:15	7.68	14.40	10.37	7.26	8.90	6.78	70.00	0.12	5.35	75.61	80.96	122.66	41.70	34.00%	126.58	1.30	3.04
11	0.00	09:45	7.70	13.80	4.43	3.10	2.65	8.28	79.50	1.02	0.24	59.04	59.28	96.41	37.13	38.51%	102.18	0.95	2.75
12MQ										0.01	0.40	0.19		ND			2.82	0.03	

Results of the Plym Estuary samples collected on 12 - 11 - 2002

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	10:30	8.10	15.00	44.30	31.00	27.96	7.30	81.00	0.43	3.32	13.04	16.36	54.60	38.24	70.04%	159.15	0.14	4.16
2	5.05	10:35	8.15	14.60	39.20	27.40	25.02	8.40	94.00	1.28	6.53	33.05	39.58	77.28	37.70	48.78%	153.75	0.11	4.08
3	4.50	10:40	8.06	15.00	31.50	22.00	20.15	8.70	94.00	11.30	8.44	54.94	63.38	240.71	177.33	73.67%	290.45	0.64	1.64
4	3.80	10:45	8.16	14.70	38.50	27.00	24.24	7.82	89.00	1.46	8.02	33.55	41.57	87.73	46.16	52.62%	188.75	0.30	4.09

5	3.35	10:50	8.15	14.00	26.50	18.50	14.98	8.50	90.00	2.04	6.85	60.69	67.54	124.49	56.95	45.75%	164.65	0.25	2.89
6	2.85	10:55	8.11	13.90	13.84	9.72	6.92	9.80	97.00	1.63	4.83	63.39	68.22	154.40	86.18	55.82%	181.05	0.25	2.10
7	1.90	11:00	8.15	13.40	3.77	2.64	1.82	10.20	97.00	2.18	2.33	62.69	65.02	143.79	78.77	54.78%	180.95	0.36	2.30
8	1.15	11:10	7.85	13.40	0.34	0.24	0.44	10.10	94.00	1.57	0.64	56.22	56.86	132.79	75.93	57.18%	199.35	0.70	2.63
9	0.85	11:15	7.70	13.20	0.08	0.06	0.19	10.30	95.00	1.28	1.23	56.81	58.04	125.81	67.77	53.87%	204.55	0.28	3.02
10	0.55	11:20	7.65	13.40	0.02	0.01	0.05	10.68	98.00	0.28	0.06	89.94	90.00	110.73	20.73	18.72%	274.50	0.36	13.24
11	0.00	12:05	7.15	13.30	0.02	0.01	0.03	9.93	90.00	0.28	0.28	88.53	88.81	106.76	17.95	16.81%	255.45	Lost	14.23
12MQ										0.03	0.16	0.23	0.39	ND			2.35	ND	

Results of the Plym Estuary samples collected on 08 - 01 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	08:45	8.35	7.40	39.50	19.80	31.44	9.40	100.0	0.83	3.90	32.46	36.36	45.62	9.26	20.30%	85.34	0.16	9.22
2	5.05	08:55	8.36	6.80	33.80	16.90	28.01	8.95	93.0	2.49	6.88	75.77	82.65	112.73	30.08	26.68%	89.65	0.20	2.98
3	4.50	09:00	8.41	7.00	34.20	17.10	28.50	9.40	96.00	1.19	4.53	37.15	41.68	70.01	28.33	40.47%	92.99	0.13	3.28
4	3.80	09:05	8.43	6.40	29.70	14.80	22.95	9.80	98.00	1.93	5.63	46.44	52.07	83.16	31.09	37.39%	90.05	0.11	2.90
5	3.35	09:15	8.44	6.50	31.00	15.50	24.74	9.80	100.0	1.49	4.25	52.73	56.98	78.22	21.24	27.15%	90.38	0.16	4.26
6	2.85	09:20	8.45	7.00	29.70	14.87	24.14	10.20	97.00	1.89	5.05	48.23	53.28	83.26	29.98	36.01%	87.03	0.17	2.90
7	1.90	09:30	8.45	6.80	30.40	15.20	24.21	9.60	98.00	1.52	3.68	38.33	42.01	82.65	40.64	49.17%	86.17	0.19	2.12
8	1.15	09:40	8.27	6.10	15.09	7.55	17.26	10.80	95.00	1.22	3.71	65.17	68.88	100.68	31.80	31.59%	95.08	0.16	2.99
9	0.85	09:50	7.82	4.80	3.23	1.61	2.23	11.70	97.20	0.20	1.20	83.09	84.29	127.62	43.33	33.95%	105.27	0.03	2.43
10	0.55	09:55	7.72	5.20	1.82	0.91	1.23	12.20	100.6	0.01	1.13	52.49	53.62	135.00	81.38	60.28%	96.89	0.12	1.19
11	0.00	10:25	7.41	5.40	0.08	0.04	0.05	8.50	69.00	0.25	0.63	112.98	113.61	130.03	16.42	12.63%	104.68	0.17	6.38
12mq										0.03	0.01	0.53		0.99			1.90		

Results of the Plym Estuary samples collected on 26 - 02 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	01:15	8.04	9.20	43.40	30.40	32.46	9.60	98.00	0.62	4.27	18.43	22.70	41.48	18.78	45.27%	71.16	0.28	3.79
2	5.05	01:25	8.26	9.10	30.10	21.50	32.46	9.40	99.00	1.27	6.20	21.33	27.53	43.81	16.28	37.16%	74.39	0.37	4.57
3	4.50	01:30	8.30	9.10	41.10	28.18	31.02	8.99	92.90	1.91	8.57	23.54	32.11	73.88	41.77	56.54%	111.45	0.44	2.67
4	3.80	01:40	8.31	9.00	42.90	30.10	32.66	9.38	96.20	0.95	5.67	22.04	27.71	42.06	14.35	34.12%	85.18	0.28	5.94
5	3.35	01:45	8.32	9.20	34.40	24.10	20.74	8.09	82.60	3.77	3.80	87.22	91.02	104.69	13.67	13.06%	103.31	0.17	7.56
6	2.85	01:50	7.59	9.60	14.89	10.41	10.93	7.13	72.60	10.80	6.49	171.59	178.08	188.04	9.96	5.30%	125.73	0.25	12.62
7	1.90	01:55	8.01	9.40	26.30	18.41	19.32	8.80	78.60	5.27	5.86	78.95	84.81	119.50	34.69	29.03%	101.61	0.42	2.93
8	1.15	02:00	7.99	9.20	29.30	20.50	20.05	8.90	85.00	4.11	4.59	68.32	72.91	102.33	29.42	28.75%	99.35	0.60	3.38
9	0.85	02:05	8.30	9.50	3.87	7.71	3.48	9.86	91.50	20.25	4.28	127.0	131.26	138.41	7.15	5.17%	177.06	0.56	24.76
10	0.55	02:10	8.10	9.40	1.79	1.26	2.33	10.05	89.50	0.65	3.11	94.25	97.36	113.49	16.13	14.21%	107.43	0.37	6.66
11	0.00	02:45	7.22	9.80	0.03	0.02	0.06	10.90	96.00	0.79	1.75	90.01	91.76	113.08	21.32	18.85%	104.55	0.51	4.90
12mq										ND	0.21	0.36		0.99			1.33	ND	

Results of the Plym Estuary samples collected on 10 - 04 - 2003

Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	10:20	8.11	11.60	55.60	38.90	33.40	#	#	1.59	4.76	17.65	22.41	28.05	5.64	20.11%	74.65	0.10	13.24
2	5.05	13:15	8.09	11.60	52.90	37.00	31.57	#	#	1.52	5.17	21.58	26.75	38.70	11.95	30.88%	87.57	0.10	7.33
3	4.50	13:10	8.08	12.20	47.90	33.50	30.22	#	#	3.16	4.40	37.41	41.81	54.54	12.73	23.34%	83.65	0.10	6.57
4	3.80	13:05	8.09	12.10	50.20	35.20	29.77	#	#	2.94	3.80	36.65	40.45	46.99	6.54	13.92%	87.02	0.15	13.31
5	3.35	13:00	8.08	12.40	49.90	34.90	29.65	#	#	3.08	4.37	38.67	43.04	49.16	6.12	12.45%	96.50	0.18	15.77
6	2.85	12:50	8.08	12.30	51.10	35.80	30.77	#	#	2.46	4.51	26.25	30.76	43.35	12.59	29.04%	85.64	0.34	6.80
7	1.90	12:45	8.07	12.20	50.60	35.40	30.85	#	#	2.31	5.10	25.77	30.87	45.15	14.28	31.63%	88.39	0.37	6.19
8	1.15	12:40	8.05	13.00	48.90	34.40	30.46	#	#	2.34	5.87	31.24	37.11	44.33	7.22	16.29%	102.46	0.40	14.19
9	0.85	14:35	7.04	13.60	5.06	3.54	1.52	#	#	0.22	3.05	80.13	83.18	89.49	6.31	7.05%	104.42	3.09	16.55
10	0.55	14:40	7.22	12.20	0.84	0.60	0.54	#	#	0.13	2.63	84.38	87.01	90.24	3.23	3.58%	106.20	5.14	32.88
11	0.00	14:50	7.35	10.90	0.07	0.05	0.15	#	#	0.42	1.40	78.70	80.10	89.74	9.64	10.74%	96.01	4.29	9.96
12mq										ND	0.79	0.86		0.75			0.85	0.04	

Results of the Plym Estuary samples collected on 04 - 06 - 2003																			
Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	10:25	8.04	14.30	36.60	18.20	33.30	9.60	115.0	0.69	0.39	5.25	5.64	11.80	6.16	52.20%	84.25	#	13.68
2	5.05	10:15	8.05	14.30	35.50	17.80	33.14	9.62	114.0	0.74	1.45	6.04	7.49	13.81	6.32	45.78%	107.00	#	16.93
3	4.50	10:10	8.05	14.40	32.00	16.00	29.86	9.50	110.0	1.57	1.88	14.56	16.44	22.88	6.44	28.14%	107.00	#	16.62
4	3.80	10:00	8.06	14.40	35.30	17.60	32.07	9.56	116.0	0.86	1.29	9.87	11.16	17.80	6.64	37.29%	78.89	#	11.88
5	3.35	09:55	8.04	14.40	32.20	16.20	29.47	10.09	107.0	2.25	3.49	17.72	21.21	33.05	11.85	35.84%	96.07	#	8.11
6	2.85	09:50	8.02	14.50	31.70	15.90	26.89	9.11	105.0	2.99	5.47	26.18	31.65	43.41	11.76	27.09%	113.20	#	9.63
7	1.90	09:40	8.01	14.50	28.60	14.34	24.57	10.30	109.0	4.48	9.35	42.09	51.44	71.59	20.15	28.14%	87.64	#	4.35
8	1.15	09:35	7.92	14.30	25.24	13.10	8.79	9.80	107.0	6.48	8.86	96.68	105.54	140.35	34.81	24.80%	113.20	#	3.25
9	0.85	09:30	6.76	14.70	2.50	1.25	15.95	10.80	108.0	5.90	4.17	96.81	100.98	123.93	22.95	18.52%	95.95	#	4.18
10	0.55	09:20	6.70	14.60	0.67	0.33	1.20	9.32	92.00	0.98	1.57	65.35	66.92	86.77	19.85	22.87%	75.66	#	3.81
11	0.00	11:20	7.09	12.90	0.62	0.30	0.12	7.52	72.00	1.38	8.43	63.61	72.04	100.96	28.92	28.64%	110.00	#	3.80
12mq										ND	ND	ND		0.25			4.63		

Results of the Plym Estuary samples collected on 04 - 08 - 2003																			
Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	11 55	8.12	18.50	52.80	37.00	33.19	#	#	0.70	1.95	5.55	7.50	12.64	5.14	40.66%	75.03	#	14.60
2	5.05	11 50	8.10	18.50	51.80	36.40	32.85	#	#	1.10	6.13	8.44	14.57	92.40	77.83	84.23%	81.03	#	1.04
3	4.50	11 45	8.09	19.20	47.50	33.20	30.60	#	#	1.30	3.13	13.62	16.75	22.18	5.43	24.48%	99.02	#	18.24
4	3.80	11 40	8.07	19.40	47.00	32.90	29.21	#	#	1.88	5.96	19.76	25.72	42.50	16.78	39.48%	89.14	#	5.31
5	3.35	11 35	8.06	19.20	45.40	31.80	28.35	#	#	2.26	6.05	24.98	31.03	87.74	56.71	64.63%	89.93	#	1.59
6	2.85	11 30	8.04	18.70	46.90	32.90	29.96	#	#	1.65	8.51	17.02	25.53	69.60	44.07	63.32%	87.47	#	1.98
7	1.90	11 25	7.98	18.40	29.50	20.70	16.88	#	#	3.72	5.31	63.65	68.96	78.86	9.90	12.55%	102.82	#	10.39
8	1.15	11 20	7.52	18.20	25.40	17.80	27.06	#	#	2.61	8.16	26.22	34.38	108.21	73.83	68.23%	93.58	#	1.27
9	0.85	11 15	7.12	18.10	10.28	7.20	9.47	#	#	4.42	6.61	93.12	99.73	130.71	30.98	23.70%	100.02	#	3.23
10	0.55	11 10	6.98	18.70	3.61	2.53	2.11	#	#	2.32	4.23	65.27	69.50	89.28	19.78	22.16%	111.42	#	5.63
11	0.00	12 40	7.42	17.80	0.02	0.01	0.06	#	#	2.01	1.09	76.59	77.68	93.11	15.43	16.57%	119.62	#	7.75
12mq										ND	0.19	0.19	0.38	0.28			1.08		

Results of the Plym Estuary samples collected on 16 - 09 - 2003																			
Station	Distance km-weir	Time	pH	Temperature	Cond. mS/cm	TDS gm L^{-1}	Salinity ‰	DO mg L^{-1}	DO %	PO_4 µM P	NH_4 µM N	NO_3 +NO_2 µM N	DIN µM N	TDN µM N	DON µM N	%DON /TDN	DOC µM C	CHL a µg L^{-1}	DOC:DON
1	6.18	10:30	7.47	17.50	55.00	38.50	34.10	#	#	1.58	7.13	7.97	15.10	35.54	20.44	57.51%	100.79	#	4.93
2	5.05	10:20	7.46	17.50	54.90	38.40	33.98	#	#	1.40	8.04	9.72	17.76	60.34	42.58	70.57%	104.09	#	2.44
3	4.50	10:15	7.43	17.40	53.50	37.50	32.40	#	#	2.19	8.92	25.58	34.50	77.55	43.05	55.51%	120.79	#	2.81
4	3.80	10:10	7.41	17.00	54.20	37.90	33.53	#	#	1.87	2.89	10.56	13.45	24.93	11.48	46.05%	97.04	#	8.45
5	3.35	10:05	7.21	15.40	38.40	26.90	19.52	#	#	7.34	6.97	94.75	101.72	140.08	38.36	27.38%	134.39	#	3.50
6	2.85	10:00	7.21	15.20	29.30	20.50	19.61	#	#	8.15	7.01	115.89	122.90	167.81	44.91	26.76%	138.69	#	3.09
7	1.90	09:50	7.08	15.70	28.30	19.80	14.50	#	#	17.75	5.64	227.18	232.8	327.6	94.80	28.94%	175.49	#	1.85
8	1.15	09:45	6.77	14.80	16.20	11.33	30.32	#	#	4.11	7.98	35.06	43.04	114.50	71.46	62.41%	113.99	#	1.60
9	0.85	09:40	7.10	13.60	8.82	6.17	3.16	#	#	5.09	9.21	127.06	136.27	246.4	110.08	44.68%	118.09	#	1.07
10	0.55	09:35	7.20	13.80	3.15	2.21	2.40	#	#	3.25	8.02	90.77	98.79	158.83	60.04	37.80%	102.79	#	1.71
11	0.00	11:35	7.16	18.70	4.91	3.40	1.71	#	#	3.12	8.96	57.05	66.01	134.34	68.33	50.86%	105.19	#	1.54
12mq										0.02	0.07	0.44	0.51	0.19			2.21		

Appendix 3.5

Pearson's product moment correlation matrix for the Plym Estuary samples for different seasons

		SALINITY	PH	PO4	NH4	NO3	TDN	DON	DON/TDN	DOC	CHLA	DOC : DON
SALINITY	Pearson Correlation	1	.721**	-.284	.454*	-.746**	-.851**	-.863**	-.080	-.259	.775**	.619**
	Sig. (2-tailed)	.	.000	.201	.034	.000	.000	.000	.722	.245	.000	.002
	N	22	22	22	22	22	22	22	22	22	22	22
PH	Pearson Correlation	.721**	1	-.004	.584**	-.454*	-.492*	-.493*	.029	-.014	.464*	.355
	Sig. (2-tailed)	.000	.	.985	.004	.034	.020	.020	.900	.952	.029	.105
	N	22	22	22	22	22	22	22	22	22	22	22
PO4	Pearson Correlation	-.284	-.004	1	.150	.735**	.658**	.236	-.422	.075	-.479*	-.306
	Sig. (2-tailed)	.201	.985	.	.506	.000	.001	.290	.051	.741	.024	.166
	N	22	22	22	22	22	22	22	22	22	22	22
NH4	Pearson Correlation	.454*	.584**	.150	1	-.251	-.176	-.090	.306	-.545**	-.088	-.121
	Sig. (2-tailed)	.034	.004	.506	.	.261	.433	.691	.166	.009	.698	.590
	N	22	22	22	22	22	22	22	22	22	22	22
NO3	Pearson Correlation	-.746**	-.454*	.735**	-.251	1	.957**	.569**	-.403	.099	-.732**	-.530*
	Sig. (2-tailed)	.000	.034	.000	.261	.	.000	.006	.063	.662	.000	.011
	N	22	22	22	22	22	22	22	22	22	22	22
TDN	Pearson Correlation	-.851**	-.492*	.658**	-.176	.957**	1	.779**	-.142	.084	-.869**	-.685**
	Sig. (2-tailed)	.000	.020	.001	.433	.000	.	.000	.528	.710	.000	.000
	N	22	22	22	22	22	22	22	22	22	22	22
DON	Pearson Correlation	-.863**	-.493*	.236	-.090	.569**	.779**	1	.453*	.099	-.869**	-.784**
	Sig. (2-tailed)	.000	.020	.290	.691	.006	.000	.	.034	.661	.000	.000
	N	22	22	22	22	22	22	22	22	22	22	22
DON/TDN	Pearson Correlation	-.080	.029	-.422	.306	-.403	-.142	.453*	1	-.174	-.233	-.443*
	Sig. (2-tailed)	.722	.900	.051	.166	.063	.528	.034	.	.439	.296	.039
	N	22	22	22	22	22	22	22	22	22	22	22
DOC	Pearson Correlation	-.259	-.014	.075	-.545**	.099	.084	.099	-.174	1	.195	.301
	Sig. (2-tailed)	.245	.952	.741	.009	.662	.710	.661	.439	.	.385	.173
	N	22	22	22	22	22	22	22	22	22	22	22
CHLA	Pearson Correlation	.775**	.464*	-.479*	-.088	-.732**	-.869**	-.869**	-.233	.195	1	.830**
	Sig. (2-tailed)	.000	.029	.024	.698	.000	.000	.000	.296	.385	.	.000
	N	22	22	22	22	22	22	22	22	22	22	22
DOC : DON	Pearson Correlation	.619**	.355	-.306	-.121	-.530*	-.685**	-.784**	-.443*	.301	.830**	1
	Sig. (2-tailed)	.002	.105	.166	.590	.011	.000	.000	.039	.173	.000	.
	N	22	22	22	22	22	22	22	22	22	22	22

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Pearson's product moment correlations matrix for the Plym Estuary summer 2002 samples.

		SALINITY	PH	PO4	NH4	NO3	TDN	DON	DON/TDN	DOC	CHLA	DOC : DON
SALINITY	Pearson Correlation	1	.309	.018	.583**	-.697**	-.635**	-.367	.331	-.638**	.444*	.073
	Sig. (2-tailed)	.	.161	.936	.004	.000	.002	.093	.132	.001	.044	.747
	N	22	22	22	22	22	22	22	22	22	21	22
PH	Pearson Correlation	.309	1	.293	.614**	-.346	.079	.392	.662**	-.102	-.556**	-.668**
	Sig. (2-tailed)	.161	.	.186	.002	.115	.727	.071	.001	.653	.009	.001
	N	22	22	22	22	22	22	22	22	22	21	22
PO4	Pearson Correlation	.018	.293	1	.469*	.151	.627**	.781**	.302	.397	.010	-.395
	Sig. (2-tailed)	.936	.186	.	.028	.502	.002	.000	.172	.067	.964	.069
	N	22	22	22	22	22	22	22	22	22	21	22
NH4	Pearson Correlation	.583**	.614**	.469*	1	-.220	.086	.265	.314	-.154	.037	-.404
	Sig. (2-tailed)	.004	.002	.028	.	.326	.703	.234	.154	.493	.873	.062
	N	22	22	22	22	22	22	22	22	22	21	22
NO3	Pearson Correlation	-.697**	-.346	.151	-.220	1	.755**	.251	-.667**	.539**	-.177	-.022
	Sig. (2-tailed)	.000	.115	.502	.326	.	.000	.259	.001	.010	.442	.923
	N	22	22	22	22	22	22	22	22	22	21	22
TDN	Pearson Correlation	-.635**	.079	.627**	.086	.755**	1	.823**	-.074	.728**	-.383	-.420
	Sig. (2-tailed)	.002	.727	.002	.703	.000	.	.000	.743	.000	.086	.052
	N	22	22	22	22	22	22	22	22	22	21	22
DON	Pearson Correlation	-.367	.392	.781**	.265	.251	.823**	1	.471*	.623**	-.431	-.588**
	Sig. (2-tailed)	.093	.071	.000	.234	.259	.000	.	.027	.002	.051	.004
	N	22	22	22	22	22	22	22	22	22	21	22
DON/TDN	Pearson Correlation	.331	.662**	.302	.314	-.667**	-.074	.471*	1	-.065	-.282	-.516*
	Sig. (2-tailed)	.132	.001	.172	.154	.001	.743	.027	.	.775	.215	.014
	N	22	22	22	22	22	22	22	22	22	21	22
DOC	Pearson Correlation	-.638**	-.102	.397	-.154	.539**	.728**	.623**	-.065	1	-.660**	.088
	Sig. (2-tailed)	.001	.653	.067	.493	.010	.000	.002	.775	.	.001	.698
	N	22	22	22	22	22	22	22	22	22	21	22
CHLA	Pearson Correlation	.444*	-.556**	.010	.037	-.177	-.383	-.431	-.282	-.660**	1	.244
	Sig. (2-tailed)	.044	.009	.964	.873	.442	.086	.051	.215	.001	.	.286
	N	21	21	21	21	21	21	21	21	21	21	21
DOC : DON	Pearson Correlation	.073	-.668**	-.395	-.404	-.022	-.420	-.588**	-.516*	.088	.244	1
	Sig. (2-tailed)	.747	.001	.069	.062	.923	.052	.004	.014	.698	.286	.
	N	22	22	22	22	22	22	22	22	22	21	22

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Pearson's product moment correlations matrix for the Plym Estuary autumn 2002 samples.

		SALINITY	PH	PO4	NH4	NO3	TDN	DON	DON/TDN	DOC	CHLA	DOC : DON
SALINITY	Pearson Correlation	1	.713**	-.231	.704**	-.716**	-.785**	-.169	.399	-.578**	-.082	-.281
	Sig. (2-tailed)	.	.000	.302	.000	.000	.000	.453	.066	.005	.717	.205
	N	22	22	22	22	22	22	22	22	22	22	22
PH	Pearson Correlation	.713**	1	.030	.532*	-.538**	-.569**	-.082	.266	-.206	-.191	-.031
	Sig. (2-tailed)	.000	.	.895	.011	.010	.006	.715	.232	.358	.395	.891
	N	22	22	22	22	22	22	22	22	22	22	22
PO4	Pearson Correlation	-.231	.030	1	.240	.604**	.486*	-.357	-.522*	.856**	.464*	.874**
	Sig. (2-tailed)	.302	.895	.	.283	.003	.022	.103	.013	.000	.030	.000
	N	22	22	22	22	22	22	22	22	22	22	22
NH4	Pearson Correlation	.704**	.532*	.240	1	-.219	-.264	-.201	.139	-.046	.276	.030
	Sig. (2-tailed)	.000	.011	.283	.	.327	.235	.369	.537	.838	.213	.896
	N	22	22	22	22	22	22	22	22	22	22	22
NO3	Pearson Correlation	-.716**	-.538**	.604**	-.219	1	.911**	-.277	-.773**	.718**	.164	.573**
	Sig. (2-tailed)	.000	.010	.003	.327	.	.000	.212	.000	.000	.467	.005
	N	22	22	22	22	22	22	22	22	22	22	22
TDN	Pearson Correlation	-.785**	-.569**	.486*	-.264	.911**	1	.141	-.467*	.663**	.064	.338
	Sig. (2-tailed)	.000	.006	.022	.235	.000	.	.532	.028	.001	.776	.124
	N	22	22	22	22	22	22	22	22	22	22	22
DON	Pearson Correlation	-.169	-.082	-.357	-.201	-.277	.141	1	.755**	-.192	-.276	-.594**
	Sig. (2-tailed)	.453	.715	.103	.369	.212	.532	.	.000	.391	.214	.004
	N	22	22	22	22	22	22	22	22	22	22	22
DON/TDN	Pearson Correlation	.399	.266	-.522*	.139	-.773**	-.467*	.755**	1	-.532*	-.229	-.690**
	Sig. (2-tailed)	.066	.232	.013	.537	.000	.028	.000	.	.011	.305	.000
	N	22	22	22	22	22	22	22	22	22	22	22
DOC	Pearson Correlation	-.578**	-.206	.856**	-.046	.718**	.663**	-.192	-.532*	1	.421	.810**
	Sig. (2-tailed)	.005	.358	.000	.838	.000	.001	.391	.011	.	.051	.000
	N	22	22	22	22	22	22	22	22	22	22	22
CHLA	Pearson Correlation	-.082	-.191	.464*	.276	.164	.064	-.276	-.229	.421	1	.372
	Sig. (2-tailed)	.717	.395	.030	.213	.467	.776	.214	.305	.051	.	.088
	N	22	22	22	22	22	22	22	22	22	22	22
DOC : DON	Pearson Correlation	-.281	-.031	.874**	.030	.573**	.338	-.594**	-.690**	.810**	.372	1
	Sig. (2-tailed)	.205	.891	.000	.896	.005	.124	.004	.000	.000	.088	.
	N	22	22	22	22	22	22	22	22	22	22	22

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Pearson's product moment correlations matrix for the Plym Estuary winter 2003 samples.

		SALINITY	PH	PO4	NH4	NO3	TDN	DON	DON/TDN	DOC	CHLA	DOC : DON
SALINITY	Pearson Correlation	1	.856**	.123	-.070	-.863**	-.816**	-.406	.535*	-.340	-.971**	-.032
	Sig. (2-tailed)	.	.000	.585	.757	.000	.000	.061	.010	.121	.000	.887
	N	22	22	22	22	22	22	22	22	22	11	22
PH	Pearson Correlation	.856**	1	.116	.117	-.768**	-.707**	-.332	.429*	-.136	-.929**	.035
	Sig. (2-tailed)	.000	.	.608	.605	.000	.000	.131	.046	.547	.000	.876
	N	22	22	22	22	22	22	22	22	22	11	22
PO4	Pearson Correlation	.123	.116	1	.649**	.307	.448*	.642**	-.049	.138	-.860**	-.536*
	Sig. (2-tailed)	.585	.608	.	.001	.165	.037	.001	.828	.542	.001	.010
	N	22	22	22	22	22	22	22	22	22	11	22
NH4	Pearson Correlation	-.070	.117	.649**	1	.255	.435*	.666**	-.130	.264	-.848**	-.468*
	Sig. (2-tailed)	.757	.605	.001	.	.251	.043	.001	.564	.234	.001	.028
	N	22	22	22	22	22	22	22	22	22	11	22
NO3	Pearson Correlation	-.863**	-.768**	.307	.255	1	.969**	.520*	-.664**	.338	.942**	-.066
	Sig. (2-tailed)	.000	.000	.165	.251	.	.000	.013	.001	.124	.000	.772
	N	22	22	22	22	22	22	22	22	22	11	22
TDN	Pearson Correlation	-.816**	-.707**	.448*	.435*	.969**	1	.709**	-.541**	.346	.930**	-.258
	Sig. (2-tailed)	.000	.000	.037	.043	.000	.	.000	.009	.114	.000	.247
	N	22	22	22	22	22	22	22	22	22	11	22
DON	Pearson Correlation	-.406	-.332	.642**	.666**	.520*	.709**	1	.089	.204	-.434	-.742**
	Sig. (2-tailed)	.061	.131	.001	.001	.013	.000	.	.693	.362	.182	.000
	N	22	22	22	22	22	22	22	22	22	11	22
DON/TDN	Pearson Correlation	.535*	.429*	-.049	-.130	-.664**	-.541**	.089	1	-.172	-.716*	-.319
	Sig. (2-tailed)	.010	.046	.828	.564	.001	.009	.693	.	.444	.013	.148
	N	22	22	22	22	22	22	22	22	22	11	22
DOC	Pearson Correlation	-.340	-.136	.138	.264	.338	.346	.204	-.172	1	.669*	.241
	Sig. (2-tailed)	.121	.547	.542	.234	.124	.114	.362	.444	.	.025	.280
	N	22	22	22	22	22	22	22	22	22	11	22
CHLA	Pearson Correlation	-.971**	-.929**	-.860**	-.848**	.942**	.930**	-.434	-.716*	.669*	1	.660*
	Sig. (2-tailed)	.000	.000	.001	.001	.000	.000	.182	.013	.025	.	.027
	N	11	11	11	11	11	11	11	11	11	11	11
DOC : DON	Pearson Correlation	-.032	.035	-.536*	-.468*	-.066	-.258	-.742**	-.319	.241	.660*	1
	Sig. (2-tailed)	.887	.876	.010	.028	.772	.247	.000	.148	.280	.027	.
	N	22	22	22	22	22	22	22	22	22	11	22

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Pearson's product moment correlations matrix for the Plym Estuary spring 2003 samples.

		SALINITY	PH	PO4	NH4	NO3	TDN	DON	DON/TDN	DOC	CHLA	DOC : DON
SALINITY	Pearson Correlation	1	.532*	-.296	-.011	-.641**	-.561**	-.227	.566**	-.375	.	.189
	Sig. (2-tailed)	.	.011	.182	.961	.001	.007	.310	.006	.086	.	.398
	N	22	22	22	22	22	22	22	22	22	0	22
PH	Pearson Correlation	.532*	1	-.464*	-.319	-.561**	-.579**	-.392	.199	-.634**	.	.457*
	Sig. (2-tailed)	.011	.	.030	.148	.007	.005	.072	.376	.002	.	.032
	N	22	22	22	22	22	22	22	22	22	0	22
PO4	Pearson Correlation	-.296	-.464*	1	.125	.905**	.855**	.467*	-.340	.883**	.	-.284
	Sig. (2-tailed)	.182	.030	.	.580	.000	.000	.028	.121	.000	.	.200
	N	22	22	22	22	22	22	22	22	22	0	22
NH4	Pearson Correlation	-.011	-.319	.125	1	.098	.366	.650**	.489*	.116	.	-.760**
	Sig. (2-tailed)	.961	.148	.580	.	.663	.094	.001	.021	.606	.	.000
	N	22	22	22	22	22	22	22	22	22	0	22
NO3	Pearson Correlation	-.641**	-.561**	.905**	.098	1	.925**	.471*	-.517*	.846**	.	-.288
	Sig. (2-tailed)	.001	.007	.000	.663	.	.000	.027	.014	.000	.	.194
	N	22	22	22	22	22	22	22	22	22	0	22
TDN	Pearson Correlation	-.561**	-.579**	.855**	.366	.925**	1	.770**	-.184	.767**	.	-.539**
	Sig. (2-tailed)	.007	.005	.000	.094	.000	.	.000	.412	.000	.	.010
	N	22	22	22	22	22	22	22	22	22	0	22
DON	Pearson Correlation	-.227	-.392	.467*	.650**	.471*	.770**	1	.442*	.357	.	-.753**
	Sig. (2-tailed)	.310	.072	.028	.001	.027	.000	.	.040	.103	.	.000
	N	22	22	22	22	22	22	22	22	22	0	22
DON/TDN	Pearson Correlation	.566**	.199	-.340	.489*	-.517*	-.184	.442*	1	-.416	.	-.493*
	Sig. (2-tailed)	.006	.376	.121	.021	.014	.412	.040	.	.054	.	.020
	N	22	22	22	22	22	22	22	22	22	0	22
DOC	Pearson Correlation	-.375	-.634**	.883**	.116	.846**	.767**	.357	-.416	1	.	-.255
	Sig. (2-tailed)	.086	.002	.000	.606	.000	.000	.103	.054	.	.	.252
	N	22	22	22	22	22	22	22	22	22	0	22
CHLA	Pearson Correlation	.	.	.	.	.	.	.	.	.	.	.
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.
	N	0	0	0	0	0	0	0	0	0	0	0
DOC : DON	Pearson Correlation	.189	.457*	-.284	-.760**	-.288	-.539**	-.753**	-.493*	-.255	.	1
	Sig. (2-tailed)	.398	.032	.200	.000	.194	.010	.000	.020	.252	.	.
	N	22	22	22	22	22	22	22	22	22	0	22

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson's product moment correlations matrix for the Plym Estuary summer 2003 samples.

Appendix 3.6

The River Plym flow data close to the tidal limit during January 2002 – October 2003

Date	FQ (m3/s	Date	FQ (m3/s	Date	FQ (m3/s	Date	FQ (m3/s	Date	Q (m3/s	Date	FQ (m3/s
01/01/2002	1.847	27/02/2002	5.259	25/04/2002	0.708	21/06/2002	3.346	17/08/2002	0.51	13/10/2002	2.183
02/01/2002	1.815	28/02/2002	5.077	26/04/2002	0.976	22/06/2002	2.527	18/08/2002	0.59	14/10/2002	0.723
03/01/2002	1.089	01/03/2002	4.031	27/04/2002	1.538	23/06/2002	2.025	19/08/2002	0.49	15/10/2002	4.149
04/01/2002	0.792	02/03/2002	3.494	28/04/2002	1.463	24/06/2002	1.738	20/08/2002	0.48	16/10/2002	0.923
05/01/2002	0.77	03/03/2002	3.132	29/04/2002	0.971	25/06/2002	1.656	21/08/2002	0.46	17/10/2002	0.577
06/01/2002	0.782	04/03/2002	2.917	30/04/2002	7.584	26/06/2002	1.442	22/08/2002	0.44	18/10/2002	0.527
07/01/2002	0.745	05/03/2002	2.641	01/05/2002	1.982	27/06/2002	1.364	23/08/2002	0.45	19/10/2002	0.442
08/01/2002	0.705	06/03/2002	2.439	02/05/2002	1.134	28/06/2002	1.285	24/08/2002	0.43	20/10/2002	0.526
09/01/2002	0.768	07/03/2002	2.14	03/05/2002	0.964	29/06/2002	1.232	25/08/2002	0.42	21/10/2002	1.037
10/01/2002	0.755	08/03/2002	1.955	04/05/2002	0.987	30/06/2002	1.33	26/08/2002	0.4	22/10/2002	1.715
11/01/2002	0.738	09/03/2002	1.846	05/05/2002	0.945	01/07/2002	1.224	27/08/2002	0.39	23/10/2002	0.731
12/01/2002	0.764	10/03/2002	1.863	06/05/2002	0.935	02/07/2002	4.068	28/08/2002	0.39	24/10/2002	0.62
13/01/2002	1.536	11/03/2002	2.542	07/05/2002	0.911	03/07/2002	2.588	29/08/2002	0.4	25/10/2002	1.643
14/01/2002	1.814	12/03/2002	2.741	08/05/2002	0.878	04/07/2002	2.033	30/08/2002	0.44	26/10/2002	2.566
15/01/2002	1.336	13/03/2002	2.774	09/05/2002	0.879	05/07/2002	2.211	31/08/2002	0.41	27/10/2002	1.62
16/01/2002	1.072	14/03/2002	1.934	10/05/2002	0.802	06/07/2002	1.442	01/09/2002	0.39	28/10/2002	0.902
17/01/2002	4.436	15/03/2002	2.527	11/05/2002	0.947	07/07/2002	1.967	02/09/2002	0.38	29/10/2002	0.801
18/01/2002	3.561	16/03/2002	1.712	12/05/2002	1.405	08/07/2002	4.058	03/09/2002	0.38	30/10/2002	0.747
19/01/2002	2.61	17/03/2002	3.079	13/05/2002	3.249	09/07/2002	2.737	04/09/2002	0.41	31/10/2002	0.726
20/01/2002	6.382	18/03/2002	7.405	14/05/2002	1.437	10/07/2002	2.246	05/09/2002	0.39	01/11/2002	3.532
21/01/2002	4.229	19/03/2002	6.509	15/05/2002	1.107	11/07/2002	1.936	06/09/2002	0.39	02/11/2002	6.635
22/01/2002	4.131	20/03/2002	8.857	16/05/2002	1.3	12/07/2002	1.899	07/09/2002	0.4	03/11/2002	2.424
23/01/2002	8.969	21/03/2002	5.054	17/05/2002	5.779	13/07/2002	1.623	08/09/2002	0.38	04/11/2002	1.791
24/01/2002	4.783	22/03/2002	4.011	18/05/2002	3.184	14/07/2002	1.535	09/09/2002	0.42	05/11/2002	4.837
25/01/2002	12.13	23/03/2002	3.627	19/05/2002	2.581	15/07/2002	1.396	10/09/2002	0.41	06/11/2002	8.667
26/01/2002	17.55	24/03/2002	3.392	20/05/2002	2.67	16/07/2002	1.339	11/09/2002	0.36	07/11/2002	4.836
27/01/2002	19.2	25/03/2002	2.933	21/05/2002	8.143	17/07/2002	1.207	12/09/2002	0.35	08/11/2002	7.767
28/01/2002	9.688	26/03/2002	2.542	22/05/2002	4.571	18/07/2002	1.206	13/09/2002	0.33	09/11/2002	4.771
29/01/2002	8.009	27/03/2002	2.29	23/05/2002	8.306	19/07/2002	1.15	14/09/2002	0.32	10/11/2002	5.253
30/01/2002	8.072	28/03/2002	2.099	24/05/2002	7.997	20/07/2002	1.041	15/09/2002	0.32	11/11/2002	3.986
31/01/2002	8.135	29/03/2002	1.859	25/05/2002	5.217	21/07/2002	1.012	16/09/2002	0.31	12/11/2002	5.478
01/02/2002	8.205	30/03/2002	1.677	26/05/2002	6.882	22/07/2002	0.954	17/09/2002	0.3	13/11/2002	13.25
02/02/2002	13.11	31/03/2002	2.554	27/05/2002	6.024	23/07/2002	0.992	18/09/2002	0.29	14/11/2002	9.48
03/02/2002	9.537	01/04/2002	2.598	28/05/2002	8.466	24/07/2002	0.909	19/09/2002	0.29	15/11/2002	6.349
04/02/2002	25.64	02/04/2002	1.847	29/05/2002	5.26	25/07/2002	0.914	20/09/2002	0.29	16/11/2002	4.599
05/02/2002	11.22	03/04/2002	1.793	30/05/2002	4.402	26/07/2002	0.871	21/09/2002	0.29	17/11/2002	3.835
06/02/2002	7.975	04/04/2002	1.687	31/05/2002	3.68	27/07/2002	0.893	22/09/2002	0.29	18/11/2002	3.912
07/02/2002	7.903	05/04/2002	1.42	01/06/2002	3.092	28/07/2002	0.805	23/09/2002	0.28	19/11/2002	5.527
08/02/2002	6.777	06/04/2002	1.378	02/06/2002	2.729	29/07/2002	0.764	24/09/2002	0.27	20/11/2002	6.999
09/02/2002	6.167	07/04/2002	1.206	03/06/2002	2.715	30/07/2002	0.815	25/09/2002	0.27	21/11/2002	10.31
10/02/2002	5.756	08/04/2002	1.063	04/06/2002	2.401	31/07/2002	0.849	26/09/2002	0.27	22/11/2002	9.222
11/02/2002	11.67	09/04/2002	0.984	05/06/2002	2.059	01/08/2002	0.723	27/09/2002	0.27	23/11/2002	7.228
12/02/2002	7.036	10/04/2002	0.935	06/06/2002	1.829	02/08/2002	0.681	28/09/2002	0.27	24/11/2002	5.745
13/02/2002	5.637	11/04/2002	0.973	07/06/2002	2.889	03/08/2002	0.65	29/09/2002	0.26	25/11/2002	4.707
14/02/2002	4.767	12/04/2002	1.003	08/06/2002	2.308	04/08/2002	0.624	30/09/2002	0.26	26/11/2002	4.921
15/02/2002	4.335	13/04/2002	0.912	09/06/2002	5.133	05/08/2002	0.62	01/10/2002	0.29	27/11/2002	10.08
16/02/2002	3.831	14/04/2002	0.902	10/06/2002	2.527	06/08/2002	0.591	02/10/2002	0.33	28/11/2002	5.957
17/02/2002	3.498	15/04/2002	0.821	11/06/2002	2.19	07/08/2002	0.597	03/10/2002	0.33	29/11/2002	4.864
18/02/2002	3.135	16/04/2002	0.865	12/06/2002	2.207	08/08/2002	0.719	04/10/2002	0.29	30/11/2002	6.202
19/02/2002	3.857	17/04/2002	1.023	13/06/2002	1.982	09/08/2002	0.776	05/10/2002	0.28	01/12/2002	6.868
20/02/2002	4.991	18/04/2002	0.935	14/06/2002	2.057	10/08/2002	0.653	06/10/2002	0.3	02/12/2002	4.758
21/02/2002	2.929	19/04/2002	0.879	15/06/2002	6.467	11/08/2002	0.707	07/10/2002	0.31	03/12/2002	4.202
22/02/2002	2.869	20/04/2002	0.811	16/06/2002	6.107	12/08/2002	0.636	08/10/2002	0.29	04/12/2002	4.054
23/02/2002	2.527	21/04/2002	0.787	17/06/2002	3.756	13/08/2002	0.561	09/10/2002	0.27	05/12/2002	3.402
24/02/2002	2.432	22/04/2002	0.758	18/06/2002	2.899	14/08/2002	0.538	10/10/2002	0.26	06/12/2002	2.899
25/02/2002	13.73	23/04/2002	0.675	19/06/2002	2.45	15/08/2002	0.546	11/10/2002	0.29	07/12/2002	2.77
26/02/2002	8.008	24/04/2002	0.66	20/06/2002	2.229	16/08/2002	0.49	12/10/2002	0.31	08/12/2002	2.63

Date	FQ (m3/s	Date	FQ (m3/s	Date	FQ (m3/s	Date	Q (m3/s	Date	Q (m3/s	Date	FQ (m3/s)
09/12/2002	2.595	04/02/2003	2.215	02/04/2003	0.793	29/05/2003	1.13	25/07/2003	3.99	20/09/2003	0.335
10/12/2002	2.34	05/02/2003	1.958	03/04/2003	0.716	30/05/2003	1.07	26/07/2003	1.21	21/09/2003	0.341
11/12/2002	2.079	06/02/2003	2.363	04/04/2003	0.672	31/05/2003	1.03	27/07/2003	0.88	22/09/2003	0.413
12/12/2002	3.395	07/02/2003	2.152	05/04/2003	0.636	01/06/2003	1.09	28/07/2003	2.33	23/09/2003	0.372
13/12/2002	2.709	08/02/2003	3.689	06/04/2003	0.661	02/06/2003	1.05	29/07/2003	3.88	24/09/2003	0.349
14/12/2002	8.03	09/02/2003	5.308	07/04/2003	0.617	03/06/2003	0.98	30/07/2003	1.8	25/09/2003	0.335
15/12/2002	4.573	10/02/2003	6.294	08/04/2003	0.587	04/06/2003	1.03	31/07/2003	2.04	26/09/2003	0.328
16/12/2002	2.873	11/02/2003	5.279	09/04/2003	0.578	05/06/2003	1.1	01/08/2003	3.94	27/09/2003	0.306
17/12/2002	2.464	12/02/2003	3.614	10/04/2003	0.56	06/06/2003	3.35	02/08/2003	1.5	28/09/2003	0.308
18/12/2002	3.052	13/02/2003	3.18	11/04/2003	0.63	07/06/2003	1.79	03/08/2003	1.27	29/09/2003	0.31
19/12/2002	2.958	14/02/2003	2.717	12/04/2003	0.54	08/06/2003	1.97	04/08/2003	1.17	30/09/2003	0.303
20/12/2002	3.06	15/02/2003	2.515	13/04/2003	0.544	09/06/2003	1.82	05/08/2003	1.06	01/10/2003	0.291
21/12/2002	16.87	16/02/2003	2.268	14/04/2003	0.518	10/06/2003	1.94	06/08/2003	1.01	02/10/2003	0.3
22/12/2002	8.72	17/02/2003	1.987	15/04/2003	0.497	11/06/2003	1.4	07/08/2003	0.98	03/10/2003	0.289
23/12/2002	7.95	18/02/2003	1.767	16/04/2003	0.476	12/06/2003	1.32	08/08/2003	0.86	04/10/2003	0.288
24/12/2002	6.207	19/02/2003	1.754	17/04/2003	0.46	13/06/2003	1.25	09/08/2003	0.83	05/10/2003	0.292
25/12/2002	6.721	20/02/2003	1.66	18/04/2003	0.452	14/06/2003	1.16	10/08/2003	0.82	06/10/2003	0.293
26/12/2002	11.72	21/02/2003	1.556	19/04/2003	0.446	15/06/2003	1.1	11/08/2003	0.78	07/10/2003	0.294
27/12/2002	8.268	22/02/2003	1.712	20/04/2003	0.455	16/06/2003	1.05	12/08/2003	0.73	08/10/2003	0.292
28/12/2002	6.512	23/02/2003	1.658	21/04/2003	0.497	17/06/2003	1	13/08/2003	0.67	09/10/2003	0.288
29/12/2002	10.2	24/02/2003	1.381	22/04/2003	0.474	18/06/2003	1	14/08/2003	0.68	10/10/2003	0.302
30/12/2002	9.477	25/02/2003	1.267	23/04/2003	0.449	19/06/2003	1.08	15/08/2003	0.66	11/10/2003	0.307
31/12/2002	15.79	26/02/2003	1.922	24/04/2003	0.532	20/06/2003	0.94	16/08/2003	0.62	12/10/2003	0.289
01/01/2003	27.13	27/02/2003	3.067	25/04/2003	0.684	21/06/2003	0.85	17/08/2003	0.6	13/10/2003	0.294
02/01/2003	14.45	28/02/2003	11.26	26/04/2003	0.561	22/06/2003	0.91	18/08/2003	0.58	14/10/2003	0.284
03/01/2003	9.154	01/03/2003	6.462	27/04/2003	2.425	23/06/2003	0.88	19/08/2003	0.54	15/10/2003	0.273
04/01/2003	7.022	02/03/2003	3.772	28/04/2003	5.885	24/06/2003	0.82	20/08/2003	0.53	16/10/2003	0.261
05/01/2003	5.994	03/03/2003	3.796	29/04/2003	2.105	25/06/2003	0.78	21/08/2003	0.53	17/10/2003	0.258
06/01/2003	5.005	04/03/2003	4.255	30/04/2003	1.668	26/06/2003	0.77	22/08/2003	0.53	18/10/2003	0.254
07/01/2003	4.305	05/03/2003	6.61	01/05/2003	3.866	27/06/2003	0.81	23/08/2003	0.52	19/10/2003	0.252
08/01/2003	3.766	06/03/2003	3.99	02/05/2003	7.298	28/06/2003	0.74	24/08/2003	0.5	20/10/2003	0.246
09/01/2003	3.188	07/03/2003	5.022	03/05/2003	2.612	29/06/2003	0.75	25/08/2003	0.47	21/10/2003	0.252
10/01/2003	2.807	08/03/2003	3.666	04/05/2003	1.83	30/06/2003	0.74	26/08/2003	0.5	22/10/2003	0.835
11/01/2003	2.526	09/03/2003	3.218	05/05/2003	1.598	01/07/2003	0.72	27/08/2003	0.46	23/10/2003	0.658
12/01/2003	2.207	10/03/2003	2.899	06/05/2003	1.407	02/07/2003	0.7	28/08/2003	0.47	24/10/2003	0.416
13/01/2003	2	11/03/2003	2.719	07/05/2003	1.317	03/07/2003	0.64	29/08/2003	0.52	25/10/2003	0.326
14/01/2003	1.944	12/03/2003	2.316	08/05/2003	1.242	04/07/2003	0.61	30/08/2003	0.48	26/10/2003	0.317
15/01/2003	2.034	13/03/2003	2.111	09/05/2003	1.096	05/07/2003	0.59	31/08/2003	0.47	27/10/2003	0.292
16/01/2003	1.601	14/03/2003	1.95	10/05/2003	1.055	06/07/2003	0.58	01/09/2003	0.44	28/10/2003	0.33
17/01/2003	1.869	15/03/2003	1.783	11/05/2003	1.01	07/07/2003	0.57	02/09/2003	0.43	29/10/2003	0.804
18/01/2003	5.578	16/03/2003	1.667	12/05/2003	1.012	08/07/2003	0.57	03/09/2003	0.42	30/10/2003	2.933
19/01/2003	6.926	17/03/2003	1.496	13/05/2003	0.921	09/07/2003	0.55	04/09/2003	0.4	31/10/2003	0.884
20/01/2003	14.14	18/03/2003	1.373	14/05/2003	0.846	10/07/2003	0.53	05/09/2003	0.39	01/11/2003	1.485
21/01/2003	7.392	19/03/2003	1.259	15/05/2003	1.276	11/07/2003	0.51	06/09/2003	0.4	02/11/2003	3.383
22/01/2003	4.796	20/03/2003	1.205	16/05/2003	4.251	12/07/2003	0.49	07/09/2003	0.46	03/11/2003	1.15
23/01/2003	3.591	21/03/2003	1.2	17/05/2003	2.057	13/07/2003	0.46	08/09/2003	0.46	04/11/2003	0.724
24/01/2003	3.185	22/03/2003	1.183	18/05/2003	1.873	14/07/2003	0.44	09/09/2003	0.41	05/11/2003	0.689
25/01/2003	3.004	23/03/2003	1.111	19/05/2003	2.981	15/07/2003	0.44	10/09/2003	0.44	06/11/2003	0.699
26/01/2003	2.711	24/03/2003	1.046	20/05/2003	1.881	16/07/2003	0.51	11/09/2003	0.4	07/11/2003	0.522
27/01/2003	2.333	25/03/2003	0.96	21/05/2003	1.684	17/07/2003	0.47	12/09/2003	0.38	08/11/2003	0.474
28/01/2003	2.33	26/03/2003	0.927	22/05/2003	1.838	18/07/2003	0.48	13/09/2003	0.37	09/11/2003	0.486
29/01/2003	2.266	27/03/2003	0.904	23/05/2003	1.804	19/07/2003	0.45	14/09/2003	0.36	10/11/2003	0.527
30/01/2003	2.833	28/03/2003	0.901	24/05/2003	1.607	20/07/2003	0.57	15/09/2003	0.35	11/11/2003	1.379
31/01/2003	1.923	29/03/2003	0.947	25/05/2003	1.528	21/07/2003	0.56	16/09/2003	0.35	12/11/2003	1.364
01/02/2003	2.516	30/03/2003	0.851	26/05/2003	1.505	22/07/2003	0.47	17/09/2003	0.34	13/11/2003	0.795
02/02/2003	2.537	31/03/2003	0.773	27/05/2003	1.404	23/07/2003	0.72	18/09/2003	0.34	14/11/2003	0.814
03/02/2003	3.184	01/04/2003	0.911	28/05/2003	1.232	24/07/2003	0.76	19/09/2003	0.34	15/11/2003	0.617

References

References

Abril, G., Nogueira, M., Etcheber, H., Cabecadas, G., Lemaire, E. and Brogueira, M. J. Behaviour of Organic Carbon in Nine Contrasting European Estuaries. 2002. Estuarine, Coastal and Shelf Science **54**: 241-262.

Abril, G., Riou, S. A., Etcheber, H., Frankignoulle, M., de Wit, R. and Middelburg, J. J. Transient, tidal time-scale, nitrogen transformations in an estuarine turbidity maximum-fluid mud system (The Gironde, south-west France). 2000. Estuarine Coastal and Shelf Science **50**: 703-715.

Aiken, G., Kaplan, L. A. and Weishaar, J. Assessment of relative accuracy in the determination of organic matter concentrations in aquatic systems. 2002. Journal of Environmental Monitoring **4**: 70-74.

Al-Azri, A. R. N. Seasoanl variation of dissolved organic carbon (DOC) and nitrogen (DON) in relation to primary production and yellow substance in the Menai Strait, North Wales. 2003. PhD thesis, University of Wales-Bangor.
Ref Type: Thesis/Dissertation

Alvarez-Salgado, X. A. and Miller, A. E. J. Dissolved organic carbon in a large macrotidal estuary (the Humber, UK): Behaviour during estuarine mixing. 1998a. Marine Pollution Bulletin **37**: 216-224.

Alvarez-Salgado, X. A. and Miller, A. E. J. Simultaneous determination of dissolved organic carbon and total dissolved nitrogen in seawater by high temperature catalytic oxidation: conditions for precise shipboard measurements. 1998b. Marine Chemistry **62**: 325-333.

American Public Health Association 1995. Standard methods for Examination of water and wastewater. American Public Health Association, Washington.

Aminot, A., Kirkwood, D. S. and Kerouel, R. Determination of ammonia in seawater by the indophenol-blue method: Evaluation of the ICES NUTS I/C 5 questionnaire. 1997. Marine Chemistry **56**: 59-75.

Amon, R. M. W. and Benner, R. Bacterial utilization of different size classes of dissolved organic matter. 1996. Limnology and Oceanography **41**: 41-51.

Avery, G. B., Willey, J. D., Kieber, R. J., Shank, G. C. and Whitehead, R. F. Flux and bioavailability of Cape Fear River and rainwater dissolved organic carbon to Long Bay, southeastern United States. 2003. Global Biogeochemical Cycles **17**: art-1042.

Axler, R. P. and Reuter, J. E. A Simple Method for Estimating the N-15 Content of Dissolved Organic-Matter (do N-15) in N-Cycling Studies. 1987. Canadian Journal of Fisheries and Aquatic Sciences **44**: 130-133.

Badr, E. A., Achterberg, E. P., Tappin, A. D., Hill, S. J. and Braungardt, C. B. Determination of dissolved organic nitrogen in natural water using high-temperature catalytic oxidation. 2003. Trac-Trends in Analytical Chemistry **22**: 819-827.

Badr, E. A., Cashmore, M. and Cobb, D. The consideration of impacts upon the aquatic environment in environmental impact statements in England and Wales. 2004. Journal of Envionmental Assessment Policy and Management **6**: 19-49.

Balbi, D. M. Suspended chlorophyll in the River Nene, a small nutrient-rich river in eastern England: long-term and spatial trends. 2000. The Science of The Total Environment **251-252**: 401-421.

Becquevort, S., Bouvier, T., Lancelot, C., Cauwet, G., Deliat, G., Egorov, V. N. and Popovichev, V. N. The Seasonal Modulation of Organic Matter Utilization by Bacteria in the Danube-Black Sea Mixing Zone. 2002. Estuarine, Coastal and Shelf Science **54**: 337-354.

Benner, R. 2002. Chemical compostion and reactivity. Pages 59-90 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Benner, R., Biddanda, B., Black, B. and Mccarthy, M. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. 1997. Marine Chemistry **57**: 243-263.

Benner, R. and Hedges, J. I. A Test of the Accuracy of Fresh-Water Doc Measurements by High- Temperature Catalytic-Oxidation and Uv-Promoted Persulfate Oxidation. 1993. Marine Chemistry **41**: 161-165.

Benner, R. and Strom, M. A Critical-Evaluation of the Analytical Blank Associated with Doc Measurements by High-Temperature Catalytic-Oxidation. 1993. Marine Chemistry **41**: 153-160.

Berman, T. and Bronk, D. A. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. 2003. Aquatic Microbial Ecology **31**: 279-305.

Bjornsen, P. K. Phytoplankton Exudation of Organic-Matter - Why do Healthy Cells do It. 1988. Limnology and Oceanography **33**: 151-154.

Blough, N. V. and Del Vecchio, R. 2002. Chromophoric DOM in the coastal environment. Pages 509-546 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Borch, N. H. and Kirchmann, D. L. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. 1997. Marine Chemistry **57**: 85-95.

Borsheim, K. Y., Myklestad, S. M. and Sneli, J. A. Monthly profiles of DOC, mono- and polysaccharides at two locations in the Trondheimsfjord (Norway) during two years. 1999. Marine Chemistry **63**: 255-272.

Bouloubassi, I., Lipiatou, E., Saliot, A., Tolosa, I., Bayona, J. M. and Albaiges, J. Carbon sources and cycle in the western Mediterranean - The use of molecular markers to determine the origin of organic matter. 1997. Deep-Sea Research Part Ii-Topical Studies in Oceanography **44**: 781-799.

Bowden, K. F. 1980. Physical factors: salinity, temperature, circulation, and mixing processes. Pages 37-70 *In*: E. Olausson and I. Cato, Editors. Chemistry and biogeochemistry of estuaries. John Wiley & Sons Ltd, Chichester.

Bowers, D. G., Evans, D., Thomas, D. N., Ellis, K. and Williams, P. J. Interpreting the colour of an estuary. 2004. Estuarine, Coastal and Shelf Science **59**: 13-20.

Boyer, E. W., Goodale, C. L., Jaworsk, N. A. and Howarth, R. W. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA. 2002. Biogeochemistry **57**: 137-169.

Bricelj, V. M. and Lonsdale, D. J. Aureococcus anophagefferens: Causes and ecological consequences of brown tides in US mid-Atlantic coastal waters. 1997. Limnology and Oceanography **42**: 1023-1038.

Bronk, D. A. 2002. Dynamic of DON. Pages 153-247 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Bronk, D. A., Glibert, P. M., Malone, T. C., Banahan, S. and Sahlsten, E. Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. 1998. Aquatic Microbial Ecology **15**: 177-189.

Bronk, D. A. and Ward, B. B. Gross and net nitrogen uptake and DON release in the euphotic zone of Monterey Bay, California. 1999. Limnology and Oceanography **44**: 573-585.

Bronk, D. A., Lomas, M. W., Glibert, P. M., Schukert, K. J. and Sanderson, M. P. Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. 2000. Marine Chemistry **69**: 163-178.

Brookes, A. 1999. Environmental impact assessment for water projects. *In*: J. Petts, Editor. Handbook of environmental impact assessment, volume 2 - Environmental impact assessment in practice: impact and limitations. Blackwell Science, Oxford.

Brooks, E. A study of aluminium speciation in the River Plym. 2003. Plymouth, BSc Environmental Science project, University of Plymouth.
Ref Type: Report

Buck, A. L. An inventory of UK estuaries, Volume 6 Southern England. 1997. Join Nature Conservation Committee.

Ref Type: Report

Buesseler, K. O., Bauer, J. E., Chen, R. F., Eglinton, T. I., Gustafsson, O., Landing, W., Mopper, K., Moran, S. B., Santschi, P. H., VernonClark, R. and Wells, M. L. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: Overview and organic carbon results. 1996. Marine Chemistry **55**: 1-31.

Buffam, I., Galloway, J. N., Blum, L. K. and McGlathery, K. J. A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream. 2001. Biogeochemistry **53**: 269-306.

Burdige, D. J. 2002. Sediment pore waters. Pages 611-683 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Burdige, D. J. and Zheng, S. L. The biogeochemical cycling of dissolved organic nitrogen in estuarine sediments. 1998. Limnology and Oceanography **43**: 1796-1813.

Bushaw, K. L., Zepp, R. G., Tarr, M. A., SchulzJander, D., Bourbonniere, R. A., Hodson, R. E., Miller, W. L., Bronk, D. A. and Moran, M. A. Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. 1996. Nature **381**: 404-407.

Butler, E. I., Knox, S. and Liddicoat, M. I. The relationshipe between inorganic and organic nutrients in seawater. 1979. Journal of the Marine Biological Association of the United Kingdom **59**: 239-250.

Carlson, C. A. 2002. Production and removal processes. Pages 91-152 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Castro, M. S., Driscoll, C. T., Jordan, T. E., Reay, W. G. and Boynton, W. R. Sources of nitrogen to estuaries in the United States. 2003. Estuaries **26**: 803-814.

Cauwet, G. HTCO Method for Dissolved Organic-Carbon Analysis in Seawater - Influence of Catalyst on Blank Estimation. 1994. Marine Chemistry **47**: 55-64.

Cauwet, G. 1999. Determination of dissolved organic carbon and nitrogen by high temperature combustion. Pages 407-420 *In*: K. Grasshoff, K. Kremling and M. Ehrhardt, Editors. Methods of seawater analysis. Wiley-vch, Germany.

Cauwet, G. 2002. DOM in the coastal zone. Pages 579-609 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Cauwet, G., Miller, A., Brasse, S., Fengler, G., Mantoura, R. F. C. and Spitzy, A. Dissolved and particulate organic carbon in the western Mediterranean Sea. 1997. Deep-Sea Research Part Ii-Topical Studies in Oceanography **44**: 769-779.

Cauwet, G., Deliat, G., Krastev, A., Shtereva, G., Becquevort, S., Lancelot, C., Momzikoff, A., Saliot, A., Cociasu, A. and Popa, L. Seasonal DOC accumulation in the

Black Sea: a regional explanation for a general mechanism. 2002. Marine Chemistry **79**: 193-205.

Chang, C. C. Y., Kendall, C., Silva, S. R., Battaglin, W. A. and Campbell, D. H. Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River Basin. 2002. Canadian Journal of Fisheries and Aquatic Sciences **59**: 1874-1885.

Chapman, D. 1996. Water quality assessments- A guide to the use of biota, sediments and water in environmental monitoring. E & FN SPON An Imprint of Routledege on behalf of UNSCO, WHO and UNEP, London New York.

Chapman, P. J., Edwards, A. C. and Cresser, M. S. The nitrogen composition of streams in upland Scotland: some regional and seasonal differences. 2001. The Science of The Total Environment **265**: 65-83.

Chatfield, C. and Collins, A. J. 1980. Introduction to multivariate analysis. Chapman and Hall, London.

Chen, R. F., Zhang, Y., Vlahos, P. and Rudnick, S. M. The fluorescence of dissolved organic matter in the Mid- Atlantic Bight. 2002a. Deep-Sea Research Part Ii-Topical Studies in Oceanography **49**: 4439-4459.

Chen, W. H., Zhao, Z. Z., Koprivnjak, J. F. and Perdue, E. M. A mechanistic study of the high-temperature oxidation of organic matter in a carbon analyzer. 2002b. Marine Chemistry **78**: 185-196.

Chen, W. and Wangersky, P. J. A high-temperature catalytic oxidation method for the determination of marine dissolved organic carbon and its comparison with the UV photo-oxidation method. 1993. Marine Chemistry **42**: 95-106.

Chester, R. 2003. Marine geochemistry. Blackwell Science, Oxford.

Christian, R. R. and Thomas, C. R. Network analysis of nitrogen inputs and cycling in the Neuse River Estuary, North Carolina, USA. 2003. Estuaries **26**: 815-828.

Church, M. J., Ducklow, H. W. and Karl, D. M. Multiyear increases in dissolved organic matter inventories at station ALOHA in the North Pacific Subtropical Gyre. 2002. Limnology and Oceanography **47**: 1-10.

Cifuentes, L. A. and Eldridge, P. M. A mass- and isotope-balance model of DOC mixing in estuaries. 1998. Limnology and Oceanography **43**: 1872-1882.

Cifuentes, L. A., Sharp, J. H. and Fogel, M. L. Stable Carbon and Nitrogen Isotope Biogeochemistry in the Delaware Estuary. 1988. Limnology and Oceanography **33**: 1102-1115.

Clark, C. D., Jimenez-Morais, J., Jones, I. I., Zanardi-Lamardo, E., Moore, C. A. and Zika, R. G. A time-resolved fluorescence study of dissolved organic matter in a riverine to marine transition zone. 2002. Marine Chemistry **78**: 121-135.

Collos, Y. Nitrogen Budgets and Dissolved Organic-Matter Cycling. 1992. Marine Ecology-Progress Series **90**: 201-206.

Copping, A. E. and Lorenzen, C. J. Carbon budget of a marine phytoplankton-herbivore system with carbon-14 as a tracer. 1980. Limnology and Oceanography **25**: 873-882.

Cornell, S., Rendell, A. and Jickells, T. Atmospheric Inputs of Dissolved Organic Nitrogen to the Oceans. 1995. Nature **376**: 243-246.

Cornell, S. E., Jickells, T. D., Cape, J. N., Rowland, A. P. and Duce, R. A. Organic nitrogen deposition on land and coastal environments: a review of methods and data. 2003. Atmospheric Environment **37**: 2173-2191.

Dafner, E. V. and Wangersky, P. J. A brief overview of modern directions in marine DOC studies - Part I. Methodological aspects. 2002a. Journal of Environmental Monitoring **4**: 48-54.

Dafner, E. V. and Wangersky, P. J. A brief overview of modern directions in marine DOC studies - Part II - Recent progress in marine DOC studies. 2002b. Journal of Environmental Monitoring **4**: 55-69.

Dalsgaard, T., Canfield, D. E., Petersen, J., Thamdrup, B. and Acuna-Gonzalez, J. N-2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. 2003. Nature **422**: 606-608.

de Haas, H., van Weering, T. C. E. and de Stieger, H. Organic carbon in shelf seas: sinks or sources, processes and products. 2002. Continental Shelf Research **22**: 691-717.

Downs, M., Michener, R., Fry, B. and Nadelhoffer, K. Routine measurement of dissolved inorganic N-15 in precipitation and streamwater. 1999. Environmental Monitoring and Assessment **55**: 211-220.

Druffel, E. R. M. Comments on the importance of black carbon in the global carbon cycle. 2004. Marine Chemistry **92**: 197-200.

Eatherall, A., Warwick, M. S. and Tolchard, S. Identifying sources of dissolved organic carbon on the River Swale, Yorkshire. 2000. Science of the Total Environment **251**: 173-190.

Elliott, M. and McLusky, D. S. The Need for Definitions in Understanding Estuaries. 2002. Estuarine, Coastal and Shelf Science **55**: 815-827.

English Nature. Campaign for a living coast "Important areas for marine wildlife around England". 1996. English Nature.

Ref Type: Report

Environment Agency. Local environmental agency plan "Tamar Estuary & Tributaries" Consultation report. 1996. Environment Agency.
Ref Type: Report

Environment Agency. Local environmental agency plan "Tamar Estuary & Tributaries" first annual report. 1-29. 1998. Environment Agency.
Ref Type: Report

Escobar, I. C. and Randall, A. A. Assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC): Complementary measurements. 2001. Water Research **35**: 4444-4454.

Eyre, B. and Balls, P. A comparative study of nutrient behavior along the salinity gradient of tropical and temperate estuaries. 1999. Estuaries **22**: 313-326.

Eyrolle, F., Benedetti, M. F., Benaim, J. Y. and Fevrier, D. The distributions of colloidal and dissolved organic carbon, major elements, and trace elements in small tropical catchments. 1996. Geochimica et Cosmochimica Acta **60**: 3643-3656.

Fairbridge, R. W. 1980. The estuary: its defintion and geodynamic cycle. Pages 1-36 *In*: E. Olausson and I. Cato, Editors. Chemistry and biogeochemsitry of estuaries. John Wiley & Sons Ltd, Chichester.

Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Hogberg, P., Linder, S., Mackenzie, F. T., Moore, B., Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V. and Steffen, W. The global carbon cycle: A test of our knowledge of earth as a system. 2000. Science **290**: 291-296.

Feuerstein, T. P., Ostrom, P. H. and Ostrom, N. E. Isotopic biogeochemistry of dissolved organic nitrogen: A new technique and application. 1997. Organic Geochemistry **27**: 363-370.

Fisher, T. R., Hagy, J. D. and Rochelle-Newall, E. Dissolved and particulate organic carbon in Chesapeake Bay. 1998. Estuaries **21**: 215-229.

Frankovich, T. A. and Jones, R. D. A rapid, precise and sensitive method for the determination of total nitrogen in natural waters. 1998. Marine Chemistry **60**: 227-234.

Frias, J., Ribas, F. and Lucena, F. A Method for the Measurement of Biodegradable Organic-Carbon in Waters. 1992. Water Research **26**: 255-258.

Fry, B., Garritt, R., Tholke, K., Neill, C., Michener, R. H., Mersch, F. J. and Brand, W. Cryoflow: Cryofocusing nanomole amounts of CO2, N-2, and SO2 from an elemental analyzer for stable isotopic analysis. 1996. Rapid Communications in Mass Spectrometry **10**: 953-958.

Fuhrman, J. A. Marine viruses and their biogeochemical and ecological effects. 1999. Nature **399**: 541-548.

Galloway, J. N. The global nitrogen cycle: changes and consequences. 1998. Environmental Pollution **102**: 15-24.

Gardolinski, P. C. F. C., Hanrahan, G., Achterberg, E. P., Gledhill, M., Tappin, A. D., House, W. A. and Worsfold, P. J. Comparison of sample storage protocols for the determination of nutrients in natural waters. 2001. Water Research **35**: 3670-3678.

Glasson, J., Therivel, R. and Chadwick, A. 1999. Introduction to environmental impact assessment. UCL Press, London.

Gobler, C. J. and Sanudo-Wilhelmy, S. A. Cycling of colloidal organic carbon and nitrogen during an estuarine phytoplankton bloom . 2003. Limnology and Oceanography **48**: 2314-2320.

Goldman, J. C. and Dennett, M. R. Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation. 2000. Limnology and Oceanography **45**: 789-800.

Goni, M. A., Teixeira, M. J. and Perkey, D. W. Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA). 2003. Estuarine, Coastal and Shelf Science **57**: 1023-1048.

Hanrahan, G., Gardolinski, P., Gledhill, M. and Worsfold, P. 2002. Environmental monitoring of nutrients. 8.1-8.16 *In*: F. R. Burden, I. D. Mckelvie, U. Forstner and A. Guenther, Editors. Environmental monitoring handbook. McGraw-Hill, New York.

Hansell, D. A. Results and Observations from the Measurement of Doc and Don in Seawater Using A High-Temperature Catalytic-Oxidation Technique. 1993. Marine Chemistry **41**: 195-202.

Hansell, D. A. and Carlson, C. A. 2002. Biogeochemistry of Marine Dissolved Organic Matter. Academic press, New York.

Hansell, D. A., Bates, N. R. and Gundersen, K. Mineralization of dissolved organic carbon in the Sargasso Sea. 1995. Marine Chemistry **51**: 201-212.

Hansen, H. P. 1999. Determination of oxygen. Pages 75-90 *In*: K. Grasshoff, K. Kremling and M. Ehrhardt, Editors. Methods of seawater analysis. WILEY-VCH, Weinheim.

Hansen, H. P. and Koroleff, F. 1999. Determination of nutrients. Pages 159-228 *In*: K. Grasshoff, K. Kremling and M. Ehrhardt, Editors. Methods of seawater analysis. WILEY-VCH, Weinheim.

Hart, B. T. 2002. Water quality guidelines. 1.1-1.26 *In*: F. R. Burden, I. D. Mckelvie, U. Forstner and A. Guenther, Editors. Environmental monitoring handbook. McGraw-Hill, New York.

Hasegawa, T., Koike, I. and Mukai, H. Release of dissolved organic nitrogen by size-fractionated natural planktonic assemblages in coastal waters. 2000. Marine Ecology-Progress Series **198**: 43-49.

Head, P. C. 1985. Data presentation and interpretation. Pages 278-330 *In*: P. C. Head, Editor. Practical estuarine chemistry - A handbook. Cambridge University Press, Cambridge.

Heaton, T. H. E. Isotopic Evidence for the Diverse Origins of Nitrate Minerals. 1987. South African Journal of Science **83**: 118-119.

Heaton, T. H. E. Procedure and notes on the "diffusion" method for $^{15}N/^{14}N$ analysis of nitrate and ammonium. 176, 1-5. 2001. NGL Report Series.
Ref Type: Report

Hedges, J. I. Global Biogeochemical Cycles - Progress and Problems. 1992. Marine Chemistry **39**: 67-93.

Hedges, J. I., Bergamaschi, B. A. and Benner, R. Comparative Analyses of Doc and Don in Natural-Waters. 1993a. Marine Chemistry **41**: 121-134.

Hedges, J. I. and Keil, R. G. Sedimentary organic matter preservation: an assessment and speculative synthesis, Marine Chemistry Discussion Paper. 1995. Marine Chemistry **49**: 81-115.

Hedges, J., Lee, C. and Wangersky, P. Comments from the editors on the suzuki statement. 1993b. Marine Chemistry **41**: 289-290.

Hiscock, K. Marine nature conservation review " Benthic marine ecosystems of Great Britain and the north-east Atlantic"_. 1998. Join Nature Conservation Committee.
Ref Type: Report

Hiscock, K. and Moore, J. Surveys of harbours, rias, and estuaries in southern Britian-Plymouth area including the Yealm. Volume 1, 1-143. 1986. Pembroke, Dyfed, A report to the Nature Conservancy Council from the Field Studies Council Oil Pollution Research Unit.
Ref Type: Report

Holmes, R. M., McClelland, J. W., Sigman, D. M., Fry, B. and Peterson, B. J. Measuring N-15-NH4+ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. 1998. Marine Chemistry **60**: 235-243.

Hopkinson, C., Cifuentes, L., Burdige, D., Fitzwater, S., Hansell, D., Henrichs, S., Kahler, P., Koike, I., Walsh, T. and Bergamaschi, B. Measurement of Dissolved Organic-Carbon

and Nitrogen in Natural-Waters - Don Subgroup Report. 1993. Marine Chemistry **41**: 23-36.

Hopkinson, C. S., Buffam, I., Hobbies, J., Vallino, J., Perdue, M., Eversmeyer, B., Prahl, F., Covert, J., Hodson, R., Moran, M. A., Smith, E., Baross, J., Crump, B., Findlay, S. and Foreman, K. Terrestrial inputs of organic matter to coastal ecosystems: an intercalibration of chemical characteristics and bioavailability. 1997a. Biogeochemistry **43**: 211-234.

Hopkinson, C. S., Fry, B. and Nolin, A. L. Stoichiometry of dissolved organic matter dynamics on the continental shelf of the northeastern USA. 1997b. Continental Shelf Research **17**: 473-489.

Howell, K. A. In situ measurement of trace metals in coastal waters. 2003. PhD thesis, School of Environmental Sciences, Faculty of Science, Plymouth University .
Ref Type: Thesis/Dissertation

Howland, R. J. M., Tappin, A. D., Uncles, R. J., Plummer, D. H. and Bloomer, N. J. Distributions and seasonal variability of pH and alkalinity in the Tweed Estuary, UK. 2000. The Science of The Total Environment **251-252**: 125-138.

Hubberten, U., Lara, R. J. and Kattner, G. Refractory Organic-Compounds in Polar Waters - Relationship Between Humic Substances and Amino-Acids in the Arctic and Antarctic. 1995. Journal of Marine Research **53**: 137-149.

Ivancic, I. and Degobbis, D. An Optimal Manual Procedure for Ammonia Analysis in Natural- Waters by the Indophenol Blue Method. 1984. Water Research **18**: 1143-1147.

Jarvie, H. P., Neal, C., Tappin, A. D., Burton, J. D., Harrow, M., Hill, L. and Neal, M. The water quality of the River Trent: from the lower non-tidal reaches to the freshwater tidal zone. 2000. The Science of The Total Environment **251-252**: 335-367.

Johnston, P. and et al. Nutrient Pollution. 4. 1998. University of Exeter, Exeter, Greenpeace Research Laboratories, Department of Biological Sciences. Greenpeace Report on the world Oceans.
Ref Type: Report

Jones, V., Ruddell, C. J., Wainwright, G., Rees, H. H., Jaffe, R. and Wolff, G. A. One-dimensional and two-dimensional polyacrylamide gel electrophoresis: a tool for protein characterisation in aquatic samples. 2004. Marine Chemistry **85**: 63-73.

Kahler, P. and Koeve, W. Marine dissolved organic matter: can its C : N ratio explain carbon overconsumption? 2001. Deep-Sea Research Part I-Oceanographic Research Papers **48**: 49-62.

Kaplan, L. A. and Newbold, J. D. Measurement of Streamwater Biodegradable Dissolved Organic- Carbon with A Plug-Flow Bioreactor. 1995. Water Research **29**: 2696-2706.

Karl, D. M. and Bjorkman, K. M. 2002. Dynamics of DOP. Pages 250-366 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Keil, R. G., Montlucon, D. B., Prahl, F. G. and Hedges, J. I. Sorptive Preservation of Labile Organic-Matter in Marine- Sediments. 1994. Nature **370**: 549-552.

Keller, E. A. 1999. Introduction to Environmental Geology. Prentice Hall, New Jersey.

Kelley, K. R., Ditsch, D. C. and Alley, M. M. Diffusion and Automated N-15 Analysis of Low-Mass Ammonium Samples. 1991. Soil Science Society of America Journal **55**: 1016-1020.

Kendall, C. 1998. Tracing Nitrogen Sources and Cycling in Catchments. Pages 519-576 *In*: C. Kendall and J. J. McDonnell, Editors. Isotope Tracers in Catchment Hydrology. Elsevier Science.

Kepkay, P. E. 2000. Colloids and the Ocean Carbon Cycle. Pages 35-56 *In*: O. Hutzinger, Editor. Handbook of environmental chemistry. Springer.

Kerner, M. and Spitzy, A. Nitrate regeneration coupled to degradation of different size fractions of DON by the picoplankton in the Elbe estuary. 2001. Microbial Ecology **41**: 69-81.

Koike, I. and Tupas, L. Total Dissolved Nitrogen in the Northern North Pacific Assessed by A High-Temperature Combustion Method. 1993. Marine Chemistry **41**: 209-214.

Kroeze, C. and Seitzinger, S. P. Nitrogen inputs to rivers, estuaries and continental shelves and related nitrous oxide emissions in 1990 and 2050: a global model. 1998. Nutrient Cycling in Agroecosystems **52**: 195-212.

Krom, M. D. Spectrophotometric determination of ammonia: A study of a modified berthelot reaction using salicylate and dichloroisocyanurate. 1980. Analyst **105**: 305-316.

Krug, E. C. and Winstanley, D. The need for comprehensive and consistent treatment of the nitrogen cycle in nitrogen cycling and mass balance studies: I. Terrestrial nitrogen cycle. 2002. Science of the Total Environment **293**: 1-29.

Kuypers, M. M. M., Sliekers, A. O., Lavik, G., Schmid, M., Jorgensen, B. B., Kuenen, J. G., Damste, J. S. S., Strous, M. and Jetten, M. S. M. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. 2003. Nature **422**: 608-611.

Lake, J. L., McKinney, R. A., Osterman, F. A., Pruell, R. J., Kiddon, J., Ryba, S. A. and Libby, A. D. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. 2001. Canadian Journal of Fisheries and Aquatic Sciences **58**: 870-878.

Langston, W. J., Chesman, B. S., Burt, G. R., Hawkins, S. J., Readman, J. and Worsfold, P. Site characterisation of the South West European marine sites: Plymouth Sound and Estuaries cSAC, SPA. Occasional publication number 9. 2003. Plymouth, The Marine Biological Association.
Ref Type: Report

Lee, C. and Henrichs, S. M. How the Nature of Dissolved Organic-Matter Might Affect the Analysis of Dissolved Organic-Carbon. 1993. Marine Chemistry **41**: 105-120.

Lehmann, M. F., Bernasconi, M. and McKenzie, J. A. A method for the extraction of ammonium from freshwaters for nitrogen isotope analysis. 2001. Analytical Chemistry **73**: 4717-4721.

Lory, J. A. and Russelle, M. P. Evaluation of A Diffusion Method for Preparing Low-Nitrogen Samples for N-15 Analysis. 1994. Soil Science Society of America Journal **58**: 1400-1404.

Lucena, F., Frias, J. and Ribas, F. A New Dynamic Approach to the Determination of Biodegradable Dissolved Organic-Carbon in Water. 1991. Environmental Technology **12**: 343-347.

Mahaffey, C., Williams, R. G., Wolff, G. A., Mahowald, N., Anderson, W. and Woodward, M. Biogeochemical signatures of nitrogen fixation in the eastern North Atlantic. 2003. Geophysical Research Letters **30**: art-1300.

Mantoura, R. F. C. and Woodward, E. M. S. Conservative Behavior of Riverine Dissolved Organic-Carbon in the Severn Estuary - Chemical and Geochemical Implications. 1983. Geochimica et Cosmochimica Acta **47**: 1293-1309.

Mason, C. 2002. Biology of freshwater pollution. Prentice Hall, Harlow.

McCarthy, M., Hedges, J. and Benner, R. Major biochemical composition of dissolved high molecular weight organic matter in seawater. 1996. Marine Chemistry **55**: 281-297.

Mchale, M. R., Mitchell, M. J., McDonnell, J. J. and Cirmo, C. P. Nitrogen solutes in an Adirondack forested watershed: Importance of dissolved organic nitrogen. 2000. Biogeochemistry **48**: 165-184.

Mckelvie, I. D. 2002. In-Situ measurement of physicochemical water quality parameters. 3.1-3.21 *In*: F. R. Burden, I. D. Mckelvie, U. Forstner and A. Guenther, Editors. Environmental monitoring handbook. McGraw-Hill, New York.

McKenna, J. H. and Doering, P. H. Measurement of dissolved organic carbon by wet chemical oxidation with persulfate: influence of chloride concentration and reagent volume. 1995. Marine Chemistry **48**: 109-114.

Meybeck, M. 1993. C, N, P, and S in Rivers: from sources to global inputs. Pages 163-193 *In*: R. Wollast, F. T. Mackenzie and L. Chou, Editors. Interactions of C, N, P and S biogeochemical cycles and global change. Springer-Verlag, Berlin.

Middelburg, J. J. and Nieuwenhuize, J. Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. 2000a. Marine Ecology-Progress Series **203**: 13-21.

Middelburg, J. J. and Nieuwenhuize, J. Uptake of dissolved inorganic nitrogen in turbid, tidal estuaries. 2000b. Marine Ecology-Progress Series **192**: 79-88.

Middelburg, J. J. and Nieuwenhuize, J. Nitrogen isotope tracing of dissolved inorganic nitrogen behaviour in tidal estuaries. 2001. Estuarine Coastal and Shelf Science **53**: 385-391.

Mikhail, S. K. Toxic red tide species are on rise in Alexandria waters In Harmful Algae News: An IOC Newsletter on toxic algae and algal blooms. Wyatt, T. 22, 1-12. 2001. France, The Intergovernmental Oceanographic Commission of UNESCO.
Ref Type: Report

Miller, A. E. J. Seasonal investigations of dissolved organic carbon dynamics in the Tamar Estuary, UK. 1999. Estuarine Coastal and Shelf Science **49**: 891-908.

Miller, A. E. J., Mantoura, R. F. C. and Preston, M. R. Shipboard Investigation of Doc in the Ne Atlantic Using Platinum-Based Catalysts in A Shimadzu Toc-500 Htco Analyzer. 1993a. Marine Chemistry **41**: 215-221.

Miller, A. E. J., Mantoura, R. F. C., Suzuki, Y. and Preston, M. R. Preliminary-Study of Doc in the Tamar Estuary, Uk, Using Uv- Persulfate and Htco Techniques. 1993b. Marine Chemistry **41**: 223-228.

Mopper, K. and Kieber, D. 2002. Photochemistry and the cycling of carbon, sulfur,nitrogen and phosphorus. Pages 455-507 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Moran, M. A., Sheldon, W. M. and Sheldon, J. E. Biodegradation of riverine dissolved organic carbon in five estuaries of the southeastern United States. 1999. Estuaries **22**: 55-64.

Moran, M. A. and Zepp, R. G. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. 1997. Limnology and Oceanography **42**: 1307-1316.

Morris, P. and Biggs, J. 2001. Water. Pages 161-196 *In*: P. Morris and R. Therivel, Editors. Methods of environmental impact assessment. UCL press, London.

Mortazavi, B., Iverson, R. L. and Huang, W. R. Dissolved organic nitrogen and nitrate in Apalachicola Bay, Florida: spatial distributions and monthly budgets. 2001. Marine Ecology-Progress Series **214**: 79-91.

Muller, T. J. 1999. Determination of salinity. Pages 41-47 *In*: K. Grasshoff, K. Kremling and M. Ehrhardt, Editors. Methods of seawater analysis. WILEY-VCH, Weinheim.

Murrell, M. C. and Hollibaugh, J. T. Distribution and composition of dissolved and particulate organic carbon in northern San Francisco Bay during low flow conditions. 2000. Estuarine Coastal and Shelf Science **51**: 75-90.

Myklestad, S. M. 2000. Dissolved Organic Carbon from phytoplankton. Pages 111-148 *In*: O. Hutzinger, Editor. Handbook of environmental chemistry. Springer.

Nagata, T. 2000. Production mechanisms of dissolved organic matter. Pages 121-152 *In*: D. L. Kirchman, Editor. Microbial Ecology of the Oceans. Wiley, New York.

Nagata, T. and Kirchman, D. L. Release of dissolved organic matter by heterotrophic protozoa: implications for microbial food webs. 1992. Archiv fur Hydrobiologie **35**: 99-109.

Nagata, T. and Kirchman, D. L. Bacterial degradation of protein adsorbed to model submicron particles in seawater. 1996. Marine Ecology-Progress Series **132**: 241-248.

National River Authority. The quality of rivers and canals in England and Wales (1990 to 1992) as assessed by a new general quality assessment scheme. 1994. London, HMSO. Ref Type: Report

Neal, C., Jarvie, H. P., Whitton, B. A. and Gemmell, J. The water quality of the River Wear, north-east England. 2000a. The Science of The Total Environment **251-252**: 153-172.

Neal, C., Jarvie, H. P., Williams, R. J., Pinder, L. C., Collett, G. D., Neal, M. and Bhardwaj, L. The water quality of the Great Ouse. 2000b. The Science of The Total Environment **251-252**: 423-440.

Nedwell, D. B., Jickells, T. D., Trimmer, M. and Sanders, R. Nutrients in estuaries. 1999. Advances in Ecological Research **29**: 43-92.

Nelson, N. B. and Siegel, D. A. 2002. Chromophoric DOM in the open ocean. Pages 547-578 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Norrman, B. Filtration of Water Samples for Doc Studies. 1993. Marine Chemistry **41**: 239-242.

Ogawa, H., Amagai, Y., Koike, I., Kaiser, K. and Benner, R. Production of refractory dissolved organic matter by bacteria. 2001. Science **292**: 917-920.

Ogawa, H., Fukuda, R. and Koike, I. Vertical distributions of dissolved organic carbon and nitrogen in the Southern Ocean. 1999. Deep-Sea Research Part I-Oceanographic Research Papers **46**: 1809-1826.

Palenik, B. and Morel, F. M. M. Amino-Acid Utilization by Marine-Phytoplankton - A Novel Mechanism. 1990. Limnology and Oceanography **35**: 260-269.

Pantoja, S., Repeta, D. J., Sachs, J. P. and Sigman, D. M. Stable isotope constraints on the nitrogen cycle of the Mediterranean Sea water column. 2002. Deep-Sea Research Part I-Oceanographic Research Papers **49**: 1609-1621.

Parsons, T. R., Maita, Y. and Lalli, C. M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Peltzer, E. T. and Brewer, P. G. Some Practical Aspects of Measuring Doc - Sampling Artifacts and Analytical Problems with Marine Samples. 1993. Marine Chemistry **41**: 243-252.

Peltzer, E. T., Fry, B., Doering, P. H., McKenna, J. H., Norrman, B. and Zweifel, U. L. A comparison of methods for the measurement of dissolved organic carbon in natural waters. 1996. Marine Chemistry **54**: 85-96.

Perakis, S. S. and Hedin, L. O. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. 2002. Nature **415**: 416-419.

Perdue, E. M., Mantoura, F., Ertel, J., Lee, C., Mopper, K., Peyton, G., Tanoue, E., Williams, P. M., Zafirou, O. and Coble, P. Measurement of Dissolved Organic-Carbon and Nitrogen in Natural-Waters - Mechanisms Subgroup Report. 1993. Marine Chemistry **41**: 51-60.

Peterson, B., Fry, B., Hullar, M., Saupe, S. and Wright, R. The Distribution and Stable Carbon Isotopic Composition of Dissolved Organic-Carbon in Estuaries. 1994. Estuaries **17**: 111-121.

Peterson, M. L., Lang, S. Q., Aufdenkampe, A. K. and Hedges, J. I. Dissolved organic carbon measurement using a modified high- temperature combustion analyzer. 2003. Marine Chemistry **81**: 89-104.

Petts, J. 1999. Introduction to environmental assessment in practice: Fulfilled potential or wasted opportunity. *In*: J. Petts, Editor. Handbook of environmental impact assessment, volume 2 - Environmental impact assessment in practice: impact and limitations . Blackwell Science, Oxford.

Prahl, F. G. and Coble, p. G. 1994. Inputs and behaviour of dissolved organic carbon in the Columbia River estuary. Pages 451-457 Changes in fluxes in estuaries: implications from science to management. Olsen and Olsen, Fredensborg.

Probst, J. L., Faure, H. and Veizer, J. Global carbon cycle. 1999. Chemical Geology **159**: 1-2.

Qian, J. G. and Mopper, K. Automated high performance, high-temperature combustion total organic carbon analyzer. 1996. Analytical Chemistry **68**: 3090-3097.

Radojevic, M. and Bashkin, V. N. 1999. Practical environmental analysis. The Royal Society of Chemistry, Cambridge .

Raymond, P. A. and Bauer, J. E. DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes . 2001. Limnology and Oceanography **46**: 655-667.

Ribas, F., Frias, J. and Lucena, F. A New Dynamic Method for the Rapid-Determination of the Biodegradable Dissolved Organic-Carbon in Drinking-Water. 1991. Journal of Applied Bacteriology **71**: 371-378.

Rocha, C. Rhythmic ammonium regeneration and flushing in intertidal sediments of the Sado estuary. 1998. Limnology and Oceanography **43**: 823-831.

Rochelle-Newall, E. J. and Fisher, T. R. Chromophoric dissolved organic matter and dissolved organic carbon in Chesapeake Bay. 2002. Marine Chemistry 77: 23-41.

Rump, H. H. 1999. Laboratory manual for the examination of water, wastewater and soil. WILEY-VCH, Weinheim.

Sanders, R., Klein, C. and Jickells, T. Biogeochemical Nutrient Cycling in the Upper Great Ouse Estuary, Norfolk, U.K. 1997a. Estuarine, Coastal and Shelf Science **44**: 543-555.

Sanders, R. J., Jickells, T., Malcolm, S., Brown, J., Kirkwood, D., Reeve, A., Taylor, J., Horrobin, T. and Ashcroft, C. Nutrient fluxes through the Hamber estuary. 1997b. Journal of Sea Research **37**: 3-23.

Sawyer, C. N., McCarty, P. L. and Parkin, G. F. 1994. Chemistry for environmental engineering. McGraw-Hill, Inc., Singapore.

Searle, P. L. The Berthelot Or Indophenol Reaction and Its Use in the Analytical-Chemistry of Nitrogen - A Review. 1984. Analyst **109**: 549-568.

Seitzinger, S. P. Denitrification in Fresh-Water and Coastal Marine Ecosystems - Ecological and Geochemical Significance. 1988. Limnology and Oceanography **33**: 702-724.

Seitzinger, S. P. Linkages Between Organic-Matter Mineralization and Denitrification in 8 Riparian Wetlands. 1994. Biogeochemistry **25**: 19-39.

Seitzinger, S. P. and Sanders, R. W. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. 1997. Marine Ecology-Progress Series **159**: 1-12.

Seitzinger, S. P. and Sanders, R. W. Atmospheric inputs of dissolved organic nitrogen stimulate estuarine bacteria and phytoplankton. 1999. Limnology and Oceanography **44**: 721-730.

Seitzinger, S. P., Sanders, R. W. and Styles, R. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. 2002. Limnology and Oceanography **47**: 353-366.

Sharp, J. H. Marine dissolved organic carbon: Are the older values correct? 1997. Marine Chemistry **56**: 265-277.

Sharp, J. H. 2002. Analytical method for total DOM pools. Pages 35-58 *In*: D. A. Hansell and C. A. Carlson, Editors. Biogeochemistry of Marine Dissolved Organic Matter. Academic press.

Sharp, J. H., Beauregard, A. Y., Burdige, D., Cauwet, G., Curless, S. E., Lauck, R., Nagel, K., Ogawa, H., Parker, A. E. and Primm, O. A direct instrument comparison for measurement of total dissolved nitrogen in seawater. 2004. Marine Chemistry **84**: 181-193.

Sharp, J. H., Benner, R., Bennett, L., Carlson, C. A., Fitzwater, S. E., Peltzer, E. T. and Tupas, L. M. Analyses of Dissolved Organic-Carbon in Seawater - the Jgofs Eqpac Methods Comparison. 1995. Marine Chemistry **48**: 91-108.

Sharp, J. H., Carlson, C. A., Peltzer, E. T., Castle-Ward, D. M., Savidge, K. B. and Rinker, K. R. Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. 2002a. Marine Chemistry **77**: 239-253.

Sharp, J. H., Peltzer, E. T., Alperin, M. J., Cauwet, G., Farrington, J. W., Fry, B., Karl, D. M., Martin, J. H., Spitzy, A., Tugrul, S. and Carlson, C. A. Measurement of Dissolved Organic-Carbon and Nitrogen in Natural-Waters - Procedures Subgroup Report. 1993a. Marine Chemistry **41**: 37-49.

Sharp, J. H., Rinker, K. R., Savidge, K. B., Abell, J., Benaim, J. Y., Bronk, D., Burdige, D. J., Cauwet, G., Chen, W. H., Doval, M. D., Hansell, D., Hopkinson, C., Kattner, G., Kaumeyer, N., McGlathery, K. J., Merriam, J., Morley, N., Nagel, K., Ogawa, H., Pollard, C., Pujo-Pay, M., Raimbault, P., Sambrotto, R., Seitzinger, S., Spyres, G., Tirendi, F., Walsh, T. W. and Wong, C. S. A preliminary methods comparison for measurement of dissolved organic nitrogen in seawater. 2002b. Marine Chemistry **78**: 171-184.

Sharp, J. H., Suzuki, Y. and Munday, W. L. A Comparison of Dissolved Organic-Carbon in North-Atlantic Ocean Nearshore Waters by High-Temperature Combustion and Wet Chemical Oxidation. 1993b. Marine Chemistry **41**: 253-259.

Sigleo, A. C. and Macko, S. A. Carbon and nitrogen isotopes in suspended particles and colloids, Chesapeake and San Francisco estuaries, USA. 2002. Estuarine Coastal and Shelf Science **54**: 701-711.

Sigman, D. M., Altabet, M. A., McCorkle, D. C., Francois, R. and Fischer, G. The delta N-15 of nitrate in the Southern Ocean: Consumption of nitrate in surface waters. 1999. Global Biogeochemical Cycles **13**: 1149-1166.

Sigman, D. M., Altabet, M. A., McCorkle, D. C., Francois, R. and Fischer, G. The delta N-15 of nitrate in the Southern Ocean: Nitrogen cycling and circulation in the ocean interior. 2000. Journal of Geophysical Research-Oceans **105**: 19599-19614.

Sigman, D. M., Altabet, M. A., Michener, R., McCorkle, D. C., Fry, B. and Holmes, R. M. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic

nitrate: an adaptation of the ammonia diffusion method. 1997. Marine Chemistry **57**: 227-242.

Skoog, A., Thomas, D., Lara, R. and Richter, K. U. Methodological investigations on DOC determinations by the HTCO method. 1997. Marine Chemistry **56**: 39-44.

Slawyk, G. and Raimbault, P. Simple Procedure for Simultaneous Recovery of Dissolved Inorganic and Organic Nitrogen in N-15-Tracer Experiments and Improving the Isotopic Mass-Balance. 1995. Marine Ecology-Progress Series **124**: 289-299.

Smith, D. C., Simon, M., Alldredge, A. L. and Azam, F. Intense Hydrolytic Enzyme-Activity on Marine Aggregates and Implications for Rapid Particle Dissolution. 1992. Nature **359**: 139-142.

Sondergaard, M., Williams, P. J. L., Cauwet, G., Riemann, B., Robinson, C., Terzic, S., Woodward, E. M. S. and Worm, J. Net accumulation and flux of dissolved organic carbon and dissolved organic nitrogen in marine plankton communities. 2000. Limnology and Oceanography **45**: 1097-1111.

Sondergaard, M. and Worm, J. Measurement of biodegradable dissolved organic carbon (BDOC) in lake water with a bioreactor. 2001. Water Research **35**: 2505-2513.

Sorensen, P. and Jensen, E. S. Sequential Diffusion of Ammonium and Nitrate from Soil Extracts to A Polytetrafluoroethylene Trap for N-15 Determination. 1991. Analytica Chimica Acta **252**: 201-203.

Spyres, G., Nimmo, M., Worsfold, P. J., Achterberg, E. P. and Miller, A. E. J. Determination of dissolved organic carbon in seawater using high temperature catalytic oxidation techniques. 2000. Trac-Trends in Analytical Chemistry **19**: 498-506.

Stedmon, C. A., Markager, S. and Bro, R. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. 2003. Marine Chemistry **82**: 239-254.

Stepanauskas, R., Leonardson, L. and Tranvik, L. J. Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. 1999. Limnology and Oceanography **44**: 1477-1485.

Stumm, W. and Morgan, J. J. 1996. Dissolved Carbon Dioxide,and Global cycling. Pages 148,914-205,934 Aquatic chemistry.

Sugimura, Y. and Suzuki, Y. A High-Temperature Catalytic-Oxidation Method for the Determination of Non-Volatile Dissolved Organic-Carbon in Seawater by Direct Injection of A Liquid Sample. 1988. Marine Chemistry **24**: 105-131.

Sun, L., Perdue, E. M., Meyer, J. L. and Weis, J. Use of elemental compostion to predict bioavailability of dissolved organic matter in a Georgia River. 1997. Limnology and Oceanography **42**: 714-721.

Suzuki, Y., Sugimura, Y. and Itoh, T. A Catalytic-Oxidation Method for the Determination of Total Nitrogen Dissolved in Seawater. 1985. Marine Chemistry **16**: 83-97.

Suzuki, Y., Tanoue, E. and Ito, H. A High-Temperature Catalytic-Oxidation Method for the Determination of Dissolved Organic-Carbon in Seawater - Analysis and Improvement. 1992. Deep-Sea Research Part A-Oceanographic Research Papers **39**: 185-198.

Suzuki, Y. On the measurement of DOC and DON in seawater. 1993. Marine Chemistry **41**: 287-288.

Tanoue, E. Three vertical profiles of dissolved organic carbon in the North Pacific. 1993. Marine Chemistry **41**: 261-264.

Tipping, E., Marker, A. F. H., Butterwick, C., Collett, G. D., Cranwell, P. A., Ingram, J. K. G., Leach, D. V., Lishman, J. P., Pinder, A. C., Rigg, E. and Simon, B. M. Organic carbon in the Humber rivers. 1997. The Science of The Total Environment **194-195**: 345-355.

Toda, H., Uemura, Y., Okino, T., Kawanishi, T. and Kawashima, H. Use of nitrogen stable isotope ratio of periphyton for monitoring nitrogen sources in a river system. 2002. Water Science and Technology **46**: 431-435.

Treguer, P. and Queguiner, B. Seasonal-Variations in Conservative and Nonconservative Mixing of Nitrogen-Compounds in A West European Macrotidal Estuary. 1989. Oceanologica Acta **12**: 371-380.

Trier, C. An environmrntal appraisal of the Chelson Meadow Landfill site situated on the outskirts of the City of Plymouth, South West England. ENV307 waste management course. 2004. Plymouth, University of Plymouth.
Ref Type: Report

Tugrul, S. Comparison of TOC concentrations by persulphate-UV and high-temperature catalytic oxidation techniques in the Marmara and Black Seas. 1993. Marine Chemistry **41**: 265-270.

Tupas, L. M., Popp, B. N. and Karl, D. M. Dissolved Organic-Carbon in Oligotrophic Waters - Experiments on Sample Preservation, Storage and Analysis. 1994. Marine Chemistry **45**: 207-216.

Turley, C. M., Bale, A. J., Barquero-Molina, S., Groom, S. B., Lavender, S. J., Lewis, R. E., Lowe, D. M., Tappin, A. D., Uncles, R. J. and Widdows, J. A scientific perspective of estuarine complexity and the use of classification indices. Aquatic Conservation . 2005.
Ref Type: In Press

Turner, A., Le Roux, S. M. and Millward, G. E. Speciation and partitioning of cadmium and zinc in two contrasting estuaries: The role of hydrophobic organic matter. 2004. Limnology and Oceanography **49**: 11-19.

Turner, S. J. and Kendall, M. A. A comparison of vegetated and unvegetated soft-sediment macrobenthic communities in the River Yealm, south-western Britain. 1999. Journal of the Marine Biological Association of the United Kingdom **79**: 741-743.

Uncles, R. J., Easton, A. E., Griffiths, M. L., Harris, C., Howland, R. J. M., Joint, I., King, R. S., Morris, A. W. and Plummer, D. H. Concentrations of suspended chlorophyll in the tidal Yorkshire Ouse and Humber Estuary. 1998a. The Science of The Total Environment **210-211**: 367-375.

Uncles, R. J., Howland, R. J. M., Easton, A. E., Griffiths, M. L., Harris, C., King, R. S., Morris, A. W., Plummer, D. H. and Woodward, E. M. S. Concentrations of dissolved nutrients in the tidal Yorkshire Ouse River and Humber Estuary. 1998b. The Science of The Total Environment **210-211**: 377-388.

Uncles, R. J., Stephens, J. A. and Smith, R. E. The dependence of estuarine turbidity on tidal intrusion length, tidal range and residence time. 2002. Continental Shelf Research **22**: 1835-1856.

USEPA. Method 445.0 "In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence. 445.0-0-445.0-22. 1997. United State Environmental Protection Agency.
Ref Type: Report

Velinsky, D. J., Fogel, M. L., Pennock, J. R. and Sharp, J. H. Biogeochemistry of Nitrogen Isotopes in the Delaware Estuary and Coastal Salt Marshes. 1990. Abstracts of Papers of the American Chemical Society **199**: 51-GEOC.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H. and Tilman, D. G. Human alteration of the global nitrogen cycle: Sources and consequences. 1997. Ecological Applications **7**: 737-750.

Volk, C., Wood, L., Johnson, B., Robinson, J., Zhu, H. W. and Kaplan, L. Monitoring dissolved organic carbon in surface and drinking waters. 2002. Journal of Environmental Monitoring **4**: 43-47.

Volk, C. J., Volk, C. B. and Kaplan, L. A. Chemical composition of biodegradable dissolved organic matter in streamwater. 1997. Limnology and Oceanography **42**: 39-44.

Wallace, B., Purcell, M. and Furlong, J. Total organic carbon analysis as a precursor to disinfection byproducts in potable water: Oxidation technique considerations. 2002. Journal of Environmental Monitoring **4**: 35-42.

Walsh, T. W. Total Dissolved Nitrogen in Seawater - A New High-Temperature Combustion Method and A Comparison with Photo-Oxidation. 1989. Marine Chemistry **26**: 295-311.

Wangersky, P. J. Dissolved Organic-Carbon Methods - A Critical-Review. 1993. Marine Chemistry **41**: 61-74.

Wangersky, P. J. 2000. Intercomparisons and Intercalibrations. Pages 167-191 *In*: O. Hutzinger, Editor. Handbook of environmental chemistry. Springer.

Ward, B. B. and Bronk, D. A. Net nitrogen uptake and DON release in surface waters: importance of trophic interactions implied from size fractionation experiments. 2001. Marine Ecology-Progress Series **219**: 11-24.

Wells, M. L. and Goldberg, E. D. Occurrence of Small Colloids in Sea-Water. 1991. Nature **353**: 342-344.

Westerhoff, P. and Mash, H. Dissolved organic nitrogen in drinking water supplies: a review. 2002. Journal of Water Supply Research and Technology-Aqua **51**: 415-448.

Whitehead, R. F., de Mora, S., Demers, S., Gosselin, M., Monfort, P. and Mostajir, B. Interactions of ultraviolet-B radiation, mixing, and biological activity on photobleaching of natural chromophoric dissolved organic matter: A mesocosm study. 2000. Limnology and Oceanography **45**: 278-291.

Wiebinga, C. J. and de Baar, H. J. W. Determination of the distribution of dissolved organic carbon in the Indian sector of the Southern Ocean. 1998. Marine Chemistry **61**: 185-201.

Wiegner, T. N. and Seitzinger, S. P. Photochemical and microbial degradation of external dissolved organic matter inputs to rivers. 2001. Aquatic Microbial Ecology **24**: 27-40.

Wiegner, T. N. and Seitzinger, S. P. Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. 2004. Limnology and Oceanography **49**: 1703-1712.

Williams, P. J. L., Bauer, J., Benner, R., Hegeman, J., Ittekkot, V., Miller, A., Norrman, B., Suzuki, Y., Wangersky, P. and Mccarthy, M. Measurement of Dissolved Organic-Carbon and Nitrogen in Natural-Waters - Doc Subgroup Report. 1993a. Marine Chemistry **41**: 11-21.

Williams, P. M., Bauer, J. E., Robertson, K. J., Wolgast, D. M. and Occelli, M. L. Report on DOC and DON measurements made at Scripps Institution of Oceanography, 1988-1991. 1993b. Marine Chemistry **41**: 271-281.

Williams, P. J. Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. 1995. Marine Chemistry **51**: 17-29.

Wu, J. P., Calvert, S. E. and Wong, C. S. Carbon and Nitrogen Isotope Ratios in Sedimenting Particulate Organic Matter at an Upwelling Site off Vancouver Island. 1999. Estuarine, Coastal and Shelf Science **48**: 193-203.