University of Plymouth

PEARL **https://pearl.plymouth.ac.uk**

04 University of Plymouth Research Theses 01 Research Theses Main Collection

2002

In situ monitoring and biogeochemical cycling of nutrients in estuarine waters

Gardolinski, Paulo Cesar Ferreira da Costa

http://hdl.handle.net/10026.1/497

http://dx.doi.org/10.24382/3565
University of Plymouth

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Copyright

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with the author and that no quotation from the thesis and no information derived from it may be published without the author's prior consent.

Signed.....

Date.....03/09/02

IN SITU MONITORING AND BIOGEOCHEMICAL CYCLING OF NUTRIENTS IN ESTUARINE WATERS

by

Paulo Cesar Ferreira da Costa Gardolinski

A thesis submitted to the University of Plymouth
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

Department of Environmental Sciences
Faculty of Science

Funded by CNPq –Brazil

September 2002

REFERENCE ONLY

90 0524983 2

LIBRARY STORE

REFERENCE ONLY

30 JAN 2003
16 JUL 2003

UNIVERSITY OF PLYMOUTH

PLYMOUTH LIBRARY

Tel: (01752) 232323
This book is subject to recall if required by another reader
Books may be renewed by phone
CHARGES WILL BE MADE FOR OVERDUE BOOKS

REFERENCE ONLY

UNIVERSITY OF PLYMOUTH
Item No. 9005249832
Date 13 NOV 2002
Class No. THESIS 594.526365 GAR
Cont. No. X704498358
PLYMOUTH LIBRARY

LIBRARY STORE

ABSTRACT

In situ monitoring and biogeochemical cycling of nutrients in estuarine waters

Paulo Cesar Ferreira da Costa Gardolinski

This thesis describes the use of *in situ* and laboratory techniques for monitoring nitrate, phosphate and master variables in the environment. Chapter One presents a general overview of nutrients, how they are essential for aquatic biota, their general characteristics and the importance of their behaviour in estuaries. Commonly used analytical methods for nutrient determinations in natural waters are also evaluated.

Chapter Two reports the use of chemometrics to determine trends in historical time-series physico-chemical datasets (*e.g.* nitrate+nitrite, phosphate, river flow, precipitation, suspended solids, water temperature and chlorophyll a) from the Tamar catchment, UK. The results provided useful information about correlations between the studied variables. The influence of time delay to correlate variables, such as rainfall and river flow, rainfall and nitrate+nitrite, river flow and nitrate+nitrite, rainfall and phosphate, river flow and phosphate, demonstrated the interaction between variables over time, and increased their correlation coefficients. Seasonal trends were separated using PCA. Models for nitrate +nitrite and phosphate were built to predict their behaviour based on the other five physico-chemical variables, and explained 91.3 and 72.9 % of the variance for nitrate+nitrite and phosphate, respectively.

In Chapter Three evaluation of different natural waters sample storage techniques is reported. Results indicate that it is generally not possible to develop a single storage protocol for different kinds of natural waters. During sample storage the preservation of nutrients was found to be extremely matrix dependent, *e.g.* phosphate co-precipitated when calcium rich samples were frozen, and chemical treatment of samples rich in dissolved organic carbon resulted in a slight increase in phosphate concentration. In order to overcome these problems, practical guidelines on how to elaborate a site-specific protocol for sample storage are presented.

Chapter Four reports the development and deployment of a submersible flow injection spectrophotometric (submersible FI) analyser for determination of nitrate in estuarine waters. Key features of the analyser are its portability and ease of deployment owing to its small size, weight and low buoyancy. The analyser was optimised to perform accurately in the laboratory, on board ship (during the Impact Cruise) and *in situ* during field surveys. The detection limit achieved was 2.8 $\mu g\ L^{-1}$ N whilst the linear range could be varied from 2.8 – 100 $\mu g\ L^{-1}$ N up to 100 – 2000 $\mu g\ L^{-1}$ N over a salinity range of 0 – 35 ‰ requiring only small changes in the manifold. The good instrument performance was assured during an intercomparison exercise (32 laboratories) for the determination of nitrate in seawater (assigned tolerance 327.6 ± 33.6 $\mu g\ L^{-1}$, submersible FI 351.4 ± 28.0 $\mu g\ L^{-1}$) and comparison with the University of Plymouth reference method.

The results from eight surveys within the Tamar Estuary for the monitoring of nitrate, phosphate, pH, dissolved oxygen, salinity, conductivity and temperature, are presented in Chapter Five. These results were comparable with previous literature datasets, *e.g.* nitrate+nitrite and phosphate concentrations. This suggests that estuarine conditions have remained fairly consistent over the last 20 years.

Laboratory experiments undertaken to help understand some of the environmental processes previously described are presented in Chapter Six. The aim of the investigation was to monitor dissolved (<0.45 μm) organic and inorganic phosphorus (Po, Pi) forms that could be released into the estuarine mixing zone. Results indicate that a measurable and significant release of Po, from suspended sediments, occurred when salinity was increased to 5 and 10 ‰ prior to the release of Pi. The analytical method proved to be very sensitive and reproducible (RSD < 3%) for this type of matrix, achieving limits of detection ranging from 1.8 – 3.0 $\mu g\ L^{-1}$ $P\text{-}PO_4$ with recovery of the organic phosphorus model compound typically between 76 – 87 %.

ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude to my very understanding supervisor, Professor Paul Worsfold, whose friendly advice, comments and subtle hints (wherever the place...) throughout my Ph.D. made the journey much more enjoyable. As one would say: "Excellent, ...it's coming along!" Many, many thanks to Alan Tappin, whose advice as always provided in a brief space of time, although accompanied with a huge number of ideas. Thanks also to Martha Gledhill, for helping with the first steps of this project.

I am very grateful to Tim Fileman, principal scientist of the IMPACT Cruise, for the opportunity to take part in and work (and laugh) a lot during the cruise. Also thanks for all those involved in the cruise and in one way or another helped with my work.

I would like to acknowledge Tony David for his immense scientific, professional and 'food wise' support during all the surveys of the Tamar, especially on those cold and dark winter mornings and during storm conditions under gale force winds...

A big 'Thank You' for all the friendly and always helpful ladies in the Environmental Sciences Department Tamsin, Donella, Lee, Debbie and Elaine, also to the technical support group - Ian, Sally, Andrew, Andy, Jeremy, Adrian and, Derek (good luck mate).

Thanks a lot also for all the ex-members of The Complex (T, Vinc, Phil, Cyril, Grady), you guys made that two years pass like two months... Now, how to start with the "106/102 crowd"??? All the oldies thanks for all the advice: Matt (first housemate), Andy Bowie (and the last drink...), Simon (now Captain') Coles (great help on the night life) and Richard Sandford (great partner for long talks...). To those incredible guys, friends and research partners, Grady (long lab hours) and Vinc (professional victim...), all the best for your careers, whatever they will be... To all those 'foreigners' that just stayed for a short while, but were nevertheless an important presence: JoJo (The big friend), Bellen, Denise-Denise, Gregor, Rebeca (always joking, laughing, 'oiiiiie'), Christina, Raquel, Luis (Pre-Dr No, the men of the numbers and the graphs...), Paula (the random girl) and Stephan ('did I tell u about my system???') thanks for your time and friendship! 'Our 106 Group now' Simon (follow your heart dude!), Laura (don't let that trip escape, enjoy down under), Utra (you better take care of Jennifer, ok?) and Omaka (Sr. keep your happiness!) best wishes for all of you! To all the friends of the Department, especially Tony, Andy C., Rob, Andy B., Emma (keep learning pool...), Paul (you showed me the way to the Clifton, and now... you are the best reviser...) all the best for all of you!!!.

Big cheers to Ian McKelvie for his patience, advice and help in Monash, Australia. Also good luck for Amanda, Sarah, Nana, Belinda (always smiling) and for Peter (the problem solver man).

Special thanks for the Brazilian community, for all the parties (even if I was not there very often) but mainly: "Galera, Valeu pelo Penta-Brasil !!!" Thanks a lot for my 'English family' from the Clifton, 4 years of Tuesday support, cheers Ged, Trish, Caroline, Helen, Lucy, Lez, Gloria and George!

Thanks for the best flatmate ever! Nui, this year has been great in our flat, you are 'sooo' patient...

The most special and full of love thanks to Kate, who has made my life in the last year extremely enjoyable and funny, and don't worry, because our dreams are coming true already...

..And finally, Thanks with all my heart to my family, which even being spread around the world managed to "send me" love over the last 4 years. "Mãe, Pai, sem a ajuda, o amor e a confiança de vocês em mim, eu nunca teria chegado ate aqui, e ainda, não seria a metade do homem que sou!!! Muito amor a todos vocês!!!"

"I respect a man who knows how to spell a word more than one way." – Mark Twain

AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award. The work was fully financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. A research collaboration program was developed under the basis of the 'Memorandum of Agreement' between the University of Plymouth and Monash University, Australia. The exchange had financial support, in the form of subsistence grant, from the International Research Exchange Program (IREX), Australia.

A programme of advanced study related to nutrients was undertaken, which included the participation in a scientific cruise (IMPACT Cruise), research of publicly accessible data bases, an investigation into methods for the storage of water samples, several surveys of the Tamar Estuary, development and deployment of *in situ* instrumentation, and development of an incubation experiment. Relevant scientific seminars and conferences were regularly attended, at which work was presented, and three papers and two book chapters were prepared for publication.

The work presented in Chapter Two was prepared with the collaboration of Luis A. Tortajada-Genaro (Universitat de Valencia, Spain), and the work presented in Chapter Three was performed in collaboration with Dr Grady Hanrahan (Louisiana Universities Marine Consortium). All other data presented in this thesis was prepared by the author, whom the ownership rests with. Before using this data in any presentation or printed publication, please contact the author and include full acknowledgements.

Signed .....

Date 03/09/2002

PUBLICATIONS (see Appendices)

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. *Water Research*, 2001; 35: 3670-3678.

Hanrahan, G., Gardolinski, P.C.F.C., Gledhill, M., and Worsfold, P., 2002, Environmental Monitoring of Nutrients. In *'Handbook of Environmental Monitoring'* (Eds. Burden, F.R., McKelvie, I., Förstner, U. and Guenther, A.) pp 8.1-8.16. (McGraw-Hill, New York) ISBN 0 07 135176 0

Worsfold, P.J., Achterberg, E.P., Bowie, A.R., Sandford, R., Cannizzaro, V. and Gardolinski, P.C.F.C. (2002) Flow injection techniques for the in situ monitoring of marine processes. In *'Chemistry of Marine Water and Sediments'* (Eds. Gianguzza A., Pelizzetti, E., and Sammartano S.) pp. 385-402, ISBN 3-540-42055-X.

Gardolinski, P.C.F.C., David, A.R.J. and Worsfold, P.J. Miniature flow injection analyser for laboratory, shipboard and *in situ* monitoring of nitrate in estuarine and coastal waters. *Talanta* – in press.

Gardolinski, P.C.F.C., Worsfold, P.J. and McKelvie I.D. Salinity induced release and transformation of organic phosphorus from river sediments. *Water Research* - submitted.

PRESENTATIONS AND CONFERENCES ATTENDED

36th R&D in Analytical Chemitry – Royal Society of Chemistry, University of Greenwich (London). Poster presentation: "*Biogeochemistry of nutrients in the Tamar Estuary using a submersible Flow Injection Analyser*". April 1999.

III PICO - Progress in Chemical Oceanography, University of Plymouth (Plymouth). Poster presentation: "*Biogeochemistry of nutrients in the Tamar estuary using submersible FIA*". September 1999.

3rd Euroconference on Environmental Analytical Chemistry, Sani Beach Hotel – Chalkidiki, Greece. Poster presentation: "*Estuarine biogeochemistry of nitrogen using submersible flow injection*". October 1999

37th R&D in Analytical Chemistry at Young Researchers' Meeting and Specialist Symposia, at the Annual Conference, UMIST – University of Manchester Institute of Science and Technology (Manchester). Poster presentation: "*Estuarine biogeochemistry of nitrogen using submersible flow injection*". July 2000.

IV PICO - Progress in Chemical Oceanography, Bangor - Wales. University of Wales. Poster presentation: "*Behaviour of dissolved organic and inorganic phosphorus released from suspended sediments under estuarine conditions*"*. September 2001, *Awarded prize for best poster presentation.

Analytical Research Forum - Incorporating R&D Topics, Norwich. Royal Society of Chemistry. Poster presentation: '*Behaviour of dissolved organic and inorganic phosphorus released from suspended sediments under estuarine conditions*'. June 2001.

International Phosphorus Transfer Workshop, Plymouth. Connecting Phosphorus Transfer from Agriculture to Impacts in Surface Waters. Institute of Grassland & Environmental Research. Co-author of oral presentation: "*Phosphorus in fluvial systems: speciation, transformations and dynamics*". June 2001.

International Symposium of Environmental Analytical Chemistry - ISEAC 32, University of Plymouth and Plymouth Marine Laboratories (Plymouth). Poster presentation: "*Miniature flow injection analyser for laboratory, shipboard and in situ monitoring of nitrate in estuarine and coastal waters*". June 2002.

Analytical Research Forum - Incorporating R&D Topics, Kingston - Kingston University. Royal Society of Chemistry. Poster presentation: "*Miniature flow injection analyser for laboratory, shipboard and in situ monitoring of nitrate in estuarine and coastal waters*". July 2002.

TABLE OF CONTENTS

1 CHAPTER ONE

INTRODUCTION 1

1.1 NUTRIENTS 1

1.1.1 The Nitrogen cycle 1

1.1.2 The Phosphorus Cycle 4

1.1.3 Eutrophication 9

1.1.4 Legislation Relating to Nutrients in Aquatic Environments 12

1.2 ESTUARIES 13

1.2.1 General characteristics and classifications 13

1.2.2 Physico-chemical estuarine processes 16

1.2.3 Ecological importance of estuaries 17

1.3 ANALYTICAL METHODS FOR DETERMINATION OF NUTRIENTS IN NATURAL WATERS 18

1.3.1 Nitrate Determination 18

1.3.2 Phosphorus Determination 23

1.3.3 Flow Injection 26

1.3.4 Sampling and sample storage 28

1.3.4.1 Site selection 29

1.3.4.2 Sampling frequency and cost 29

1.3.4.3 Sample collection and storage 30

1.3.4.4 Filtration procedure 30

1.3.4.5 Storage procedure 31

1.3.4.6 Storage container 32

1.4 RESEARCH AIMS AND OBJECTIVES 33

2 CHAPTER TWO

IDENTIFICATION OF ENVIRONMENTAL TRENDS IN TIME SERIES DATA FROM THE TAMAR CATCHMENT

2.1 INTRODUCTION 34

2.1.1 Historical background of the Tamar Area 34

2.1.2 Current Environmental Regulations 35

2.1.5 Time series studies 36

2.1.4 Predictive models 37

2.1.5 Aims 39

2.2 EXPERIMENTAL 39

2.2.1 Description of River Tamar Catchment and Tamar Estuary 39

2.2.2 Time series data 41

2.3 Methods 42

2.3.1 Data matrix preparation 42

2.4 RESULTS AND DISCUSSION 43

2.4.1 Study of the distributions of the measured parameters 43

2.4.2 Study of variables correlations and seasonal trends 49

2.4.3 Modelling of nutrients 55

2.5 CONCLUSIONS 58

2.5.1 Study of the distributions of the measured parameters 60

2.5.2 Study of correlation and seasonal trends 60

2.5.3 Modelling of nutrients 61

3 CHAPTER THREE
COMPARISON OF SAMPLE STORAGE PROTOCOLS FOR THE DETERMINATION OF NUTRIENTS IN NATURAL WATERS
3.1 INTRODUCTION ... 62
3.1.1 Sample matrix ... 62
3.1.2 Previous studies ... 63
3.2 EXPERIMENTAL ... 65
3.2.1 Sampling sites ... 65
3.2.2 Cleaning procedure ... 66
3.2.3 Sampling and storage procedures ... 66
3.2.4 Instrumentation ... 69
3.2.5 Reagents and standards ... 72
3.3 RESULTS AND DISCUSSION ... 73
3.3.1 TON and FRP in controls (February, 1999) ... 74
3.3.2 TON in natural water sub-samples (February, 1999) ... 76
3.3.3 FRP in natural water sub-samples (February, 1999) ... 76
3.3.4 TON in natural water sub-samples (October, 1999 ... 79
3.3.5 FRP in natural water sub-samples (October, 1999) ... 81
3.4 CONCLUSIONS ... 81

4 CHAPTER FOUR
DESIGN AND DEVELOPMENT OF A SUBMERSIBLE FLOW INJECTION ANALYSER FOR THE DETERMINATION OF NITRATE IN ESTUARINE AND COASTAL WATERS
4.1 INTRODUCTION ... 85
4.2 EXPERIMENTAL ... 85
4.2.1 Reagents ... 85
4.2.2 Cleaning protocol ... 86
4.2.3 Instrumentation ... 86
4.2.4 Deployments ... 88
4.3 RESULTS AND DISCUSSION ... 93
4.3.1 Laboratory optimisation ... 93
4.3.2 Instrument validation ... 96
4.3.3 Operating modes ... 98
4.3.4 Shipboard deployment ... 100
4.3.5 Submersible deployments ... 106
4.4 CONCLUSIONS ... 108

5 CHAPTER FIVE
BIOGEOCHEMISTRY OF NUTRIENTS IN THE TAMAR ESTUARY
5.1 INTRODUCTION ... 109
5.2 EXPERIMENTAL ... 110
5.2.1 Field campaigns ... 110
5.2.2 Sampling sites ... 110
5.2.3 Laboratory preparation ... 111
5.3.4 Data analysis from the Environment Agency and South West Water ... 113
5.3 RESULTS AND DISCUSSION ... 116
5.3.1 Axial transects ... 116
Transect 1 ... 116
Transect 2 ... 118

Transect 3.....121
Transect 4.....123
5.3.2 Tidal cycles.....125
Tidal cycle 1.....125
Tidal cycle 2.....127
Tidal cycle 3.....127
Tidal cycle 4.....130
5.4 CONCLUSIONS.....132

6 ***CHAPTER SIX***
SALINITY INDUCED RELEASE AND TRANSFORMATION OF ORGANIC PHOSPHORUS FROM RIVER SEDIMENTS
6.1 **INTRODUCTION**.....134
6.2 **EXPERIMENTAL**.....136
6.2.1 Cleaning procedure.....136
6.2.2 Sampling.....136
6.2.3 Reagents.....138
6.2.4 Preparation of slurries.....139
6.2.5 Instrumentation.....139
6.2.6 Experiment design.....141
6.3 **RESULTS AND DISCUSSION**.....143
6.4 **CONCLUSIONS**.....146

7 ***CHAPTER SEVEN***
CONCLUSIONS AND FUTURE WORK
7.1 **GENERAL CONCLUSIONS**.....147
7.1.1 Historical time series datasets.....147
7.1.2 Nutrients sampling and storage.....147
7.1.3 The use of a submersible FI analyser for nitrate determination.....148
7.1.4 Tamar Estuary surveys.....148
7.1.5 Organic and inorganic phosphorus release from sediments.....149
7.2 **SUGGESTIONS FOR FUTURE WORK**.....149
7.2.1 Time series datasets.....149
7.2.2 Sample storage protocols.....150
7.2.3 Development of the submersible FI analyser for phosphate Determination.....150
7.2.4 Estuarine biogeochemistry of nutrients.....150
7.2.5 Release of phosphorus species from suspended sediments.....150
7.3 **GUIDELINES FOR MONITORING AND MODELLING THE BIOGEOCHEMICAL CYCLING OF NUTRIENTS IN ESTUARIES**.....150

REFERENCES.....153

APPENDICES

TABLE OF FIGURES

CHAPTER 1

Figure 1.1 Nitrogen speciation in natural waters, showing typical concentrations expressed as mg L^{-1} N.....2

Figure 1.2 The aquatic Nitrogen Cycle 3
Figure 1.3 Operationally defined phosphorous fractions in natural waters 5
Figure 1.4 The aquatic Phosphorus Cycle 5
Figure 1.5 Phosphorus species 7
Figure 1.6 Structural formula of phytic acid 8
Figure 1.7 Example of one of the effects of eutrophication in the Sea of Azov, north of Black Sea 10
Figure 1.8 Distribution of chlorophyll in waters worldwide, image from the week 20 – 27/07/2002 12
Figure 1.9 Diagram showing the diazotisation and coupling reaction 24
Figure 1.10 Block diagram of principal FI components 27

CHAPTER TWO

Figure 2.1 The Tamar Valley and some of the mining locations 34
Figure 2.2 Tamar Area located in UK 40
Figure 2.3 Distribution of weekly values fro nitrate and phosphate during the period 1979-2002 44
Figure 2.4 Boxplots for studied variables 47
Figure 2.5 3-D representations of the data 1974-1998 using triangular interpolation for response curves 48
Figure 2.6 PCA score plots showing the seasonal separation of the week periods, corresponding to the seasons of the year 50
Figure 2.7 Loading plots showing similar distributions due to the main contribution of PC1 51
Figure 2.8 Representation of the flow periods using median values for the complete 28 years dataset 53
Figure 2.9 Correlation coefficient for several parameters: nitrate+nitrite, phosphate, flow and suspended solids, versus some delayed weeks of expressed parameters 54
Figure 2.10 Regression coefficients for the best models 57
Figure 2.11 Comparison of real concentrations (mg/L) vs. predicted concentrations (mg/L) data for all weeks with complete data in the period 1974-1998, for nitrate+nitrite and phosphate 59
Figure 2.12 Comparison of results for the period 1999-2002, for real nitrate+nitrite, predicted nitrate+nitrite, real phosphate and predicted phosphate 60

CHAPTER THREE

Figure 3.1 River Frome Catchement 65
Figure 3.2 Schematic allocations of sub-samples during storage experiment 68
Figure 3.3 Flow diagram for TON determination using segmented flow analysis 70
Figure 3.4 Flow diagram for FRP determination using segmented flow analysis 71
Figure 3.5 Measured concentrations (μM) of TON and FRP in controls for the February, 1999 study 75
Figure 3.6 Measured concentrations (μM) of TON in sub-samples for the February, 1999 study 77
Figure 3.7 Measured concentrations (μM) of FRP in sub-samples for the February, 1999 study 78
Figure 3.8 Measured concentrations (μM) of TON in sub-samples for the

October, 1999 study 80
Figure 3.9 Measured concentrations (μM) of FRP in sub-samples for the October, 1999 study 82

CHAPTER FOUR

Figure 4.1 Schematic diagram of the FI manifold for nitrate determination 87
Figure 4.2 Schematic diagram of the submersible FI analyser showing key components and flow paths of the reagent and sample lines 88
Figure 4.3 Representation of the flow through solid state-detector 90
Figure 4.4 Shipboard deployment in the North Sea, during IMPACT Cruise I, 15 - 27th of September 1999 91
Figure 4.5 RRS Challenger during the IMPACT Cruise 91
Figure 4.6 Deployment of the (12 x 10 L 'Go-Flow' bottle) rosette (CTD) 92
Figure 4.7 Analyser ready for deployment, on board the research vessel *John Dory* 92
Figure 4.8 Daviron Instrument's research vessel *Tealia*, detail of the analyser inside the car and boxes containing samples and field instrumentation 93
Figure 4.9 Analysers body (manifold and electronics) and housing. Detail of a 30 cm ruler in the middle 94
Figure 4.10 Z scores for each of the 32 laboratories participants of the intercomparison for nutrients in seawater 99
Figure 4.11 Example of submersible FI analyser output 100
Figure 4.12 Depth profiles for nitrate in 31 North Sea stations 103
Figure 4.13 Tidal cycle at station 8 showing the inverse correlation between nitrate concentration and salinity, at the mouth of Humber estuary 104
Figure 4.14A Contour plot of surface nitrate distribution of North Sea during the IMPACT cruise September 1999 105
Figure 4.14B Contour plot of surface nitrate distribution of North Sea during the North Sea project Sept/Oct 1989 105
Figure 4.15 Concentration of nitrate measured in situ (Submersible FI) and in the laboratory (Skalar) in the Tamar Estuary 107

CHAPTER FIVE

Figure 5.1 Location of the 11 stations visited during the Tamar Estuary surveys 112
Figure 5.2 Location of wastewater treatment works and sampling stations in the Tamar Estuary 115
Figure 5.3 Behaviour of all the in situ and laboratory measured variables during the Tamar Estuary Axial Transect 1 (06/08/2001) 117
Figure 5.4 Behaviour of all the in situ and laboratory measured variables during the Tamar Estuary Axial Transect 2 (24/09/2001) 120
Figure 5.5 Behaviour of all the in situ and laboratory measured variables during the Tamar Estuary Axial Transect 3 (05/10/2001) 122
Figure 5.6 Behaviour of all the in situ and laboratory measured variables during the Tamar Estuary Axial Transect 4 (18/04/2002) 124
Figure 5.7 Behaviour of all the in situ and laboratory measured variables during the Tidal Cycle 1 at Weir Quay (10/11/2001) 126
Figure 5.8 Behaviour of all the in situ and laboratory measured variables during the Tidal Cycle 2 at Barn Pool (22/11/2001) 128
Figure 5.9 Behaviour of all the in situ and laboratory measured variables during the Tidal Cycle 3 at Calstock (06/12/2001) 129

Figure 5.10 Behaviour of all the in situ and laboratory measured variables during the Tidal Cycle 4 at Barn Pool (16/04/2002)) 131

CHAPTER SIX

Figure 6.1 Schematic distribution of organic P in soils 135
Figure 6.2 Location of the seawater sampling site in Wilsons Promontory, Victoria Australia 137
Figure 6.3 Stainless steel Ekman grab sampler used for collection of surface sediments 138
Figure 6.4 Glass reactor, probes and controller 140
Figure 6.5 Schematic diagram of the FI manifold for organic P determination 141
Figure 6.6 Physico-chemical parameters measured in the sample and control reactors 144
Figure 6.7 Release of inorganic (Pi) and organic (Po) phosphorus from oxic river sediment as a function of increasing salinity 145

TABLE OF TABLES

CHAPTER ONE

Table 1.1 Suggested methodologically defined classification on P forms in waters, with their equivalent established terms 6
Table 1.2 Examples of methods for the determination of nitrate in natural waters 19
Table 1.3 Techniques for the determination of phosphorus in natural waters 25

CHAPTER TWO

Table 2.1 Classification of the chemometrics methods 38
Table 2.2 Descriptive statistics of data set parameters 45
Table 2.3 Correlation matrix for data set parameters 45
Table 2.4 *t*-test of slope for all data 45
Table 2.5 Number of weeks for Kolgomorov-Sminorv test classified according to the normal statistical distribution 46
Table 2.6 Pearson coefficient for median values 52
Table 2.7 PLS-models for nitrate+nitrite 56

CHAPTER THREE

Table 3.1 Conversion chart for micromolar (μM) to μg L^{-1} 63
Table 3.2 Storage protocols for the determination of dissolved nutrients in natural waters (adapted from Maher and Woo, 1998) 64
Table 3.3A Physico-chemical parameters and grid references of sub-samples at day 0 (February 1999) 67
Table 3.3B Physico-chemical parameters and grid references of sub-samples at day 0 (October 1999) 67
Table 3.4 Operational specifications of the segmented flow analyser 72
Table 3.5A Original concentrations of TON and FRP in controls and samples on day 0 (day of sampling) for February, 1999 study 73
Table 3.5B Original concentrations of TON and FRP in controls and samples on day 0 (day of sampling) for October, 1999 study 73

CHAPTER FOUR

Table 4.1 Specifications for the submersible nutrient analyser 89

Table 4.2 Location, time of day, distance from tidal limit, salinity and nitrate concentrations for samples from stations in the Tamar Estuary, Devon, UK 90
Table 4.3 Analytical figures of merit for 20 mm and 10 mm optical path length detectors using nitrate standards in ultra pure water 95
Table 4.4 Effect of LED and flow cell design on baseline noise and response for nitrate 97
Table 4.5 Daily shipboard calibration data during the North Sea IMPACT Cruise in September 1999 102
Table 4.6 Time, concentration of nitrate in the samples and LOD for the in situ deployment 106

CHAPTER FIVE

Table 5.1 Name, national grid reference, distance from tidal limit and salinity Range at sampling stations 111
Table 5.2 Range of salinity of samples and corresponding standard used in the segmented flow analyser 113
Table 5.3 Consented dry weather flows (DWF) and maximum discharge rates for the main wastewater treatment works (WWTW) in the Tamar estuary 114
Table 5.4 Date, type of survey, river discharge, tidal condition and time of all eight surveys 116

CHAPTER SIX

Table 6.1 Physico-chemical parameters of the samples 112
Table 6.2 Analytical figures of merit for the FI system, salinity of the standards and samples, time of sampling and time of analysis 112

CHAPTER ONE

Introduction

Main topics in this chapter:

Nutrients and legislation
Estuaries
Analytical methods
Research aims and objectives

1. INTRODUCTION

1.1 Nutrients

A nutrient element is one which is functionally involved in the process of living organisms (Parsons, 1975). Nitrogen (N) is an essential nutrient for life, because it is a component of amino acids and therefore is present in all cell proteins, vitamins and nucleic acids. Phosphorus (P) is also essential for all life forms. It is a component of nucleic acids, several intermediary metabolites, and is involved in the synthesis of all complex molecules necessary for life. Life is powered by the energy released by cleaving the phosphate bond when adenosine triphosphate (ATP) is converted to adenosine diphosphate (ADP). The essential role that both nutrients play in life has lead to great interest in their behaviour in the environment. An overview of nitrogen and phosphorus in natural waters is given below.

1.1.1 The Nitrogen cycle

The elemental gas dinitrogen (N_2) is the most abundant but least reactive form of nitrogen in the global environment. However, many biochemical transformations can convert dinitrogen into dissolved inorganic species; *e.g.* nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and organic nitrogen compounds, in both dissolved and particulate forms. Nitrogen speciation can be operationally defined (Figure 1.1) as total particulate nitrogen (TPN), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) (Robards et al., 1994). Total oxidised nitrogen (TON) can be used to refer to $NO_2^- + NO_3^-$.

Figure 1.2 illustrates the aquatic nitrogen cycle, including biochemical transformations. Between those processes, fixation is the one that converts gaseous nitrogen into the bioavailable chemical form of ammonia; assimilation is the process that is well represented by primary production, during nitrification, nitrifying bacteria converts ammonia into nitrate; ammonification is the process where organic nitrogen is transformed into ammonia; and during denitrification fixed nitrate is converted back into gaseous species by denitrifying bacteria. The

atmosphere is the principal nitrogen reservoir, with over 99% of the total in the form of N_2. Nitrogen in terrestrial systems occurs mainly as soil organic matter, with litter and soil inorganic nitrogen accounting for the majority (97%), although uncertainties in the terrestrial nitrogen cycle budget have recently been reported (Krug and Winstanley, 2002).

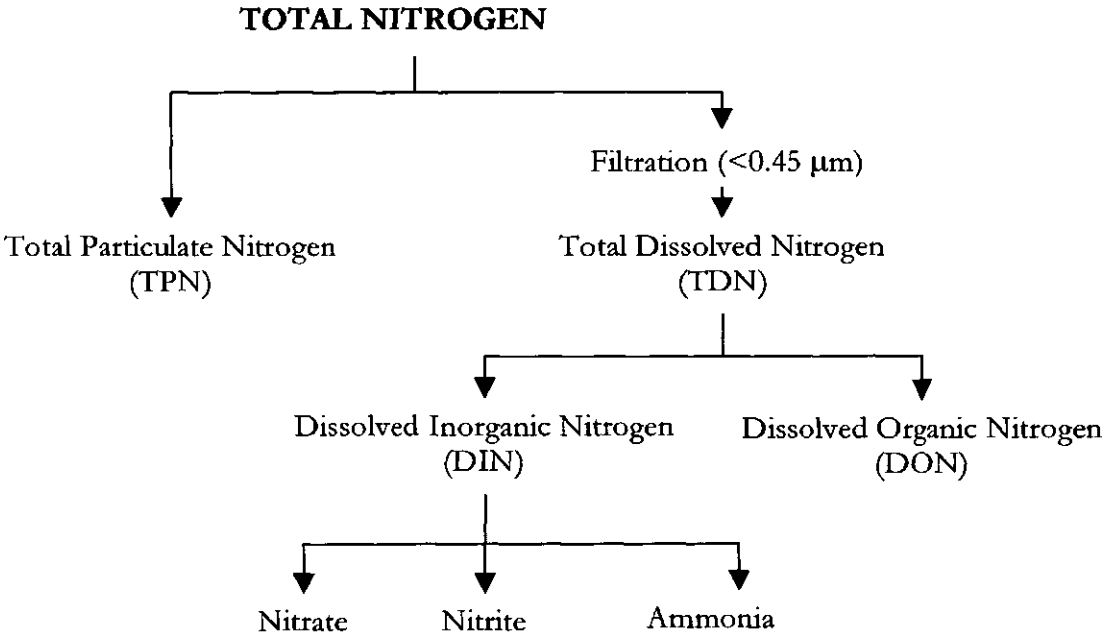


Figure 1.1 Operational definition of Nitrogen speciation and typical concentrations in natural waters expressed as mg L^{-1} N (Robards *et al.*, 1994)

N_2, in dissolved form, is the most abundant form in the world's oceans. Nitrogen also occurs in various inorganic forms, *e.g.* nitrate, nitrite, ammonia, hydrazine, nitrous oxide, and nitrogen dioxide and organic forms, *e.g.* amino acids, amines and amides. Nitrogen has five valence electrons and can take on oxidation states between +5 and -3; *e.g.* are HNO_3, N(V), NO_2, N(IV), HNO_2, N(III), NO, (II), N_2O, N(I), N_2, N(0), NH_3 and NH_4^+, N(-III). For the study of biogeochemical processes the most important forms are the dissolved inorganic species (*e.g.* nitrate, nitrite and ammonia) and organic nitrogen compounds (in dissolved and particulate forms). The organic

fraction is not well characterized yet (an analytical challenge) but a recent study showed that approximately one third of the total dissolved bioavailable nitrogen to estuarine phytoplankton was dissolved organic nitrogen (Seitzinger *et al.*, 2002).

Nitrate concentrations in many fresh water systems are increasing (Burt *et al.*, 1996). This rise is attributed to intensive agricultural practices with the use of N-fertilizers but also from industrial and domestic wastewaters.

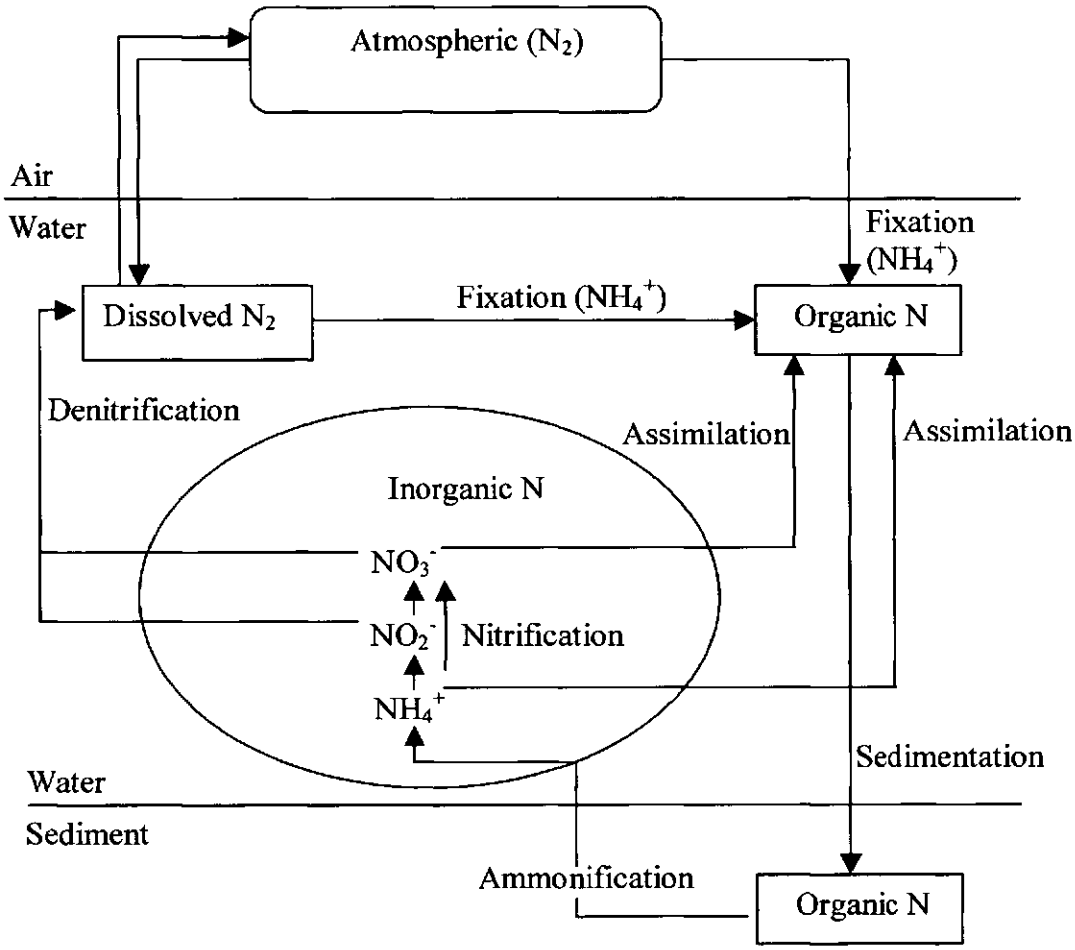


Figure 1.2 The aquatic Nitrogen Cycle (adapted from Hanrahan *et al.*, 2002).

Domestic waste water is not a negligible source of N in the environment. In Japan, the average daily human diet contains 13.3 g N and about 85 % is released in domestic wastewaters (Ukita *et al.*, 1986), almost all as dissolved ammonia (Reneau *et al.*, 1989). However, the excessive use of N-fertilizers remains one of the main sources of nitrate to water systems. For example, in the UK, the annual usage of N-fertilizers has increased from about 400 kt in 1960 to 1.3 Mt in 1980 (Gasser, 1982),

althought, in the mid-1980's, the use of N-fertilizers reached a plateau in the UK (Parkinson, 1993). The problem of using excessive amounts of N-fertilizers comes from the fact that nitrate is not totally bound to the soil and is very soluble in water, therefore it can leach into water systems, a process known as runoff (Haygarth and Sharpley, 2000). Not only will inorganic nitrate fertilizers applied to soils leach the nutrient, but also the application of organic manures can contribute to runoff with concentrations of concern in sensitive catchments (Smith *et al.*, 2001). Nitrate runoff is one of the loss processes of concern for both economic reasons and its impact on water quality. As an example, agriculture accounted for between 37 and 84 % of nitrogen emission into surface waters in western Europe (Isermann, 1990).

In a broader perspective, anthropogenic activities, especially agriculture, have both depleted terrestrial fixed nitrogen and have increased the inputs of highly mobile and reactive forms of nitrogen to the aquatic ecosystem causing alteration to the global nitrogen cycle (Galloway, 1998). A recent review on the terrestrial nitrogen cycle (Krug and Winstanley, 2002) has highlighted that there are great uncertainties in the nitrogen cycling and mass-balance in global models, and recommended that more comprehensive and consistent investigations have to be made considering geographical and temporal scales, as well as improvement in quantification of the large fluxes or organic N and NH_4-N by headwater in rivers during storm events.

1.1.2 The Phosphorus Cycle

Phosphorus occurs in aquatic systems in both particulate and dissolved forms and can be operationally defined (Figure 1.3) as total phosphorus (TP), total reactive phosphorus (TRP), filterable reactive phosphorus (FRP), and total filterable phosphorus (TFP).

The distribution and transformation of phosphorus in aquatic systems is shown in Figure 1.4. Phosphorus can enter natural water systems from several point and diffuse sources including weathering of the earth's crust, sediment release, animal and plant wastes, agricultural runoff, effluents from industry and sewage treatment

works, and atmospheric deposition. Unlike nitrogen, the phosphorus cycle does not have a significant atmospheric component. A chemical distribution of phosphorus between aquatic and particulate components occurs, *via e.g.* adsorption and precipitation processes.

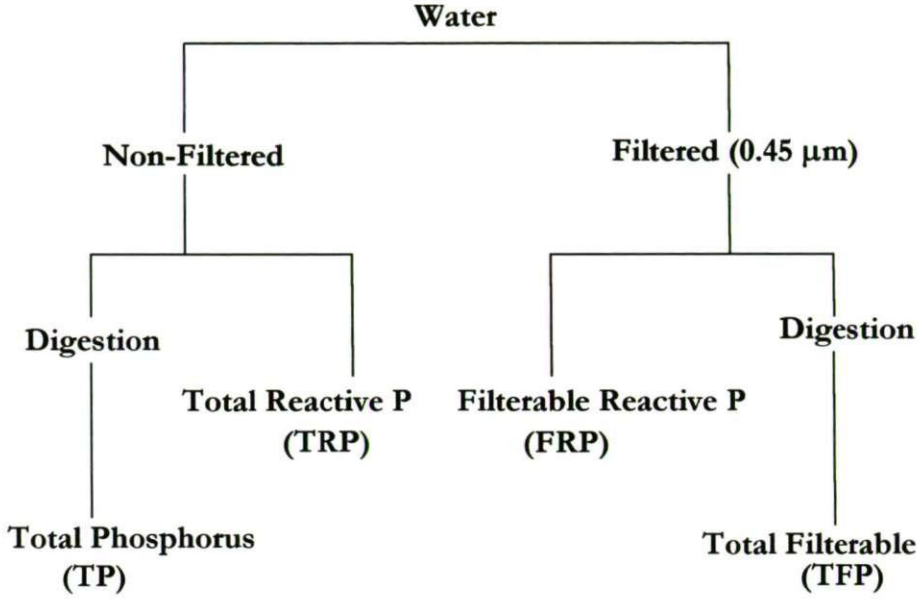


Figure 1.3 Operationally defined phosphorus fractions in natural waters (adapted from Maher and Woo, 1998).

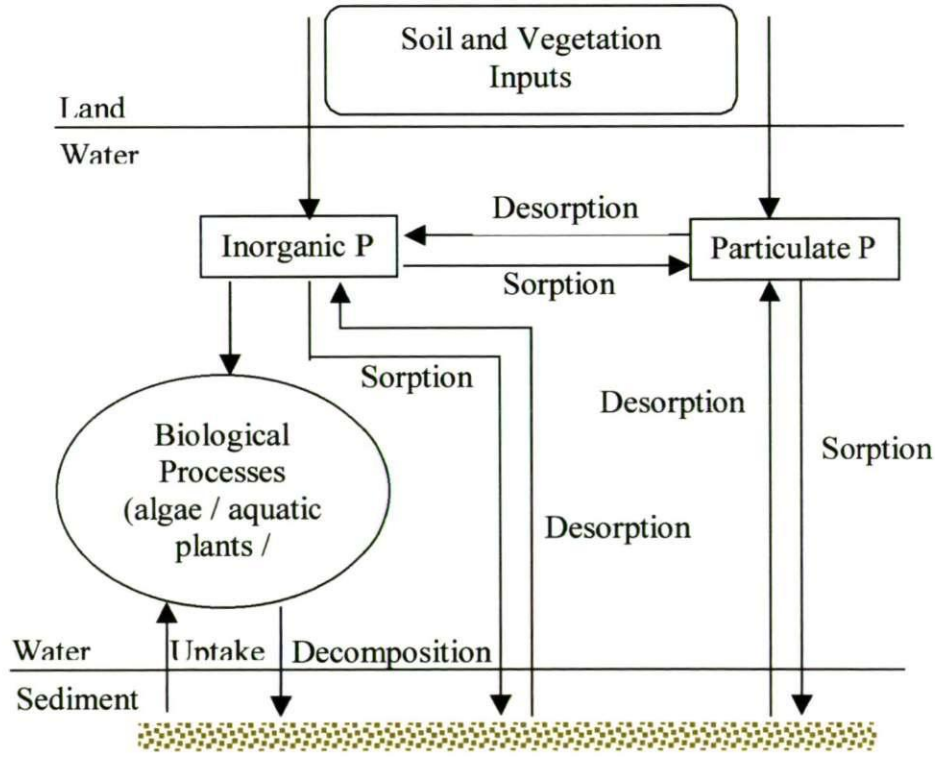


Figure 1.4 The aquatic Phosphorus Cycle (adapted from Hanrahan *et al.*, 2002).

In the literature, it is common to find authors using different abbreviations and nomenclatures to define the different phosphorus fractions (McKelvie *et al.*, 1995; Haygarth and Sharpley, 2000; Hens and Merkx, 2002; Jarvie *et al.*, 2002). Aiming at a standardisation of terminology Haygarth and Sharpley (2000) suggested a simple methodological-based classification of nomenclature. Table 1.1 presents the proposed nomenclature and the literature most cited equivalents.

Table 1.1 Suggested methodologically defined classification on P forms in waters, with their equivalent established terms, from Haygarth and Sharpley (2000).

Proposed classification	Equivalent 'established' terms*
RP (<0.45)	Molybdate-reactive P (MRP), dissolved- reactive P (DRP), soluble reactive P (SRP), filterable reactive P (FRP), dissolved molybdate-reactive P, orthophosphate, inorganic P, phosphate
RP (unf)	Total reactive P (TRP), raw unfiltered sample
TP (<0.45)	Total dissolved P (TDP)
TP (unf)	Total P on a raw unfiltered sample
UP (<0.45)	Dissolved organic P (DOP), soluble organic P (SOP), dissolved non-reactive P (DNRP)
TP (>0.45)	Particulate P (PP)
RP (>0.45)	Molybdate-reactive particulate P (MRPP), particulate-reactive P

*May not necessarily be correct.

RP = reactive phosphorus, unf = unfiltered, TP = total phosphorus, UP = unreactive phosphorus.

The inorganic forms of phosphorus are mainly iron and aluminium phosphates in acid soils and calcium phosphates in alkali soils. They are extremely insoluble in water and levels of soluble phosphorus in soil leacheate from surface soils are typically in the range from 0.01 to 0.1 mg L^{-1} P. Inorganic phosphorus exhibits nine oxidation states from +5 to -3. Oxoacids, such as orthophosphate, P(V), phosphite,

P(III), hypophosphite, P(I) and diphosphate, P(IV) are shown in Figure 1.5. Diphosphonate is known to be a phosphorylation agent for biological substances (Fujiwara, 1994). Pyrophosphates occur in soil and are involved in biological cycling, polyphosphates are also known to occur in soils and could be of microbial origin (Haygarth and Jarvis, 1999) but can also originate from detergents *e.g.* sodium tripolyphosphate (STPP) (Clark *et al.*, 1992).

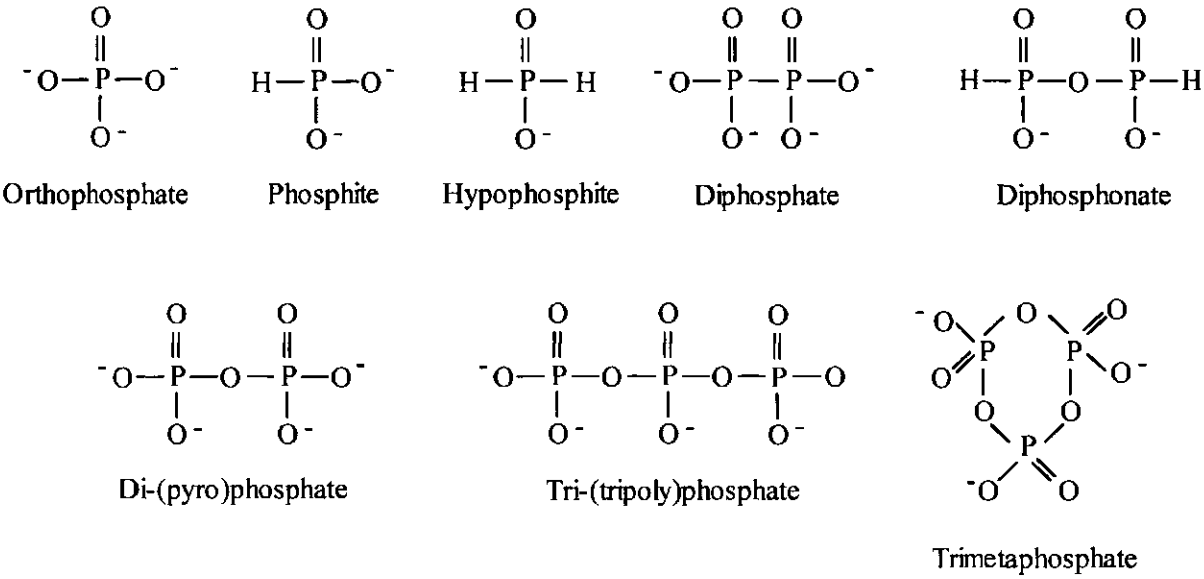


Figure 1.5 Major phosphorus species

The majority of organic phosphorus compounds contain either a P-C bond or a P-O-C bond. They occur naturally, *e.g.* inositol hexaphosphate ($(C_6H_6O_6)(PO_3)_6$) also known as phytic acid (Figure 1.6), in soils or natural waters (Broberg and Persson, 1988 ; Rydin, 2000), and can be formed from orthophosphate in biological treatment processes. They can also come from fertilizers, herbicides, insecticides and fungicides.

Organic phosphorus compounds may be necessary for plant growth and are found mostly in seeds as mixed calcium-magnesium-potassium salts, where they are believed to act as a store for phosphate and trace metals (Haygarth and Jarvis, 1999). Phosphate levels in fresh water systems have increased in the past 50 years, for example the level of orthophosphate has increased in the river Frome (Dorset) by

21% between 1965-1972 (Heathwaite *et al.,* 1996) and by 15% from 1980 to 1986 in the Slapton Catchment in South West England (Burt *et al.,* 1996). Different sources of phosphorus can be distinguished, such as constituents of industrial disharges *e.g.* commercial cleaning solutions, livestock manure, agriculture (P-fertilizers), effluents from sewage treatment works, and soil particles.

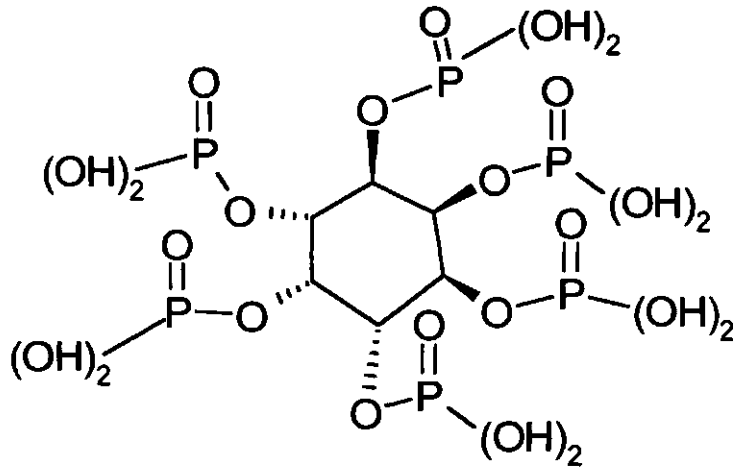


Figure 1.6 Structural formula of inositol hexaphosphate (phytic acid).

Domestic sources of P have been estimated as contributing 53% of total P released to water in the UK (Lund and Moss, 1990). In Japan, an average of 1.8 g P person^{-1} day^{-1} is released into domestic wastewaters (Goda, 1986). In the USA detergents have been estimated to account for about 46% of total P content of domestic effluents (Alhajjar *et al.,* 1989). The composition of final effluents from domestic septic tanks has been investigated (Whelan and Titamni, 1982) and showed that the two major contaminants were N and P. Total P levels were around 17 mg L^{-1}, almost all present as dissolved orthophosphate.

Traditionally, soil phosphorus has been considered as insoluble in water and past agronomic studies considered P leaching as insignificant (Gardwood and Tyson, 1973; Marrs *et al.,* 1991). For this reason, farmers were encouraged to use phosphatic fertilizers, thereby transferring small amounts of phosphate from agricultural lands to water systems. This transfer depends on rainfall and is not strongly influenced by the quantity of P applied to the land. However, it has

recently been shown that transfer of P as small as 10 μg L^{-1} (2-3 kg ha^{-1} yr^{-1} P) from agricultural land can contribute to eutrophication (Foy and Withers, 1995; Haygarth, 1997).

1.1.3 Eutrophication

Nutrient enrichment of natural waters affects both freshwater and marine ecosystems (Henderson-Sellers and Markland, 1987). Though widely associated with pollution, eutrophication is also a natural process in lakes as they gradually accumulate carbon, nitrogen and phosphorus. Lake ecosystems can recycle some of the nitrogen and phosphorus compounds deposited in the lake bed and this promotes more production of organic material. The organic material builds up on the lakebed and contributes to the lake's own demise. Bogs are shallow lakes that have filled with detritus (often peat) in this manner. The process may take hundreds or thousands of years and lead to new land formation when the bogs themselves are colonised by trees. Eutrophication of a lake may therefore be considered as an increase in organic matter in the system. This may be a result of direct river or sewage discharges but is most commonly associated with the increase in plant production caused by an increased supply of nitrogen and phosphorus compounds, essential nutrients for plant growth. The problem is that human intervention is accelerating this natural process, leading ultimately to the accelerated decline of lakes. The anthropogenic input of nutrients can cause a rapid growth of algae and other aquatic plants which may be toxic to shellfish and fish and may be harmful to mammals, *e.g. aphanizomenon flos-aquae* blue-green alga. In 1989 the National Rivers Authority (NRA), now the Environmental Agency (EA), detected blue-green algal blooms in 90 % of the waters monitored, and up to 70 % of these blooms had produced toxins (NRA, 1990). The toxicity of the algal blooms not only directly affects other living organisms in the environment, but also has indirect consequences. When the excess of vegetation dies the oxygen available is dramatically reduced, causing further environmental damage (Hutchinson, 1969).

In the marine environment, eutrophication has only been recognised in the past three decades. It was first identified in coastal lagoons of the US eastern seaboard through the presence of unusually intense blooms of phytoplankton. Since eutrophication has been documented in coastal seas throughout the world, it is seen as a non-natural phenomenon associated with an increase of essential nutrients, nitrogen and phosphorus compounds, utilised for phytoplankton growth. Figure 1.7 illustrates an example of one of the consequences of eutrophication, the algal bloom covering the entire surface of Sea Azov, north of the Black Sea. The extent of the algal bloom is shown by all green area in the centre of the picture.

Figure 1.7 Example of one of the effects of eutrophication in the Sea of Azov, north of the Black Sea. Satellite image provided by the Sea-viewing Wide Field-of-view Sensor - SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE (http://seawifs.gsfc.nasa.gov/SEAWIFS.html).

Assessment of the extent of eutrophication can be in the form of direct measurements of nutrients or the effects of nutrient enrichment (*e.g.* increased algal

biomass), but no single variable is accepted as being the sole indicator. Thus, the degree of eutrophication is assessed in terms of reference criteria. Several classification systems based on trophic state have been developed to characterise lakes (Carlson, 1977; Forsberg and Ryding, 1980; OECD, 1982) and coastal waters (Vollenweider *et al*, 1998) in terms of nutrients, chlorophyll and oxygen saturation. From the Greek vocabulary the words oligotrophic ('oligos' = few, a little and 'trophe' = food) meaning nutrient poor and eutrophic ('eu' = well, good) meaning nutrient rich, were words originally used by limnologists to characterize the trophic conditions of inland waters, although have become more frequent in the marine literature (Vollenweider *et al*, 1998). It is of general agreement nowadays that oligotrophic conditions have low nutrient levels and low productivity levels, eutrophic conditions are characterised by high nutrient levels and high productivity levels, and mesotrophic conditions have intermediate characteristics.

For coastal waters the proposed classification of trophic state, such as the Trophic Index - Trix (Vollenweider *et al*, 1998) have component parameters of the index that represent production and production dynamics, include major casual factors and are a routine measurement in most marine surveys. The productivity factors are chlorophyll a and oxygen as absolute deviation from saturation; the nutritional factors are total nitrogen and total phosphorus, and the available dissolved inorganic nitrogen as N-(NO_3+NO_2+NH_4) and dissolved inorganic phosphorus as P-PO4; and as a supplementary water quality factor, the transparency. The calculated results are scaled from 0 to 10, and intend to identify similar functions regarding the trophic state of coastal waters.

Since 1997, visual assessment of chlorophyll a distribution in waters worldwide has being possible (Figure 1.8). Satellite images are available, for research purposes, from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) Project web site (http://seawifs.gsfc.nasa.gov/SEAWIFS.html).

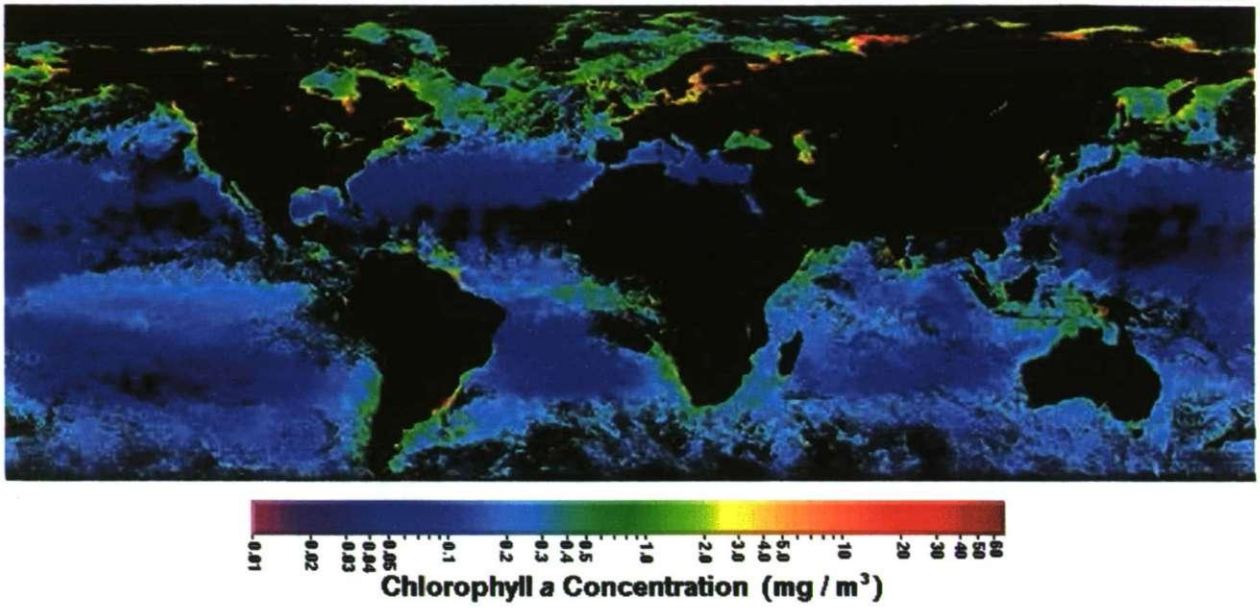


Figure 1.8 Distribution of chlorophyll in waters worldwide, image from the week 20 – 27/07/2002. Satellite image provided by the Sea-viewing Wide Field-of-view Sensor - SeaWiFS Project, NASA / Goddard Space Flight Center and ORBIMAGE
(http://bluefin.gsfc.nasa.gov/cgi/level3.pl?DAY=&PER=&TYP=chlo_r4)

1.1.4 Legislation Relating to Nutrients in Aquatic Environments

Concerns regarding the environmental impact of elevated nutrient concentrations are reflected in recent legislation enacted in many parts of the world. For example, the main European Union (EU) Directives relating to the quality of surface and seawaters, with special consideration for nitrogen and phosphorus are as follows (Council of the European Communities, A-D).

Directive 75/440 - Abstraction of drinking water in Member States.

Surface waters abstracted for drinking water purposes are grouped in three classes: A1, A2 and A3, based upon the type and degree of treatment, with A3 being the most advanced. The maximum value for nitrate is 11.3 mg L^{-1} NO_3-N for all classes. For phosphates, set values are 0.17 mg L^{-1} PO_4-P for A1 and 0.31 mg L^{-1} PO_4-P for A2 and A3.

Directive 80/778 – Quality of water intended for human consumption.

This covers all water intended for human consumption, treated or untreated regardless of origin. Both guide levels (G) and maximum admissible concentrations (MAC) are listed for most parameters. For nitrates, values of 5.35 mg L^{-1} NO_3-N (G) and 11.3 mg L^{-1} NO_3-N (MAC) are stated. Values for phosphate are 0.17 mg L^{-1} PO_4-P (G) and 2.18 mg L^{-1} PO_4-P (MAC).

Directive 91/271-Urban wastewater treatment.

This concerns the determination of the required treatment of urban wastewater, prior to discharge into a given body of water, which is based upon the receiving water characteristics and sensitivity to eutrophication. In the case of sensitive areas, the aim is to reduce nitrogen and phosphorus concentration levels from effluents below 10 mg L^{-1} NO_3-N and 1 mg L^{-1} PO_4-P, respectively.

Directive 91/676-Protection of waters from nitrates from agricultural sources.

This prescribes surveys for the identification of sensitive surface waters, with respect to nitrogen inputs, especially of an agricultural origin. It aims to promote sound agricultural practices in order to reduce pollution caused by nitrogen inputs.

1.2 Estuaries

1.2.1 General characteristics and classifications

From a physico-chemical standpoint, an estuary is a semi-enclosed coastal body of water having a free connection with the open sea and containing a measurable quantity of seawater (Pritchard, 1952). Each estuary has its own particular characteristics, which can directly influence its ecology, such as the geological composition of the catchment, seasonal riverine inputs, dimension and intensity of tidal intrusion, flushing time and anthropogenic stress.

In order to compare different estuaries and to attempt a prediction of characteristics of estuaries a scheme of classification is required (Dyer, 2000). The

main factors that will affect the rate and extent of the mixing between freshwater and seawater, within an estuary, are topography, river flow, tidal action and salinity structure. Consequently, estuaries can be classified accordingly to each one of factors mentioned above. Further details of the physical classification of estuaries can be found in the work of Dyer (2000) and Dyer *et al.* (2000), although the general classification is briefly presented below.

Classification by tides; according to the tidal ranges (Davies, 1964):

Microtidal	< 2 m range
Mesotidal	< 4 m, > 2 m
Macrotidal	< 6 m, > 4 m
Hypertidal	> 6 m

Classification by topography; presented by Pritchard (1952):

Coastal plain estuaries. The topography is very much like that of a river valley, maximum depth of 30 m, width-depth ratio is usually large, extensive mudflats and salting, central channel is often sinuous, generally restricted to temperate latitudes, river flow is normally small, *e.g.* Thames, Southampton Water and Mersey.

Fjords. Can present a wide range of sills depths, ranging from of 4 m at the fjords mouth in Norway, up to 40 – 150 m in British Columbia, small width-depth ratio, can reach 100 km length, occurrence is restricted to high latitudes in mountainous areas, *e.g.* Loch Etive (Scotland), Sogne Fjord (Norway) and Alberni Inlet (British Columbia).

Bar-built Estuaries. Have a characteristic bar across their mouths, have large amounts of sediment available, only few meters deep and often have extensive lagoons and shallow waterways just inside the mouth, river flow is large and seasonal variable, mouth position can vary over the years, generally found in tropical areas, *e.g.* Vellar Estuary (India) and Roanoke River (USA).

Others. All estuaries that do not conveniently fit elsewhere, including estuaries formed by faulting, landslides and volcanic eruptions, *e.g.* San Francisco Bay.

Morphological Classification; according to Dalrymple *et al.* (1992).

Wave dominated. The waves are significant at the mouth, extensive mudflats and marshes in the middle section, likely to occur in micro- or meso-tidal conditions, *e.g.* Exe Estuary (UK).

Tide dominated. Result of the large tidal currents relative to the wave effects, mouth contains sandbanks aligned to the current flow, salt marshes occur in macro-tidal areas, *e.g.* Severn Estuary (UK).

Classification on Salinity Structure; according to Pritchard (1955) and Cameron and Pritchard (1963).

Highly stratified, salt edge type. The interface between layers of different salinity (halocline) is very sharp, along the estuary salinity is almost constant in surface and bottom layers except for zones at the tip wedge and where the halocline meets the surface, large ratio of river flow to tidal flow, small width to depth ratio, tidal range is generally micro-tidal, *e.g.* Mississippi and Vellar estuaries.

Highly stratified, fjord type. Similar aspects to the above, isohaline layer is very deep, river flow is dominant over tidal flow, the thin upper layer is commonly of constant thickness from head to mouth, upper layer thickness is restricted to the depth of the sill, circulation over sills can promote mixing, reducing stratification, *e.g.* Alberni Inlet (British Columbia), Silver Bay (Alaska).

Partially mixed estuary. Turbulent eddies, two layer flow, middle sector with linear horizontal salinity gradient, significantly changes in tidal range between neap and spring, *e.g.* James River, the Mersey.

Vertically homogeneous estuary. Tidal range is large relative to the water depth producing turbulence, tidal flow much larger than river flow, macrotidal conditions, can be further subdivided into laterally inhomogeneous or homogeneous.

All these classification criteria help to improve the general understanding of physical processes within an estuary. Nevertheless, further investigation is needed regarding the monitoring of physico-chemical variables in such environments.

1.2.2 Physico-chemical estuarine processes

The estuarine mixing zone, where freshwater and its components are retained during the mixing process with seawater before reaching the open sea, also promotes changes in the water composition such that the masses of water that reach the sea will have different compositions than the ones transported by the river. The front of the interface between fresh / seawater (FSI) (Millward, 1995) is also the region of elevated concentrations of suspended particulate matter, which is caused and maintained by tidal energies (Bale *et al.,* 1985). Other distinguishable evidence for formation of the FSI includes, the depletion of dissolved oxygen, caused by the oxygen-demanding bacteria during the degradation of dissolved organic carbon released by riverine plytoplankton that suffer osmotic stress when reaching saline waters (Morris *et al.,* 1982a, Millward, 1995) and decrease in chlorophyll a concentrations and particulate carbon (Zwolsman and van Eck, 1993). The turbidity maximum zone (TMZ) is the location where particulate-water interactions predominantly occur, thereby controlling the internal cycling and retention of elements, characterizing a crucial component of an estuary (Morris *et al.,* 1986a). In the TMZ uptake of dissolved constituents onto particles is enhanced not only because of the mass of material in suspension but also because of increases in specific surface area of the suspended particles (Millward *et al.,* 1990), arising from a decrease in particle diameters due to disaggregation and / or particle selection processes during remobilisation from the bed (Bale *et al.,* 1989). Freshwaters have calcium and carbonate as major ions, and generally lower pH and dissolved oxygen concentration then seawater, which has sodium and chloride as major ions (Mommaerts, 1969). During the mixing process, particle and chemical species distributed in those waters promote significant changes in the distribution of those species between compartments. Physical and chemical properties, such as particles size, adsorption / desorption of water constituents to particles, and sedimentation / resuspension rates, also characterize the turbidity maximum zone.

1.2.3 Ecological importance of estuaries

The tidal, sheltered waters of estuaries support unique communities of plants and animals, specially adapted for life at the margin of the sea. Estuarine environments are among the most productive on earth, creating more organic matter each year than comparably sized areas of forest, grassland, or agricultural land (NERRS / CDMO, 2002). Many different habitat types are found in and around estuaries, including shallow open waters, freshwater and salt marshes, sandy beaches, mud and sand flats, rocky shores, oyster reefs, mangrove forests, river deltas, tidal pools, sea grass beds, and wooded swamps. The productivity and variety of estuarine habitats promote the abundance and diversity of wildlife. Shore birds, fish, crabs and lobsters, marine mammals, clams and other shellfish, marine worms, sea birds, and reptiles are just some of the animals that make their homes in and around estuaries. These animals are linked to one another and to an assortment of specialized plants and microscopic organisms through complex food webs and other interactions. In summary, estuaries can provide (NERRS / CDMO, 2002):

Habitat: Tens of thousands of birds, mammals, fish, molluscs, crustaceans and other wildlife depend on estuaries.

Nursery: Many marine organisms and most commercially valuable fish species included, depend on estuaries at some point during their development.

Productivity: A healthy estuary is able to produce more organic matter than the same area of forest, grassland or some agricultural areas.

Water filtration: Water draining from the river catchment carries a load of sediments and nutrients. As the water flows through the mixing zone, much of the sediment and nutrient load is precipitated or coagulated. This sedimentation process removes most of the suspended solids, therefore, delivering clearer water for coastal zones.

For all the reasons mentioned above, protective legislation for estuarine areas is becoming more specific, rigorous and frequently updated (see Section 2.1.2).

1.3 Analytical methods for determination of nutrients in natural waters

In order to better understand nutrient utilization and transport in aquatic systems, there is a need to develop sensitive and robust analytical measurement technologies. Monitoring techniques must be able to provide the necessary detection limit and linear range to meet all environmental situations. The need to measure low levels of the analytes has, in many cases, contributed to problems of poor precision and reduced accuracy (Kirkwood *et al.,* 1991). It is therefore necessary to adopt an analytical method that meets all of the above requirements, and ideally is not costly, time consuming or neither of extremely operational difficulty.

1.3.1 Nitrate Determination

Determinations of nitrate in natural waters as the single ion and combined with nitrite as total oxidised nitrogen (TON) have been carried out by a variety of methods as shown in Table 1.2, and are described below.

Chemiluminescence (CL) techniques have been used to determine trace amounts of nitrate and nitrite in seawater (Garside, 1982; Aoki and Wakabayashi, 1995). This method depends on selective reduction of each species to nitrogen oxide which is swept from the sample by a helium carrier gas. The nitrogen oxide is then reacted with ozone to form excited nitrogen dioxide. The return of the nitrogen dioxide to the ground state is accompanied by the release of a photon, which is subsequently detected by a photomultiplier tube.

The sequence of reactions involved in the CL determination of nitrate are as follows:

1. $NO_3^- + H^+ <----> NO_2^-$
2. $NO_2^- + H^+ <----> HONO \qquad NO$
3. $NO + O_3 \rightarrow NO_2^* \rightarrow NO_2 + hv$

Table 1.2 Examples of methods for the determination of nitrate in natural waters (adapted from David, 1996)

Technique	Sample	Method	Linear Range	Reference
Continuous Flow	seawater	Indirect spectrophotometric	0 - 400 μg L^{-1}	(Riley, 1965)
Manual	seawater	Indirect spectrophotometric	0 - 60 μg L^{-1}	(Wood *et al.*, 1967)
Flow Injection	seawater, potable, waste	Indirect spectrophotometric	1 - 25 μM	(Anderson, 1979)
Flow Injection	natural waters	Indirect spectrophotometric	0 - 5 mg L^{-1}	(Gine-Rosias *et al.*, 1980)
Manual	natural waters	Indirect spectrophotometric	2 - 100 μg L^{-1}	(Gaugush and Heath, 1984)
Flow Injection	surface, ground waters	Indirect spectrophotometric	0 - 2 mg L^{-1}	(vanStaden *et al.*, 1986)
Flow Injection	natural water	Indirect spectrophotometric	0 - 12 mg L^{-1}	(Clinch *et al.*, 1987)
Flow Injection	natural water	Indirect spectrophotometric	0.1 – 5.5 mg L^{-1}	(Chen *et al.*, 2000)
Continuous Flow	seawater	Indirect spectrophotometric	0 – 35 μM	(Oudot and Montel, 1988)
Flow Injection	natural water	Indirect spectrophotometric	N/A	(Maimo *et al.*, 1989)
Continuous Flow	seawater	Indirect spectrophotometric	0 - 100 nM	(Raimbault, 1990)
Continuous Flow	natural waters	Indirect spectrophotometric	0 – 12 μM	(Zhang, 2000)
Flow Injection	seawater	Indirect spectrophotometric	1 - 20 μM	(Pai and Riley, 1994)
Potentiometry	water	Nitrate selective electrode	1 - 1000 mg L^{-1}	(Ebdon, 1991)
Fluorimetry	natural waters		10^{-4} - 3.0 mg L^{-1}	(Motomizu, 1987)
Polarography	drinking and river waters	Reaction, nitrate and uranyl ion	10^{-2} - 0.1 mg L^{-1}	(Noufi *et al.*, 1990)
Polarography	hydroponic waters	Nitration of organics	10^{-1} - 70 mg L^{-1}	(Fogg *et al.*, 1988)
Direct UV	natural waters			(Brown and Bellinger, 1978)
Chemiluminescence	seawater			(Garside, 1982)
Chemiluminescence	natural water		5 – 1400 μg L^{-1}	(Aoki and Wakabayashi, 1995)
Chromatographic	seawater			(Verma and Verma, 1992)
Amperometric				(Fogg *et al.*, 1991)
Amperometric				(Doherty, 1996)

Ion selective electrodes for nitrate, develop a potential across a thin, inert porous membrane behind which a water immiscible liquid ion-exchanger is held. The electrode responds to nitrate ion activity between 0.14 and 1400 mg L^{-1} NO_3-N but is currently not sensitive enough for levels found in seawater (Crompton, 1989). A further drawback is that chloride and bicarbonate ions interfere when their weight ratios to nitrate ions are > 10:1 and > 5:1, respectively. The electrodes behave satisfactorily in buffered solutions over the pH range of 3 to 9 but can be erratic when pH is not stable. The electrode responds to NO_3^- activity rather than concentration. Therefore, solution ionic strength must remain constant in all samples and standards. This can be achieved by using a buffer solution of silver sulphate to eliminate Cl^-, Br^-, I^-, S^{2-} and CN^{-1}, sulfamic acid to remove NO_2^-, and pH 3 buffer to eliminate HCO_3^- and maintain a constant ionic strength.

Voltammetry Recent developments in electrode membrane technology, such as the [Ru (bipy)$_2$poly-(4-vinylpyridine)$_{10}$Cl]Cl modified glassy electrode (Doherty *et al.,* 1996), have overcome previous interference problems and improved sensitivity This device has been used as a detector for nitrate after first reducing it to nitrite in a flow injection based system.

Chromatographic techniques have been proposed for the determination of nitrate and nitrite, including ion chromatography (Verma and Verma, 1992), ion exclusion chromatography (Kim and Kim, 1989) and ion-interaction chromatography (Genaro *et al.,* 1990). The major advantage of chromatographic techniques is the ability to determine a range of different ions simultaneously, *e.g.* nitrate, chloride and sulphate. A comparison of ion chromatography, segmented flow analysis and flow injection for the determination of nitrate found ion chromatography and segmented flow analysis to have better detection limits. However, the sampling rate of 30 - 60 samples per hour for FI is significantly better than that of ion chromatography at 10 samples per hour (Burke *et al.,* 1989).

Direct spectrophotometric methods. There have been several methods proposed for direct UV absorption of the nitrate ion at 210 nm for use in natural waters. The sample is first filtered and then acidified with sulphuric acid to remove hydrogen carbonate. Nitrite is then removed by the addition of sulphamic acid. Reliable results have been obtained for natural waters low in organic matter such as humic acid. For waters containing higher levels of organic matter and/or iron, corrections have to be made for the resultant positive bias. The correction factor is usually determined by measuring the solution at absorbance at 275 nm where the nitrate ion has negligible absorbance. The absorbance of the solution at 275 nm is then multiplied by an empirical correction factor. Nitrate concentration is relative to the difference of the two absorbances but the main problem is that the correction factor is dependent on the nature of the water. Different manual correction methods have been proposed to overcome interference from organic substances such as coagulation with aluminium hydroxide followed by filtration (Cawse, 1967) and a zinc-copper reduction of nitrate to assess non-nitrate absorption contribution (Brown and Bellinger, 1978). Measurement at several different wavelengths followed by empirical correction has also been reported (Morries, 1971). The problem of interference was also overcome by selective removal of the interfering species by the addition of sodium hydroxide solution to give a sample pH of 12.6. The sample was then passed through an activated charcoal filter (Rennie *et al.*, 1979). The main drawback with all these manual correction techniques is that they are not easily automated.

Indirect spectrophotometric methods have been based on first reacting the sample with reagents such as 2,6 xylenol (Montgomery and Dymcock, 1962) or brucine (Jenkins and Medsker, 1964). Other indirect methods require an initial reduction stage of nitrate to nitrite either by homogeneous or heterogeneous reduction methods.

Homogeneous reduction: has been effected by the use of hydrazine (Mullin and Riley, 1955), where the time to reach equilibrium can be in the region of 20 hours.

More recent work on the hydrazine reduction methods has reduced reaction times to around 1½ - 2 hours (Bower and Holm-Hansen, 1980; Kempers and Luft, 1988).

Heterogeneous reduction: methods have utilised reduction columns of various dimensions containing a variety of powdered or granular metals such as zinc (Chow and Johnstone, 1962; Matsunaga and Nishimura, 1969), amalgamated zinc (Bajic and Jaselkis, 1985), cadmium (Margeson *et al.,* 1980), amalgamated cadmium (Morris and Riley, 1963; Gaugush and Heath, 1984) and copperised-cadmium (Wood *et al.,* 1967; Lambert and DuBois, 1977; Oudot and Montel, 1988). Reduction methods utilising cadmium powder and granules have been investigated and optimised by Nydahl (1976). The nitrite yield from the reduction of nitrate using various types of reductors was compared, and the effect of pH, temperature, chloride concentration and contact time (flow rate) through a packed reduction column was investigated. Maximum yields approached 100% at pH 9.5 and temperature was reported to have little effect between 20 - 30 ^{0}C, but at 10 ^{0}C the reduction was significantly slower (Nydahl, 1976). Copperised-cadmium wire (Sainton, 1974) and copperised cadmium-silver wire (95% Cd) (Willis, 1980) have also been used successfully in the reduction stage. Hydes and Hill (1985) described the use of a copperised granulated 50:50 cadmium-copper alloy reduction column and van Staden (1982) reported the successful use of a copper tube pre-column followed by a copperised-cadmium tube reduction column. The use of copperised-cadmium is now well established for the nitrate reduction stage for the determination of TON. The reduction conditions are adjusted so that the nitrate is quantitatively reduced to nitrite and no further. The principle reaction is:

$$NO_3^- + Me_{(S)} + 2H^+ \rightarrow NO_2^- + Me^{2+} + H_2O$$

The reduction yield is dependent on the metal used in the reductor, the solution pH, and the activity of the metal surface. The use of a reaction solution which is too alkaline or a metal reductor with an inactive surface will result in partial

reduction of the nitrate. Conversely, if the reaction solution is too acidic or the metal reductor is too electronegative or highly active, the reduction process will proceed further than the nitrite step. In both situations low nitrate values will be obtained.

Copperised-cadmium filings or granules are very effective in the heterogeneous reduction stage. However, in weak alkaline or neutral conditions the cadmium ions formed during the nitrate reduction stage react with the hydroxyl ions to form a precipitate. Furthermore, the reduction potential required for the reduction of nitrate to nitrite is dependent on the hydrogen ion activity within the solution. This suggests that the pH is changed if the solution is not buffered especially in the vicinity of the metal reductor surfaces. The pH of seawater rarely falls outside the limits of 7.7 - 8.2 (Parsons *et al.,* 1975) and therefore ammonium chloride is added to act as a buffer and as a complexant:

$$2NH_4^+ \leftrightarrow 2NH_3 + 2H^+$$

$$Cd^{2+} + 2NH_3 \leftrightarrow [Cd(NH_3)_2]^{2+}$$

The ammonia is bound in the diammine cadmium complex. Under controlled conditions, nitrite that is present in the seawater passes through the reduction stage without further reduction. This will therefore contribute to the total value as total oxidised nitrogen (TON) at the subsequent diazotisation and coupling stage.

The most commonly used chemistry involves the diazotisation of the nitrite and subsequent coupling with sulphanilamide and N-(1-naphthyl)ethylene diammine (Benshneider and Robinson, 1952) to form an intensely pink colored azo dye, as shown in Figure 1.9.

1.3.2 Phosphorus Determination

Several methods have been developed (Table 1.3) which are capable of determining well-defined phosphorus species including: atomic spectrometry (Broberg and Pettersson, 1988; Crompton, 1992), chromatography (Halliwell *et al.,* 1996; Colina and Gardiner, 1999; Vorob'eva and Gurskii, 2000) and

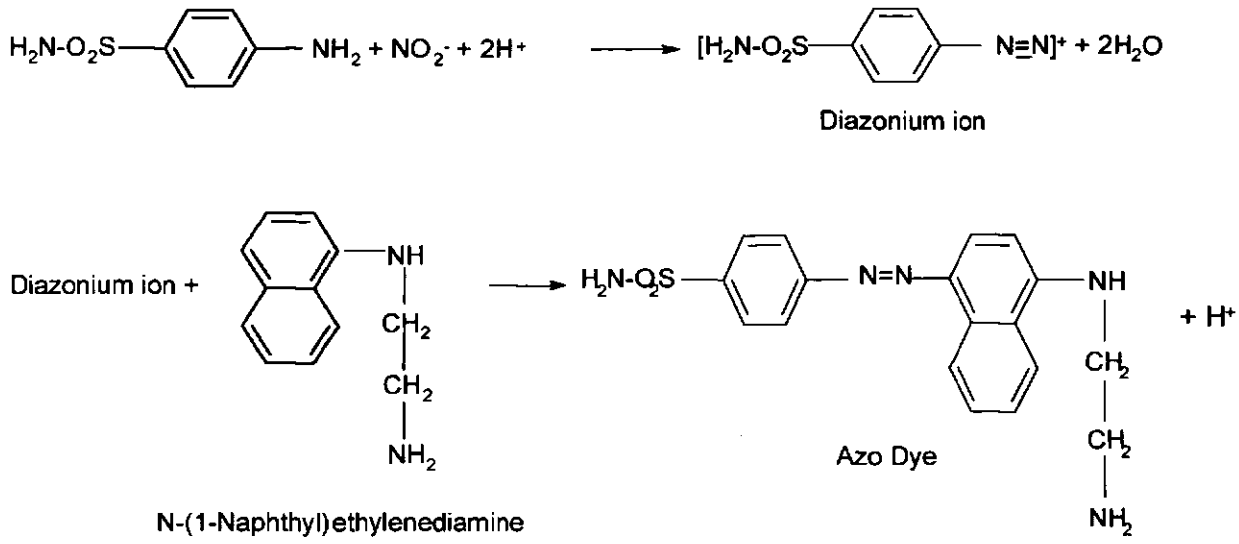


Figure 1.9 Diagram showing the diazotisation and coupling reaction.

electroanalytical techniques (Coetzee and Gardner, 1986 and Davey *et al.,* 1990). Spectrophotometry offers several advantages over such methods in that it allows for a wider range of analytes to be determined (Robards *et al.,* 1994), and, when coupled with techniques such as flow injection analysis (Section 1.4.3), lends itself to *in situ* environmental monitoring (McKelvie *et al.,* 1995; Hanrahan *et al.,* 2001a).

The majority of analytical methods for the determination of phosphate are spectrophotometric and based on the formation of 12-molybdophosphoric acid from phosphate and molybdate in acid solution and subsequent reduction by either ascorbic acid or tin(II) chloride with antimony as a catalyst to form a blue heteropoly phosphomolybdenum compound first described by Murphy and Riley (1962).

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

$$H_3PO_4\ (MoO_3)_{12} \rightarrow \text{Phosphomolybdenum blue Mo(V)}$$

There have been several modifications of the original method and examination of the literature reveals a range of molybdate and antimony concentrations, pH and reductants. Studies have shown that changes in the hydrogen ion $[H^+]$ and molybdate [Mo] concentrations play key roles in the formation of the heteropoly compound (Crouch and Malmstadt, 1967; Drummond and Maher, 1995). Ascorbic

Table 1.3 Techniques for the determination of phosphorus in natural waters.

Technique	Species detected	Detection limit (μM)	Comments	References
Enzymatic with spectrophotometric detection	FRP	0.16	Reaction of FRP with glyceraldehyde-3-phosphate to produce 1,3-diglycero-phosphate. Spectophotometrically detected as formazin and used as a measure of FRP.	Stevens, 1979
Voltammetry	FRP	0.29	Differential-pulse polarographic detection. Catalytic reduction of perchlorate or nitrate by solvent extracted phosphomolybdate.	Hight *et al.*, 1982
High-performance liquid chromatography (HPLC)	H_2PO_4	24	Determination of hypozanthine produced by nucleoside phosphorylase catalysed reaction of phosphate with inosine.	Morgan and Danielson, 1983
Flow injection analysis (FIA) with spectrophotometric detection	FRP	0.40	LED detection. Dual photometric detector.	Worsfold *et al.*, 1987
Inductively coupled plasma mass spectrometry (ICP-MS)	FRP	0.26	Liquid chromatographic separation of model phosphorus compounds.	Jiang and Houk, 1988
Inductively coupled plasma atomic emission spectrometry (ICP-AES)	TP	16	Sample (200μL) introduced by FIA mode.	Manzoori *et al.*, 1990
Ion chromatography (IC) coupled with FIA	FRP	0.32	Indirect UV detection.	Halliwell *et al.*, 1996
Flow injection analysis (FIA)	FRP	0.67	*In situ* monitoring system employing a miniature CCD spectrometer.	Hanrahan *et al.*, 2001a

acid with an antimony tartrate catalyst is generally the preferred method in the reduction step (Broberg and Pettersson, 1988). Use of tin(II) chloride as a reductant has been shown to be temperature-dependent, produce unstable colour formation and suffer from AsO_4^{3-} and Cu(II) interferences (Ron Vaz *et al.*, 1992). However, others have reported that tin(II) chloride allows higher sensitivity due to faster reaction kinetics (Janse *et al.*, 1983; Pauer *et al.*, 1988). Other interferences in the determination of phosphate by the molybdenum blue method include Ca^{2+}, Fe(III), SiO_2 and organic ions such as acetate, citrate and oxalate (Ciavatta, 1990). Studies have also shown that spectrophotometric determination can overestimate the concentration of orthophosphate as a result of the hydrolysis of labile organic phosphates (Chamberlain and Shapiro, 1969; Broberg and Pettersson, 1988).

1.3.3 Flow Injection

Flow injection (FI) has been described as an unsegmented flow technique in which a volume of liquid sample is inserted into a moving liquid carrier stream, whereupon it undergoes physical dispersion as it is transported to a flow-through detector for measurement (Ruzicka and Hansen, 1988). The transient response is usually in the form of a peak, with a sharp rising edge and a more gradual decay, the shape being due to axial dispersion and radial diffusion of the sample zone as it travels through the FI manifold. The height and area of the peak are usually directly related to analyte concentration but for convenience peak height is usually the measured parameter. The degree of sample dispersion is controlled by factors such as sample volume, carrier flow rate, length and diameter of the manifold tubing and manifold geometry. Under most conditions sample dispersion is highly reproducible (relative standard deviations are typically less than 5 %). The technique is now widely used in analytical laboratories for the automation of wet chemical methods and has considerable potential for use on board ship (Worsfold *et al.*, 2002) and in submersible analysers (David *et al.*, 1998)

A block diagram of a simple, single channel FI manifold is shown in Figure 6 and typically consists of a means of propulsion (*e.g.* a peristaltic pump), a rotary

injection valve for sample introduction (similar to High Performance Liquid Chromatography valves but low pressure) and a flow-through detector (*e.g.* a spectrophotometer). In this manifold the carrier stream transports the sample to the detector. Polytetrafluoroethilene (PTFE) tubing (typically 0.8 mm i.d.) is used throughout the manifold for sample and reagent transport, with tightly-wound coils often included to enhance mixing. If the method requires more than one reagent, additional streams can be merged with the carrier stream at suitable points in the manifold. Similarly, if in-line physical treatment of the sample is required the necessary components can easily be incorporated. Solid phase reaction columns in which the injected sample reacts with a solid material, *e.g.* copperized cadmium, gel filtration (McKelvie et al. 1993), packed in a column can also be incorporated.

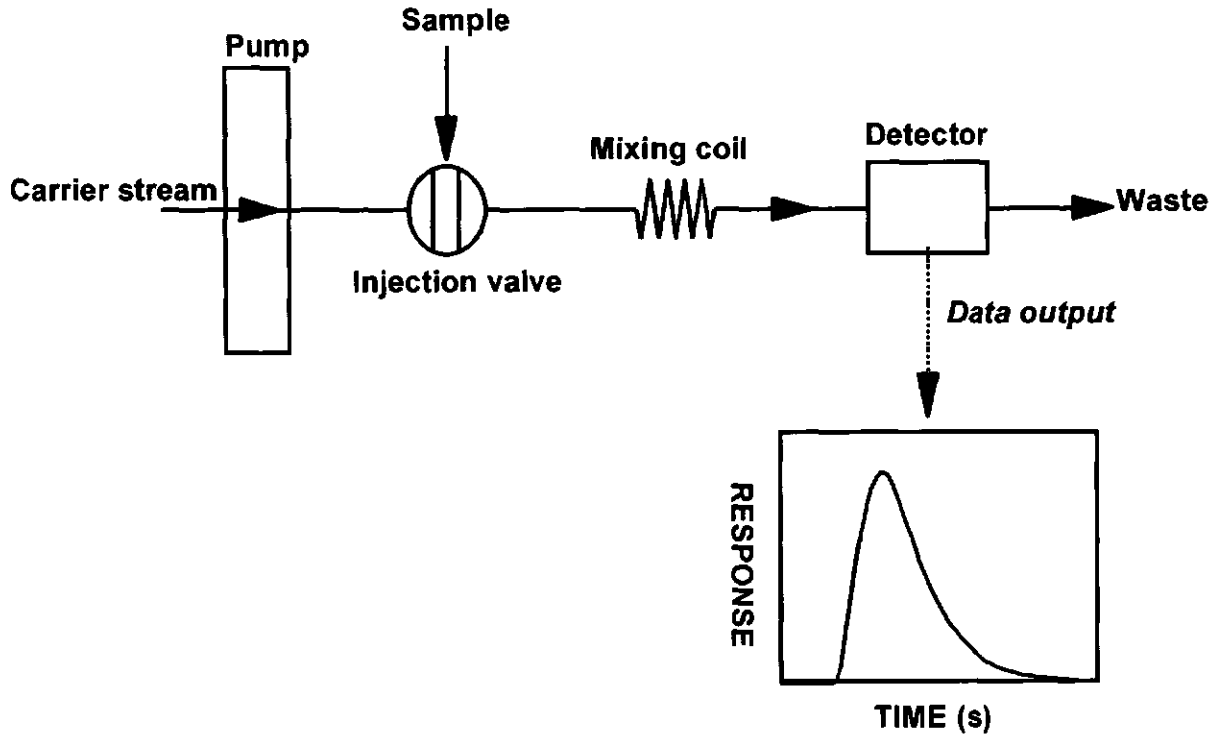


Figure 1.10 Block diagram of principal FI components.

Reagent consumption is generally low (0.2 – 1.2 mL min^{-1}) in FI systems (an important factor for shipboard and submersible applications) and can be reduced still further by using a reagent injection manifold, whereby a discrete volume of

reagent is injected into a continuously flowing sample stream. This option is suitable for applications in which the sample is in abundant supply (as in many marine situations) and is particularly beneficial when expensive reagents are required. Designing manifolds in which the sample is injected into more than one flow channel, undergoing different reaction chemistries in each, can perform simultaneous FI determinations (Maimo *et al.*, 1989; Martelli *et al.*, 1995). FI systems are easily automated using off-the-shelf components and a notebook PC to control the operation of the valves and pumps and data acquisition and processing.

Spectrophotometry (SPEC) was the first detection system used in conjunction with FI (Ruzicka and Hansen, 1981) and remains the most popular in terms of applications and published papers. The determination of nutrients in natural waters is the most common environmental application of the technique. In recent years the advent of solid state detectors has made FI-SPEC a viable system for deployment in the field, at sea and *in situ*, due to its compact nature when incorporated in a suitable housing. This provides the possibility of autonomous long-term operation at remote sites for days or even weeks at a time, *e.g.* at a river gauging station monitoring daily differences during winter. The possibility of acquiring high temporal data resolution during short-term transient events, such as storms or periodic events such as tidal cycles, is of great attraction

1.3.4 Sampling and sample storage for nutrient analysis

Once the most suitable analytical technique for the determination of nutrients in a particular aquatic environment has been selected, it is then necessary to define a sampling and storage strategy (assuming *in situ* measurements are not being made).

Most aquatic systems are dynamic in nature, changing randomly and/or systematically over time and space. Through different seasons, tides and river flows, nutrient levels will fluctuate as will other variables, *e.g.* water temperature, pH, dissolved oxygen, major ions composition, salinity and biological activity (Casey, 1992). As soon as the sample is isolated from its original environment and placed in a closed container, it is possible that physical, chemical and biological

alterations will occur in a short or long period of time after the sampling. Therefore, a well-organized sampling and storage strategy should accomplish the most important aim, to retain the original chemical composition of the sample and to preserve the concentrations of the analyte for as long as it is needed before analysis.

1.3.4.1 Site selection

Selecting representative sampling sites is one of the most important factors in any nutrient monitoring program. The number and location of potential sites should be determined in the initial phase of the sampling campaign and will be dependent on the problem that needs to be addressed. If the purpose of the program is to monitor the impact of point sources, monitoring sites should be clustered where nutrients are likely to enter the water body. To help ensure scientific validity, sites upstream and downstream from the pollutant inflow, as well as the point of entry, should be monitored to provide comparative data, and monitor dispersal/dilution. Other considerations include avoiding boundary areas (*e.g.*, confluence of streams or rivers), convenience and overall accessibility (Lambert *et al.*, 1992).

1.3.4.2 Sampling frequency and cost

Nutrient concentrations fluctuate with changes in physico-chemical conditions and biological activity on a seasonal and diurnal basis. The rate and transport of nutrients in surface waters varies depending on sources, pathways, interactions with particulate matter and the inherent biology of the water body. Other factors include in-stream velocity (flow rate), the proportion of surface run-off and the blending of water from tributaries of different quality. Effective monitoring therefore involves sampling at adequately frequent intervals so that the data set spans the inherent changes. However, continuous sampling is not always a viable option. Most sampling programs are a compromise in that information is obtained in the most cost-effective way.

1.3.4.3 Sample collection

Sample collection should be simple and avoid the possibility of contamination, or interference from foreign substances, and degradation. Today, there are several types of automatic sampler that can be programmed to take samples at specific time intervals or locations. Individual grab samples can also be taken at specific times and locations. Whatever method is chosen, it is important that it minimizes contamination or alteration of the sample. All sample bottles should be clean and rinsed at least twice with the water of interest prior to analysis. Care must be taken to avoid the surface film, which can be enriched with nutrients. The sample should be collected half way between the surface and the bottom of the water body and upstream of where you are standing (if collecting grab samples) to avoid disturbing underlying sediments.

1.3.4.4 Filtration procedure

For nutrients, preliminary treatment often involves filtration. This process differentiates between the dissolved phase, operationally defined as that fraction which passes through a 0.45 μm filter (Hurd and Spencer, 1991), although this pore size allows bacteria to pass through, only then being retained using 0.2 μm filters (Currie and Kalff, 1984; Baretta-Becker, 1998). Polycarbonate and cellulose acetate membrane filters are reported to be best for dissolved constituents in natural waters (Hall *et al.,* 1996). High concentrations of suspended solids can cause analytical interference, *e.g.*, scattering of light in spectrophotometry. Filtering also removes the majority of bacteria and plankton that may alter nutrient concentrations during storage, but may not eliminate colloidal particulate matter that can remove or release nutrients (Horowitz *et al.,* 1992). Continuous filtering, for example, can lead to clogging of the membrane by retained particles or colloids leading to a gradual decrease in pore size, thus entrapping particles that would normally pass through the filter. As the pore size decreases, the filtration rate drops, ultimately leading to an increase in filtration pressure (Laxen and Chandler,

1982). An increase in pressure can in turn lead to lysis of biological cells, releasing enzymes that could liberate nutrients (*e.g.* phosphate). To avoid cell lysis, pressures less than 1 atm across the membrane are recommended (Gardolinski *et al.*, 2001).

1.3.4.5 Storage procedure

The effectiveness of preservation methods depends on various factors including filtration technique, composition of sample, container type and size, temperature and type of chemical addition or treatment. Both physical and/or chemical treatments aim to slow down the biological processes that cause nutrient depletion. Cooling (both refrigeration and freezing) of the samples with storage is widely accepted as a preservation process, with freezing being the preferred technique (Zhang and Ortner, 1998). Although nitrate and phosphate can be adequately preserved by the freezing process, silicate recoveries have been reported as low (Zhang and Ortner, 1998). This can be attributed to the conversion of reactive silicate to a non-reactive, polymeric form (Macdonald *et al.*, 1986). Refrigeration (4°C) has been favourably recommended for samples (including those for silicate determination) that will be analysed within a few hours of collection (Morse *et al.*, 1982). Assessment of heat treatment (pasteurisation) (Kirkwood, 1992; Brezonik and Lee, 1996; Dore *et al.*, 1996; Zhang and Ortner, 1998) for the preservation of nitrate and phosphate revealed stable levels for more than a year, whereas ammonia levels decreased within three days due to heat alteration (Aminot and Kerouel, 1997).

Chloroform and mercuric chloride are the most widespread chemical additions made for sample preservation, but contradictions are reported in the literature about their effectiveness. Chloroform has been reported to be effective in nutrient concentration stabilisation if samples are saturated of it and stored at 4°C (Pichete *et al.*, 1979; Haygarth *et al.*, 1995). However, other studies have shown that chloroform produces an increase in FRP and TP concentration, possibly due to phosphorus release from particles such as algal cells (Fitzgerald and Faust, 1967). Mercuric chloride is the less effective of the two as it can interfere with the

molybdenum blue reaction for phosphorus determination when ascorbic acid is used as the reducing agent (Skjemstad and Reeve, 1978). In addition, mercuric chloride can result in the precipitation of bacteria and proteins (Maher and Woo, 1998).

1.3.4.6 Storage container

It is also important to consider what actually happens during the storage process. Biological activity does not stop when samples are collected and stored, as bacteria and micro-plankton continue to digest and excrete nutrient species. Walls of bottles and containers are excellent substrates for bacteria and micro-plankton, often enhancing biological growth, which continue to digest and excrete nutrient species after collection. Thus, containers for sample storage must be made of suitable material (*i.e.* soda glass, pyrex, polythene, polypropylene, PTFE). Both soda glass (Grasshoff, 1976) and polythene (Morse *et al.,* 1982; Macdonald *et al.,* 1986) containers have been recommended for the analysis of phosphate. Soda glass should not be used in the case of deep-freezing, and, in the case of silicate analysis, dissolution of silicate from the glass containers can contaminate the samples (Zhang and Ortner, 1998). In addition, aqueous solutions containing 0.03 - 0.112 mg L^{-1} orthophosphate can be stored with less than 0.5 % adsorption (first 24 h) in either soda glass or polyethylene containers after first soaking in 1 M HCl and later rinsed with deionised water (Latterell *et al.,* 1974). Acid washing has the advantage of halting biological growth and reducing phosphorus adsorption by container walls (Maher and Woo, 1998). This is usually done by washing with nutrient-free detergent, soaking in 10% HCl overnight, then final rinsing with ultra-pure water.

Breakdown of organic compounds and changes in the speciation of inorganic constituents may also alter measured nutrient concentrations. For example, it has been shown that both organosphosphorus compounds and inorganic polyphosphates are hydrolysed in acidic conditions such as those used in the molybdate colorimetric method (Clesceri and Lee, 1965; Tarapchak, 1983). Other

considerations include adsorption of nutrients to container walls, contamination from sampling/transfer procedures and sample matrix characteristics.

1.4 Research aims and objectives

The overall aims of this research were to design and optimise analytical techniques for the determination of nutrients in natural waters (both *in situ* and in the laboratory) and to investigate estuarine biogeochemical processes using acquired and historical nutrient data (together with other master variable data).

The specific objectives of this research were to:

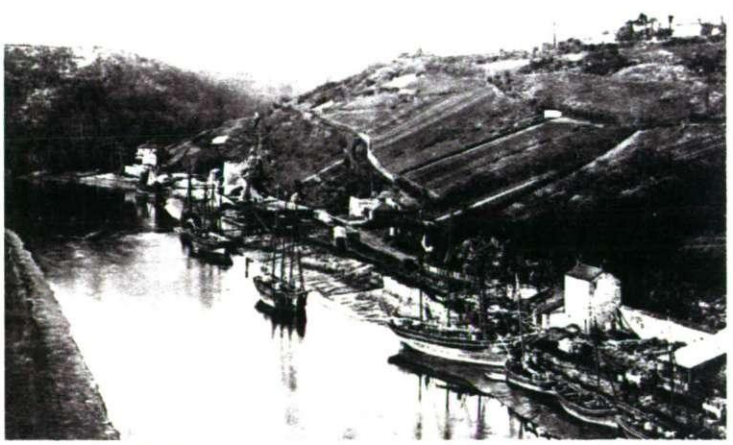
1. Acquire, verify and process publicly available historical environmental data sets to extract as much information as possible (using chemometrics techniques) and to elaborate predictive models for the behaviour of nitrate and phosphate inputs to the Tamar Estuary (Chapter 2).
2. Investigate sample storage techniques for the subsequent determination of nutrients in natural waters with contrasting matrix compositions. In addition, to compare late winter and early autumn samples (from same sites) to assess the effects of seasonal variations on storage (Chapter 3).
3. Optimise, validate and deploy a submersible FI monitor for laboratory, on board and *in situ* determinations of nitrate in estuarine and coastal waters (Chapter 4).
4. Perform surveys in the Tamar estuary using the submersible FI monitor and a laboratory reference method for the determination of nutrients and other techniques for monitoring master variables to study estuarine biogeochemical cycling of nitrate and phosphate (Chapter 5).
5. Use a laboratory incubator and a flow injection manifold to quantify, using real samples, the release of organic and inorganic dissolved phosphorus from suspended sediments exposed to a salinity gradient (Chapter 6).

CHAPTER TWO

Identification of Environmental Trends in Time Series Data from the Tamar Catchment

Main topics in this chapter:

Historical aspects
Catchments description
Physico-chemical parameters
Seasonal trends
Correlations
Nutrient predictions



Schooners and barges in the river Tamar at Calstock c. 1907.

2.1 INTRODUCTION

2.1.1 Historical background of the Tamar Area

The Tamar estuary and tributaries have played a vital part in the agrarian, and later in the industrial life, of the community inhabiting the rough, hilly but also fertile terrain on both banks for more than a thousand years (Merry, 1980). For centuries the importance of the area was related to agriculture and a strong mining activity, with tin, copper, lead, silver, arsenic and granite (Paige, 1982) being the main products of extraction (Figure 2.1). Channels and ferries were commonly used to transport these products *via* land and water, until the end of 19th century and beginning of the 20th century, when mining activity was ending (Booker, 1971). For nearly a hundred years most of these areas have been left to regenerate, and have been used for animal production or for grazing.

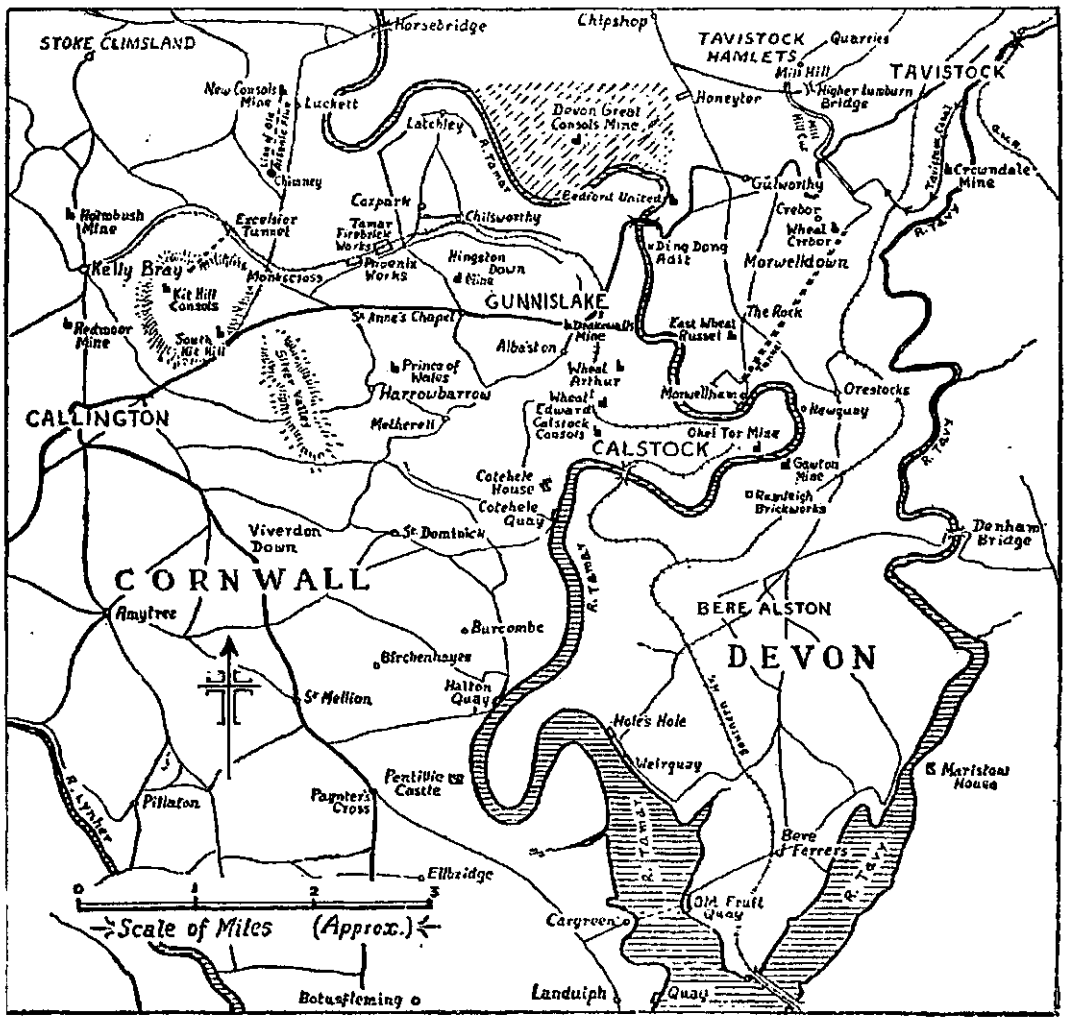


Figure 2.1 The Tamar Valley and some of the mining locations (Booker, 1971).

Agriculture in the Tamar catchment has been for centuries an important activity and has influenced not only the economy of the region, but also the environment. The first applications of artificial manure were reported as early as 1835, and consisted of bone flour and dust. Chilean nitrates, guano and phosphate of soda were easily available and largely utilized from 1850 onwards. The effects of one single application of any of these manures were noticeable for ten years afterwards (Booker, 1971). Modern agricultural activities still have seasonal effects in catchments all over the United Kingdom (EA, 2000). Fortunately, significant areas of the Tamar were left unaltered by mining activity and agriculture, and are still a refuge and natural habitat for several species of plants, insects, molluscs, reptiles, birds, fish and small mammals (EA, 2000).

2.1.2 Current Environmental Regulations

Nowadays the Tamar estuary is part of an area considered by the European Union to be a Special Area of Conservation under the Habitats Directive (Estuary Management Plans (EMPs)), as well as a Special Protection Area under the Birds Directive. These Directives contain international agreements that set out a number of actions to be taken towards nature conservation, actions known as "Natura 2000" (English Nature, 2000). Regulation 33(2) of the Conservation (Natural Habitats) Regulations 1994 translates these Directives into law in Great Britain. This recognition by law requires that relevant authorities exercise their functions in order to ensure compliance with the Habitats and Birds Directives. The management scheme adopted includes: shoreline management plans, Local Environmental Agency (EA) Plans, 'Site of Special Scientific Interest' management plans, local and national 'Biodiversity Action Plans' and sustainable development strategies for estuaries. The Environment Agency has to support the requirements of other European Directives, such as those controlling 'Nitrates from Agricultural Sources', 'Urban Waste Water Quality Treatment', 'Water Framework', in addition the 'River Quality Objectives' which targets all rivers in England and Wales [EA, Standards].

Owing to the environmental importance of the Tamar Estuary, monitoring and controlling the water quality of the river and estuary catchments through various physico-chemical parameters, has produced an extensive and complex set of environmental data. Data used in this chapter (details in Section 2.2.2) cover the last 28 years (1974 – 2002) and was provided by several organisations including the Environment Agency, Centre of Ecology and Hydrology (CEH - National River Flow Archive), and the Meteorological Office - British Atmospheric Data Centre (BADC).

2.1.3 Time series studies

The time sequence of nutrient runoff is one of the important components of the response of a catchment to a rainfall event. Since water is the primary transport medium of many chemical substances, runoff pathways from the land surface to the exit point of the catchment have an important relationship with water quality response patterns (Kim & Delleur, 2001). The hydrologic response includes several flow pathways: subsurface flow, tile flow, saturation excess flow (Dunne & Black, 1970) and infiltration excess runoff (Horton, 1933), each having different residence times in different soil horizons. Therefore, the role of hydrology in time series data is complicated because it is four dimensional, with variations in space and time. Temporal variation in amount, intensity and location of rainfall are important considerations because they affect the levels of discharge activity (*e.g.* high-frequency low magnitude or low-frequency high-impact) (Haygarth and Jarvis, 1999).

A common approach to identify whether monotonic trends exist in hydro-meteorological time series data sets, such as temperature, precipitation and streamflow, is to use non-parametric statistical tests (Yue *et al.*, 2002). The advantage of these statistical tests over parametric tests, such as the *t*-test, is that the non-parametric tests are more suitable for non-normally distributed, censored, and missing data, which are frequently encountered in hydrological time series data sets (Hirch and Slack, 1984). Trend series data sets have been widely analysed

through theoretical and applied water resource studies (Hirch and Slack, 1984; Chiew and McMahon, 1993; Burn, 1994; Lettenmaier *et al.,* 1994; Gan and Kwong, 1998; Yulianti and Burn, 1998; Douglas *et al.,* 2000; Zang *et al.,* 2001), and the majority of these studies have assumed that observed data are serially independent. However, certain hydrological time series frequently display statistically significant serial correlation. In such cases the existence of serial correlation will increase the probability that the non-parametric test detects a significant trend (Von Storch, 1995).

The time series analysed in this chapter contain physico-chemical and hydrological variables, measured over the last 28 years in the Tamar catchment. It is difficult to understand, or graphically plot, the environmental trends in water quality described by so many variables (multivariate data), because many dimensions as there are variables would be needed. In this case, there are many more variables than can be visualised in three dimensions, so straightforward ways of plotting the data are necessary. Chemometrics offers methods that allow the display of such multi-dimensional data by reducing the dimensionality to only a few dimensions (Massart *et al,* 1997). One such technique in this respect is principal component analysis (PCA). Table 2.1 (adapted from Tortajada-Genaro, 2002) summarizes the relations between the principal chemometrics methods and their application in environmental analysis.

2.1.4 Predictive models

An important part of biogeochemical research in catchments is the development of mathematical models of different types. Crucial is simulation of the water cycle, but most models also take into account geochemical, hydrochemical, soil and biological processes. Models help to establish new understanding and integrate information, as the findings of many disciplines are exploited and synthesized. Consequently, models can be formulated, developed, tested (validated) and finally used for forecasting of future events and changes (Moldan and Cerny, 1994).

Most environmental models include parameters which must be tuned or adjusted to obtain a reasonable match between model predictions and observed conditions.

Table 2.1 Classification of the chemometrics methods.

Method	Objectives
Statistic tests (hypothesis verification)	
Comparison of averages, variances, localisation of outliers, ANOVA, normality tests	Establish comparison of results, evaluation of quality assurance
Experiment design and optimisation	
Factorial design, simplex and response surface	Finding the best conditions to achieve the best results or interpretation
Self-directed learning methods	
Cluster analysis, non-linear mapping, principal components analysis (PCA)	Finding of structures or similarities (groups or classes) in the data
Directed learning methods	
(Multivariate) analysis of variance, discrimination analysis, Bayes classification, SIMCA, classification UNEQ	Quantitative selection of principal classes, relation between classes properties and variables
Factorial methods	
Factor analysis, principal components analysis (PCA)	Finding and quantification of relations between variables and objects
Correlations and regressions	
Multiple linear regression, principal components regression, partial least squares regression (PLS), HSPAM and GHPSAM	Quantitative description between variable relations

All models require checking and testing to evaluate how well they perform. The first activity is referred to as model calibration, and the latter as model validation. Without calibration and validation, a modelling application is only an educated

guess. This may be adequate to provide a qualitative picture, but generally is not sufficiently precise to enable decision-making by environmental managers. Given the objectives of most model applications, validation aims for a quantitative statement that the model adequately describes observed behaviour so that it will be a useful predictive method (Reckhow *et al.*, 1990; Munson *et al.*, 2002).

Once a model is calibrated and verified, on a particular catchment, it provides a multipurpose tool for further analysis. The model can be used to test hypotheses and gain a better understanding of how the catchment might behave under different conditions in the future, *i.e.*, to make predictions (Moldan and Cerny, 1994; Brezonik and Stadelmann, 2002).

2.1.5 Aims

The aims in this chapter are:

1. To bring together the most relevant available data used in biogeochemical studies for the Tamar catchment;
2. To investigate these data in different forms to extract as much information as possible;
3. To elaborate, test and verify the resolution of a predictive environmental models;

2.2 EXPERIMENTAL

2.2.1 Description of River Tamar Catchment and Tamar Estuary

The Environment Agency defines as the Tamar Area (Figure 2.2), the River Tamar catchment and the Tamar Estuary. In this chapter the separation between the catchment and the estuary is made at Gunnislake (the tidal limit) and a description of each is as follows.

The freshwater Tamar and Tributaries catchment covers an area of 465 km^2 and extends from the tidal limit at Gunnislake Weir to within 10 km of the north coast of Devon. The population in the catchment in 1991 was estimated at 32,900 but,

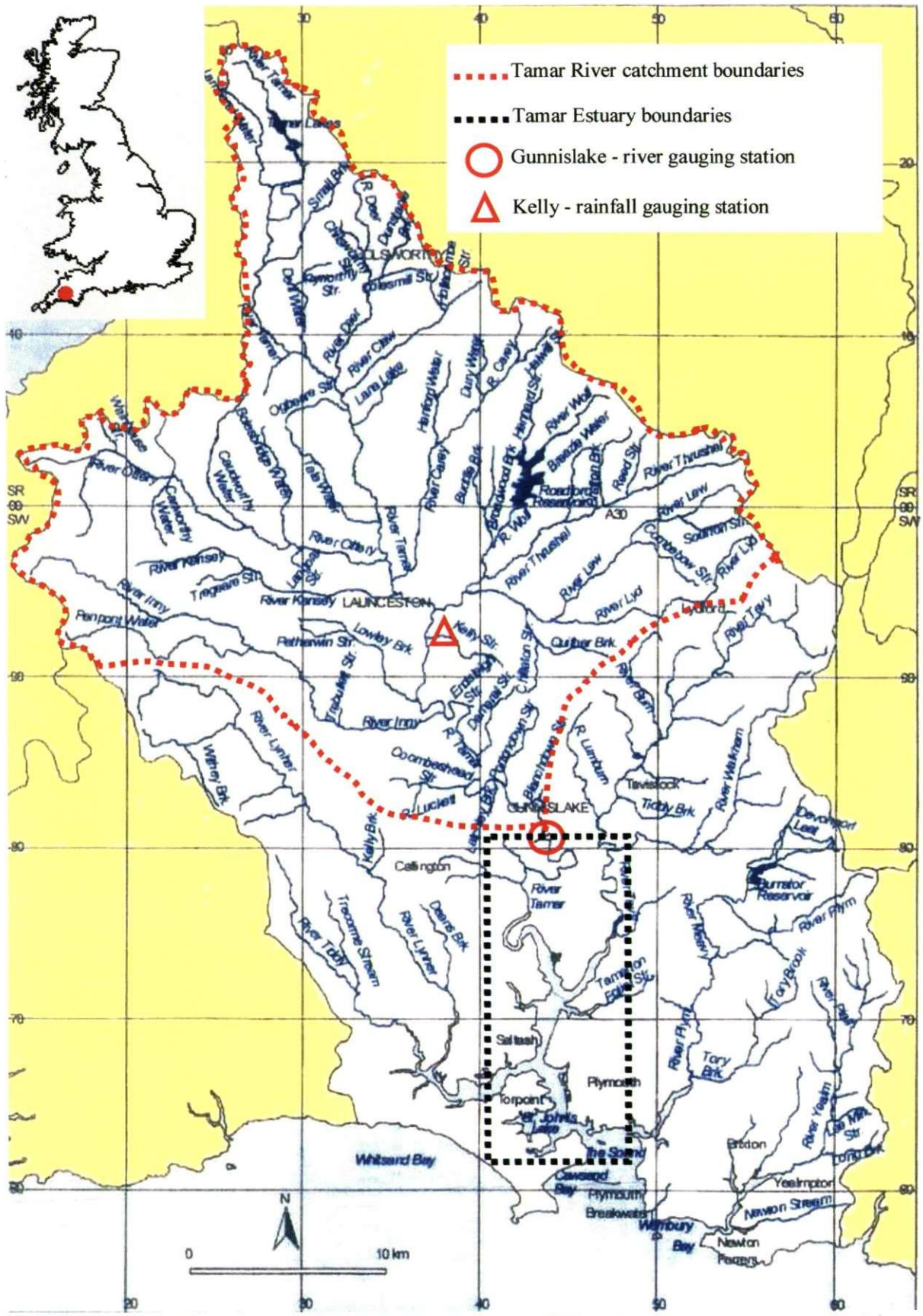


Figure 2.2 Tamar Area located in UK (● in detail map), Tamar catchment (red dotted line) and Tamar Estuary (considered in this work - black dotted line), where ○ represents Gunnislake tidal limit and gauging station, and △ represents Kelly rainfall gauging station (adapted from EA, 2002).

with the popularity of the area as a holiday destination, population grows substantially (nearly four times higher) (EA-SW, 1999) over the summer period.

The catchment has no large industry, but from the extensive historical mining activity in the area, drainage from these workings sites still affect rivers and sediments (EA, 2000).

The Tamar Estuary drainage area, as used in this thesis, is 590 km^2. The length of the estuary, from Gunnislake (tidal limit) down to the mouth of the estuary in Plymouth Sound (Barn Pool station) is 31.5 km. The geology of the region is mainly composed of underlying slates, limestones and grits; at the north-western and eastern areas there is a metamorphic aureole with associated tin, copper, arsenic and local tungsten mineralisation (EA, 1996). Estuarine flushing time is around one week, reducing to one day under summer conditions when the river Tamar reaches a minimum flow of 5 m^3s^{-1} in the low salinity region. The annual average flow of the Tamar River is 30 m^3s^{-1} whilst instantaneous flow can exceed 100 m^3s^{-1}(Miller, 1999). Specific classification of the Tamar estuary based on the types of mudflats (Dyer *et al.*, 2000) sub-divides the estuary into two areas, Calstock (macro/mesotidal with very steep slope) and South Hooe - Weir Quay (macro/mesotidal with low slope). It also has characteristics of a partially mixed and flood dominant estuary (Dyer, 2000). The population estimated to live in the area is well over 250,000, and the economic health of Plymouth, situated at the mouth of the estuary, is closely related with the Defence industries (EA, 2000).

2.2.2 Time series data

Several sets of publicly available data were used in this research. The period selected was between July 1974 and January 2002. The Environment Agency provided data from the gauging station in the Tamar river at Gunnislake (Figure 2.2), which is at the tidal limit (SX 426 725). This data comprised dissolved (<0.45 μm) nitrate + nitrite and orthophosphate, chlorophyll a, suspended solids (dried at 105 °C) and temperature. The Centre of Ecology and Hydrology provided

data for river flow at the same station. Rainfall in the catchment was based on data collected at Kelly rainfall gauging station (SX 396 819) and was provided by the British Atmospheric Data Centre.

2.3 Methods

2.3.1 Data matrix preparation

After receiving the data sets from the organisations described above, a detailed screening process took place on each set. The intention was to format all variables in the same way by the elimination of symbols, such as the representation of limits of detection or non-available data, and by the standardisation of units, which varied over the years (*e.g.* mg L^{-1}, μg L^{-1}, and mm^3 d^{-1}). Over the studied interval the periodicity of sampling and analysis of the samples, for the determination of nitrate + nitrite, orthophosphate, chlorophyll a, suspended solids and temperature varied from a maximum of twice a week to only once a month. There were also periods of months without any data for some of the variables. River flow rates and precipitation were the only variables with daily values throughout the study period. Having converted the data into a common format, single variable plots were made in order to visualise their distributions over time.

Next, a matrix of monthly averages of the measured values over the years was prepared. Also, a matrix with the averages of the monthly values over the 28 years was prepared, *e.g.* all the January data over 28 years was averaged, in order to identify differences or trends across the years. Visual evaluation of the data was performed by univariate analysis as well as normal distribution plots. Finally, multivariate analysis was applied to the data with the aim of identifying, correlating and separating seasonal inputs of nutrients into the Tamar catchment.

The final matrix for detailed chemometrics analysis was prepared using weekly values for all the variables, thereby maximising the resolution of the data but also increasing the number of periods with gaps in the data. The data treatment applied was as described above.

For the elaboration of predictive models based on chemometrics, only periods of uninterrupted data (174 weeks x 7 variables) were used. The models were elaborated to predict future behaviour of nitrate + nitrite and phosphate. For all calculations Unscrambler (7.6 CAMO, Norway) and SPSS (10.0 SPSS Inc.) were used.

2.4 RESULTS AND DISCUSSION

In this section all variables are represented in the tables and plots by abbreviations, nitrate + nitrite (NIT), orthophosphate (PHO), chlorophyll a (CHL), suspended solids (SS), temperature (TEM), river flow (FLW) and rainfall (RAI).

Although all univariate and multivariate analysis for the matrix prepared with monthly values was carried out, this section only discusses the results achieved with the matrix prepared with weekly values. The latter provided greater resolution in order to define seasonal trends, increased the correlations between variables and produced models which predicted results more accurately when compared with the matrix prepared with monthly values.

2.4.1 Study of the distributions of the measured parameters

The distributions of the weekly values of nitrate+nitrite and phosphate over the period July 1974 - March 2002 are shown in Figure 2.3. The pink line in the plot represents the annual mean concentration of the variable over the years. It is noticeable that there is a significant variation in nitrate concentration (Figure 2.3a) over the period 1996 –1998, while phosphate (Figure 2.3b) presented more stable mean concentration values over the entire period. From the analysis of the existing data set, it was not possible to find a direct reason for the variation in nitrate concentration over that period.

The descriptive statistics for all the variables are reported in Table 2.2 for the period July 1974 - March 2002. This table presents, for all variables, the mean values, standard deviations, minimum and maximum values during the period, median, percentiles (25 and 75 %) and relative standard deviation. It is observable

that there is a discrepancy in the number of measurements between the variables. High standard deviation values are evident, represented by the magnitude of the relative standard deviations, demonstrating the natural variability of the data. The only minimum value of zero is for rain.

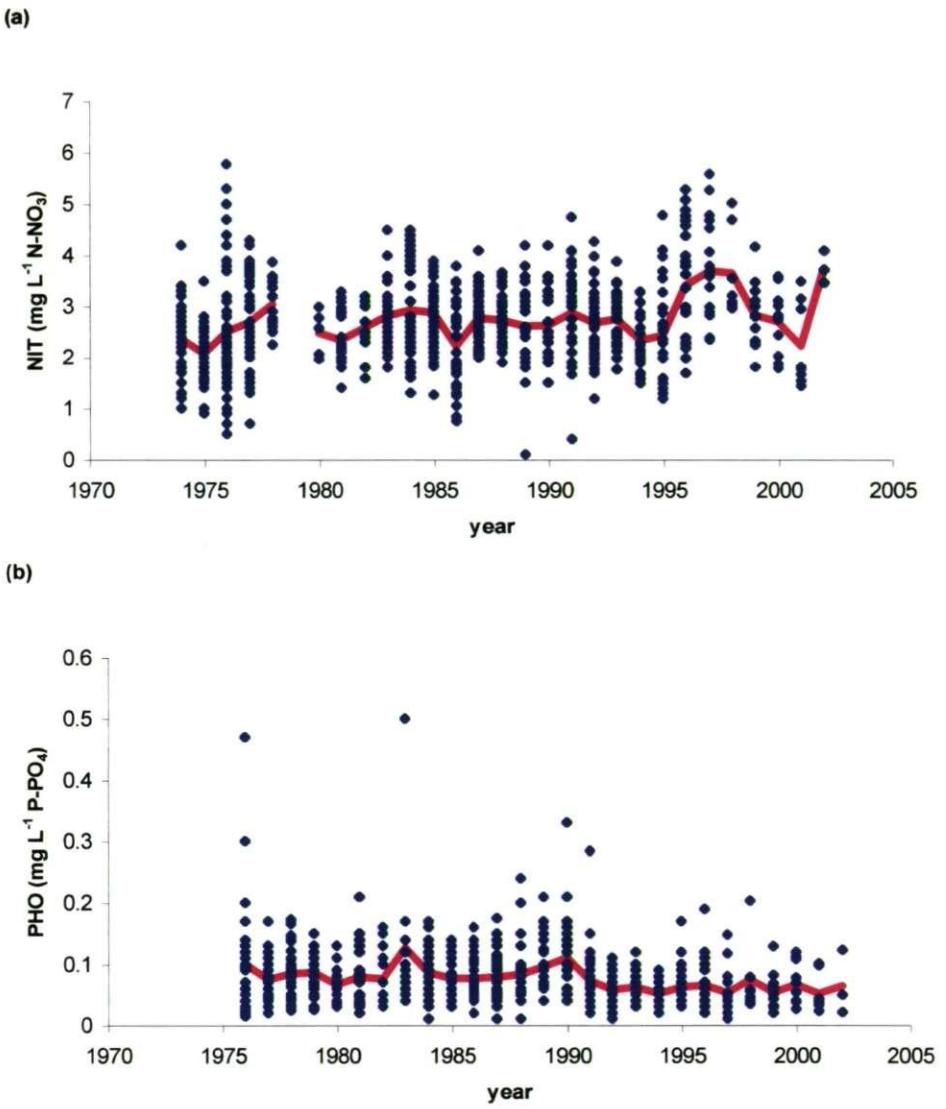


Figure 2.3 Distribution of weekly values for nitrate (a) and phosphate (b) during the period 1974 – 2002. The pink line is the interpolation of the mean annual values.

Examination of the correlation matrix (Table 2.3) shows that linear relationships exist between some parameters. A *t*-test was applied to the slopes of the regressions to check their significance, and showed that most of the slopes were different from zero (Table 2.4) at 95 % confidence level, confirming the linear correlations.

Table 2.2 Descriptive statistics of data set variables

	RAI	TEM	FLW	SS	NIT	PHO	CHL
Unit	mm day^{-1}	°C	m^3s^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	µg L^{-1}
No. of data samples	1455	881	1497	900	758	708	280
Mean	2.96	11.3	23.0	24.0	2.67	0.08	9.10
Standard deviation	3.20	4.20	25.5	50.7	0.85	0.06	13.2
Minimum	0.00	0.75	0.61	0.60	0.10	0.01	0.89
Maximum	18.9	24.0	147	580	5.77	0.91	156
Median	1.89	10.8	12.8	7.10	2.60	0.07	5.00
percentile 25	0.41	8.10	5.10	4.00	2.07	0.05	2.28
percentile 75	4.55	14.7	31.6	19.0	3.20	0.10	11.0
RSD %	108	37	111	210	32	72	146

Table 2.3 Correlation matrix for data set variables

r^2	RAI	TEM	FLW	SS	NIT	PHO	CHL
RAI	1.00						
TEM	0.03	1.00					
FLW	0.40	0.20	1.00				
SS	0.14	0.02	0.18	1.00			
NIT	0.01	0.25	0.11	0.00	1.00		
PHO	0.00	0.11	0.02	0.05	0.08	1.00	
CHL	0.00	0.05	0.01	0.04	0.09	0.03	1.00

Table 2.4 *t*-test of slope for all data

	RAI	TEM	FLW	SS	NIT	PHO	CHL
RAI		-6.57	31.35	15.86	3.9	*(1.41)*	*(-1.56)*
TEM	-4.92		-14.73	-4.13	-17.21	10.6	7.15
FLW	32.59	-21.31		19.88	14.74	-6.7	-3.68
SS	12.82	-4.12	13.92		*(-0.79)*	7.22	7.12
NIT	2.76	-16.02	9.64	*(-0.76)*		-8.12	-9.16
PHO	*(0.98)*	9.38	-4.22	7.51	-7.51		3.92
CHL	*(-0.68)*	3.66	-1.49	3.37	-4.89	2.7	

Boxplot is a visual representation of the data. To produce a boxplot one constructs a box with ends corresponding to the lower and upper fourths in which the median is represented by a horizontal bar (percentile 25 and 75 %). From each end of the box a vertical line is then drawn to the most remote point that is not an outlier. These most remote, non-extreme values are pictured with a small horizontal line, called a whisker. If no outliers are identified the whiskers

correspond to the lowest and the highest value in the data set. Outliers are indicated by an asterisc or a circle outside the whiskers, according to the required confidence level.

Figure 2.4, boxplots were produced with weekly data for each variable for the 28 years period. It is clear that there are outliers for all the variables, but in particular for RAI (a), FLW (b), PHO (d, e), SS (f, g) and CHL (h). The presence of seasonal trends is also strongly evident in most of the parameters studied

Figure 2.5 is a 3-dimension visual representation, which uses a triangular interpolation of all the data. Therefore, these plots facilitate visualisation of the temporal distribution of the data, but it must be interpreted with care. The interpolation fills in gaps in the matrix and produces a continuous surface in the plot, which can mislead the interpretation of the real distribution.

A Kolmogorov-Smirnov test was applied to test whether the data for different years followed a normal distribution for each week and each variable. Table 2.5 indicates the number of weeks that follows a normal distribution and the number of weeks that are not normally distributed, by one (d1) or both limits (d2).

From the observation of Table 2.5, the variables TEM and NIT can be considered to follow a normal distribution for different years whereas SS is not normally distributed.

Table 2.5 Number of weeks for Kolgomorov-Smirnov test classified according to the normal statistical distribution.

	normal	d1	d2
RAI	21	19	13
TEM	49	4	0
FLW	17	9	27
SS	3	6	44
NIT	51	2	0
PHO	36	13	4
CHL	45	8	0

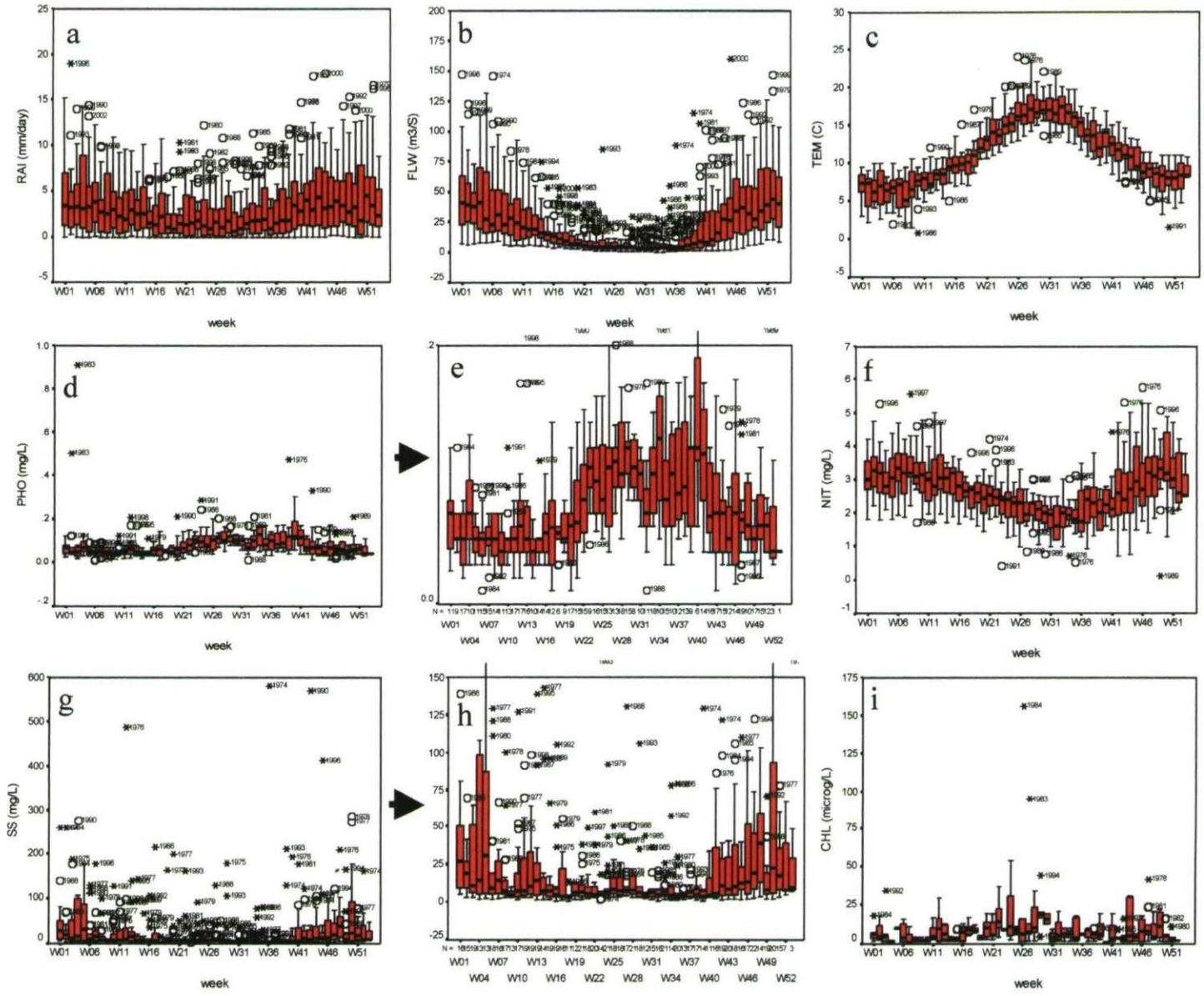


Figure 2.4 Boxplots for studied variables (a) RAI, (b) FLW, (c) TEM, (d) PHO, (e) PHO expanded, (f) NIT, (g) SS, (h) SS expanded and (i) CHL.

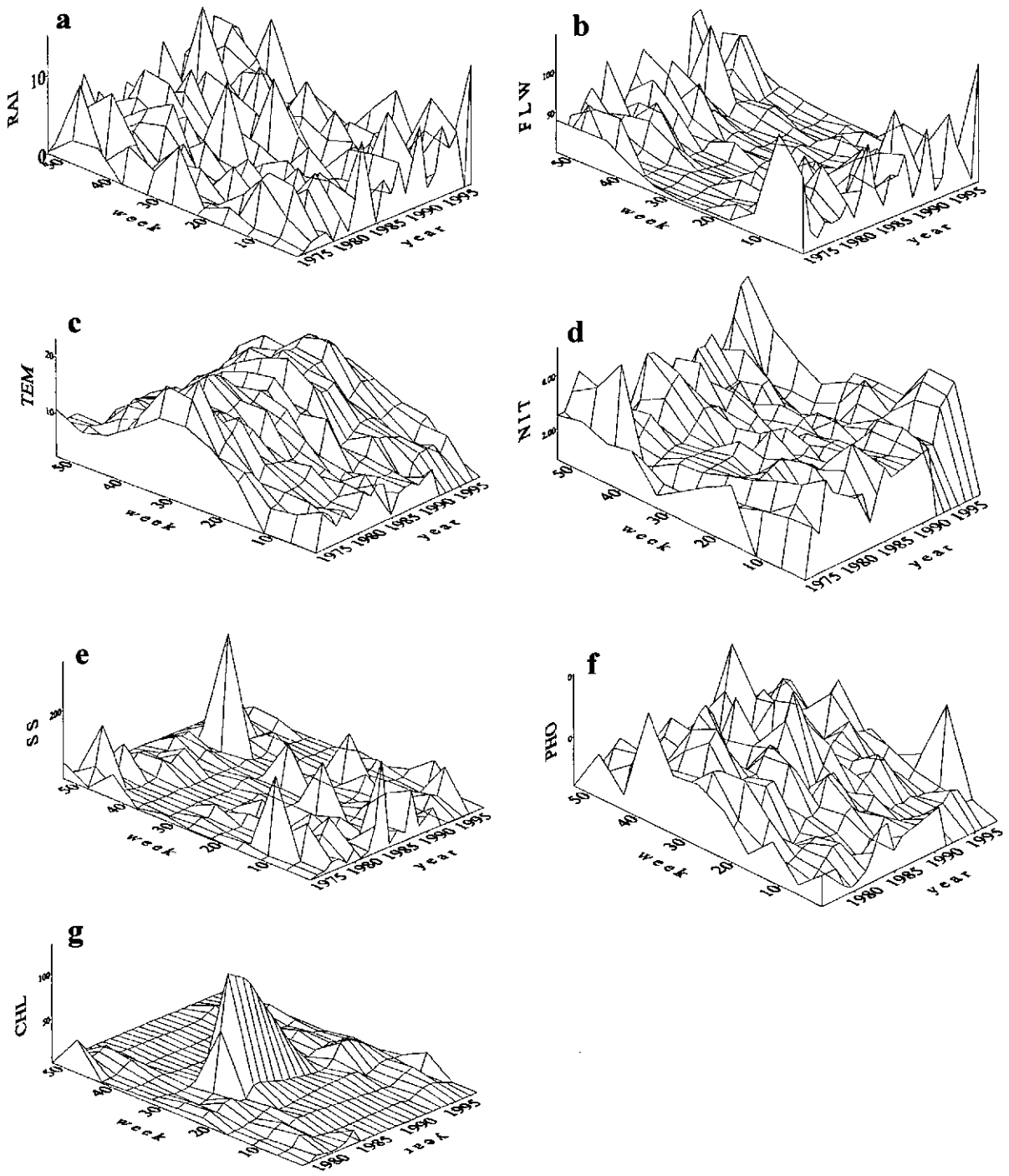


Figure 2.5 3-D representations of the data 1974-1998 using triangular interpolation for response curves. In all plots X, Y and Z-axis are year, week and variable, respectively. (a) RAI, (b) FLW, (c) TEM, (d) NIT, (e) SS, (f) PHO and (g) CHL.

The Kolmogorov-Smirnov test is based on comparison of the observed distribution with the expected or theoretical distribution (normal distribution). If the data can be considered to follow a normal distribution, it can be described by a standard deviation and a mean. This test, which is not suitable for small data sets and is applicable only to continuous distributions, consists of determining the largest difference between two cumulative relative frequency distributions. These are the observed distribution (upper and lower) and the expected distribution. The largest differences are compared with the critical value at n values and probability α.

For the studies described below, robust parameters (such as median, lower percentile 25 % or upper percentile 75 %) were used for the calculations. The reason for that was the presence of outliers and different distributions in the data.

2.4.2 Study of variables correlations and seasonal trends

Median values for different years were calculated for each variable and each week, producing a matrix with dimensions of 53 weeks × 7 variables. Different exploratory analyses were performed on this matrix.

In order to verify associations among variables and to use multivariate data classification, principal component analysis (PCA) was chosen. This chemometric approach, which eliminates redundant information, allows one to produce a limited number of factors that best describe the data, and at the same time permits a better visualisation of the key variables.

Principal component analysis was performed for normalised variables, because the columns of the matrix involved different units. The variance explained for the principal components was PC1 62 %, PC2 13 % and PC3 12 %. Figure 2.6a (PC1 vs. PC2) and Figure 2.6b (PC1 vs. PC3) show the scores plots obtained, where weeks are separated according to season. It can be observed that a plot of PC1 versus PC3 (Figure 2.6b) provided a cyclic representation, where the different weeks are distributed as a function of the season during the year. From loading plots (Figure 2.7a and b), it is possible to establish similar behaviour between some variables.

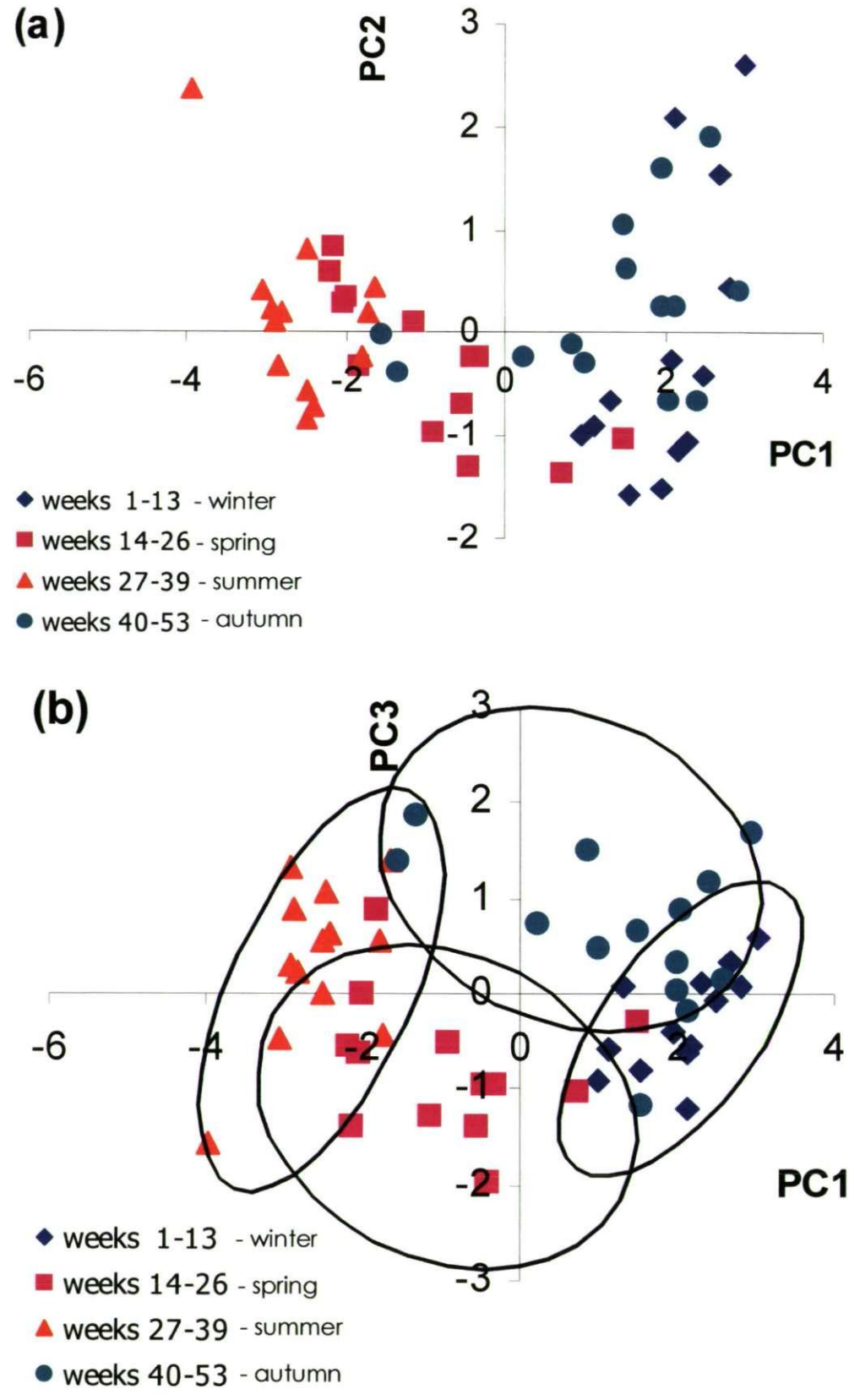


Figure 2.6 **PCA score plots showing the seasonal separation of the week periods, corresponding to the seasons of the year. (a) PC1 was plotted versus PC2 and (b) PC1 was plotted versus PC3.**

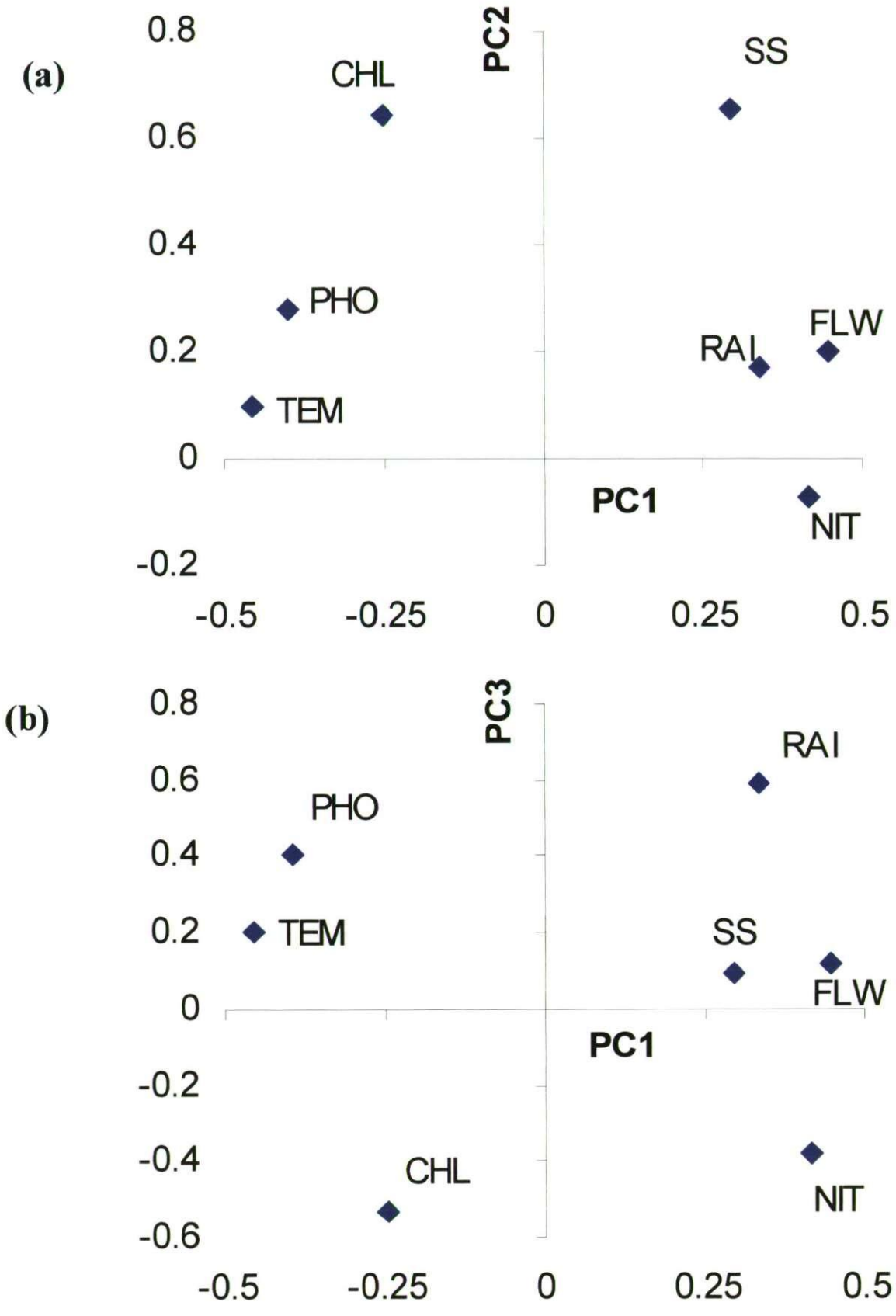


Figure 2.7 Loading plots showing similar distributions due to the main contribution of PC1. (a) PC1 versus PC2 and (b) PC1 versus PC3.

The contribution to PC1 is important and similar for NIT, RAI and FLW and inversely related to TEM and PHO. Thus, the seasonal trends for those variables should be similar.

The correlation between variables was evaluated using different coefficients; parametric (Pearson correlation) and non-parametric (τ_b of Kendall and ρ of Spearman). Table 2.6 shows that the Pearson correlation coefficients were significant, at the 0.01 level (bilateral, i.e. positive or negative), for most of the variables. Also from Table 2.6, expected correlations between variables are evident; *e.g.* a direct correlation between rainfall and river flow, and inverse correlations between temperature and river flow and between nitrate and temperature. The importance of the table is that it also shows correlations that one would not intuitively expect, such as the correlation between phosphate and temperature and the poor inverse correlation between phosphate and suspended solids.

Table 2.6 Pearson coefficient for median values

	RAI	TEM	FLW	SS	NIT	PHO	CHL
RAI	1.00						
TEM	-0.57	1.00					
FLW	0.75	-0.84	1.00				
SS	0.41	-0.48	0.66	1.00			
NIT	0.45	-0.89	0.73	0.41	1.00		
PHO	-0.33*	0.83	-0.69	-0.34*	-0.77	1.00	
CHL	-0.39	0.43	-0.39	-0.11**	-0.30*	0.41	1.00

* significative correlation at 0.05 level (bilateral)
** no correlation

According to Haygarth and Jarvis (1999) nutrient behaviour can be characterised by different flow regimes. Flow properties in soils change according to the levels of rainfall that they receive, therefore altering the different pathways by which nutrients can runoff (Haygarth and Sharpley, 2000) from the soil. In Figure 2.8, the different flow periods can been observed independently of the use of mean, median or percentile values. For winter and summer periods (represented in Figure

2.8 by the letters **a** and **c**, respectively), flow value is relatively constant. For spring (**b**) and autumn (**d**) periods, the flow shows a linear decrease and a fairly linear increase, respectively. Therefore, the weeks were grouped as a function of flow periods in order to detect associations between variables or responses specific for each period. The weeks were grouped into **a**-period (weeks 1-5 and 51-53), **b**-period (weeks 6-24), **c**-period (weeks 25-34) and **d**-period (weeks 35-50), with week 1 the first week of January.

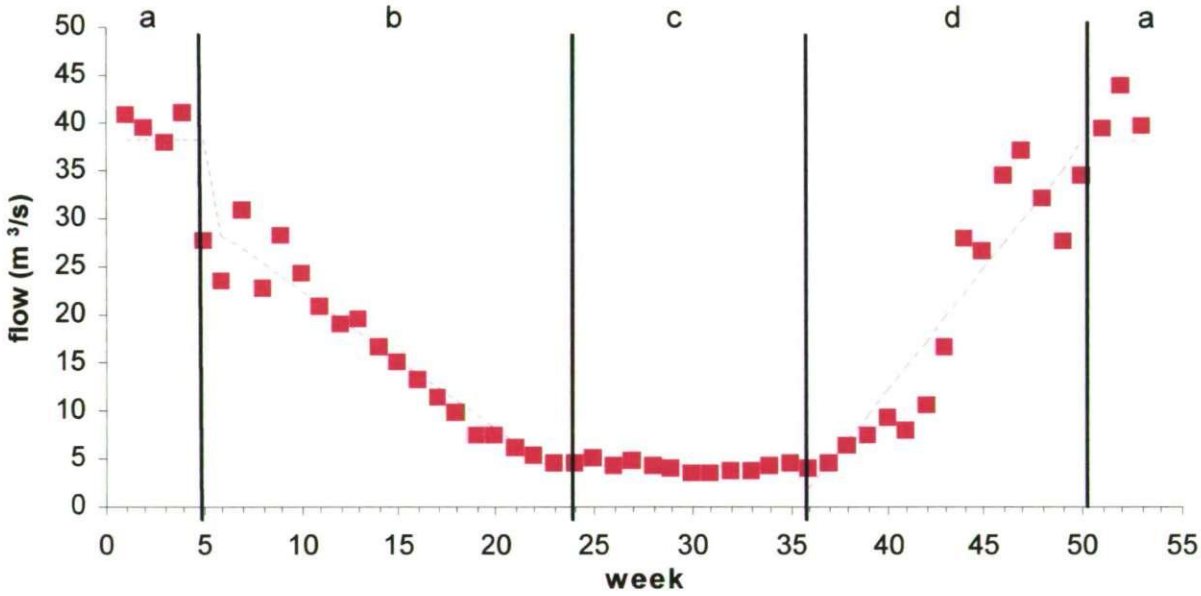


Figure 2.8 Representation of the flow periods using median values for the complete 28 years dataset. The letters indicate the distinguishable flow regimes (a-period weeks 1-5 and 51-53, winter; b-period weeks 6-24, spring; c-period weeks 25-34, summer; d-period weeks 35-50, autumn).

The correlation between variables was also studied by considering different delays in time, *i.e.* possible delayed interactions between variables. The correlation coefficient between particular variables was studied considering a range of weeks. Trend in correlation between some variables over time was observed (Figure 2.9a-d). The representation of the correlation coefficients for nitrate+nitrite with rain and flow and temperature, considering different delayed periods is shown in Figure 2.9a. For nitrate+nitrite the correlation was higher with rain using a 4 - 10 weeks delay

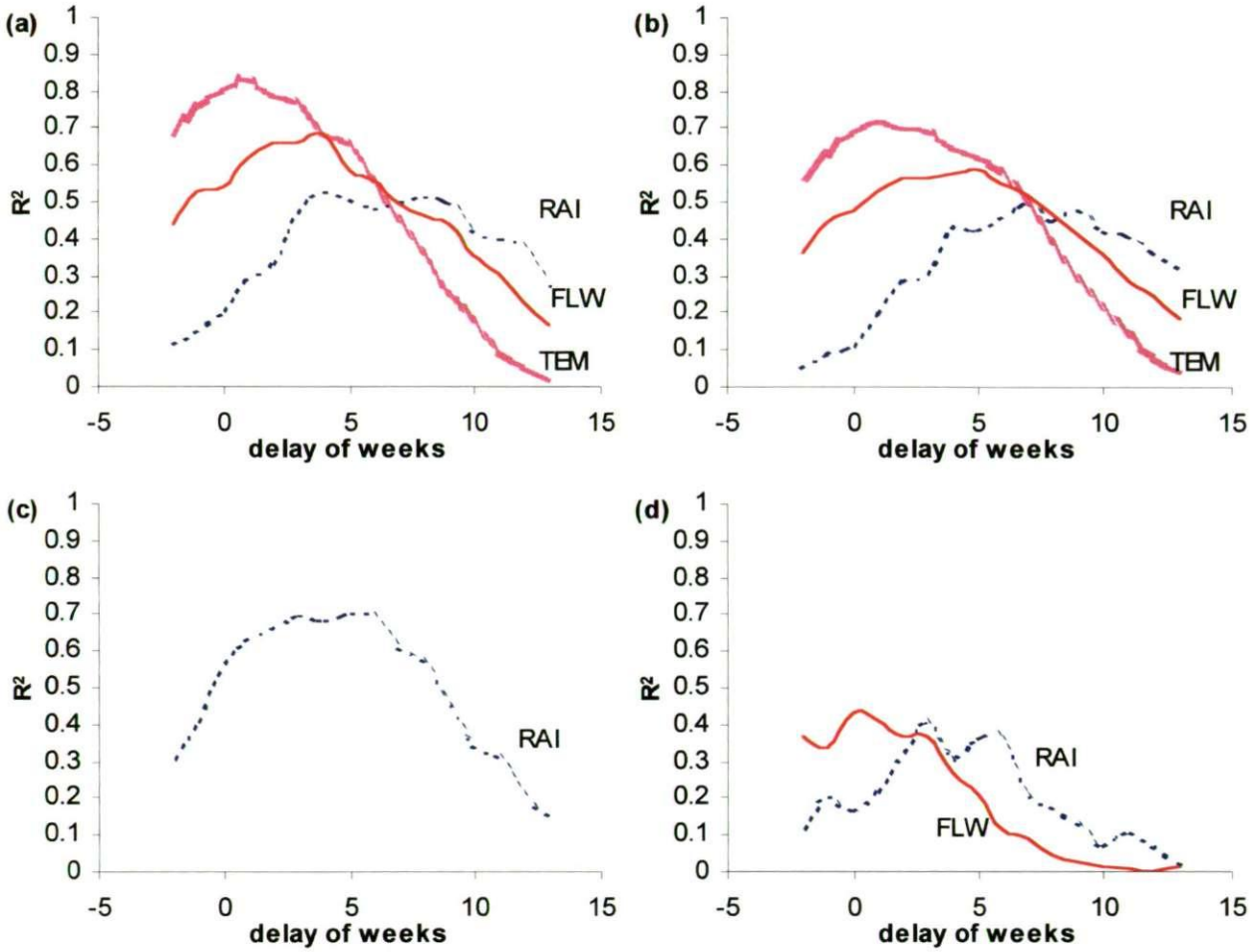


Figure 2.9 Correlation coefficient for several parameters: (a) nitrate+nitrite, (b) phosphate, (c) flow and (d) suspended solids, versus some delayed weeks of expressed parameters

before (*i.e.* rain earlier than nitrate+nitrite), and with flow using 4 weeks delay (flow earlier than nitrate+nitrite). However, the best correlation between nitrate+nitrite and temperature was obtained when no delay was considered in temperature. Phosphate (Figure 2.9b) correlation with rain was greater when considering a delay of 7 weeks, with flow the correlation was higher considering 5 weeks of delay, and with temperature 2 weeks of delay presented higher correlation. Figure 2.9c shows the significant increase in correlation between rain and flow using 4 – 5 weeks of delay. Suspended solids (Figure 2.9d) showed increase in correlation with rain using

3 weeks of delay, although the higher correlation with flow happened without any delay.

2.4.3 Modelling of nutrients

In the above sections, temporal variations for all studied variables and significant correlations between some variables were observed. The aim of this section is to obtain a model predicting nutrient behaviour based on other meteorological, biological and physico-chemical parameters. The application of multivariate models is used to provide a description of significant variables and a prediction of nutrient behaviour in future conditions.

Different models were built using information from 1974-1998. The models were validated by predicting real weeks (those weeks with complete data for all variables) from 1974-1998 period and also from 1999-2002. The different approaches were based on the use of different descriptors such as mean, median, percentiles and their combinations. The inclusion of delay times (as discussed above) was also evaluated.

Nine Partial Least Square regression (PLS-1) models were obtained using RAI, TEM, FLW, SS and CHL as X-block (column normalised) variables. For delay models, variables considering a delay of 4 and 10 weeks were included. NIT or PHO values formed the Y-block in all cases.

The characteristics and the results of all the models investigated are summarised in Table 2.7. Columns 2 to 4 indicate the descriptor of the variable used (mean, median and/or percentiles). Column 5 indicates if delay information was included in the X-block. The number of factors and percent variance explained are in Column 6 and 7, respectively. Columns 8 and 9 present the standard error of prediction (SEP). The best models can be established according to the following criteria: the lowest number of latent variables, the highest percentage of explained variance and the lowest prediction error.

For nitrate+nitrate, the best model was obtained using the mean values and including a delay of four weeks in X-block data. For phosphate, the best model was

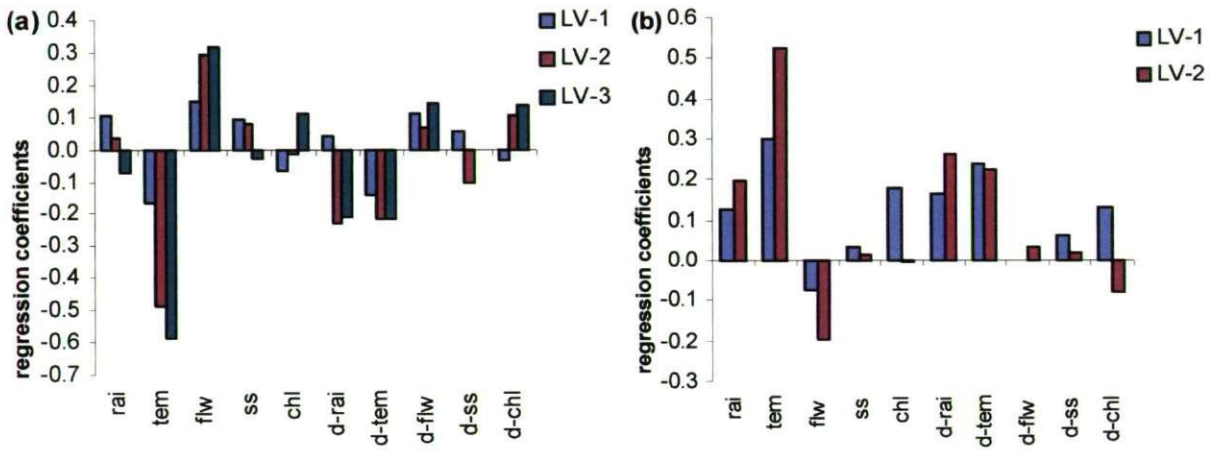
Table 2.7 PLS-models for nitrate+nitrite. Bold in indicates the best model

Model no.	mean	median	percentile 25 and 75%	delay	factors	% variance explained	sep/mean*100 1974-1998	sep/mean*100 1999-2002
1	x			0	2	90.8	27.7	27.0
2		x		0	3	81.5	30.0	31.9
3			x	0	3	81.9	39.1	29.4
4	x		x	0	2	80.6	35.7	27.6
5		x	x	0	3	78.5	38.8	30.2
6	x	x	x	0	2	77.8	34.9	27.5
7	**x**			**0,4**	**3**	**91.3**	**30.1**	**27.3**
8	x			0,10	3	90.3	35.1	30.1
9	x			0,4,10	3	91.0	34.1	32.3

Table 2.7 PLS-models for phosphate. Bold indicates the best model

Model no.	mean	median	percentile 25 and 75 %	delay	factors	% variance explained	sep/mean*100 1974-1998	sep/mean*100 1999-2002
1	x			0	3	59.6	66.1	70.9
2		x		0	2	72.6	69.7	68.8
3			x	0	3	74.6	52.6	54.4
4	x		x	0	3	70.4	53.3	55.4
5		x	x	0	3	74.0	52.9	55.5
6	x	x	x	0	3	70.7	53.5	56.2
7		**x**	**x**	**0,4**	**2**	**72.9**	**53.1**	**59.3**
8		x	x	0,10	4	76.2	50.8	54.3
9		x	x	0,4,10	4	77.3	50.9	53.5

obtained using the median values and percentiles 25 % and 75 % and including a delay of four weeks in the X-block data. The reason for using median and percentiles to produce this model was the lower normal distribution of this variable (see Table 2.5, section 2.4.1). In a general way, the models obtained for phosphate are not as good as those for nitrate+nitrite (*i.e.* lower % of explained variance and higher standard error prediction). Figures 2.10a and 2.10b show the regression coefficients for both models. As can be seen, the variables with the highest contributions to the model are RAI, TEM and FLW. The importance of contribution of delay for the variables in the models is shown by the higher regression coefficient values, for the variables, when compared with original values.



LV – Latent Variable

Figure 2.10 Regression coefficients for the best models (according to Table 2.7): (a) nitrate+nitrite and (b) phosphate. The letter 'd' in front of the name of the variable, represents the variable with a delay of 4 weeks.

The separation into distinct flow regimes did not provide better results for the models. This is probably because the variability of data is inherently included in variables such as RAI, TEM, or FLW (high correlation, see Table 2.4).

The models, built using mean, median and/or percentiles for the different years in the period 1974-1998 have been used for the prediction of several weeks in the period 1974-1998 and in the period 1999-2002. The explained variance for the best model

was 91.33 and 72.91 % for nitrate+nitrite and phosphate, respectively. Explained variance values for phosphate were lower then nitrate+nitrite, possibly due to the more complex mechanisms of interaction and transport of phosphate within the catchment.

The robustness of the models was evaluated by comparing the similarity of the prediction errors for two different sub-sets of data. In this case, prediction errors for nitrate+nitrite were 30.11 and 33.44 % for the periods 1974-1998 and 1999-2002, respectively, and for phosphate were 53.12 and 56.89 %, for 1974-1998 and 1999-2002, respectively. Therefore, the produced models were quantitatively superior when compared to a recent study, using similar methodological and predictive approaches (Brezonik and Stadelmann, 2002), made on storm water runoff. In the referred study the maximum explained variance for nitrate and phosphate ranged between 14 and 41%, while the standard prediction errors were 73 and 135 % for nitrate and phosphate respectively.

The prediction using the best models (Table 2.7, nitrate+nitrite model 7 and phosphate model 7) and real values are plotted in Figure 2.11 for the period 1974-1998. Figure 2.12 shows the predicted surface response curves for all weeks in the period 1999-2002 compared with the response curves for the real data. Good predictions were obtained for most of the weeks. Some poor predictions were related to weeks where variables such RAI, TEM, FLW, SS or CHL had values outside the 25 - 75% percentile interval. Also, part of the visual difference between predicted and observed, in this particular period, was due to the amount of gaps from the matrix, *i.e.* where variables presented only 12 values per year, instead of 53 values per year.

2.5 CONCLUSIONS

All data provided by the organisations listed in Section 2.2.2, for the entire 28 years period, proved to be useful, and despite the structural differences within all data files, standard matrices were successfully constructed.

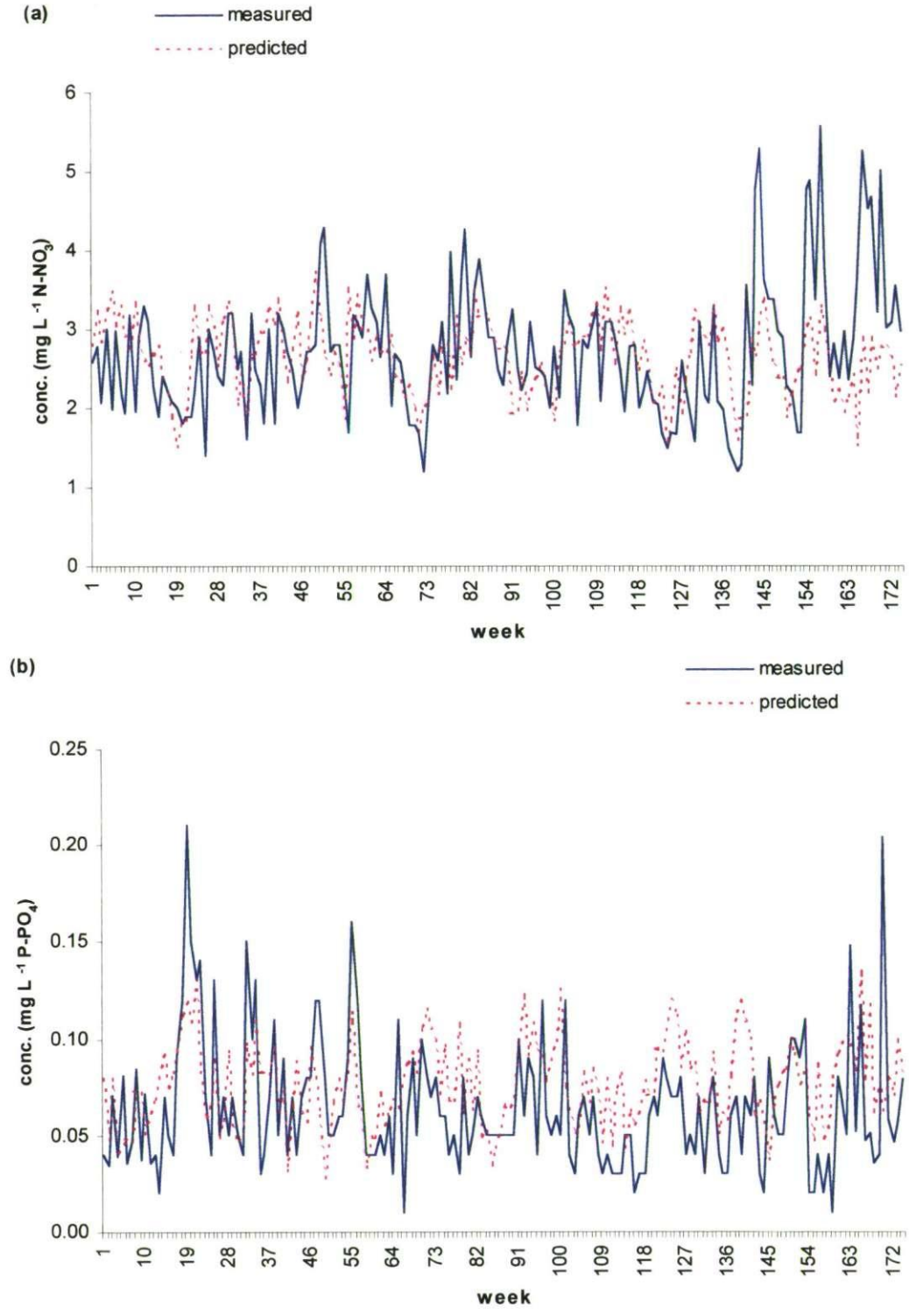


Figure 2.11 Comparison of measured concentrations (mg/L) vs. predicted concentrations (mg/L) data for all weeks with complete data in the period 1974-1998, (a) nitrate+nitrite and (b) phosphate.

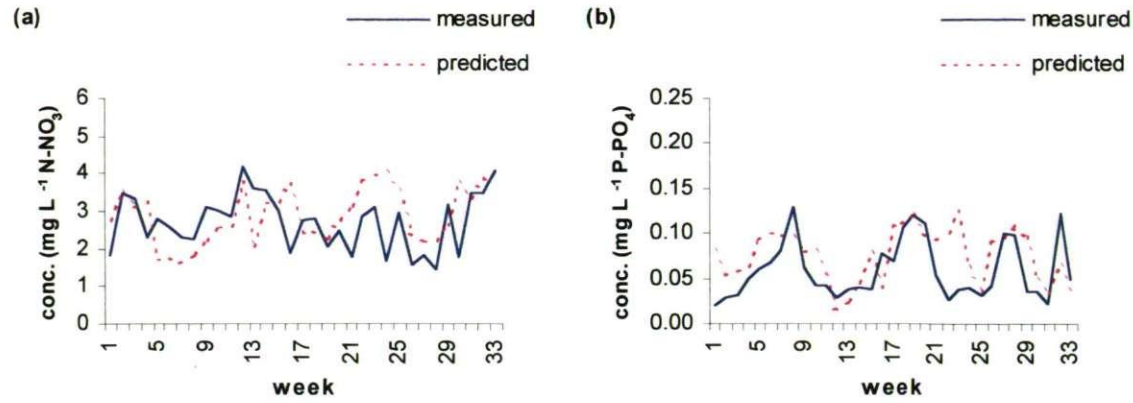


Figure 2.12 Comparison of results for the period 1999-2002, (a) real nitrate+nitrite, (b) predicted nitrate+nitrite, (c) real phosphate and (d) predicted phosphate.

The weekly average matrix improved the resolution of seasonal trends, increased the correlations between variables, allowed one to perform delayed correlation studies and produced models which predicted results more accurately, when compared with the matrix prepared with monthly values.

2.5.1 Study of the distributions of the measured parameters

The preliminary statistics showed and confirmed the existence of linear correlations between some variables.

The distribution of the data using Boxplot enabled the identification of seasonal trends for most of the variables and visualisation of outliers within the distribution.

Use of Kolmogorov-Smirnov test allowed identification of variables which could be further analysed using mean and standard deviation values, or using median and percentiles.

2.5.2 Study of correlation and seasonal trends

The use of principal component analysis, in the matrix with weekly median values, separated groups of weeks that represented the seasons of the year.

The use of Pearson's test established which associations of variables had significant correlations at 0.01 and 0.05 % levels, as well as distinguishing only one association of variables that did not correlate (*e.g.* chlorophyll a vs. suspended solids).

Four seasonal river flow regimes were identified independently of the use of mean, median or percentile values for the period of 1974-2002.

Correlation coefficients between particular variables increased when a delay of four weeks between them was considered, *e.g.* nitrate+nitrite with rain, nitrate+nitrite with river flow, phosphate with rain, phosphate with flow, and flow with rain.

2.5.3 Modelling of nutrients

Multivariate models were produced using the information from the period of 1974-1998, and were able to predict the behaviour of nitrate+nitrite and phosphate based on meteorological, physico-chemical and biological variables. The explained variances by the models were 91.33 and 72.91 % for nitrate+nitrite and phosphate, respectively. The prediction errors for the best model of nitrate+nitrite (30.11 and 33.44 %) and phosphate (53.12 and 56.89 %) were similar for both sub-sets of real data (1974-1998 and 1999-2002) proving the robustness of the models.

The results achieved with PLS models during this study (*i.e.* % of explained variance and standard prediction errors) for nitrate+nitrite and phosphate were qualitatively better than the results reported in the literature, using similar models applied to similar conditions.

CHAPTER THREE

Comparison of Sample Storage Protocols for the Determination of Nutrients in Natural Waters

Operation of a 5 L *in situ* filtration unit at the gauging station in the river Frome.

Main topics in this chapter:

Overview of storage techniques
Sampling and filtration procedure
Matrix effects
Seasonal differences for samples
Overtime nutrients behaviour
Guidelines for the design of storage protocols

3.1 INTRODUCTION

As shown in Chapter 2, long term monitoring of environmental parameters can produce a large volume of data and it is essential that such data is both accurate and consistent over time. High quality environmental monitoring depends on the optimisation of key factors, such as sampling sites, frequency and season of sampling, analytical techniques, storage of samples before analysis. Accurate and representative measurements are also needed for the validation of any environmental monitoring programme. If manual sampling and laboratory analysis are used to provide the necessary data, a suitable sample preservation protocol which minimises the physical, chemical and biological processes that can alter the speciation of nutrients during storage must be used. The quality of data for such protocols depends on numerous factors including sampling procedure, filtration, storage container, physical and chemical treatment, sample matrix and determination method. A recent review [Jarvie *et al.*, 2002] has extensively addressed the importance of these factors for the monitoring of phosphorus in river waters, facts that substantiate the Conclusions from this chapter.

This chapter presents all nutrient concentrations in the text, tables and graphs in μM, also the determinants are nominated filterable reactive phosphate as $P\text{-}PO_4$ (FRP) and total oxidised nitrogen as $N\text{-}NO_2+NO_3$ (TON), which represent an exception from the others chapters in this thesis. The main reason for this was to facilitate the comparison of results with the related literature. A nutrient concentration conversion table (from μM to μg L^{-1} N and P) is presented bellow for the concentration range of samples analysed in this chapter.

3.1.1 Sample matrix

The biological and physico-chemical characteristics between different water types (*i.e.* surface, groundwater, estuarine or open ocean) can vary considerably in composition and what is suitable for preserving nutrient concentrations in one system may not apply to others. Most studies in the literature have been carried out

on a single sample matrix obtained from one sampling campaign. Thus, a more thorough investigation (as described in this chapter) is needed to further investigate sample matrix effects.

Table 3.1 Conversion chart for micromolar (μM) to μg L^{-1}.

μM Nitrate - N	μg L^{-1} Nitrate - N	μM Phosphate - P	μg L^{-1} Phosphate - P
1	14	0.1	3.1
2	28	0.2	6.2
5	70	0.5	15.5
10	140	1.0	31
15	210	1.5	46.5
35.72	500	3.0	93
71.43	1000	6.0	186

3.1.2 Previous studies

Table 3.2 summarises reported methods of storage and maximum storage time for nutrient determinations in the dissolved fraction. The major conclusions were that freezing (-20°C) or the addition of preservatives is indispensable for any given sample storage protocol. However, few of these studies focused on riverine and estuarine waters and none on seasonal variations. It is quite likely that these factors will lead to changes in suspended particulate and dissolved organic matter, biological populations, major ions and extracellular enzymes between sample matrices over time.

Based on these findings, it was thought necessary to augment previous studies by examining the long-term trends in measured nutrient (total oxidised nitrogen (TON) and FRP) concentrations in natural waters with contrasting matrix compositions. In addition, the possible effect of seasonal variations on nutrient behaviour in sub-samples and controls were examined by comparing late winter (February, 1999) and early autumn (October, 1999) samples. Five different storage

Table 3.2 Storage protocols for the determination of dissolved nutrients in natural waters (adapted from Maher and Woo, 1998).

Authors	Nutrient Species[a]	Matrix	Method of storage	Maximum storage time	Comments
Ryden *et al.* (1972)	FRP	Distilled, tap and lake water	Refrigerator (4°C)	1 day	Polypropylene and polycarbonate containers were suitable for storage. Glass containers sorbed phosphorus within 1-6 h.
Skjemstad and Reeve (1978)	FRP	Standards added to rain water	Room temperature with $HgCl_2$ (0-50 mg L^{-1})	3 days	$HgCl_2$ interfered with the molybdenum blue method when ascorbic acid was used as reducing agent.
Pichete *et al.* (1979)	FRP	River water	-10°, 4°, 20°C with/without thymol (0.01%), KF (0.01%), TBT (0.001%), H_2SO_4 (0.05M) or $CHCl_3$ (5 mL L^{-1})	14 days	Samples showed no decrease in FRP if chloroform added and samples stored at 4°C.
Morse *et al.* (1982)	FRP, TP	Open ocean water	Frozen (quick and slow), cooled (2°C) with/without $HgCl_2$ (120 mg L^{-1}), phenol (4 mg L^{-1}) and acid (pH 5)	60 days	No significant change in TP concentration when samples frozen with/without acid.
MacDonald and McLaughlin (1982)	FRP	Coastal and estuarine waters	-10°C, slow and quick freezing	365 days	Small change in FRP when samples were frozen. Quick freezing reduced losses.
Vesely (1990)	NH_4 and NO_3	Precipitation and lake waters	Refrigerator (4°C)	19 days	Significant changes in concentration were observed after 1 day.
Clementson and Wayte (1992)	FRP and NO_3	Open ocean water	Frozen at -40°C initially, then stored at -20°C	147-210 days	FRP concentration decreased in samples stored longer than 4 months.
Lambert *et al.* (1992)	TP, TDP, FRP and TRP	Lake water	Refrigerator (4°C)	180 days	No change in TP in samples for up to 6 months.
Avanzino and Kennedy (1993)	FRP	Stream water	Frozen at -16°C	4-8 years	No significant change in FRP concentration.
Haygarth *et al.* (1995)	FRP	Soil leachates	Room temperature (5-19°C), refrigeration (4°C) frozen (-20°C) with/without $HgCl_2$ (40-400 mg L^{-1}) and H_2SO_4	1-2 days	Changes occurred within 2 days for all samples with smallest changes in samples stored at room temperature or 4°.
Aminot and Kerouel (1997)	TON, NH_4 and FRP	Open ocean and coastal waters	Pasteurization and stored at room temperature	18 months	TON and FRP remained constant for 1 year. NH_4 losses after 3 days.

[a] FRP-filterable reactive phosphorus, TDP-total dissolved phosphorus, TON-total oxidised nitrogen, TP-total phosphorus, TRP-total reactive phosphorus.

treatments: refrigeration (4°C), refrigeration (4°C) with chloroform addition, freezing (-20°C), freezing (-20°C) with chloroform addition and deep-freezing (-80°C) were investigated. All five protocols were applied to samples from four UK sites with contrasting salinities and catchment characteristics.

3.2 EXPERIMENTAL

3.2.1 Sampling sites

Four UK sampling sites were chosen, as described bellow:

The River Frome, starts on the North Dorset Downs near Evershot and flows into Poole Harbour in the south-west of the UK (Figure 3.1). The most important geological formation is chalk, which comprises nearly 50 % of the 41,429 ha catchment (Casey and Newton, 1973). The catchment has a braided network of channels, both naturally occurring and constructed for flood relief. The soils are shallow and well drained with a few areas of clay-influenced soils in the lower

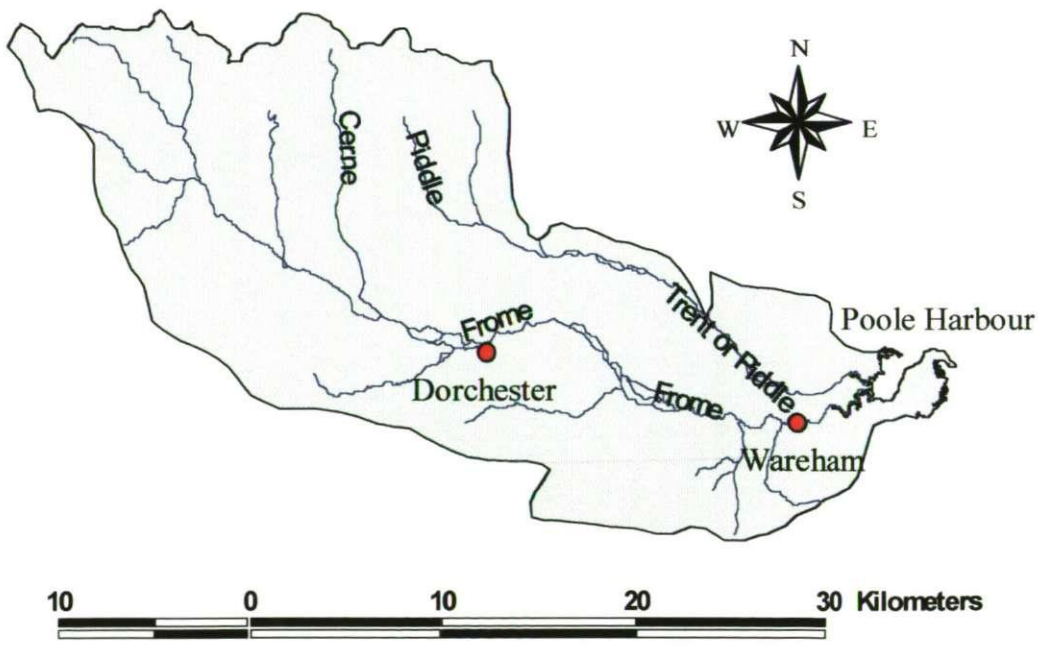


Figure 3.1 River Frome Catchement.

catchment. Land use in the catchment is predominately meadow / verge /semi-natural (31.0 %), tilled land (28.0 %) and mown / grazed turf (19.4 %).

The hydrology of the Frome catchment is dominated by an aquifer passing beneath the lower catchment and Poole Harbour (National Rivers Authority, 1995).

Tamar Estuary, three sites, near the tidal limit, at Calstock, 0.5 ‰, Halton Quay, 10 ‰ and Drake Island in Plymouth Sound, 34 ‰. See Tamar Estuary description in section 2.1.2.

3.2.2 Cleaning procedure

All containers, bottles and glassware used during this study for manipulating and storing reagents, samples, controls and standards were first cleaned overnight with nutrient free detergent (Neutracon®, Decon Laboratories, UK), rinsed three times with ultra-pure water (distilled, double deionised and UV irradiated) (Elga Maxima®, 18.2 MΩ) and soaked in 10 % (v/v) HCl for at least 24 h. All were then rinsed three times with ultra-pure water and dried at room temperature. As an extra precaution, cellulose acetate membrane filters (0.45 µm, Whatman, UK) were also soaked in 10 % (v/v) HCl for at least 24 h and rinsed three times with ultra-pure water before use.

3.2.3 Sampling and storage procedures

Table 3.3A (February, 1999) and 3.3B (October, 1999) list the field data for both sampling campaigns for all four sites at the time of sampling. Samples were collected in 5 L high-density polyethylene (HDPE) containers (Nalgene®) and immediately filtered in the field using a 5 L nitrogen pressurised (< 1 atm) filtration unit housing a 0.45 µm cellulose acetate membrane filter (142 mm diameter, Whatman®). Figure 3.2 shows the distribution of sub-samples during the storage experiment. All 125 mL sub-sample bottles (HDPE, Nalgene®) were first rinsed twice with filtrate then filled with 50 mL aliquots. Bottles were then labelled, placed

Table 3.3A Physico-chemical parameters and grid references of sub-samples at day 0 (February 1999).

Site	Sampling date	pH	Temperature (°C)	Salinity (‰)	Calcium (mM)	Grid reference
River Frome	22nd February, 1999	7.9	6.6	0.5	2.40	SY 872869
Tamar Estuary	23rd February, 1999	7.3	7.6	0.5	0.50	SX 434687
Tamar Estuary	25th February, 1999	7.5	9.2	10	-	SX 413645
Tamar Estuary	25th February, 1999	7.7	9.6	34	-	SX 470530

Table 3.3B Physico-chemical parameters and grid references of sub-samples at day 0 (October 1999).

Site	Sampling date	pH	Temperature (°C)	Salinity (‰)	Calcium (mM)	Grid reference
River Frome	18th October, 1999	7.6	10.7	0.5	2.40	SY 872869
Tamar Estuary	19th October, 1999	6.4	11.6	0.5	1.60	SX 434687
Tamar Estuary	21st October, 1999	7.7	11.5	10	-	SX 413645
Tamar Estuary	21st October, 1999	7.9	13.9	34	-	SX 470530

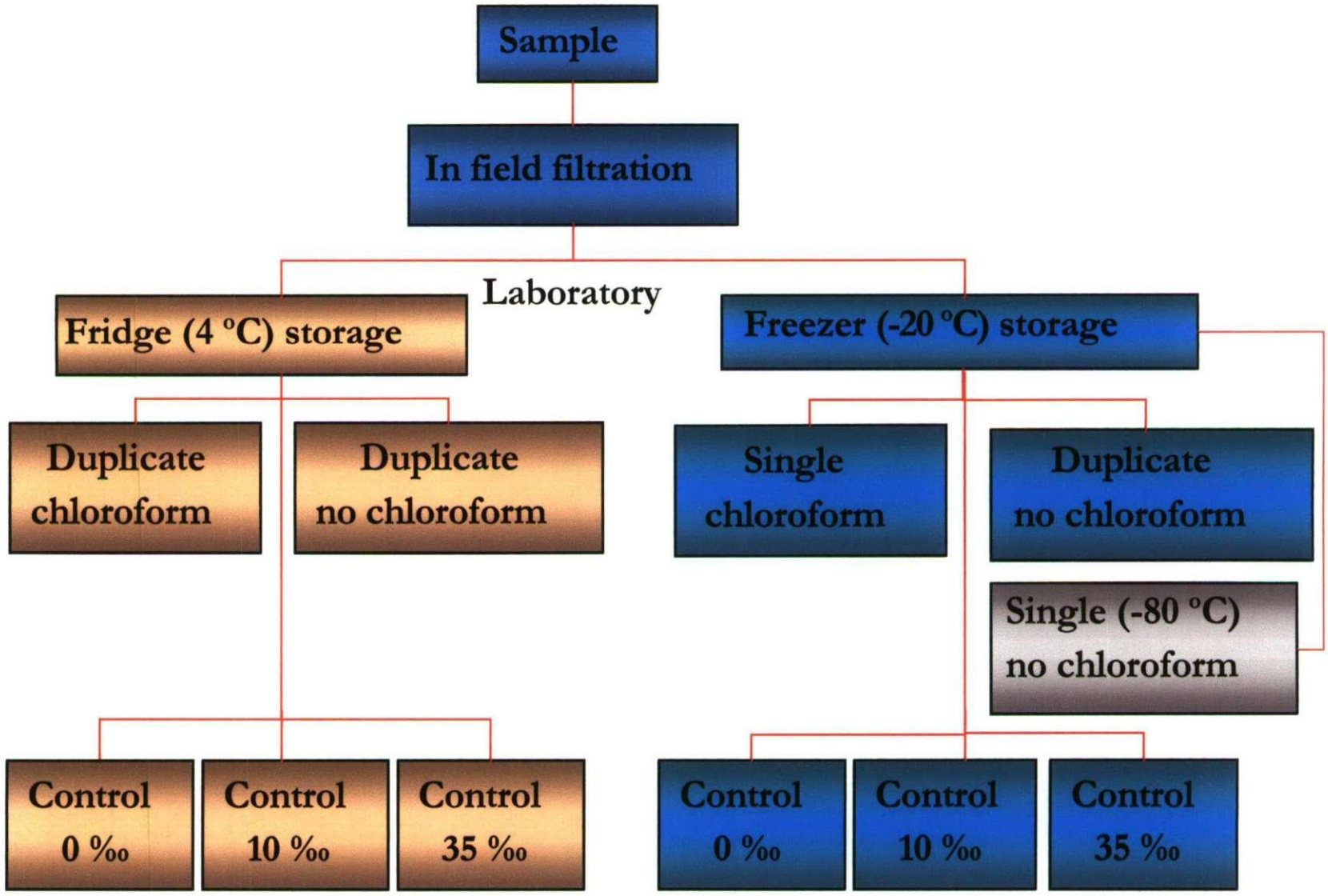


Figure 3.2 Schematic allocations of sub-samples during storage experiment.

in resealable plastic bags and stored under specified conditions: 4°C with/without chloroform (0.1 % v/v) addition, -20°C with/without chloroform (0.1 % v/v) addition and -80°C. Controls were prepared in 0, 10, and 35 ‰ water using appropriate volumes of ultra-pure water and low nutrient seawater (LNS, Ocean Scientific International, UK) with known nutrient concentrations (1.47 μM PO_4, 74 μM NO_2 + NO_3) prepared from stock solutions of 3 mM PO_4 and 7.17 mM NO_3, respectively. All sub-samples and controls (treated in the same way as samples) were placed in appropriate storage conditions within 6 - 8 h. On day 0 (same day as sampling), samples from each site and a single control were analysed in triplicate. Further analyses were performed on days 1, 2, 4, 8, 14, 21, 56, 84, 247, and on day 380 for Tamar 34 ‰ sub-samples.

3.2.4 Instrumentation

A segmented flow analyser (Skalar SAN^{plus}, The Netherlands) incorporating a four-channel module holder and two 16 channel proportioning pumps was used in all nutrient determinations. Figures 3.3 and 3.4 show the analytical chemistry modules and appropriate flow diagrams for TON and FRP, respectively. Automatic background corrections were achieved for each determinant (TON and FRP) by the use of the Matrix (6250) photometer. Refractive index effects were eliminated by subtracting the absorbance at a reference wavelength (TON - 620 nm, FRP – 1100 nm) from the absorbance at the analyte wavelength (TON – 540 nm, FRP – 880 nm). All data handling was performed using the Skalar Data Processing (SDP) package (Version 6.0). An interface (SA 8600) was used to convert analog signals from the detector to digital signals read by the computer. Table 3.4 lists the overall technical and operational specifications of the system. A double beam flame atomic absorption spectrophotometer (GBC Model 902, GBC) was used for all calcium concentration determinations.

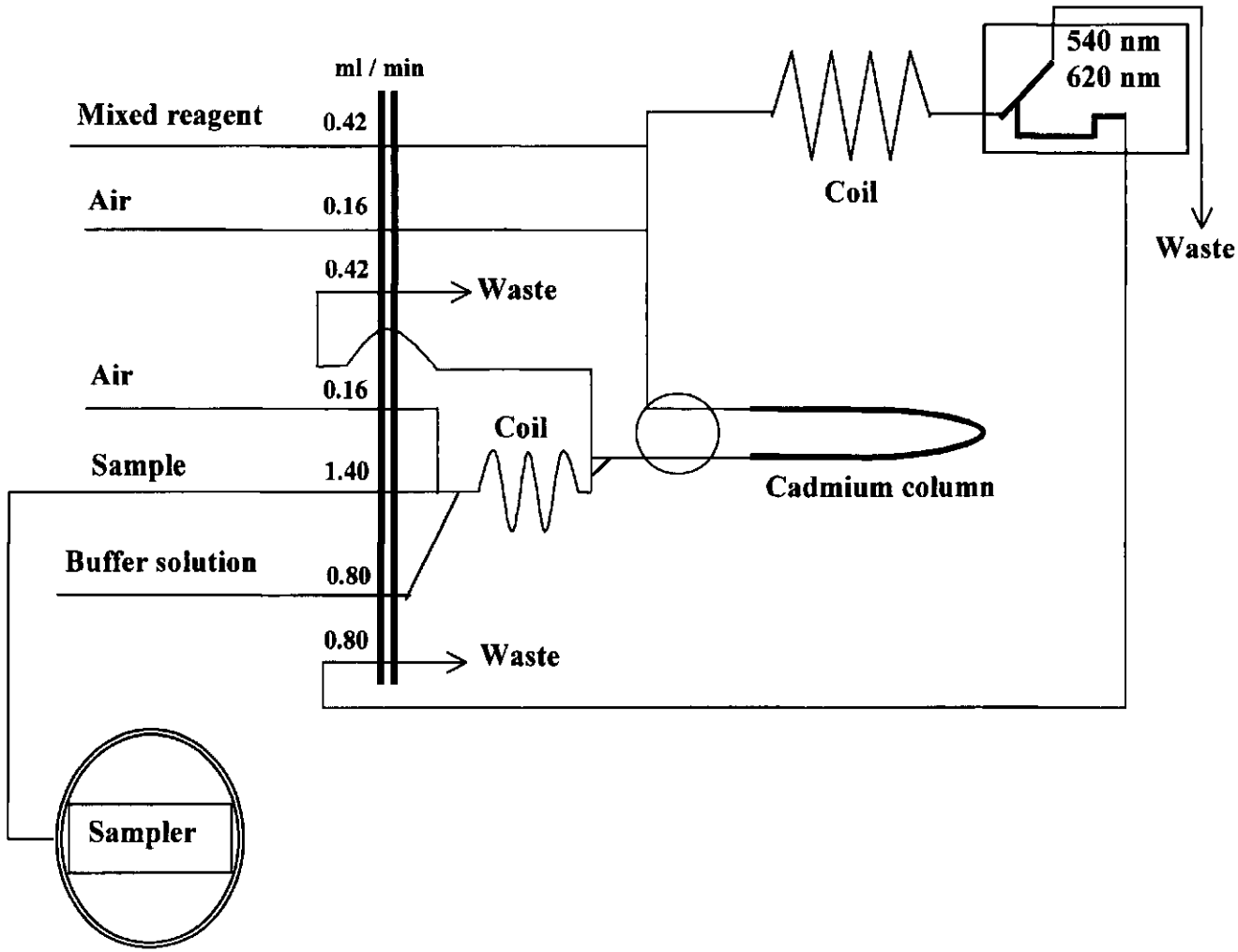


Figure 3.3 Flow diagram for TON determination using segmented flow analysis.

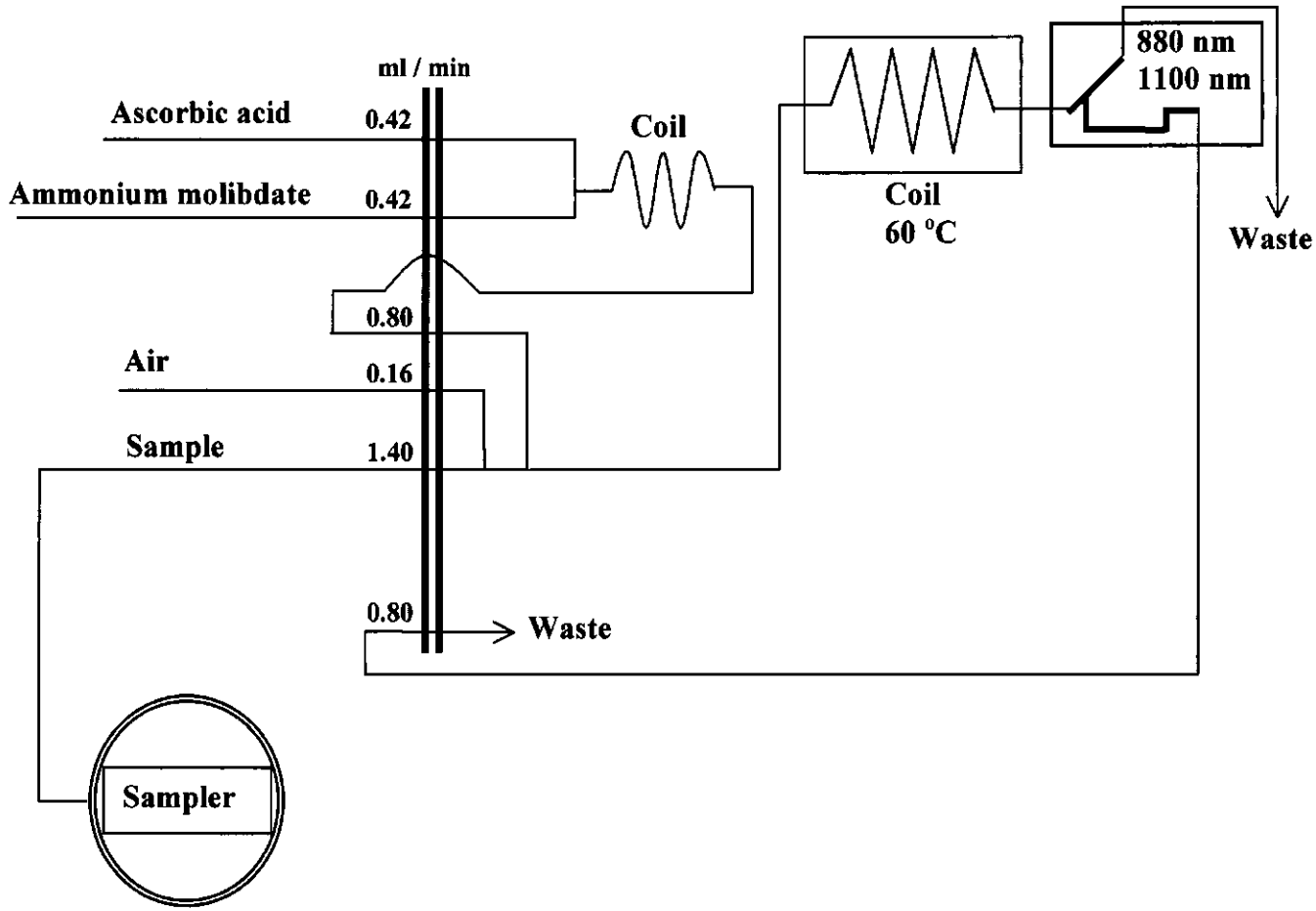


Figure 3.4 Flow diagram for FRP determination using segmented flow analysis.

3.2.5 Reagents and standards

All solutions were prepared in ultra-pure water with all reagents being AnalaR (or equivalent) grade (BDH, UK), otherwise indicated. Working standard solutions (0.1-8.0 μM PO_4 and 9.0-360 μM NO_3) were prepared from stock solutions (3 mM PO_4 and 7.14 mM NO_3) by appropriate dilution with ultra-pure water and LNS prior to analysis.

Table 3.4 Operational specifications of the segmented flow analyser.

Parameter	Specification
Lamp	Halogen, 6V / 10 W cooled
Spectral range	340 - 1100 nm
Optical path length	50 mm
Concentration range (reported)	20 - 100 μg L^{-1} (N and P) 100 - 5000 μg L^{-1} (N)
Sample time	60 s
Wash time	60 s
Injected air	30 bubbles/min
Sample through-put	140 samples/h

(a) TON

Buffer solution contained 25 g L^{-1} ammonium chloride, 1 mL L^{-1} 25 % (v/v) ammonia solution and 3 mL L^{-1} 30 % (v/v) Brij 35 nutrient-free detergent (Skalar). The colour reagent contained 10 g L^{-1} sulphanilamide, 0.5 g L^{-1} α-naphthylethylene diamine dihydrochloride and 150 mL L^{-1} low nitrite *o*-phosphoric acid.

(b) FRP

Ammonium molybdate solution, 230 mg L^{-1} potassium antimony tartrate, 6 g L^{-1} ammonium molybdate, 69.4 mL L^{-1} sulphuric acid and 2 mL L^{-1} FFD6 nutrient-free detergent (Skalar). Ascorbic acid solution, 11 g L^{-1} ascorbic acid, 60 mL L^{-1} acetone and 2 mL L^{-1} FFD6.

3.3 RESULTS AND DISCUSSION

The original concentrations of TON and FRP in controls and samples corresponding to measurements on day 0 (February, 1999 and October, 1999) are shown in Tables 3.5A and 3.5B, respectively. Blanks of ultra-pure water filtered through acid washed cellulose acetate filters gave no detectable response.

Table 3.5A Original concentrations of TON and FRP in controls and samples on day 0 (day of sampling) for February, 1999 study.

Site	Salinity (‰)	TON ± 3s (μM)	FRP ± 3s (μM)
Control	0.0	77.0 ± 0.9	1.53 ± 0.02
Control	10	74.2 ± 4.4	1.52 ± 0.06
Control	35	74.8 ± 4.8	1.51 ± 0.01
River Frome	0.5	480 ± 44	2.88 ± 0.43
Tamar Estuary	0.5	229 ± 23	1.73 ± 0.01
Tamar Estuary	10	146 ± 8.7	1.22 ± 0.03
Tamar Estuary	34	21.5 ± 1.2	0.98 ± 0.01

Table 3.5B Original concentrations of TON and FRP in controls and samples on day 0 (day of sampling) for October, 1999 study.

Site	Salinity (‰)	TON ± 3s (μM)	FRP ± 3s (μM)
Control	0.0	76.0 ± 5.2	1.45 ± 0.04
Control	10	78.8 ± 6.3	1.50 ± 0.06
Control	35	69.1 ± 4.8	1.63 ± 0.06
River Frome	0.5	413 ± 33	4.86 ± 0.21
Tamar Estuary	0.5	196 ± 27	1.25 ± 0.12
Tamar Estuary	10	168 ± 11	1.37 ± 0.03
Tamar Estuary	34	23.2 ± 2.5	1.10 ± 0.08

3.3.1 TON and FRP in controls (February, 1999)

Figure 3.5 shows the measured concentrations of TON and FRP over the duration of each study in control samples (0, 10, and 35 ‰) stored at 4 °C and -20 °C for February and October 1999, respectively. Dashed lines represent ± 3 s of the mean of each of three measurements of two replicates (n=6) analysed on day 0. The error bars for all control data points represent ± 2 s of the mean of three replicate measurements (n=3). Nutrients concentrations are defined as stable if the data point error bars overlapped with the ± 3 s confidence interval for the day 0 data point. As shown in February (Figure 3.5), all TON controls remained stable throughout the duration of the study (247 days) with the exception of the 35 ‰ control stored at 4 °C, which showed a decrease in concentration between day 84 and 247. Overall, 23.1 μM TON (31 % of initial value) was lost after 247 days. FRP controls stored in the freezer remained stable, but there was a more marked deterioration than was observed for TON when stored at 4 °C after 247 days (0.76 μM loss, 51 % of the initial 35 ‰ control). TON and FRP losses from the 35 ‰ controls (Figures 3.5E and 3.5K) are the result of nutrient uptake by the possible presence of bacteria in the low nutrient seawater (LNS) used in the preparation of controls. A 0.20 μm membrane filter was used in the LNS filtration process, which has been reported to not retain unicellular bacteria (Cotner and Wetzel, 1992). The bacteria remaining in the LNS could thus promote the consumption of TON and FRP as reported in previous studies involving bacterial uptake of inorganic nutrients in natural waters (Jansson, 1988; Gronlund *et al.*, 1996). Molar losses were calculated and showed a N:P depletion ratio of 17.4 on day 84 in the 35 ‰ control. This value (17.4) lies within the range of 1.4-18.8 reported for bacterial growth in oceanic waters (Rivkin and Anderson, 1997). The results from the 28 day October study showed no important differences for the controls when compared to the February study.

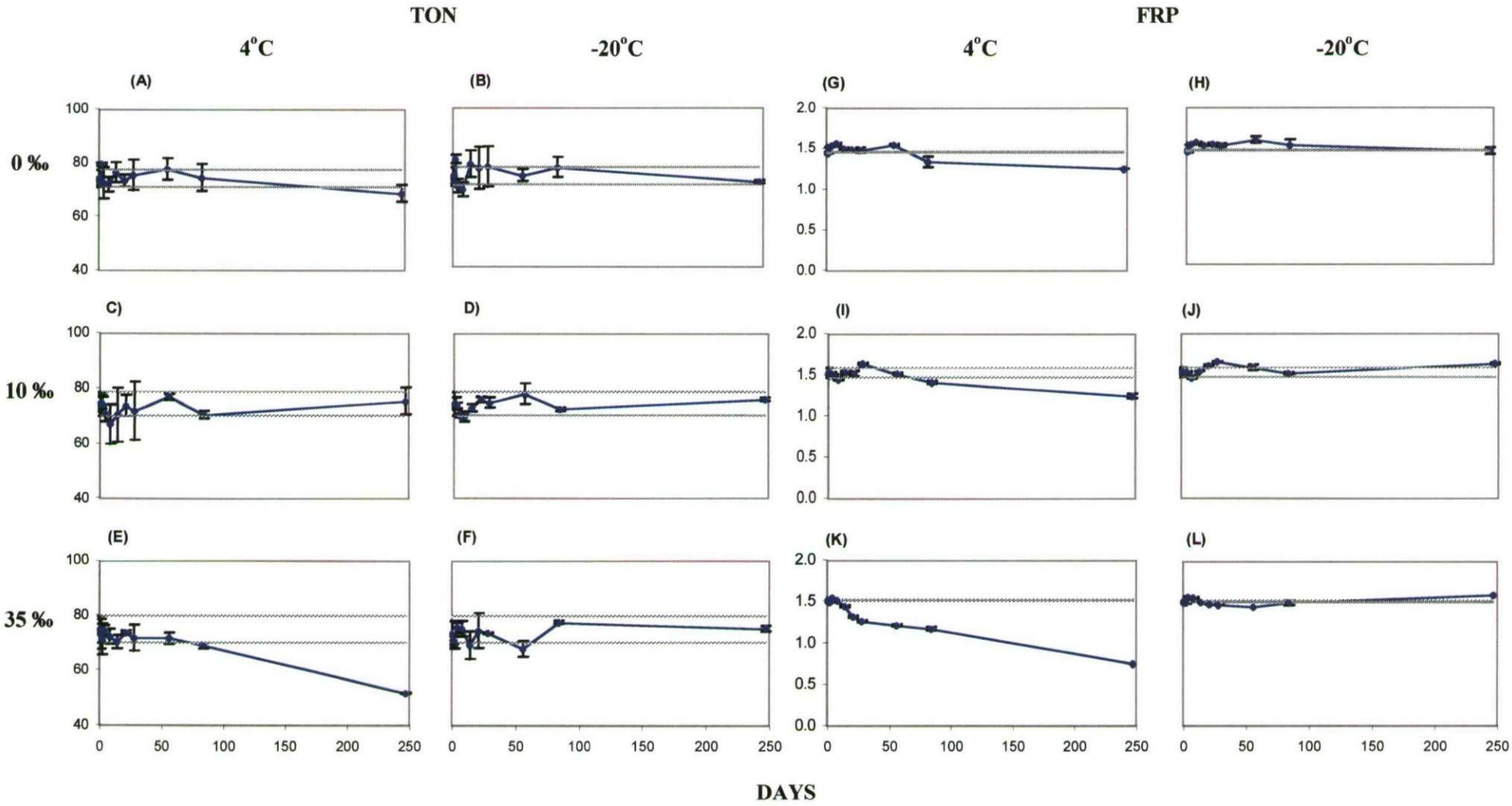


Figure 3.5 Measured concentrations (μM) of TON and FRP in controls for the February, 1999 study.

3.3.2 TON in natural water sub-samples (February, 1999)

As shown in Figure 3.6, measured TON concentrations remained stable for all storage treatment and sites with the exception of Tamar 34 ‰ sub-samples stored at 4 °C without chloroform (Figure 3.6 N). The first noticeable decrease was observed on day 28 and continued steadily to day 247, where the overall loss in concentration was 11.1 μM (52 % loss). As with the controls, this is believed to be partly due to bacterial presence in the sub-samples. In addition, a higher level of bacterioplankton (0.2 – 10.0 μm) could have been present in the sub-samples compared to the controls due to filtration through a 0.45 μm membrane filter (Cotner and Wetzel, 1992).

3.3.3 FRP in natural water sub-samples (February, 1999)

Figure 3.7 shows the measured concentrations of FRP in sub-samples for all sites and treatments for February, 1999. The River Frome sub-samples (Figure 3.7A-D) showed noticeable differences between treatments over time. The greatest nutrient loss occurred in sub-samples stored at 4 °C with no chloroform. The initial FRP concentration (2.88 ± 0.43 μM) was maintained up to day 28, but steadily decreased thereafter to almost complete reduction (0.05 ± 0.01 μM) by day 247. As with the TON sub-samples and controls, this decrease can likely be explained by bacterial uptake. Both freezer treatments (Figure 3.7C and D) showed immediate decreases, which can be explained by physico-chemical alteration of the sub-samples. A white precipitate was present after the freezing/thawing process, probably calcite, which coprecipitated inorganic phosphate, thus removing FRP from the dissolved phase (House *et al.,* 1986; Afif *et al.,* 1993). This coprecipitation reaction, caused by the interaction between inorganic phosphorus and the calcite surface during crystal growth, promoted a reduction in FRP concentration in sub-samples to a stable value of approximately 1.95 ± 0.34 μM. River Frome sub-samples stored at 4 °C with chloroform (Figure 3.7A) remained stable for up to 247 days. The stability in FRP concentration is believed to be the result of chloroform

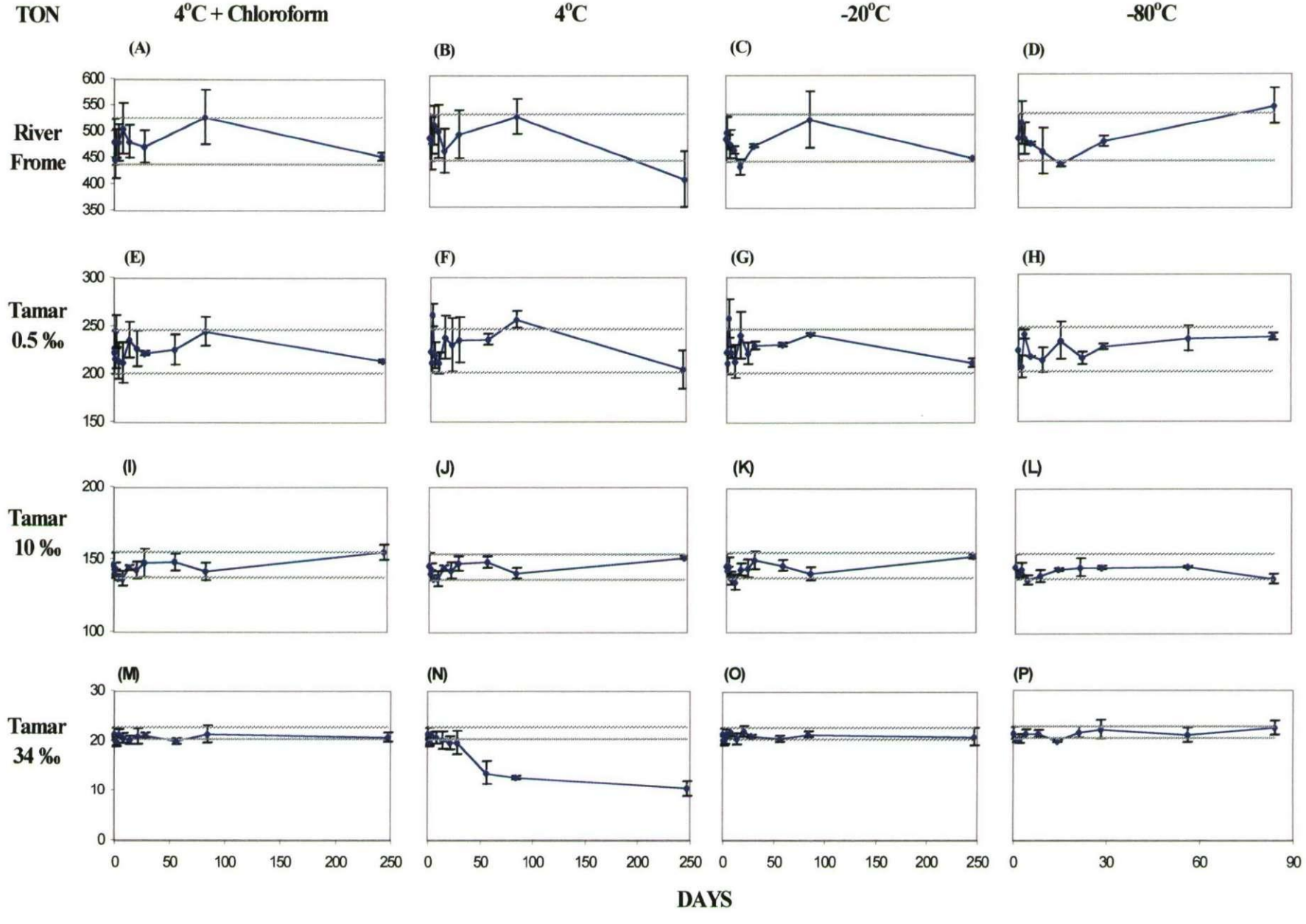


Figure 3.6 Measured concentrations (μM) of TON in sub-samples for the February, 1999 study.

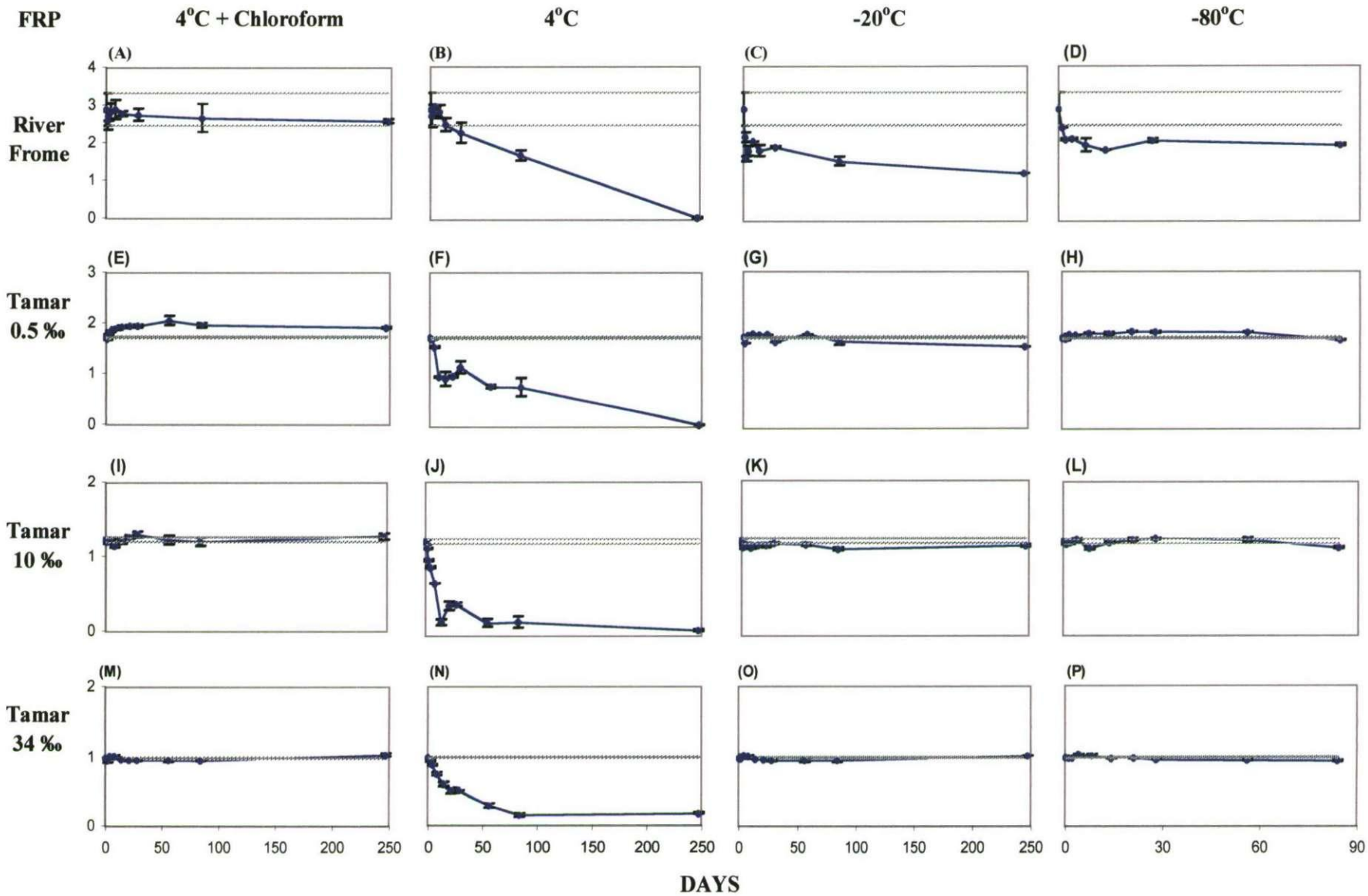


Figure 3.7 Measured concentration (μM) of FRP in sub-samples for the February, 1999 study.

acting as a toxic agent preventing bacterial growth during storage. Tamar 0.5, 10 and 34 ‰ sub-samples stored at 4 °C (Figure 3.7 F, J and N) showed immediate decreases after day 0, probably due to the presence of bacteria. This trend lead to near complete consumption of FRP by day 86 for Tamar 34 ‰ and by day 247 for both Tamar 0.5 ‰ and 10 ‰. Sub-samples from the same sites stored in –20 °C (Figure 3.7G, K and O) and –80 °C (Figure 3.7H, L and P) were stable over the 247 day study. Unlike the River Frome sub-samples, Tamar 0.5 ‰ sub-samples stored at 4 °C with chloroform (Figure 3.7E) showed an increase from the initial FRP concentration (1.73 ± 0.01 μM) to a stable mean value of 1.92 ± 0.16 μM. It is believed that the cause of the increase in FRP concentration is possibly due to the lysis of bacteria as a result of chloroform addition. Chloroform is volatile and may have been partially lost to an ill-fitting closure or through the matrix of the container wall. Once the initial dose of chloroform is lost, the processes that lead to nutrient depletion may restart. These processes could release enzymes, which could decompose colloidal organic matter, thus liberating FRP (Haygarth *et al.,* 1995). In the case of released phosphatases, the enzymes could hydrolyse polyphosphates and phosphorus containing organic compounds, again liberating FRP (Denison *et al.,* 1998).

3.3.4 TON in natural water sub-samples (October, 1999)

To investigate possible seasonal effects (*i.e.* changes in biological and chemical matrix composition), a second sampling campaign was undertaken in early autumn (October, 1999). Figure 3.8 shows the measured concentrations of TON in sub-samples for all sites and treatments for the October, 1999 study. With the exception of a few data point fluctuations, the River Frome sub-sample (Figure 3.8A-D) concentrations remained stable throughout the 28 days. These minor fluctuations could be the result of systemic instrumental errors, as a few points show rather large error bars. However, all data points (with the exception of day 8, -80 °C treatment) fell within the 99 % confidence interval. With the exception of a

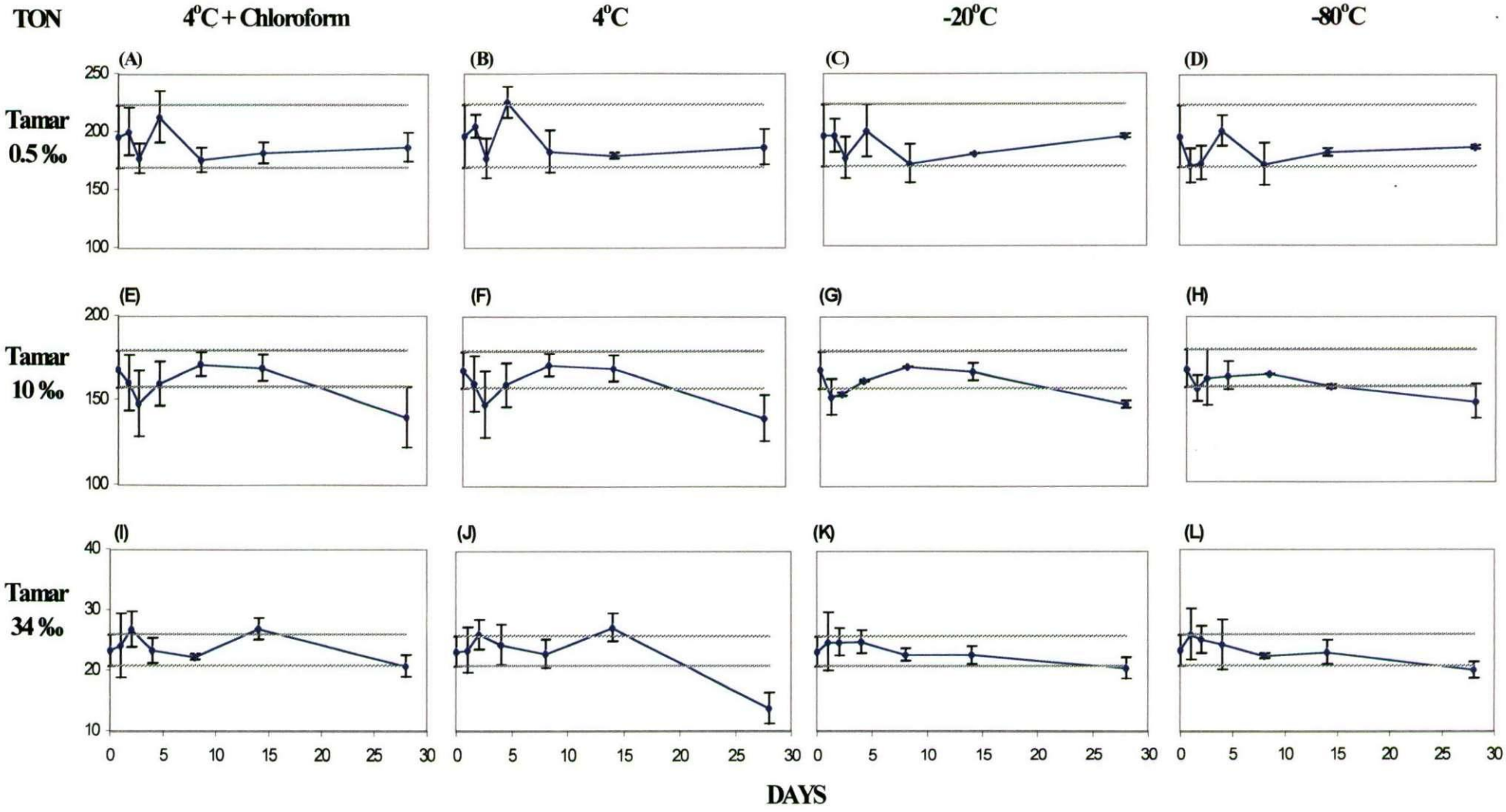


Figure 3.8 Measured concentration (μM) of TON in sub-samples for the October, 1999 study.

small fluctuation (increase) in concentration on day 4, for all treatments, the Tamar 0 ‰ sub-samples (Figure 3.8E-H) remained stable. The Tamar 10 ‰ and Tamar 35 ‰ sub-samples showed similar behaviour as the February, 1999 study.

3.3.5 FRP in natural water sub-samples (October, 1999)

Figure 3.9 shows the measured concentrations of FRP in sub-samples for all sites and treatments for the October, 1999 study. The depletion of FRP from the River Frome sub-samples (Figure 3.9A-D) occurred in a similar way to the first study, with the exception of a delay for the initial decrease. As calcium concentrations in the River Frome were the same for both study periods (2.40 mM), it is believed that changes in biological activity and/or populations are responsible for the differences shown.

Tamar 0, 10 and 35 ‰ sub-samples for the freezer treatments (both -20 °C and -80 °C) showed complete stability over the 28 day study. Sub-samples stored at the 4 °C treatment also showed similar results to the first study, again with a slight delay in nutrient loss from day 0. For Tamar 35 ‰ sub-samples, the observed loss in nutrient concentration (9.38 μM for TON; 0.89 μM for FRP) represented a molar loss ratio for N:P of 10.5 compared with 11.3 for the first study.

3.4 CONCLUSIONS

Variability in the physico-chemical parameters, *e.g.* salinity, calcium concentration and bacterial presence/nutrient uptake existed between all four sites studied, thus making it difficult to select one reliable storage treatment. River Frome sub-samples proved to be the most problematic for maintaining FRP concentrations, especially during freezer treatment, as coprecipitation of inorganic phosphate with calcite occurred after thawing of the samples (House *et al.*, 1986). The most effective storage treatment proved to be 4 °C with chloroform, which showed complete stability for the duration of both studies (February and October 1999).

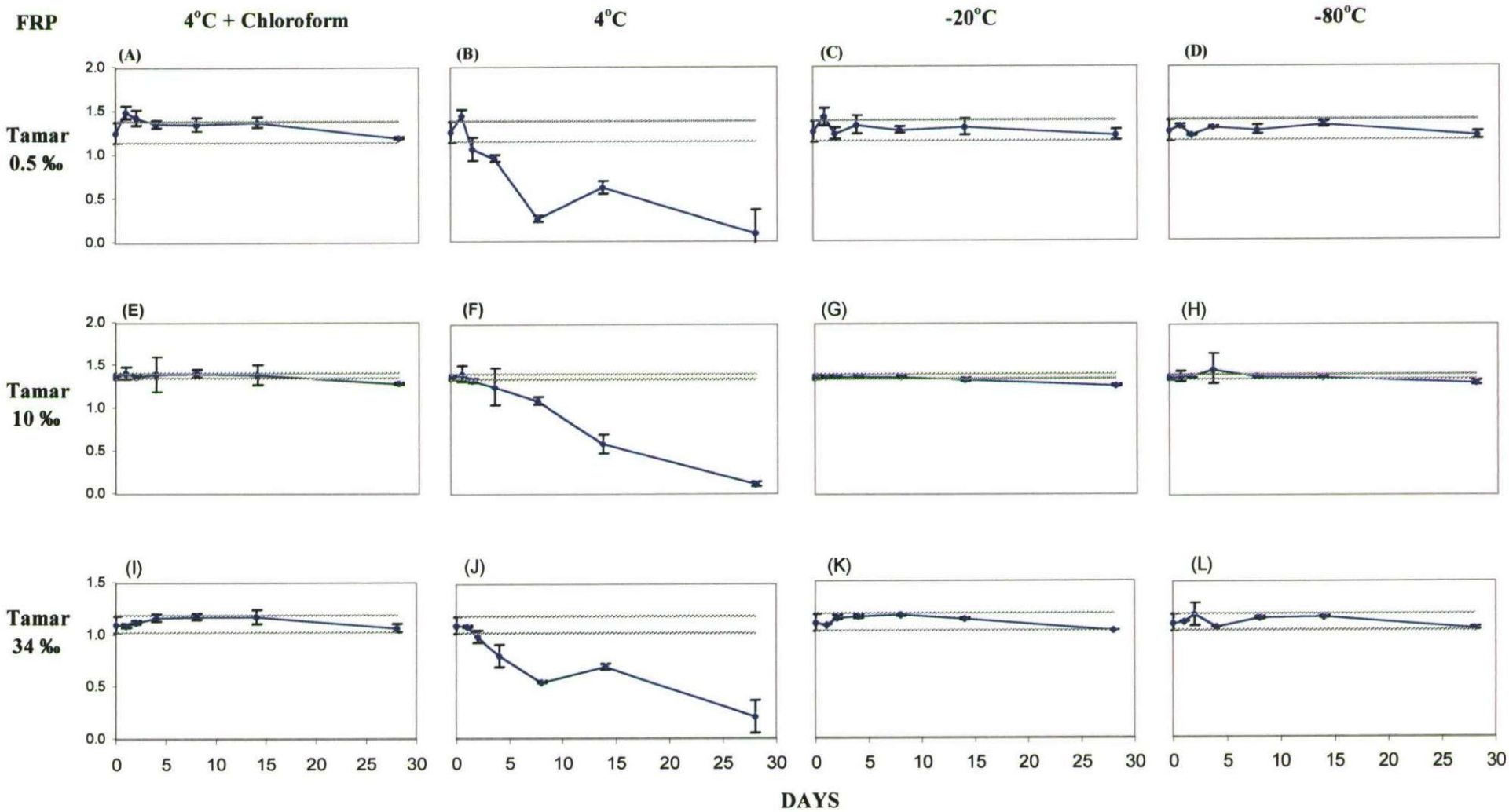


Figure 3.9 Measured concentrations (μM) of FRP in sub-samples for the October, 1999 study.

TON and FRP concentrations in Tamar 0, 10 and 35 ‰ sub-samples remained stable using both freezer treatments (-20 °C and -80 °C) throughout the duration of both studies (February and October 1999). Sub-samples stored at 4 °C showed immediate decreases after day 0, leading to near complete consumption of FRP by day 86 for Tamar 34 ‰ and by day 247 for both Tamar 0.5 ‰ and 10 ‰ (February, 1999). The 4 °C treatment proved to be inefficient in maintaining TON and FRP concentrations in all three controls, while freezing (-20 °C) provided complete stability for the duration of the 247 day study.

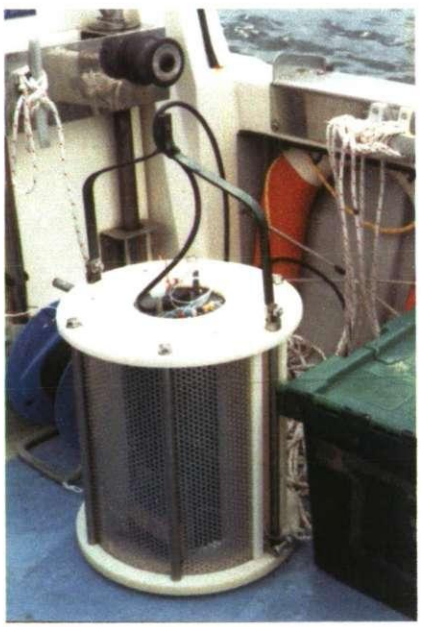
Overall, the optimum storage conditions for the determination of TON and FRP were highly matrix dependent, with differences in FRP stability between the Frome and Tamar catchments. This disparity is believed to be the result of differences in calcium concentrations (Frome 2.40 mM; Tamar 0.50 mM) and varying salinity (due to different bacterial populations and/or dissolved organic matter). Therefore, in the absence of *in situ* measurements, it is essential to design an appropriate site-specific protocol before undertaking any sampling programme for the determination of nutrients in natural waters, especially in the case of FRP. Based on the findings presented in this chapter, such a protocol should take into account the following general guidelines:

1. Design and implementation of a rigorous cleaning procedure to avoid nutrient contamination (as presented in this chapter).
2. All sample containers should be of high quality, inert material such as HDPE.
3. Filtration should be performed in the field with a low-pressure gradient (<1 atm across membrane) to avoid lysis of biological cells, releasing enzymes that could liberate nutrients.
4. Storage at 4 °C without chemical treatment is not recommended for samples, and calcium rich sample matrices should not be frozen in order to avoid precipitation of phosphate.
5. Filtered samples should be analysed as quickly as possible, ideally within 8 h of collection.

6. Chemical treatment should not be used in samples rich in dissolved organic matter to avoid the release of cellular enzymes.
7. A thorough investigation of the biology of the sample (eg. chlorophyll a and bacteria characterization) should be undertaken.

CHAPTER FOUR

Design and Development of a Submersible Flow Injection Analyser for the Determination of Nitrate in Estuarine and Coastal Waters



Submersible Flow Injection Analyser ready for in situ deployment.

Main topics in this chapter:

Design of submersible FI analyser
Validation of results
(intercomparison exercise)
Shipboard deployment
(North Sea)
Submersible deployments
(Tamar Estuary)

4.1 INTRODUCTION

As discussed in the previous chapter, sample integrity may not be preserved during storage for the subsequent action of determine nutrient concentrations. Therefore a field deployable instrument is the ideal solution in order to eliminate the collection, transport and storage of samples prior to analysis.

Nutrient analysers based on Flow Injection (FI) manifolds with spectrophotometric detection have been reported as being sufficiently robust for long-term, high temporal resolution field deployments for the determination of nitrate (Clinch *et al.,* 1987; Casey *et al.,* 1989; Blundell *et al.,* 1995) and phosphate (Worsfold *et al.,* 1987; Hanrahan *et al.,* 2001) in rivers. For marine monitoring the demand for more remote deployments with high spatial resolution has led to the design of *in situ* analysers for nitrate in coastal (Daniel *et al.,* 1995a and 1995b; Finch *et al.,* 1998; David *et al.* 1998) and estuarine (David *et al.,* 1999) waters and silicate in sea water (Floch *et al.,* 1998).

This chapter presents the laboratory optimisation and field deployment of a submersible FI analyser for monitoring nitrate in estuarine and coastal waters. The instrument has significant advantages over the prototype described by David *et al.* (1998, 1999), not least in terms of reduced size, weight and buoyancy, flexibility of use and ease of deployment and communication. The instrument has been shown to operate reliably in the laboratory, on board ship (North Sea deployment) and in submersible mode (Tamar Estuary).

4.2 EXPERIMENTAL

4.2.1 Reagents

All reagents used were AnalaR grade and purchased from BDH (Merck) unless otherwise specified. Low nutrient seawater (LNS; Ocean Scientific, Hampshire, UK) was used to prepare matrix matched nitrate standards. Nitrate standards were

prepared by dilution of a stock solution of 1000 μg L^{-1} N (6.0681 g of dried sodium nitrate in 1 L of ultra pure water (Elga Maxima®, 18.2 MΩ cm^{-1})) in appropriate proportions of LNS and ultra pure water, in order to match the salinity of samples. The carrier used was prepared by dissolving 10 g ammonium chloride in 1 L of LNS, or in appropriate proportions of LNS and ultra pure water in order to match the salinity of samples. Sulphanilamide solution was prepared by adding 100 mL ortho-phosphoric acid to 700 mL of ultra pure water, then adding 25 g sulphanilamide and making up to 1 L with ultra pure water. The α-naphthylethylene diamine dihydrochloride (N1NED) solution was prepared by adding 100 mL ortho-phosphoric acid to 700 mL ultra pure water, then adding 0.5 g N1NED and making up to 1 L with ultra pure water.

4.2.2 Cleaning protocol

All containers, bottles and laboratory ware used during the experiments and for storage of reagents, samples and standards were first soaked with nutrient free detergent (Decon® – Neutracon) overnight, then rinsed with ultra pure water three times. They were then soaked in 10% HCl for at least 24 h, rinsed three times with ultra pure water, stored at room temperature until dry, then packed in zip lock plastic bags to prevent contamination.

4.2.3 Instrumentation

The FI manifold (Figure 4.1), which can also be operated as a stand alone bench top instrument, is mounted, together with control and processing boards, within a pressure housing engineered from a single block of PVC. The instrument consists of two battery powered 12 V Ismatec® peristaltic pumps, a 6 port injection valve (Rheodyne), a 3 way switching valve (Biochem Valve Corporation), a 1 m reaction coil, an acrylic "T" piece for mixing reagents, a variable volume sample loop, a copperised cadmium reduction column and a flow through, solid-state detector (Daviron Instruments).

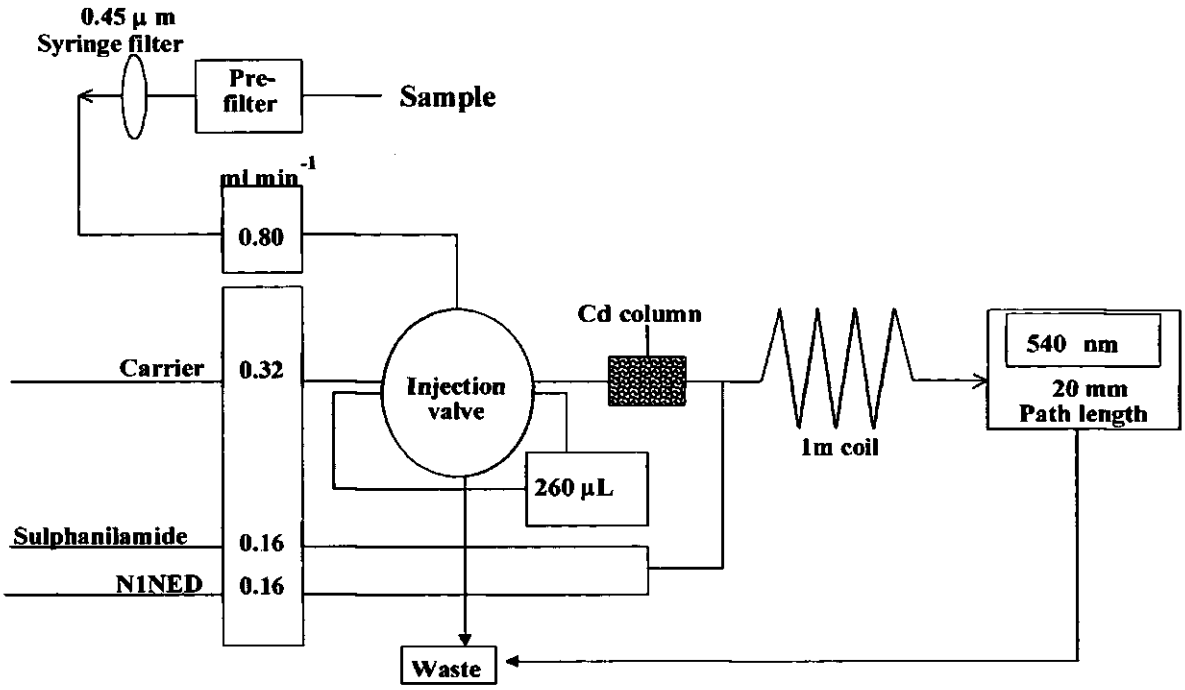


Figure 4.1. Schematic diagram of the FI manifold for nitrate determination

Figure 4.2 shows a schematic representation of the instrument and Table 4.1 describes the physical specifications of the analyser. Communication with the analyser was via the RS232 port using Windows compatible software (Procomm Plus).

The FI chemistry is based on the reduction of nitrate to nitrite in a copperised cadmium column followed by diazotisation and subsequent coupling with sulphanilamide and N-(1-naphthylethylene diamine - N1NED) to produce a pink-purple azo dye ($\lambda_{max.}$ = 540nm). This is quantified using a flow-through, solid-state detector (Figure 4.3) incorporating an ultrabright green light emitting diode (LED) as the light source (Dasgupta *et al.*, 1993) and a photodiode (Radio Spares BPW21 – peak response 540 nm) to detect the transmitted radiation. The chemistry will determine nitrate plus nitrite but for all of the deployments reported, the nitrite concentrations were insignificant and therefore nitrate is quoted throughout. All data are reported as µg L^{-1} of N.

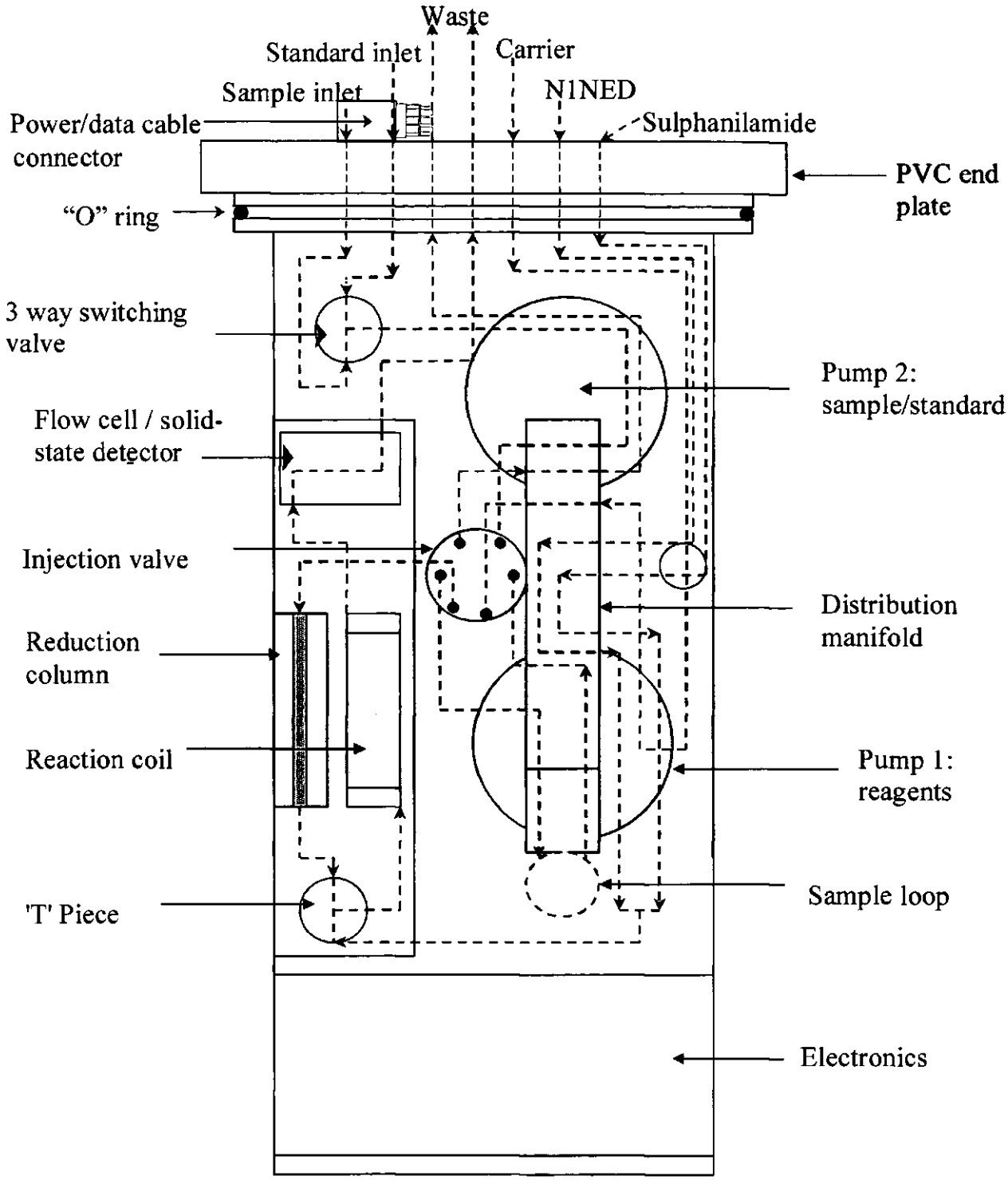


Figure 4.2 Schematic diagram of the submersible FI analyser, showing key components and the flow paths of the reagent and sample lines

4.2.4 Deployments

During 1999 - 2002 the FI monitor was deployed at the following locations:

Table 4.1 Specifications for the submersible nutrient analyser.

Physical attribute	Specification
Housing material	PVC
Analyser size	200 mm diameter x 500 mm height
Analyser weight	12.6 kg
Frame size	400 mm diameter x 515 mm height
Frame weight	12.6 kg
Total weight (including reagents)	35 kg
Operational depth rating	50 m
Reagent consumption per injection	1 ml of carrier and 0.5 ml of each reagent
One analytical cycle (n = 3)	10 min
Central process unit	Intel® 8032
Analogue inputs	2
Analogue-to-digital converter	AD 78715 14-Bit
Real-time clock	Hitachi HD146818P
Communications	RS 232 @ 9600 Baud.
Supply voltage	12 V DC
Number of storable readings	1500

1. Shipboard (Figure 4.4) deployment on the RRS Challenger (Figure 4.5) in the North Sea, during IMPACT Cruise I, 15 - 27th of September 1999. Samples were analysed immediately after being sampled with a CTD-rosette (Figure 4.6) with modified Go-Flo from 32 stations at 3 different depths and passed through polycarbonate membrane filters (0.45 μm).

2. Drakes Island, Plymouth Sound, Plymouth, UK, 3rd of July 2001. Deployed *in situ* for 5 h from the side of the University of Plymouth research vessel *John Dory* (Figure 4.7), at 1 m depth, using double 6 mm diameter rope. Samples were filtered on-line using acid washed (10% HCl) quartz wool packed pre-filter and a Nalgene 0.45 μm syringe filter.

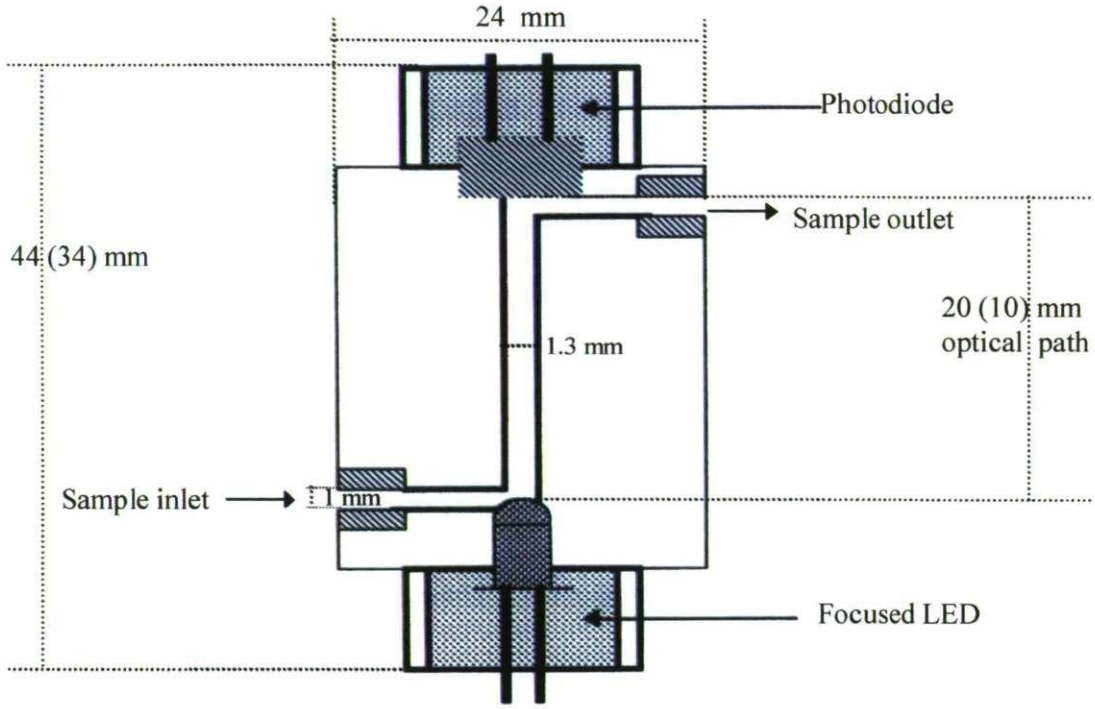


Figure 4.3 Representation of the flow through solid state-detector, dimensions in brackets represent the sizes for the 10 mm optical path size.

3. Tamar Estuary, Devon, UK, 24th of September 2001. Deployed *in situ* from the side of Daviron Instrument's research vessel *Tealia* (Figure 4.8), at 1 m depth, using double 6 mm diameter rope at 10 stations, with salinity varying from 0 (Moorwellham Quay) to 32 ‰ (Tamar Bridge). Samples were pre-filtered and filtered as described above. Station co-ordinates, time of analysis and salinity of samples are shown in Table 4.2.

Table 4.2 Location, time of day, distance from tidal limit, salinity and nitrate concentrations for samples from stations in the Tamar Estuary, Devon, UK. High tide at Calstock was at 11:21.

Station	Latitude N	Longitude W	Time (h)	Distance (km)	Salinity (‰)
Morwellham Quay	50° 30.38	4° 11.42	12:00	3.6	0
Calstock	50° 29.66	4° 12.31	12:40	8.1	5
South Ward Farm	50° 28.85	4° 13.02	13:40	9.9	8
Halton Quay	50° 28.12	4° 13.85	15:00	13.3	15
Weir Quay	50° 27.45	4° 12.44	15:40	17.7	20
Weir Point	50° 26.06	4° 11.84	16:30	20.6	25
Tamar Bridge	50° 24.56	4° 12.19	17:05	23.9	32

Figure 4.4 Shipboard deployment in the North Sea, during IMPACT Cruise I, 15 - 27th of September 1999. Detail of the monitor and pc tied on vessel's bench.

Figure 4.5 RRS Challenger during the IMPACT Cruise.

Figure 4.6 Deployment of the (12 x 10 L 'Go-Flo' bottle) rosette (CTD).

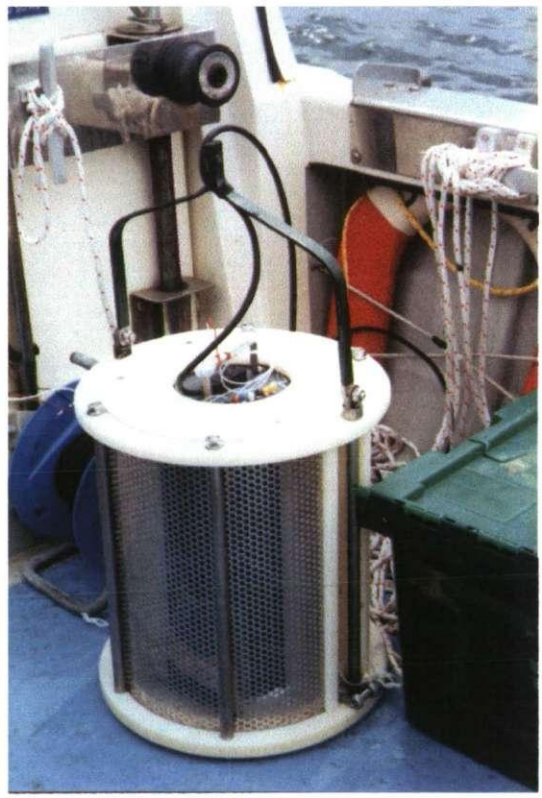


Figure 4.7 Analyser ready for deployment, on board the research vessel *John Dory*.

Figure 4.8 Daviron Instrument's research vessel *Tealia*, detail of the analyser inside the car and boxes containing samples and field instrumentation.

4.3 RESULTS AND DISCUSSION

4.3.1 Laboratory optimisation

The instrument has significant advantages, in size, weight and buoyancy reduction, when compared with the prototype (David *et al.*, 1998, 1999). A reduction in size of the analyser housing (from 270 mm diameter x 510 mm height to 200 mm x 500 mm) and weight (from 23 kg to 12.6 kg) was achieved by miniaturisation of the electronic components, removal of the internal battery pack and radical redesign of the FI manifold layout (Figure 4.9).

The complete assembly, including reagent housing and protective cage, was also smaller (from 370 mm diameter x 700 mm height to 400 mm x 515 mm) and lighter (from 56 kg, including 20 kg weight to reduce buoyancy, to 35 kg). This resulted in the complete instrument being truly portable rather than transportable, which had major logistical advantages for *in situ* monitoring. One person could directly deploy the analyser from a small research vessel without the need for a winch or an A-frame. The new design also had much lower buoyancy, due to less airspace inside

the housing, and this improved handling in submersible mode, particularly in condition of high water flows. The use of separated reagent lines for sulphanilamide and N1NED significantly reduced the baseline response that resulted from the storage of a mixed reagent. This led to improved accuracy at the lower end of the linear range and therefore enhanced the quality of data for samples from nutrient depleted waters. Results from laboratory based experiments using reagents and standards prepared in ultra pure water and the submersible FI analyser operated in stand alone mode, without the housing or protective cage, are shown in Table 4.3.

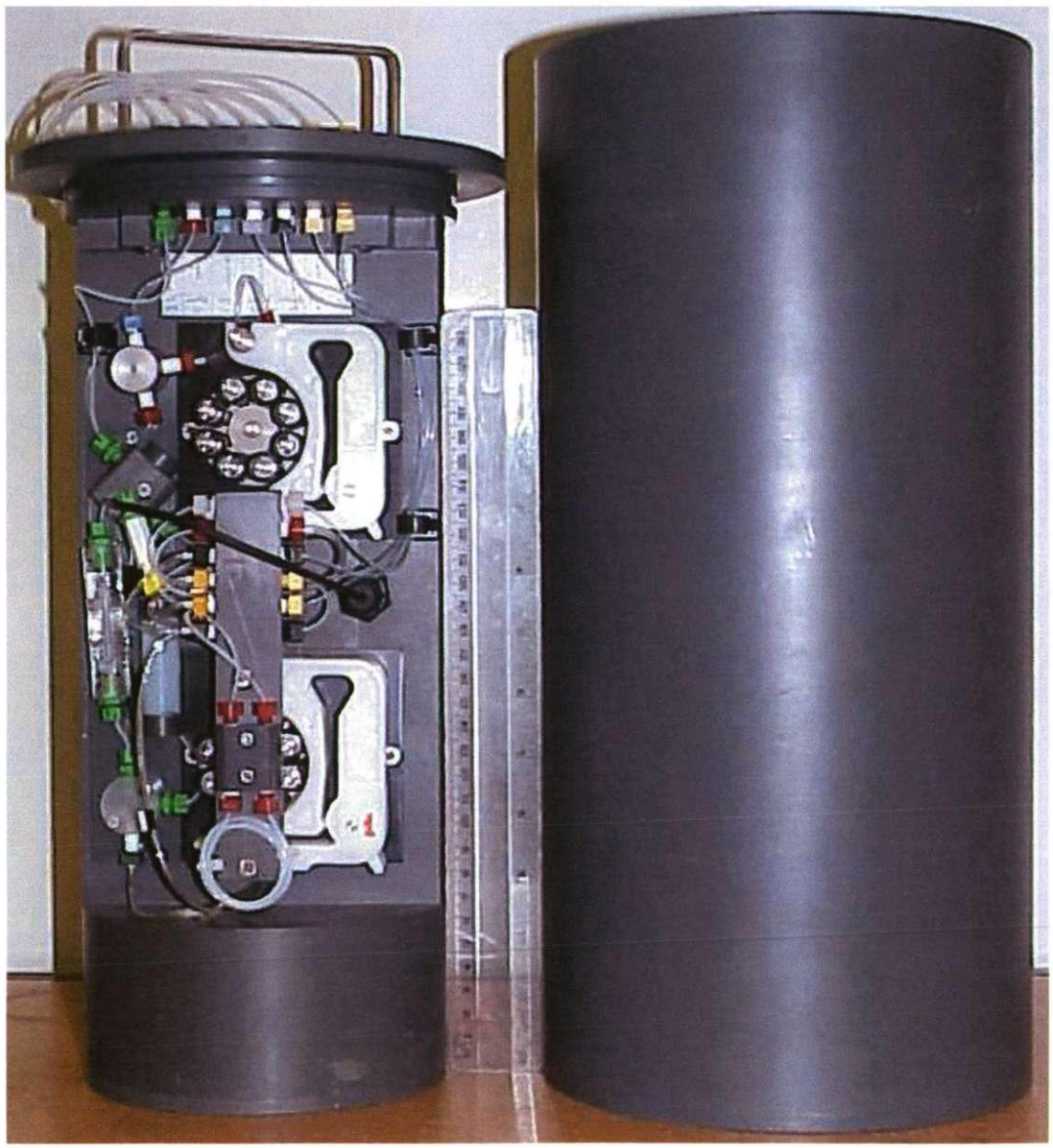


Figure 4.9 Analysers body (manifold and electronics) and housing. Detail of a 30 cm ruler in the middle.

Table 4.3 Analytical figures of merit for 20 mm and 10 mm optical path length detectors using nitrate standards in ultra pure water.

Path length	20 mm		Path length	10 mm	
Injection volume	250 µL		Injection volume	86 µL	
NO_3-N ($\mu g\ L^{-1}$)	Signal (ADU*)	RSD (n=3) (%)	NO_3-N ($\mu g\ L^{-1}$)	Signal (ADU*)	RSD (n=3) (%)
0	163.3	11	0	169.8	2.8
5	397.8	4.5	100	611.0	2.8
10	513.8	1.4	250	1118	0.40
25	766.4	3.3	500	1764	5.0
50	1365	1.9	1000	3300	4.1
100	2500	0.40	1500	4683	0.40
			2000	6394	3.2
R^2	0.9987		R^2	0.9969	
LOD**	2.8 $\mu g\ L^{-1}$		LOD**	85 $\mu g\ L^{-1}$	

* Arbitrary Digital Units. **Limit of Detection.

The layout of the FI manifold meant that the detector flow cell and the sample injection loop could be changed very quickly, which allows the instrument to be rapidly reconfigured in the field to suit local environmental conditions. Table 4.3 shows calibration data for a 20 mm path length flow cell with a 250 µL sample loop and for a 10 mm flow cell with a 86 µL loop. The former had a linear range of 2.8 - 100 $\mu g\ L^{-1}$ N and a detection limit (baseline plus three standard deviations of the baseline) of 2.8 $\mu g\ L^{-1}$ N and is the manifold of choice for deployment in nutrient depleted waters. The 10 mm flow cell had a linear range of 100 - 2000 $\mu g\ L^{-1}$ N and would be used for monitoring nutrient rich waters. It is important to note that the detection limit is much improved by using 'low in nitrite' orthophosphoric acid (BDH) rather than AnalaR grade.

The solid-state detector incorporates a commercially available green LED and a photodiode with peak response at 540nm and the signal-to-noise ratio is significantly affected by the specifications of these components. The LED originally

used was one of low power, producing only 700 mcd of luminous intensity. This was later replaced by a high-powered device with a narrow beam (6000 mcd/15° beam angle), which focused the source more efficiently through the central bore of the flowcell. In the final flow cell design, the internal bore surfaces were polished and then microscopically deburred prior to assembly, which was very effective for preventing air bubbles being trapped in the flow cell. Table 4.4 shows the improvement in baseline signal (measured over a 2 min period), the reduction in peak-to-peak background noise and the gain in sensitivity for nitrate standards in ultra pure water (laboratory experiments) or LNS (*in situ* trials) for each design modification. In addition, the alignment of the photodiode and the LED is critical for the performance of any single detector and has to be optimised when the detector is first assembled, prior to sealing in the components with epoxy resin.

4.3.2 Instrument validation

Participation in an international interlaboratory comparison study for nutrients in seawater (Willie and Clancy, 2000) using a segmented flow nutrient analyser (Skalar SAN^{plus}) and the submersible FI analyser provided independent evidence of analytical quality assurance. The study, organised by the National Research Council of Canada (NRCC) in collaboration with the National Oceanic and Atmospheric Administration (NOAA), was established to assess the capabilities of various laboratories worldwide to analyse seawater for orthophosphate, dissolved silica, nitrite and nitrate. For this research, only the nitrate data are presented.

Seawater samples were collected at a depth of 200 metres off Cape Breton Island, Canada (Lat. 47.062833 °N, Long. 59.982333 °W) using a rosette containing 22 Niskin bottles on 24 June 1996. The contents of each bottle were transferred into 50 L carboys by pumping through a 0.05 µm cartridge filter and later homogenised in a 400 L tank at the NRCC laboratories. Sub-samples (50 mL) were allocated into pre-cleaned plastic bottles, sealed and gamma irradiated (25 kGy). Participating laboratories were sent two bottles of the sub-samples (MOOS-1) and performed duplicate analysis on each bottle.

Table 4.4 Effect of LED and flow cell design on baseline noise and response for nitrate.

Detector	Deployment	LED		Flow through detector		Mean baseline signal	Peak-to-peak baseline noise	Nitrate	Mean response	RSD (n=3)
		Shape	Intensity (mcd)	Path length (mm)	Design	(ADU)	(ADU)	(μgL^{-1})	(ADU)	(%)
1	Laboratory	Spherical	700	20	Machined	2719	668	100	2073	1.1
2	Laboratory	Spherical	6000	20	Polished	4849	572	100	2347	2.0
3	Laboratory	Focused	6000	20	Microscopically deburred	9502	662	100	2342	1.9
3	*In situ*	Focused	6000	20	Microscopically deburred	10700	596	100	2216	3.1 (n=7)
1	Laboratory	Spherical	700	10	Machined	3043	663	500	2615	2.1
2	Laboratory	Spherical	6000	10	Polished	7331	690	500	3742	0.8
3	Laboratory	Focused	6000	10	Microscopically deburred	16382	1	500	3249	1.8
3	*In situ*	Focused	6000	10	Microscopically deburred	16382	1	1000	4663	4.3 (n=7)

Thirty two sets of results were received with the laboratories numbered sequentially upon receipt of the data. A tolerance level (determined by multiplying the calculated standard deviation for the pooled data by an expansion factor (between 2 and 3) was calculated to cover at least 95 % of the bottle population with 95 % probability. Z-scores were then calculated and used in the intercomparison study to assess bias as follows:

$$Z = \frac{(X_i - \overline{X})}{\sigma_{TARGET}}$$ where X_i is the individual laboratory mean, $\bar{X}$ is the accepted (or assigned) mean and σ_{TARGET} is the target value for standard deviation. The σ_{TARGET} was defined as one-half the assigned tolerance interval and calculated from the results of several expert laboratories that analysed the samples prior to the intercomparison. Figure 4.10 shows the results (each bottle separately) of the laboratory z-scoring system for nitrate. The samples analysed by Plymouth with a segmented flow analyser (SFA a, SFA b) and the submersible FI analyser (FI a, FI b) had z-scores of 1.1 and 1.5 and 1.1 and 1.7, respectively.

The assigned tolerance interval was 327.6 ± 33.6 μg L^{-1}, the consensus mean and standard deviation for nitrate were 315.0 and 43.4 μg L^{-1} respectively, and the results achieved using the segmented flow analyser were 343.0 ± 35.0 μg L^{-1} and using the submersible FI analyser were 351.4 ± 28.0 μg L^{-1} for the mean and 3 standard deviations of the mean respectively were considered satisfactory.

4.3.3 Operating modes

The monitor has the versatility to operate as a laboratory bench top instrument, on board research vessels or *in situ* (i.e. submersible) and with two different modes for control and data acquisition, i.e. automatic and manual modes. The former is designed for long term monitoring and the latter for method development and short deployments. In automatic mode, after sample or standard injection, the instrument data acquisition protocol samples the baseline signal for a set period of time and records the average value for the baseline and one value for the peak

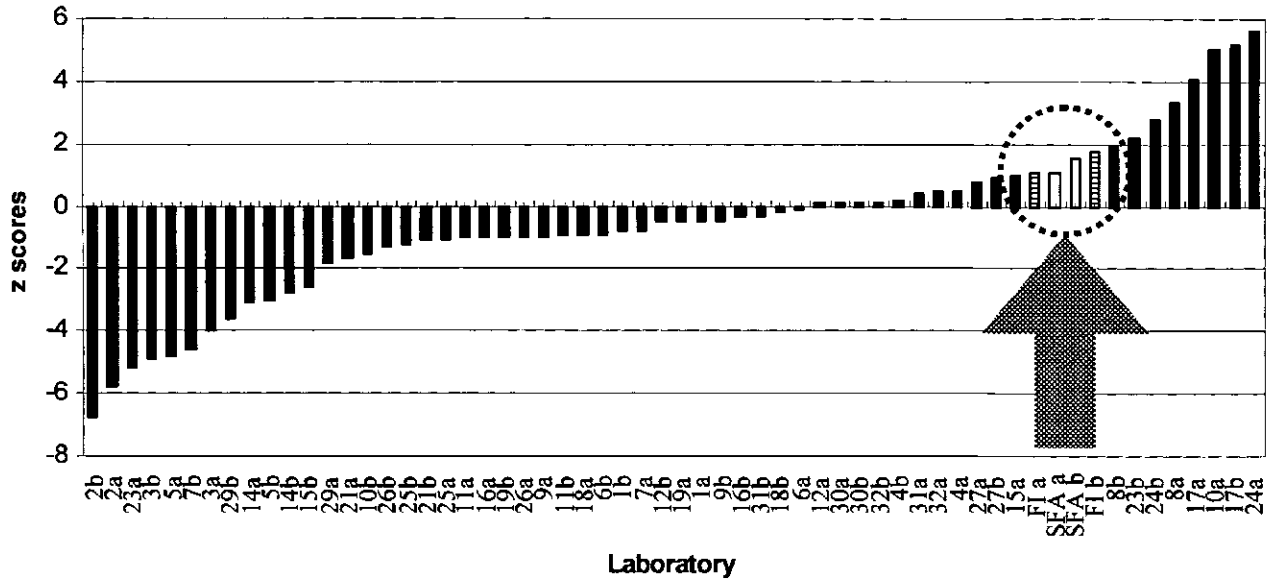


Figure 4.10 Z scores for each of the 32 laboratories participants of the intercomparison for nutrients in seawater (the arrow indicates the results obtained with the submersible FI analyser and Plymouth reference method).

maximum. The nutrient concentration, sample ID, date and time are also stored in protected memory. For long term deployments it is advisable to operate in this mode, although the disadvantage is that any unexpected variation of the baseline or transient peak maximum cannot be monitored for diagnostic purposes. However, the quality of the long-term data can be assessed by regularly analysing the on-board standard and by carrying out a post-deployment evaluation of the recorded baseline and peak data. In manual mode, the instrument acquires a large amount of raw data (in arbitrary digital units) originating from the 14 – bit analogue-digital converter (ADC) of the detector, which is memory intensive. The total acquisition of data can however be controlled by selecting the number of readings per second that the detector provides (maximum 2 s^{-1}), which is therefore a compromise between number of samples analysed and baseline quality/precision. Figure 4.11 is an example of the instruments output for a calibration run, with three repetitions of each standard (0, 25, 50, 100, 150 $\mu g\ L^{-1}$). This mode also allows the immediate diagnosis of possible

malfunctions such as leaks, air bubbles and lost of reduction column efficiency, and is therefore the mode used for all the deployments reported below.

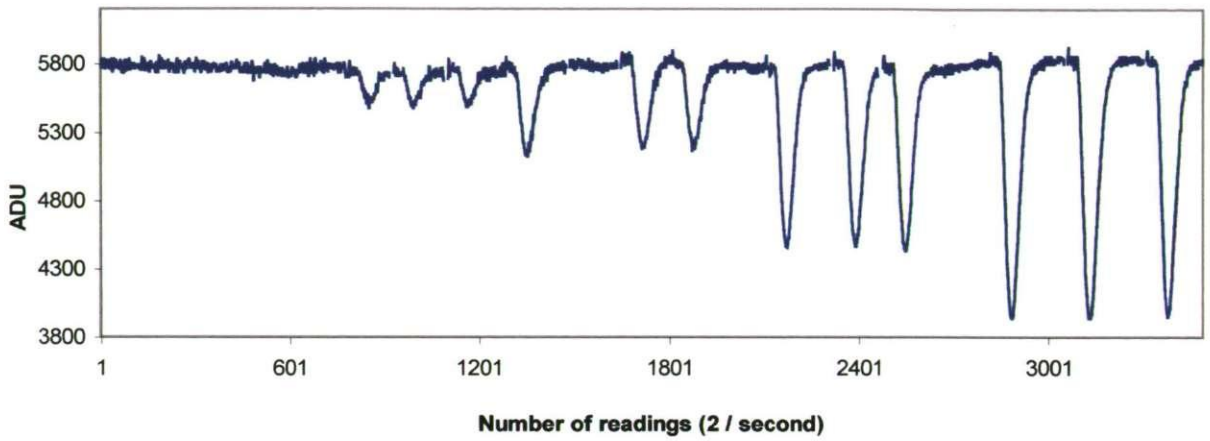


Figure 4.11. Example of submersible FI analyser output.

4.3.4 Shipboard deployment

The main scientific objectives of IMPACT Cruise I were to enhance knowledge of contaminant behaviour, including nutrients, in the North Sea and to generate information on the dissipation / diffusion, degradation and cycling of contaminants, in order to predict toxic effects. The hypothesis was to determine whether pollutants dissipating from the Humber contaminate the Dogger Bank. The cruise focused on the use of newly developed techniques for shipboard determination of trace metals, for high temporal resolution monitoring of nutrients, for the in situ pumping and solid phase extraction of organic complexes in seawater and the use of sensitive markers of toxicological response.

The on-board deployment of the submersible FI analyser involved intense preparation and laboratory work to assure that the best operational conditions were provided. All reagents were prepared 48 h before the cruise and the standards were prepared during the cruise according to the range of concentrations found in the samples.

An experiment to assess the possible use of an artificial seawater salt (*Instant Ocean,* Tropical Fishery, Plymouth, UK) as a low cost alternative to LNS in the preparation of the carrier solution was made. However the nitrate level in the artificial seawater was 20 μg L^{-1} N (compared with published data stating that it contained nil nitrate/nitrite) and this gave rise to an elevated background. Therefore LNS is recommended for the preparation of standards and carrier stream when the analyser is deployed in nutrient depleted waters.

Deployments of a 12 x 10 L 'Go-Flo' bottle rosette (CTD) (Figure 4.6), allowed the collection of water samples from a range of depths at 32 stations. All stations (except station 8) had three or four depths sampled. The associated suite of master variable data, i.e. salinity, temperature, turbidity and fluorescence were also recorded for each station. All collected water samples were pressure filtered through 37 mm diameter, 0.4 μm pore size acid washed (0.1 % HCl) polycarbonate Nuclepore® filters.

The analytical strategy designed for the cruise was daily calibrations before analysis of the samples, with analysis of one standard after every three samples (9 injections), to monitor the efficiency of the reduction column. The manual operating mode was used with maximum acquisition of data, registering two readings per second from the detector. All samples and standards were analysed in triplicate, using a FI manifold configured to determine concentrations in the linear range of 0 – 100 μg L^{-1}, with the exception of samples collected during the tidal cycle from station 8 when the linear range was 100 – 2000 μg L^{-1}. This was achieved by simply changing the 20 mm flow cell and 250 μL sample loop to a 10 mm flowcell and 86 μL sample loop combination, as discussed above.

The analytical figures of merit for the submersible FI analyser during the cruise are summarized in Table 4.5 and show good reproducibility over time. The data plotted in Figure 4.12 shows the nitrate concentration at each depth (m) for every station, where the lowest depth sampled was always above the seabed, which is represented in the graphs as the lower line of each plot. For station 8, Figure 4.13 shows the

inverse correlation between nitrate concentration and salinity over a half-tidal cycle at the mouth of the Humber Estuary (53 32.89 N 00 05.41 E) on 25/09/99. The nitrate concentrations in the depth profiles and the tidal cycle showed a significant variability with water column composition and are good examples of the effectiveness of the shipboard analyser for acquiring high resolution data.

Table 4.5 Daily shipboard calibration data during the North Sea IMPACT Cruise in September 1999.

Date	Loop size (μ L)	Path length (mm)	Gradient (ADU/μg L^{-1})	Intercept (ADU)	R^2	LOD (μg L^{-1} N)
17.09.99	250	20	12.5	60.1	0.9892	8.3
18.09.99	250	20	10.4	83.0	0.9989	4.3
19.09.99	250	20	12.9	22.0	0.9959	2.8
20.09.99	250	20	12.3	11.2	0.9946	3.9
21.09.99	250	20	12.2	39.1	0.9931	2.8
22.09.99	250	20	9.57	49.1	0.9921	3.8
23.09.99	250	20	13.9	63.3	0.9958	4.0
24.09.99	250	20	20.3	27.1	0.9885	2.8
25.09.99	125	20	6.85	847	0.9955	3.4
25.09.99	125	10	7.37	487	0.9888	85
25.09.99	86	10	3.39	2160	0.9938	76

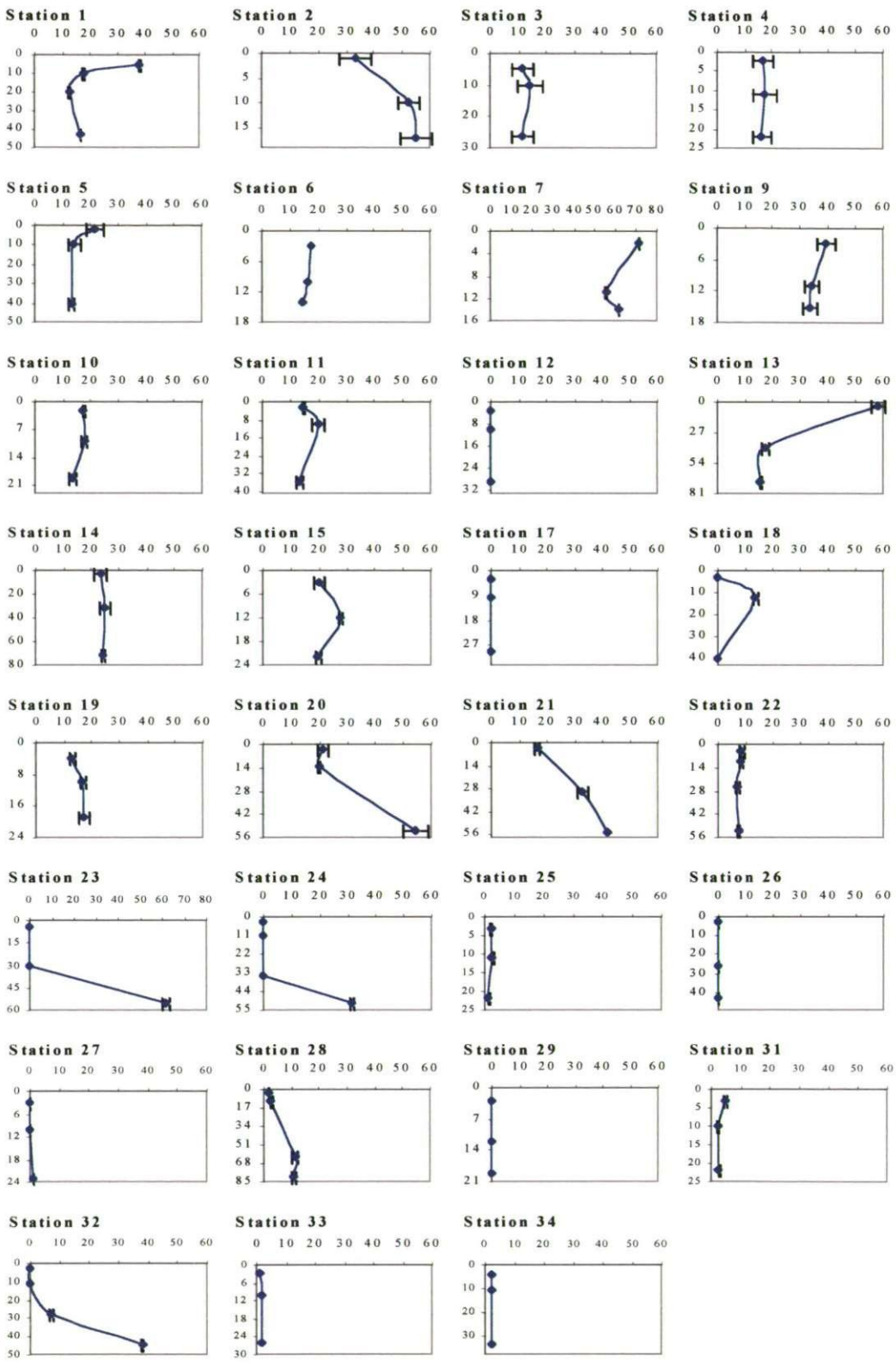


Figure 4.12 Depth profiles for nitrate in 31 North Sea stations. Depths (y axes) are in meters and concentrations (x axes) are in μg L^{-1} N. Error bars represent 3s (n=3). The bottom line of each graph represents the depth of sea bed.

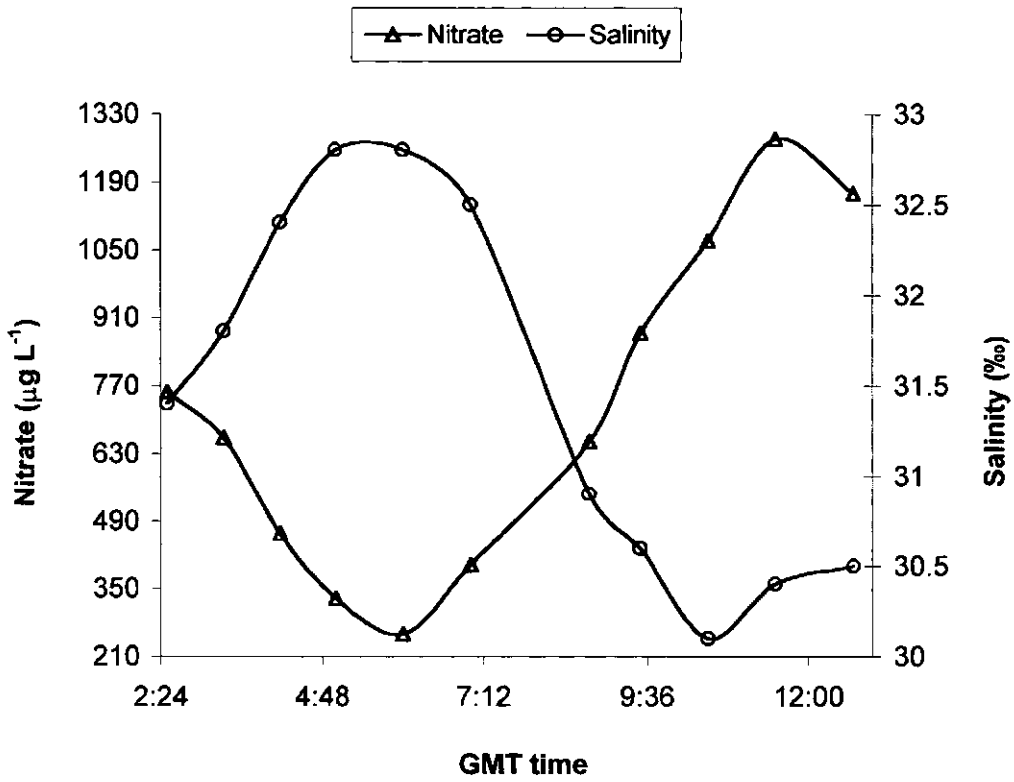


Figure 4.13 Tidal cycle at station 8 showing the inverse correlation between nitrate concentration and salinity, at the mouth of Humber estuary.

A plot of the surface distribution of nitrate in the sampled area is shown in Figures 4.14A and B (expressed as µg L^{-1}), where Figure 4.14A represents the data acquired with the submersible monitor during the IMPACT Cruise, September 1999 and Figure 4.14B the data acquired with a shipboard segmented flow analyser during the North Sea Project, September / October 1989 (Lowry *et al.,* 1992). The decrease in nitrate concentration over the last ten years is probably due to lower nutrient runoff from land into the Humber estuary. The stations with higher nitrate concentrations are all in North Sea subdivision 3b (as defined by the North Sea Task Force (NSTF, 1993; Howarth *et al.,* 1993), which is an area of limited biological productivity (uptake of nutrients) due to high levels of suspended sediment and low light conditions in the water column (Eisma and Kalf, 1987; Hydes *et al.,* 1999). Conversely, at stations in the shallow waters of the Dogger Bank (all in North Sea subdivision 7b), the nitrate concentrations were lower due to higher water clarity and lower suspended sediment, and therefore higher biological productivity.

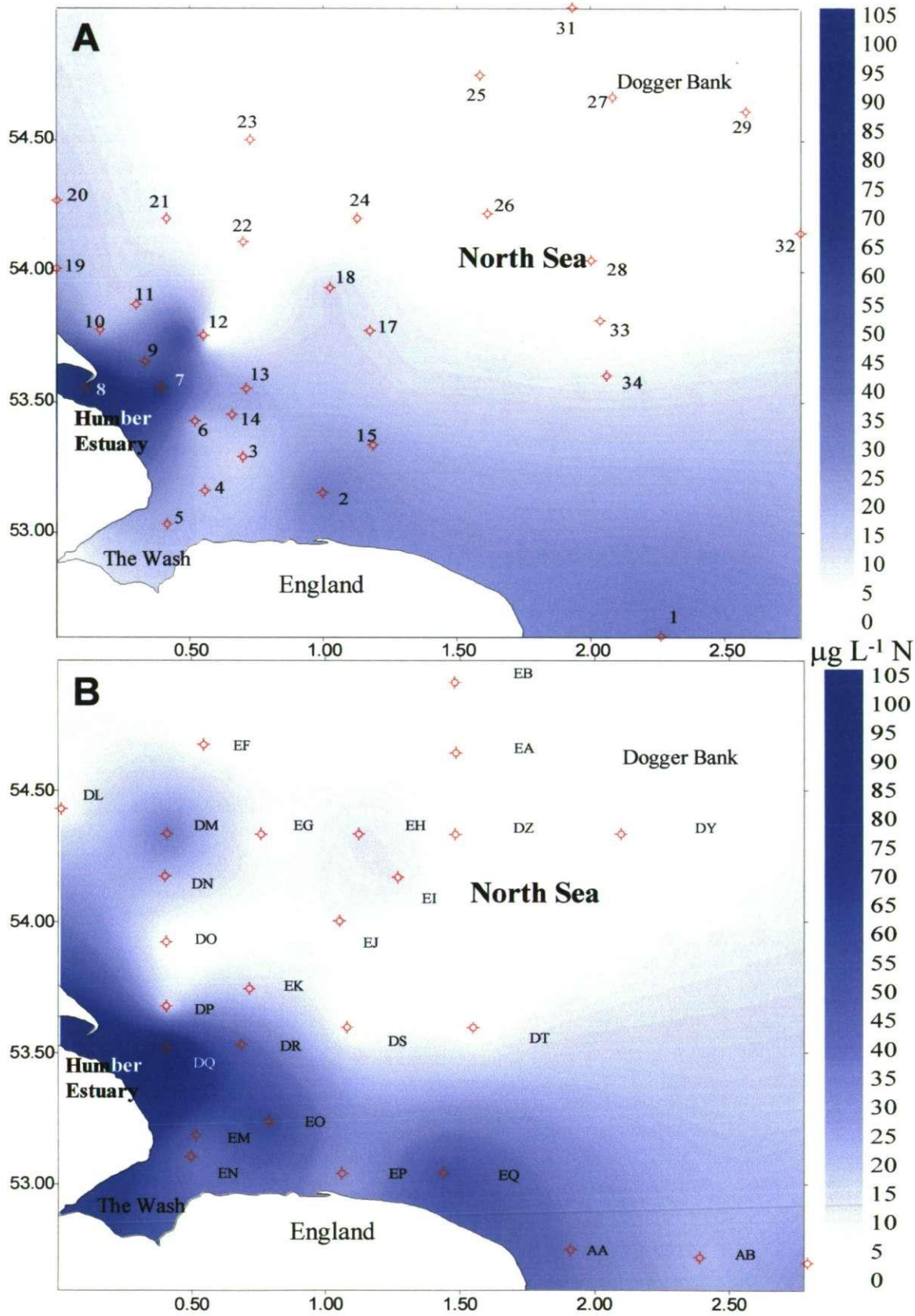


Figure 4.14 Contour plot of surface nitrate distribution in the North Sea during the IMPACT Cruise – September 1999 (A) and during the North Sea Project – Sept / Oct 1989 (B). The numbers and the letters show the locations of the sampling stations.

4.3.5 Submersible deployments

Drakes Island: The objective of this initial *in situ* deployment was to establish a protocol for deployment from small vessels and to determine parameters such as reagent and internal standard consumption and time for one analytical cycle (see Table 4.1), lifetime of the pre-filter and filter (at least 5 h) and nitrate concentration at the mouth of the Tamar Estuary. Nitrate was therefore determined every 45 min for a period of 5 h, using the instrument in manual mode, and found to be in the range 150 – 220 μg L^{-1} N. Time of analysis and concentrations measured in this deployment for each sample are listed in Table 4.6.

Table 4.6 Time, concentration of nitrate in the samples and LOD for the in situ deployment.

Time	Concentration (μg L^{-1})	RSD (%)
11:06	153.0	1.9
11:40	158.0	2.3
12:30	172.0	2.2
13:30	221.0	2.1
LOD*	80.0	

*Limit of Detection

Tamar Estuary: Calibrations for both linear ranges were run in the laboratory the night before the deployment and stored in the instrument memory. Ammonium chloride carrier and QC standards (100 and 1000 μg L^{-1} N) were prepared at two different salinities (17 and 35 ‰) using ultra pure water and LNS. The transect started at high tide at the tidal limit (salinity 0 ‰) close to Gunnislake (Morwellham Quay) and moved down river with the tide (until the Tamar Bridge). The submersible FI analyser was deployed from the side of the vessel, at 1 m depth, left for 15 min (for one analytical cycle plus one quality control standard), and then brought back on board while the vessel moved to the next station. The flow cell,

sample loop and salinity of the carrier and QC standard were changed at Weir Quay, as discussed above, to determine the lower nitrate concentrations at the seawater end of the Estuary. Concurrently with the in situ deployment, discreet samples were collected, filtered and preserved (0.1 % v/v chloroform), for later laboratory analysis using the University of Plymouth reference method (air segmented continuous flow analyser described on Chapter 3).

The comparison between *in situ* and laboratory results (Figure 4.15) shows a small discrepancy at the higher concentration range. This difference was due to the calibration of the submersible FI being made using a set of standards with a background concentration of nitrate (around 240 $\mu g\ L^{-1}$), causing an overestimation of the real concentration of the lower salinity samples. Nevertheless, from the time that the instrument was adjusted to measure higher salinity and lower nitrate concentration samples (Weir Quay), there was a good agreement with the reference method.

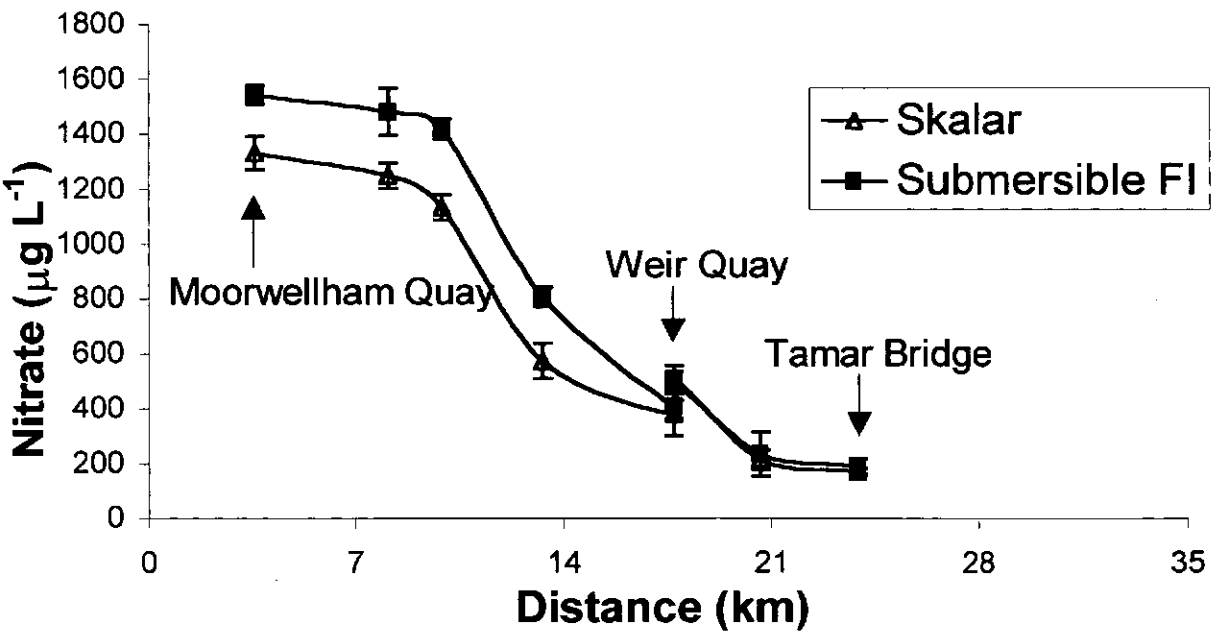


Figure 4.15 Concentration of nitrate measured in situ (Submersible FI) and in the laboratory (Skalar) in the Tamar Estuary. The distance is represented from the tidal limit at Gunnislake (0 km) to the breakwater in Plymouth Sound (34.2 km).

4.4 CONCLUSIONS

A submersible FI analyser is described for the determination of nitrate in estuarine and coastal waters. Validation of the instrument was achieved during an international interlaboratory comparison study for nutrients in seawater, and the instrument produced results in agreement with the proposed assigned tolerance interval. The analyser can be used in manual (diagnostic) or automatic (long term monitoring) mode and operate as a bench-top, shipboard or submersible instrument. The limit of detection with a 20 mm path length flow cell and a 250 μL loop was 2.8 μg L^{-1} N but the linear range can easily be changed in the field to suit local conditions by changing the flow cell path length and/or sample loop volume. The analyser has been proven to successfully operate in shipboard mode for mapping nitrate concentration in the North Sea and in submersible mode for a Tamar Estuary transect. The effects of salinity changes, throughout the estuarine mixing zone, can be overcome using salinity-matched reagents and standards prepared using appropriate dilutions of low nutrient seawater.

CHAPTER FIVE

Biogeochemistry of Nutrients in the Tamar Estuary

Main topics in this chapter:

Tamar Estuary surveys
Four axial transects
Four tidal cycles
Estuarine nutrient biogeochemistry
Correlation with master variables

On board the research vessel *Tealia*, passing Calstock

5.1 INTRODUCTION

The Tamar River catchment and the Tamar Estuary were defined and described previously in Section 2.1, and a geological description of the region is given in Section 2.2.1. The current environmental importance of both areas is reflected by all of the legislation described in Section 2.1.2. The Tamar Estuary is probably one of the most studied systems of its kind in the United Kingdom. Most notable are the works of Morris and co-workers in the late 1970's and early 1980's which provided a broad description of local estuarine biogeochemical processes (Miller, 1999).

Various mechanisms are involved in the biogeochemical behaviour of nutrients in riverine and estuarine systems. The River Tamar provides the main supply of nitrates and phosphates to the upper part of the estuary (Morris *et al.,* 1981). Significant point source inputs of nutrients can, however, be identified in axial transects throughout the estuary. These are mainly related to agricultural runoff, wastewater treatment works discharges, and some industrial processes. Previously identified (Section 2.4.2) seasonal trends in riverine nutrient inputs to the estuary can be important indicators of water quality status for the entire estuary. However, conclusions drawn for the estuarine environment based only from riverine inputs are incomplete and can give a misleading view. Several other master variables within the estuary, such as salinity or conductivity, pH, dissolved oxygen (DO), together with the main anthropogenic sources of nutrients along the estuary, also need to be considered to enable a better understanding of the biogeochemical behaviour of nutrients in the estuarine system.

This chapter presents and discusses the results from eight field campaigns in the Tamar Estuary to monitor nitrate, phosphate, pH, dissolved oxygen, conductivity and salinity. Four axial transects were performed, at different times of the year and under different tidal regimes, from the tidal limit near Gunnislake downstream to the mouth of the estuary. Also, four stationary tidal cycle monitoring campaigns were carried out at stations of contrasting salinity gradients, namely, Calstock, Weir Quay and Barn Pool. Data provided by the Environment Agency and South

West Water, regarding flow rates and concentrations of nitrate and phosphate from the outflows of the main Waste Water Treatment Works (WWTW) in the estuary, are also presented and discussed.

5.2 EXPERIMENTAL

5.2.1 Field campaigns

Four axial transects and four tidal cycles were performed during the period July 2001 - May 2002 in the Tamar Estuary, using the research vessel *Tealia* (Figure 3.8), from Daviron Instruments. Conductivity was measured using a conductivity meter (model HI9635, Hanna Instruments Ltd, Happy Valley Industrial Park, Herts). pH measurements were performed using a pH meter (model HI9025, Hanna Instruments Ltd) fitted with a Gelplas probe (BDH). The pH meter was calibrated at the beginning of each survey using buffer solutions of pH 7.0 and 4.0 (NIST), and calibrated for temperature. Temperature and dissolved oxygen concentrations were determined using a combined meter (model 55, Yellow Springs Industries [Y.S.I.] U.K. Ltd, Lynford House, Farnborough, Hampshire).

5.2.2 Sampling sites

The locations of the sampling sites for the field campaigns were based on previous studies (Morris *et al.,* 1981 and 1982; David *et al.,* 1999; Gardolinski *et al.,* 2001) and are described below:

Axial transects; eleven stations were visited during the four campaigns. The transects always started as close as possible to the tidal limit at Gunnislake, at high tide, and proceeded down the river with the ebb tide at a constant speed of 5 knots, usually stopping for 15 minutes at each site for *in situ* monitoring (nitrate, pH, dissolved oxygen, conductivity and salinity) and discrete sample collection (on-board filtration 0.45 μm cellulose acetate membrane filters) for subsequent laboratory determination of phosphate. Other details of the procedures for instrument deployment are given in Section 4.2.4 item 3.

Tidal cycles; three stations with contrasting salinity ranges were chosen for the stationary monitoring, Calstock (salinity range 0 – 8.0 ‰), Weir Quay (6.0 –

25.0 ‰) and Barn Pool (32.0 – 36.0 ‰) (see name, grid reference, distance from tidal limit and salinity ranges on Table 5.1, location on Figure 5.1). The sampling and analysis procedures were the same as described for the *axial transects*. The average duration of these surveys was 10 to 11 hours, with the first *in situ* analysis and discrete sampling being performed at high tide and then at intervals of approximately 30 minutes.

Table 5.1. Name, national grid reference, distance from tidal limit and salinity range at sampling stations.

	Station	Latitude N	Longitude W	National Grid Reference (SX)	Distance (km)	Salinity (‰)
1	Morwellham Rocks	50° 30.60	4° 12.06	43958 70181	1.2	0.0
2	Moorwellham Quay	50° 30.38	4° 11.42	44702 69751	3.6	0.0 – 4.0
3	Calstock	50° 29.66	4° 12.31	43611 68448	8.1	0.0 – 8.0
4	South Ward Farm	50° 28.85	4° 13.02	42727 66972	9.9	3.5 – 12.0
5	Halton Quay	50° 28.12	4° 13.85	41705 65649	13.3	5.0 – 20.0
6	Weir Quay	50° 27.45	4° 12.44	43335 64358	17.7	6.0 – 25.0
7	Weir Point	50° 26.06	4° 11.84	43969 61761	20.6	23.0 – 30.0
8	Tamar Bridge	50° 24.56	4° 12.19	43472 58994	23.9	27.0 – 33.0
9	Carew Point (Lynher)	50° 23.60	4° 12.25	43349 57218	25.6	30.0 – 35.0
10	St. Johns Lake (entr.)	50° 22.08	4° 12.44	43040 54408	29.1	31.0 – 35.0
11	Barn Pool	50° 21.32	4° 10.20	45653 52922	31.5	32.0 – 36.0

5.2.3 Laboratory preparation

All laboratory preparation before field campaigns followed the same protocol. Cleaning of all laboratory ware was made as described in Section 3.2. Reagents were prepared for the reference method and the submersible FI as described in Sections 3.2.5 and 4.2.1, respectively.

Calibrations for the submersible FI analyser were made the night before each field campaign according to the concentrations expected, *e.g.* for axial estuarine transect two calibrations were made, one for samples in the low salinity range (0 – 15 ‰) of 0.5 – 2.0 mg L^{-1} NO_3-N and the other for the higher salinity range (15 - 35 ‰) of 0.002 – 0.5 mg L^{-1} NO_3-N. For the stationary monitoring of tidal cycles,

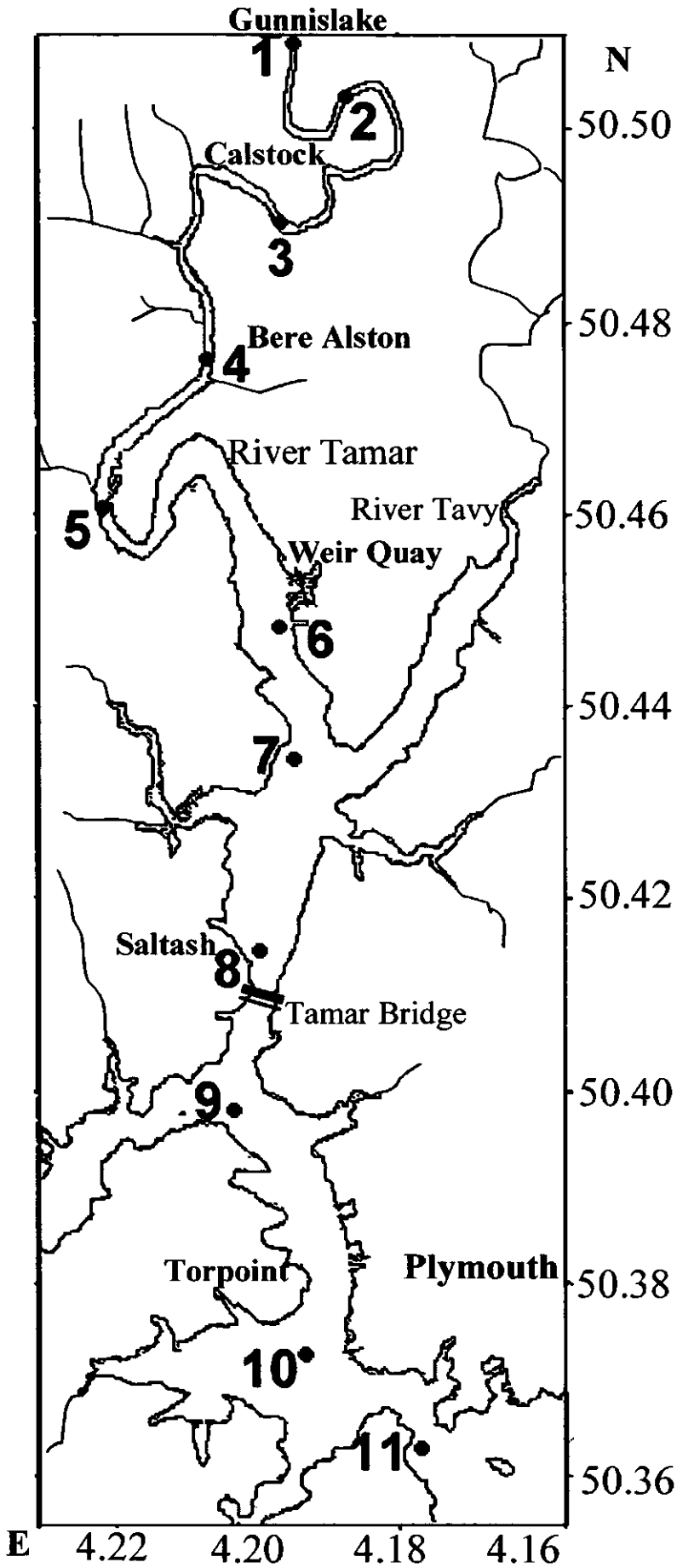


Figure 5.1 Location of the 11 stations visited during the Tamar Estuary surveys.

calibrations were made according to the maximum salinity of the site, *i.e.* for Calstock standards and carrier were prepared in ultra pure water; Weir Quay standards and carrier were prepared with appropriate volumes of ultra pure water and low nutrient seawater to achieve 17 ‰ salinity; Barn Pool standards and carrier were prepared only with low nutrient seawater of 35 ‰ salinity. Field instruments such as the pH and DO meters were calibrated in the laboratory and in the field, whereas the conductivity meter was only calibrated in the laboratory.

Calibrations for the segmented flow analyser (Section 3.2.4) for the determination of nitrate and phosphate in discrete samples collected during the field campaigns were made in two concentration ranges according to the analyte concentrations and the salinity ranges. Salinity matched standards were prepared using ultra pure water and low nutrient seawater to cover three ranges of salinity in the samples, as described in Table 5.2.

Table 5.2 Range of salinity of samples and corresponding standards used in the segmented flow analyser.

Salinity (‰)		Concentration range (mg L^{-1})	
sample	standards	NO_3-N	PO_4-P
0.0 - 12.0	0.0	0.1 – 5.0	0.0 – 0.1
12.0 - 25.0	17.0	0.1 – 5.0	0.0 – 0.1
25.0 - 36.0	35.0	0.1 – 5.0	0.0 – 0.1

5.2.4 Data analysis from the Environment Agency and South West Water

Data provided from the Environment Agency and South West Water, regarding flow rates and concentrations of nitrate and phosphate from the outflows of the main Waste Water Treatment Works (WWTW) in the estuary (Figure 5.2), were tabulated and analysed.

Consented dry weather flows (DWF) and maximum discharge rates are listed in Table 5.3. The definition of DWF is 'the average daily flow to the treatment works during seven consecutive days without rain (excluding a period which includes

public holidays) following seven days during which the rainfall did not exceed 0.25 mm on any one day' (EA/SWW). The flows presented relate to the final effluent discharges. These are flows that have received the full treatment that is available at the works (treatment processes can vary). Wet weather will produce additional (storm) flows from combined sewers (foul and surface water) from the WWTWs and from sewer overflows; these discharges are also consented (maximum discharge rate).

Table 5.3 Consented dry weather flows (DWF) and maximum discharge rates for the main wastewater treatment works (WWTW) in the Tamar estuary.

WWTW		Consented		
		DWF		Max. Discharge Rate
Site No. in Fig 5.2	Name	m^3d^{-1}	Ls^{-1}	(Ls^{-1})
1	Gunnislake	254	2.9	11.0
2	Calstock	259	3.0	7.5
3	Bere Alston	520	6.0	13.8
4	St. Mellion	41	0.5	3.0**
5	Harrow Barrow	166	1.9	11.5**
6	Metherell	87	1.0	6.0
7	St. Dominic	118	1.4	8.3
8	Cargreen	100	1.2	7.2**
9	Ernesettle	17950	208	624*

* = Consent does not state a maximum discharge rate therefore estimate as 3 x DWF.

** = Consent does not state a maximum discharge rate therefore estimate as 6 x DWF due to high diurnal variation.

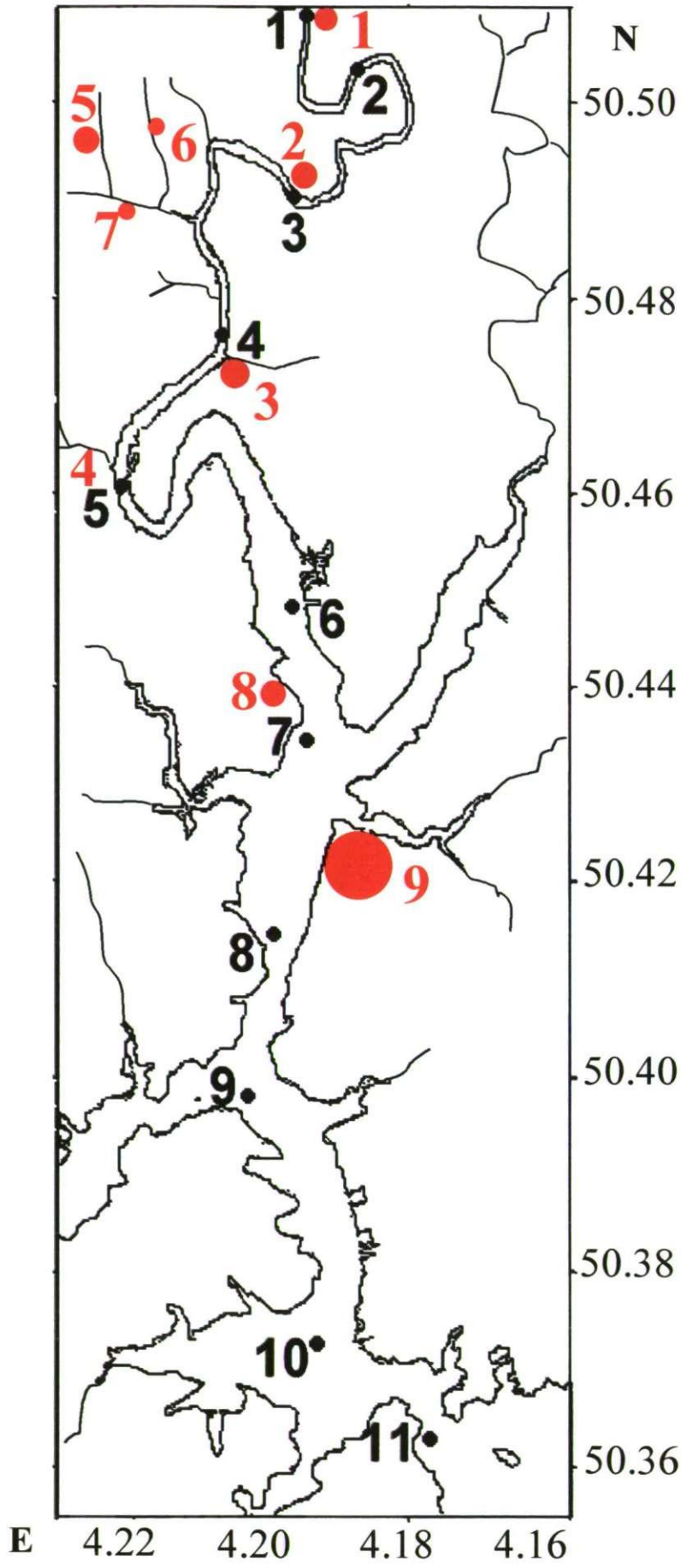


Figure 5.2 Location of wastewater treatment works (red circles) and sampling stations (black circles) in the Tamar Estuary. The size of red circles is proportional to maximum discharge rate as described in Table 5.3.

5.3 RESULTS AND DISCUSSION

Survey details including date, type of survey, river discharge, tidal condition and time of high tide are summarised in Table 5.4. In this section all references to time are British Summer Time (BST). Also, all nitrate and phosphate concentrations correspond to the element (mg L^{-1} NO_3-N or PO_4-P). In all the transect plots Distance (km) is referering to the distance from the tidal limit Gunnislake.

Table 5.4 Date, type of survey, river discharge, tidal condition and time of all eight surveys.

Date	Type of survey	River discharge (m^3s^{-1})	Tidal conditions (days relative to peak spring (S) or neap (N) tide)	Time of high tide (Barn Pool)
06/08/2001	Transect	3.6	S	07:56
24/09/2001	Transect	2.0	N – 3	10:56
05/10/2001	Transect	4.3	S + 2	08:15
10/11/2001	Tidal cycle	14.2	N	13:00
22/11/2001	Tidal cycle	8.3	N – 3	09:35
06/12/2001	Tidal cycle	45.0	N – 3	09:15
16/04/2002	Tidal cycle	7.3	S + 4	08:46
18/04/2002	Transect	7.7	N – 3	09:45

5.3.1 Axial transects

Transect 1 (06/08/2001)

The transect started at Morwellham Quay at 8:15 h (high tide in Calstock at 8:21 h) and, following the description in Section 5.2.2, progressed down the estuary, but only as far as the Tamar Bridge due to strong winds closer to the mouth of the estuary. Nevertheless, all the first seven stations were visited and monitored. Figure 5.3 shows data for all of the *in situ* and laboratory measured variables.

It is clear from Figure 5.3a the similar trend between salinity, conductivity, pH and distance from the head of the estuary, which one would

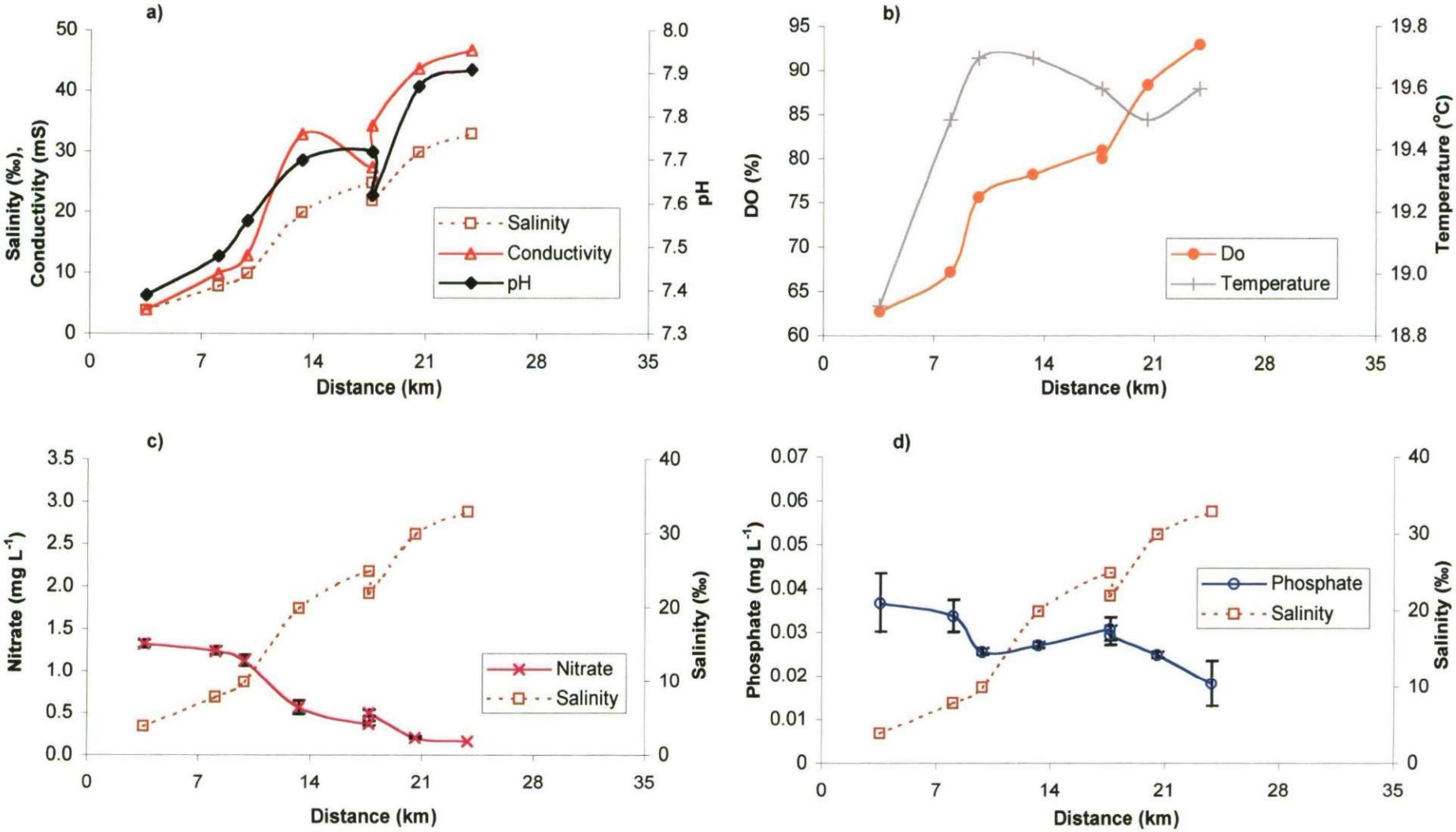


Figure 5.3 Behaviour of all the *in situ* and laboratory measured variables during the Tamar Estuary Axial Transect 1 (06/08/2001).

expect from conservative mixing of fresh with saline waters. Estuarine field studies have predominantly utilized variable-salinity correlations, with the assumption that salinity is conservative (Morris *et al.*, 1982a). Fluctuations from linearity within a particular estuarine segment indicate chemical interactions involving the variables (Boyle *et al.*, 1974). The slight decrease in all variables at Weir Quay (17.7 km) between two measurements represents the time the vessel remained static (about 45 minutes for changes in the manifold of the submersible FI system), coincidently at the time of the maximum ebb of a spring tide. The 45 minutes difference between measurements was enough to show a proportional increase in the concentration of nitrate with increase of riverine water (Figure 5.3c), which is indicative of conservative behaviour and inversely proportional to the salinity behaviour within the estuary. Also noticeable is the increase in dissolved oxygen concentration with seawater inlet (Figure 5.3b). The non-conservative temperature behaviour in the upper - middle estuary (8.1 – 17.7 km) could be attributable to differential heating or cooling where comparatively shallow waters moved tidally over extensive, temporarily exposed mudflats (Morris *et al.*, 1982a). Phosphate (Figure 5.3d) showed a conservative trend although its behaviour is much more influenced by factors such as resuspension, adsorption / desorption, precipitation and anthropogenic inputs throughout the estuary, when compared with nitrate behaviour. These influences on phosphate behaviour are much more pronounced in the turbidity maximum zone (8.1 – 13.3 km)(Mommaerts, 1969 and 1970; Morris *et al.*, 1981 and 1982a; Morris, 1985).

Transect 2 (24/09/2001)

This transect started as far upstream as it was possible to navigate in the upper Tamar, at Moorwell Rocks (1.2 km from tidal limit). As the river got narrower and shallower the river bed also got extremely irregular, being subjected to unpredicted changes in depth and location of sand banks. In this part of the river bathymetric readings from the vessel's instrument were rarely in agreement with the information printed on a recent chart for the area (Imray, 2001). Therefore, further advance towards the tidal limit was not attempted for safety reasons.

The start time at the first station was 11:40 h (high tide at 10:57 h in Calstock) and the survey was performed in the same way as described above, except for the changes in the submersible FI manifold, which were made at South Ward Farm (9.9 km from the tidal limit). As happened in the previous transect, the vessel remained stationary for approximately 45 minutes and the *in situ* difference between measurements was enough to show an increase in the concentration of nitrate with an increase of riverine water flow (Figure 5.4c). Figure 5.4a shows a gradual increase of salinity along the estuary, probably due to the proximity of neap tides (N – 3), which could result in a less intense tidal intrusion, therefore promoting more mixing between riverine and estuarine water in the upper part of the estuary. A stepwise increase in pH was noticed throughout the transect, correlating with the increase in salinity, but not in a regular manner. This has been previously reported (Morris *et al.*, 1982a) and could be associated with the late-summer low river flow conditions (only 2.0 m^3s^{-1}), producing patterns of higher fresh water pH than normally encountered. Phosphate showed a relatively stable distribution all the way through the transect (Figure 5.4d), with the exception of both extreme stations. It is probable that the higher concentration at Moorwell Rocks (1.1 km) was due to the very low flow conditions of the river and also the holiday season (as discussed in Section 2.2.1), when phosphate inputs are significantly higher in the upper part of the river catchment. The constant phosphate values (around 0.04 mg L^{-1}) showed an unusual distribution for estuarine waters, also reported by Mommaerts (1970) and Morris *et al.* (1981), although neither author could attribute the fact to one specific reason. Lau and Chu (1999) observed in the laboratory (15 °C) a significant phosphate release from estuarine suspended sediments when salinity was increased from 5 to 10 ‰ but no further increase with further salinity increase to 25 ‰. Another possibility for that increase could be originated from high phosphate values from the outputs of WWTW, situated in the middle estuary (Figure 5.2). Morris *et al.* (1981) verified that the late summer values for phosphate in the estuary were significantly higher when compared with other periods of the year. Riverine temperatures were less conservative than the seawater input, which remained warmer in the late summer (Figure 5.4b).

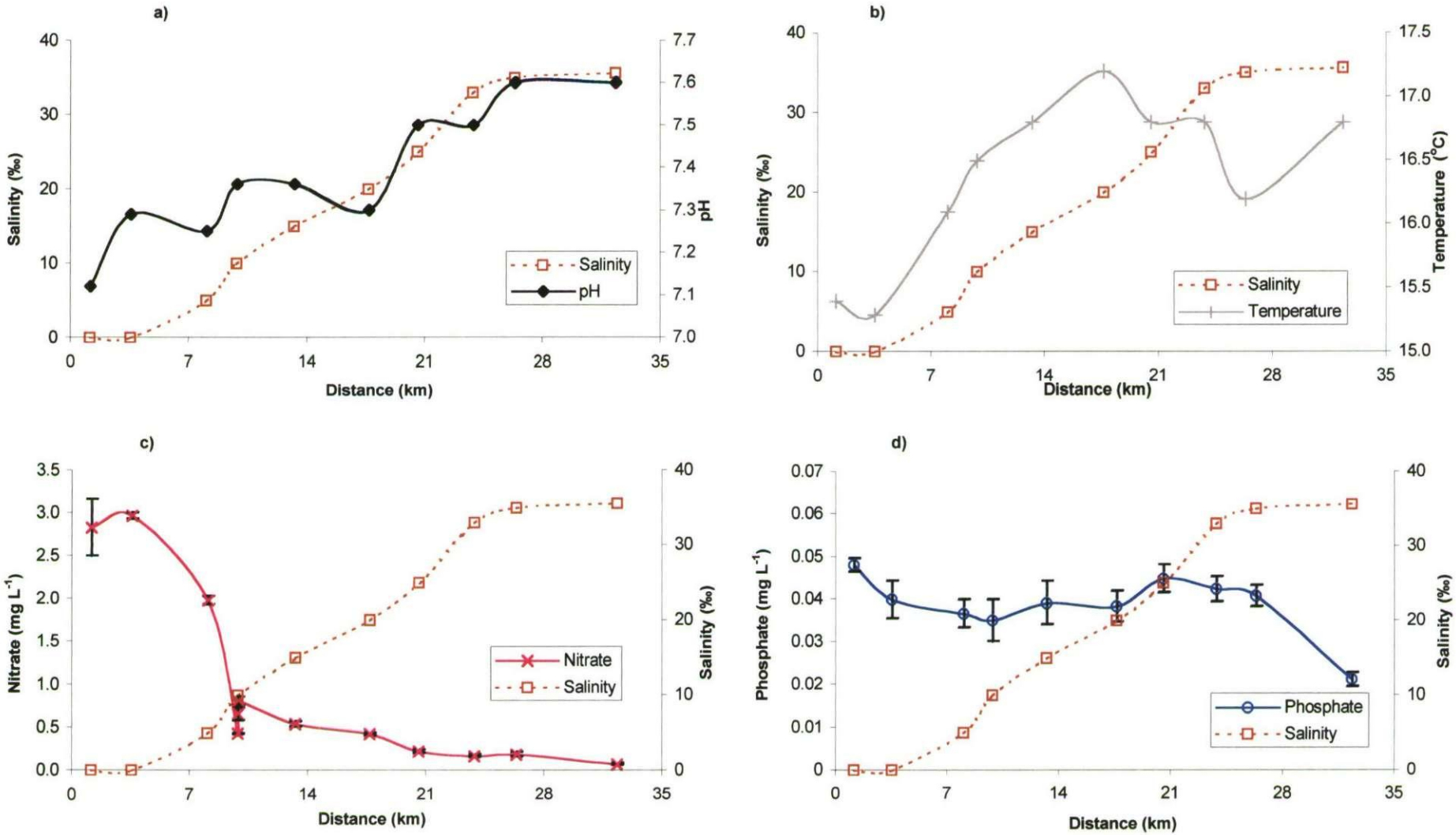


Figure 5.4 Behaviour of all the *in situ* and laboratory measured variables during the Tamar Estuary Axial Transect 2 (24/09/2001).

Transect 3 (05/10/2001)

The survey started at Morwellham Quay at 8:40 h (high tide at 8:40 h in Calstock). Extremely severe weather conditions were faced in the middle of the estuary (Weir Quay). Gale force winds blowing in the opposite direction to the maximum ebb flow during spring tide conditions created waves of 0.75 to 1.00 m, and continuation of survey was thought to be unsafe. At this point the skipper decided to find a sheltered spot by the Tamar Bridge, anchor the vessel and wait until the tide changed direction and improved conditions for navigation. For these reasons monitoring of the station at Weir Point (20.6 km from tidal limit) and the stations downstream of the Tamar Bridge were not performed. Nevertheless, all other stations were effectively monitored. During the wait under the Tamar Bridge two *in situ* samples were analysed for nitrate using the submersible FI monitor, even under those appalling weather conditions.

Figure 5.5a shows a steady increase in salinity and conductivity values with the increase in distance from the tidal limit, demonstrating a regular mixing process. An atypical behaviour for pH is noted in this plot when compared with the 'normal' behaviour exhibited by the other variables, raising suspicions about the reliability of the instrument and data acquired. The seawater intrusion up the estuary contributed to slightly higher temperature values in the mixing zone (Figure 5.5b). Dissolved oxygen levels increased dramatically with salinity. The highest value was recorded at Weir Quay where the waves were highest. Nitrate once again demonstrated a conservative distribution throughout the estuary and an inverse correlation with salinity (Figure 5.5c), with a slight increase in concentration when the vessel remained anchored over a period of 90 minutes. Phosphate concentrations through the estuary (Figure 5.5d) were probably affected by the intensity of the spring tide, washing out the river margins and resuspending sediments that could retain adsorbed phosphate, which would be released into solution with the increase in ionic strength. This effect was more pronounced at Calstock and South Ward Farm stations, areas of very intense agricultural activity. The lowest phosphate concentration in the transect (Halton Quay) could be attributed to an error during the sampling method, *i.e.* as the boat

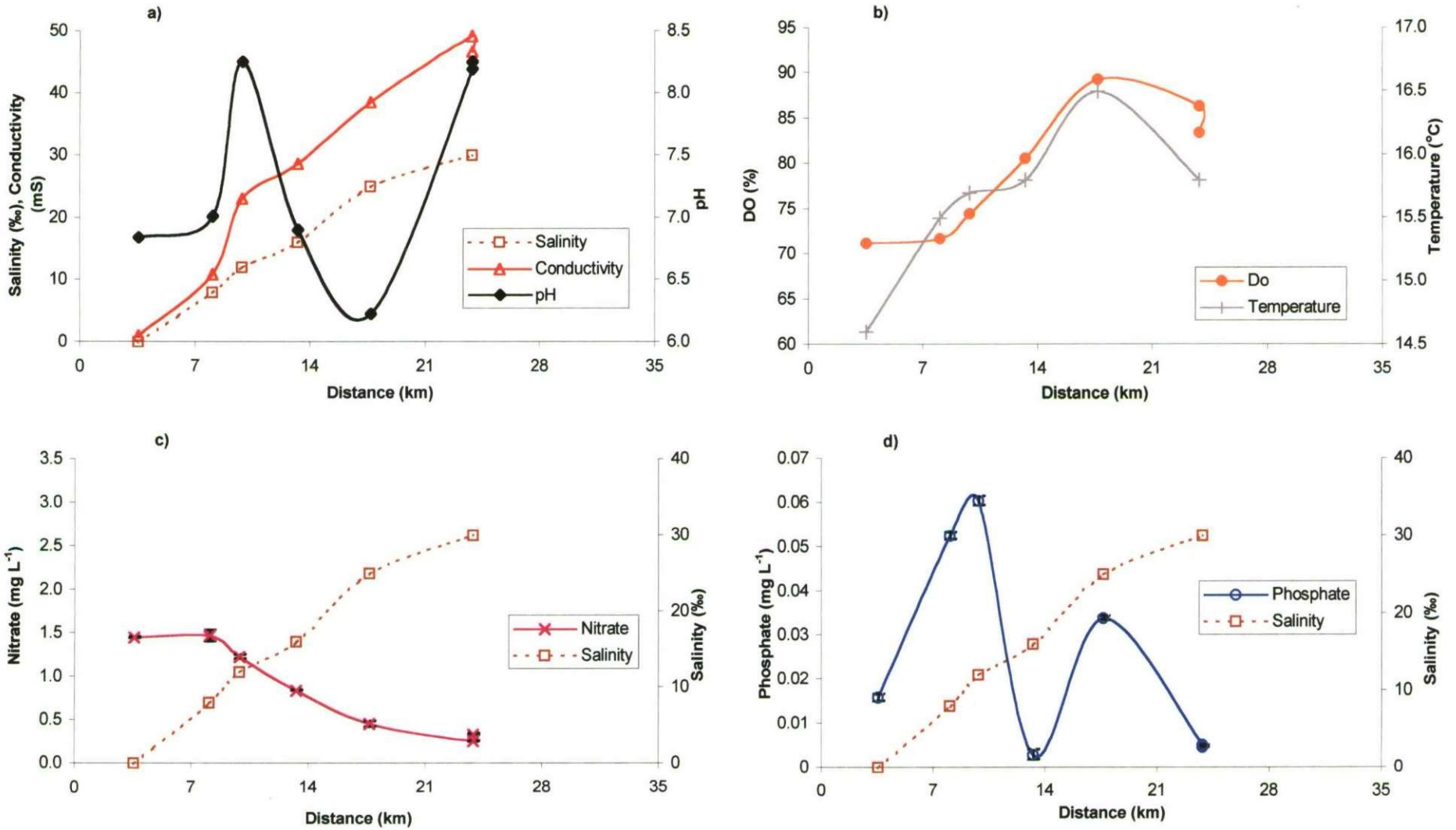


Figure 5.5 Behaviour of all the *in situ* and laboratory measured variables during the Tamar Estuary Axial Transect 3 (05/10/2001).

was in movement across a section of very turbulent waters, the sample was taken closer to the surface, therefore resulting in a sample with less suspended solids. Unfortunately, quantitative measurements of suspended solids were not performed during the surveys, however visual observation of the filter indicated a significant reduction in the amount of particulate matter retained.

Transect 4 (18/04/2002)

This survey started at Moorwellham Quay at 10:30 h (high tide at 10:10 h in Calstock) and successfully travelled down the estuary to Barn Pool station. As the submersible FI monitor was not in use during this survey, there was no need to stop at each station for *in situ* measurements of nitrate, a process that would normally take 20 to 30 minutes. Consequently, discrete samples were collected and master variables were measured with the vessel constantly moving at 5 knots.

Figure 5.6a shows a similar behaviour for salinity and conductivity values to the previous transects, except for the fact that values for both variables started to increase further down the estuary. In other words, the vessel was moving down the estuary with, and at the same speed, as the freshwater / seawater interface (FSI). More evidence of this can be found from the behaviour of other variables. For example, pH values fluctuated greatly in relation to salinity, reaching values close to freshwater levels, while in the turbidity maximum zone (approximately between 8 and 20 km from tidal limit). Further evidence is the dissolved oxygen depletion that normally takes place across the FSI. This behaviour is clearly noticeable in Figure 5.6b, and is due to freshwater phytoplankton being unable to withstand the osmotic stress and their degradation leads to the release of dissolved organic carbon which is degraded by oxygen-utilising bacteria (Morris, *et al.* 1978; Millward, 1995), a process also described for the Weser estuary (Zwolsmann and van Eck, 1993). Temperature values remained constant all through the transect. Once again, nitrate (Figure 5.6c) demonstrated a inverse correlation with salinity. Phosphate values across the estuary (Figure 5.6d) showed a gradual increase with increasing salinity up to nearly 10 ‰, and possibly within the TMZ, then a gradual decrease in concentration with

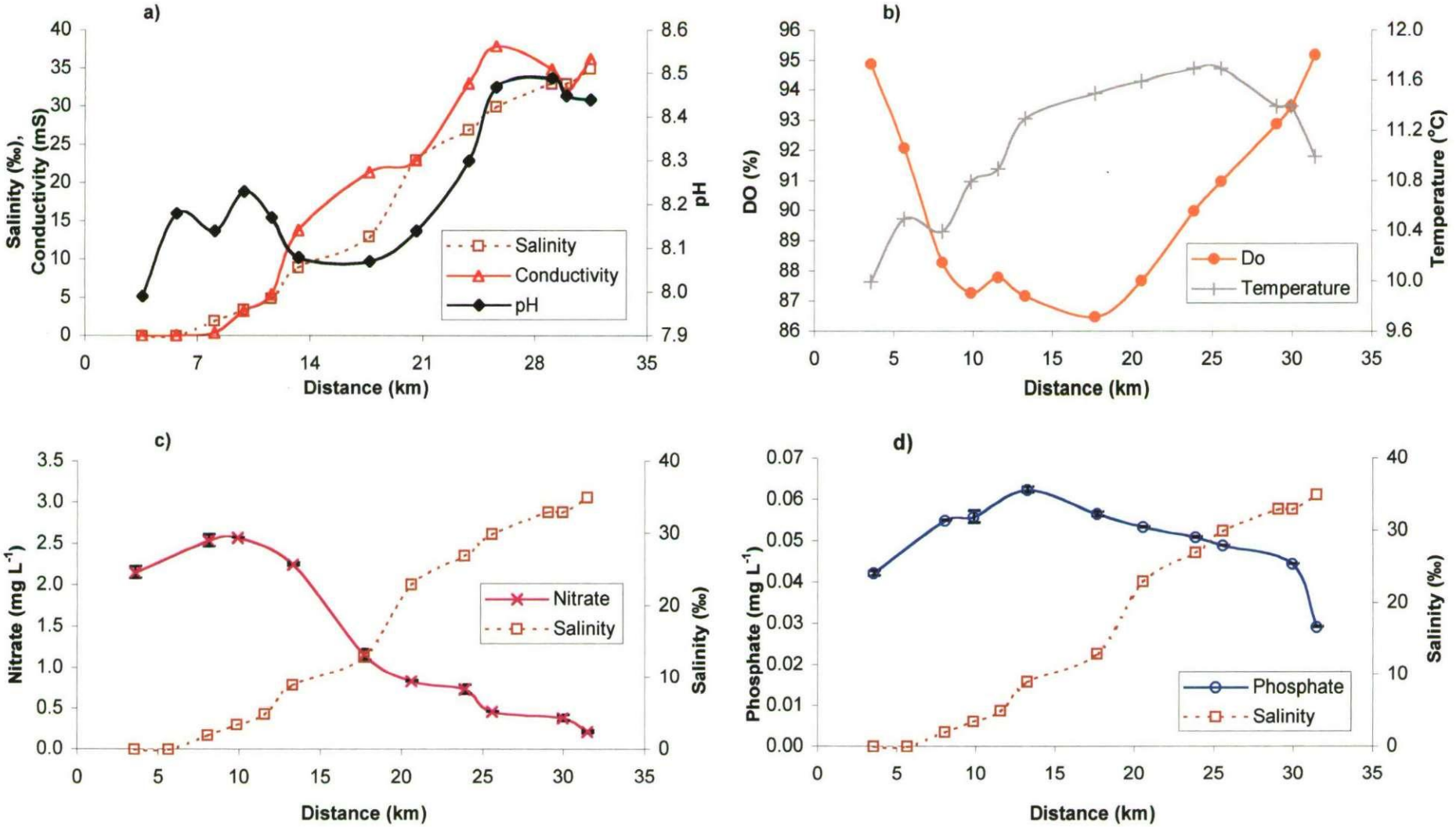


Figure 5.6 Behaviour of all the *in situ* and laboratory measured variables during the Tamar Estuary Axial Transect 4 (18/04/2002).

distance downstream from the tidal limit. The initial increase in phosphate concentration with salinity raised questions about the mechanisms of phosphorus release and the physico-chemical form and resulted in the specific investigation described in Chapter 6.

5.3.2 Tidal cycles

Tidal cycle 1 (10/11/2001)

This survey was performed at Weir Quay station (Figure 5.1) and started at 13:20 h (high tide at 13:00 h and low tide at 19:38 h in Barn Pool).

Figure 5.7a shows a slow decrease of salinity and conductivity during the tide outflow until the maximum ebb flow reached the station (between 16:30 and 17:00 h), then the recorded values decreased extremely quickly. A similar trend was noted for pH. The contribution of more freshwater from the upper estuary than seawater was evident with all the variables including temperature, values for which also dropped and remained lower during low tide (Figure 5.7b). Nitrate behaviour over time (Figure 5.7c) demonstrated a very strong inverse relation with salinity and was not affected, unlike the other variables (DO, temperature, phosphate), by different water masses. For the entire duration of the tidal cycle dissolved oxygen values remained stable apart from one measurement at 20:20 h, which was slightly lower. At the same time (20:20 h), variation in temperature, salinity and pH also occurred. From the observation of phosphate behaviour (Figure 5.7d) also at 20:20 h, a large increase in concentration (0.01 mg L^{-1} PO_4-P) was also observed. A possible explanation for the simultaneous changes in all these variables could be that a mass of water from the upper estuary, which received discharge from the WWTW at Bere Alston (Figure 5.2) during high tide, passed the sampling station. This water mass was visually identified *in situ*, when debris and scum were observed in the surface layer of the water. This type of event was previously reported (Morris *et al.,* 1982a) as a regular occurrence, particularly during neap tides and at a similar estuarine location.

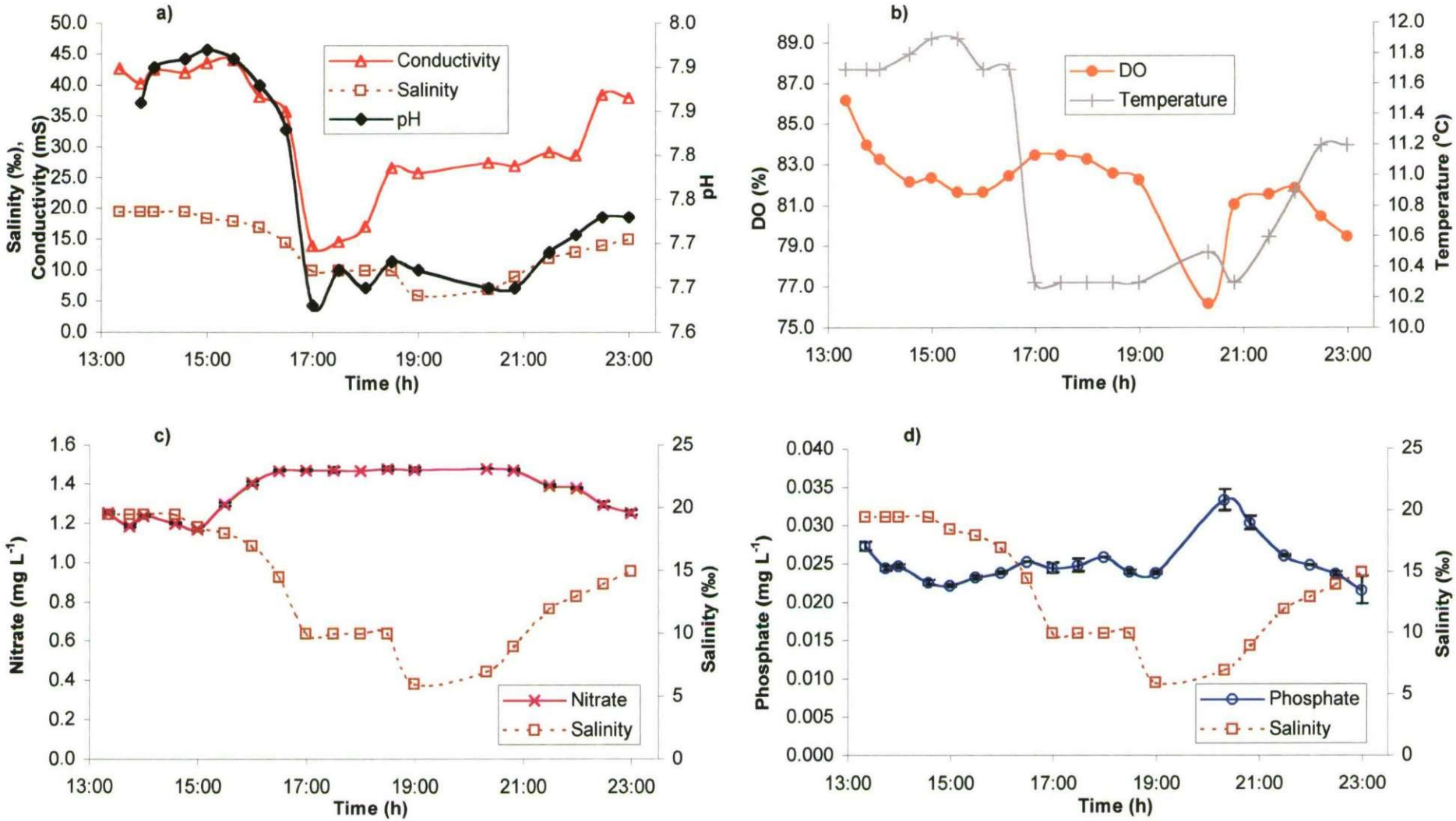


Figure 5.7 Behaviour of all the *in situ* and laboratory measured variables during the Tidal Cycle 1 at Weir Quay (10/11/2001).

Tidal cycle 2 (22/11/2001)

This tidal cycle was carried out at Barn Pool station (Figure 5.2), started at 10:00 h, just after high tide (09:35 h) and went through low tide conditions at 15:39 h.

Figure 5.8a,b shows that after the sample of 12:00 h (maximum ebb flow) significant changes were noted for most of the variables. Salinity, conductivity, temperature and DO exhibited slight decreases, but nitrate and phosphate increased in concentration, with higher values at low tide (Figure 5.8c,d). Both nutrient concentrations started a slow decrease at approximately the same time, *i.e.* the beginning of the high tide push (around 18:00 h), followed by a more intense decrease, nearly reaching the high tide values again. Also with the push of the high tide, salinity and conductivity values started a slow increase, which was not followed by temperature, values possibly due to a small diurnal temperature difference.

Tidal cycle 3 (06/12/2001)

This survey was performed at Calstock station, started at 09:45 h (high tide at 9:40 h at the site) and went through low tide conditions at 15:00 pm.

Figure 5.9a shows clearly that there was no salinity contribution during this tidal cycle, firstly due to the very strong river flow conditions (45.0 m^3L^{-1}) and secondly, due to the proximity of neap tide conditions (N – 3). These conditions suggest that only freshwater was retained in this sector of the estuary. During the first 3 hours of monitoring no pH values were obtained due to instrument failures, measurements were made only after the entire instrument was replaced. For pH, there was an indication that higher values were observed until the river flow was at its maximum speed (15:00 h) and then reduced to normal pH freshwater values. Conductivity (Figure 5.9a) increased slightly with the high river flow, although was still in the μS range. Considering that the South West has a rain composition with a strong seawater influence (370, 358, 92 μeq L^{-1} of Cl^-, Na^+ and Mg^{2+},

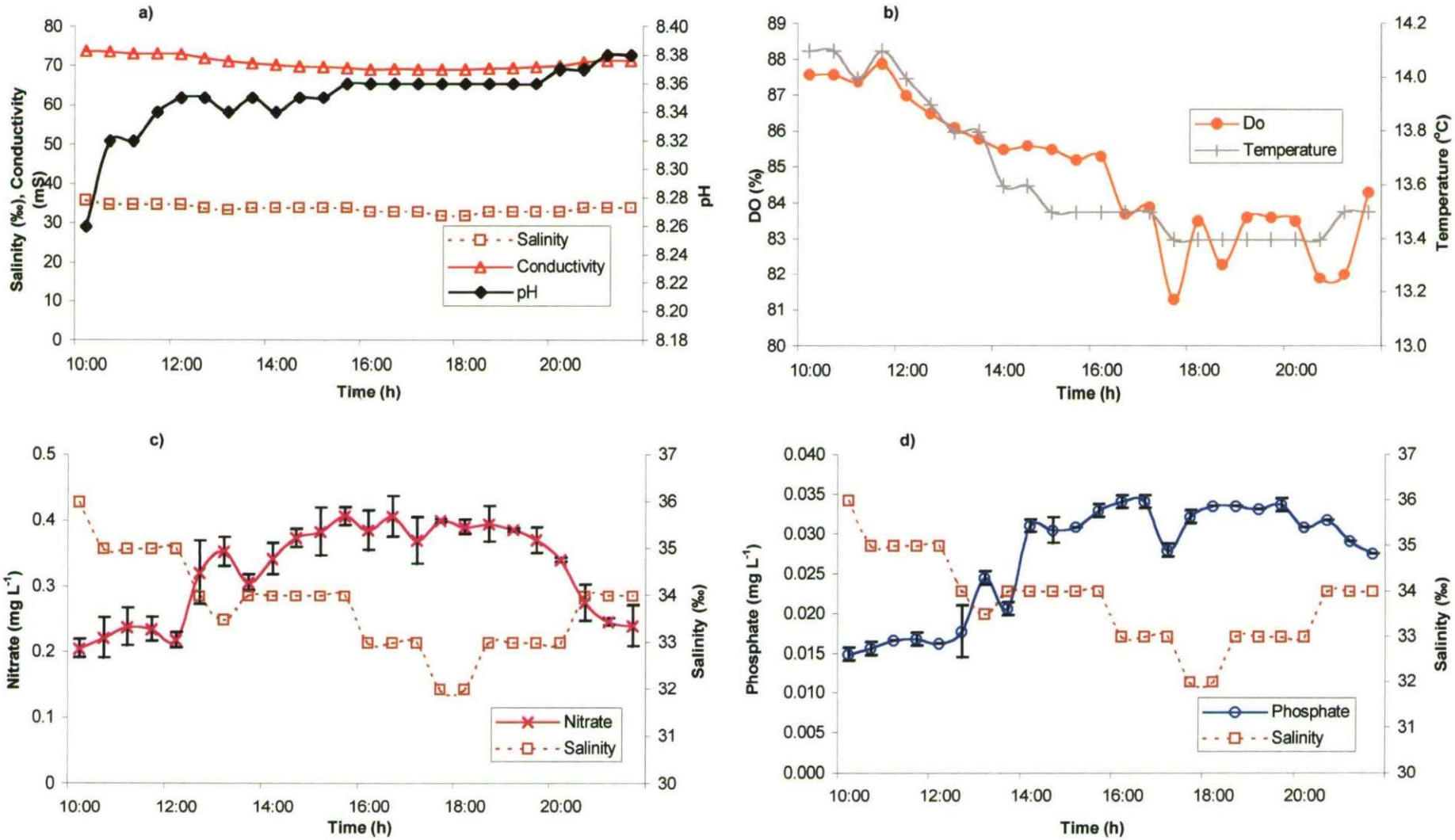


Figure 5.8 Behaviour of all the *in situ* and laboratory measured variables during the Tidal Cycle 2 at Barn Pool (22/11/2001).

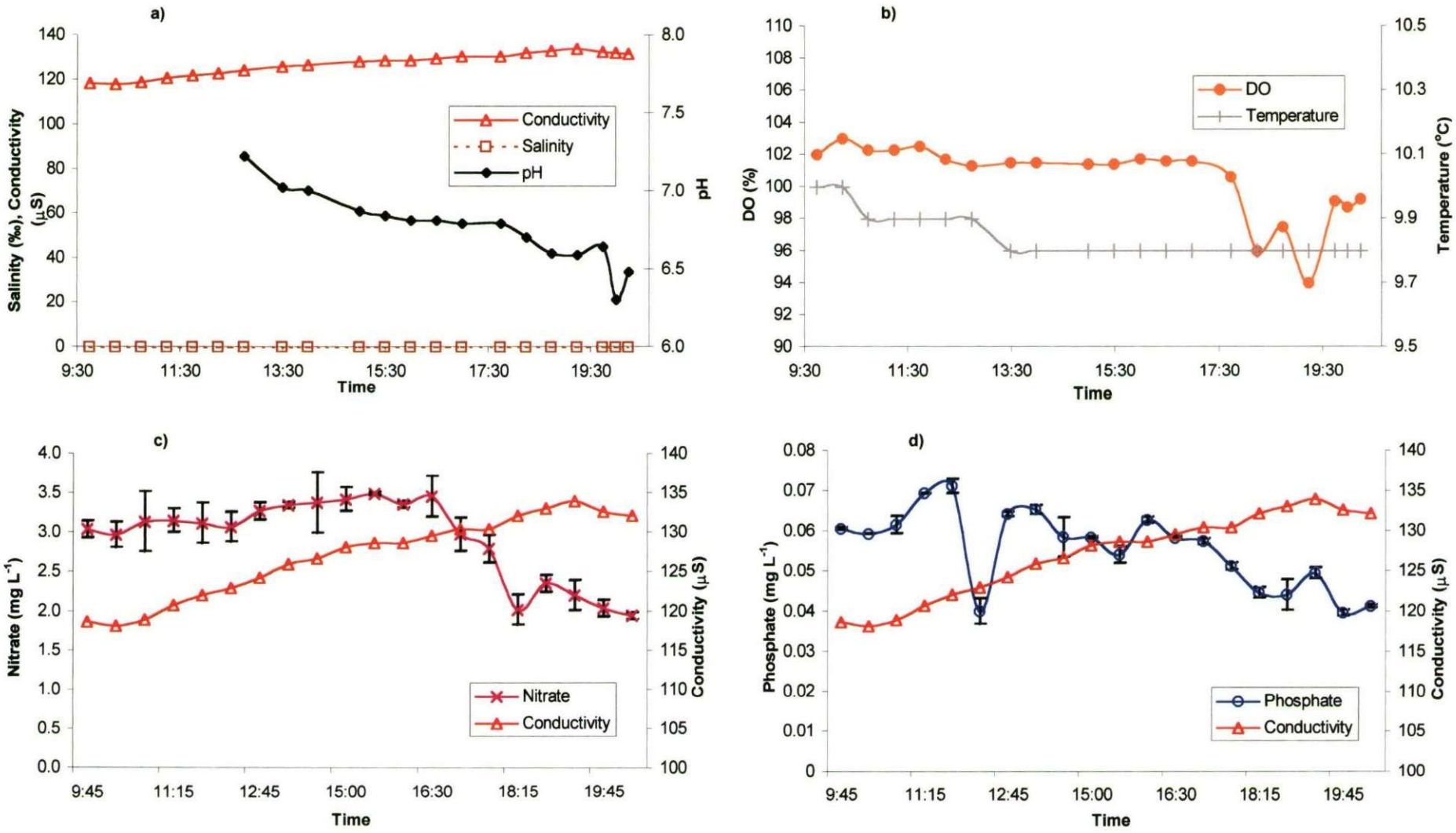


Figure 5.9 Behaviour of all the *in situ* and laboratory measured variables during the Tidal Cycle 3 at Calstock (06/12/2001).

respectively)(Coles, 1999), and the heavy rainfall in the previous week (daily average of 8.3 mmd^{-1} in Kelly station) could have input a large amount of ions and as a result, slightly increased freshwater conductivity. Dissolved oxygen values were close to saturation, unusual for this sector of the estuary, but probably the high flow rate of the river created turbulence that oxygenated the water. Nitrate values remained reasonably constant (Figure 5.9c) until the measurement at 16:30 h, after which nitrate values started to decrease, reaching a stable average value at 18:15 h coincident with the time of maximum push of the tide at this site. It is probable that the tide forces holding the strong river flow increased the mixing effect of the water masses, allowing nitrate concentrations to remain constant. A similar trend was observed for phosphate, except that a slow decrease in concentration started during the maximum ebb flow (Figure 5.9d).

Tidal cycle 4 (16/04/2002)

This tidal cycle was performed at Barn Pool station, started at 10:45 h (high tide at 9:45 h at the site) and experienced low tide at 15:28 h.

Figure 5.10a shows the similar behaviour between salinity and conductivity. The higher values started to decrease during the maximum ebb flow (at 12:30 h), then reaching values typical of the lower end of the estuary. The initial push of the tide, at 17:00 h, made values of salinity and conductivity increase nearly as fast as during the ebb flow. Dissolved oxygen data (Figure 5.10b) exhibited a similar trend to salinity, however with more fluctuation, probably due to the effect of wind producing swells, or the motion of large vessels oxygenating surface waters. The lowest value was recorded just before the push of the high tide, and after this values reached seawater levels extremely rapidly. Figure 5.10c,d shows similar behaviour for nitrate and phosphate, *i.e.* inversely correlated with salinity. However phosphate exhibited more short-term temporal variability than nitrate.

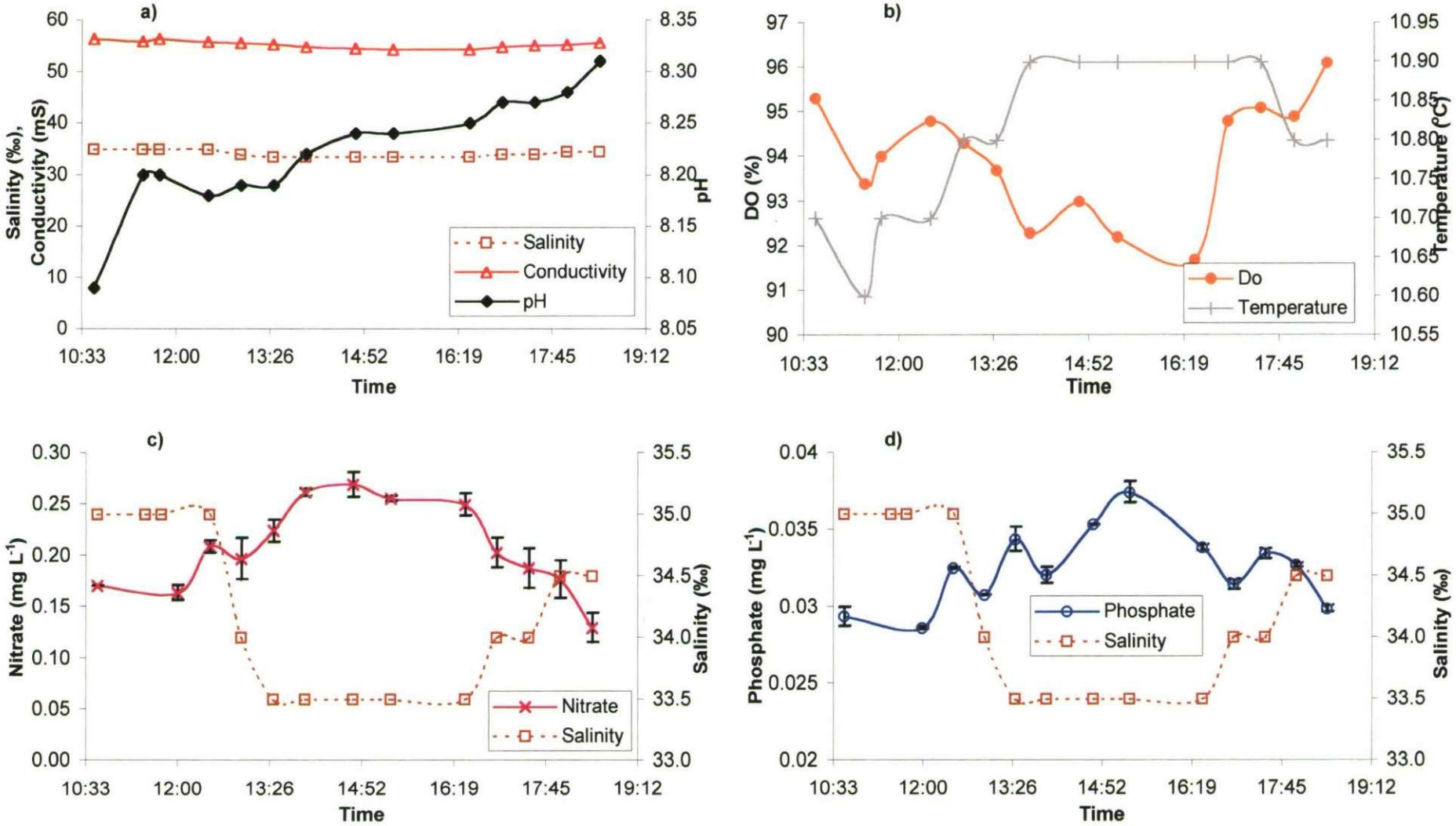


Figure 5.10 Behaviour of all the *in situ* and laboratory measured variables during the Tidal Cycle 4 at Barn Pool (16/04/2002).

5.4 CONCLUSIONS

The results from all surveys performed in the Tamar Estuary reiterate the importance of reliable measurements of master variables (salinity, conductivity, pH, temperature, dissolved oxygen and suspended solids) to help understand estuarine nutrient biogeochemical processes.

The overall behaviour of nitrate, during axial transects of the estuary and tidal cycle at fixed stations, showed its conservative nature during the mixing process and its inverse relationship with salinity. Maximum nitrate concentrations were measured in the freshwater sector of the estuary and varied from 1.3 to 3.5 mg L^{-1} NO_3-N, and in the lower part of the estuary nitrate varied between 0.08 and 0.20 mg L^{-1} NO_3-N, due to the influence river flow rates and tidal regimes. The River Tamar is the major source of nitrate to the estuary (Mommaerts, 1969; Morris *et al.*, 1981; Morris *et al.*, 1985; Knox et al. 1986) and no evidence of other significant sources of nitrate were identified during this work.

Phosphate demonstrates a more complex behaviour than nitrate along the estuary because several variables influence phosphate behaviour. It was noticeable that the Tamar River inputs (concentrations varying between 0.016 and 0.060 mg L^{-1} PO_4-P) were not always the major source of phosphate to the upper sector of the estuary. Under specific conditions within the turbidity maximum zone, the concentration of phosphate was up to 0.20 mg L^{-1} PO_4-P higher than the riverine contribution (Figure 5.7d). Although release of phosphate from sediments with salinity increase has been previously described (Chambers *et al.*, 1995; Rizzo and Christian, 1996; Van Beusekom and Brockmann, 1998; Lau and Chu, 1999), anthropogenic inputs of phosphate, mostly from wastewater treatment works and agriculture runoff, in the turbidity maximum zone were also identified during this study and are consistent with previous reports (Mommaerts, 1969; Morris *et al.*, 1981).

As a general conclusion, nutrient concentrations reported above are in good agreement with previously reported data (Morris *et al.*, 1981). They reported concentrations ranging between 0.03 and 3.0 mg L^{-1} for N and P, respectively, for

the upper estuary and between 0.06 and 1.0 mg L^{-1} for N and P, respectively, in the lower 10 km of the estuary.

Salinity and conductivity showed a linear correlation in all the surveys, although occasionally both variables demonstrated short-term fluctuations in relation to distance from the tidal limit, possibly due to mixing of different water masses.

Seawater is likely to retain slightly higher temperatures then freshwater during middle and late summer. Also during summer the estuary experiences different heating and cooling processes affecting shallow water masses, principally over exposed mudflat areas (Morris *et al.,* 1982a). At Weir Quay station during winter it was possible to identify a measurable increase in water temperature, when debris and scum from a primary wastewater treatment work reached the sampling station and caused alterations to most measured variables (Figure 5.7).

Both pH and dissolved oxygen were at all times non-conservative variables and were influenced throughout the mixing zone by several dynamic factors. As a dynamic open system the mixing zone is subjected to variable boundary fluxes, transient localised perturbations produced by chemical and biological processes (Morris *et al.,* 1982a), strong alterations of flow, wind and tidal energy inputs that generate internal fluxes, and resuspension of sediments as well as geographical movement of the turbidity maximum zone (Morris, 1985). For all these reasons several studies (Morris *et al.,* 1981; Morris *et al.,* 1982a,b; Ackroyd *et al.,* 1986; Morris *et al.,* 1985; Morris, 1986; Morris et al. 1986; West *et al.,* 1990; Millward, 1995; Miller, 1999) have focused a great part of their research monitoring turbidity.

It was considered important to investigate the turbidity maximum zone and its interactions with phosphate. Specifically, the aim of Chapter 6 is therefore to identify the effect of changing salinity (*e.g.* during a tidal cycle) on the release of dissolved organic and inorganic phosphorus from sediments in suspension.

The effects of salinity changes in the analysis, throughout the estuarine mixing zone, can be overcome using salinity-matched reagents and standards prepared using appropriate dilutions of low nutrient seawater.

CHAPTER SIX

Salinity Induced Release and Transformation of Organic Phosphorus from River Sediments

Temperature controlled slurry reactor.

Main topics in this chapter:

FI manifold for organic P analysis
Experimental design
Stabilization of the slurries
Dissolved organic and inorganic phosphorus release

6.1 INTRODUCTION

In the discussion of the previous chapter, phosphorus behaviour in the mixing zone of the estuary appeared to be influenced by sediment processes. In this chapter, a more detailed investigation was made to analyse the non-conservative behaviour of phosphorus with increase of salinity in the mixing zone of an estuary.

Inorganic phosphorus, in the form of orthophosphate, is most frequently the limiting nutrient for algal photosynthesis in fresh water systems. Consequently, high priority is given to minimisation of phosphorus inputs to rivers and waterways from agricultural runoff and wastewater point sources. However, algal blooms can continue to occur on a seasonal basis long after phosphorus inputs to water bodies have ceased, and it is thought that this is due to periodic remobilisation of phosphorus bound in the sediments. In this chapter the abbreviations Po, for organic phosphorus, and Pi, inorganic phosphorus or orthophosphate were adopted to facilitate the observations of behaviour between the two forms of phosphorus.

Stevenson (1982) indicated that the range of Po species derived from plant and animal remains that occur in sediment and soil systems would degrade through different mechanisms, therefore suggesting a degree of stability. This implies that several different species of soil Po (Figure 6.1) will ultimately degrade to Pi. Recent data have shown that organic and condensed phosphates may undergo this conversion to orthophosphate, either through abiotic (Baldwin *et al.,* 1995, 2001) or microbial hydrolysis (Halliwell *et al.,* 2001) or arguably even through photochemical oxidation reactions. Sediment release of Pi, as bioavailable orthophosphate or filterable reactive phosphorus is known to occur under a range of biogeochemical conditions. When the Pi concentration in water overlying sediments decreases, the phosphorus concentration may be buffered by the release of "exchangeable" phosphate from the large sediment / interstitial water P reservoir (Aminot and Andrieux, 1996). Under anoxic conditions reduction of Fe(III) hydroxyoxide minerals by iron or sulfate reducing bacteria (Baldwin *et al.,* 2002) can lead to release of Pi associated with the iron

mineral phases. Anoxic conditions also favour the release of luxury Pi from facultative anaerobic sediment bacteria (Gächter *et al.*, 1988).

Increased salinity, such as that which occurs during estuarine mixing, has also been reported to cause rapid release of Pi from sediment and suspended particulate material. For example, Fox *et al.* (1986) reported that Amazon sediments released significant amounts of Pi when suspended in waters of increasing salinity, and suggested that this process contributed to more than half of the mean annual input of inorganic phosphorus to the outer estuary of the Amazon.

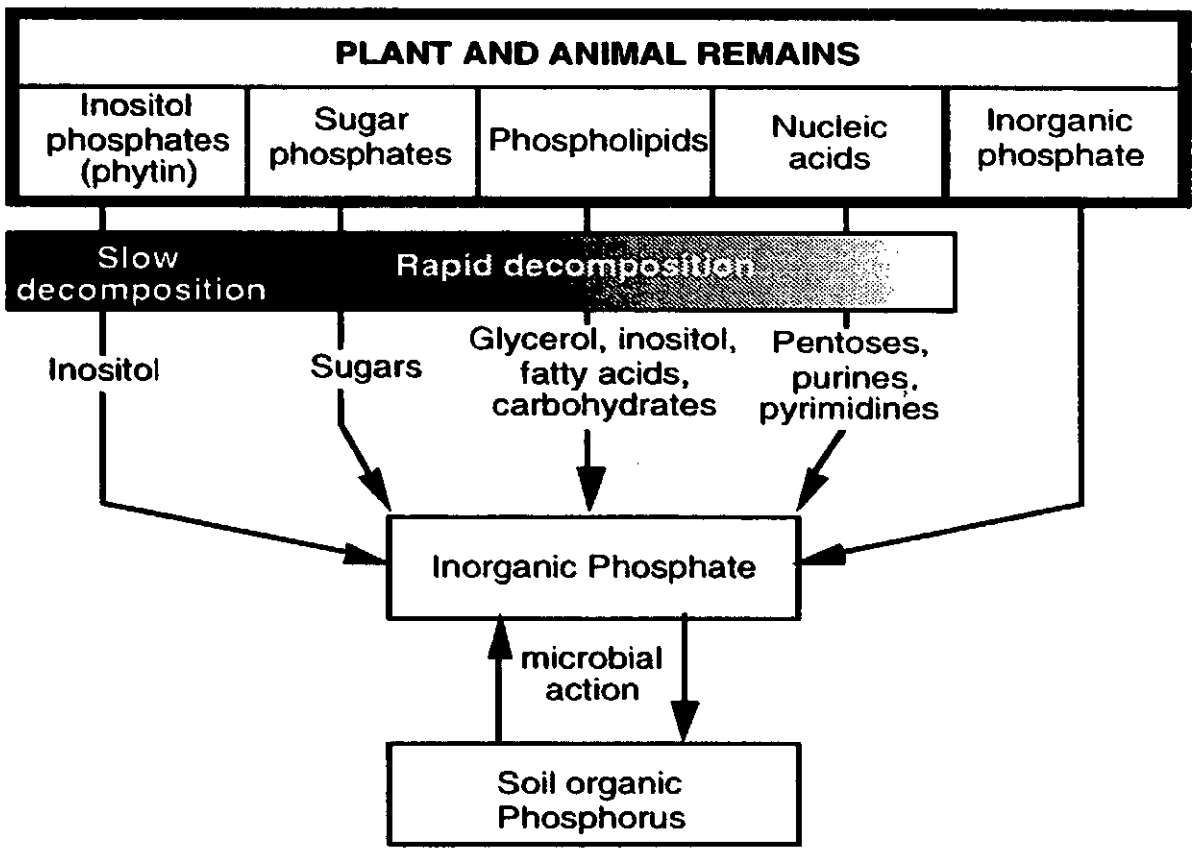


Figure 6.1 Schematic distribution of organic P in soils (modified from Stevenson, 1982).

The majority of sediment phosphorus release studies have concentrated on the behaviour of inorganic phosphorus (Chambers *et al.*, 1995; Lau and Chu, 1999; Petticrew and Arocena, 2001). However, the potential role and algal bioavailability of organic phosphorus (Po) species (comprising compounds such as inositol phosphates, sugar phosphates, phospholipids and nucleic acids) in aquatic systems

has been largely ignored, perhaps because the techniques for their measurement are either undeveloped, or are effectively inaccessible to those conducting the studies.

This chapter describes a modified version of an automated on-line UV-photo-digestion FI system with spectrophotometric detection (McKelvie *et al.,* 1989) for the determination of dissolved organic phosphorus in estuarine waters. Results presented are from an experiment showing that rapid release of soluble organic phosphorus occurred when freshwater riverine sediment was exposed to increasing salinity, such as would occur when suspended particulate material is transported into an estuary, and that liberated Po was quickly converted to bioavailable inorganic phosphorus.

6.2 EXPERIMENTAL

6.2.1 Cleaning procedure

All the labware and sampling material used for this work were cleaned using the same protocols described in detail in section 3.2.2.

6.2.2 Sampling

River water and seawater samples were collected in March 2001 from the Yarra River at Fairfield Park - Melbourne and Norman Bay (Figure 6.2), Wilsons Promontory (Victoria, SE Australia), respectively. All samples were then filtered, at 0.2 μm using 47mm diameter cellulose acetate filters and a vacuum pump, and analysed for dissolved inorganic and organic P within 24 hours of sampling.

Riverine sediment samples were collected from Yarra River at Fairfield Park using a stainless steel Ekman grab sampler (Figure 6.3). Care was taken when removing the grab from the water to minimise the runoff from surface sediment. Only the top 1 cm from each sample was separated and stored in resealable polyethylene bags to be used in the preparation of the slurries. The sediments were wet sieved at 150 μm, with a nylon sieve, using river water and then stored at 4° C until the preparation of the slurries.

Subsamples from the slurries were collected with disposable 10 mL syringes, immediately filtered at 0.2 μm with cellulose acetate Nalgene syringe filters and analysed for Po and Pi. Exemptions on filtration were made for subsamples taken from slurries for the analysis of Total P just before the first addition of seawater.

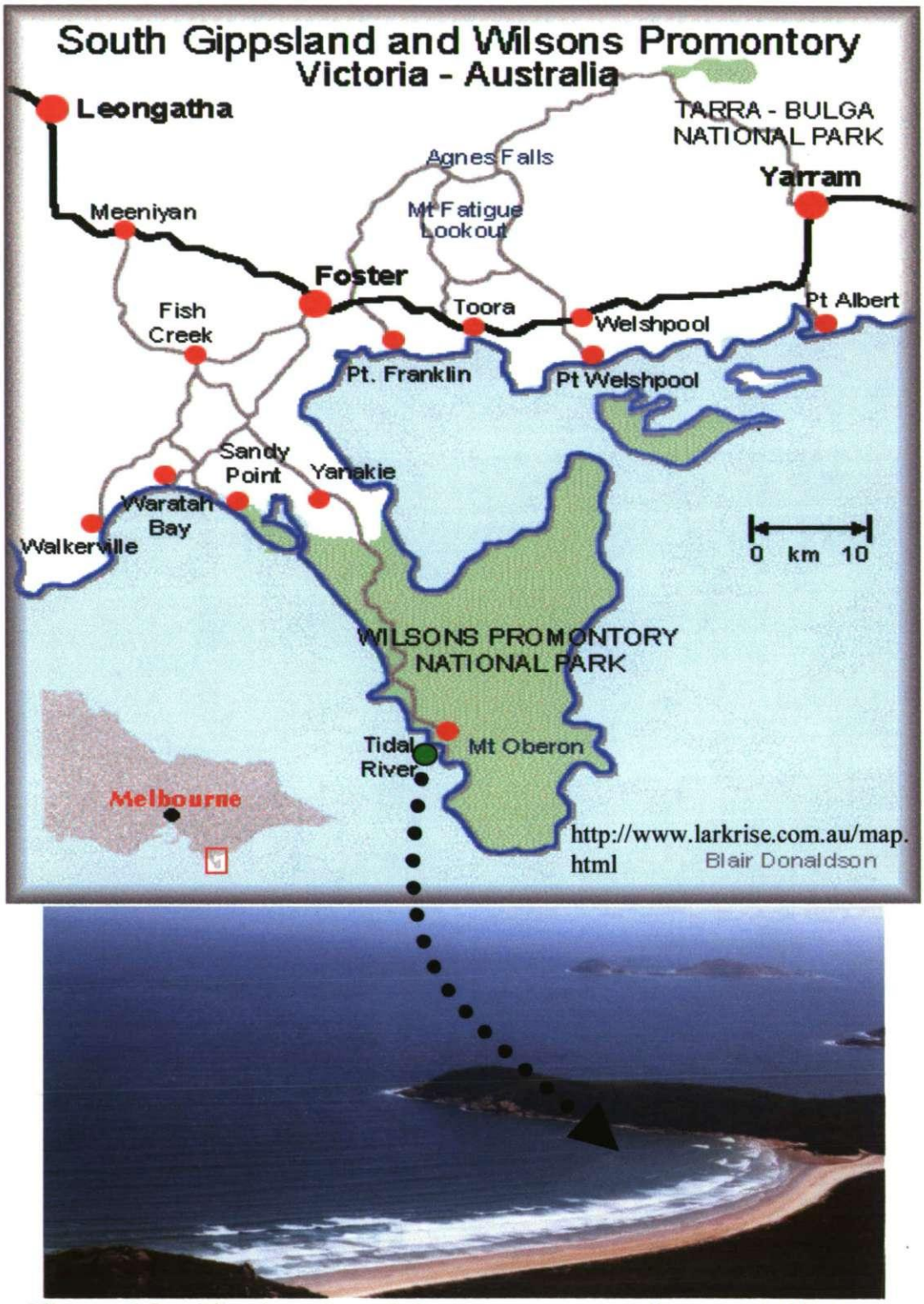


Figure 6.2 The location of the seawater sampling site in Wilsons Promontory, Victoria Australia (the green dot represents the exact site).

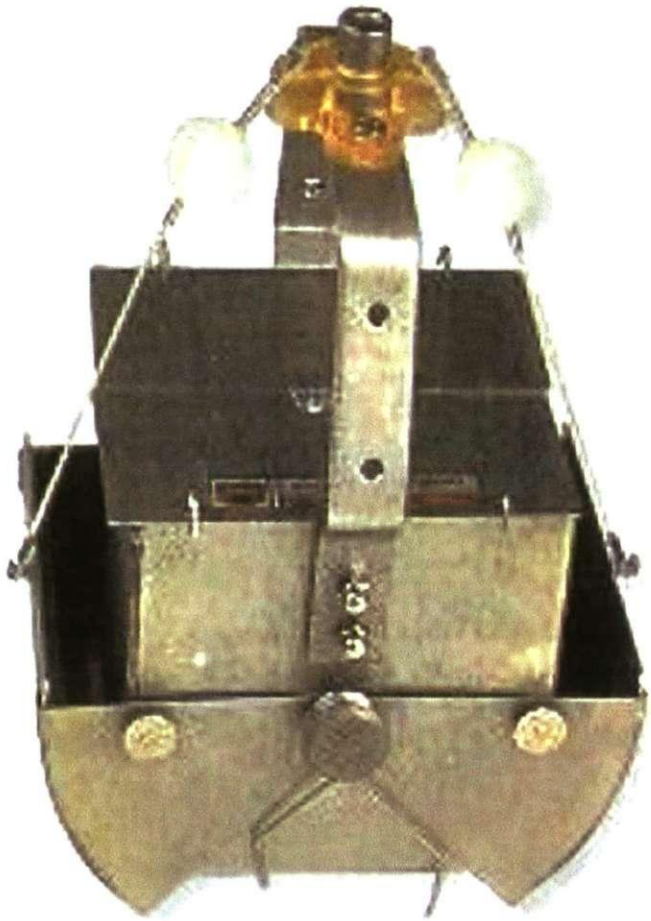


Figure 6.3 Stainless steel Ekman grab sampler used for collection of surface sediments.

6.2.3 Reagents

All solutions were prepared in ultra-pure water and all reagents were of AnalaR (or equivalent) grade and were purchased from BDH, unless otherwise indicated. The alkaline persulphate solution contained 40 g L^{-1} of potassium persulphate and 9.5 g L^{-1} of sodium tetraborate decahydrate (May and Baker). The ammonium molybdate solution contained 10 g L^{-1} of reagent and 34.2 mL v/v of sulphuric acid (Ajax Chemicals). The tin(II) chloride solution (May and Baker) contained 0.2 g L^{-1} of this reagent, 1.95 g L^{-1} of hydrazine sulphate and 27.2 ml (v/v) of sulphuric acid. Working standard solutions (1.0-100 μg L^{-1} $P\text{-}PO_4$) were prepared from stock solutions (100 mg L^{-1} $P\text{-}PO_4$) by dilution with appropriate volumes of ultra-pure water immediately prior to analysis. The model phosphorus compounds used to

prepare the 100 mg-P L^{-1} stock solutions were potassium dihydrogen orthophosphate and inositol hexaphosphoric acid magnesium-potassium salt desiccate (Phytic acid 95% pure - SIGMA)(Stevenson, 1982).

6.2.4 Preparation of slurries

The slurries used in the experiment for both control and sample were prepared by mixing 150 mL of the sieved wet sediment (or 17 g dried sediment) with 850 mL of filtered river water.

6.2.5 Instrumentation

The equipment used to contain and monitor the slurry (Figure 6.4) consisted of two thermostatted (20.8 ± 0.2°C) glass reactors with a capacity of 1800 mL each and with fitted probes to monitor pH, Eh, temperature and dissolved oxygen. A stirrer at 160 RPM kept the sediments in suspension.

The Flow Injection manifold (adapted from McKelvie et al., 1993) with spectrophotometric detection (ABI 757 UV-VIS detector) was assembled using PTFE tubing of 0.5 mm i.d (Figure 6.5). An automated Flow Injection Station, comprising two peristaltic pumps (Ismatec MS-CA 4 820) and one PTFE rotary four-port valve (Rheodyne 5041) was connected to a 40 W UV-photo-digestion unit. Before the detector, a debubbler (5 cm of microporous polypropylene tubing - Accurel S6/2, Enka) was used to remove any formed bubbles from the system. To control the FI Station, as well as to record the readings from the detector, the software package FCS was used.

The alkaline UV oxidation approach has been shown to provide a suitable means of discriminating between dissolved organic and condensed phosphates because condensed phosphates are not measured using this technique (Solórzano and Strickland, 1968; McKelvie *et al.,* 1989). The flow injection method used is sensitive to chloride (McKelvie *et al.,* 1995), and for this reason, salinity matched standards of orthophosphate were used for calibration of both inorganic (Pi) and organic (Po) phosphorus measurements, for each set of samples obtained.

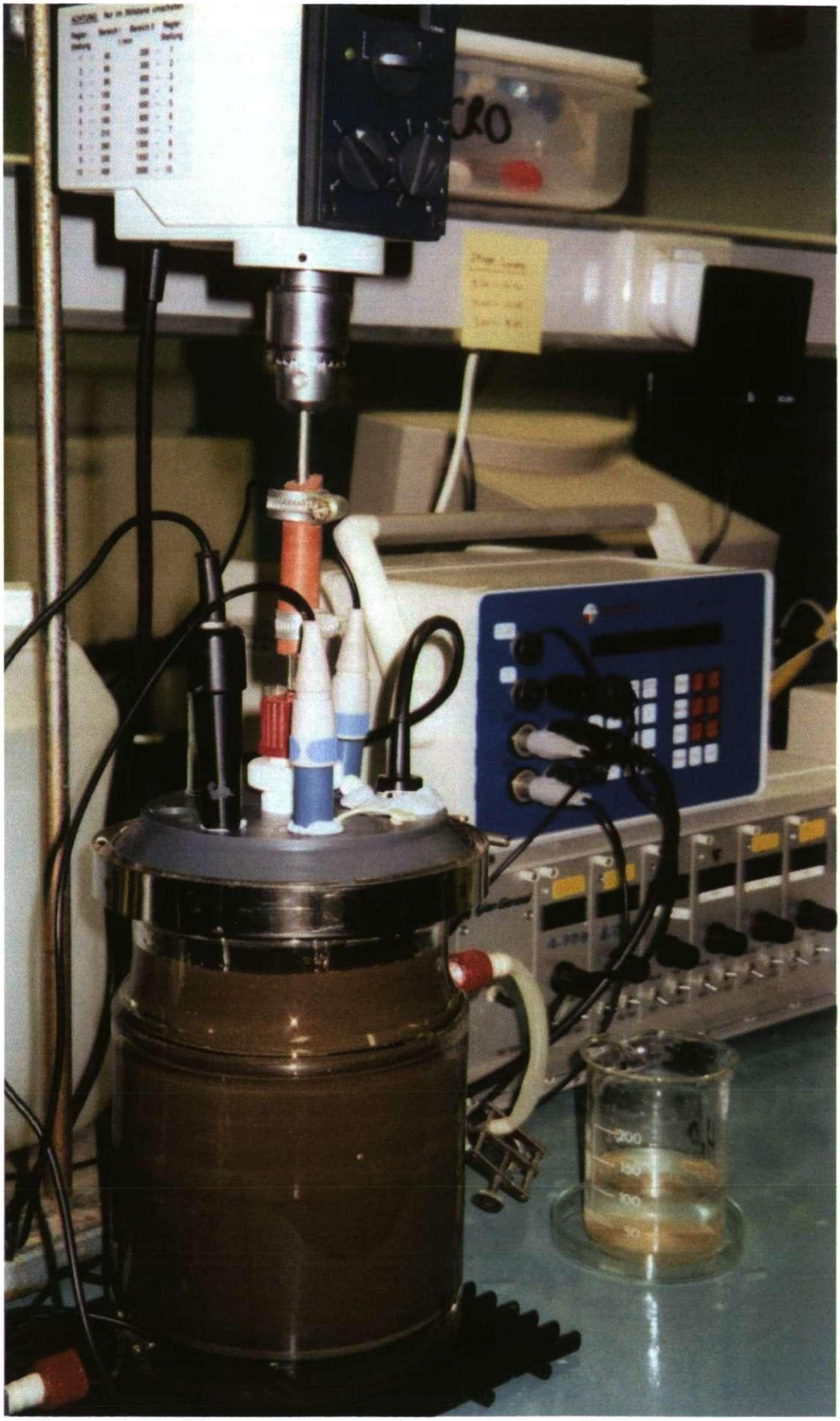


Figure 6.4 Glass reactor, probes and controller.

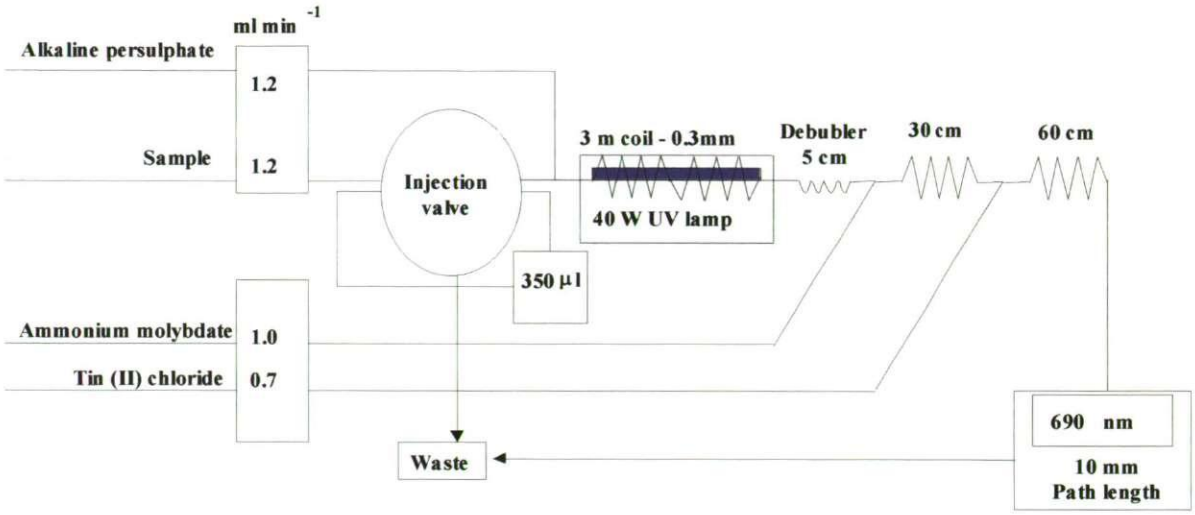


Figure 6.5. Schematic diagram of the FI manifold for organic P determination

6.2.6 Experiment design

The experiment had two slurries prepared following exactly the same procedure (Section 6.2.2). In both of them, after mixing wet sediment with filtered river water, it was necessary to allow a period of time to reach a dynamic equilibrium between the concentration of phosphorus in the water and in the suspended sediment (Aminot & Andrieux, 1996), which can take between 25-30 hours of contact time depending on the type of sediment.

The main focus of the experiment was the effect of stepwise increases in salinity (5, 10, 15 and 20 ‰) on the Pi and Po content of the slurry. To achieve this stepwise increase, volumes of filtered low nutrient seawater were added to the slurry, and the same volumes of ultra pure water were added to the control slurry. The total P content of sample and control slurries was determined by nitric / sulfuric acid digestion and spectrophotometric detection (APHA, 1998).

6.3 RESULTS AND DISCUSSION

The samples physico-chemical parameters of the samples measured at the sampling sites are shown in Table 6.1.

Table 6.1 Physico-chemical parameters of the samples.

	pH	Conductivity (μS)	Dissolved O_2 (mg L^{-1})	Salinity ‰	*Pi (μg L^{-1}) (n=3 ± 2s)	*Po (μg L^{-1}) (n=3 ± 2s)
River water	7.8	0.19	6.7	0.0	7.6 ± 2.0	8.7 ± 1.8
Sea water	7.7	54.1	7.9	35.8	5.5 ± 0.7	4.4 ± 0.9

* Analysed in laboratory within 24h.

The FI system was calibrated each time it was used and achieved calibrations typically within the range of R^2 = 0.999 – 1.0000 (Table 6.2), with a limit of detection (3s) of 1.5 - 3.0 μg L^{-1}. The organic P digestion efficiency (% recovery) was 76 – 87 % for a standard of 20 μg L^{-1}, using phytic acid as a model compound.

During the experiment all samples and standards were analysed in triplicate for Pi and Po. All concentration values for the samples collected from the slurry are background corrected for the concentration of Pi and Po added with the seawater.

Table 6.2 Analytical figures of merit for the FI system, salinity of the standards and samples, time of sampling and time of analysis

Calibration	R^2	LOD (μg L^{-1})	% Recovery	Salinity (‰)	Time sample (h)	Time analysis (h)
1	0.9994	2.9	87.3	0	70.3	70.3
2	0.999	2.0	82.0	5	71.3, 73.3	73.3
4	0.9991	2.1	81.2	5	89.1	89.1
6	0.9992	1.5	87.2	10	90.9, 93.0	93.0
7	0.9991	1.7	85.0	10	117.3	117.3
8	0.9993	2.5	76.9	15	118.3, 121.3	121.3
11	0.9993	3.0	77.8	15	139.1, 160.3	160.3
12	1.0000	1.8	75.8	20	161.3, 163.3	163.3
14	0.9995	2.3	75.8	20	184.3	184.3

After mixing of sediment and filtered river water in the reactors, a period of ca. 3 days was allowed for pH and redox conditions to stabililize (faded area in Figure 6.6). Prior to the first salinity increase at 70.3 hours from the beginning of the experiment, the concentrations of Pi and Po in the sample reactor were 11.7 ± 0.8 and 15.4 ± 1.1 μg L^{-1} P respectively. Sub-samples taken from the control and

sample slurries for total P determination contained 7.1 ± 0.9 and 6.8 ± 0.8 mg L^{-1} P (mean ± $2\sigma_{n-1}$), respectively. Seawater was only added (red vertical lines in Figure 6.6) when the redox and pH values were stable in both reactors.

As the salinity was increased to 5 and 10 ‰ over a period of 16 h, Pi showed no significant change. However, rapid (<1 h) release of 14 and 9 μg L^{-1} Pi did occur following salinity increases to 15 and 20 ‰ respectively (Figure 6.7). This observation concurs with data previously reported for wetland sediments (Lau and Chu, 1999) and Amazon River suspended sediments (Fox *et al.*, 1986) exposed to waters of increasing salinity.

However, release of *organic* phosphorus (7.7 μg L^{-1} P) was observed at the lower salinity of 10 ‰, and similar measurable Po increases of 6.0 and 5.5 μg L^{-1} P occurred at 15 ‰ and 20 ‰.

Given that Po and Pi releases are initiated at different salinities, it is possible that there are different release mechanisms for the two fractions. A likely explanation for the observed Po behaviour is that salinities of 10 ‰ and greater cause plasmolysis of sediment bacteria which results in cell rupture and release of dissolved Po. Similar reasoning has been invoked to explain the inhibition of carbon removal by non-halophilic bacteria in wastewater treatment plants with salinities of ≥ 10, and it is likely that similar processes occur in sediment suspensions (Kargi and Dincer, 2000).

While it is evident from Figure 6.6 that there is a stepwise decrease in redox potential that follows each addition of seawater, this is in response to the corresponding and expected incremental increase in pH that occurs as seawater is added to river water. The final redox potential of 190 mV (vs Standard Hydrogen Electrode) is still well within the range considered to be oxidising (Stumm and Morgan, 1996; Petticrew and Arocena, 2001). Thus the release of Pi that occurs at ≥ 15 ‰ is unlikely to be associated with reductive processes, *i.e.* associated with reduction and solubilization of Fe (III) oxides, or the release of Pi from facultative anaerobes (Gächter and Meyer, 1993), but more probably due to desorption or ion

exchange processes associated with the increasing pH and ionic strength (Baldwin *et al.*, 2002).

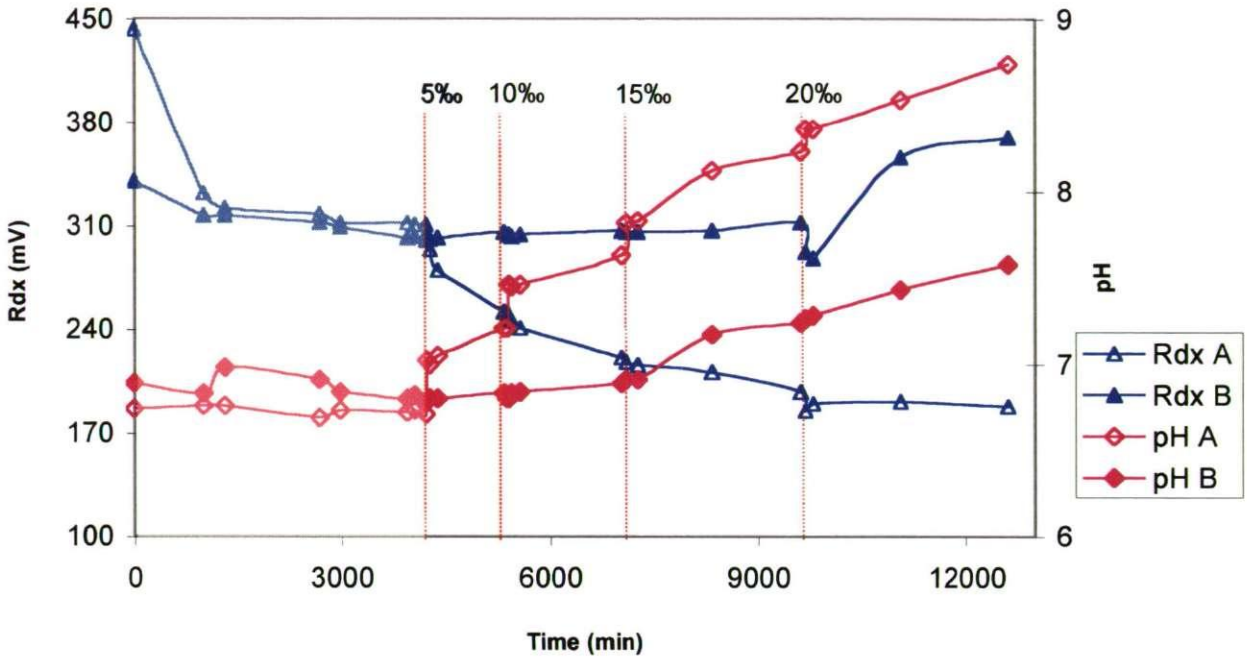


Figure 6.6 Physico-chemical parameters measured in the sample (A) and control (B) reactors. Faded area represents the stabilisation time and red vertical lines represent the addition of seawater.

Figure 6.7 also shows that released Po was quantitatively converted to Pi during the 24 h following the initial release, and that this process was repeated after each incremental salinity increase. This is most likely due to hydrolysis of Po by the action of phosphohydrolytic enzymes such as alkaline phosphatase that are associated predominantly with sediment bacteria, and to a lesser extent present in solution (Herbes *et al.*, 1975; Boon, 1993). The bioavailable phosphorus derived from the release and rapid hydrolysis of Po contributed ca. 37 % of the total bioavailable fraction released during the experiment. This equates to a total Po release of ca. 2.3 $\mu g\ g^{-1}$ (dry wt) from suspended sediment when exposed to an estuarine salinity gradient, and while this is small compared with the total P content of the sediment (360 $\mu g\ g^{-1}$ P (dry wt)), it nevertheless represents an important and labile pool of readily bioavailable P. However, not all of the released Po was converted to Pi, as indicated by the elevated final Po value. Presumably this

residual was comprised of organic P species such as myo-inositolhexakisphosphate that is found in high concentrations in sediments and is not readily hydrolysed by exocellular phosphatases (Turner *et al.*, 2002).

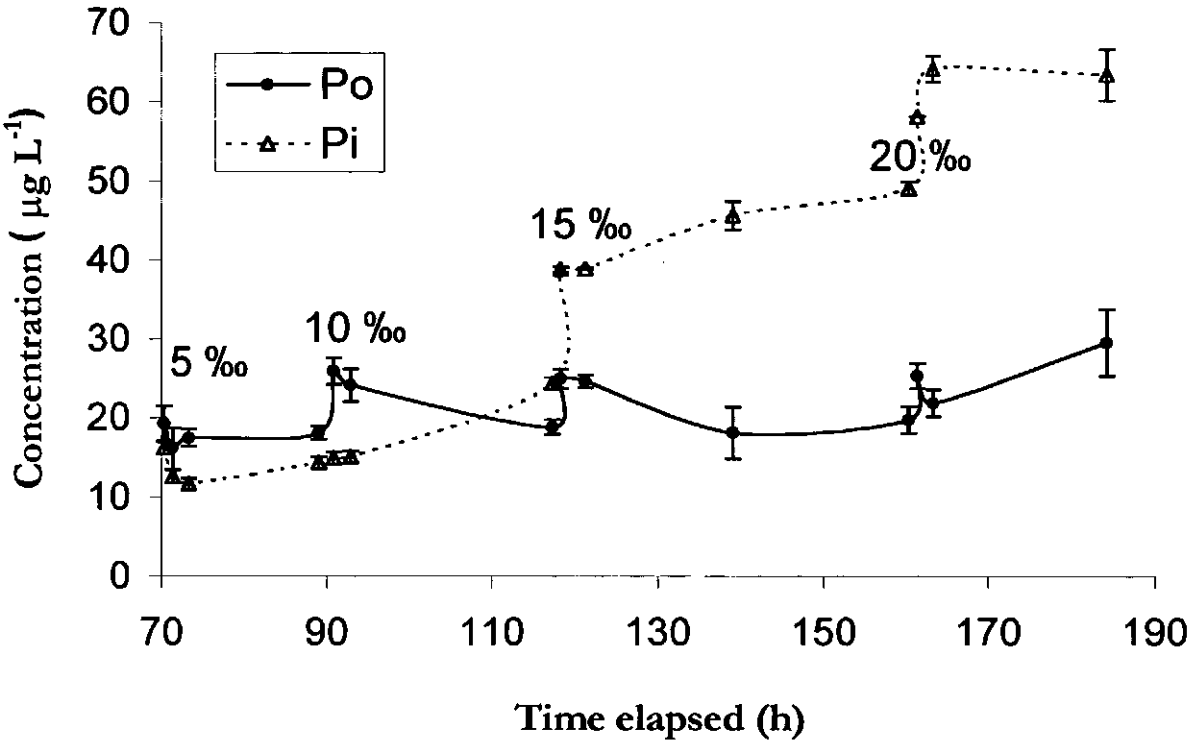


Figure 6.7 Release of inorganic (Pi) and organic (Po) phosphorus from oxic river sediment as a function of increasing salinity. Elapsed time refers to the total time of the experiment and includes the equilibration time prior to salinity additions. Concentration data are volume corrected and error bars are ± $2\sigma_{n-1}$ (n = 3). Mean control concentrations for Pi and Po over the 4-day experiment were 17.5 ± 4.6 and 17.0 ±4.8 µg L^{-1} P respectively (mean ± $2\sigma_{n-1}$ (n = 16))

The estimated release rates for Pi and Po averaged over the 4 day experimental period were 10.8 µg L^{-1} d^{-1} (0.35 µM d^{-1}) and 2.8 µg L^{-1} d^{-1} (0.09 µM d^{-1}) respectively, and it is noted that this Pi release rate is similar to the value of 0.2 µM d^{-1} reported for Amazon River sediments (Fox *et al.*, 1986). However, the estimated maximum release rates immediately following the salinity increases were somewhat larger ($\Delta Pi/\Delta t$ = 11 µM d^{-1} (340 µg L^{-1} d^{-1}) and $\Delta Po/\Delta t$ = 4.6 µM d^{-1} (140 µg L^{-1} d^{-1})), emphasizing the very episodic nature of these P releases.

It might also be expected that salinity induced plasmolysis of facultative bacteria would result in the release of polyphosphates (poly-Pi). Poly-Pi are not degraded by UV photo-oxidation and thus are not measured in the Po fraction. Similarly they are not molybdate reactive and are not detected as Pi unless first hydrolysed to orthophosphate. However, in the presence of phosphohydrolytic enzymes, degradation is rapid (within hours) (Halliwell *et al.*, 2001), and it is probable that some of the P release reported as Pi (e.g. 117.3 h - 118.3 h, and 160.3 h -163.3 h) has originated from the hydrolysis of poly-Pi.

6.4 Conclusions

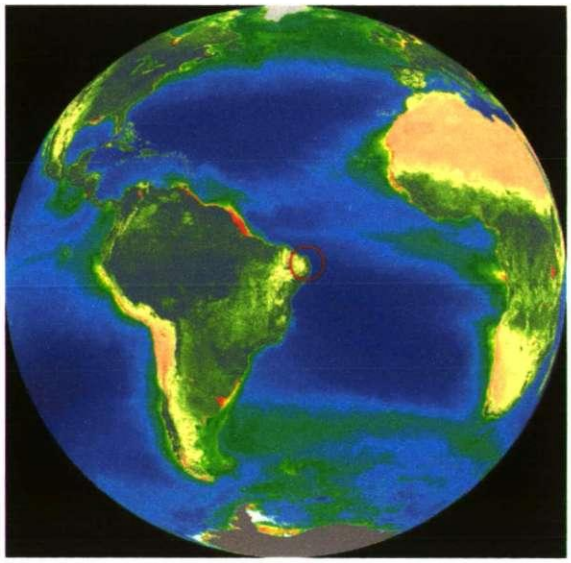
The findings of this experiment show that substantial concentrations of Po and Pi are rapidly released from a freshwater sandy-silt sediment, under conditions of increasing salinity (10 - 20 ‰) similar to that which applies when sediment and suspended particulate materials are transported into an estuary. Furthermore, a large proportion of the released Po is rapidly hydrolysed to the bioavailable Pi form (ca. 37 % of the total Pi release). It is assumed that the observed Po release is due to plasmolysis of sediment bacteria in response to rapid changes in salinity. Pi derived from Po in this manner represents a potentially important source of bioavailable phosphorus in environments of variable salinity that has hitherto been largely ignored. This highlights the need for more attention to be given to speciation in studies of the biogeochemical behaviour of nutrients, and for further research into the nature and behaviour of organic phosphorus in the aquatic environment.

CHAPTER SEVEN

Conclusions and Future Work

Main topics in this chapter:

General conclusions
Future work
Methodological template for monitoring nutrients in estuaries



Somewhere around there...

7. CONCLUSIONS AND FUTURE WORK

Specific conclusions covering the studies performed during this research are presented at the end of each of the previous chapters. This chapter presents the overall conclusions from the research, provides suggestions for further work, and proposes general guidelines for studying the biogeochemistry of nutrients in estuaries.

7.1 General conclusions

7.1.1 Historical time series datasets

Historical physico-chemical datasets are an invaluable resource for time series studies. These datasets when analysed and interpreted using appropriate chemometrics tools, such as PCA and PLS, can provide enhanced information recovery in a variety of ways. The best way to maximize the information obtained is to use multidisciplinary teams, including environmental analytical chemists, ecologists, hydrologists and specialists in chemometrics. In these terms, the best achievements made were relating the inclusion of a time delay while considering the correlation between variables, and the capability of the produced PCS models in predicting the concentration of nutrients based on meteorological, hydrological, and physico-chemical datasets.

It is always important to assure the quality of data originally produced and therefore, independent monitoring programs are necessary to 'ground truth' the data. Public access to the datasets is also important, as are frequency of data collection and the absence of gaps in the data.

7.1.2 Nutrients sampling and storage

The results of the comparison of sample storage protocols showed that the optimum storage conditions for the determination of FRP and TON were highly matrix dependent, with significant differences in FRP stability between the Frome and Tamar catchments (due to different calcium concentrations) and between samples of different salinities (due to different bacterial populations and/or dissolved organic matter). For the River Frome sub-samples, the most effective

storage treatment was shown to be 4 °C with chloroform. Freezing was shown to be the least effective storage treatment because of the coprecipitation of inorganic phosphate with calcite after thawing of the samples. In the absence of *in situ* measurements, it is thus essential to design an appropriate site specific protocol before undertaking any sampling campaign. General guidelines for such a protocol include: the development of a rigorous cleaning procedure; the use of inert sample containers; (*e.g.* HDPE), the analysis of filtered (< 1 atm) samples within 8 h of collection, avoiding frozen storage for calcium rich sample matrices; and chemical addition to those samples rich in dissolved organic matter.

7.1.3 The use of a submersible FI analyser for nitrate determination.

Flow injection with spectrophotometric detection can be used to monitor nitrate in estuaries. The effects of salinity changes, throughout the mixing zone, can be overcome using salinity-matched reagents and standards prepared using appropriate dilutions of low nutrient seawater. The FI analyser can be used in the laboratory, on board research vessels and in submersible modes. The quality of results was assured by participation in an intercomparison exercise for nutrient analysis and also by continual comparison of data obtained in the study with the University of Plymouth reference method. During field and on board deployments, the analyser achieved a limit of detection of 2.8 $\mu g\ L^{-1}$ NO_3-N and two operational linear ranges were defined 0 - 100 and 100 - 2000 μgL^{-1} NO_3-N. The FI analyser proved to be reliable, robust and truly one-person deployable. In addition, it has been demonstrated to be able to respond to changes in environmental conditions by on board adjustments, such as changing the flow cell, sample loop and carrier, to match local conditions.

7.1.4 Tamar Estuary surveys.

A comparison between data acquired during surveys in the Tamar Estuary in 2001-2002 with data from the period 1978-1980 showed that nitrate and phosphate concentrations have not increased over this period. This is most likely to changes in

fertilizer application practices and improvements in process quality in wastewater treatment works. Nevertheless, monitoring dissolved nutrient concentrations in the estuarine mixing zone requires accurate and reliable analytical instrumentation with detection limits below 80 μgL^{-1} NO_3-N and 5 μgL^{-1} PO_4-P, and linear ranges of 0 – 4000 μgL^{-1} NO_3-N and 0 – 100 μgL^{-1} PO_4-P. For an extensive interpretation of nutrient behaviour in an estuary, other master variables should be monitored *in situ*, *i.e.* pH, dissolved oxygen, salinity, conductivity, temperature and suspended solids, in addition to the location and characterisation of point source inputs such as wastewater treatment works.

7.1.5 Organic and inorganic phosphorus release from sediments

To be able to monitor the rapid release of dissolved organic and inorganic phosphorus from suspended sediments with increasing salinity, instrumentation is needed that interfaces directly with an incubation chamber. FI is ideal for this purpose because samples can be collected, filtered and analysed rapidly (within a few minutes) using the same manifold. The analytical method used was accurate and reproducible, with detection limit of 1.5 μgL^{-1} PO_4-P and organic P digestion efficiency (% recovery) of 76- 87 %. These phosphorus experiments highlighted the importance of phosphorus speciation studies in the aquatic environment.

7.2 Suggestions for future work

Future work arising from the research reported in this thesis are made below.

7.2.1 Time series datasets

To search for pathways to improve the predictive models, *e.g.* to investigate differences between data from other rain gauges within the catchment and their relation with the other variables. Also, to perform a study only with the outliers of the variables, searching for correlations.

7.2.2 Sample storage protocols

To investigate a wider range of samples for shorter storage time, and perform a detailed investigation of sample composition on Day 0, including the characterisation of the sample biota in different fractions of the sample, *e.g.* unfiltered, > 0.45 μm and > 0.2 μm - < 0.45 μm filtrates.

7.2.3 Development of the submersible FI analyser for phosphate determination

To develop and test in the laboratory and in the field the submersible FI analyser with a manifold for determination of phosphate in estuarine waters. To test different methods, *e.g.* ascorbic acid or tin(II) chloride, with the use or otherwise of a heating block to assist the reaction.

7.2.4 Estuarine biogeochemistry of nutrients

To increase the short-term resolution of analysis during tidal cycles, particularly in the turbidity maximum zone and during maximum ebb flow. To increase the duration of deployments to acquire long-term resolution data, *e.g.* to monitor daily variations of nitrate or phosphate within one month, at a river gauge station.

7.2.5 Release of phosphorus species from suspended sediments

To use sediments of different origins and compositions, and evaluate the behaviour of inorganic and organic phosphorus under the same stepwise increase of salinity.

7.3 Guidelines for monitoring and modelling the biogeochemical cycling of nutrients in estuaries

From the overall conclusions given above, one can produce a set of guidelines for the monitoring and modelling of the biogeochemical cycling of nutrients in estuaries. A methodological approach for studying any estuarine environment, would take into consideration the following aspects;

1) Assess the available laboratory and field instrumentation and sampling storage, and sample treatment facilities. This information will clarify several important aspects before planning any surveys, such as the amount of samples to collect, acceptable delay time between sampling and analysis, and sample analysis throughput.
2) Acquire any available data regarding the estuary, *e.g.* Environment Agency reports, previous scientific literature, historical description, geology and hydrology of the area, main natural and anthropogenic influences to the area, legislation and socio-economical aspects of the area.
3) After analysing existing datasets, set up a monitoring strategy. This would consist of a preliminary visit to the entire estuarine area followed by strategic visits to selected sectors of the estuary, *e.g.* main freshwater contributors, three sites in the middle-up sector of the estuary, three sites in the middle-down sector and finally the mouth of the estuary. Following identification of sites where access for sampling from the riverbank is safe, plan a sampling campaign. Some 'precautionary practices' should be followed for sampling and filtration procedures, choice of containers and cleaning laboratory ware (such as described in Sections 3.1.1 – 3.1.3 and 3.2.2). From each sampling site, measure master variables *in situ* (pH, Eh, dissolved oxygen, conductivity, salinity and turbidity) and collect discrete samples to determine 'typical levels' as soon as possible, preferably within a few hours. Analyse should include dissolved and total nutrients (nitrogen, phosphorus, silicate and carbon), chlorophill a, suspended solids, and a brief characterisation of phytoplankton/bacteria contents.
4) After determining the 'typical concentration levels' of the nutrients at each of the specific sites, a plan for a significant sampling campaign, for a short to medium term sample storage exercise, should be undertaken. This strategy is of fundamental importance to the reliability of the data that will be produced. The experiment design should incorporate different storage protocols, such as refrigeration and freezing, and

addition or otherwise of chemical preservatives. The duration of the experiment should reflect the reality of the delay time necessary to perform the analysis of all the samples. Although, this experiment is only valid if the sample taken on Day 0 will be analysed immediately, or within a few hours after being sampled. This will set the basis of the experiment, so should be performed as soon as possible.

5) After analysing the results of the storage experiment, and concluding which storage protocol is the more reliable for maintaining original levels of nutrients concentration in the sample, surveys can be planned according to sampling sites and tide conditions. Always start axial transects at the same point, fresh or seawater ends, and proportionally at the same time in the tide regime, *i.e.* beginning of slack water during high or low tide. Tidal cycles surveys should be firstly performed during the same tide conditions and at different sectors of the estuary; secondly, should be performed under different tide regimes and at the same sector.
6) Analysis and interpretation of all data acquired (from previous available data and from data acquired during the surveys) should be carried out together. Previous acquired information can help explain or lead the discussion towards to common conclusions. Advanced statistical analytical methods, such as chemometrics can help to visualise and establish correlations between the variables, as well as provide predictive models of the nutrient behaviour in the estuarine environment studied.

REFERENCES

Ackroyd, D. R., Bale, A. J., Howland, R. J. M., Knox, S., Millward, G. E., Morris, A. W., 1986, Distributions and behavior of dissolved Cu, Zn and Mn in the Tamar Estuary, *Estuarine Coastal And Shelf Science,* **23** , 621-640.

Afif, E., Matar, A. and Torrent, J., 1993, Availability of phosphate applied to calcareous soils of West Asia, *Soil Science Society of America Journal,* **57**, 756-760.

Alhajjar, B. J., Harkin, J. M. and Chester, G., 1989, Detergent formula and characteristics of wastewater in septic tanks, *Journal of the Water Pollution Control Federation,* **61**, 605-613.

Aminot, A. and Andrieux, F., 1996, Concept and determination of exchangeable phosphate in aquatic sediments, *Water Research,* **30**, 2805-2811.

Aminot, A. and Kerouel, R., 1997, Assessment of heat treatment for nutrient preservation in seawater samples, *Analytica Chimica Acta,* **351**, 299-309.

Anderson, L., 1979, Simultaneous spectrophotometric determination of nitrite and nitrate by flow injection analysis, *Analytica Chimica Acta.,* **110,** 123-128.

Aoki, T. and Wakabayashi, M., 1995, Simultaneous flow injection determination of nitrate and nitrite in water by Gas-Phase Chemiluminescence, *Analytica Chimica Acta,* **308**, 308-312.

APHA-AWWA-WEF, 1998, *Standard methods for the examination of water and wastewater.* American Public Health Association. Washington, DC, 1220 p.

Avanzino, R. J. and Kennedy, V. C., 1993, Long-term frozen storage of stream water for dissolved orthophosphate, nitrate plus nitrite, and ammonia analysis. *Water Resources Research,* **16**, 3357-3362.

Bajic, S. J. and Jaselskis, B., 1985, Spectrophotometric determination of nitrate and nitrite in natural-water and seawater, *Talanta,* **32,** 115-118.

Baldwin, D. S., Beattle, J. K., Coleman, L. M. and Jones, D. R., 1995, Phosphate ester hydrolysis facilitated by mineral phases, *Environmental Science and Technology,* **29**, 1706-1709.

Baldwin, D. S., Beattle, J. K., Coleman, L. M. and Jones, D. R., 2001, Hydrolysis of an organophosphate ester by manganese dioxide, *Environmental Science and Technology,* **35**, 713-716.

Baldwin, D. S., Mitchell, A. M. and Olley, J. M., 2002, Pollutant-sediment interactions: sorption, reactivity and transport of phosphorus, In: *Agriculture, Hydrology and Water Quality* (Eds. Haygarth, P. M. and Jarvis, S.), CABI Press International, Wallingford, UK.

Bale, A. J., Barret, C. D., West, J. R. and Oduyemi, K. O. K., 1989, *Developments in Estuarine and Coastal Study Techniques* (Eds. McManus, J. and Elliot, M.) Olsen and Olsen, Fredensborg, 133-138.

Bale, A. J., Morris, A. W. and Howland, R. J. M., 1985, Seasonal sediment movement in the Tamar Estuary, *Oceanology Acta*, **8**, 1-6.

Baretta-Becker, J. G., Baretta, J. W., Hansen, A. S. and Riemann, B., 1998, An improved model of carbon and nutrient dynamics in the microbial food web in marine enclosures, *Aquatic Microbial Ecology*, **14**, 91-108.

Bendschneider, K. and Robinson, R. J., 1952, *Journal of Marine Science*, **11**, 87.

Biggs, B. J. F., 2000, Eutrophication of streams and rivers: Dissolved nutrient-chlorophyll relationships for benthic algae, *Journal of the North American Benthological Society*, **19**, 17-31.

Blundell, N. J., Worsfold, P. J., Casey, H. and Smith S., 1995, The design and performance of a portable, automated flow injection monitor for the in-situ analysis of nutrients in natural waters, *Environment International*, **21**, 205-209.

Booker, F., 1971, *Industrial Archaeology of The Tamar Valley*, 2nd Impression, David & Charles Publishers, Newton Abbot, 303 p.

Boon, P. I., 1993, Organic matter degradation and nutrient regeneration in Australian fresh waters: III. Size Fractionation of Phosphatase Activity, *Archive fur Hydrobiologie*, **126**, 339-360.

Bower, C. E., and Holm-Hansen, T., 1980, Simplified hydrazine-reduction method for determining high concentrations of nitrate in recirculated seawater, *Aquaculture*, **21**, 281-286.

Boyle, E., Collier, R., Dengler, A. T., Edmond J. M., Ng, A. C. and Stallard, R. F., 1974, On the chemical mass-balance in estuaries, *Geochimica et Cosmochimica Acta*, **38**, 1719-1728.

Brezonick, P. L. and Stadelmann, T. H., 2002, Analysis and predictive models of stormwater runoff volumes, loads, and pollutant concentrations from watersheds in the twin cities metropolitan area, Minessota, USA, *Water Research*, **36**, 1743-1757.

Brezonik, P. L. and Lee, G. F., 1966, Preservation of water samples for inorganic nitrogen analysis with mercuric chloride, *International Journal of Air and Water Pollution*, **10**, 549-553.

Broberg, O. and Persson, G., 1998, Particulate and dissolved phosphorus forms in freshwater: composition and analysis, *Hydrobiologia*, **170**, 61-90.

Brown, L. and Bellinger, E. G., 1978, Nitrate determination in fresh and some estuarine waters by ultraviolet light absorption: a new proposed method, *Water Research,* **12,** 223-229.

Burke, E. M., Suarez, F. X., Hillman, D. C., and Helthmer, E. M., 1989, The evaluation and comparison of ion chromatography, segmented flow-analysis and flow-injection analysis for the determination on nitrate in natural surface waters, *Water Research,* **23**, 519-521.

Burn, D. H., 1994, Hydrological effects of climatic change in the west central Canada. *Journal of Hydrology,* **160**, 53-70.

Burt, T. P., Heathwaite, A. L. and Johnes, P. J., (1996), Stream water quality and nutrient export in the Slapton catchments, *Field Studies,* **8**, 613.

Cameron, W. M. and Pritchard, D. W., Estuaries. In: *The Sea* (Ed. Hill, M. N.), Vol. 2, Wiley, New York, 306-324.

Carlson, R. E., 1977, A trophic state index for lakes, *Limnology and Oceanography,* **22,** 361-369.

Casey, H. and Newton, P. V. R., 1973, The chemical composition and flow of the River Frome and it's main tributaries, *Freshwater Biology,* **3**, 317-333.

Casey, H., 1992, Collection, filtration and storage of samples for water quality monitoring, *Analytical Proceedings,* **29**, 401-402.

Casey, H., Clarke, R. T., Smith, S. M., Clinch, J. R. and Worsfold, P. J., 1989, Spectrophotometric field monitor for the determination of nitrate in river water, statistical analysis of the results from nine-month field trial, *Analytica Chimica Acta,* **227**, 379-385.

Cawse, P. A., 1967, *Analyst,* **92**, 311.

Chamberlain, W. M. and Shapiro, J., 1969, On the biological significance of phosphate analysis: comparison of standard and new methods with a bioassay, *Limnology and Oceanography,* **14**, 921-927.

Chambers, R. M., Fourqurean, J. W., Hollibaugh, J. T., Vink. S. M., 1995, Importance of terrestrially-derived, particulate phosphorus to phosphorus dynamics in a west-coast estuary, *Estuaries,* **18,** 518-526.

Chen, H., Fang, Y, Taicheng, A. N., Zhu, K. and Lu, J., 2000, Simultaneous spectrophotometric determination of nitrite and nitrate in water samples by flow-injection analysis, *International Journal of Environmental Analytical Chemistry*, **76**, 89-98.

Chiew, F. H. S. and McMahon, T. A., 1993. Detection of trend or change in annual flow of australian rivers, *International Journal of Climatology*, **13**, 643-653.

Chow, J. J., and Johnstone, M. S., 1962, Determination of nitrate in sea water, *Analytica Chimica Acta*, **27**, 446-453.

Ciavatta, C., Antisari, L.V. and Sequi, P., 1990, Interference of soluble silica in the determination of orthophosphate-phosphorus, *Journal of Environmental Quality*, **19**, 761-764.

Clarke, S., Towner, J. V. and Yeoman, S., 1992, *Freshwater Quality: Pollution Impact of Cleaning Products*, Additional reports undertaken for the Royal Commission of Environmental Pollution, London, 133-183.

Clementson, L. A. and Wayte, S. E., 1992, The effect of frozen storage of open-ocean seawater samples on the concentration of dissolved phosphate and nitrate, *Water Research*, **26**, 1171-1176.

Clesceri, N. J. and Lee, G. F., 1965, Hydrolysis of condensed phosphates-I: non-sterile environment, *International Journal of Air and Water Pollution*, **9**, 723-742.

Clinch, J. R., Worsfold, P. J and Casey, H., 1987, An automated spectrophotometric field monitor for water quality parameters, determination of nitrate, *Analytica Chimica Acta*, **200**, 523-531.

Coetzee, J. F. and Gardner, C. W., 1986, Determination of sulfate, orthophosphate and triphosphate ions by flow injection analysis with the lead Ion Selective Electrode as Detector. *Analytical Chemistry*, **58**, 608-611.

Colina, M. and Gardiner, P. H. E., 1999., Simultaneous determination of total nitrogen, phosphorus and sulphur by means of microwave digestion and ion chromatography, *Journal of Chromatography A*, **847**, 285-290.

Crompton, T. R., 1992, *Comprehensive Water Analysis, Vol 1:Natural Waters*. Elsevier Applied Science, London, 524 p.

Crompton, T. R., 1989, *Analysis of Seawater*, Butterworths, London, 423p.

Crouch, S. R. and Malmstadt, H. V. A., 1967, A mechanistic investigation of molybdenum blue method for determination of phosphate. *Analytical Chemistry*, **39**, 1084-1089.

Currie, D. J. and Kalff, J., 1984, A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus, *Limnology and Oceanography*, **29**, 298-310.

Dalrymple, R. W., Zaitlin, B. A. and Boyd, R., 1992, A conceptual model of estuarine sedimentation, *Journal of Sedimentology and Petrology*, **62**, 1130-1146.

Daniel, A., Birot, D., Blain, S., Tréguer, P., Leildé and Menut, E., 1995a, A submersible flow-injection analyser fro the in-situ determination of nitrite and nitrate in coastal waters, *Marine Chemistry*, **51**, 67-77.

Daniel, A., Birot, Lehaitre, M. and Poncin, J., 1995b, Characterization and reduction of interferences in flow injection analysis for the in situ determination of nitrate and nitrite in sea water, *Analytica Chimica Acta,* **308**, 413-424.

Dasgupta P. K., Bellamy H. S., Liu H., Lopez J. L., Loree J. L., Morris K., Petersen, K. and Kalan K. M., 1993, Light-emitting diode based flow-through optical-absorption detectors, *Talanta*, **40**, 53-74.

Davey, D. E., Mulcahy, D. E. and O'Connel, G. R., 1990, Flow-injection determination of phosphate with a cadmium ion-selective electrode. *Talanta,* **37**, 683-687.

David, A. R. J., McCormack, T., Morris, A. W. and Worsfold, P. J., 1998, A submersible flow injection-based sensor for yhe determination of total oxidised nitrogen in coastal waters, *Analytica Chimica Acta,* **361**, 63-72.

David, A. R. J., McCormack, T., Worsfold, P. J., 1999, A submersible battery-powered flow injection (fi) sensor for the determination of nitrate in estuarine and coastal waters, *Journal of Automated Methods and Management in Chemistry*, **21,** 1-9.

Davies, J. H., 1964, A morphogenetic approah to world shorelines, *Z. Geomorphology*, **8**, 127-142.

Denison, F. H., Haygarth, P. M., House, W. A. and Bristow, A. W., 1998, The measurement of dissolved phosphorus compounds: evidence for hydrolysis during storage and implications for analytical definitions in environmental analysis. *International Journal of Environmental Analytical Chemistry,* **69**, 111-123.

Dodds, W. K.; Jones, J. R.; Welch, E. B, 1998, Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus, *Water Research*, **32** ,1455-1462.

Doherty, A. P., 1996, Oxidative detection of nitrite at an electrocatalytic [Ru $(bipy)_2$poly-(4-vinylpyridine)$_{10}$Cl]Cl electrochemical sensor applied for the flow

injection determination of nitrate using a Cu/Cd reductor column, *Analytica Chimica Acta,* **319,** 111-120.

Dore, J. F., Houlihan, T., Hebel, D. V., Tien, G., Tupas, L. and Karl, D. M., 1996, Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater, *Marine Chemistry,* **53**, 173-185.

Douglas, E. M., Vogel, R. M. and Kroll, C. N., 2000, Trends in floods and low flows in the united states: impact of spatial correlation, *Journal of Hydrology,* **240**, 90-105.

Drummond, L. and Maher, W., 1995, Determination of phosphorus in aqueous solution via formation of the phosphoantimonylmolybdenum blue complex: re-examination of optimum conditions for the analysis of phosphate. *Analytical ChimicaActa,* **302**, 69-74.

Dunne, T. and Black, R. D., 1970, Partial area contributing to storm runoff in a small New England watershed, *Water Resources Research,* **6**, 1296-1311.

Dyer, K. R., 2000, *Estuaries: A Physical Introduction*, 2nd Edition, John Wiley & Sons, Chichester, 195p.

Dyer, K. R., Christie, M. C. and Wright, E. W., 2000, The classification of intertidal mudflats, *Continental Shelf Research*, **20**, 1039-1060.

EA, 2000, *Tamar Annual Review*, Local Environment Agency Plan, Bodmin, 100 p.

EA standards, Web Reference: www.environment-agency.gov.uk

EA, 1996, *Tamar Estuary and Tributaries*, Consultation Report, Local Environmental Agency Plan. Environment Agency, Bodmin, 215 p.

EA-SW, 1999, *The South West's Environment*, Environment Agency, Exeter, 38 p.

Ebdon, L., Braven, J. and Frampton, N.C,.1991, Nitrate-selective electrodes containing immobilized ion-exchangers within a rubbery membrane with controlled cross-link density, *Analyst,* **116,** 1005-1010.

Eisma, D. and Kalf, J., 1987, Dispersal, Concentration and deposition of suspended matter in the North Sea, *Journal of the Geological Society of London*, **144**, 161-178.

English Nature, 2000, *Plymouth Sound and Estuaries*, European Marine Site, 83 p.

Finch, M. S., Hydes, D. J., Clayson, C. H., Weigl, B., Dakin, J. and Gwilliam, P., 1998, a Low power ultra violet spectrophotometer for measurement of nitrate in

seawater: introduction, calibration and initial seatrials, *Analytica Chimica Acta,* **377**, 167-177.

Fitzgerald, G. P. and Faust, S. L., 1967, Effects of water sample preservation methods on the release of phosphorus from algae, *Limnology and Oceanography,* **12**, 332-335.

Floch, J., Blain, S., Birot, D. Treguer, P., 1998, In situ determination of silicic acid in sea water based on fia and colorimetric dual-wavelength measurements, *Analytica Chimica Acta*, **377**, 157-166.

Fogg, A. G., Scullion, S. P. and Edmonds, T. E., 1988, Differential-pulse polarographic-determination of nitrate as 3-nitrobenzoic acid and other nitro-derivatives, *Analyst,* **113,** 979-981.

Fogg, A. G., Scullion, S. P., Edmonds, T. E. and Birch, B. J., 1991, Direct reductive amperometric determination of nitrate at a copper electrode formed in *situ* in a capillary-fill sensor device, *Analyst,* **116,** 573-579.

Forsberg, C. and Ryding, S. O., 1980, eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. *Archaeology Hydrobiology,* **89,** 189-207.

Fox, L. E., Sager, S. L. and Wofsy, S. C., 1986, The chemical control of soluble phosphorus in the Amazon Estuary, *Geochimica Cosmochimica Acta*, **50**, 783-94.

Foy, R. H. and Withers, P. J. A., 1995, *The Contribution of Agricultural Phosphorus to Eutrophication*, Proceeding-Fertiliser Society, London, 4-26.

Fujiwara, K., 1994, Encyclopedia of Analytical Chemistry: *Phosphorus*, Townshend, A. (Ed.), Academic Press, London, **7**, 3950-3956.

Hanrahan, G., Gledhill, M., Fletcher, P. J. and Worsfold, P. J., 2001, High temporal resolution field monitoring of phosphate in the river frome using flow injection with diode array detection, *Analytica Chimica Acta*, **440**, 55-62.

Gächter, R., Meyer, J. S. and Mares, A., 1988, contribution of bacteria to release and fixation of phosphorus in lake sediments, *Limnology and Oceanography*, **33**, 1542-1558.

Galehouse, J. S., 1971, Sedimentation analysis. In: Carver RE, ed *Procedures in Sedimentary Petrology*, John Wiley & Sons, New York, 69-94.

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

Gan, T. Y., Kwong, Y. T. S., 1998, Identification of warming trends in northen

Alberta and southem northwest territories by the non-parametric Kendall's test. In: *Using hydrometric data to detect and monitor climatic change.* Proceedings of NHRI Workshop No. 8. Gite GW. (Ed. Harvey, K. D.). National Hydrology Research Institute: Saskatoon, Sask.; 43-56.

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

Gardwood, E. A. and Tyson, K. C., 1973, Losses of nitrogen and other plant nutrients to drainage from soil under grass, *Journal of Agricultural Science*, **80**, 303.

Garside, C., 1982, A chemi-luminescent technique for the determination of nanomolar concentrations of nitrate and nitrite in sea-water, *Marine Chemistry*, **11**, 159-167.

Gasser, J. K. R., (1982), Agricultural productivity and the nitrogen cycle, *Philosophical Transanctions Royal Society London* B, **296**, 303-314.

Gaugush, R. F. and Heath, R. T., 1984, A rapid manual method for nitrate determination in small volumes by a modification of the cadmium reduction method, *Water Research,* **18,** 449-450.

Gennaro, M. C., Bertolo, P. L. and Cordero, A., 1990, Determination of nitrite and nitrate in venice lagoon water by ion interaction reversed-phase liquid-chromatography, *Analytica Chimica.Acta.*, **239**, 203-209.

Gine, M. F., Bergamin., F., Zagatto, E. A. G., and Reis, B. F., 1980, Simultaneous determination of nitrate and nitrite by flow injection analysis, *Analytica Chimica Acta,* **114**, 191-197.

Goda, T., 1986, General review and new concepts regarding the development of human wastewater treatment in japan, *Water Science and Technology*, **18**, 137-.

Grasshoff, K. 1976. Filtration and storage. *In:* Grasshoff, K. (Ed), *Methods of Seawater Analysis,* Verlag Chemie, Weinheim, 21-30.

Hall, G. E. M., Bonham-Carter, G. F., Horowitz, A. J., Lum, K., Lemieux, C., Quemarais, B. and Garbarino, J. R. 1996, The effect of using different 0.45 μm filter membranes on dissolved element concentrations in natural waters, *Applied Geochemistry*, **11**, 243-249.

Halliwell, D. J., McKelvie, I. D., Hart, B. T. and Dunhill, R. H. 1996. Separation and detection of condensed phosphates in waste waters by ion chromatography coupled with flow injection, *Analyst,* **121,** 1089-1093.

Halliwell, D. J., McKelvie, I. D., Hart, B. T. and Dunhill, R. H., 2001, Hydrolysis of triphosphate from detergents in a rural waste water system, *Water Research*, **35**, 448-454.

Hanrahan, G., Gardolinski, P. C. F. C., Gledhill, M., and Worsfold, P. J., 2002, Environmental Monitoring of Nutrients. In *'Handbook of Environmental Monitoring'* (Eds. Burden, F.R., McKelvie, I., Förstner, U., and Guenther, A.) 8.1-8.16. McGraw-Hill, New York. 1100 p.

Hanrahan, G., Gledhill, M., Fletcher, P. J. and Worsfold, P.J., 200la, High temporal resolution field monitoring of phosphate in the river frome using flow injection with diode array detection, *Analytica Chimica Acta,* **440**, 55-62.

Hanrahan, G., Gledhill, M., House, W. A. and Worsfold, P. J., 2001b, Phosphorus loading in the Frome catchment, UK: seasonal refinement of the coefficient modelling approach, *Journal of Environmental Quality,* **30**, 1738-1746.

Haygarth, P. M. and Jarvis, S. C., 1999, Transfer of phosphorus from agricultural soils, *Avances in Agronomy*, **66**, 196-249.

Haygarth, P. M. and Sharpley, A. N., 2000, Terminology for phosphorus transfer, *Journal of Environmental Quality*, **29**, 10-15.

Haygarth, P. M., 1997, *Phosphorus Losses from Grassland Soils*: New understanding and concepts, IGER Innovations, 60-63.

Haygarth, P. M., Ashby, C. D. and Jarvis, S. C., 1995, Short-term Changes in the Molybdate Reactive Phosphorus of Stored Soil Waters, *Journal of Environmental Quality,* **24**, 1133-1140.

Heathwaite, A. L., Johnes, P. J. and Peters, N. E., 1996, Trends in Nutrients, *Hydrological processes*, **10**, 263-293.

Henderson-Sellers, B. and Markland, H., 1987, *Decaying Lakes*: Origin and control of eutrophication, Wiley, Chichester, 272 p.

Hens, M. and Merckx, R., 2002, The role of colloidal particles in the speciation and analysis of "dissolved" phosphorus, *Water Research*, **36**, 1483-1492.

Herbes, S. E., Allen, H. E., Mancy, K.H., 1975, enzymatic characterization of soluble organic phosphorus in lake water. *Science*, **187**, 432-434.

Hight, S. C., Betpera, F. and Jaselskis, B., 1982, Differential pulse polarographic determination of orthophosphate in aqueous media, *Talanta,* **29**, 72 1-724.

Hirsch, R. M. and Slack, J. R., 1984. Non-parametric trend test for seasonal data with serial dependence. *Water Resources Research,* **20**, 727-732.

Horowitz, A. J., Elrick, K. A. and Colberg, M. R., 1992, The effect of membrane filtration artifacts on dissolved trace element concentrations, *Water Research,* **26**, 753-763.

Horton, R. E., 1933, The Role of Infiltration in the Hydrologic Cycle. *Eos Transactions, American Geophysical Union,* **14**, 446-460.

House, W. A., Casey, H. and Smith, S. 1986, Factors affecting the coprecipitation of phosphate with calcite in hardwaters - II. Recirculating experimental stream system. *Water Research,* **20**, 923-927.

Howarth, M. J., Dyer, K. R., Joint, I. R., Hydes, D. J., Purdie, D. A, Edmunds, H., Jones, J. E., Lowry, R. K., Moffat, T. J., Pomroy, A. J. and Proctor, R., 1993, Seasonal cycles and their spatial variability, *Philosophical Transactions Of The Royal Society Of London Series A-Mathematical Physical And Engineering Sciences,* **343,** 383-403.

Hurd, D. C. and Spencer, D. W. (Eds), 1991, *Marine Particles: Analysis and Characterization,* American Geophysical Union, Geophysical Monograph, 63p.

Hutchinson, G. E., 1969, *Eutrophication: Causes, Consequences, Correctives,* National Academy of Sciences, Washington, D.C., 1-13.

Hydes, D. J., Kelly-Gerrey, B. A., Le Gall, A. C. and Proctor, R., 1999, The balance of supply of nutrients and demands of biological production and denitrification in a temperate latitude shelf sea – a treatment of the southern North Sea as an extended estuary, *Marine Chemistry,* **68**, 117-131.

Hydes, D. J., and Hill, N. C., 1985, Determination of nitrate in seawater - nitrate to nitrite reduction with copper-cadmium alloy, *Estuarine, Coastal and Shelf Science,* **21,** 127-130.

Imray, 2001, Plymouth Harbour and Rivers Chart (C14), Imray Laurie Norie & Wilson Ltd, Huntingdon.

Iserman, K., 1990, Share of nitrogen and phosphorus emissions into the surface water of western europe against the background of their euthrophication, *Fertilizer Research,* **26**, 253-269.

Janse, T. A. H. M., Van Der Wiel, P. F. A. and Kateman, G., 1983, Experimental optimization proceduresin the determination of phosphate by flow-injection analysis. *Analytica ChimicaActa,* **155**, 89-102.

Jarvie, H. P, Withers, P. J. A. and Neal, C., 2002, Review of robust measurement of phosphorus in river water: sampling, storage, fractionation and sensitivity, *Hydrology and Earth System Sciences*, **6**, 113-132.

Jenkins, D., and Medsker, L. L., 1964, Brucine method for the determination of nitrate in ocean, estuarine, and fresh waters, *Analytical Chemistry*, **36**, 610-612.

Jiang, S. J. and Houk, R. S., 1988, Inductively Coupled Plasma Mass Spectrometric detection for phosphorus and sulfur compounds separated by Liquid Chromatography, *Spectrochimica Acta*, **43B**, 405-411.

Kargi, F., Dincer, A. R., 2000, Use of halophilic bacteria in biological treatment of saline wastewater by fed-batch operation, *Water Environment Research*, **72**, 170-174.

Kelly, M. G.; Whitton, B. A., 1995, The trophic diatom index: A new index for monitoring eutrophication in rivers, *Journal of Applied Phycology*, **7**, 4232-4244.

Kempers, A. J. and Luft, A. G., 1988, Re-examination of the determination of environmental nitrate as nitrite by reduction with hydrazine, *Analyst*, **113**, 1117-1120.

Kim, H. J. and Kim, Y. K., 1989, determination of nitrite in drinking-water and environmental-samples by ion exclusion chromatography with electrochemical detection, *Analytical Chemistry*, **61**, 1485-1489.

Kim, S. H. and Delleur, J. W., 2001, Stochastic structures between quantity and quality responses of rainfall-runoff at an upland agricultural watershed, *Water Science and Technology*, **44**, 91-104.

Kirkwood, D. S., 1992, Stability of solutions of nutrient salts during storage, *Marine Chemistry*, **38**, 151-164.

Knox, S., Whitfield, M., Turner, D. R., Liddicoat, M. I., 1986, Statistical-analysis of estuarine profiles .3. application to nitrate, nitrite and ammonium in the Tamar Estuary, *Estuarine Coastal and Shelf Science*, **22**, 619-636.

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

Lambert, D. W. Maher and I. Hogg, 1992, Changes in phosphorus fractions during storage of lake water, *Water Research*, **26**, 645-648.

Lambert, R. S., and DuBois, R. J., 1977, *Analytical Chemistry*, **47**, 955.

Latterell, J. J., Thomas, D. R., Holt, R. F. and Sherstad, E. M., 1974, Sorption of

orthophosphate on the surface of water sample containers, *Water Resources Research,* **10**, 865-869.

Lau S. S. S. and Chu, L. M., 1999, Contaminant release from sediments in a coastal wetland, *Water Research,* **33**, 909-918.

Laxen, D. P. H. and Chandler, I. M., 1982, Comparison of filtration techniques for size distribution in fresh-waters, *Analytical Chemistry,* **54**, 1350-1355.

Lettenmaier, D. P., Wood, E. F. and Wallis, J. R., 1994. Hydro-climatological trends in the continental United States, 1948-88. *Journal of Climate,* **7**, 586-607.

Lowry, R., Cramer, R. N. and Richards, L. J., 1992, *North Sea Project CD ROM and Users Guide,* British Oceanographic Data Centre, Natural Environment Research Council, Swindon, 168 p.

Lund, J. W. E. and Moss, B., 1990, *Eutrophication in the United Kingdom-Trends in the 1980s,* The Soap and Detergent Industry Association.

Macdonald, R. W. and Mclaughlin, F. A., 1982, The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate for samples from coastal and estuarmne waters, *Water Resources Research,* **29**, 95-104.

Macdonald, R. W., Mclaughlin, F. A. and Wong, C. S., 1986, The storage of reactive silicate samples by freezing, *Limnology and Oceanography,* **31**, 1139-1141.

Maher W. and Woo L., 1998, Procedures for the storage and digestion of natural waters for the determination of filterable reactive phosphorus, total filterable phosphorus and total phosphorus, *Analytica Chimica Acta,* **375**, 5-47.

Maimó, J., Cladera, A., Mas, F., Forteza, R., Estela, L. M. and Cerdá, 1989, automatic system for simultaneous determination of nitrates and nitrites in waters, *International Journal of Environmental Analytical Chemistry,* **35**, 161-167.

Manzoori, J. L., Miyazakia, A. and Tao, H., 1990, Rapid differential flow injection of phosphorus compounds in waste-water by Sequential Spectrophotometry and Inductively Coupled Plasma Atomic Emission Spectrometry using a vacuum Ultraviolet Emission Line, *Analyst,* **115**, 1055-1058.

Margeson, J. H., Suggs, J. C., and Midgett, M. R., 1980, Reduction of nitrate to nitrite with cadmium, *Analytical Chemistry,* 52, 1955-1957.

Marrs, R. H., Gough, M. W. and Griffiths, M., 1991, Soil chemistry and leaching losses of nutrients from semi-natural grassland and arable soils on three contrasting parent materials, *Biological Conservation,* **57**, 257-271.

Martelli, P. B., Neto, J. A. G., Zagatto, E. A. G., Brienza, S. M. B., Montenegro, M. C. B. S. M. and Lima, J. L. F. C., 1995, Sequential analyte removal in flow analysis: determination of nitrogen, phosphorus and potassium in fertilizers, *Analytica Chimica Acta*, **317**, 239-245.

Massart, D. L., Vandeginste, B. G. M., Buydens, L. M. C., De Jong, S., Lewi, P. J. and Smeyers-Verbeke, J., 1997, *Handbook of Chemometrics and Qualimetrics*: Part A, Data Handling in Science and Technology, Vol. **20A**, Amsterdam, 867 p.

Matsunanga, K., and Nishimura, M., 1969, Determination of nitrate in sea water, *Analytica Chimica Acta,* **45,** 350-353.

McKelvie, I. D., Hart, B. T., Cardwell, T. J. and Cattrall, R. W., 1989, Spectrophotometric determination of dissolved organic phosphorus in natural waters using in-line photo-oxidation and flow injection, *Analyst*, **114**, 1459-1463.

McKelvie, I. D., Peat, D. and Worsfold, P. J., 1995, Techniques for the quantification and speciation of phosphorus in natural waters. *Analytical Procedures*, **32**, 437-445.

McKelvie, I. D., Hart, B. T., Cardwell, T. J. and Cattrall, 1993, Speciation of dissolved phosphorus in environmental samples by Gel Filtration and Flow-Injection Analysis, *Talanta*, **40**, 1981-1993.

Merry, I., 1980, *The Shipping and Trade of the River Tamar*, Part I, Maritime Monographs and Reports, no. 46. National Maritime Museum, Greenwich, London, 38 p.

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

Millward, G. E., 1995, Processes affecting trace element speciation in estuaries, *Analyst*, **120**, 609-614.

Millward, G. E., Turner, A., Glasson, D. R. and Glegg, G. A., 1990, Intra-estuarine and inter-estuarine variability of particle microstructure, *Science of the Total Environment*, **97/98**, 289-300.

Moldan, B. and Cerný, J.,1994, *Biogeochemistry of Small Catchements, A Tool for Environmental Research*, SCOPE 51, John Wiley & Sons, Chichester, 419 p.

Mommaerts, J. P., 1969, On the distribution of major nutrients and phytoplankton in the Tamar Estuary, *Journal of Marine Biological Association*, **49**, 749-765.

Mommaerts, J. P., 1970, On the unususal patterns of phosphate vertical distribution in the Tamar Estuary, *Journal of the Marine Biological Association*, **50**, 849-855.

Montgomery, H. A. C. and Dymcock, J. F., 1962, *Analyst*, **87**, 374.

Morgan, D. K. and Danielson, N. D., 1983, enzymatic determination of phosphate in conjunction with high-performance liquid chromatography, *Journal of Chromatography*, **262**, 265-276.

Morries, P., 1971, *Water Treatment Exam.*, **20**, 132.

Morris, A. W., 1986, removal of trace-metals in the very low salinity region of the Tamar Estuary, England. *Science of the Total Environment* **49**, 297-304.

Morris, A. W., Bale, A. J.; Howland, R. J. M., Millward, G. E., Ackroyd, D. R. Loring, D. H. and Rantala, R. T. T., 1986a, Sediment mobility and its contribution to trace-metal cycling and in a macrotidal estuary, *Water Science and Technology*, **18**, 111-119.

Morris, A. W., Bale, A.J.; Howland, R. J. M., 1986b, Dissolved aluminium in the Tamar Estuary, Southwest England, *Geochimica et Cosmochimica Acta*, **50**, 189-197.

Morris, A. W., 1985, Chemical and ecological responses to variable river discharge in The Tamar Estuary. *Estuaries*, **8**, A54

Morris, A. W., Howland, R. J. M. Woodward, E. M. S., Bale, A. J. and Mantoura, R. F. C., 1985, Nitrite and ammonia in the Tamar Estuary. *Netherlands Journal of Sea Research*, **19**, 217-222.

Morris, A. W., Bale, A. J. and Howland, R. J. M., 1982a, Chemical variability in the Tamar Estuary, Southwest England. *Estuarine Coastal and Shelf Science*, **14**, 649-661.

Morris, A. W., Bale A. J., and Howland R. J. M., 1982b, The dynamics of estuarine manganese cycling. *Estuarine Coastal and Shelf Science* **14**, 175-192.

Morris, A. W., Bale, A. J. and Howland, R. J. M., 1981, Nutrient distributions in an estuary - evidence of chemical precipitation of dissolved silicate and phosphate. *Estuarine Coastal and Shelf Science*, **12**, 205-216.

Morris, A. W., and Riley, J. P., 1963, The determination of nitrate in sea water, *Analytica Chimica Acta*, **29**, 272-279.

Morse, J. W., Hunt, M., Zulling, J., Mucci, A. and Mendez, T., 1982, A comparison of techniques for preserving dissolved nutrients in open ocean seawater samples. *Ocean Science and Engineering*, **7**, 75-106.

Motomizu, S., Mikasa, H. and Toei, K., 1987, Fluorometric-determination of nitrate in natural-waters with 3-amino-1,5-naphthalenedisulphonic acid in a flow-injection system, *Analytica Chimica Acta.*, **193,** 343-347.

Mullin, J. B. and Riley, J. P., 1955, The spectrophotometric determination of nitrate in natural waters, with particular reference to sea-water, *Analytica Chimica Acta,* **12,** 464-480.

Munson, R. K., Roy, S. B., Gherini, S. A., Macneill, A. L., Hudson, R. J. M. and Blette, V. L., 2002, Model prediction of the efects of changing phosphorus loads on the Everglades Protection Area, *Water, Air, and soil Pollution*, **134**, 255-273.

Murphy, J. and Riley, P.A., 1962, A modified single solution method for the determination of phosphorus in natural waters, *Analytica Chimica Acta,* **27**, 31-36.

National Rivers Authority. 1995, *The Frome and Piddle Catchment Management Plan Consultation Report*, NRA, Blandford Forum, Dorset.

Noufi, M., Yarnitzky, C. and Ariel, M., 1990, Determination of nitrate with a flow-injection system combining square-wave polarographic detection with online deaeration, *Analytica Chimica Acta,* **234,** 475-478.

NRA Report – National Rivers Authority, 1990, Water Quality Series No. 2, *Toxic Blue-Green Algae.*

NRSS/CDMO, 2001. National Estuarine Research Reserve System (NERRS), Centralized Data Management Office (CDMO), http://cdmo.baruch.sc.edu/

NSTF, 1993, North Sea Task Force – *Quality Status Report*, Oslo and Paris Commissions, London. Olsen and Olsen, Fredensborg, Denmark, 132 p.

Nydahl, F., 1976, On the optimum conditions for the reduction of nitrate to nitrite by cadmium, *Talanta,* **23**, 349-357.

OECD (Organization for Economic Cooperation and Development), 1982, *Eutrophication of Waters. Monitoring assessment and control*, Final Report. OECD Cooperative Programme on Monitoring of Inland Waters, Environment Directorate, OECD, Paris, 154p.

Oudot, C. and Montel, Y., 1988, A high-sensitivity method for the determination of nanomolar concentrations of nitrate and nitrite in seawater with a Technicon Autoanalyzer-II, *Marine Chemistry,* **24**, 239-259.

Pai, S.C. and Riley. J.P., 1994, Determination of nitrate in the presence of nitrite in natural-waters by flow-injection analysis with a non-quantitative online cadmium reductor, *International Journal of Environmental Analytical Chemistry,* **57**, 263-277.

Paige, R. T., 1982, *The Upper Tamar Valley A Century Ago,* Dartington Amenity Research Trust, Tavistock, 95 p.

Parkinson R. J., 1993 Nitrate Processes, Patterns and Management: In '*Change in agricultural practice*', Burt T. P., Heathwaite, A. L. and Trudgill (Eds.), John Wiley & Sons, Chichester, 321-323.

Parsons, T. R., 1975, in: Riley, J. P. and Skirrow, G., (eds.), *Chemical Oceanography,* Vol.2., Academic Press, London, 647 p.

Pauer, J. J., Vanvliet, H. R. and Vanstaden, J. F., 1988, Determination of phosphate at low concentrations in surface waters by flow injection analysis. *Water SA,* **14,** 125-130.

Petticrew, E. L. and Arocena, J. M., Evaluation of iron-phosphate as a source of internal lake phosphorus loadings, *The Science of The Total Environment,* **266**, 87-93.

Pichete, P., Jamati, K. and Goulden, P. D., 1979, Preservation du contenu en o-phosphate D'Echantillons D'eau du fleuve Saint-Laurent, *Water Research,* **13**, 1187-1191.

Pritchard, D. W., 1952, Salinity distribution and circulation in the chesapeake bay estuarine system, *Journal of Marine Research,* **11**, 106-123.

Pritchard, D. W., 1955, Estuarine circulation patterns, *Proceeding of American Society of Civil Engineering,* **81**, No.717.

Raimbault, P., Slawyk, G., Coste, B. and Fry, J., 1990, feasibility of using an automated colorimetric procedure for the determination of seawater nitrate in the 0 to 100 nM range - examples from field and culture, *Marine Biology,* **104,** 347-351.

Reckhow, K. H., Clements, J. T. and Dodd, R., 1990, Statistical evaluation of mechanistic water-quality models, *Journal of Environmental Engineering,* **116**, 250-268.

Reneau, R. B., Hagedorn, C. and Degen, M. J., 1989, Fate and transport of biological and inorganic contaminants from on-site disposal of domestic wastewater, *Journal of Environmental Quality,* **18**, 135-144.

Rennie, P. J., Sumner, A. M., and Basketter, F. B., 1979, Determination of nitrate in raw, potable and waste waters by ultraviolet spectrophotometry. *Analyst,* **104,** 837-845.

Riley, J. P., 1965, The automatic determination of nitrate in sea water. *Deep Sea Research and Oceanographic Abstracts,* **12,** 765-772.

Rizzo, W. M. and Cristian, R. R., 1996, Significance of subtidal sediments to heterotrophically-mediated oxygen and nutrient dynamics in a temperate estuary, *Estuaries,* **19**, 2B, 457-487.

Robards, K., I. D. McKelvie, R. L. Benson, P. J. Worsfold, N. J. Blundell and H. Casey, 1994, Determination of carbon, phosphorus, nitrogen and silicon species in waters, *Analytical Chimica Acta,* **287**, 147-190.

Ron Vaz, M. D., Edwards, A. C., Shand, C. A. and Cresser, M., 1992, Determination of dissolved organic phosphorus in soil solutions by an improved automated photo-oxidation procedure, *Talanta,* **39**, 1479-1487.

Ruzicka J. and Hansen, E. H., 1988, *Flow injection analysis.* 2nd ed. [Chemical Analysis, 62] New York: John Wiley & Sons. 498pp.

Ryden, J. C., Syers, J. K. and Harris, R. F., 1972, Sorption of inorganic phosphorus by laboratory ware, implications in environmental phosphorus techniques, *Analyst,* **97**, 903-908.

Rydin, E., 2000, Potentially mobile phosphorus in Lake Erken sediment, *Water Research,* **34**, 2037-2042.

Seitzinger, S. P., Sanders, R. W. and Styles R., 2002, Bioavailability of DON from natural and anthropogenic sources to estuarine plankton, *Limnology and Oceanography,* **47**, 2, 353-366.

Shepard, F. P., 1954, Nomenclature based on sand-silt-clay ratios, *Journal of Sedimentary Petrology,* **24**, 151-158.

Skjemstad, J. O. and Reeve, R., 1978, The automatic determination of ppb levels of ammonia, nitrate plus nitrite, and phosphate in water in the presence of added mercury(Ll) chloride, *Journal of Environmental Quality,* **7**, 137-141.

Smith, K. A., Jackson, D. R. and Pepper, T. J., 2001, Nutrient losses by surface run-off following the application of organic manures to arable land. 1 Nitrogen, *Environmental Pollution,* **112**, 41-51.

Solórzano, L. and Strickland J. D., 1968, Polyphosphate in Seawater, *Limnology and Oceanography,* **13**, 515-518.

Stainton, M. P., 1974, Simple, efficient reduction column for use in the automated determination of nitrate in water, *Analytical Chemistry,* **46**, 1616.

Stevens, R. J., 1979, Evaluation of an enzymatic method for orthophosphate determination in freshwaters, *Water Research,* **13**, 763-770.

Stevenson, F. J., 1982, Organic Phosphorus Sulfur Compunds, In: *Humus Chemistry. Genesis, Composition, Reactions,* John Wiley & Sons, New York,. 120-145.

Stumm, W. and Morgan, J. J., 1996, *Aquatic Chemistry,* Second Ed., Wiley, New York, 703p.

Tarapchak, S. J., 1983, soluble reactive phosphorus measurements in lake water: evidence for molybdate-enhanced hydrolysis, *Journal of Environmental Quality,* **12**, 105-108.

Tortajada-Genaro, L. A., 2002, *Soluciones Quimiométricas para Optimizar el Análisis de Parámetros Químicos en Aguas,* Tesis Doctoral Europeia, Universitat de Valencia, 305p.

Turner, B. L., Papházy, M. J., Haygarth, P.M., McKelvie, I.D., 2002, Inositol Phosphates in the Environment. *Philosophical Transactions Royal Soc: Biological Sciences,* **357**, 449-470.

Ukita, M., Nakanishi, H. and Sekine, M., 1986, the pollutant load factor of household wastewater, *Water Science and Technology,* **18**, 157-167.

van Staden, J. F., 1982, Automated simultaneous determination of nitrate and nitrite by pre-valve reduction of nitrate in a flow-injection system, *Analytica Chimica Acta,* **138**, 403-408.

van Staden, J. F., Joubert, A. E. and van Vliet, H. R., 1986, Flow-Injection determination of nitrate in natural-waters with copper-cadmium and copperized cadmium tubesin the reaction manifold system, *Fresenius Z. Analytical Chemistry,* **325**, 150-152.

Vau Beusekom, J. E. E. and Brockmann, U. H., 1998, Transformation of phosphorus in the Elbe Estuary, *Estuaries,* **21**, 4A, 518-526.

Verma, K. K. and Verma, A., 1992, determination of nitrite by precolumn derivatization and high-performance liquid-chromatography, *Analytical Letters,* **25**, 2083-2093.

Vesely, J., 1990, Stability of the pH and the contents of ammonium and nitrate in precipitation samples, *Atmospheric Environment,* **24A**, 3085-3089.

Vollenweider, R. A., Giovanardi, F., Montanari, G. and Rinaldi, A., 1998, Characterization of the trophic conditions of marine coastal waters with special

reference to the NW Adriatic Sea: Proposal for a trophic scale, turbidity and generalized water quality index. *Environmetrics*, **9** (3), 329-357.

Von Starch, V. E., 1995, Misuses of statistical analysis in climate research. In *Analysis of Climate Variability: Applications of Statistical Techniques*, (Eds. von Starch, H., Navarra, A). Springer-Verlag: Berlin, 11-26.

Vorob'eva, I. S. and Gurskil, V. S. 2000. Determination of the total content of N, P, and S in water using ion-chromatography. *Industrial Laboratory Diagnostics of Materials,* **66,** 505-507.

West, J. R., Oduyemi, K. O. K., Bale, A. J. and Morris A. W., 1990, The field measurement of sediment transport parameters in estuaries. *Estuarine Coastal and Shelf Science* **30**, 167-183.

Whelan, B. R. and Titamni, Z. V., 1982, Daily chemical variability of domestic septic tank effluent, *Water, Air and Soil Pollution*, **17**, 131-139.

Willie, S. and Clancy, V., 2000, NOAA/NRC Intercomparison for nutrients in seawater. NOAA Technical Memorandum NOS, NCCOS, CCMA 143. Silver Springs, Maryland. 36p.

Willis, R. B., 1980, Reduction column for automated determination of nitrate and nitrite in water, *Analytical Chemistry,* **52**, 1376-1377.

Wood, E. D., Armstrong, F. A. J. and Richards, F. A., 1967, Determination of nitrate in sea water by cadmium-copper reduction to nitrite , *Journal of Marine Biological Association, 47,* 23-31.

Worsfold, P. J., Achterberg, E. P., Bowie, A. R., Sandford, R., Cannizzaro, V., Gardolinski, P. C. F. C., 2002, Flow injection techniques for the in situ monitoring of marine processes. In *'Chemistry of Marine Water and Sediments'* (Eds. Gianguzza A., Pelizzetti, E., and Sammartano S.), 385-402.

Worsfold, P. J., Clinch, J. R. and Casey H., 1987, Automated spectrophotometric field monitor for water quality parameters, determination of phosphate, *Analytica Chimica Acta*, **197**, 43-50.

Yue, S., Pilon, P., Phinney, B. and Canavadis, G., 2002, The influence of autocorrelation on the ability to detect trend in hydrological series, *Hydrological Process*, **16**, 1607-1829.

Yulianti, J. S. and Burn, D. H., 1998, Investigating links between climatic warming and low streamflow in the Prairies region of Canada. *Canadian Water Resources Journal,* **23**, 45-60.

Zhang, J. and Ortner, P. B., 1998, Effect of thawing condition on the recovery of reactive silicic acid from frozen natural water samples, *WaterResearch,* **32**, 2553-2556.

Zhang, J. Z., 2000, Shipboard automated determination of trace concentrations of nitrite and nitrate in oligotrophic water by gas-segmented continuous flow analysis with a liquid waveguide capillary flow cell, *Deep-Sea Research I,* **47**, 1157-1171.

Zhang, X., Harvey, K. O., Hogg, W. D. and Yuzyk, T. R., 2001, Trends in Canadian streamflow. *Water Resources Research,* **37,** 987-998.

Zwolsman, J. J. G. and van Eck, G. T. M., 1993, Dissolved and particulate trace metal geochemistry in the Scheldt Estuary, S.W. Netherlands (water column and sediments), *Netherlands Journal of Aquatic Ecology,* **27**, 287-300.

APPENDICES